THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON EXERCISE METABOLISM

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SUMMARY

The endurance capacity of individuals is diminished during exercise in the heat, such that heat stress leads to an earlier onset of fatigue. Chronic exposure to hot conditions, however, may reverse this process. Exercise in cool conditions can either improve or reduce exercise tolerance depending on the severity of the environmental temperature and/or the intensity of the exercise. Research examining the effect of exercise in different ambient temperatures on muscle metabolism, in particular carbohydrate metabolism, has produced conflicting results. The difinitive mechanisms whereby exercise in different environmental conditions affects muscle metabolism have yet to be determined. The studies reported in this dissertation examined muscle metabolism during exercise in different environmental conditions and in different states of heat acclimation. Furthermore, possible mechanisms proposed for such metabolic alterations were investigated.

A total of 27 subjects participated in four separate studies which involved bicycle ergometry. The first three studies utilised endurance trained subjects, who cycled at a submaximal workload at between 65 and 70% VO_2max for a period of 40 minutes. The fourth study, which aimed to answer a specific question, utilised active, untrained individuals who cycled at a supramaximal workload (115% VO_2max) for a period of two minutes. In all trials blood samples were taken from a vein in the antecubital space and analysed for metabolites and hormones. Pre- and post-exercise muscle biopsies were also taken in all studies and analysed for glycogen, lactate, creatine, creatine phosphate, total adenine nucleotides and their degradation products.

Results from the first two studies indicate that muscle metabolism is altered when comparing exercise in the heat with that in a thermoneutral

environment. Furthermore, heat acclimation results in a partial reversal in these changes during exercise in the heat. The results indicate that the combination of exercise and heat stress results in an augmented increase in muscle glycogenolysis, muscle lactate accumulation and muscle creatine phosphate degradation when comparing exercise in the heat with that in a thermoneutral environment. Following acclimation muscle lactate accumulation and muscle glycogenolysis is reduced during exercise in the heat, whilst adenine nucleotide and ammonia metabolism are unaltered by environmental temperature and acclimation status. Muscle temperature, histochemical and hormonal analyses revealed that the metabolic changes were influenced by the sympatho-adrenal response and direct effect of intramuscular temperature (Q_{10}). Fibre type recruitment, as measured by the histochemical Periodic-acid Schiff (PAS) staining method, revealed that the pattern of recruitment is unaltered by changes in temperature and/or acclimation state.

Results from study three confirmed the previously observed findings with respect to exercise in the heat in the unacclimated state. Furthermore, when the thermoregulatory load is diminished by lowering the environmental temperature such that the exercise-induced rise in core temperature and heart rate is attenuated, the intramuscular carbohydrate utilisation is also reduced. A relationship between the exercise-induced rise in core temperature and the exercise-induced change in muscle glycogenolysis was found when comparing the two parameters in cool, thermoneutral and hot conditions. The lowering of environmental temperature confirmed earlier findings that adenine nucleotide and ammonia metabolism were not temperature dependant in trained athletes. Hormonal and muscle temperature measurements confirmed the previous observation that these are mechanisms for alterations in metabolism. Results from study four indicated that during whole body exercise, muscle metabolism is influenced by intramuscular temperature changes in the absence of any changes in core

temperature or sympatho-adrenal activity. Although a Q_{10} effect cannot totally account for changes observed in muscle metabolism during exercise performed in different environmental temperatures, it may contribute, in part, to the increased metabolic response.

Based upon the results obtained in the four studies reported in this dissertation the following conclusions were obtained: 1) exercise in the heat results in a greater carbohydrate utilisation; 2) heat acclimation results in a partial reversal of the metabolic response during exercise in the heat; 3) a positive relationship exists between the rise in body temperature and muscle glycogen utilisation; 4) the changes in muscle metabolism are largely influenced by an altered sympatho-adrenal response and a direct effect of intramuscular temperature; 5) adenine nucleotide data suggest that fatigue during exercise in the heat may be related to factors other than muscle metabolism.

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DECLARATION

This dissertation summarises original, previously unpublished work conducted in the Human Performance Laboratory, Department of Physical Education and Recreation at Victoria University of Technology. With the exception of isotope analyses, which were conducted at St. Vincents Hospital and the University of Melbourne, all analyses were conducted in the Department of Chemistry and Biology, Victoria University of Technology. With the exception of data collection which required collaboration and invasive procedures which were conducted by qualified medical personnel, this dissertation is the result of work performed solely by the author.

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PREFACE

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CHAPTER 1

INTRODUCTION

It is well established that the capacity for exercise endurance is reduced when exercise is performed in hot compared with cool conditions (Astrand & Rodahl, 1977; MacDougall et al., 1974; Nielsen, 1992; Saltin et al., 1972). The precise mechanisms for the reduction in performance during exercise in the heat are not fully understood, but may be related to the metabolic processes in the contracting muscle. The performance of individuals exercising in high environmental temperatures has been studied extensively with respect to body fluid changes, cardiovascular adaptations and temperature regulation. Less research, however, has investigated the effect of prolonged exercise in a hot environment on the metabolic processes within the muscle. Although some studies (Fink et al., 1975; Nielsen et al., 1990; Yaspelkis et al. 1993; Young et al. 1985) have examined the effect of exercise in the heat on glycolytic and glycogenolytic processes in contracting muscle, no studies have examined high energy phosphate and/or adenine nucleotide metabolism in humans exercising in different environmental conditions.

The relationship between performance and muscle glycogen availability during prolonged exercise is well established (Coggan & Coyle, 1987; Constantin-Teodosiu et al., 1992; Coyle et al., 1986; Spencer & Katz, 1991). It has been established that the lack of carbohydrate results in reduced pyruvate levels leading to a redution in levels of tricarboxylic acid cycle intermediates (Sahlin et al., 1990). The relatively low levels of carbohydrate result in an inability to generate adequate adenosine 5'-triphosphate (ATP)

required to maintain forceduring exercise (Broberg & Sahlin, 1989; Sahlin et al., 1990). In these circumstances, the myokinase reaction shown below

$$ADP + ADP \text{-------} > ATP + AMP$$

where ADP = adenosine 5'- diphosphate and AMP = adenosine 5'-monophosphate is activated in an effort to maintain the cell's energy sources (Tullson & Terjung, 1991). Since AMP inhibits this reaction, this product is subsequently converted to inosine 5'-monophosphate (IMP). It is surprising, therefore, that no research has examined the relationship between exercise in elevated environmental temperatures and these metabolites in humans. Furthermore, the relationship between exercise and glycogen availability in these conditions remains equivocal. If the reduced performance during exercise in the heat is metabolic in origin, the intramuscular concentrations of glycogen and ATP would be low whilst IMP concentration would be high. Thus, an examination of muscle glycogen levels, the high energy phosphates and adenine nucleotide metabolism during exercise at different environmental temperatures is warranted. Such an investigation could determine the possible role of the metabolic processes in the etiology of fatigue during exercise in the heat. The studies reported in this dissertation aim to examine the adaptations which take place in the contracting muscle when the individual is subjected to different environmental temperatures.

The mechanisms for alterations in muscle metabolism during exercise in different environmental conditions have not been widely examined. Researchers have proposed a number of hypotheses for the increased glycolytic and glycogenolytic rate which has sometimes been observed place in humans during exercise in the heat compared with that in a cooler environment. These include:

a) an increased sympatho-adrenal response to exercise in the heat leading to an increase in glycogenolysis (Powers et al. 1985; Yaspelkis et al., 1993);

b) a temperature or Q_{10} effect on enzyme reaction rate resulting in an increase in glycolysis due to an increased muscle temperature (Young et al., 1985; Kozlowski et al., 1985);

c) a change in the fibre type recruitment pattern favouring greater use of fast- than slow-twitch fibres during exercise in the heat, thereby increasing lactate production (Young et al., 1985); and

d) a reduction in O_2 delivery, secondary to a redistribution of blood flow away from the contracting muscle during exercise in the heat, leading to an increase in anaerobic metabolism (Rowell, 1974).

This dissertation examines the possible role the first three have in any metabolic changes which may take place during exercise at different environmental temperatures.

Much of the previous research which has investigated the effect of different thermoregulatory loads on physiological and performance parameters has focused on acute heat exposure. The effect of exercise in cooler than normal temperatures and the effect of acclimation to hot environments have also been the subject of some interest. As with acute expose to exercise in the heat, a relative paucity of research exists with respect to muscle metabolism in these areas. The effect of exercise in cool conditions on muscle metabolism

is in fact, quite unclear, since cooling has been found to increase (Jacobs et al., 1985) or decrease (Fink et al., 1975) muscle glycogenolysis. The discrepancy between these studies is likely to be related the difference in respective workloads. The workload in the study by Jacobs et al. (1985) was not adequate enough to produce a rise in core temperature and the authors postulated that the increased glycogenolysis during exercise in the cold was due to the added energy cost of shivering. In contrast, Fink et al. (1975) had subjects work at 85% VO_2max which produced an attenuated rise in core temperature rather than resulting in the need to shiver to maintain core temperature. Interestingly, Fink et al. (1975) compared exercise at 41^{o}C (hot) with that at 9^{o}C (cool) and found an increase in glycogenolysis during exercise in the heat. Others(Yaspelkis et al., 1993; Nielsen et al., 1990) observed an unaltered glycogenolysis when comparing exercise in the heat with that in a thermoneutral environment. These observations have lead to the hypothesis that exercise in cool conditions will attenuate glycogenolysis but exercise in the heat will not increase it (Yaspelkis et al., 1993). No studies have examined muscle glycogenolysis in cool, hot and thermoneutral environments in the same experiment.

Heat acclimation, or chronic exercise in hot conditions, results in an increase in performance (Nielsen et al., 1993). Numerous studies have observed a decrease in glycogenolysis (King et al., 1985; Kirwan et al., 1987) and lactate accumulation (Nielsen et al., 1993; Young et al., 1985), during exercise in the heat after a period of heat acclimation. The effect of heat acclimation on high energy phosphate and adenine nucleotide metabolism, however, has not been investigated. In addition, the mechanisms for the previously observed changes in muscle metabolism are also unclear. This thesis attempts to clarify the metabolic adaptations to heat acclimation, and the mechanisms associated with such adaptations.

The purpose of this dissertation, therefore, was to examine the effect environmental temperature and acclimation status on muscle metabolism during submaximal exercise in trained individuals. It was hypothesised that:

1 Muscle metabolism is different when comparing exercise in the heat with similar exercise in a thermoneutral environment.

2. Muscle metabolism is altered during exercise in the heat following a period of heat acclimation.

3. The changes in muscle metabolism are influenced by sympatho-adrenal activity, changes in muscle temperature and changes in muscle fibre type recruitment patterns.

4. Exercise in a cooler environment results in a lesser reliance upon muscle carbohydrate utilisation compared with exercise in the heat or in a thermoneutral environment.

Throughout this dissertation the term thermoneutral environment is used to imply thermoneutral for man at rest, rather than imply a situation whereby heat is neither gained nor lost during exercise. Previous literature has defined thermoneutral as comfortable ambient conditions typically in the range of 18-28^{o}C. The terms cool and hot environments refer to the ambient temperatures outside this range. A cool environment in most studies, including those in this thesis, is an ambient temperature in the range of 0-10^{o}C and a hot environment is usually in excess of 33^{o}C.

CHAPTER 2

REVIEW OF LITERATURE

Although the published literature relevant to the effect of temperature on muscle metabolism during exercise is somewhat limited, numerous studies have examined the physiological, cardiovascular and thermal response to exercise in different ambient conditions. Since the experiments described in this dissertation are concerned with the metabolic response to exercise, this review focuses primarily on the published literature examining metabolism both during exercise in thermoneutral environments and at different ambient temperatures. Specifically, this review will be broken into two major areas:

1) Muscle metabolism during exercise;

2) Muscle metabolism during exercise in different ambient conditions.

2.1 MUSCLE METABOLISM DURING EXERCISE

The immediate energy source for muscular contraction is the splitting of ATP to ADP and inorganic phosphate (Pi). The ability of the muscle to meet the energy demand placed upon it depends upon the rate of ATP utilisation and the rate at which ATP can be rephosphorylated. Thus, the intensity of exercise and

the associated rate of energy demand will be an important determinant of metabolic processes within the muscle.

2.1.1 Anaerobic Pathways

During high intensity activity above 90% of maximal aerobic capacity (VO_2max) an increasing proportion of the energy requirement is covered by anaerobic processes (Sahlin, 1992). The rate of ATP utilisation increases more than 100 times compared with non-contracting muscle. In order to maintain force, hydrolysis of ATP to ADP must be paralleled by an equal rate of ATP formation (Sahlin & Broberg, 1990). The maximal rate of ATP generation from high energy phosphate stores has been estimated to be a comparatively fast 9 $mmol.kg^{-1}.sec^{-1}$ (Sahlin, 1992). These stores, however, can only provide approximately 30kJ of energy (Hultman, 1969; Saltin, 1973) and are rapidly depleted during this type of exercise. The subsequent anaerobic energy demands are met through glycolysis, primarily as the result of intramuscular glycogen breakdown (glycogenolysis). The products of anaerobic glycolysis are ATP and lactate. As outlined in Fig. 2.1, lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to lactate:

$$\text{pyruvate} + \text{NADH} + H^+ \text{ --> } \text{lactate} + NAD^+$$

and is considered to be close to equilibrium under most conditions (Katz & Sahlin, 1990). Thus, due to mass action, an increase in the production of lactate will occur whenever an increase occurs in the concentration of pyruvate, hydrogen ion concentration or cytosolic NADH. Based on the equilibrium of LDH, the lactate/pyruvate ratio in a tissue is an index of the cytosolic redox state (Sahlin et al., 1987). Since the cytosolic redox state is

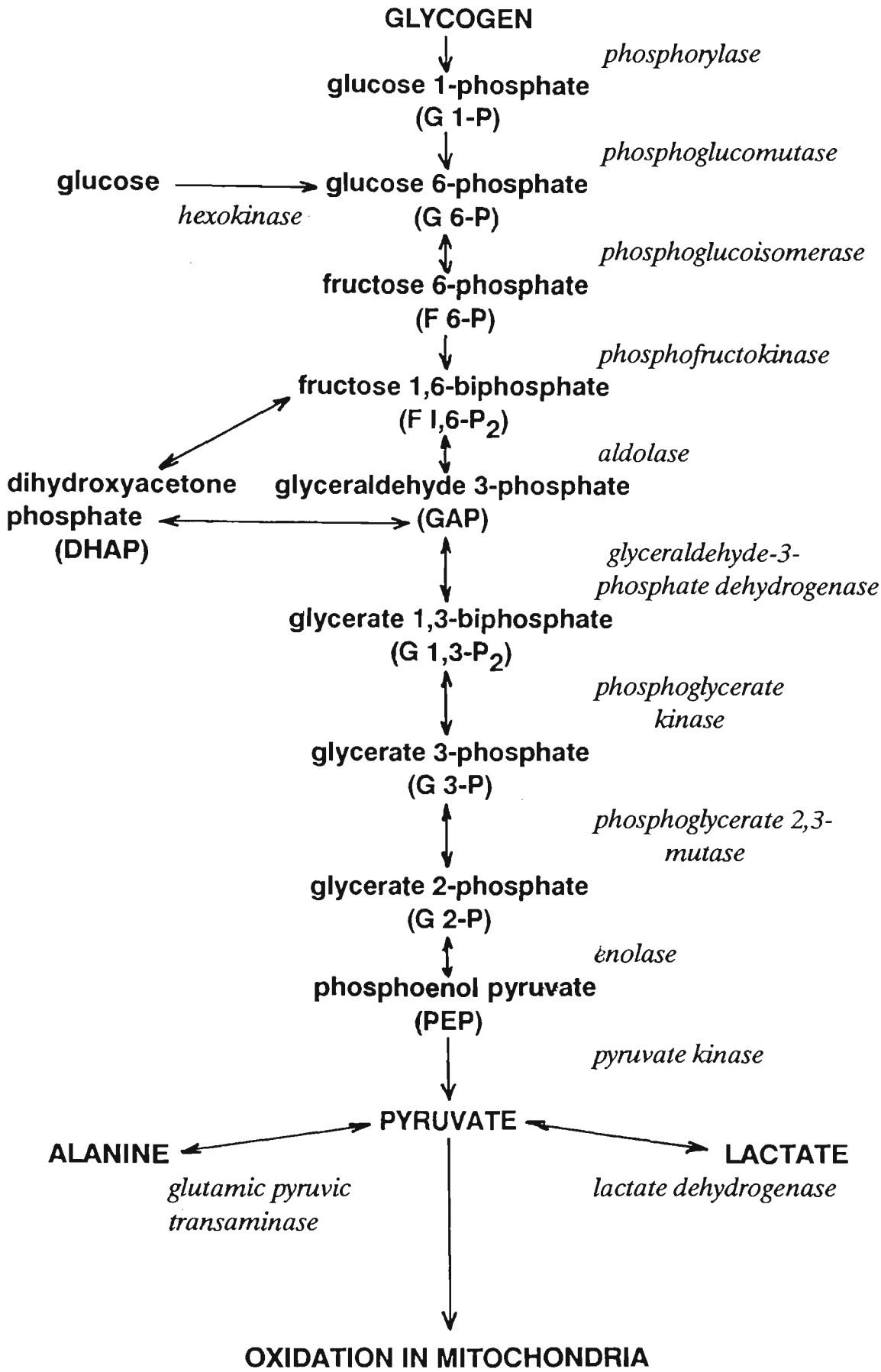


Fig. 2.1 Reactions involved in the degradation of glucose and glycogen.

influenced by mitochondrial respiration, oxygen (O_2) availability is an important factor regulating lactate production during exercise (Katz & Sahlin, 1987), although it is not the only determinant of lactate metabolism (Stainsby & Brooks, 1992). Lactate metabolism will be discussed in greater detail subsequently (2.1.2.1).

When the rate of ATP utilisation exceeds the rate of ATP rephosphorylation, the transient increases in muscle ADP content will act as powerful stimulus for activation an ATP-generating processes (Sahlin & Broberg, 1990), namely the myokinase reaction. This reaction

$$ADP + ADP \text{ ---> } ATP + AMP$$

allows for the rephosphorylation of ATP and the production of AMP (Aragon et al., 1980). Consequently, there is little change in the concentration of intramuscular free ADP (*f*ADP), although the ATP levels will decline (Katz et al., 1986b). Likewise, there is little change in free AMP (*f*AMP), since this metabolite is a potent stimulator of AMP deaminase (Wheeler & Lowenstein, 1979) which catalyses the deamination of AMP to IMP and ammonia (NH_3). Since further metabolism of IMP is slow (Tullson & Terjung, 1991), the intramuscular concentration of both IMP and NH_3 generally reflects the AMP and ADP transients and the magnitude of ATP degradation (Sahlin, 1992). NH_3 accumulation within skeletal muscle occurs via the AMP deaminase pathway (Fig. 2.2) during high intensity, anaerobic exercise (Graham et al., 1990; Katz et al., 1986a; Katz et al., 1986b; Meyer & Terjung, 1979; Meyer et al., 1980), since there is close to a 1:1 stiochiometric relationship between IMP and NH_3 accumulation and total adenine nucleotide degradation (TAN = ATP + ADP + AMP). It is well established, therefore, that during high intensity exercise these pathways represent the only source of NH_3 production (Graham & MacLean, 1992), which may not be the case during aerobic exercise.

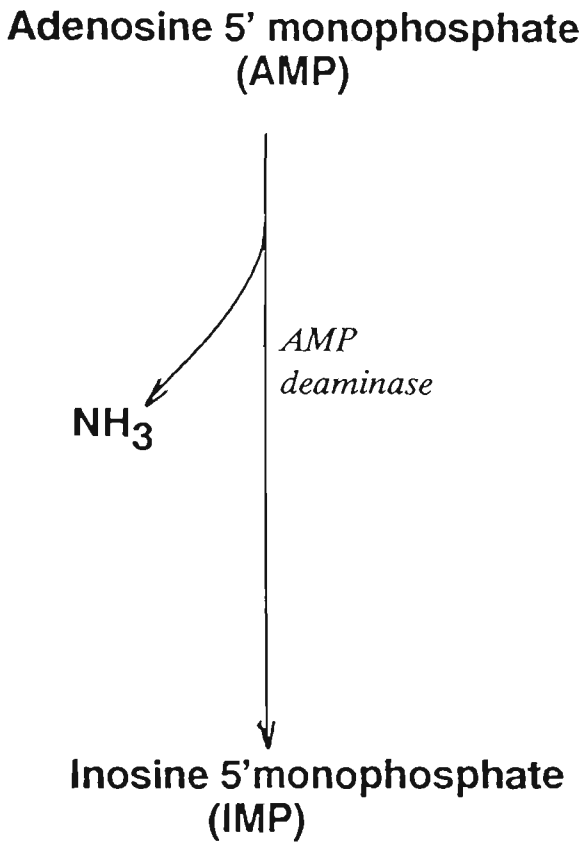


Fig. 2.2 Biochemical pathway for the production of ammonia from adenosine 5'monophosphate (AMP) catabolism.

The biochemical pathways utilised in the transduction of energy during high intensity activity result, therefore, in the accumulation of metabolic end products such as lactate, hydrogen ions (Sahlin et al., 1975; Sahlin et al., 1976; Sahlin et al., 1989), NH_3 (Bangsbo et al., 1992a; Broberg, 1989; Katz et al., 1986a; Katz et al., 1986b; Graham et al., 1990), IMP and concomitant low levels of ATP (Katz et al., 1986a; Katz et al., 1986b), but in adequate glycogen levels. The ability to maintain anaerobic exercise appears, therefore, to be related to factors other than substrate availability, such as acidosis and/or the accumulation of adenine nucleotide degradation products.

2.1.2 Oxidative Pathways

During steady-state exercise the rates of energy provision are carefully controlled to match the rates of energy expenditure with the energy being derived predominantly from aerobic metabolism from the oxidation of carbohydrate, fat and to a far lesser extent, protein in the mitochondria. Although lipids play an important role as an energy substrate, fatigue during submaximal exercise has not been associated with lipid availability, since, the energy which could be derived from lipid metabolism is essentially infinite (Sahlin, 1992). The metabolic power of lipid oxidation is low compared with the oxidation of carbohydrate (Sahlin, 1992). At workloads in excess of approximately 65% $\dot{V}O_2$max the oxidation of lipids alone would provide insufficient energy to meet the requirements of the exercise (Hultman et al., 1990). It is therefore evident that the ability to sustain exercise above this workload is dependant upon the availability of carbohydrate. The importance of lipid and protein metabolism, nevertheless, should not be overlooked when examining metabolism during prolonged exercise since energy is derived from the catabolism of these substrates.

2.1.2.1 Carbohydrate Metabolism

The relationship between carbohydrate availabilty and endurance performance was established in the 1930's (Christensen & Hansen, 1939a; Christensen & Hansen 1939b) and confirmed in the 1960's (Ahlborg et al., 1967; Bergstom & Hultman, 1967; Bergstrom et al., 1967; Hermansen et al., 1967) with the application of the needle biopsy technique (Bergstrom, 1962). This work established the theoretical basis for glycogen loading (Bergstrom et al., 1967). Research has since focussed on the effects of exercise on skeletal muscle carbohydrate metabolism, mechanisms which may affect its metabolism and the relationship between carbohydrate availability and fatigue.

Muscle glycogen and blood glucose are important substrates for ATP synthesis within contracting skeletal muscle. The importance of carbohydrate during prolonged exercise is demonstrated by the observation that fatigue during prolonged exercise is associated with muscle glycogen depletion and or hypoglycaemia (Coggan & Coyle, 1987; Constatin-Teodosiu et al., 1992; Coyle et al., 1986; Sahlin et al., 1990; Vϕllestad et al., 1984; Wahren, 1977). These findings suggest a dependency on glycogen for muscle function. This will be discussed in detail, subsequently.

Muscle Glycogenolysis During Exercise

As previously discussed, carbohydrate is the principal fuel source for high intensity exercise. As exercise intensity decreases, muscle glycogenolysis is reduced in an exponential relationship (Gollnick et al., 1974; Saltin & Karlsson, 1971). Muscle glycogen utilisation is also reduced during steady state exercise as exercise progresses, most likely as a result of reduced glycogen availability (Gollnick et al., 1986; Richter & Galbo, 1986), but

possibly due to alterations in glycogen phosphorylase activity and/or increased availability of alternative fuels such as blood-borne glucose or lipids. Muscle glycogenolysis occurs principally as a result of the activation of glycogen phosphorylase and phosphofructokinase (PFK), which are the key enzymes for glycolytic and glycogenolytic activation (Fig. 2.1). PFK activity is sensitive to changes in substrate availability, adenine nucleotide concentration, pH, citrate and ammonium ion concentration (Stanley & Connett, 1991). When muscle glycogen availability is reduced, leading to a reduction in fructose 6-phosphate (F-6-P), the rate of muscle glycolysis during exercise is maintained because PFK activity is increased by rises in *f*ADP and *f*AMP (Spencer & Katz, 1991).

Glycogen phosphorylase activity is influenced by local and hormonal regulatory mechanisms. During excitation-contraction coupling calcium increases the activity of phosphorylase by stimulating the conversion of the inactive *b* form to the active *a* form by activating phosphorylase *b* kinase (Drummond et al., 1969; Fischer et al., 1971; Gross & Meyer, 1974). The calcium which is released from the lateral sacs of the sarcoplasmic reticulum binds to the calmodulin subunit of the phosphorylase kinase which activates the enzyme by lowering the Km for phosphorylase (Picton et al., 1981; Cohen, 1982). In addition further activation occurs as a result of calcium binding to troponin C, troponin C in turn activating phosphorylase kinase (Picton et al., 1981). The conversion of phoshorylase *b* to *a* is transient, however, and is reversed despite the maintenance of contractile force (Conlee et al., 1979; Richter et al., 1982; Rennie et al., 1982; Chasiotis et al., 1982). Although this phenomenon is not fully understood, glycogen depletion (Entman et al., 1980), changes in phosphorylase and/or phosphorylase kinase (Constable et al., 1986) and increased muscle acidity (Chasiotis et al., 1983a) have been hypothesised as mechanisms underlying such a reversal. Interestingly, although

phosphorylase*a* activity is reduced as exercise progresses (Chasiotis et al., 1982), glycogen breakdown can continue until depletion (Coyle et al., 1986). An increase in phosphorylase *b* activity has been hypothesised (Aragon et al., 1980) as a mechanism for continual glycogenolysis despite low phosphorylase *a* levels. It is possible that this phenomenon is due to an increase in the glycogenolytic activity of the phosphorylase *b* form in the presence of increases in *f*ADP, *f*AMP and IMP and Pi which occur during high intensity activity (Meyer & Terjung, 1979; Ren & Hultman, 1990). Muscle glycogen levels can also influence phosphorylase activity. It has been suggested that glycogen can bind to phosphorylase to increase its activity (Johnson, 1992). This may explain the observation that the rate of muscle glycogengenolysis during exercise is usually observed to be directly related to pre-exercise glycogen concentration and that as exercise progresses the glycogenolytic rate decreases (Gollnick et al., 1981; Richter & Galbo, 1986).

The activity of phoshosphorylase during exercise is under external regulation by hormones including adrenaline and insulin. Insulin is an activator of glycogen synthase and an inhibitor of phosphorylase. When insulin secretion is inhibited during exercise with mannoheptulose, an increase in hepatic glucose production has been observed (Issekutz, 1980). Thus, it is suggested that the decrease in plasma insulin which occurs during prolonged exercise greater than 50% $\dot{V}O_2max$ (Galbo et al., 1979a), may therefore, play a role in increasing glycogenolysis during exercise (Hargreaves & Richter, 1988), although a direct role for insulin in the regulation of muscle glycogenolysis has not been demonstrated. Indeed, in cases where insulin is elevated prior to exercise, muscle glycogenolysis increases at the onset of exercise (Costill et al., 1977; Hargreaves et al., 1985), suggesting that other factors overide the potential inhibitory effect of insulin on glycogen phosphorylase. Similarly glucagon stimulates hepatic glycogenolysis (Bjorkman et al., 1981; Issekutz &

Vranic, 1980; Wasserman et al., 1984) but has little effect on muscle carbohydrate metabolism. Interestingly, during prolonged exercise, the effect of glucagon on hepatic carbohydrate metabolism decreases with time (Ahlborg et al., 1974; Issekutz, 1980) indicating that it does not play an essential role in carbohydrate metabolism during aerobic exercise.

Of far greater significance for muscle glycogenolysis is the circulating adrenaline concentration. The mechanisms by which adrenaline concentration enhances muscle glycogenolysis appears to be related to cyclic AMP (*c*AMP) and its effect upon phosphorylase activity. Adrenaline infusion causes a rise in *c*AMP activity, increased phosphorylase *a* activity, increased glycogenolytic rate and decreased glycogen synthase activity (Chasiotis et al., 1983b). Although increased circulating adrenaline can increase hepatic glycogenolysis by mechanisms which do not involve a rise in *c*AMP activity (Exton & Harper, 1975; Sherline et al., 1972: Tolbert et al., 1973), evidence suggests that adrenaline mediated muscle glycogenolysis arises from phosphorylase kinase activation (Chasiotis et al., 1983b).

The most conclusive evidence regarding the potency of adrenaline on glycogenolysis can be found in studies examining adrenodemedullated rats and in epinephrine infusion studies. Adrenodemedullation reduces muscle glycogen breakdown both in moderate (Richter et al., 1981a; Sonne et al., 1985) and high (Marker et al., 1986) intensity exercise. When adrenaline levels have been restored in adrenodemedullated rats by adrenaline infusion, the rate of glycogenolysis increases to levels observed prior to adrenodemedullation (Arnall et al., 1986; Richter et al., 1981b). In addition to these studies, Hashimoto et al. (1982) observed a greater muscle and hepatic glycogenolytic rate in control rats compared with rats which were adrenodemedullated, sympathectomised or adenodemedullated and sympathectomised. These results

indicate that both the adrenal medullary response and the sympathetic neural activity influence glycogen breakdown during exercise.

Adrenaline infusion studies also provide evidence of the role of catecholamines on glycogenolysis. Twenty minutes of continuous adrenaline infusion results in an increased glycogenolytic rate in resting human muscle (Chasiotis et al., 1983b). Furthermore, adrenaline infusion during exercise increases glycogen breakdown in both dogs (Issekutz, 1985) and humans (Greenhaff et al., 1991; Jansson et al., 1986). Studies have also examined the effect of circulating adrenaline on the glycogenolytic rate of specific fibre types during exercise. During high frequency electrical stimulation Richter et al. (1982) observed adrenaline infusion to increase the rate of glycogen use in slow twitch rat fibres but have little effect on the fast twich fibres. Conversly, during low frequency stimulation, the slow twitch fibres were not affected whilst the glycogenolytic rate of the fast twich fibres was enhanced. Greenhaff et al. (1991) observed that whilst type II fibres were predominantly utilised during high frequency electrical stimulation in humans, the infusion of adrenaline increased the glycogenolytic rate of the type I fibres but had no effect on type II fibre glycogen use. Studies which have examined glycogenolysis in specific fibres during prolonged exercise with adrenodemedullation and/or sympathectomy report the rate of glycogenolysis in adrenodemedullated/sympathectomised soleus muscle to be significantly lower when compared with the glycogenolytic rate in control rat soleus. The glycogenolytic rate of the vastus lateralis and white portions of the gastrocnemius muscles were, however, not different between the treated and the control rats (Galbo et al., 1978; Hashimoto et al., 1982).

Studies utilising beta-adrenergic blockade to elucidate the role of adrenaline in the regulation of muscle glycogenolysis have produced conflicting

results. Muscle glycogenolysis is either decreased (Chasiotis et al., 1983a; Gorski & Pietrzyk, 1982; Issekutz, 1984) or increased (Juhlin-Dannfelt et al., 1982; Nazar et al., 1972) with beta-blockade, although the effects of blockade on lipolysis, cardiac output, blood flow and hepatic glycogenolysis make the direct effects of beta-adrenergic blockade on muscle glycogenolysis difficult.

The rate of muscle glycogenolysis may also be influenced by the availability of blood-borne substrates, in particular glucose and free fatty acids (FFA). Elevated plasma FFA levels result in either an attenuated (Costill et al., 1977; Hickson et al., 1977; Rennie et al., 1976) or unchanged (Hargreaves et al., 1991) rate of muscle glycogenolysis, although in the latter study muscle glucose uptake was reduced when lipid levels were high. Conversly, a reduced FFA availability results in an augmented rate of glycogenolysis (Juhlin-Dannfelt et al., 1982). It is postulated that the reduced glycogenolysis observed in the earlier studies results from a citrate-mediated inhibition of PFK arising from an increase lipid utilisation in the muscle (Rennie & Holloszy, 1977). Intramuscular lipid utilisation will be discussed further in a subsequent section of this review (2.1.2.2).

Blood glucose may influence muscle glycogenolysis in circumstances where muscle glucose uptake is increased. It is possible that elevated blood glucose levels may affect muscle glycogenolysis because changes in muscle glucose uptake are influenced by both delivery and extraction (Ahlborg & Felig, 1982; Ahlborg et al., 1974; Hargreaves et al., 1992; Katz et al., 1991). Blood glucose levels may be increased during exercise by oral ingestion prior to (Hargreaves et al., 1985) or during (Coyle et al., 1986) exercise or by infusing adrenaline (Hakanson et al., 1986). Although ingestion of glucose during prolonged exercise at 30% VO_2max results in enhanced muscle glucose uptake (Ahlborg & Felig, 1976), at higher intensities muscle glucose uptake may

not be increased when glycogen stores are adequare to meet energy demand (Gollnick et al., 1981; Jansson et al., 1986; Richter & Galbo, 1986). Such an effect may be due to a higher rate of glycogenolysis resulting in an elevated intramuscular glucose-6-phosphate (G-6-P) concentration. In these circumstances, factors such as the inhibition of hexokinase by G-6-P may play a role in the regulation of muscle glucose uptake. No difference in glycogenolytic rate has been observed when blood glucose was elevated by carbohydrate ingestion prior to (Devlin et al., 1986; Fielding et al., 1987) or during (Coyle et al., 1986) steady state exercise. In contrast, elevations in blood glucose by oral ingestion of carbohydrate prior to exercise has been found to increase muscle glycogenolysis (Costill et al., 1977; Hargreaves et al., 1985). In the latter studies, the blood glucose levels immediately prior to exercise were elevated to such a magnitude to result in an insulin-mediated hypoglycaemia at the onset of exercise, possibly resulting in a reduced lipid oxidation and a resultant increase in muscle carbohydrate metabolism. This was not the case in the former studies. Hargreaves et al. (1984) observed a reduced muscle glycogenolysis during exercise accompanied by carbohydrate feeding. Of note, however, the exercise protocol in this study required subjects to cycle at 50% $\dot{V}O_2$max in combination with intermittent high intensity (100% $\dot{V}O_2$max) exercise. It is possible that glycogen synthesis occurred during the low intensity exercise which may account for such a result. Interestingly, when muscle glycogen levels are depleted, elevated blood glucose levels result in a prolongation of exercise (Coyle et al., 1986), since muscle glucose uptake and subsequent glucose oxidation is increased when the contracting muscle is low in carbohydrate stores (Coggan & Coyle, 1987; Coggan et al., 1991). It appears, therefore, that glycogen utilisation and muscle glucose uptake are not affected during moderate intensity exercise when muscle glycogen stores are adequate.

Other factors which may influence muscle glycogenolysis include endurance training and environmental conditions (i.e. altitude and temperature). It is well documented that endurance training reduces muscle glycogenolysis at a given exercise intensity (Hurley et al., 1986; Jansson & Kaijser, 1987) and hypoxic exercise is associated with increased glycolysis and muscle lactate accumulation (Brooks et al., 1991; Green et al., 1992b). The effect of environmental temperature on muscle carbohydrate metabolism will be reviewed in detail, subsequently.

Glycogenolysis in Specific Muscle Fibre Types

Glycogen has been found in all types of human skeletal muscle fibres at rest (Gollnick et al. 1972). During exercise, although the glycogen content of muscle is gradually reduced (Saltin, 1973), a pattern of glycogenolysis in different fibre types has been established with respect to exercise intensity and duration. During prolonged, steady state exercise muscle glycogenolysis occurs primarily in the type I fibres. Glycogenolysis in the type II fibres occurs only when type I fibres are glycogen depleted (Gollnick & Hermensen, 1973; Saltin, 1973). After a 30 km running race, marked glycogen depletion in the type I fibres with only a slight glycogenolysis in the type II fibres has been observed (Costill et al., 1973). During prolonged exercise, type I fibre glycogenolysis is substantial in the early phases of exercise, but as time progresses, type II fibre glycogenolysis increases (Armstrong et al., 1974; Gollnick et al., 1973a).

Muscle fibre type recuitment patterns have been determined by glycogenolysis in specific fibre types (Armstrong et al., 1974; Baldwin et al., 1977; Costill et al., 1973; Costill et al., 1974; Gollnick et al., 1973a; Gollnick et al., 1973b). Consequently, the Periodic-acid Schiff (PAS) staining

technique described by Pearse (1961) and single fibre biochemical analysis of glycogen content described by Essen et al. (1975) have historically been assumed to be an indicator of fibre recruitment. Studies in rats using forelimb treadmill with hindlimb suspension (Bonen et al., 1985; McDermott et al., 1987; McDermott et al., 1991), however, have questioned the validity of such an assumption, since enhanced glycogenolysis in non-contracting muscle was observed. It is hypothesised that circulating adrenaline can cause glycogenolysis in both active and inactive muscle and that glycogen mobilised in the inactive muscle is released as lactate, making it available for gluconeogenesis and/or oxidation (McDermott et al., 1991). This hypothesis is supported by the work of Ahlborg & Felig (1982) who observed lactate release from inactive forearm muscle during prolonged leg exercise in humans. The forearm release of lactate in this study could not be entirely accounted for by forearm glucose uptake, implying that muscle glycogen was mobilized from inactive forearm muscle, although muscle glycogenolysis was not measured. The level of inactivity may be critical, however, when assessing this hypothesis. In the experiment by McDermott et al. (1987) rat hindlimb suspended muscle was classed as inactive. Although electromyogram (EMG) recordings placed on this muscle during forelimb treadmill running showed no observable activity, the authors acknowledged that minimal movement was observed which may have influenced the results. Relatively few studies have examined muscle glycogenolysis in inactive human muscle. Muscle glycogenolysis in inactive muscle has mostly (Ball-Burnett et al., 1991; Bergstrom & Hultman, 1967; Koivisto et al., 1985) but not always (Bonen et al., 1985) been observed to be unchanged during exercise which has produced marked glycogenolysis in contracting muscle. Hence, the use of fibre type glycogenolysis as a measure of fibre type recruitment pattern in human muscle would appear justified.

PAS staining as a measure of fibre type recruitment is also limited by the

qualitative method of measurement. Piehl (1974) reported that maximal PAS staining intensity in both fibre types was at a glycogen concentration between 70-80 mmol. glucosyl units. kg^{-1}. Beyond this concentration all fibres stain dark and are considered unused. It is possible that the PAS method is not sensitive enough to detect changes in muscle fibre glycogenolysis when muscles are fully loaded with glycogen. Nevertheless, whilst somewhat limiting, PAS staining provides a qualitative indicator of motor unit recruitment patterns.

Lactate Metabolism During Prolonged Exercise

Although lactate accumulation has long been used as an indicator of glycolysis, the lack of increase in lactate during prolonged exercise does not necessarily mean that glycolysis is not increased (Katz & Sahlin, 1990). Exercise at below 50% $\dot{V}O_2max$ results in a 25 fold increase in glycolysis compared with rest (Katz et al., 1988), despite there being no increase in muscle lactate accumulation (Sahlin et al., 1987) or lactate efflux from the muscle (Anderson & Saltin, 1985; Jorfeldt et al. 1978), but with an increased rate of pyruvate production (Newsholm & Leech, 1984). The exercise intensity will determine if the pyruvate is oxidised in the mitochondria or further reduced to lactate (Fig. 2.1). As previously discussed, lactate is primarily considered a metabolic waste product of anaerobic glycolysis. There is growing evidence, however, to suggest that lactate is utilised both as an oxidative substrate for contracting skeletal muscle (Stanley et al., 1986) and in hepatic gluconeogenolytic processes (Wasserman et al., 1991). This is achieved by the conversion of lactic acid to pyruvic acid and utilised as a source of energy (Brooks, 1991).

It is well recognised that the causes of increased lactic acid production are several including; the contraction pattern of the muscle, substrate

availability, circulating hormones and hypoxia (Stainsby & Brooks, 1990; Stainsby et al., 1990). Considerable interest has focussed on whether hypoxia is a necessary prerequisite for lactate production. A large body of literature supports the hypothesis that lactate production is primarily dependent upon oxygen availability (Duboc et al., 1988; Katz & Sahlin, 1987; Katz & Sahlin, 1988; Knuttgen & Saltin, 1973; Linarrson et al., 1974). It is appears, however, that enhanced glycolysis and lactate production can occur in the absence of tissue hypoxia (Connett et al., 1984; Connett et al., 1986). Jobsis & Stainsby (1968) observed increased lactate production in spite of adequate mitochondrial O_2 supply by measuring the redox state in resting dog muscle. More recent studies (Stainsby et al., 1989; Wolfe et al., 1987) which have examined mitochondrial redox state also suggest that lactate can form under normoxic conditions.

2.1.2.2 Lipid Metabolism

Although the ability to sustain aerobic exercise is not dependent upon lipid availability, lipid oxidation provides significant energy from beta oxidation of FFA and glycerol catabolism. There are significant stores of lipids in both adipocytes and skeletal muscle. Approximately 80% of FFA taken up by the muscle are esterified in the sarcoplasmic reticulum to triglycerides (TG) and incorporated into lipid droplets in the muscle (Linder et al., 1976; Tan et al., 1977). The remaining FFA enter the cell to form a pool of non-esterified fatty acids (Terjung et al., 1982; Terjung et al., 1983). These non-esterified fatty acids and the FFA released through intracellular lipolysis are transported to the mitochondria where they undergo beta-oxidation to supply energy (Oscai et al., 1990). Fat combustion by skeletal muscle increases when the energy demands are increased during prolonged exercise (Gorski, 1992). It is well established that that FFA derived from lipolysis of triglycerides in

adipocytes are a major source of energy for muscle contraction (Jones et al., 1980). Indeed, it has been suggested, that the oxidation of fat during exercise is derived entirely from the oxidation of blood-borne FFA (Baldwin et al., 1973). The calculations of the amount of fat combusted during prolonged exercise in both humans (Havel et al., 1967) and dogs (Issekutz & Paul, 1968) indicate, however, that blood-borne FFA do not account for all fat oxidation as determined from respiratory quotient measurements. It is hypothesised that muscle TG are the most likely source for this discrepency (Gorski, 1992). This hypothesis is supported by the observations that the size of intramuscular fat vacuoles is reduced following prolonged exercise (Gollnick & Saltin, 1988) and that 15% of energy derived from the total combustion of fat during submaximal exercise came from TG (Jansson & Kaijser, 1987).

The mechanisms involved regulation of lipid metabolism, in particular TG metabolism is unclear. Although it is clear that the enzyme lipoprotein lipase (LPL) plays a regulatory role in the metabolism of lipid in the capillary beds of extra-hepatic tissue (Borensztajn, 1979; Robinson, 1970), it is unclear whether the enzyme regulates intramuscular TG hydrolysis. It has been hypothesised that LPL functions in the intracellular regulation of stored TG in skeletal muscle (Oscai, 1979; Oscai et al., 1982; Palmer et al., 1981). This hypothesis has been questioned (Gorski, 1992), however, because the optimum pH for enzyme activity is 8.5 and its activity at pH 7.0 is negligible (Strohfeld & Heugel, 1984) whereas the pH in the muscle cell during contraction is less than 7.0 (Sahlin, 1978). Furthermore, LPL synthesised in the muscle is transported to its surface in vescicles, thereby depriving it of contact with TG particles (Gorski, 1992).

The hydrolysis of lipids are influenced by hormonal changes and the *c*AMP cascade, although conflicting findings on the precise role of the

sympatho-adrenergic system have been reported. Noradrenaline infusion results in a reduction in TG in humans (Froberg et al., 1975) but not in dogs (Carlson et al., 1965). Adrenodemedullated, sympathectomized (Sembrowich et al., 1974) and immunosympathectomised (Luyckx et al., 1978) rats have lower plasma FFA concentration compared with sham treated controls. In contrast, FFA and glycerol levels increase during exercise in adrenalectomised humans (Barwich et al., 1981). In addition, FFA and glycerol levels are unaffected by glucose infusion during exercise, in spite of the reduction in adrenaline secretion which this induces (Galbo et al., 1979a). In contrast, glucose ingestion suppresses plasma FFA levels (Coyle et al., 1985). This process appears to be mediated by insulin, plasma levels of which remain high with glucose ingestion. It is well established that insulin supresses FFA mobilisation from adipocytes (Coyle et al., 1985). Although lipolysis may be influenced by circulating catecholamines, the relationship between the two is not as strong as that between adrenaline and carbohydrate metabolism. Other factors which may influence lipid metabolism include muscle glycogen concentration (Stankiewicz-Choroszucha & Gorski 1978), lactate (Issekutz & Miller, 1962) and pH (Gorski, 1977).

2.1.2.3 Protein Metabolism

Protein catabolism is often overlooked when discussing substrate metabolism during exercise, because it only contributes a minor portion of energy and the continuation of exercise is not protein dependant. Protein has been reported to contribute from 1% (Callow et al., 1986; Hood & Terjung, 1990) to 10% (Lemon & Mullin, 1980) of total energy demand during exercise. The metabolism of amino groups such as branched chain amino acids (BCAA) and NH_3 may, however, influence the overall regulation of metabolism. BCAA, in particular, may be a continual source of energy during prolonged, submaximal exercise (Wagenmakers

et al., 1990). Their catabolism, however, may produce NH_3 (Golberg & Chang, 1978), which may interfere with cellular function.

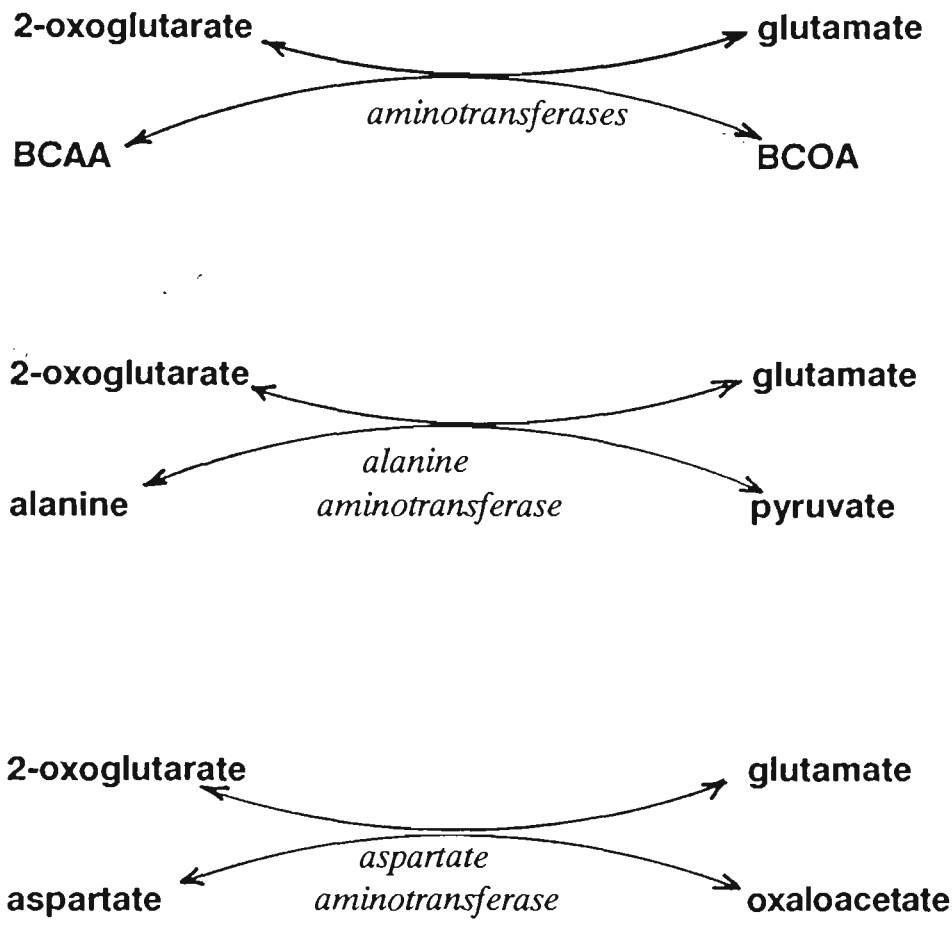
Several investigators have reported increased protein degradation in response to exercise (Booth & Watson, 1985; Dohm et al., 1987; Kasperek et al., 1982; Kasperek & Snider, 1987; Rennie et al., 1981; Wolfe et al., 1982). The literature generally supports the contention that protein degradation occurs principally in the liver and non-contracting muscle whilst contractile protein degradation is suppressed (Graham & MacLean, 1992). Tyrosine and phenylalanine have been used as indicators of non-contractile protein degradation since they are not subject to intermediary metabolism in skeletal muscle (Smith & Rennie, 1990). Several studies have reported an increase in muscle release of tyrosine and phenylalanine during exercise (Dohm et al., 1980; Felig & Wahren, 1971; Kasperek et al., 1982). The amino acid 3-methylhistidine is used in the formation of the contractile proteins, actin and myosin. 3-methylhistidine cannot be further catabolised, but rather, is transported to the kidney and excreted when the contractile proteins are broken down. Consequently, plasma and urinary concentration of 3-methylhistidine has been used as a marker of contractile protein degradation. Conflicting results have been reported with respect to 3-methylhistidine excretion with exercise (Decombaz et al., 1979; Dohm et al., 1982; Rennie et al., 1981). These results are likely, however, to be due to the observation that 3-methylhistidine excretion is decreased during exercise but increased in recovery (Dohm et al., 1987).

There are many regulators of protein catabolism during exercise. Protein degradation is increased in response to fasting (Fryburg et al., 1990) and decreased by amino acid influsion (Nair et al., 1992), lipid infusion (Beaufrere et al., 1985; Graham et al., 1991) and decreases in dietary protein (Tawa & Goldberg, 1992). In addition, endocrine factors regulate protein

metabolism. Studies of the relationship between catecholamines and protein turnover have produced conflicting results. Goodman (1987) observed a 27% increase in protein degradation when rat muscle was stimulated *in vitro* and infused with adrenaline. In contrast, Nie et al. (1989) report that adrenaline has no effect on protein catabolism. The increase in glucocorticoids and glucagon and supression of insulin which usually occur during prolonged exercise, may also influence protein catabolism. Protein synthesis increases in response to increased insulin (Marshal & Monzon, 1989) and growth hormone (Fryburg et al., 1991) whilst protein degradation is increased with elevated glucagon and glucocorticoid levels (Beaufrere et al., 1985).

During exercise muscles principally metabolise protein and amino acids stored within the muscle and make less use of circulating amino acids (Graham & MacLean, 1992). As previously discussed, amino acid catabolism, whilst producing energy, also produces NH_3. During prolonged exercise, the rate of ATP synthesis matches the rate of ATP degradation, hence, *f*ADP and *f*AMP are not present in large concentrations. The production of NH_3 from AMP deamination during prolonged exercise occurs only when glycogen levels are low (Sahlin et al., 1990). This point will be discussed in detail in a subsequent section (2.1.2.4). As a concenquence NH_3 production during prolonged exercise could potentially be used as a marker of amino acid catabolism.

There are seven amino acids that can be oxidised by skeletal muscle, namely; alanine, glutamine, glutamate, aspartate and the three BCAA leucine isolucine and valine (Wagenmakers et al., 1990). The oxidation of these amino acids results in the formation of NH_3 which has, therefore, been used as a marker of amino acid catabolism. The formation of NH_3 from amino acid catabolism is summarised in Fig. 2.3. Although the BCAA are the main amino



1) glutamine + ADP <----------> glutamate + NH_3 + ATP
glutamine synthetase

2) glutamate + NAD^+ + H_2O
glutamate dehydrogenase
2-oxoglutarate + NADH + NH_4^+

3) aspartate + IMP + GTP <----------> adenylosuccinate + GDP + Pi
AS synthetase

adenylosuccinate <----------> AMP + fumarate
AS lyase

AMP + H_20 <----------> IMP + ammonia
AMP deaminase

Fig. 2.3. The formation of ammonia from amino acid catabolism.

acids which are catabolised in the muscle (Wagenmakers et al., 1990), they are not directly oxidised. The BCAA undergo an aminotranferase reaction, whereby the amino group is donated to 2-oxoglutarate to form glutamate (Golberg & Chang, 1978; Randle et al., 1984) and the branched chain oxoacid (BCOA) is oxidised. Although most evidence suggests that alanine and glutamine are released from the active muscle and metabolised by the liver (Felig, 1975; Wolfe et al., 1982; Wolfe et al., 1984) some alanine is transferred to 2-oxoglutarate to form glutamate. Likewise, glutamine is converted to glutamate in the glutamine synthetase reaction (Goodman & Ruderman, 1982). NH_3 is produced in the conversion of glutamate to 2-oxogluterate via the enzyme glutamate dehydrogenase (Terjung & Tullson, 1992). Aspartate can be transaminated to form glutamate and produce NH_3 by glutamate dehydrogenase reaction (Goodman & Ruderman, 1982). Alternatively, it can enter the purine nucleotide cycle (PNC) (Fig. 2.4) in which its amino group is converted to free NH_3 (Lowenstein, 1972). Likewise, glutamate can be transaminated to form aspartate and enter the PNC (Wagenmakers et al., 1990).

There is much evidence to suggest that during prolonged exercise, amino acids are a major source of NH_3 production. MacLean et al. (1991) found no change in AMP, ADP, ATP and TAN despite a rise in NH_3 during 103 min of exercise at 75% VO_2max. Furthermore, Norman et al. (1987) reported an increase in muscle NH_3 accumulation despite no change in IMP concentration after 45 minutes of submaximal exercise. Interestingly, in the study by Norman et al. (1987) IMP levels rose as exhaustion approached and carbohydrate levels decreased. A recent study by Snow et al. (1993b) provides further evidence that amino acid catabolism is a major contributor to muscle NH_3 accumulation during exercise. A lower muscle NH_3 accumulation was observed in subjects cycling at 70% VO_2max for 120 min when fed carbohydrate compared with a placebo. In contrast, IMP accumulation was not different between the two

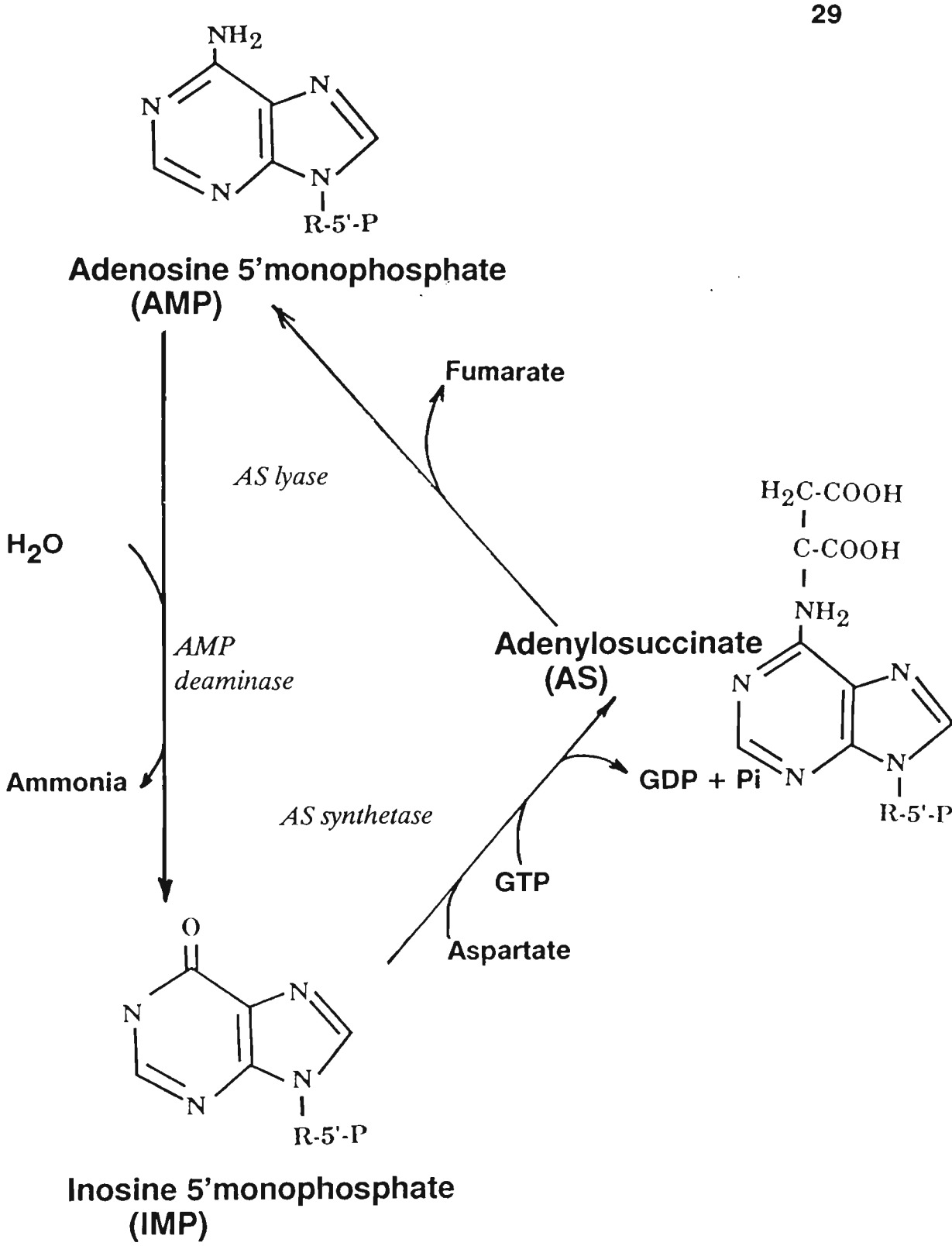


Fig. 2.4 The purine nucleotide cycle.

trials. Furthermore, the exercise-induced increase in IMP was less than 0.1 mmol. kg^{-1} dry weight in both trials, compared with a rise in NH_3 of 1.65 and 0.90 mmol. kg^{-1} dry weight for placebo and CHO fed, respectively. Further evidence that amino acid catabolism contributes to NH_3 accumulation can be found when reviewing amino acid feeding studies. A higher plasma NH_3 accumulation has been observed in individuals who consumed an amino acid supplement prior to prolonged cycling when compared with placebo feeding (MacLean & Graham, 1992; Wagenmakers et al., 1991).

Interestingly, at the onset of fatigue during prolonged exercise, amino acid catabolism may decrease and NH_3 production from AMP deamination increases (Sahlin et al., 1990). As previously discussed, ATP turnover at the point of fatigue may be compromised by a lack of carbohydrate. At this point, a rise in IMP formation has been observed (Norman et al., 1987; Sahlin et al, 1990; Spencer et al., 1991b). Thus, the production of NH_3 at fatigue comes from the AMP deaminase production pathway or a combination of this pathway and amino acid catabolism. Although there is paucity of research examining amino acid catabolism during prolonged exercise, Sahlin et al. (1990) found arterial concentrations of leucine, isoleucine, alanine and glutamine to rise during exercise prior to fatigue but plateau or fall as fatigue approached. In contrast, aterial NH_3 accumulation rose sharply as fatigue approached. These data indicate that, perhaps, the major source of NH_3 production during submaximal exercise when energy supply is sufficient is amino acid catabolism, but as fatigue approaches and ATP turnover is hindered, NH_3 is produced by the AMP deaminase pathway.

Protein, therefore, is an important component of metabolism during prolonged exercise, despite its minor contribution as a supplier of ATP. Protein may function to maintain metabolic homeostasis, since amino acid

catabolism is involved in processes such as the maintenance of the concentrations of TCA cycle intermediates, essential for oxidative metabolism. As previously discussed, NH_3 can be produced by AMP deamination or by amino acid catabolism. During prolonged exercise, amino acid catabolism appears to be the major source of muscle NH_3 accumulation, although the regulation of NH_3 production needs further clarification. It appears, however, that the appearance of NH_3 in the contracting muscle during prolonged exercise, where ATP rephosphorylation is adequate to meet ATP hydrolysis, is associated with amino acid catabolism. Hence, during this type of exercise, NH_3 may be used as a marker of protein catabolism.

2.1.2.4 Metabolic Basis of Fatigue

The reasonably well established relationship between glycogen availability and work capacity during prolonged exercise (Coggan & Coyle, 1987; Constantin-Teodosiu et al., 1992; Coyle et al., 1986; Spencer & Katz, 1991) suggests that there is a dependency on glycogen for the maintenance of contractile activity. Despite the heavy attention given to this observation, the direct answer as to the neccessity for glycogen availability during prolonged exercise is not apparent although many have hypothesised on the relationship (Conlee, 1987). The likely explanation for the need for carbohydrate during prolonged exercise is related to pyruvate formation (Sahlin et al., 1990). During exercise, glycogen provides glucose moieties that are subsequently metabolised to pyruvate. In addition to being oxidised pyruvate can be carboxylated to oxaloacetate by pyruvate carboxylase. Oxaloacetate maintains the tricarboxylic acid (TCA) cycle, and in the absence of oxaloacetate and other TCA cycle intermediates, the processing of acetyl groups derived from amino acid catabolism and the beta oxidation of fat is hindered, resulting in a decrease in muscle ATP production leading to a fall in cellular

ATP concentration (Dohm et al. 1985; Sahlin et al., 1990). In these circumstances, the myokinase reaction may be activated in an effort to maintain the cell's energy sources, resulting in an increase in IMP accumulation (Spencer et al., 1991a). Whether ATP levels fall at the point of fatigue in prolonged exercise is the subject of controversy. Recent studies, which have examined prolonged cycling to exhaustion have reported marked (Sahlin et al., 1990), slight (Broberg & Sahlin, 1989) or no (Norman et al., 1987; Spencer et al., 1991a) reduction in ATP concentration within the contracting muscle. In all studies, however, IMP rose significantly at the onset of fatigue an increase not always matched by a fall in ATP concentration. The concentration of ATP is much greater than the concentration of IMP (Tullson & Terjung, 1990) and thus, when hydrolysed the small changes in cellular ATP concentration may not be detectable due to analytical variability. The changes, however, appear in the rise in IMP accumulation, reflecting an imbalance between the rate of ATP synthesis and hydrolysis. Sahlin et al. (1990) ascribed this imbalance to reduced TCA cycle intermediates, particularly oxaloacetate as referred to above, after exhaustive, prolonged exercise. It is possible, therefore, that a reduction in these intermediates leads to an impairment, not only in carbohydrate metabolism, but also in the oxidation of amino acids and free fatty acids when glycogen is depleted. Interestingly, in the studies which observed a rise in IMP at fatigue, CP values remained at 40-50% of pre-exercise values during exercise with no pronounced decline as the subjects approached fatigue. If glycogen depletion leads to a compromised ATP regeneration, subsequently leading to fatigue, a further decline in CP concentration as subjects neared fatigue might be expected. In the absence of glycolysis, however, the low intramuscular H^+ concentration may inhibit ATP formation from CP via the creatine kinase reaction (CP + ADP + H^+ -----> C + ATP).

The bases of fatigue may, however, be related to factors other than glycogen availability. It has recently been hypothesised that fatigue during prolonged exercise is related to a disruption of the excitation-contraction coupling process within the sarcomere, and/or a reduction in central drive (Green, 1991). Green et al. (1990) implicated the sarcoplasmic reticulum as a failure site during contractile activity because of an inability to maintain cytosolic calcium concentration and subsequent activation of the contractile apparatus. Large reductions in sarcoplasmic reticulum calcium uptake and calcium ATPase activity, along with a substantial glycogen depletion, have been reported in rats after prolonged treadmill exercise (Byrd et al., 1989). It is also possible that fatigue and low glycogen levels may be related due to central rather than peripheral perturbations. Green (1990), postulated a reduction in the output of the alpha-motorneuron pool to the contracting muscle could occur, possibly due to changes in central neurotransmitters. Central catecholaminergic and serotoninergic systems have been shown to affect endurance (Chaouloff, 1989). Whether these transmitters are affected late in exercise when carbohydrates are low is speculative, but may account for the relationship between carbohydrate availability and fatigue. Although the mechanisms by which low carbohydrate availabilty affects endurance are unclear, the relationship between the two is well established.

2.2 MUSCLE METABOLISM DURING EXERCISE IN DIFFERENT ENVIRONMENTAL CONDITIONS

Much of the information regarding exercise metabolism has been obtained from studies conducted in temperate environments. This review has, thus far, examined the metabolic response to exercise in a so called 'thermoneutral environment'. Although many investigators have addressed the effect of environmental stress on the cardiovascular and thermoregulatory response to

exercise at extreme temperatures and some comprehensive reviews have been published in this area (Buskirk, 1977, Gisolfi & Wenger, 1984; Hardy, 1961; Nadel, 1985; Rowell, 1974), relatively less information is available on the metabolic response.

Humans maintain their body temperature via a number of mechanisms which allow heat dissipation to match heat production. The utilisation of energy in muscle contraction coupling process liberates the energy into either mechanical work or heat. Thus, during of exercise, the rate of heat production is proportional to exercise intensity. Since the rate of heat production is in excess of the rate dissipation from the muscle, heat storage in the muscle occurs and muscle temperature increases. The heat produced is conducted along thermal gradients through surrounding tissue and in the blood to the body core. Subsequent alterations to blood flow allow for the dissipation to the environment. The body, therefore, can account for the increase in heat production by thermoregulatory mechanisms which shunt the heat from the body to the surrounding environment. The dissipation of heat produced during exercise may be expressed in terms of the standard heat exchange equation:

$$S = M \pm R \pm C - E$$

where S is stored body heat; M is metabolically heat production; R is the radient heat exchange; C is convective and conductive heat exchange; and E is evaporative heat loss (Rodahl, 1989).

The effectiveness of these thermoregulatory mechanisms may be compromised, however, by ambient temperature. When ambient temperature rises, the rate at which the body can dissipate heat during exercise is reduced because the thermal gradient between the skin and the environment diminishes, resulting in

a continual increase in core temperature. If the environmental temperature is extremely high (above 37^{o}C) and exceeds skin temperature, the gradient for heat transfer is reversed and the body gains, rather than loses, heat. Conversely, if the gradient between the skin and the environment is extremely high, resulting from extremely low ambient temperatures, the rate of endogenous heat production may be insufficient to maintain body temperature. In these circumstances, responses to reduce heat loss are invoked. In circumstances where the core temperature is either increased or decreased thermoregulatory responses are initiated. These may include a redistribution of regional blood flow and hormonal secretions. In addition, when body temperature is reduced shivering in non-contracting muscle contributes to heat production. These responses may ultimately impact on the metabolic response to exercise.

2.2.1 Exercise Metabolism in Cool Conditions

Very little research exists examining the effect of exercise in cool conditions on metabolic processes during prolonged exercise. Whilst some literature has examined carbohydrate metabolism, none exists with respect to exercise in cool conditions on lipid or protein metabolism. Whether exercise in the cool conditions is detrimental, beneficial or of any consequence to metabolic processes is the subject of controversy. The discrepancy in the observations appear, however, to be related to both the exercise intensity and the severity of the environmental condition. Both of these variables will ultimately affect core and/or muscle temperature resulting in thermoregulatory responses which may in turn impact upon the metabolic processes outlined in the first section of this review.

An increase in anaerobic glycolysis, reflected in blood lactate accumulation has been reported during low intensity activity in cold conditions

(Doubt & Hseih, 1991; Galbo et al., 1979b; Holmer & Bergh, 1974; Nadel et al., 1974). In contrast, others have reported no change in blood lactate concentration (Hessemer et al., 1984; Flore et al., 1992; Jacobs et al., 1985). Although these studies appear to be contradictory, the discrepancy may be related to the degree of cold stress. Those studies which have observed an increased blood lactate accumulation involved whole body water immersion and/or low intensity exercise which resulted in decreased core, and possibly muscle, temperature. In contrast, the studies in which the lactate response to exercise was unaltered by the cold condition, subjects were exposed to an air temperature environment and/or moderate intensity exercise protocol which produced little or no reduction in core temperature.

The effect of exposure to cool conditions on muscle glycogenolysis has also produced conflicting results. Jacobs et al. (1985) report elevated muscle glycogenolysis during low intensity exercise (55 W) in 9^{o}C compared with similar exercise in 21^{o}C. When a different group of subjects exercised in the respective environments but at a higher workload (103 W) no difference was observed in glycogen breakdown in the vastus lateralis. In contrast, when exercise induced hyperthermia was prevented in dogs by the application of cold packs to their trunks, muscle glycogenolysis was lower compared with exercise without trunk cooling (Kozlowski et al., 1985). In order to interpret such data the possible mechanisms for the alterations need to be examined. The probable increase in anaerobic glycolysis, reflected in blood lactate and the increased glycogenolysis reported in the study by Jacobs et al. (1985) have been attributed to the direct effects of shivering (Shephard, 1985), a reduced mechanical efficiency as a result of lowered muscle temperature (Blomstrand et al., 1984; Blomstrand & Essen-Gustavsson, 1987) and increased catecholamine secretion (Galbo et al., 1979b). It is likely that shivering would elevate both glycogenolysis and muscle glycolysis, since carbohydrate is the major fuel for

the increased energy demand caused by shivering (Vallerand et al., 1988; Vallerand & Jacobs, 1989). Furthermore, when blood glucose levels are reduced by insulin infusion resulting in hypoglycaemia, a suppression of the shivering response (Gale et al., 1981) and a reduced rectal temperature (Mager & Francesconi, 1983) have been observed. Both peripheral and central thermoreceptors can invoke the shivering response. In the study by Jacobs et al. (1985) the authors observed an increased glycogen breakdown during the low intensity, cold exposure trial. They hypothesised that this response was due to the increased energy demand required for shivering which was mediated by peripheral thermoreceptors as the rectal temperature did not change. Although the authors did not report the occurance of shivering, it is possible that the heat generated from the exercising muscle was in itself not enough to maintain core temperature, but a combination of heat produced from mechanical work and shivering was sufficient. In the study by Kozlowski et al. (1985), however, a reduced muscle glycogenolysis was observed in the trial which employed cooling. This resulted in an attenuated rise in both core and muscle temperature, partially offsetting the increased thermoregulatory load, indicating that an extra metabolic cost due to shivering was not present.

The negative influence of a reduced muscle temperature on power output during dynamic exercise has been well documented (Bergh & Ekblom, 1979; Blomstrand et al., 1984; Crowley et al., 1991; Davies & Young, 1983; Feretti et al., 1992). The cause of such a negative influence has been the subject of fewer investigations. Blomstrand and Essen-Gustavsson (1987) report that along with a decrement in performance, cooling the muscle increased the glucose-6-phosphate (G-6-P) and lactate concentration and the rate of ATP and CP degradation in the muscle at exhaustion. In addition most subjects showed a greater glycogenolysis in the cooled muscle compared with the control,

indicating that glycolysis from glycogen increases when exercise is performed in cooled muscles. These observations have been attributed to two possible mechanisms. Firstly blood flow to colder muscles may be reduced in order to minimise heat loss (Pendergast, 1988). If this was the case, O_2 delivery to the contracting muscle would be reduced thereby increasing the energy derived from anaerobic metabolism (Blomstrand & Essen-Gustavsson, 1987; Blomstrand et al., 1986). In addition, a reduced blood flow may reduce lactate efflux from the muscle thereby increasing intramuscular concentrations of lactate. Secondly, a reduced mechanical efficiency at subnormal muscle temperatures, possibly due to enzyme inefficiency, could lead to an increased energy demand for a given power output (Blomstrand & Essen-Gustavsson, 1987; Blomstrand et al. 1986).

The observed changes in carbohydrate metabolism during exercise in the cold have also been attributed to hormonal alterations (Galbo et al., 1979b). Swimming at 68% VO_2max in water at 21^{o}C results in increased concentrations of both plasma adrenaline and noradrenaline compared with 27^{o}C. In addition, plasma lactate concentrations followed a similar pattern, indicating there was a probable increased glycolysis. This was probably due to an increase in circulating catecholamines since an augmented rise in lactate accumulation has been found in the presence of higher circulating adrenaline (Chasiotis et al., 1983b). Interestingly, rectal temperature continued to fall whilst swimming at 21^{o}C indicating that an increased glycolysis prevails when cold stress is severe enough to allow body heat to be lost to the environment. A fall in core temperature, however, is not an essential pre-requisite for stimulating sympathetic activity since an increase in plasma noradrenaline has been found during exercise and cold exposure despite there being no fall in core temperature (Bergh et al., 1979; Johnson et al., 1977). This indicates that peripheral thermoreceptors may activate the sympatho-adrenal system if

peripheral temperature falls below a certain threshold. In contrast to these studies, no difference in plasma catecholamines was observed during water immersion when the temperature was maintained at 34.5^{o}C (Epstein et al., 1983), a temperature which was likely not to have resulted in a drop in core and/or muscle temperature.

Cold exposure during submaximal exercise appears to increase glycolysis, muscle glycogenolysis and high energy phosphate degradation when core and/or muscle temperature is significantly reduced (Blomstrand & Essen-Gustavsson, 1987; Blomstrand et al., 1986; Doubt & Hsieh, 1991; Galbo et al., 1979b). These changes have been attributed to the increased metabolic cost of shivering (Jacobs et al., 1985; Shepherd, 1985), the effects of reduced blood flow to the cold contracting muscle (Blomstrand & Essen-Gustavsson, 1987; Blomstrand et al., 1986), an increase in circulating adrenaline (Galbo et al., 1979b) and/or a reduced mechanical efficiency leading to an increased energy demand for a given power output (Blomstrand & Essen-Gustavsson, 1987; Blomstrand et al., 1986). In contrast, when cooling attenuates a rise rather than promotes a fall in core and muscle temperature, glycogenolysis, muscle glycolysis and high energy phosphate degradation appear to be lower in comparison with similar exercise in control conditions (Kozlowski et al., 1985; Kruk et al., 1985).

2.2.2 Exercise Metabolism in the Heat: Acute Exposure to Heat Stress

A far greater problem arises with exercise in hot conditions, since the environment and production of heat via mechanical work do not offset each other. As a consequence, relatively more research has been devoted to the area of heat stress and exercise metabolism compared with that in the cool. Relatively little information, however, has been published with respect to metabolic responses to exercise in the heat compared with cardiovascular and thermoregulatory responses.

It is well established that the endurance capacity of individuals exercising in the heat is reduced by comparison with their performance in room temperature (Astrand & Rodahl, 1977; Saltin et al., 1972). Work tolerance time decreased from 75 minutes down to 45.25 minutes when subjects were exposed to a hot environment (MacDougall et al., 1974). Although a high muscle temperature has been found to influence muscle metabolism (Edwards et al., 1972), a relationship between the reduced endurance capacity during exercise at higher environmental temperatures and indices of muscle fatigue is not well established. Indeed, the literature has often presented conflicting results in this area.

2.2.2.1 Carbohydrate Metabolism

Much of the literature which has examined carbohydrate metabolism during acute exposure to exercise in the heat has concentrated on lactate metabolism although some investigations have examined muscle glycogenolysis. The results from these investigations are equivocal and the effect of acute heat stress and exercise on skeletal muscle carbohydrate utilisation needs further clarification.

Blood lactate accumulation is usually observed to be higher when comparing submaximal exercise in the heat with that in a cooler environment (Dimri et al., 1980; Dolny & Lemon, 1988; Fink et al., 1975; MacDougall et al., 1974; Powers et al,. 1985; Rowell et al., 1969; Williams et al., 1962; Yaspelkis et al., 1993; Young et al, 1985). Others (Nielsen et al., 1990; Savard et al., 1988), however, have observed no difference in blood lactate when comparing exercise in these conditions. Blood lactate concentration during exercise is dependent upon lactate production, efflux from the muscle and uptake from the blood to other organs such as the heart, liver and inactive muscle (Young,

1990). Of these variables only hepatic lactate removal and lactate efflux from the muscle have been investigated. Rowell et al. (1968) observed a reduced hepatic lactate uptake when comparing exercise in the heat with that in a cooler environment. Interestingly, the reduction in hepatic lactate removal could not account for the difference in arterial lactate concentration observed between the two environmental conditions. Nielsen et al. (1990) observed no difference in either femoral venous or arterial lactate concentration and no difference in lactate release from the muscle when comparing exercise in the heat with that in a cooler environment. This study however utilised a non-counterbalanced experiment which required the subjects to exercise for 30 minutes in a thermoneutral environment prior to 60 minutes of exercise in the heat. Thus, exercise in the heat commenced with slightly elevated muscle lactate concentration and slightly lowered muscle glycogen concentration which may have affected glycolysis. Unfortunately no studies which report an increased blood lactate concentration in the heat have measured lactate efflux. Therefore, the greater blood lactate accumulation during exercise in the heat compared with a cooler environment, could possibly be due to increased lactate efflux from the muscle, decreased lactate uptake by the heart and inactive muscle or a combination of both (Young, 1990). In addition, Yaspelkis et al. (1993) hypothesise that an increase in blood lactate during exercise in the heat may be due to a sympatho-adrenal effect on glycogenolysis in inactive tissue since plasma catecholamines have been found to be elevated during exercise in the heat (Galbo et al., 1979b; Nielsen et al., 1990; Powers et al., 1982).

Muscle lactate accumulation has also been reported to be higher when comparing submaximal exercise in hyperthermic conditions with similar exercise in cooler conditions in both dogs (Kozlowski et al., 1985) and humans (Young et al., 1985). In addition, Edwards et al. (1972) found a higher muscle lactate

accumulation following isometric contractions to fatigue preceded by passive heating of the exercising limb. An increased muscle glycogenolysis, leading to greater glycolysis, may contribute to the augmented increase in muscle lactate accumulation observed during exercise and heat stress. This too is the subject of some controversy. Fink et al. (1975) reported a higher muscle glycogenolysis and blood lactate accumulation during prolonged exercise in the heat compared with similar exercise in a cool environment. Similarly, in the previously mentioned study by Kozlowski et al. (1985), the authors reported a lower glycogenolysis, muscle lactate and pyruvate concentration in exercising dogs which were externally cooled compared with similar exercise in the absence of any cooling. Although Young et al. (1985) observed a higher muscle and blood lactate accumulation during exercise in the heat, they observed no difference in muscle glycogenolysis. These authors hypothesised an alteration in neuromuscular recruitment pattern, favouring greater use of fast- than slow-twitch fibres. They based this hypothesis on the observation that the individuals in the study who had the highest proportion of fast-twitch fibres were those who showed greatest increments in muscle lactate accumulation. This hypothesis has, however, never been experimentally confirmed nor refuted, since differential fibre type glycogenolysis has never been investigated. Young (1990) alternatively proposed that a more pronounced hepatic glucose release during exercise in the heat may increase muscle glucose uptake and subsequently lead to lactate formation from blood-borne substrate. The effect of exercise in the heat on muscle glucose uptake has not been widely investigated, although Nielsen et al. (1990) found no difference in aterial or venous glucose concentration and no difference in muscle glucose uptake between exercise in the heat and that in a thermoneutral environment. Unfortunately, those studies which report higher glucose concentrations in the heat did not measure glucose uptake (Fink et al., 1975; Yaspelkis et al., 1993). It is possible that in these studies the increase in blood glucose arose from an augmented hepatic

glucose release, although a higher blood glucose may imply reduced glucose uptake.

Two recent studies do not observe any affect of exercise in the heat on muscle glycogenolysis (Nielsen et al., 1990; Yaspelkis et al., 1993). Certain methodological differences could, however, account for the discrepancies between these studies and those previously reported by Fink et al. (1975) and Kozlowski et al. (1985). As previously noted Nielsen et al. (1990) had their subject's exercise in the thermoneutral environment immediately prior to entering the hot environment. They consequently commenced exercise in the heat with a slightly reduced glycogen concentration. This may have influenced glycogenolytic rate since some studies have demonstrated that the rate of muscle glycogen utilisation is related to the pre-exercise glycogen concentration (Gollnick et al., 1972; Richter & Galbo, 1986). In the study by Yaspelkis et al. (1993) glycogenolytic rate may have been influenced by the acclimation status of the subjects and the temperature difference between the trials. The subjects who participated in the heat trial at approximately 34^{o}C were well trained in a natural environment where the daily maximum temperature ranged between 35 and 41^{o}C. Heat acclimation has been found to reduce muscle glycogenolysis during exercise in the heat by over 40% (King et al., 1985; Kirwan et al., 1987).

There are several possible mechanisms which could account for an increased glycogenolysis during exercise in the heat. The increased glycogenolysis could arise from a need for more metabolic energy to sustain muscular contraction at a higher temperature. Edwards et al. (1972) observed a greater ATP degradation during the first of a series of isometric contractions to fatigue when muscle temperature was elevated by pre-heating the exercising limb. These authors postulated a higher cost of cross-bridge cycling due to myosin head 'slippage'

at higher temperatures. In addition, Kozlowski et al. (1985) observed an elevated muscle temperature and concomitant increased muscle glycogenolysis, lactate accumulation, CP degradation and adenine nucleotide degradation in hyperthermic dogs. The effect of exercise in the heat on adenine nucleotide and high energy phosphate metabolism in humans has not been previously investigated. The hypothesis that exercise in the heat increases energy turnover in the contracting human muscle remains unresolved.

The increased glycogenolytic rate during exercise in the heat has also been hypothesised to result from a redistribution of blood flow away from the contracting muscle (Rowell, 1974), an increase in circulating adrenaline (King et al., 1985) and/or a Q_{10} effect on metabolic processes (Edwards et al., 1972; Young et al., 1985). Whether or not blood flow to the contracting muscle is compromised during exercise in the heat is the subject of some debate. The increased demand for skin circulation during exercise in hot environments, together with the blood flow requirements to the contracting muscle is met by a reduced blood flow to other organs. A reduction in splanchnic (Rowell et al., 1968), hepatic (Rowell et al., 1965), renal (Radigan & Robinson, 1949) and inactive muscle (Rowell, 1986) blood flow occurs when comparing exercise in the heat with that in a cooler environment. It is unclear, however, whether blood flow to the active muscle is compromised during exercise in the heat. Cutaneous vasodilation displaces blood volume into cutaneous veins and lowers cardiac filling pressure (Rowell, 1986). Accordingly, the cardiac output may be reduced at a time when the cardiovascular demand is at its greatest (Rowell, 1986). The demand for O_2 supply to the active muscle and blood flow to the skin are thus, likely to be more than the heart can provide. If the active muscle O_2 supply is reduced exercise performance may be reduced. Alternatively, if blood supply to the skin is diminished, as suggested by Brenglemann et al. (1977), hyperthermia must occur (Rowell, 1986) which could be fatal (Hales, 1987).

Many authors (Fink et al., 1975; Kozlowski et al., 1985; Rowell, 1974; Rowell, 1986) have suggested that, during exercise in the heat, the 'competition' for blood flow between the skin and muscle favours the skin, leading to a reduced blood supply to the contracting muscle. It has been further hypothesised that this phenomenon contributes to the reduced performance in hot conditions (Kozlowski et al., 1985). The evidence regarding alterations to blood flow to the contracting muscle during exercise in the heat is contradictory. Quantitative measurements of muscle blood flow using radioactive microspheres have revealed a reduced active muscle blood flow during exercise and heat stress in sheep (Bell et al., 1983). Direct measurements of active limb blood flow using a thermodilution technique have shown no difference in blood flow in humans during submaximal exercise at 40-50% $\dot{V}O_2$max (Savard et al., 1988) or during uphill walking at 60% $\dot{V}O_2$max to exhaustion (Nielsen et al., 1990). Furthermore, muscle blood flow has been assessed by using a technique involving the measurement of total limb blood flow via plethysmography and skin blood flow using doppler flowmetry. Muscle blood flow is assessed by the difference between these two measurements. Using this method Smolander & Louhevaara (1992) could detect no difference when comparing muscle blood flow during dynamic handgrip exercise in the heat compared with similar exercise in a control environment. Unfortunately, these experiments cannot unequivocally determine whether contracting muscle blood flow in humans is compromised during exercise in the heat because of cross species differences and methodological problems. In the study by Savard et al. (1988), the heat stress was imposed by a water-perfused suit, preventing sweat evaporation creating a skin temperature which is clamped at levels higher than those reached in naturally hot environments. In the study by Nielsen et al. (1990) plasma volume was not reduced after exercise in the heat in contrast with others who have observed a lower plasma volume after exercise in the heat (Yaspelkis et al., 1993). The lack of difference in plasma volume observed by

Nielsen et al. (1990) is likely, to have been the result of the saline infusions used in the measurement of blood flow in this experiment. Furthermore, the authors (Nielsen et al., 1990) point out the limitations of the thermodilution technique stating that differences in blood flow less than 400 to 500 ml. min^{-1} are difficult to detect by this method. It is possible, therefore, that small, as yet undetectable, differences in blood flow exist and these may affect metabolism and performance. Although the method employed by Smolander and Louhevaara (1992) appears to be a satisfactory calculation of muscle blood flow measurement, one must exercise caution when interpreting these results since this exercise involved only a single muscle group, rather than whole body exercise. The average oesophageal temperature of 37.8^{o}C and average heart rate of 99 b. min^{-1} at the end of 'heat stress' is far lower than those values reported in other experiments (Fink et al., 1975; Nielsen et al., 1990; Savard et al., 1988; Young et al., 1985). Since it has been hypothesised that muscle blood flow only decreases when the demand for blood flow to the skin and muscle exceeds the cardiac capacity (Rowell, 1986), one would not expect the thermoregulatory load in the experiment by Smolander & Louhevaara (1992) to be of sufficient magnitude to evoke thermoregulatory responses. Further research examining active muscle blood in heat stressed humans is required in order to assess its role in altering metabolic processes. If, however, blood flow is compromised, an increased glycogenolytic rate may arise from a reduced O_2 supply to the active muscle. An increase in carbohydrate metabolism and decrease in lipid oxidation may occur in the presence of a reduction in O_2 supply, increasing anaerobic metabolism and consequently resulting in increased muscle lactate accumulation and creatine phosphate degradation. Whether or not human muscle has the ability to increase the O_2 extraction from the blood during exercise and heat stress has not been investigated. Schumacker et al. (1987) report the O_2 extraction rate to increase during hypovolemia in hyperthermic dogs, indicating that a reduced blood flow to the active muscle may have little effect on O_2 kinetics.

Although Young (1990) postulates that alterations in carbohydrate metabolism brought about by exercise in the heat are primarily the result of non-endocrine factors, many authors (King et al., 1985; Kirwan et al., 1987; Powers et al., 1985; Yaspelkis et al., 1993) have hypothesised that elevated catecholamines may be responsible for an increase in glycogenolysis and/or glycolysis during exercise in the heat. Elevated plasma catecholamine levels have been observed when comparing exercise in the heat with that in a cooler environment (Dolny & Lemon, 1988; Galbo et al., 1979b; Nielsen et al., 1990). Furthermore, when heat stress during exercise was increased by sweat supression via atropinisation, an augmented rise in sympatho-adrenal activity was observed (Davies et al., 1978). Few studies, however, have examined the relationship between catecholamine secretion and carbohydrate metabolism during exercise in the heat. Dolny & Lemon (1988) observed an increase in blood lactate accumulation along with an increase in plasma adrenaline concentration when comparing exercise in the heat with that in a cooler environment. In contrast, Nielsen et al. (1990) observed an augmented rise in plasma adrenaline concentration during exercise in the heat despite there being no difference in muscle or blood lactate accumulation, lactate efflux or muscle glycogenolysis. Further research examining the relationship between carbohydrate metabolism and sympatho-adrenal activity during exercise and heat stress is required.

The mechanism regulating the increased glycolysis may be related to the increase in muscle temperature. Young et al. (1985) suggest that the greater glycolysis during exercise in the heat compared with similar exercise in a cooler environment may be related to a differential Q_{10} effect on enzyme activity. The Q_{10} value commonly found for enzyme mediated reactions is 2.0-3.0, indicating that the enzyme reaction rate is increased two- to three-fold for every $10^{o}C$ increase in temperature (Florkin & Stolz, 1968). Snow et al. (1993a) report the exercise-induced increase in muscle temperature

is 0.9°C higher during 40 min of exercise at 40°C compared with 20°C. From the Q_{10} value quoted above, one would expect, at most, a 30% differential in lactate accumulation. Values reported in the literature exceed this estimate (Young et al., 1985) indicating that Q_{10} alone is an unlikely cause for the increase in glycolysis. Alternatively, the increase in glycolysis may arise from temperature effects on phosphorylative efficiency. Brooks et al. (1971) demonstrated that as muscle temperature was increased to between 40° and 45°C phosphorylative efficiency decreased. An increase in anaerobic energy demand may take place if mitochondrial phosphorylative efficiency is reduced. In addition, higher levels of cytoplasmic NADH and increased activity of lactate dehydrogenase (LDH) as a result of increased temperature may increase glycolytic and glycogenolytic pathways (Young et al., 1985).

2.2.2.2 Lipid Metabolism

Very little research has examined the effect of an acute bout of exercise in the heat on lipid metabolism. Plasma/serum FFA (Fink et al., 1975; Nielsen et al., 1990; Yaspelkis et al., 1993) and FFA uptake (Nielsen et al., 1990) are similar when comparing exercise in the heat with that in a cool environment. This does not necessarily mean, however, that an increase in carbohydrate metabolism occurs in the absence of a reduced lipid oxidation. Although Fink et al. (1975) found no difference in serum FFA concentration, lower rates of intramuscular TG utilisation were observed when comparing exercise in the heat with that in the cooler condition. Hence, plasma FFA concentration is not necessarily a reflection of lipid oxidation in muscles. Furthermore, both the respiratory exchange ratio (RER) (Yaspelkis et al. 1993; Young et al., 1985) and carbohydrate oxidation (Yaspelkis et al., 1993) are slightly higher at all measurement times in the heat compared with the thermoneutral environment, although these measurements were not significantly different. These data are

consistent with a decreased whole body reliance on lipid metabolism during exercise and heat stress.

2.2.2.3 Protein Metabolism

Very little research has been conducted in the area of exercise in the heat on protein and NH_3 catabolism during exercise and heat stress. Snow et al. (1993a) observed a higher plasma and muscle NH_3 accumulation during exercise in the heat compared with exercise in a thermoneutral environment in untrained individuals. Unfortunately, since IMP levels were not measured in this study, it is difficult to determine if the increase in NH_3 resulted from AMP deamination or amino acid catabolism. It is possible, however, that amino acid catabolism contributed wholly, or in part, to the observed higher appearance of NH_3 in both the muscle and the blood. This hypothesis, however, is not supported by the work of Dolny & Lemon (1988) who observed protein breakdown, as measured by urea excretion, to be lower during exercise in the heat compared with exercise at lower ambient temperatures. Nevertheless, the possibility of enhanced amino acid catabolism, as reflected in NH_3 concentration, cannot be entirely excluded. The area of protein metabolism requires further investigation.

2.2.2.4 Metabolic Basis of Fatigue During Exercise in the Heat

Of the human studies which have examined exercise to exhaustion in the heat compared with a cooler environment, only Nielsen et al. (1990) have examined muscle metabolism. Since glycogen was far from depleted in the contracting muscle these authors concluded that fatigue appears to be non-metabolic in origin. In contrast, Kozlowski et al. (1985) found lower ATP and CP concentration at fatigue in hyperthermic compared with cooled dogs. In

addition, the dogs were able to exercise for an extra 26 minutes in the cooled condition, possibly due to glycogen sparing since, at fatigue muscle glycogen was approximately equal in both conditions. Further research examining the cause of fatigue during exercise in the heat is required to resolve the discrepancy in the literature. An increase in fatiguability has been observed both *in vitro* (Lannergren & Westerblad, 1987; Lannergren & Westerblad, 1988) and *in vivo* (Edwards et al., 1972) in muscle at higher temperatures. Consequently, the optimal muscle temperature for isotonically contracting muscle is between 32^{o} and $37^{o}C$ (Faulkner, 1980). The increase in fatiguability has been related to the effect of temperature on mitochondrial capacity (Brooks et al., 1971) previously mentioned (2.2.2.1) and to the partial inhibition of enzyme activity (Edwards et al., 1972).

2.2.3 Exercise Metabolism in the Heat: Effects of Chronic Exposure

Heat acclimatisation and acclimation are terms given to the relationship between physiological adjustments and improved heat tolerance which accompany continuous exposure (McArdle et al., 1981). Acclimatisation is the term given to adaptations resulting from repeated exposure to natural environments, whilst acclimation results from controlled laboratory manipulation (Young, 1990). Both acclimation and acclimatisation to exercise in the heat have been found to result in similar adaptations (Armstrong & Maresh, 1991). Although thermal tolerance can be derived from constant training in a cool environment, improved physical conditioning as a result of endurance training is not as effective as repeated exercise in the heat (Buskirk, 1977). Gisolfi (1987), has defined the parameters for acclimatisation as being mild to moderate activity in a hot environment at 20-50% of $\dot{V}O_2$ for a duration of 1.5-4 hours per day for 5-14 consecutive days. The heat acclimation protocols used by most researchers fall

within these ranges (King et al., 1985; Kirwan et al., 1987; Young et al., 1985). Although the effect of heat acclimation on high energy phosphate, adenine nucleotide, NH_3 or amino acid metabolism has not been investigated, the effect of heat acclimation on carbohydrate, and to a lesser degree lipid, metabolism has received some attention.

2.2.3.1 Carbohydrate Metabolism

Many of the metabolic changes which take place during exercise in the heat appear to be reversed by the process of heat acclimation. Blood (King et al., 1985; Kirwan et al., 1987; Nielsen et al., 1993) and muscle (Young et al., 1987) lactate accumulation has, generally, been found to be lower after acclimation, although Senay & Kok (1977) observed an unchanged blood lactate response. Kirwan et al. (1987) observed no difference in lactate efflux or leg blood flow after acclimation. This observation suggests that removal from the blood was responsible for the lower blood lactate concentration following acclimation. This is possible if, as suggested by Rowell (1986) blood flow to the hepatic, splanchnic and inactive muscle regions is increased following acclimation. This hypothesis has not been experimentally investigated. Interestingly, Young et al. (1985) observed a reduced muscle lactate accumulation following acclimation during exercise both in hot and cool conditions. This observation suggests that perhaps regional blood flow distribution is not a mechanism for increasing lactate clearance, since regional blood flow would be expected to be similar during exercise in cool conditions independent of acclimation status. Alternatively, the reduced post-acclimation muscle lactate concentration observed during exercise in the cool (Young et al., 1985) may have resulted from a training adaptation. The subjects utilised in this study were untrained ($\dot{V}O_2max$ = 44.8 ± 1.3 ml. kg^{-1}. min^{-1}) and the authors observed an increase in $\dot{V}O_2max$ following

acclimation. Green et al. (1992a) observed decreased muscle glycogenolysis and muscle lactate accumulation following 5-7 days of endurance training. It is possible that the reduced post-acclimation muscle lactate accumulation observed in both environments (Young et al., 1985) was a result of training adaptations or a combination of training and acclimation adaptations.

The increased muscle lactate concentration observed by Young et al. (1985) could have resulted from an increase in lactate production. A decreased rate of muscle glycogenolysis has been observed during exercise in the heat following acclimation (King et al., 1985). Kirwan et al. (1987) observed a 47% reduction in muscle glycogenolysis during submaximal exercise in the heat following acclimation. In addition, Young et al. (1985) observed a lower glycogenolysis following acclimation when both hot and cool trial data were pooled. The mechanisms for a reduction in muscle glycogenolysis following heat acclimation may be the reverse of those responsible for an increase in glycogenolysis when moving from exercise in room temperature to that in the heat. Since acclimation results in a lower core temperature during exercise (Young et al., 1985), it is likely that muscle temperature is reduced following acclimation. If this is the case, the Q_{10} effect would be lower, reducing the enzyme reaction rates and/or the myosin head 'slippage' theory as postulated by Edwards et al. (1972) would, potentially, be reduced leading to a more efficient ATP turnover in the cross-bridge cycle.

A reduction in circulating catecholamines may also be responsible for the decreased glycolysis and glycogenolytic rate observed following acclimation. Nielsen et al. (1993) observed a lower arterial concentration of plasma catecholamines and lactate after 9-12 days of exercise in a hot environment. Although the fluid regulating and glucocorticoid hormones have been well investigated (Galbo, 1983), limited research has been conducted on the

sympatho-adrenal response to heat acclimation. A decrease in plasma volume does, however, result in an increased catecholamine secretion (Francis, 1979). Hence, an increase in plasma volume, may decrease catecholamine secretion, thereby decreasing muscle glycogenolysis and lactate accumulation. Green et al. (1989) postulated that plasma volume expansion, arising from adaptations to endurance training, results in a blunting of the exercise-induced increase in catecholamines and a decrease in muscle glycogenolysis. In contrast, when plasma volume was expanded by albumin infusion, rather than by training or acclimation adaptations, no differences were observed in any measured physiological or metabolic parameters (Sawka et al., 1983). On the basis of the current research, it is unclear whether plasma volume expansion alone, or plasma volume expansion in conjunction with other adaptations to acclimation, results in changes in carbohydrate metabolism, secondary to a reduced sympatho-adrenal response.

An increased blood flow during exercise in the heat following acclimation may affect carbohydrate metabolism. Rowell (1986) hypothesised that the large reduction in core temperature observed following acclimation suggests that less blood would be needed to be sent to the periphery and somewhat higher values for splanchnic and renal blood flow would be expected. If blood supply to the periphery is reduced following acclimation, one may expect an increase in muscle blood flow following acclimation. Rowell, (1974) suggested that the redistribution of cardiac output arising from heat acclimation may facilitate a greater blood supply to contraction skeletal muscle. Although little research has been conducted in the area, the few results available do not support this hypothesis. Both Kirwan et al. (1987) and Nielsen et al. (1993) found no difference in leg blood flow during submaximal exercise in the heat before and after acclimation. As noted previously, however, the thermodilution technique used in these studies did not quantify muscle blood flow. This is of importance

if cutaneous vasoconstriction arises as a result of heat acclimation. A measurement which quantifies muscle blood flow during exercise in the heat following acclimation is needed to confirm these results.

2.2.3.2 Lipid Metabolism

It is possible that lipid oxidation during exercise in the heat following acclimation is increased, although very little research has examined this phenomenon. RER is lower during exercise in the heat following acclimation (Kirwan et al., 1987; Young et al., 1985). Furthermore, although plasma FFA concentration is unchanged with acclimation (Kirwan et al., 1987; Nielsen et al., 1993), Kirwan et al. (1987) observed an increase in FFA uptake during exercise in the heat following acclimation. Further research examining intramuscular TG metabolism is required to assess the effect of acclimation on lipid utilisation.

CHAPTER 3

METHODS AND PROCEDURES

3.1 INTRODUCTION

This dissertation describes four related studies designed to address specific questions examining the effect of environmental temperature on muscle metabolism. Three of the four studies were similar in design as they involved submaximal exercise testing over a 40 minute period, whilst manipulating the environmental temperature and/or the acclimation status of the subjects. In addition in these studies the subjects were endurance trained individuals. The other study undertaken was designed to address one specific question relating to those raised in Chapter 1. As often occurs with a series of studies, the methods employed in the three similar studies were slightly refined over time. For this reason, a detailed methodology has been reserved for the subsequent chapters which address each individual study.

The four studies undertaken to address the questions raised in Chapter 1 will be referred to numerically as follows:

Study 1: Muscle metabolism during exercise in the heat in trained individuals.

Study 2: The effect of acclimation on muscle metabolism during exercise in the heat.

Study 3: Muscle metabolism during exercise in a hot, cool and thermoneutral environment.

Study 4: The influence of muscle temperature on metabolism during exercise.

The data presented in this dissertation were collected over a three year period between 1990 and 1992. Since it was important that subjects were not naturally acclimatised during their participation, all the data were collected between the months of June and October when maximum daily temperature ranged between 14^{o}C and 21^{o}C. All exercise tests were conducted in the Human Performance laboratory and all biochemical and histochemical analyses conducted in the Department of Chemistry and Biology, Victoria University of Technology. Analyses of hormones were conducted by the author in the Department of Medicine, St. Vincents Hospital Melbourne, and the Departments of Zoology and Medicine, University of Melbourne. All testing and analyses were conducted principally by the author with the assistance of medical staff, supervisors and fellow postgraduate students.

3.2 SUBJECTS

A total of 27 subjects participated in the studies which form this dissertation. One subject participated in two studies, whilst the remainder were involved in only one. Five additional subjects were recruited for the studies reported in this dissertation, but for various reasons, such as being unable to complete a trial (e.g. a heat trial) after having completed a comparative trial (e.g. a room temperature trial) they did not complete a particular study. Those data are not presented in this dissertation. Subjects volunteered for the studies after being informed of the risks associated with the procedures and signing a letter of informed consent (Appendix A). Subjects

involved in study 3 were paid an honorarium of $100.00 but were only informed of this honorarium after volunteering. Subjects were free to withdraw from each study at any time. All studies were approved by the Victoria University of Technology Human Experimentation Committee.

3.2.1 Subject Selection

All subjects who participated in this study were male. Subjects recruited for studies one, two and three were endurance trained cyclists or triathletes. Since the second of these studies involved seven 90 minute acclimation rides and all involved at least three 40 minute rides, trained subjects were selected in order to reduce the likelihood of metabolic changes arising from changes in training status and/or cycling efficiency. Study four was designed to examine the influence of elevated muscle temperature on muscle metabolism during exercise by isolating the rise in temperature to muscle alone. Consequently short term, high intensity cycling was chosen to eliminate a rise in core temperature. For these reasons active, non specifically trained males were recruited from the general population. In order to meet the selection criteria, subjects had to be male, between the ages of 18 and 35 and active but not specifically endurance trained. The following were selection criteria for trained subject: an age range of 18-35 years; a $\dot{V}O_2$max above 60 ml.kg.min^{-1}; a training regime consisting of a minimum of 15 hours per week at the time of participation. These criteria were chosen since the time course for reduction in the muscle oxidative capacity during detraining is far more rapid than that in $\dot{V}O_2$ max (Henrikkson & Reitman, 1976). Information relating to the number of training hours per week was obtained from a questionnaire (Appendix A).

3.2.2 Subject Instructions

All subjects were instructed to report to the laboratory prior to the $\dot{V}O_2max$ test and experimental trials after an overnight fast and having refrained from tobacco, alcohol and caffeine for the 24 hours prior to the tests. In study two, subjects were given no specific instructions on activity pattern, apart from the daily acclimation sessions, but they were encouraged to remain well hydrated for the entire acclimation period. During this time, additional training above the 90 minute cycling session was allowed. The final acclimation session was scheduled such that it finished 24 hours prior to the experimental exercise trial. In all circumstances, subjects were instructed to refrain from strenuous activity for a period of 48 hours and any activity for a period of 24 hours prior to the experimental trials. During study one, two and four subjects were instructed to record their diet for the 24 hours prior to their first experimental exercise trial. This was copied and handed back to the subjects for them to follow before subsequent trials, thereby attempting to minimise variations in resting intramuscular substrate concentration. Despite efforts to minimise differences in resting intramuscular glycogen concentration in these studies some subjects still had variable resting levels prior to each sub/supramaximal exercise trials. As a consequence, in order to further reduce this likelihood in the final study (study 3), subjects were provided with food packages consisting of 80% carbohydrate for the 24 hours prior to their submaximal trials. The diet is shown in Appendix A.

Following the exercise trials subjects were instructed to refrain from cycling for 24 hours, running for 48 hours and swimming for 72 hours in order to avoid complications with respect to their muscle biopsy sites. In addition subjects were contacted in the evening after their trials and were given feedback letters (Appendix A).

3.3 ENVIRONMENTAL CONDITIONS

All submaximal exercise trials were conducted in a temperature and humidity chamber (Tabai Model No. TBL-4RS-S). A temperature of 40^{o}C was selected for trials in hot conditions. This temperature was chosen since subjects were able to complete similar exercise in these conditions in previous studies (Fink et al., 1975; Nielsen et al., 1990). A temperature of 20^{o}C was selected for trials in a thermoneutral environment since this temperature was deemed to be cool but comfortable. The temperature of 3^{o}C employed in study three was selected since one aim of the third study was to minimise the possibility of an increase in core temperature during exercise. Although the thermoregulatory stress was not entirely eliminated in this environmental condition in study 3, it was significantly reduced when compared with ambient conditions (see Chapter 6). Relative humidity levels during all submaximal trials were maintained at 20% in all conditions except for the 3^{o}C trials. At this temperature the chamber was unable to maintain such a low humidity. The relative humidity in this condition was approximately 50%. Previous authors have reported higher rectal temperatures, heart rates and dehydration rates when comparing exercise in hot-wet with hot-dry conditions (Shapiro et al., 1980). Low relative humidity was adopted to increase the likelihood of subjects safely completing the 40 minute trials. Circulating air fans were not used in any trials.

3.4 MEASUREMENT OF MAXIMAL AEROBIC CAPACITY

3.4.1 Equipment

Maximal oxygen uptake ($\dot{V}O_2max$) tests were conducted on an electrically braked bicycle ergometer (Mijnhart KEM 2) or a friction braked bicycle ergometer (Monark Ergomedic 814E). A monitor (Sports Tester PE3000) was used to

record heart rate. VO_2max was determined using open circuit spirometry. Expired air was directed, by a Hans-Rudolf valve and plastic tubing, into a mixing chamber and through a ventilometer (Pneumoscan 830). Aliquots of the expired air were pumped from the mixing chamber through oxygen (Applied Electrochemistry S-3A) and carbon dioxide (Applied Electrochemistry CD-3A) analysers. VO_2 and RER were calculated using standard equations (Consolazio et al., 1963). Carbohydrate oxidation and total work ($kJ.min^{-1}$)) were estimated using the VO_2 and RER data to calculate the kJ per litre of oxygen consumed and the energy contribution of fat and carbohydrate (Appendix H). Measurement of VO_2max during study one and two were conducted in the heat chamber described previously. In these tests core temperature was monitored by a thermistor probe (YSI 401) inserted 10 cm beyond the anal sphincter. The output of the probe was monitored by a digital temperature display unit (CIG 520-572). During all VO_2max tests subjects had their nasal airway occluded by a nose clip and wore running shoes, socks and shorts.

3.4.2 Protocol

All VO_2max tests consisted of an incremental test to volitional exhaustion. The VO_2peak was quantified as the maximal value achieved during exercise. Three criteria were set for the attainment of a successful VO_2max value. These were; a) volitional exhaustion, b) a plateau in VO_2 with an increase in work c) RER above 1.1. All tests successfully achieved these criteria and thus the VO_2peak is referred to in this dissertation as VO_2max.

In studies 1 and 2 the following protocol for VO_2max determination was employed. Subjects began cycling at 100 watts (W), with the resistance increased by 50 W every two minutes for ten minutes and 25 W every minute

thereafter until volitional exhaustion. Tests were conducted in heat trial conditions ($40^{o}C$, 20% relative humidity [rh]). Studies examining the effect of temperature on VO_2 max have produced conflicting results (Rowell et al., 1968; Taylor et al., 1955).In a preliminary investigation, however, examining active but untrained individuals (n=9) no difference (51.3 ± 2.2 vs. 52.8 ± 2.1 $ml.kg.min^{-1}$, $P>0.05$) was found in VO_2 max between tests conducted in 20^{o} and $40^{o}C$ respectively.

Since the cycle ergometer employed in the first two studies was unavailable, an alternative apparatus was used to determine VO_2max in studies 3 and 4. During study 3, subjects began cycling with a one kg load at 80 rpm. The resistance was increased by one kg after three minutes and by 0.5 kg every three minutes thereafter until twelve minutes. At this point, the workload was increased by 0.2 kg every minute until exhaustion. During study 4, the protocol for VO_2max determination was modified to accomodate the lower level of aerobic fitness of the participants. Subjects began cycling with a one kilogram (kg) load at 80 revolutions per minute (rpm), with the resistance increased by one kg after three minutes. Thereafter, the load was increased by 0.5 kg until the ninth minute and by 0.2kg every two minutes thereafter until exhaustion. The time spent at each workload during VO_2max tests in this study was extended for two reasons. Firstly, a supramaximal workload had to be extrapolated from submaximal workloads and secondly, the subjects were untrained. Hence, the extension was deemed necessary in order to ensure steady state values were reached at each workload.

3.5 PREDICTION OF SUBMAXIMAL AND SUPRAMAXIMAL WORK INTENSITIES

The relationship between steady state submaximal VO_2 ($ml.kg.min^{-1}$) values and corresponding workloads was determined using a linear regression equation. From these equations workloads which would elicit 70% (study 1 & 2),

65% (study 3) and 115% (study 4) of VO_2max was calculated. The criterion for a successful correlation was set at R>0.98 (workload vs. VO_2). All tests produced a correlation R>0.98.

3.6 MEASUREMENT OF PHYSIOLOGICAL PARAMETERS DURING SUBMAXIMAL AND SUPRAMAXIMAL EXERCISE TESTS

Heart rate was measured during submaximal exercise tests by the method previously described. In studies one and two expired air samples were analysed using either an on line system, as previously described, or by Douglas bag collection. During study three, all samples were collected in Douglas bags. The oxygen and carbon dioxide contents of the expired gases collected by this method were measured on the previously mentioned analysers and volumes were determined using a ventilometer (Parkinson-Cowan), calibrated against a Tissot spirometer. Carbohydrate oxidation was calculated in study one and study two from the tables reported in Peronnet & Massicotte (1991) assuming a non protein RER. Heart rate and VO_2 were not recorded during study four.

Rectal temperature and muscle temperature were recorded in all studies. Rectal temperature was monitored by the method previously described. Muscle temperature was recorded by inserting a 25 gauge needle thermistor (YSI 524) four centimetres into the vastus lateralis. The output of this thermistor was again monitored on the digital temperature display unit. The time between the cessation of exercise and the recording of muscle temperature was <30 seconds in all studies.

3.7 BLOOD AND MUSCLE SAMPLING

Blood samples were obtained from a 20 gauge indwelling teflon catheter (Jelco) inserted into a vein in the antecubital space. Following

catheterisation, subjects lay supine for at least 10 minutes before the resting blood was sampled in order to obtain basal hormone concentrations. The catheter was kept patent by flushing with a small amount of saline containing heparin (10 IU. ml^{-1}) following each sample collection.

Muscle samples were obtained from the vastus lateralis muscle using the percutaneous needle biopsy technique (Bergstrom, 1962) modified to include suction (Evans et al., 1982). Five millimetre (Stille or Trewavis) needles were used for the samples analysed for metabolites and four millimetre (Trewavis) needles were used for samples analysed histochemically. An incision was made approximately 10cm proximal to the lateral epicondyle of the femur after local anaesthetic (1 or 2% Xylocaine). If a second incision was necessary, this was made approximately 3cm proximal to the first along the belly of the muscle. Not more than two biopsies were taken from the same incision in any study. If two biopsies were taken from the same incision, an effort was made to angle the needle away from the previously biopsy site in order to avoid the influence of acute trauma on successive biopsy specimens (Costill et al., 1988). If more than one incision was made, biopsies were taken from the distal incision first.

Samples taken for analyses of muscle metabolites were quickly frozen, and stored, in liquid nitrogen (LN_2) until analysis. The estimated time from the cessation of exercise to freezing in the post exercise samples was 20 seconds. During study one and two, muscle samples were taken for histochemistry at rest prior to one of the two trials, and following exercise in both trials. These samples were orientated in mounting medium (OCT, Tissue Tek.) and rapidly frozen in isopentane maintained at its freezing point with LN_2. The embedded muscle was stored in LN_2 for later histochemical analysis.

3.8 MUSCLE SAMPLE TREATMENT AND ANALYSIS

Following the completion of each study, muscle samples to be analysed for metabolites, were divided into two portions which were weighed at -30^{o}C. One portion (8-15 mg) was set aside in a tube (Eppendorf) for analysis of ammonia (in this dissertation ammonia denotes the sum of ammonia and ammonium). The second portion was freeze-dried (Edwards, Modulyo) for 48 hours, weighed, powdered and apportioned for glycogen or other metabolite analyses. One portion (approximately 2 mg) was used for the analysis of ATP, CP, C and lactate using standard enzymatic techniques with fluorometric detection. This sample was also used to measure ATP, IMP, ADP, AMP, inosine and hypoxanthine by high performance liquid chromatography (HPLC). The ATP results reported in this dissertation are the average of both HPLC and enzymatic results. During study one and two, a second portion (approximately 1 mg) was used for measurement of muscle glycogen. Since glycogen exists in clusters within human muscle fibres (Friden et al., 1989) and where enough muscle was available, three such portions were analysed for glycogen. This condition applied only in studies three and four, which was related to the use of newer and more effective biopsy needles.

3.8.1 Glycogen Determination

The portion or portions of powdered muscle were hydrolised in 250μl of 2M hydrochloric acid (HCL) at 100^{o}C for two hours with periodical agitation. The samples were subsequently neutralised by addition of 750μl 0.667M sodium hydroxide (NaOH) and stored at -80 until analysis. This extract was analysed for glucose according to the procedure of Lowry & Passonneau (1972) (Appendix A).

3.8.2 ATP, CP, C, Lactate and Adenine Nucleotide Determination.

3.8.2.1 Extraction of Samples

A portion of powdered muscle was extracted according to the procedure of Harris et al., (1974). Tubes containing the samples were placed on ice, and to which was added 250μl of ice cold 0.5M perchloric acid (PCA) /1mMethylenediaminetetra-acetic acid (EDTA). The tubes were periodically mixed on a vortex for a period of 10 minutes. They were then spun in a centrifuge (Heraeus Sepatech Biofuge 28RS) at 28,000 RPM (51,000 G) at 0^{O}C for two minutes. Following this centrifugation, 200μl of supernatant was transferred to a pre-cooled, appropriately labelled Eppendorf tube. This extract was neutralised with 50μl of 2.1M ice-cold $KHCO_3$, mixed and kept on ice for five minutes to ensure complete precipitation of perchlorate ions. After this period, the samples were mixed again and spun on a centrifuge as before. The supernatant was transferred to a pre-cooled, labelled cryule and stored at -80^{O}C until analysis.

3.8.2.2 Analysis of ATP, CP, C and Lactate

Measurement of ATP, CP, C, and lactate were analysed using standard ezymatic techniques with fluorometric detection (Turner Model 112) according to the procedure of Lowry & Passonneau (1972) (Appendix B). The concentrations of ATP, CP and C were adjusted to the peak total CP + C concentration for each subject in each study. This procedure minimised the error in measuring non muscle components of the tissue not visible in the sample. Lactate was not corrected due to its extracellular presence.

3.8.2.2 Analysis of Adenine Nucleotides

A 50μl aliquot of extract was used to determine the concentration of

ATP, ADP, AMP, IMP, inosine and hypoxanthine. An HPLC technique as described by Wynants & Belle (1985) was used (Appendix B). HPLC analysis was performed using a BIO-RAD Multi-Channel Chromatography Data System & Controller (Model 700). Examples of chromatographs are shown in Appendix C.

3.8.3 Muscle Ammonia Determination.

The muscle portion analysed for ammonia was conducted on wet tissue and corrected for water content using the wet-weight/dry-weight ratio determined from the freeze dried portion of each sample.

3.8.3.1 Extraction

Muscle extracted for ammonia determination was done so according to the method of Broberg (1989). Initial extraction was performed at -20^{o}C. A 55μl aliquot of cold 0.6M PCA-30% methanol (MeOH) was added to the muscle samples weighing between 8 and 15 mg. After 30 minutes at this temperature, 110μl of cold 0.6M PCA was added to the samples which were placed on ice and periodically mixed for a further 15 minutes. Samples were neutralised with the addition of 68μl of cold 1.8M potassium hydroxide (KOH), mixed, and spun in a centrifuge as previously described (3.8.2.1). Samples were stored on ice and analysed within one hour of extraction.

3.8.3.2 Analysis of Ammonia

Muscle ammonia was analysed by flow injection analysis (Svennson & Anfalt, 1982) as described by Katz et al., (1986). A 200μl aliquot of supernatant was injected into a flow injection analyser (Tecator FIAstar 5020 analyser) containing a 100μl injection loop connected to a spectrophotometer (Tecator FIAstar 5023). A detailed methodology appears in appendix B.

3.8.4 Muscle Histochemistry

The embedded muscle was sectioned into 10μm slices using a cryotome (Microm Heidelberg 500OM). Sections from each sample were stained for muscle fibre type and glycogen content. After appropriate preincubation, serial sections were stained for muscle ATPase activity according to the procedure of Dubowitz (1985). A periodic acid-Schiff (PAS) stain as described by Pearse (1961) was used to stain fibres for glycogen content. Sections which underwent PAS staining were examined on a video microscope and the intensity of the stain in approximately 400 fibres per cross section was rated visually on a scale of 0 (negative) to 4 (darkly stained) according to the method of Coyle et al. (1986). This involved tracing the video image from the PAS stained fibres and rating each fibre as mentioned above. Subsequently the fibre type was determined by matching the traced image with the ATPase stains. The PAS stained sections were always examined first to ensure that they were rated independent of knowledge of the fibre type. Fibres from each section were counted individually and reported as a percentage of the total number of fibres within the particular muscle cross section. The semi-quantitative method was adopted since a microspectrophotometer was not available. Examples of muscle histochemistry are presented in Fig. 3.1.

3.9 BLOOD ANALYSIS

3.9.1 Glucose and pH

At each sampling time approximately two ml of blood was placed in a tube containing fluoride heparin and stored on ice. These samples were analysed for blood glucose within three hours of collection using an automated glucose analyser (YSI model 23AM). Two ml of blood was drawn into a syringe containing

heparin (5000 IU/ml) and stored on ice. Blood pH was determined from these samples using an automated blood gas analyser (Radiometer, Copenhagen, ABL-30 acid-base analyser). All samples were analysed in duplicate.

3.9.2 Lactate

After sampling, approximately 8 ml of blood was mixed in a tube lined with anticoagulant (Lithium Heparin). A 500μl aliquot of this blood was transferred into a tube containing 1ml of 3M PCA, mixed and spun in a centrifuge for 2 minutes at 2000 rpm. The supernatant was transferred to another tube and stored at -80^{o}C until analysis for blood lactate. The analysis was performed on a spectrophotometer (Shimadzu UV-120) using an enzymatic technique (Appendix B) (Lowry & Passonneau, 1972).

3.9.3 Ammonia

Anticoagulated blood was transferred to tubes and spun in a centrifuge (see 3.9.2). The resultant plasma was immediately transferred to a tube and stored in LN_2 until NH_3 analysis. Samples were analysed in duplicate within 72 hours of collection to minimise the possibility of increases in ammonia due to prolonged storage (Howanitz et al., 1984). Plasma NH_3 was determined by flow injection analysis (FIA) analysis according to the method described by Cardwell et al., (1987).

3.9.4 Catecholamines and Free Fatty Acids.

A preservative was prepared by dissolving 2.25g of ethyleneglycol - bis - (betaaminoethylether)N, N'- tetraacetic acid (EGTA) and 1.5g reduced glutathione (GSH) in 25ml of normal saline (0.9% sodium chloride w/v) and

adjusted to 6-7.4 pH with 5-10M NaOH. Aliquots of this preservative (20μl per 1ml of whole blood) were placed in tubes and stored on ice. The appropriate volume of blood was added to the tubes, mixed gently, and spun in a centrifuge at 4^{o}C for 15 minutes at 1500rpm (900g). The supernatant was transferred to tubes and stored at -80^{o}C until analysis. Samples were analysed for plasma catecholamines by a modification of the single isotope [^{3}H] radioenzymatic assay of Passon & Peuler, (1973) as described in the Amersham Catecholamine research assay system (code TRK 995). Each sample was counted for 10 minutes on a beta counter (Packard Tri-Carb 4000). An aliquot of the remaining plasma was analysed for free fatty acids (FFA) by a modification of the enzymatic colormetric method for determination of non-esterified fatty acids (NEFAC) of Miles et al. (1983) as described in the Wako NEFAC kit (code no. 279-75409).

3.9.5 Insulin, Cortisol and Glucagon Determination.

For the determination of these hormones plasma was separated from anticoagulated blood by centrifugation (see 3.9.2) and stored at -80^{o}C until later analysis. Plasma insulin concentration was determined by radioimmunoassay (RIA) as described in the Incstar Insulin ^{125}I RIA Kit (Cat. No. 06130). Each sample was counted for one minute on a gamma counter (LKB Wallac 1277 Gamma Master). Cortisol concentration was also determined using this plasma by RIA as described in the ICN Biomedical Immuchem. Cortisol Coated Tube ^{125}I Radioimmunoassay Kit (Cat No. 07-221102).

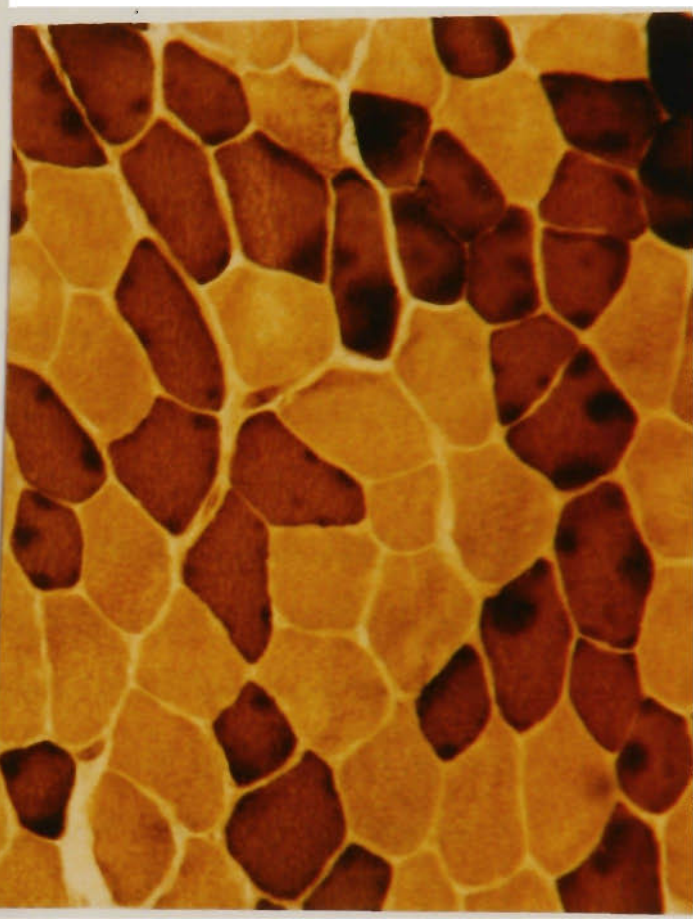
For plasma glucagon whole blood was added to a tube coated with anticoagulant (Lithium Heparin) containing the protease inhibitor Trasylol (50μl per ml). The tubes were mixed gently and centrifuged (2000rpm for 2 minutes) and the supernatant stored at -80^{o}C until analysis. Plasma glucagon was determined by RIA as described in the ICN RSL Glucagon Kit (07-152101).

Cortisol and glucagon samples were counted on a gamma counter (Packard Cobra Auto Gamma 5000 Series) for one minute.

3.10 STATISTICAL ANALYSIS

Analysis of variance with repeated measures (ANOVA) was used to compare data between trials and over time. Simple main effects and Newman-Keuls post-hoc tests were used to locate differences when ANOVA revealed a significant interation. Correlation coefficients were also determines. A BMDP computer software program was used to compute these statistics. Student t-test was used for paired comparisons (Schefler, 1979). The level of probability to reject the null hypothesis was set at $P<0.05$. All data are reported as means $\pm$ the standard error of the mean (SE). More detail on statistical analyses is presented in each experimental chapter.

Periodic Acid Schiff Stain



Myosin ATPase Stain
(pH 4.3)

Myosin ATPase Stain
(pH 10.3)

Fig. 3.1 Muscle Histochemistry

CHAPTER 4

MUSCLE METABOLISM DURING EXERCISE IN THE HEAT IN TRAINED INDIVIDUALS

4.1 INTRODUCTION

Fatigue during prolonged exercise occurs earlier at higher environmental temperatures (Saltin et al., 1972; Nielsen et al., 1993), although the precise mechanisms for the earlier onset of fatigue are unknown. The cardiovascular responses during exercise at high environmental temperatures have been studied extensively and an increased demand for blood flow to the skin, together with the requirements for blood flow to exercising muscle, have been found to reduce flow to renal, splanchnic, hepatic and non-contracting muscle (Radigan & Robinson, 1949; Rowell et al., 1965; Rowell et al., 1968; Rowell, 1986). It has been hypothesised that the increased demand for skin blood flow during exercise and heat stress may lead to a reduction in muscle blood flow to active muscle (Rowell, 1974), thereby reducing oxygen delivery and contributing to the earlier fatigue. Alternatively, it has been suggested that when exercising in the heat, the drive to exercise has little to do with local fatigue at the cellular level and is diminished by central mechanisms as a result of an increase in core temperature (Bruck & Olschewski, 1984: Nielsen et al., 1990).

Although a high muscle temperature has been found to influence muscle metabolism (Edwards et al., 1972), a relationship between fatigue during exercise in higher environmental temperatures and indices of muscle fatigue is not well established. Indeed, the effect of exercise in the heat on skeletal

muscle metabolism is under debate. When exercise induced hyperthermia in dogs was attenuated by controlled cooling, a reduction in muscle lactate concentration and increased concentrations of muscle ATP, CP and glycogen were observed during exercise (Kozlowski et al., 1985). Previous human studies have reported either increased glycogenolysis (Fink et al., 1975) and lactate accumulation (Young et al.1985) or no changes to muscle metabolism (Nielsen et al., 1990; Yaspelkis et al., 1993) when comparing exercise in the heat with that in a cooler environment. Methodological differences between these studies, for example, the acclimation status of the subjects or the temperature differential between the trials, may account for the discrepancies.

A number of mechanisms for the changes in muscle metabolism during exercise and heat stress have been suggested, if indeed, environmental temperature has an effect on muscle metabolism during exercise. It has been postulated that a change in plasma catecholamines is responsible for a change in substrate utilisation during exercise and heat stress (King et al., 1985), since, the adrenal medullary response to exercise increases in a hot environment (Galbo et al., 1979b; Powers et al., 1982) and increased circulating adrenaline enhances muscle glycogenolysis during exercise (Jansson et al., 1986). A change in fibre type recruitment pattern from type I to type II fibres when comparing exercise in the heat with that in a cooler environment has also been hypothesised since individuals with a greater proportion of type II fibres have been found to produce greater increments in muscle lactate accumulation (Young et al., 1985). Furthermore, it has been suggested that a Q_{10} effect on glycolysis (Kozlowski et al., 1985; Young et al., 1985) could possibly increase the glycolytic rate during exercise in the heat. The purpose of this study was to compare muscle metabolism during submaximal exercise in the heat with that in a cooler environment and to examine the mechanisms underlying any differences.

4.2 METHODS

Thirteen endurance trained males (21.4 ± 2.7 years; 175.5 ± 5.3 cm; 68.1 ± 6.3 kg; $\dot{V}O_2max$ = 64.8 ± 4.8 $ml.kg^{-1}.min^{-1}$; Fibre type 62 ± 2 % type I, 36 ± 2 % type IIa, 2 ± 1 % type IIb, mean ± SE) volunteered as subjects for this study. $\dot{V}O_2max$ was determined in heat trial conditions according to the protocol described in Chapter 3. Following the $\dot{V}O_2max$ test, a workload was selected which would elicit approximately 70% $\dot{V}O_2max$. Seven subjects performed a submaximal 40 min cycling trial on the bicycle ergometer used for the $\dot{V}O_2max$ test in a thermoneutral environment (20°C, 20%rh) (RT) and one week later performed a similar trial in a hot environment (40°C, 20%rh) (HT). The order was reversed for the remaining six subjects. During the HT subjects were removed from the environmental chamber if their rectal temperature exceeded 40.5°C or if their rectal temperature exceeded 39.5°C in combination with a heart rate >190b.min^{-1}. Alternatively, subjects were instructed to ask to be removed if they felt extreme discomfort. All subjects completed 40 min of exercise except for two who could not continue to exercise beyond 38 and 35 min respectively in the HT, and asked to be removed. Since these two subjects performed the HT first, the RT was terminated at the same time.

Subjects were weighed nude on arrival at the laboratory and following the exercise trial. After the initial weigh, subjects inserted a rectal thermistor probe and entered the environmental chamber, set to the designated environmental conditions. Core temperature was recorded immediately prior to exercise and at ten minute intervals until the end of the exercise period. Muscle temperature was measured prior to and immediately following exercise. Following catheterisation, subjects rested supine in the environmental chamber for approximately 10 minutes before the resting blood was sampled. Blood samples were obtained at rest and every 10 minutes until the end of exercise.

Blood samples were analysed for lactate and glucose. Plasma samples were analysed for NH_3, FFA free fatty acids, adrenaline, noradrenaline, glucagon, cortisol and insulin. Muscle samples were obtained from six subjects prior to and immediately following exercise and were analysed for ATP, ADP, IMP, AMP, NH_3, CP, C, glycogen and lactate. A further muscle sample was taken at rest during the HT, and following exercise in both trials for the histochemical estimation of glycogen depletion. A pre-exercise sample was taken in the HT only, in order to minimise the number of muscle biopsies each individual underwent. PAS staining intensity reaches a maximum at a glycogen concentration of 70-80 mmol. glucosyl units. kg^{-1} wet weight, at which concentration all type I and type II fibres stain darkly (Piehl, 1974). Since the subjects were well trained, rested for 24 hours prior to the trials and consumed their normal diet which was rich in carbohydrate, it was expected that their resting glycogen levels would be above 80 mmol. glucosyl units. kg^{-1} wet weight. Indeed, this was the case.

Heart rate was recorded during each trial at 5, 15, 25 and 35 minutes of exercise. Respiratory data were also collected at these times using either Douglas bag collection or an on-line metabolic system. Carbohydrate oxidation was estimated from these data at each measurement point. Cycling shorts and running shoes were worn for both trials. The data from the RT and HT trials were compared using ANOVA with repeated measures. Student t-tests for paired samples were used to compare muscle glycogenolysis, the percentage of glycogen depletion and the weighted mean in both type I and type II fibres.

4.3 RESULTS

There was no drift in exercise $\dot{V}O_2$ over time in either trial. There was a small (4%), but significant ($P<0.05$) difference in mean exercise $\dot{V}O_2$ between

the trials (Table 4.1). Post-exercise T_r was higher ($P<0.01$) in the HT when compared with the RT, as were mean HR, RER and CHO oxidation ($P<0.01$) (Table 4.1). Total weight loss was higher ($P<0.01$) in the HT compared with the RT (Table 4.1). Both pre-exercise and post-exercise T_m measurements were higher ($P<0.01$) in the HT compared with the RT (Table 4.1).

Blood glucose (Fig. 4.2) and lactate (Fig. 4.1) concentrations were not different at rest between the two trials. During exercise, blood glucose ($P<0.01$) and lactate ($P<0.05$) concentrations were higher in the HT at all points when compared with the RT. Plasma NH_3 concentrations were higher ($P<0.01$) during exercise when compared with resting values in both trials. No differences were observed at any point between the trials (Fig. 4.1). Plasma FFA declined ($P<0.01$) from the resting value in both trials. No difference in FFA were observed between the two trials (Fig. 4.2). Plasma adrenaline concentrations were higher prior to ($P<0.05$) and during exercise ($P<0.01$) in the HT compared with the RT (Fig. 4.3). Plasma noradrenaline concentrations were not different at rest or during exercise when comparing the two trials (Fig. 4.3). Concentrations of plasma cortisol, glucagon and insulin were not different prior to or during exercise between the two trials (Fig. 4.4).

Pre-exercise muscle lactate concentrations were not different in the HT compared with the RT. The post-exercise value in the HT was higher ($P<0.05$) compared with the resting value but exercise did not alter the muscle lactate concentration in the RT. The post-exercise muscle lactate levels were higher ($P<0.01$) in the HT when compared with RT (Fig. 4.5). There was no difference in pre-exercise muscle glycogen concentration between the two trials. Post-exercise glycogen concentration was lower ($P<0.05$) in both trials compared with pre-exercise concentrations. Muscle glycogenolysis was greater ($P<0.05$) HT compared with RT (Table 4.2). Muscle ATP levels were not affected by exercise

or temperature (Table 4.3). A main effect for time ($P<0.05$) was observed for concentrations of muscle IMP, AMP and ADP (Table 4.3). NH_3 concentration (Fig. 4.5) increased ($P<0.05$) as a result of exercise in both trials. No differences, however, were observed in these metabolites either prior to or following exercise when comparing the two trials. In contrast, post-exercise muscle CP levels were lower ($P<0.05$) and C concentration were higher ($P<0.05$) in the HT compared with the RT (Fig. 4.6). The single pre-exercise PAS stained muscle section for all subjects showed a weighted mean of 4.00 ± 0.00 (uniformly fully stained) for both type I and type II fibres. The post-exercise PAS stained muscle sections indicated that exercise in the heat had no effect on glycogen depletion in the type II fibres with the mean ratings being 3.70 ± 0.12 and 3.80 ± 0.08 and the percentage of fully stained (dark) fibres being 80.0 ± 7.1% and 82.8 ± 7.0% for the HT and RT trials, respectively (Fig 4.7). In contrast, the percentage of fully stained (dark) type I fibres was lower ($P<0.05$) in the HT when compared with the RT (29.0 ± 14.6 vs. 59.2 ± 12.6%). No significant difference ($P=0.07$) was observed for the mean rating between the HT and the RT (3.35 ± 0.23 vs. 2.64 ± 0.34) (Fig. 4.7).

	TRIAL	
	RT	HT
O_2 Uptake ($l.min^{-1}$)	3.12±0.06	2.99±0.05 *
Heart Rate ($b.min^{-1}$)	157±2	173±2 **
RER	0.89±0.01	0.93±0.01 **
CHO Oxidation ($g.min^{-1}$)	2.01±0.10	2.77±0.13 **
Energy Exp. ($kJ.min^{-1}$)	63.96±2.27	62.14±1.94
Weight Loss (Kg)	0.70±0.04	1.12±0.06 **
Rectal Temp. (^{o}C)		
PRE-EX	37.2±0.1	37.2±0.1
POST-EX	38.7±0.1	39.7±0.1 **
Muscle Temp. (^{o}C)		
PRE-EX	35.7±0.1	36.5±0.2 **
POST-EX	39.1±0.1	40.4±0.1 **

Table 4.1. *Mean O_2 uptake, mean heart rate, mean respiratory exchange ratio (RER), mean carbohydrate (CHO) oxidation, energy expenditure, total weight loss, and rectal and muscle temperature before (PRE-EX) and after (POST-EX) exercise in $20^{o}C$ RH 20% (RT) and $40^{o}C$ RH 20% (HT).*

Values are means ± SE, (n=13). * indicates difference ($P<0.05$) HT compared with RT, ** indicates difference ($P<0.01$) HT compared with RT.

TRIAL	PRE-EX	POST-EX	△
RT	488±40	360±63 [a]	128±30
HT	482±36	300±48 [a]	182±27 [*]

Table 4.2. *Muscle glycogen concentrations before (PRE-EX) and after (POST-EX) exercise and muscle glycogenolysis (△) in 20°C RH 20% (RT) and 40°C RH 20% (HT).*
Values are means ± SE (n=6) expressed in mmol.glucosyl units.kg^{-1} dry weight. [a] indicates difference ($P<0.05$) POST-EX compared with PRE-EX, [*] indicates difference ($P<0.05$) HT compared with RT.

	RT		HT	
	PRE-EX	POST-EX	PRE-EX	POST-EX
ATP	24.7±1.6	25.0±1.7	24.1±1.3	24.0±1.4
ADP +	2.03±0.13	2.24±0.12	2.12±0.09	2.47±0.08
AMP +	0.05±0.01	0.07±0.02	0.05±0.01	0.11±0.02
IMP +	0.06±0.02	0.13±0.11	0.12±0.12	0.36±0.11

Table 4.3. *Intramuscular concentrations of adenosine 5'triphosphate (ATP), adenosine 5'diphosphate (ADP), adenosine 5'monophosphate (AMP) and inosine 5'monophosphate (IMP) before (PRE-EX) and after (POST-EX) exercise in 20^{O}C RH 20% (RT) and 40^{O}C RH 20% (HT).*

Values are means ± SE (n=6) expressed in mmol. kg^{-1} dry wt. + indicates a significant ($P<0.05$) exercise effect for accumulation of ADP, AMP, IMP.

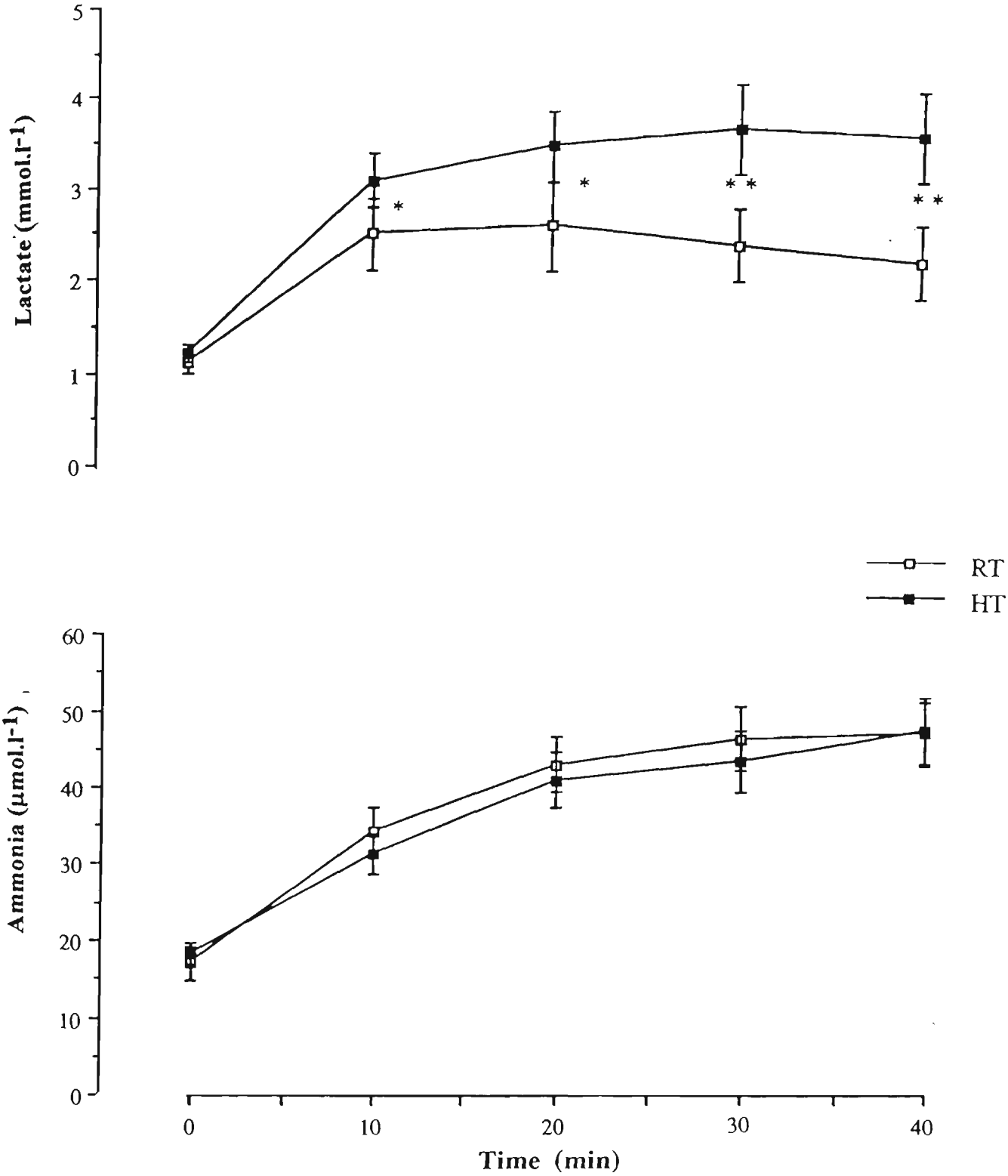


Fig. 4.1. Blood lactate and plasma ammonia concentrations during exercise in 20°C RH 20% (RT) and 40° RH 20% (HT). Values are means ± SE (n=13). * indicates difference ($P<0.05$) HT compared with RT, ** indicates difference ($P<0.01$) HT compared with RT.

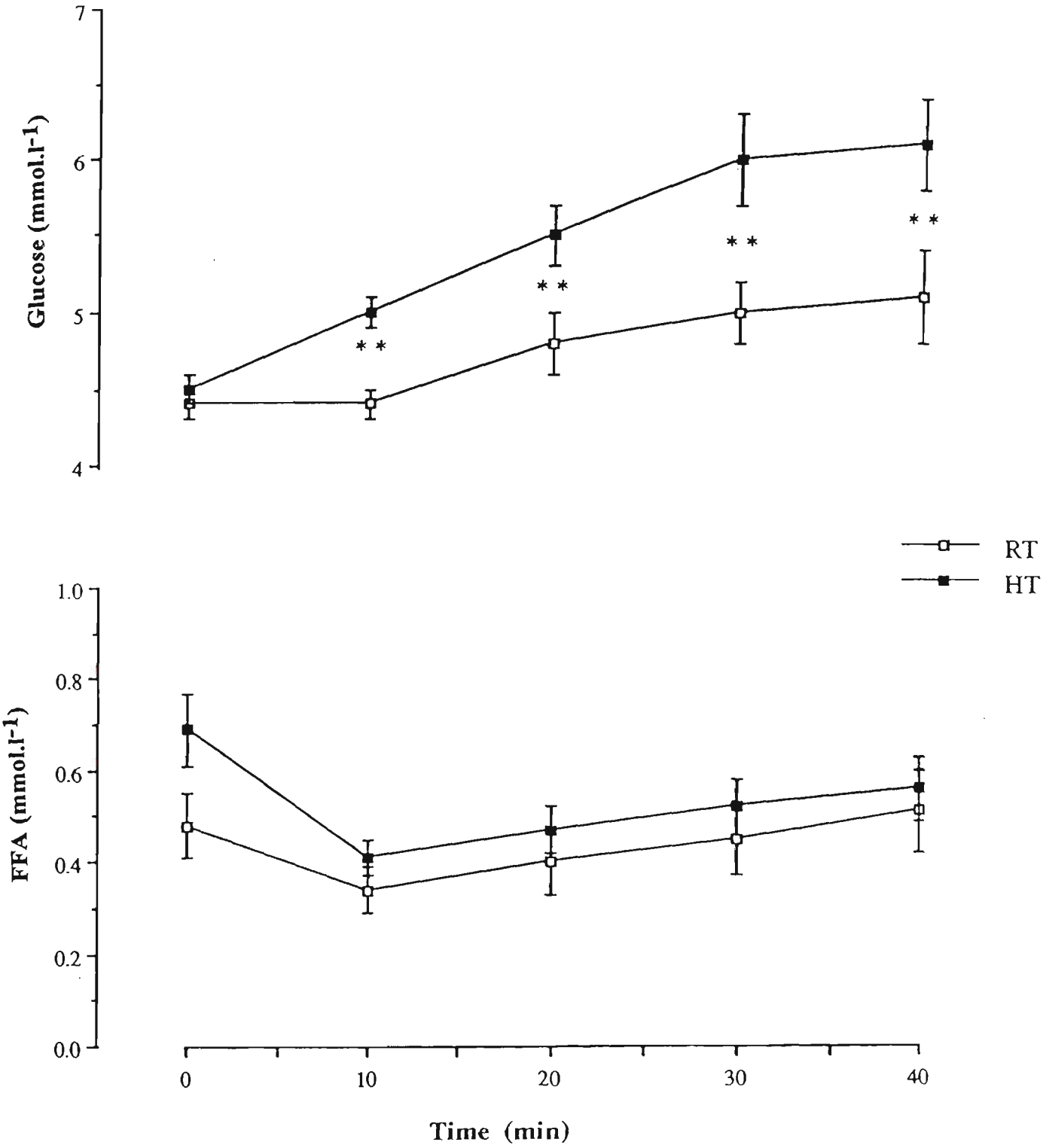


Fig 4.2. Blood glucose and plasma free fatty acid (FFA) concentrations during exercise in 20°C RH 20% (RT) and 40° RH 20% (HT). Values are means ± SE (n=13 for blood glucose, n=7 for plasma FFA). ** indicates difference ($P<0.01$) HT compared with RT.

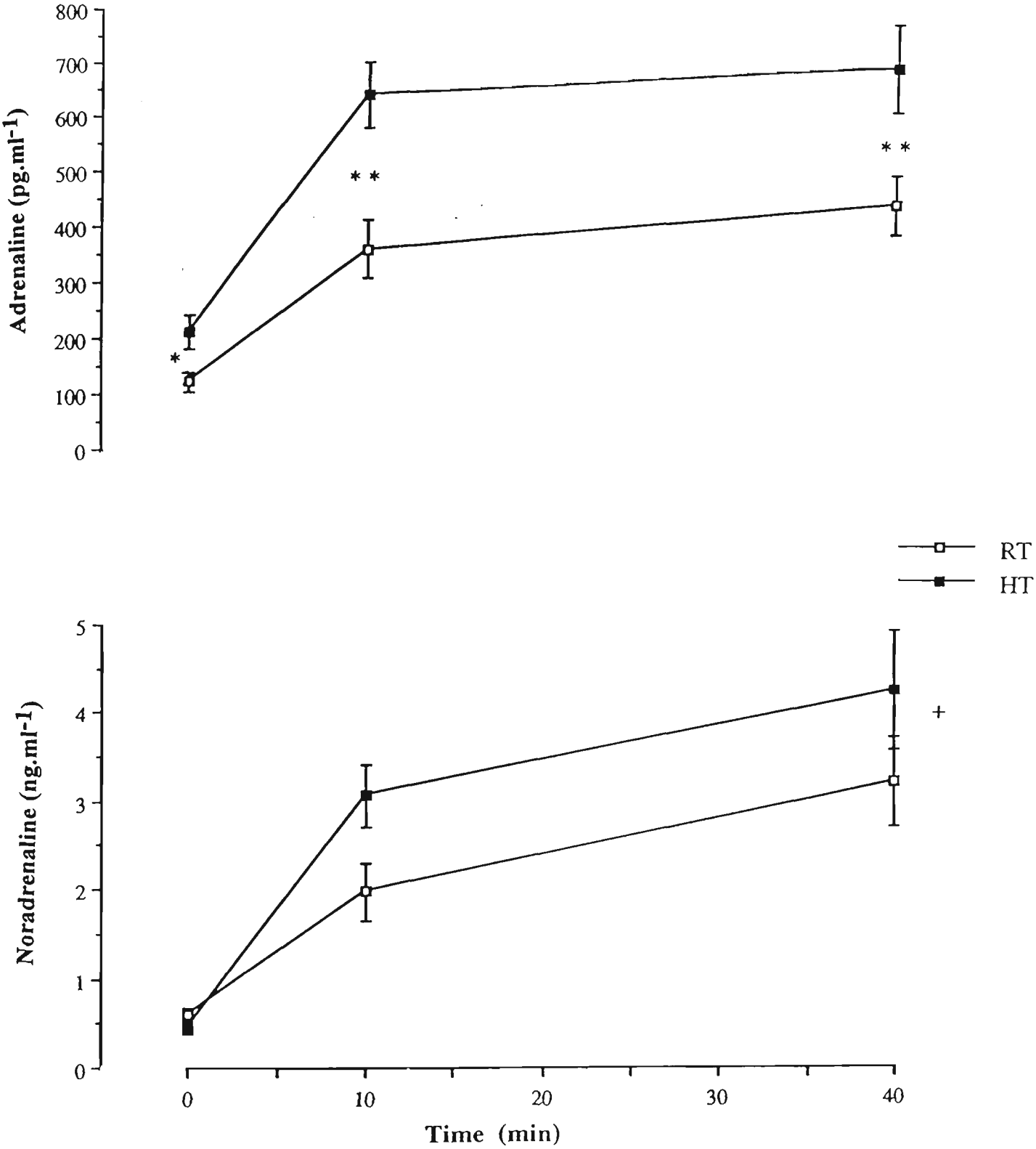


Fig. 4.3. Concentrations of plasma adrenaline and noradrenaline during exercise in 20°C RH 20% (RT) and 40°C RH 20% (HT). Values are means ± SE (n=7). * indicates difference ($P<0.05$) HT compared with RT, ** indicates difference ($P<0.01$) HT compared with RT. + indicates lower ($P<0.05$) mean noradrenaline concentration RT compared with HT.

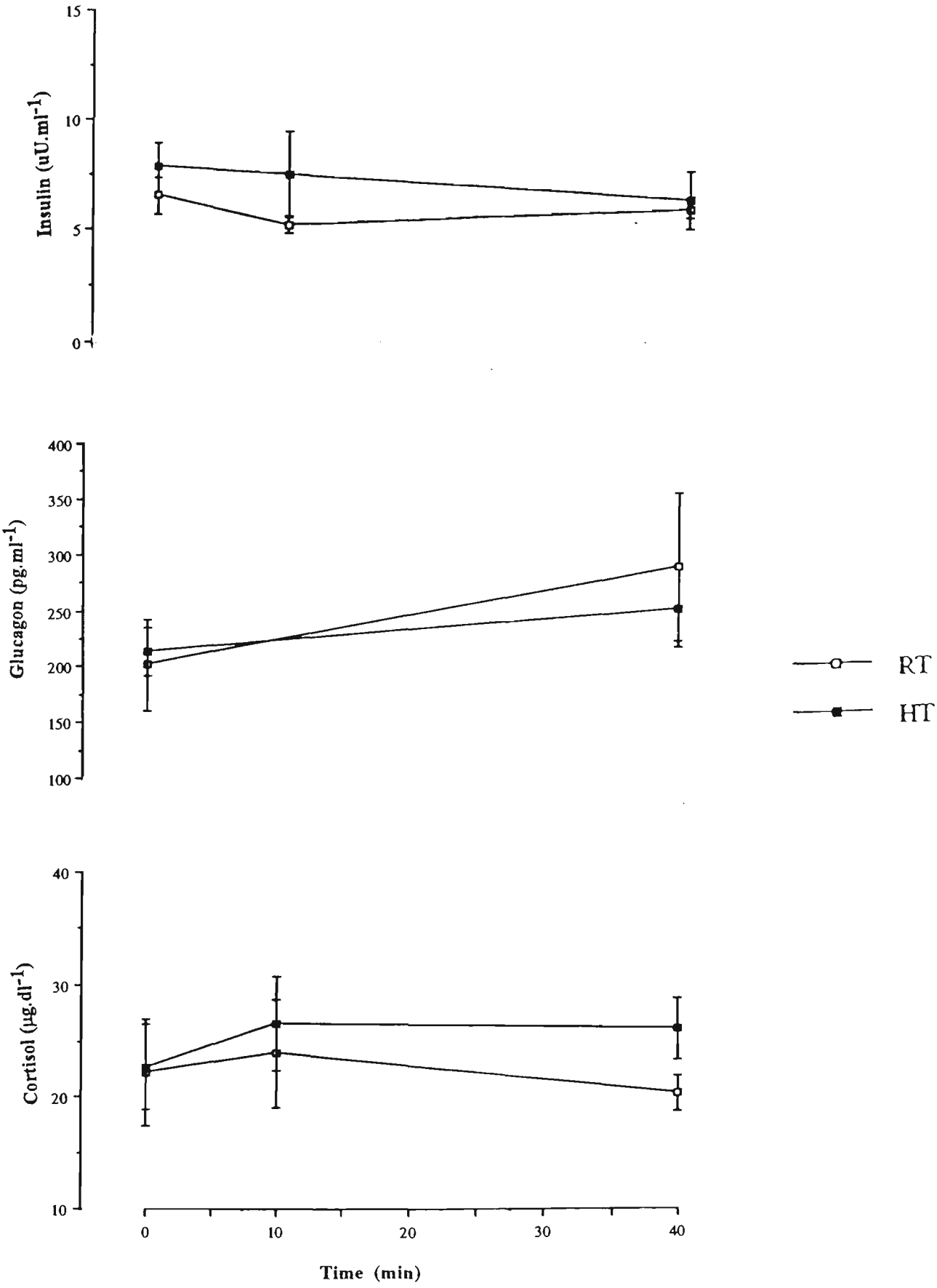


Fig. 4.4. Concentrations of plasma insulin, glucagon and cortisol during exercise in 20^{O}C RH 20% (RT) and 40^{O}C RH 20% (HT). Values are means ± SE (n=6)

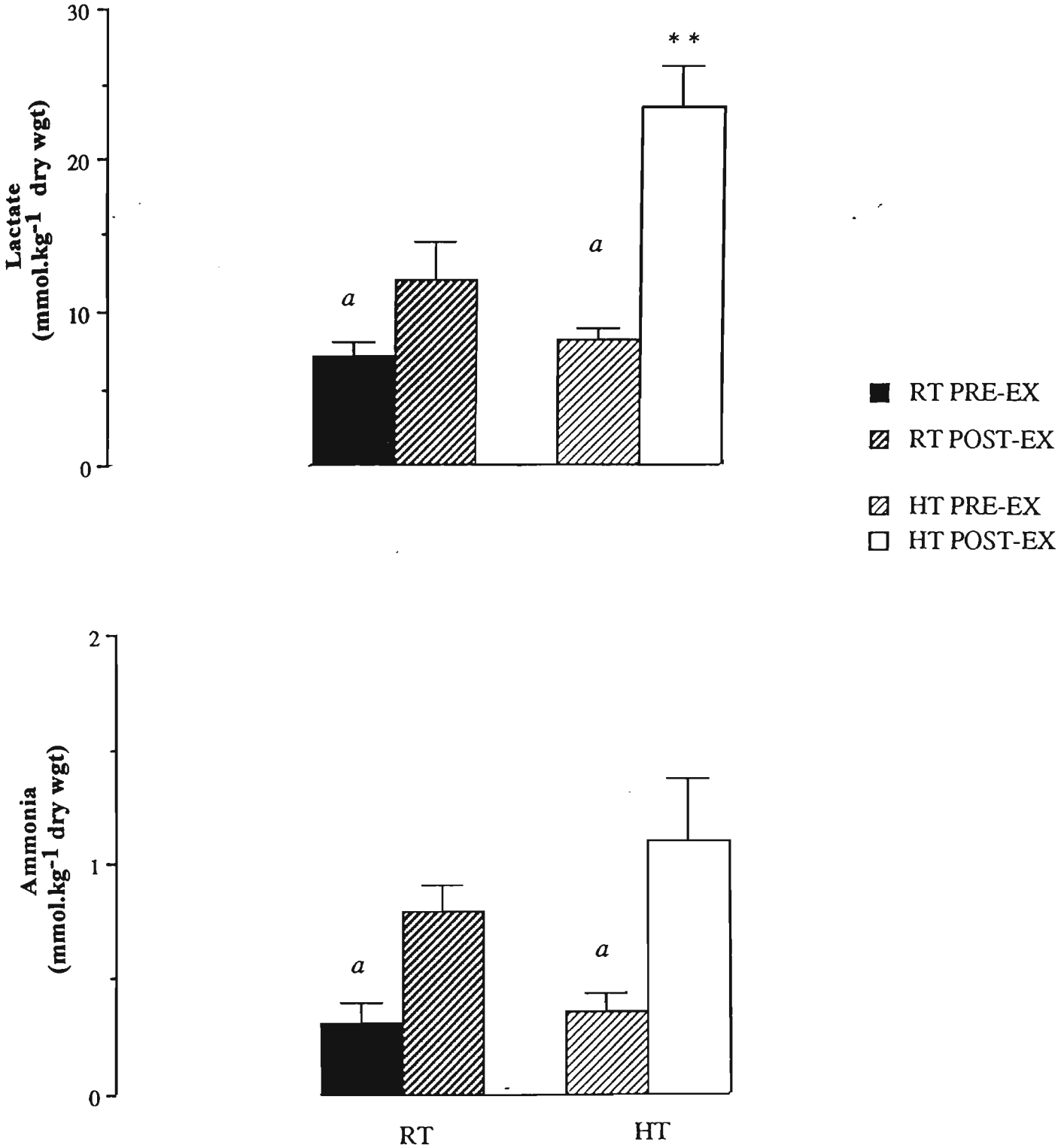


Fig. 4.5. Muscle lactate and muscle ammonia concentration before (PRE-EX) and after (POST-EX) exercise in 20°C RH 20% (RT) and 40°C RH20% (HT). Values are means ± SE (n=6). ^{a} indicates difference ($P<0.05$) PRE-EX compared with POST-EX, ** indicates difference ($P<0.01$) HT POST-EX compared with RT POST-EX.

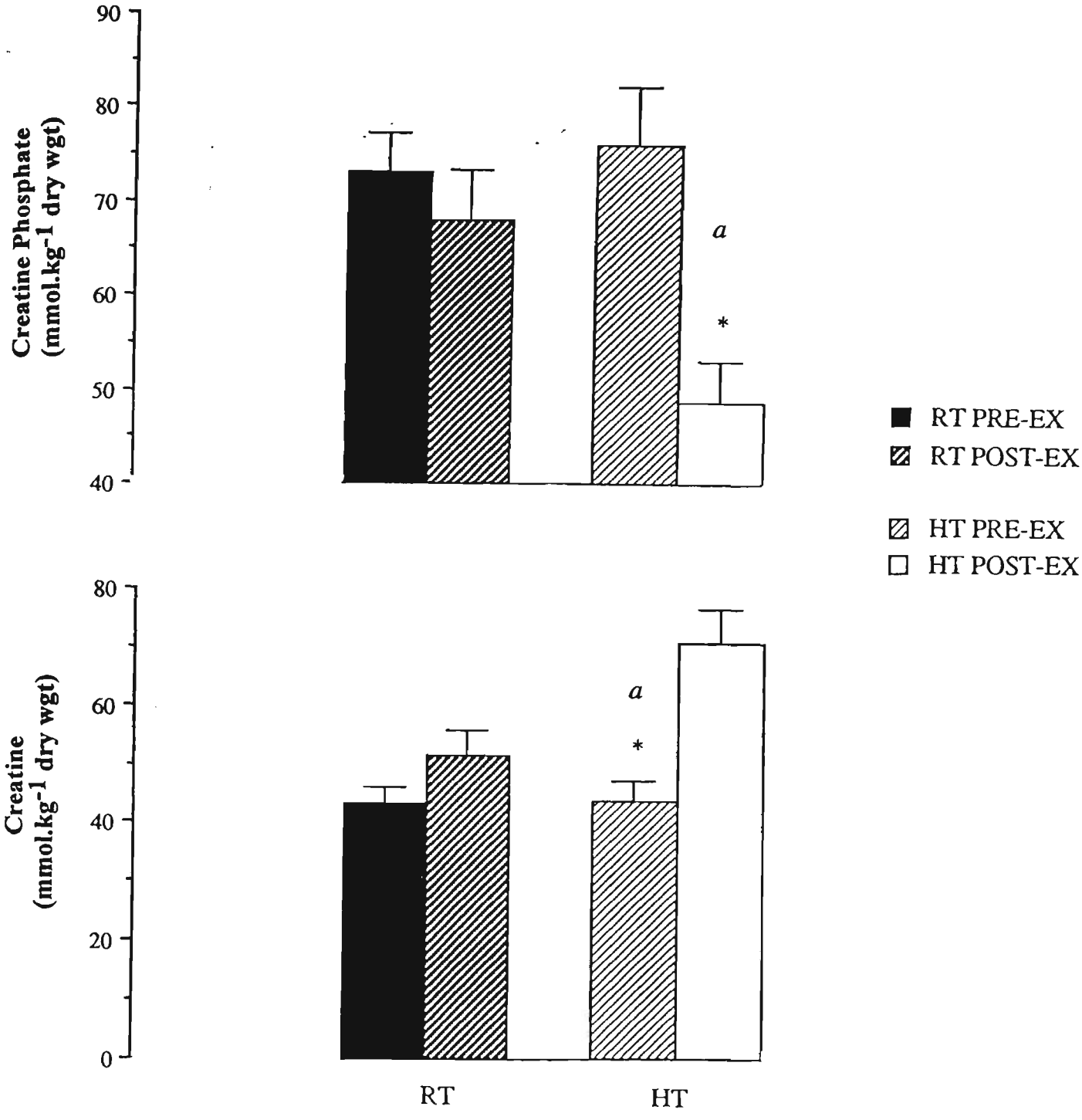


Fig. 4.6. Muscle creatine phosphate (CP) and creatine (C) concentrations before (PRE-EX) and after (POST-EX) exercise in 20°C RH 20% (RT) compared with 40°C RH 20% (HT). Values are means ± SE (n=6). ^{a} indicates difference PRE-EX compared with POST-EX, * indicates difference ($P<0.05$) HT POST-EX compared with RT POST-EX.

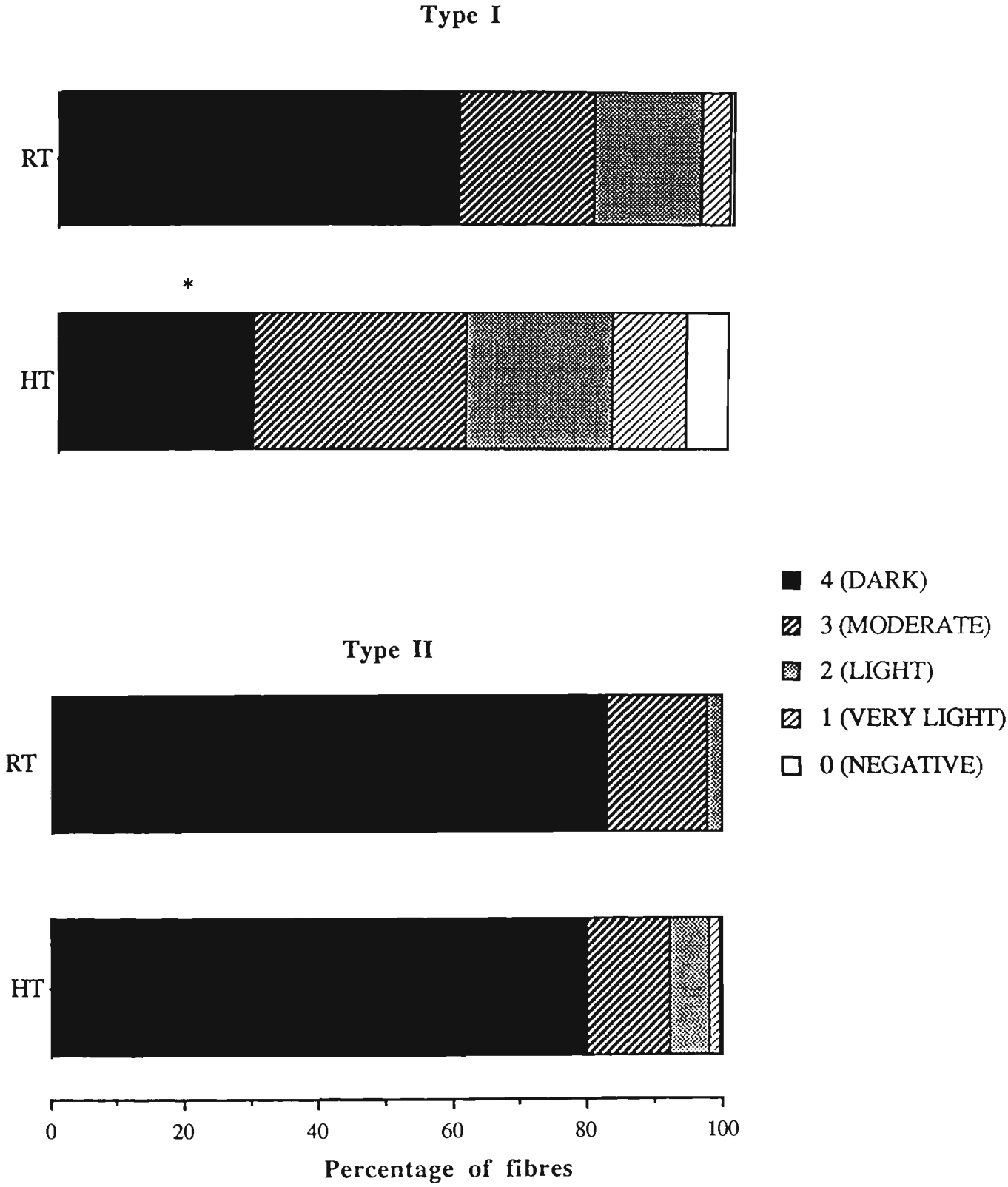


Fig. 4.7. Histochemical estimation of glycogen content in muscle sections stained with periodic acid Schiff (PAS) reagent in type I and type II fibres after exercise in 20°C RH 20% (RT) and 40°C RH 20% (HT). Values are mean ± SE (n=6). * indicates difference ($P<0.05$) in percentage of dark stained type I fibres HT compared with RT.

4.4 DISCUSSION

The results of this study support previous findings (Fink et al., 1975; Nielsen et al., 1993; Young et al., 1985) which demonstrate that exercise in the heat alters muscle metabolism. Exercise in a hot environment reduces endurance capacity (Nielsen et al., 1993) whilst exercise at room temperature with active cooling results in an increased endurance capacity (Kozlowski et al., 1985) indicating a relationship between exercise-induced hyperthermia and endurance capacity. The present results indicate that an altered muscle metabolism may influence endurance capacity during exercise in the heat, perhaps explaining the relationship referred to above. Alterations to a number of physiological variables have been proposed as explanations for the observed changes in muscle metabolism. These variables include muscle blood flow (Fink et al., 1975), muscle temperature (Kozlowski et al., 1985; Young et al., 1985), catecholamine levels (King et al., 1985) and fibre type recruitment patterns (Young et al., 1985). The data obtained in this study are consistent with the suggestion that adrenaline concentration plays a major role in altering muscle metabolism during exercise in the heat.

The effect of exercise in the heat on muscle glycogenolysis is the subject of some controversy; however, the data presented in this study indicate that exercise in the heat results in a greater reliance on carbohydrate metabolism. The observed greater glycogenolysis (Table 4.2), higher post-exercise muscle lactate concentration (Fig. 4.5), greater glycogen depletion in type I fibres (Fig. 4.7), higher mean RER and carbohydrate oxidation (Table 4.1), and possibly, the higher post-exercise C and lower post-exercise CP concentration in the HT compared with the RT (Fig. 4.6) indicate a shift in substrate utilisation. The FFA concentrations were similar between the two trials (Fig. 4.2). As previously mentioned (Chapter 2) it is not accurate to interpret these

data as indicating similar rates of fat oxidation as lipid oxidation in the contracting muscle may decrease without decreasing lipid mobilisation from the adipocytes (Fink et al., 1975)

The present data are consistent with previous work which reports an increase in muscle lactate accumulation (Young et al., 1985) and glycogenolysis (Fink et al., 1975) in humans exercising in the heat compared with similar exercise in a cooler environment. Although CP and C concentrations have never been previously measured in humans during exercise in the heat, the data reported in this study are consistent with previously reported observations in dogs (Kozlowski et al., 1985). In contrast to the data reported in this study, there are two recent studies which fail to observe any effect of exercise in the heat on muscle glycogenolysis or lactate accumulation (Nielsen et al., 1990; Yaspelkis et al., 1993). Certain methodological differences, however, could account for the discrepancies between the observations reported in these studies and those observed in this study. For example, in the study described by Nielsen et al. (1990) subjects exercised in a cool environment immediately before moving to the hot environment. The subjects therefore commenced exercise in the heat with a slightly elevated lactate and lower glycogen levels, unlike the present study. Yaspelkis et al. (1991) used a protocol which compared muscle metabolism during exercise at 33^{o}C with that at 23^{o}C, a temperature differential only half that employed in this study. Furthermore, the subjects utilised in the latter study were heat acclimated.

Although NH_3 accumulation has been used as a marker of exercise induced metabolic stress (Mutch & Banister, 1983) and fatigue has been shown to increase levels of ATP breakdown products (Sahlin et al., 1990), the effect of exercise in the heat on muscle NH_3 accumulation and adenine nucleotide concentrations has never been previously investigated. The observed differences

in post-exercise concentrations of blood (Fig. 4.1) and muscle (Fig. 4.5) lactate were not accompanied by similar changes in blood (Fig. 4.1) and muscle (Fig. 4.5) NH_3 when comparing the HT with the RT. The absence of any association between lactate and NH_3 accumulation is in contrast to previous work which has shown a relationship between these two metabolites (Babij et al., 1983; Dudley et al., 1983; Wilkerson et al., 1987). These studies, however, involved high intensity, short duration exercise where, as discussed in Chapter 2, the pathways for NH_3 production may be different from those which produce NH_3 in prolonged exercise. Two recent studies, however, support the present observation. Graham et al. (1987) found a decrease in both blood and muscle lactate accumulation during submaximal exercise with hyperoxia but no difference in the concentrations of blood or muscle NH_3. Schlict et al. (1990) also found no association between the two metabolites concluding that load threshold for lactate production is lower than that for NH_3. It is possible that the increased metabolic stress associated with exercise in the heat in trained individuals may be sufficient to exceed the load threshold for lactate but not for NH_3 production. The data from this study (Fig. 4.5) supports this hypothesis

In this study, no fluids were provided in either trial. Since the total weight loss was higher in the HT compared with the RT (Table 4.1) the level of dehydration was likely to be higher during exercise in the heat and cannot be entirely dismissed as having an influence on muscle metabolism. Plasma volume is affected during exercise by both changes in hydrostatic forces, due to changes in posture and blood pressure, and fluid loss in sweating. Gore et al. (1992) observed the former to have the greater influence during bicycle exercise. Since the hydrostatic effects were similar in both trials and the difference in weight loss between the trials, whilst being significant, was relatively small (0.42 kg^{-1}), the differences in plasma volume after 40

minutes of exercise may be small. No difference in plasma cortisol was observed between the two trials (Fig. 4.4). Plasma cortisol levels during exercise in the heat have been found to be elevated in the hypohydrated state when compared with similar exercise in the euhydrated state (Francesconi et al., 1984). Since no difference in plasma cortisol was observed in this study between the two trials it is unlikely that the magnitude of difference in hydration levels between the trials was sufficient to totally account for the observed differences in muscle metabolism between the trials.

As previously mentioned, a number of mechanisms for the observed changes in metabolism during exercise and heat stress have been suggested. One such suggestion has been a reduction in muscle blood flow, thereby reducing the O_2 delivery to the active muscles and increasing the reliance on anaerobic energy sources (Fink et al., 1975; Kozlowski et al., 1985; Rowell, 1974; Young et al., 1985). Dimri et al. (1980) have confirmed an increased reliance on anaerobic metabolism during exercise with an increase thermal stress. The evidence regarding a reduced muscle blood flow when comparing exercise in the heat with that in a cooler environment is equivocal. Studies in sheep, which have directly measured muscle blood flow, have shown that when compared with exercise in a cooler environment, muscle blood flow is compromised during exercise in the heat (Bell et al., 1983). In contrast, recent studies in humans have observed no change in leg blood flow, or leg oxygen consumption during cycling and uphill walking in the heat compared with that in a cooler environment (Nielsen et al., 1990; Savard et al., 1988). Interestingly, however, in the study of Nielsen et al. (1990) plasma volume was not reduced after exercise in the heat (due to the saline infusions necessary for the determination of leg blood flow). It is possible that the combination of temperature and accelerated dehydration associated with exercise in the heat may lead to a reduction in blood flow; however, since muscle blood flow was not

measured in this study, this suggestion is speculative. Nevertheless, many of the observed changes in muscle metabolism in this study are consistent with a reduction in muscle O_2 uptake, secondary to a reduced muscle blood flow. As previously mentioned, studies which have examined both ammonia and lactate accumulation when comparing exercise in normoxic conditions with that in hyperoxic conditions have found that ammonia accumulation, unlike lactate, appears to be independent of O_2 availability (Graham et al., 1987). Furthermore, previous studies have found an increase in both CP degradation and lactate accumulation when submaximal exercise in hypoxic conditions was compared with similar normoxic exercise (Sahlin & Katz, 1989). These observations are consistent with the findings in the present study. Of note, pulmonary $\dot{V}O_2$ was lower during exercise in the HT compared with the RT (Table. 4.1). This may also indicate a reduced O_2 supply to the working muscles; however since no direct measure of leg $\dot{V}O_2$ was available, it is difficult to determine whether the lower pulmonary measurement is due to a reduced $\dot{V}O_2$ of the contracting muscles, secondary to a reduction in O_2 delivery, or to a lower $\dot{V}O_2$ in other tissues. It is possible that a reduced blood flow during exercise in the heat may have two effects on muscle metabolism. First, a reduction in the O_2 delivery may increase glycogenolysis and lactate production and decrease the resynthesis of CP in the mitochondria. Secondly, a reduction in blood flow may also reduce the removal of lactate from the muscle, resulting in an increased accumulation of this metabolite within the muscle. Since muscle blood flow was not measured in this study, the data can neither unequivocally confirm nor refute this mechanism as having influenced the observed changes in metabolism. The observed changes in muscle metabolism, however, are consistent with a reduction in muscle blood flow. The possible influence of muscle blood flow during exercise in the heat cannot be entirely discounted.

Elevations in muscle temperature by passive heating have been shown to increase ATP and CP degradation during submaximal, isometric contractions of the quadriceps to fatigue (Edwards et al., 1972). Furthermore, elevated muscle temperature during exercise in dogs is associated with increased muscle glycogenolysis, lactate accumulation and adenine nucleotide depletion (Kozlowski et al., 1985). These authors suggested that these changes may have occurred as a result of a Q_{10} effect. A Q_{10} effect is unlikely to be responsible for the observed differences in muscle metabolism between the HT and the RT. In contrast to the observations by Edwards et al. (1972) and Kozlowski et al. (1985) no differences in adenine nucleotide metabolism were found between the HT and the RT (Table 4.3). Furthermore, no difference was found in post-exercise muscle NH_3 accumulation between the two trials. Although the exercise-induced increase in muscle NH_3 could be the result of ATP degradation leading to deamination of AMP and formation of IMP and NH_3, muscle temperature had little effect on these reactions. Although there was a difference in post-exercise muscle temperature, no changes were observed in adenine nucleotide metabolism or NH_3 accumulation. This suggests that the differences in muscle temperature was not sufficient to result in significant differences in the activity of ATPases, AMP deaminase or indeed, the adenylate kinase reaction. The Q_{10} value commonly found for enzyme mediated reactions (2.0-3.0) (Florkin & Stolz, 1968) is unlikely to be sufficient to produce the changes seen in either muscle glycogenolysis or muscle lactate accumulation. Based on the muscle temperature data (Table 4.1), the estimated difference in the enzyme reaction rates following exercise would be approximately 8% when comparing the HT with the RT. The differences in muscle glycogenolysis and post-exercise muscle lactate concentration were 30 and 120% respectively. In contrast to the observation by Kozlowski et al. (1985) no significant correlation was found between post-exercise muscle lactate concentration and post-exercise muscle temperature. The data reported in this study may be

influenced by a higher muscle temperature prior to exercise in HT compared with RT. This was most likely due to a passive warming of the muscle, since each subject spent some time in the environmental condition prior to exercise. This appears to have had little influence on resting muscle metabolism, however, since no differences in resting metabolism was observed.

Young et al. (1985) suggested that exercise in the heat may alter the neuromuscular recruitment pattern favouring a greater use of fast twitch fibres. They reported a significant correlation ($r=0.69$) between the percentage of type II fibres and the difference in post-exercise muscle lactate concentration between hot and cool environments in the unacclimated state, since type II fibres possess higher glycolytic capacities and have the capacity to produce lactate in greater concentrations (Essen et al. 1975). In contrast, no significant correlation between these two parameters was not observed in this study ($r=0.06$, $P>0.05$). As previously discussed in Chapter 2, there are some limitations to the use of histochemical estimation of glycogen depletion patterns as a measure of fibre type recruitment. Nevertheless, the data presented here suggest that type I fibres were preferentially recruited in all trials (Fig. 4.7). This is consistent with the recruitment pattern of fibre types in prolonged exercise at room temperature (Gollnick et al., 1973a). The higher lactate accumulation in the HT most likely arose as a result of enhanced glycolysis in type I fibres, since these fibres exhibited a higher level of depletion in this trial whilst no difference in depletion was observed in the type II fibres. Although resting PAS stains which showed fully loaded (mean rating 4.00 ± 0.00) type I and type II fibres were analysed during one trial only, it is highly unlikely that differences existed between this and the other trial since the resting glycogen concentrations were not different (Table 4.2) and exceeded the maximal PAS staining sensitivity concentration as described by Piehl (1974). The histochemical estimation of skeletal muscle glycogenolysis

shows a higher percentage of negatively stained type I fibres in the HT compared with the RT (6.7 ± 3.3 vs 0.7 ± 0.5%). This observation may account for the lower post-exercise CP concentration observed in HT compared with the RT, since the lack of glycogen has been found to inhibit the rate of high energy phosphate resynthesis (Hultman et al., 1967). Although the post-exercise glycogen concentrations in each trial was well above 100 mmol. glucosyl u. kg^{-1} dry wt, the totally depleted fibres in the HT could influence the post-exercise CP concentration.

In the present study an increase in circulating adrenaline during exercise in the heat in the unacclimated individual was observed. A higher pre-exercise concentration was observed in the HT compared with the RT, most likely due to the subjects spending some time in the heat before the resting blood was sampled. The difference, whilst being significant ($P<0.05$), was small (90.2 $pg.ml^{-1}$) compared with the differences observed during exercise (288.3 and 249.4 $pg.ml^{-1}$, $P<0.01$) (Fig. 4.3). The higher adrenaline concentration observed during exercise supports earlier findings that heat stress increases circulating epinephrine levels (Dolny & Lemon, 1988; Galbo et al., 1979b). During exercise, elevated adrenaline concentration has been found to increase glycogenolytic rate by stimulating the conversion of phosphorylase to its active form (Richter, 1984). Increases in adrenaline concentrations, due to adrenaline infusion, result in greater glycogenolysis (Jansson et al., 1986). Furthermore, an increase in adrenaline concentration and blood lactate accumulation were observed when exercise in the heat was compared with exercise in a cooler environment (Dolny & Lemon, 1988). The data obtained in this study support these findings, since an increase in adrenaline concentration in the HT compared with the RT was observed (Fig. 4.3) along with increased muscle glycogenolysis (Table 4.2), blood (Fig. 4.1) and muscle lactate accumulation (Fig. 4.5). These data suggest a greater reliance upon carbohydrate as an energy substrate.

Goodman & Lowenstein (1977) observed an increase in IMP and NH_3 in rat muscle perfused with adrenaline. In contrast, no differences in the concentration of these metabolites were found in this study despite a difference in circulating adrenaline levels between the trials. This discrepancy may be due to the different concentrations of adrenaline in the two studies, since the concentration of adrenaline in the fluid infused into the rat muscle was 25nM (4580 $pg.ml^{-1}$). In contrast, when adrenaline infusion in humans produced a physiological increase (0.05 $ug.kg^{-1}.min^{-1}$) in this hormone, Spencer et al. (1991a) observed no difference in resting adenine nucleotide metabolism, consistent with the present findings.

The elevated blood glucose concentration (Fig. 4.2) also suggests that hepatic glycogenolysis was increased in the HT compared with the RT, most likely the result of increases in plasma adrenaline (Galbo, 1983). Marked increases in hepatic glucose production in response to exercise have been shown to take place in the absence of changes in plasma insulin and glucagon levels (Bjorkman et al., 1981; Galbo et al., 1977b). Since no differences were observed between circulating levels of insulin or glucagon in the present study (Fig. 4.4), it is likely that hepatic glycogenolysis, probably reflected in blood glucose concentration, was stimulated by adrenaline. The exercise induced decrease in hepatic glycogen concentration has been found to be smaller in chemically sympathectomized, adrenodemedullated rats compared with sham treated rats (Galbo et al., 1978). Since we did not measure hepatic glucose production, we are unable to confirm the influence of adrenaline on hepatic glycogenolysis. The rise in blood glucose may possibly be due to alterations in splanchnic O_2 delivery as suggested by Rowell et al. (1968).

Studies examining the effect of adrenaline on the glycogenolytic rate of specific fibre types have produced conflicting results in rats (Galbo et al.,

1978; Hashimato et al., 1982; Richter, 1984). In electrically stimulated human muscle, however, glycogenolysis was elevated by adrenaline infusion in type I fibres only (Greenhalf et al.,1991). As previously mentioned, type I fibre glycogen depletion was greater in both trials when compared with type II fibres (Fig. 4.7) but showed significantly higher levels of glycogen depletion in the HT when adrenaline levels were higher (Fig. 4.2). These data support the conclusion that during submaximal exercise in trained individuals, elevations in adrenaline increase the glycogenolytic rate of type I fibres.

In summary, the observed changes in temperature regulation and heart rate which arise from exercise in the heat are also accompanied by alterations in muscle metabolism. The data from this study suggest that an alteration in neuromuscular recruitment pattern is unlikely to be responsible for the observed metabolic changes. In addition, a temperature effect on glycolysis cannot account for the magnitude of the difference observed when comparing metabolism during exercise in the heat with similar exercise at room temperature. Although the possible influence of muscle blood flow cannot be discounted, the changes appear to be largely mediated by alterations in circulating adrenaline.

CHAPTER 5

EFFECT OF ACCLIMATION ON MUSCLE METABOLISM DURING EXERCISE IN THE HEAT

5.1 INTRODUCTION

The onset of fatigue during exercise in the heat has been found to be delayed with repeated exercise in a hot environment (Adams et al., 1975, Nielsen et al., 1993). Heat acclimation which results from the repeated exposure allows the individual to maintain a lower body temperature for a given submaximal exercise intensity (Stolwijk et al., 1977). The benefits from heat acclimation result from a number of physiological adaptations including an earlier and greater sweating response (Stolwijk et al., 1977), increased peripheral blood flow (Stolwijk et al., 1977), expanded plasma volume (Harrison et al., 1981), increased stroke volume (Nielsen et al., 1993) and larger cardiac output (Nielsen et al., 1993). Since exercise in the heat alters muscle metabolism when compared with exercise in a cooler environment, does acclimation, therefore, lead to a reversal of these changes during exercise in the heat? Only limited research has been conducted in this area with conflicting results. Previous studies have observed glycogenolysis to be either reduced (King et al., 1985; Kirwan et al., 1987) or unchanged (Young et al., 1985), whilst muscle (Young et al., 1985) and blood (Nielsen et al., 1993) lactate accumulation is reduced by acclimation. It is possible that the changes in muscle metabolism during exercise in the heat following acclimation arise as a result of the reversal of the changes which are observed when comparing exercise in the unacclimated individuals in the heat with that at room temperature.

This study was undertaken to examine the effect of heat acclimation on muscle metabolism during submaximal exercise in the heat. Furthermore, the mechanisms for any such changes were examined in order to assess whether the responses exercise and heat stress are reversed by subsequent acclimation.

5.2 METHODS

The subjects used in the study described in Chapter 4 underwent a further trial after seven days of heat acclimation. The post-acclimation trial (AT) consisted of a repetition of the HT. The AT was conducted 24 hours after the final acclimation session. The protocol, sampling, biochemical and statistical analyses employed in this study were identical to those employed in the previous study. Since a pre-exercise muscle sample was taken for histochemistry in the HT, it was not taken in the AT. The rationale for this approach was described in Chapter 4. The subjects underwent a $\dot{V}O_2$ max test within three days of the AT to compare the fitness status with that observed prior to the first trial.

The heat acclimation program consisted of a 90 minute bout of exercise in heat trial conditions repeated over seven consecutive days. Subjects cycled on friction braked bicycles at a workload which elicited approximately 50% of $\dot{V}O_2$ max. This regimen was based on the original protocol as described by Robinson et al. (1945) and modified by Kirwan et al. (1987). Respiratory data were obtained on days one, three and seven by Douglas bag collection. Rectal temperatures and heart rates were monitored throughout exercise and bodyweight was recorded prior to and following each session. The criteria set for subject removal in the exercise trials was maintained for the acclimation sessions. On

one occasion, a subject asked to be removed after 60 minutes of exercise complaining of fatigue and the session was terminated. During the acclimation sessions water was consumed *ad libitum*. The attire worn by the subjects was similar to that worn in the trials.

5.3 RESULTS

The heat acclimation program lowered ($P<0.05$) final exercise T_r and HR when data from day one were compared with day seven. There was no difference in $\dot{V}O_2$ max prior to and following the testing period (64.8 ± 4.8 vs. 63.3 ± 4.2 $ml.kg^{-1}.min^{-1}$). Mean RER, and HR were lower ($P<0.01$) during the AT compared with the HT. No difference was observed in mean $\dot{V}O_2$ between the AT and the HT (Table 5.1). Weight loss was not different ($P=0.07$) when comparing the two trials. Pre-exercise T_m and post-exercise T_r and T_m were lower ($P<0.01$)in the AT compared with the HT (Table 5.1)

Resting blood glucose (Fig. 5.2) and lactate (Fig. 5.1) concentrations were not different at rest between the two trials, but were lower ($P<0.01$) at all points during exercise when comparing the AT with the HT. There was an exercise induced rise ($P<0.05$) in plasma NH_3 in both the AT and the HT although no differences were observed at rest or during exercise between the trials (Fig. 5.1). Plasma FFA fell ($P<0.01$) in the first ten minutes of both trials, but no differences in FFA concentration were observed between the two trials (Fig. 5.2). Plasma adrenaline concentration was not different between the trials at rest but was lower ($P<0.05$) during exercise in the AT when compared with the HT (Fig. 5.3). Although statistics revealed no interaction between acclimation status and time, the mean plasma noradrenaline level was lower ($P<0.05$) in the AT compared with the HT (Fig. 5.3). Although plasma glucagon rose ($P<0.05$) in both trials there was no difference in circulating concentration of this

hormone between the trials (Fig. 5.4). Plasma insulin was not affected by either time or acclimation status (Fig. 5.4). Although plasma cortisol did not differ when comparing resting with exercise values, the mean value was lower ($P<0.05$) in the AT compared with the HT (Fig. 5.4).

Pre-exercise muscle lactate concentration was not different between the trials. The post-exercise values were higher ($P<0.05$) when compared with resting values in both the AT and the HT, and post-exercise value in the AT was lower ($P<0.05$) compared with the HT (Fig. 5.5). Although there was a lower ($P<0.05$) post-exercise muscle glycogen concentration compared with pre-exercise values in both trials, there was no difference in pre or post-exercise muscle glycogen concentration between the trials. Furthermore, muscle glycogenolysis was not different between the trials (Table 5.2). Muscle NH_3 concentration was increased ($P<0.05$) as a result of exercise but no differences were observed at rest or following exercise between the trials (Fig. 5.5). Concentrations of ATP and IMP were not affected by acclimation status or exercise (Table 5.3). Although exercise altered ($P<0.05$) concentrations of AMP, ADP (Table 5.3), CP and C (Fig. 5.6) acclimation had no effect on these metabolites at rest or following exercise.

The single pre-exercise PAS stained muscle section showed a weighted mean of 4.00 ± 0.00 (uniformly fully stained) for both type I and type II fibres. The post-exercise PAS stained muscle sections revealed that acclimation had no effect on the type II fibre glycogen depletion pattern with the mean ratings being 3.84 ± 0.08 and 3.70 ± 0.12 and the percentage of dark fibres being 86.5 ± 6.8 and 80.0 ± 7.1 for the AT and the HT respectively. In contrast, the percentage of darkly stained type I fibres was higher ($P<0.05$) in the AT compared with the HT, the values being 66.5 ± 9.9 and 29.0 ± 14.6, respectively. Furthermore, a higher ($P<0.05$) mean rating following acclimation

was observed with the values being 3.43 ± 0.25 and 2.64 ± 0.34 for the AT and the HT, respectively (Fig. 5.7).

	TRIAL	
	HT	AT
O_2 Uptake ($l.min^{-1}$)	2.99±0.05	2.94±0.05
Heart Rate ($b.min^{-1}$)	173±2	162±2 **
RER	0.93±0.01	0.89±0.01 **
CHO Oxidation ($g.min^{-1}$)	2.77±0.13	2.41±0.11
Energy Exp. ($kJ.min^{-1}$)	62.14±1.94	60.43±1.79
Weight Loss (Kg)	1.12±0.06	1.34±0.10
Rectal Temp. (^oC)		
PRE-EX.	37.2±0.1	36.8±0.1
POST-EX.	39.7±0.1	39.2±0.1 **
Muscle Temp. (^oC)		
PRE-EX.	36.5±0.2	36.0±0.1 *
POST-EX.	40.4±0.1	40.0±0.1 **

Table 5.1. *Mean O_2 uptake, mean heart rate, mean repiratory exchange ratio (RER), mean carbohydrate (CHO) oxidation, energy expenditure, total weight loss, and rectal and muscle temperature before (PRE-EX) and after (POST-EX) exercise in 40^{o}C RH 20% prior to (HT) and following (AT) acclimation.*
Values are means ± SE, (n=13).* indicates difference ($P<0.05$) AT compared with HT, ** indicates difference ($P<0.01$) AT compared with HT.

TRIAL	PRE-EX	POST-EX	△
HT	482±36	300±48 [a]	182±27
AT	519±51	364±37 [a]	154±28

Table 5.2. *Muscle glycogen concentrations before (PRE-EX) and after (POST-EX) exercise and muscle glycogenolysis (△) in 40°C RH 20% prior to (HT) and following (AT) acclimation.*

Values are means ± SE (n=6) expressed in mmol.glucosyl units.kg^{-1} dry weight. [a] indicates difference ($P<0.05$) POST-EX compared with PRE-EX.

	HT		AT	
	PRE-EX	POST-EX	PRE-EX	POST-EX
ATP	24.1±1.3	24.0±1.4	24.3±1.4	24.3±1.2
ADP +	2.12±0.09	2.47±0.08	2.11±0.07	2.79±0.48
AMP +	0.05±0.01	0.11±0.02	0.15±0.10	0.26±0.16
IMP	0.12±0.05	0.36±0.11	0.24±0.10	0.28±0.09

Table 5.3. *Intramuscular concentrations of adenosine 5'triphosphate (ATP), adenosine 5'diphosphate (ADP), adenosine 5'monophosphate (AMP) and inosine 5'monophosphate (IMP) before (PRE-EX) and after (POST-EX) exercise in 40^{O}C RH 20% prior to (HT) and following (AT) acclimation.*
Values are means ± SE (n=6) expressed in mmol. kg^{-1} dry wt. + indicates exercise-induced increase ($P<0.05$) in ADP and AMP.

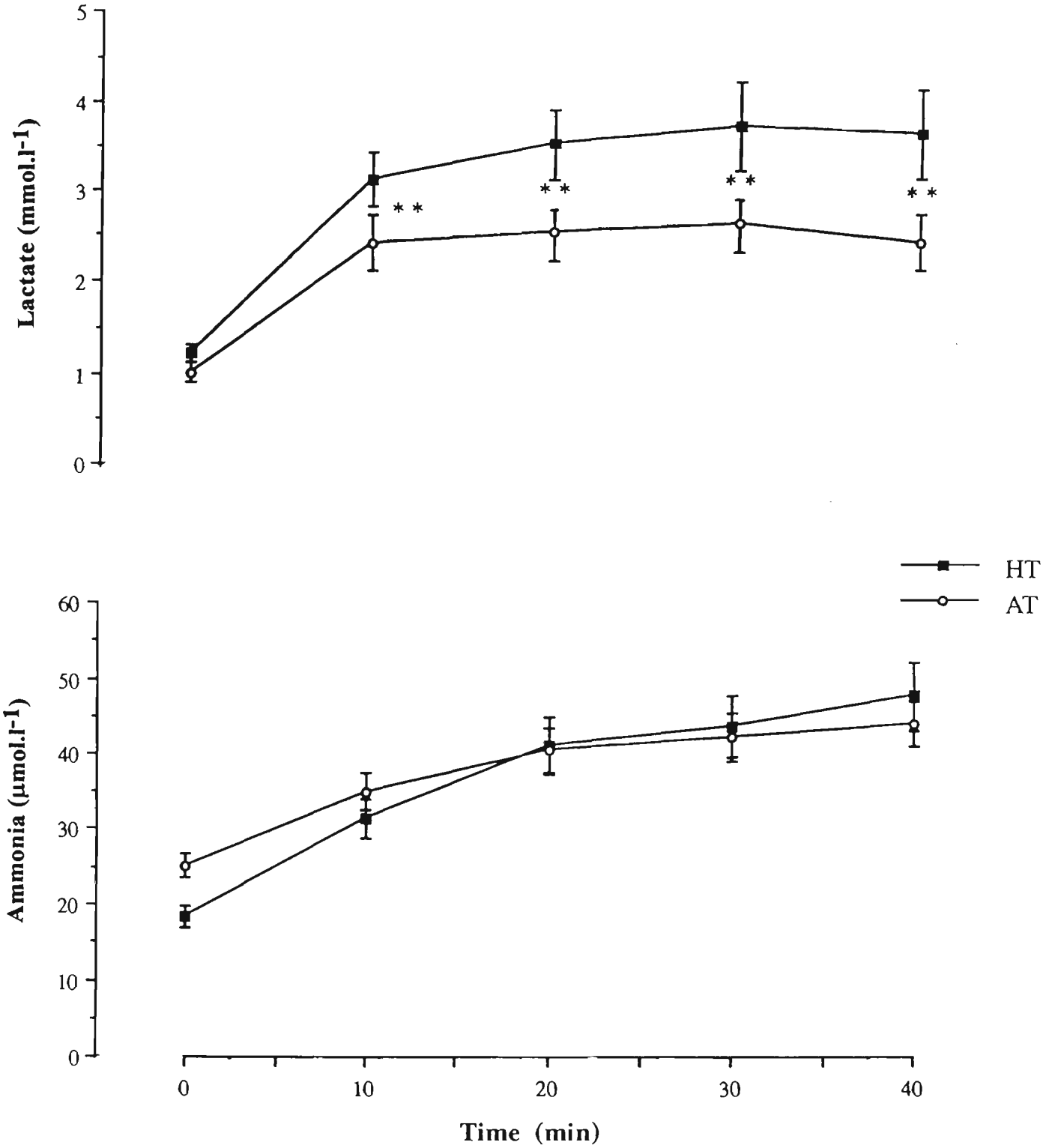


Fig. 5.1. Blood lactate and plasma ammonia concentrations during exercise in 40°C RH 20% prior to (HT) and following (AT) acclimation. Values are means ± SE (n=13). ** indicates difference ($P<0.01$) AT compared with HT.

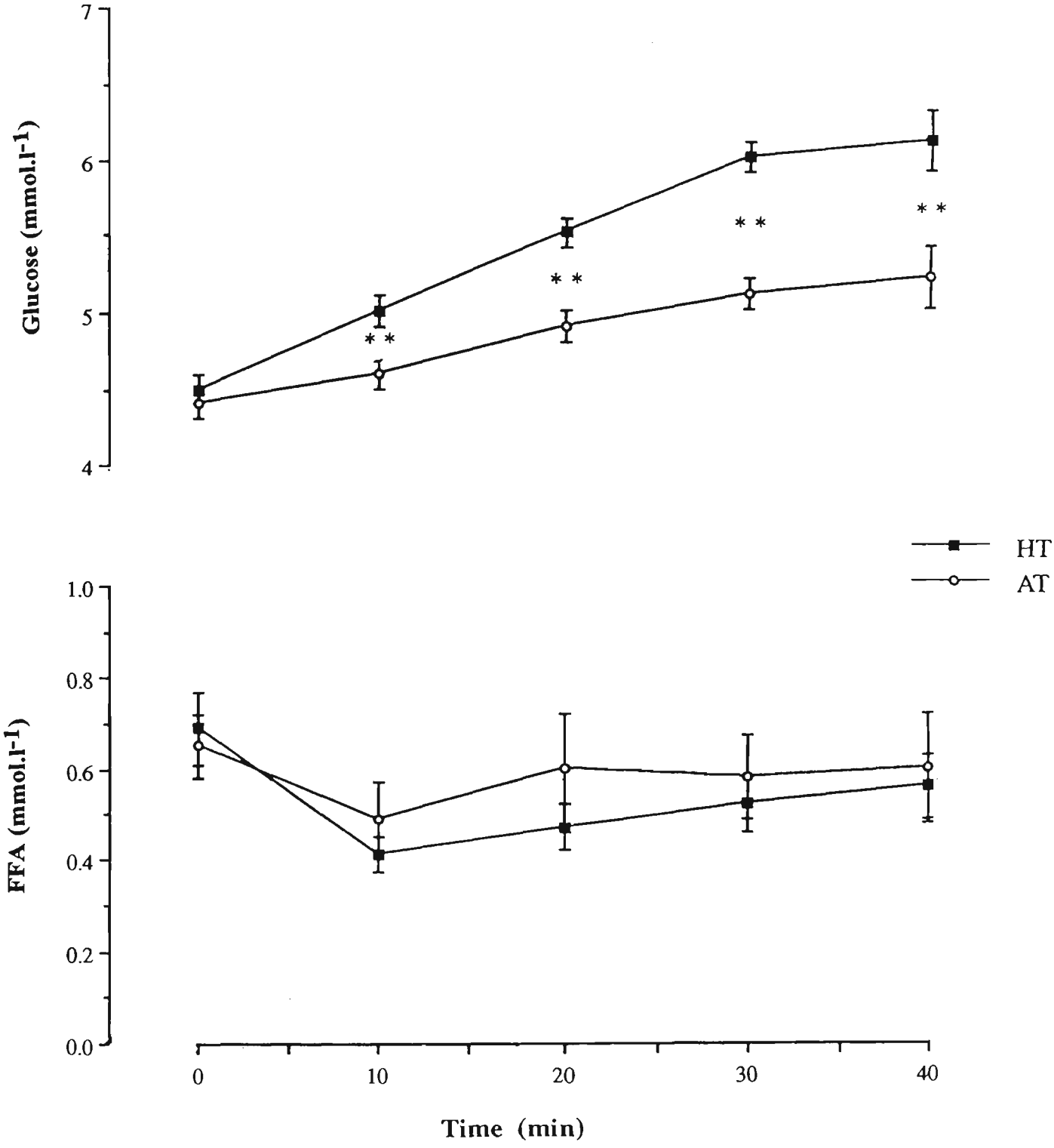


Fig. 5.2. Blood glucose and plasma free fatty acid (FFA) concentrations during exercise in 40°C RH 20% prior to (HT) and following (AT) acclimation. Values are means ± SE (n=13 for glucose, n=7 for FFA). ** indicates difference ($P<0.01$) AT compared with HT.

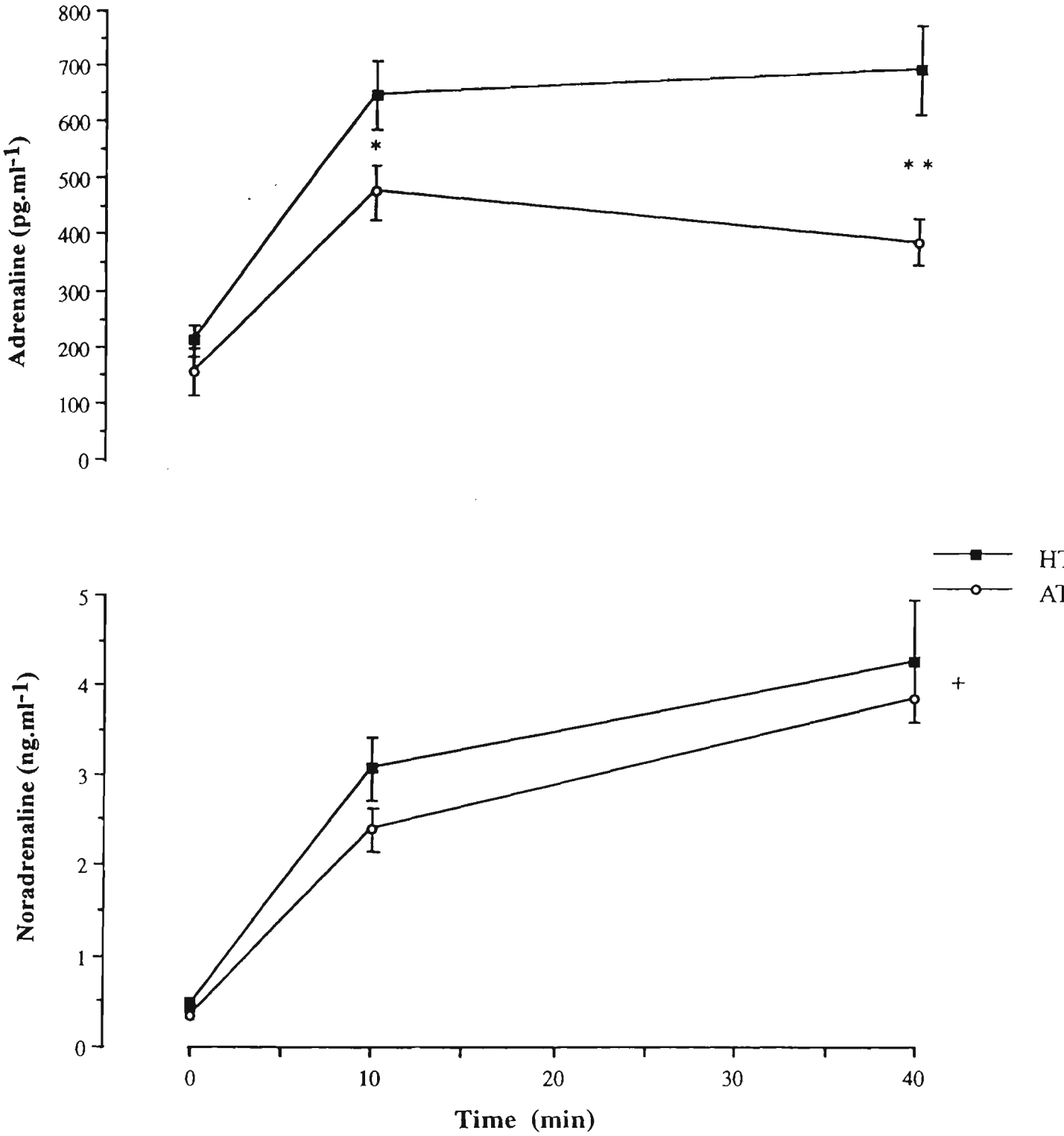


Fig. 5.3. Plasma adrenaline and noradrenaline concentrations during exercise in 40^{o}C RH 20% prior to (HT) and following (AT) acclimation. Values are means ± SE (n=7). * indicates difference ($P<0.05$) AT compared with HT, ** indicates difference ($P<0.01$) AT compared with HT. + indicates lower ($P<0.05$) mean noradrenaline concentration AT compared with HT.

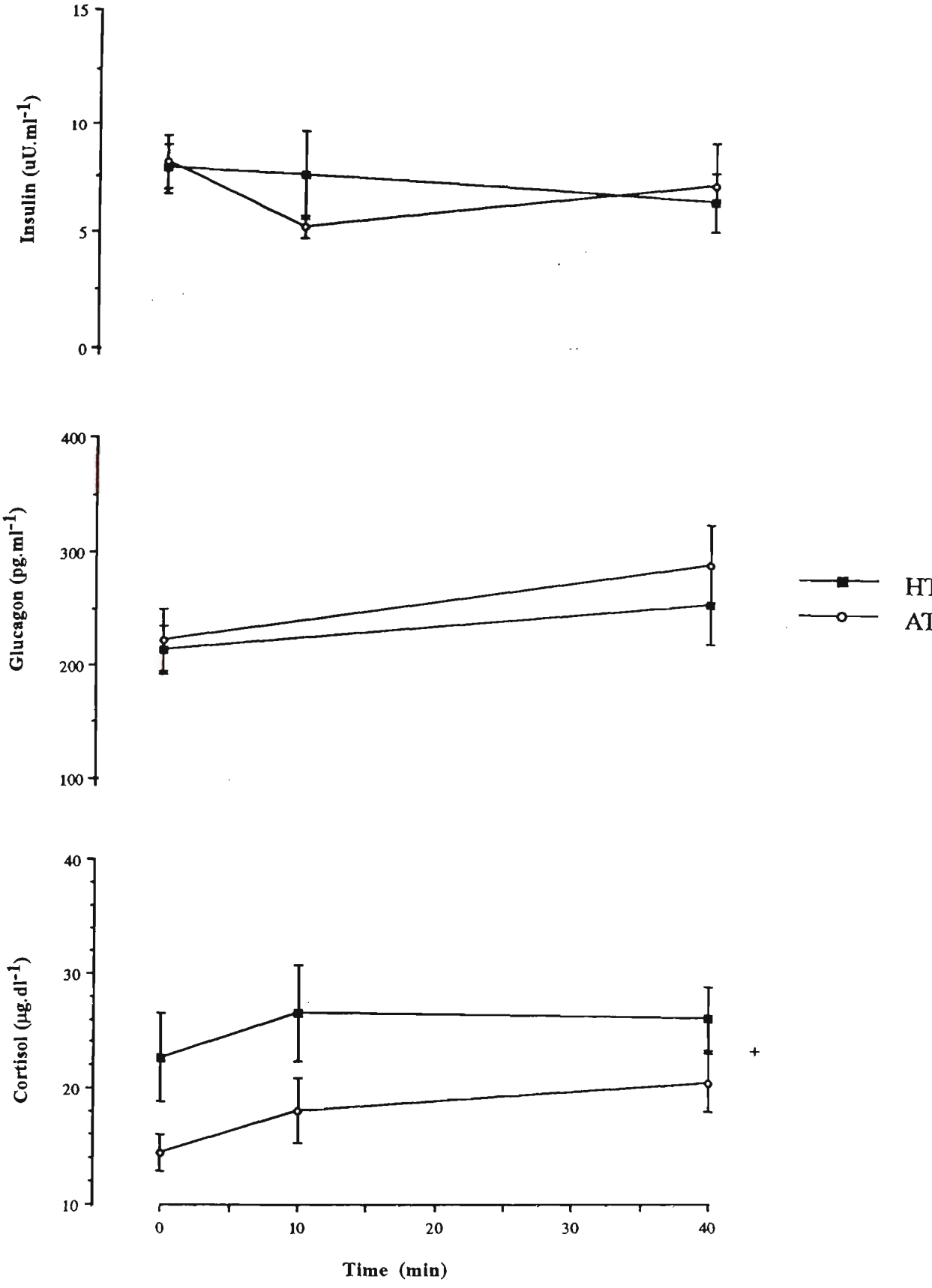


Fig 5.4. Plasma insulin, glucagon and cortisol concentrations during exercise in 40^{o}C RH 20% prior to (HT) and following (AT) acclimation. Values are means ± SE (n=6). + indicates lower ($P<0.05$) mean cortisol concentration AT compared with HT.

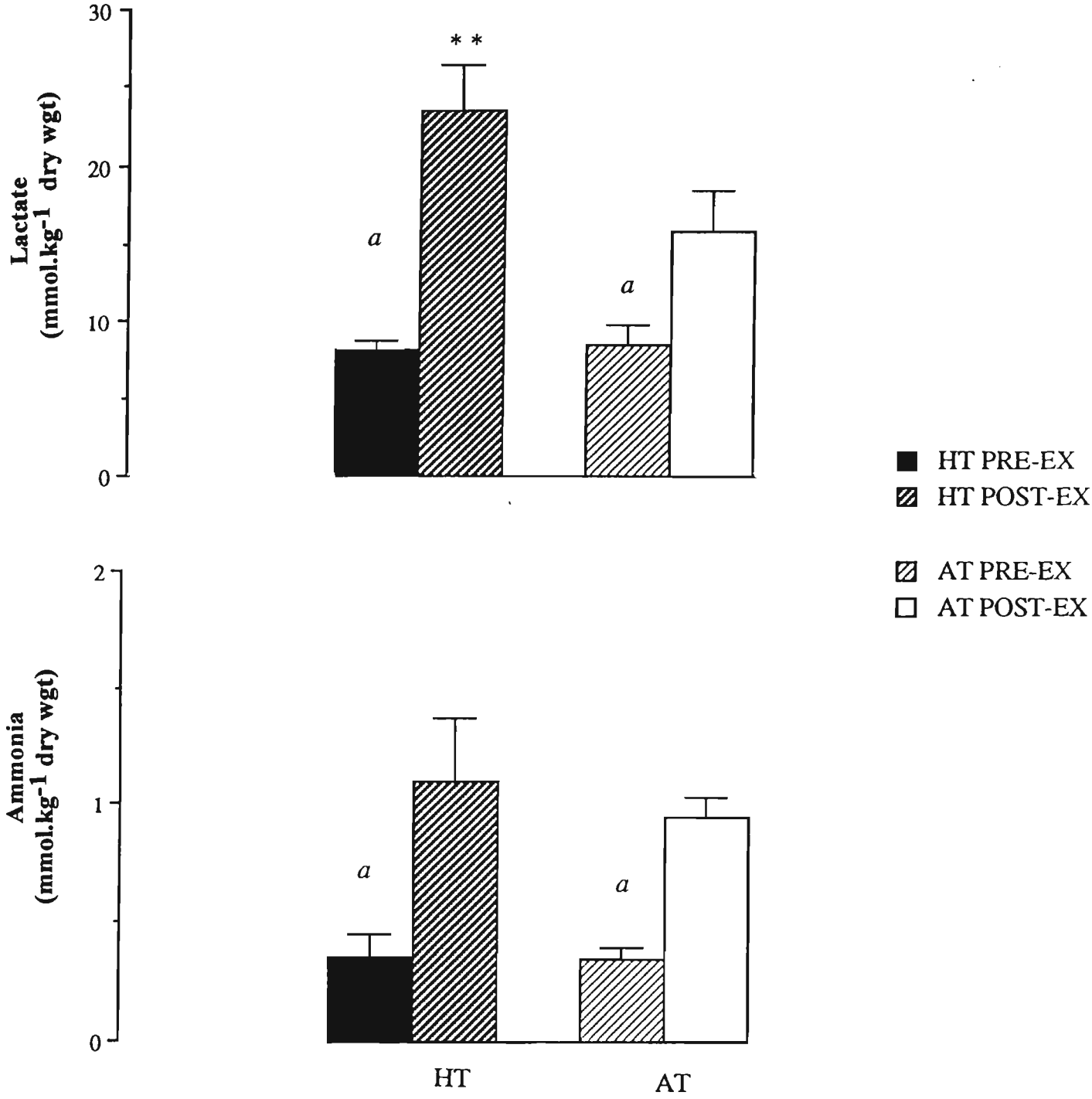


Fig. 5.5. Muscle lactate and muscle ammonia concentration before (PRE-EX) and after (POST-EX) exercise in 40°C RH 20% prior to (HT) and following (AT) acclimation. Values are means ± SE (n=6). ^{a} indicates difference ($P<0.05$) PRE-EX compared with POST-EX, ** indicates difference ($P<0.01$) AT POST-EX compared with HT POST-EX.

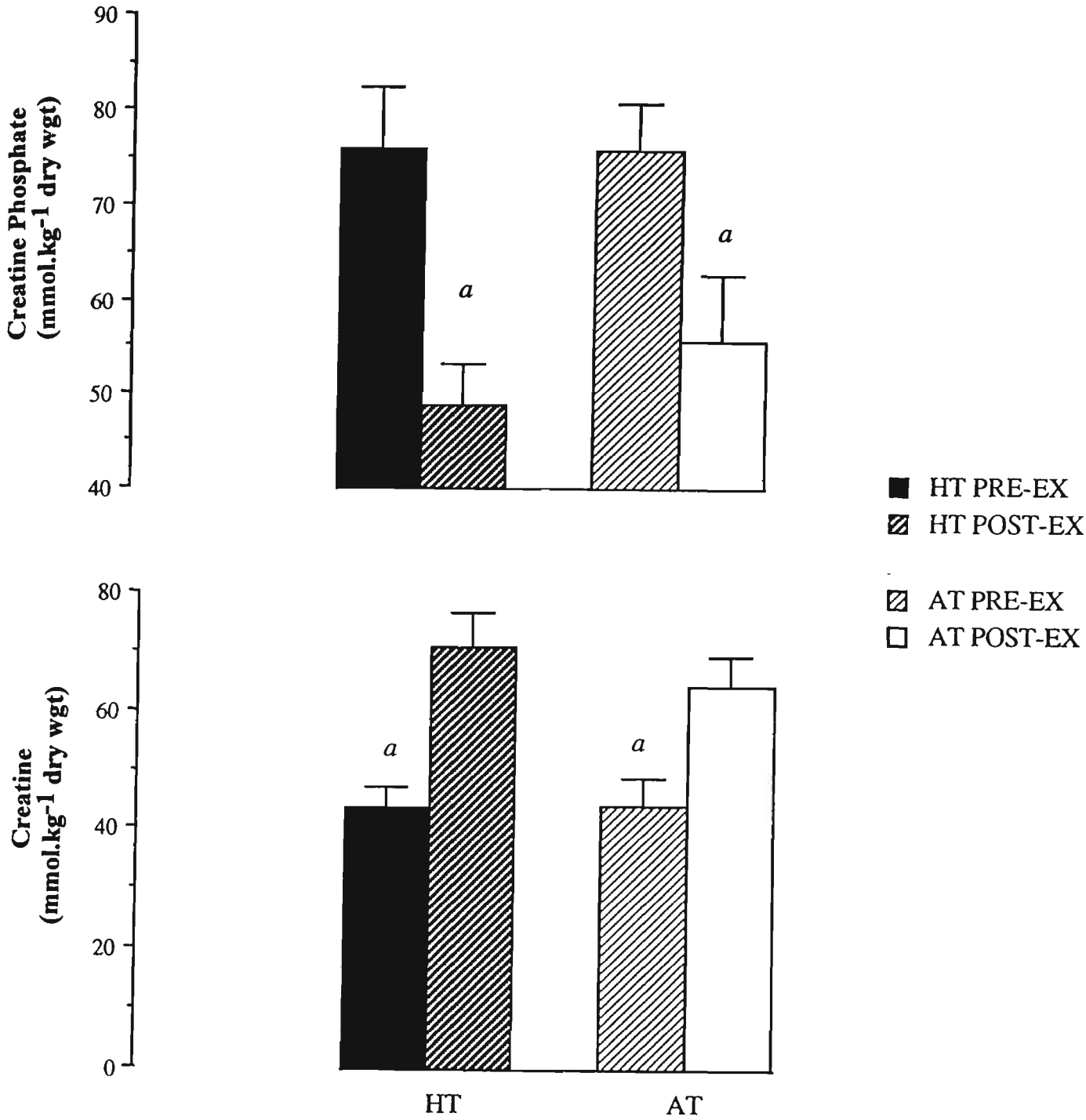


Fig. 5.6. Muscle creatine phosphate (CP) and creatine (C) concentrations before (PRE-EX) and after (POST-EX) exercise in 40^{o}C RH 20% prior to (HT) following (AT) acclimation. Values are means ± SE (n=6). ^{a} indicates difference PRE-EX compared with POST-EX.

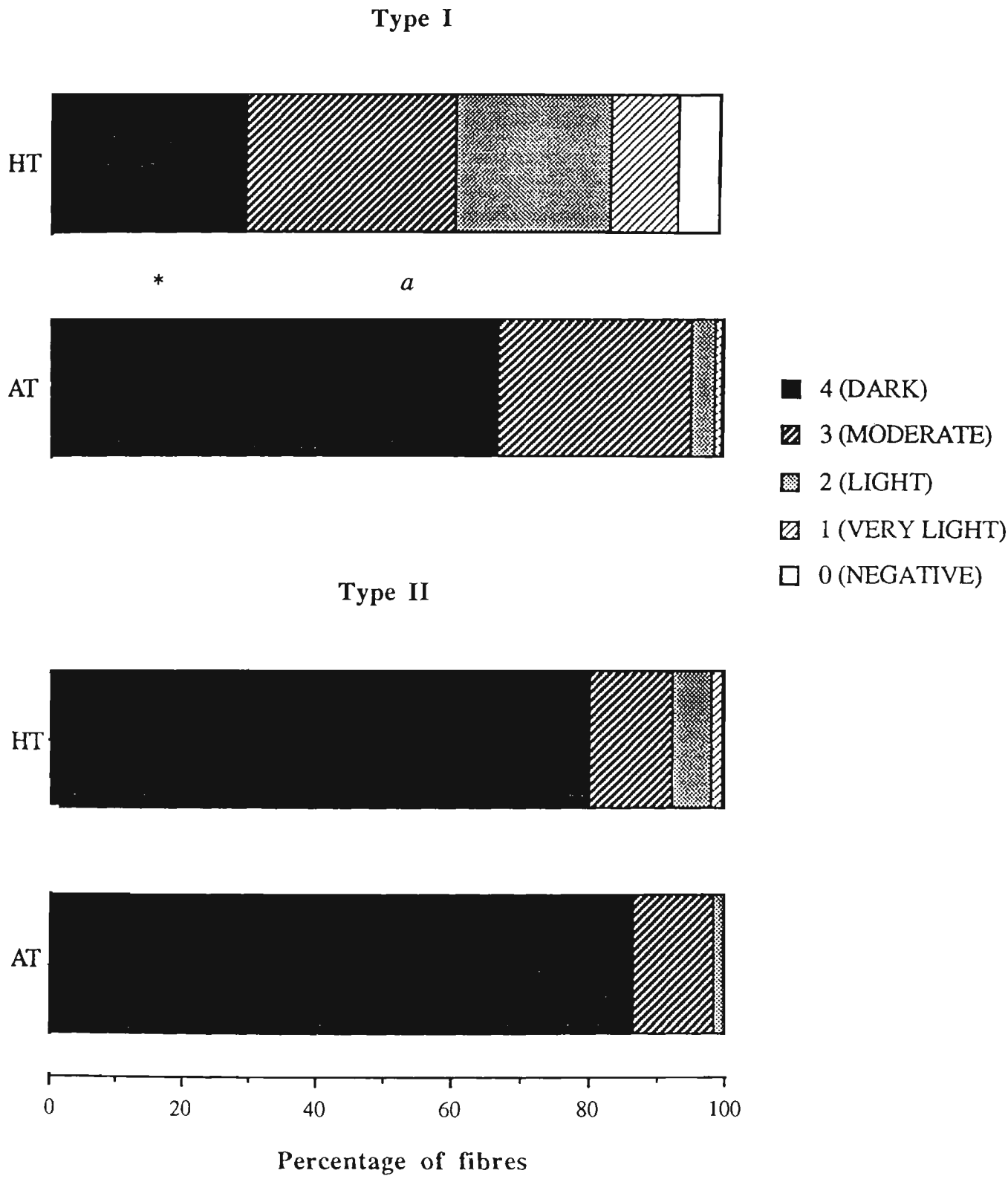


Fig. 5.7. Histochemical estimation of glycogen content in muscle sections stained with periodic acid Schiff (PAS) reagent in type I and type II fibres after exercise in 40^{o}C RH 20% prior to (HT) and following (AT) acclimation. Values are mean ± SE (n=6). * indicates difference ($P<0.05$) in percentage of dark stained type I fibres HT compared with RT, ^{a} indicates difference ($P<0.05$) in weighted mean between HT and AT.

5.4 DISCUSSION

The results of this study demonstrate that acclimation over a seven day period results in alterations to muscle metabolism during exercise in the heat. It appears that the increased reliance placed upon carbohydrate as a fuel during exercise in the heat is attenuated by acclimation. The changes in muscle metabolism observed in this study may be partly responsible for the previously reported improved performance during exercise in the heat in the acclimated individual (Adams et al., 1975; Nielsen et al., 1993).

The success of the acclimation protocol was evident from the observed reductions in exercise heart rate and rectal temperature following acclimation (Table 5.1) and the fact that both these physiological parameters were lower when comparing day one with day seven of the acclimation program. Many metabolites which were increased with heat stress were reduced during exercise in the heat following acclimation. These include blood glucose and lactate (Fig. 5.1) and muscle lactate (Fig. 5.3). A reduced muscle lactate accumulation (Young et al., 1985) blood glucose (Nielsen et al., 1993) and blood lactate (Kirwan et al., 1987; Nielsen et al., 1993) concentration have been observed following heat acclimation, consistent with the present findings. In addition, a decreased rate of glycogenolysis has been observed with acclimation (King et al., 1985; Kirwan et al., 1987). The muscle glycogen data in this study do not support these findings. There are factors, however, which may be taken into account when assessing such an observation. Of the six subjects biopsied, five had little difference in resting glycogen concentrations in the HT compared with the AT and utilised more glycogen in the HT compared with the AT. The sixth subject however, had a much higher resting glycogen concentration (a difference of 209 mmol. glucosyl u. kg^{-1} dry wt) in the AT compared with the

HT and subsequently utilised more glycogen during exercise following acclimation. It is possible that the higher resting glycogen concentration increased the rate of utilisation since muscle glycogen breakdown may vary directly with the pre-exercise muscle glycogen concentration (Gollnick et al., 1972; Richter & Galbo, 1986). When glycogenolysis was estimated by PAS staining on specific fibre types, a significant depletion of type I fibres in the HT was found compared with the AT (Fig. 5.7). This finding suggests a lower glycogen utilisation in the type I fibres during exercise in the heat following acclimation. In addition, the lower ($P<0.05$) muscle lactate concentration in the AT compared with the HT may provide evidence of a reduced glycolytic rate during exercise in the heat following acclimation. These data, along with the lower mean RER (Table 5.1) suggest a decreased reliance upon carbohydrate during exercise in the heat following acclimation. Although plasma FFA concentrations were not different between the trials (Fig. 5.4), supporting previous findings (Kirwan et al., 1987; Nielsen et al., 1993), this observation may not indicate intramuscular lipid oxidation for reasons previously discussed. Furthermore, Kirwan et al. (1987) observed increased FFA uptake during exercise in the heat following acclimation despite similar concentrations of plasma FFA.

Green et al. (1992a) reported decreased muscle glycogenolysis and muscle lactate accumulation following 5-7 days of endurance training without observing any change in $\dot{V}O_2max$ or maximal activities of enzymes involved in the citric acid cycle. These observations, however, were made in a study which utilised active, but untrained, subjects. Since all subjects recruited in this study were well trained endurance athletes the likelihood that the metabolic changes were due to a training effect is very small.

An increase in plasma volume (King et al., 1985; Nielsen et al., 1993), blood volume (Senay et al., 1976) and cardiac output during exercise (Nielsen

et al., 1993) has been observed following heat acclimation. Rowell (1974) has postulated that these adaptations may result in an increase in muscle perfusion during exercise in the heat in the acclimated individual. As previously mentioned, the evidence regarding alterations in active muscle blood flow during exercise in the heat is equivocal. Previous studies comparing leg blood flow during exercise in the heat before and after acclimation have observed no difference in this measurement (Kirwan et al., 1987; Nielsen et al., 1993). The changes in muscle metabolism in the present study indicate that blood flow is unaltered by acclimation. In contrast to the comparison between the RT and the HT, reported in the previous chapter, no differences were observed in CP or C concentrations when comparing the AT with the HT (Fig. 5.6). This indicates that acclimation did not result in greater O_2 delivery to the contracting muscle. Furthermore, no difference was observed in pulmonary $\dot{V}O_2$ in the AT compared with the HT (Table 5.1). It is therefore unlikely that an increased $\dot{V}O_2$ of the contracting leg muscles, as a result of an increase in O_2 delivery, occurred during exercise after acclimation. The reduced muscle lactate accumulation following acclimation (Fig. 5.5), may, indicate an enhanced in muscle blood flow, since a lower O_2 supply may lead to an increase in anaerobic glycolysis. A reduction in blood lactate, however, in the absence of any change in blood flow has been observed when comparing exercise in the heat before and after acclimation (Nielsen et al., 1993). Interestingly, although both the mean HR and final T_r were reduced following acclimation (Table 5.1), these measurements did not return to levels observed in the RT (Table 4.1). It is possible that the thermoregulatory stress in the AT, whilst being lower when compared with the HT, was sufficiently high to prevent an increased blood flow to the active muscle. As we did not measure blood flow in this experiment its influence is speculative. These results, however, would not support the suggestion of redistribution of blood flow during exercise in the heat after acclimation.

It is likely that an increase in plasma volume, previously observed after acclimation (Armstrong & Dziados, 1986; Nielsen et al., 1993), occurred in this study. Plasma cortisol levels have been found to be lower during exercise in the heat following acclimation (Armstrong et al., 1989; Francesconi et al., 1985), consistent with the present finding (Fig. 5.4). This phenomenon has been attributed to the level of hydration and subsequent plasma volume concentration, since, plasma cortisol secretion is lower during exercise in the heat in a euhydrated, acclimated state when compared with a hypohydrated acclimated state (Francesconi et al., 1984). The lower ($P<0.05$) mean plasma cortisol levels in the acclimated state (Fig. 5.4) tend to indicate that subjects had a higher plasma volume level both at rest and during exercise. Hence, even if the subjects experienced a greater fluid and plasma volume loss during exercise, the present results indicate that post-exercise plasma volumes were greater in the acclimated state. It is therefore possible that plasma volume differences may account for some of the observed changes in muscle metabolism. An increase in body temperature (Galbo et al., 1979b) and decrease in plasma volume (Francis, 1979) have been found to stimulate catecholamine secretion. An increase in plasma volume results in an increased sweat rate which allows for an enhanced thermoregulatory capacity and lower core temperature during exercise at a given workload (Nadel et al., 1974). The probable increase in plasma volume following acclimation may decrease catecholamine secretion, thereby decreasing muscle glycogenolysis and lactate accumulation. Green et al. (1989) postulated that plasma volume expansion, arising from adaptations to endurance training, results in a blunting of the exercise-induced increase in catecholamines and a decrease in muscle glycogenolysis. It is uncertain, however, whether the stimulus for a decrease in plasma catecholamines was the increase in plasma volume *per se* or other mechanisms associated with training adaptations. Contrary to the findings of Green et al. (1989), Sawka et al. (1983) observed no differences in

physiological or metabolic parameters when plasma volume was expanded by albumin infusion. On the basis of the current research it is not possible to assess whether plasma volume expansion alone, or plasma volume expansion in conjunction with other adaptations to acclimation, can result in a reduction in adrenaline and concomitant changes to muscle metabolism. Further research examining the effect of acute plasma volume expansion induced by albumin infusion on muscle metabolism is required to fully understand the influence of plasma volume changes.

As discussed in Chapter 4, the difference in post exercise muscle temperature between the HT and the RT was unlikely to account for the magnitude of difference observed in the metabolites. It is less likely that a change in muscle temperature was responsible for changes in metabolites when comparing the AT with the HT since the post exercise difference in muscle temperature between these trials was much smaller (Table 5.1) when compared with the difference between the HT and the RT (Table 4.1). Furthermore, the exercise-induced change in muscle temperature was higher in the AT compared with the HT (Table. 5.1). It appears that the reduction in plasma adrenaline observed in the AT compared with the HT (Fig. 5.3) had an important influence on metabolism since it was accompanied by reductions in muscle lactate accumulation (Fig. 5.5), blood glucose (Fig. 5.2) and lactate (Fig. 5.1) concentration and type I fibre glycogen utilisation (Fig. 5.7). This observation supports findings from a recent study by Nielsen et al. (1993) who found lower arterial concentration of plasma catecholamines, glucose and lactate during exercise following acclimation.

In summary, many of the metabolic changes which arise during exercise as a result of heat stress can be somewhat attenuated by the acclimation process. As

discussed in Chapter 4, the potential mechanisms responsible for the metabolic changes observed during exercise in the heat compared with that in a cooler environment are as follows: an augmented rise in catecholamines, changes in muscle temperature, changes in muscle fibre type recruitment pattern and a possible reduction in O_2 delivery as a result of reduced blood flow. The observations reported in this chapter indicate that the changes in plasma catecholamines and muscle temperature are reversed by acclimation. As discussed, however, only changes to plasma catecholamines are likely to account for the observed metabolic changes and it is unlikely that muscle temperature or fibre type recruitment patterns are involved in these changes. Furthermore, results suggest that blood flow appears to be unaltered by acclimation. The increase in plasma volume, however, associated with heat acclimation may contribute to the changes in metabolism.

CHAPTER 6

MUSCLE METABOLISM DURING SUBMAXIMAL EXERCISE AT 40, 20 AND 3°C

6.1 INTRODUCTION

The literature examining the effect of exposure to cool conditions on muscle metabolism during exercise has produced conflicting results. An increased anaerobic glycolysis, reflected in blood lactate concentration (Doubt & Hsieh, 1991; Galbo et al., 1979b; Holmer & Burgh, 1974; Nadel et al., 1974) and accelerated muscle glycogenolysis during low intensity exercise (Jacobs et al., 1985) have been previously observed during exercise in cool conditions. This increase in anaerobic glycolysis has been attributed to the direct effects of shivering thermogenesis (Shephard, 1985), increased catecholamine secretion (Galbo et al., 1979b) and reduced mechanical efficiency as a result of lowered muscle temperature (Blomstrand et al., 1986; Blomstrand & Essen-Gustavsson, 1987). In contrast, no difference in blood lactate accumulation (Hessemer et al., 1984; Flore et al., 1992; Jacobs et al., 1985) or muscle glycogenolysis (Jacobs et al., 1985) has been observed when comparing moderate intensity exercise in the cold with that in temperate conditions. Changes in blood lactate, however, may be a poor indicator of muscle metabolism during exposure to the cold. Lactate turnover increases in the absence of any change in blood lactate concentration (Minaire et al., 1971) and a similar blood lactate concentration was observed in the presence of higher noradrenaline concentration during incremental exercise in the cold (Flore et al., 1991).

Nevertheless, in experiments which involved controlled cooling during exercise, or pre-cooling prior to exercise, cooling has resulted in an increase in performance (Hessemer et al., 1984; Schmidt and Bruck, 1981) and a decrease in anaerobic glycolysis (Kozlowski et al., 1985; Kruk et al., 1985).

The discrepency in the literature appears to be related to the exercise intensity and the reduction in core and muscle temperature. On the one hand, those studies which have observed an increase in anaerobic glycolysis, have involved whole body water immersion (Doubt & Hsieh, 1991; Galbo et al., 1979b; Holmer & Burgh, 1974; Nadel et al., 1974), or low intensity exercise (Jacobs et al., 1985) in which core, and possibly muscle, temperature decreased. On the other hand, others who have studied the metabolic response to reduced air temperature (Hessemer et al., 1984; Flore et al., 1992) and/or used a relatively moderate to high intensity protocol (Jacobs et al., 1985) have observed reduced glycolysis and/or increased performance.

The conflicting reports regarding the effect of exercise in the heat on muscle metabolism, may be related to the temperature difference between trials. As discussed in Chapter 2, Fink et al. (1975) observed an increase in muscle glycogenolysis when comparing exercise at 41^{o}C with that at 9^{o}C, whilst others (Nielsen et al., 1990; Yaspelkis et al., 1993) observed similar rates of muscle glycogenolysis when the temperature difference between the respective trials was considerably less. Interestingly, during the experiment by Fink et al. (1985), core temperature rose but reached a plateau during exercise at 9^{o}C, whilst it continued to rise in the 'thermoneutral trials' in the studies reported by Neilsen et al. (1990) and Yaspelkis et al. (1993). As previously mentioned, when controlled cooling during exercise at ambient temperature was employed, a lower rate of muscle glycogenolysis along with a lower core temperature was observed in dogs (Kozlowski et al., 1985). Thus, it is apparent

that there is thermoregulatory stress associated with exercise at 'ambient' temperature. This stress may be sufficient to result in increases in circulating catecholamines and blood flow redistribution which consequently may elevate the rate of muscle glycogenolysis. It is hypothesised that a relationship exists between the rate of muscle glycogenolysis during exercise and the ambient temperature in which the exercise is performed, since both exercise and ambient temperature are additives during exercise in the heat (see section 2.2). The conflicting results in the literature with respect to muscle glycogenolysis may be related to the magnitude of the temperature difference employed in various studies. Hence, this study was undertaken to examine the effects of three different environmental temperatures on muscle metabolism during exercise.

6.2 METHODS

Seven well trained males (22.0 ± 0.9 years; 176 ± 2 cm; 72.7 ± 2.3 kg; $\dot{V}O_2max$ = 66.7 ± 1.9 ml. kg. min^{-1}) volunteered as subjects for this study. $\dot{V}O_2max$ was determined at approximately 20°C on a friction braked bicycle ergometer according to the protocol described in Chapter 3. Following the $\dot{V}O_2max$ test a workload was selected which would elicit approximately 65% of $\dot{V}O_2max$. Each subject performed three 40 min cycling trials on the bicycle ergometer used during the $\dot{V}O_2max$ test, in random order, at least one week apart. These trials were conducted in an environmental chamber set at 40°C 20%RH (HT), 20°C 20%RH (RT) or 3°C ~ 50%RH (CT). All subjects completed 40 minutes of exercise except for two who could not continue beyond 32 and 39 minutes of exercise in the HT, repectively and asked to be removed. Since this occurred during the first trial, subsequent trials were terminated at the same time. The post-exercise muscle data for this subject were treated in the same way as the post-exercise data for all subjects.

Subjects were weighed nude on arrival at the laboratory and then inserted a rectal thermistor probe. In order to assess the influence of passive exposure to hot and cool conditions on metabolic and physiological responses during inactivity, data were collected prior to 20 min of exposure (PRE-EXP) in the HT and CT. This included the measurement of rectal and muscle temperature and collection of blood and muscle samples. Since the ambient temperature was similar to the temperature of the RT, this procedure was deemed unnecessary in this trial. In addition to collection of these data, measurements were made prior to, during and immediately following each trial. Core temperature was recorded prior to exercise and at 10 min intervals until the end of the exercise period and muscle temperature was measured prior to and following exercise. Blood samples were obtained prior to exercise and every 10 min until the end of exercise. Blood samples were analysed for lactate and glucose. Plasma samples were analysed for NH_3, adrenaline and noradrenaline. Muscle samples obtained from the vastus lateralis prior to and immediately following exercise were analysed for ATP, ADP, IMP, AMP, NH_3, CP, C, glycogen and lactate.

Heart rate was recorded during each trial at 5, 15, 25 and 35 minutes of exercise. Expired gases were also collected in Douglas bags at these times. Cycling shorts and running shoes were worn in all trials. The data from the three trials were compared using ANOVA with repeated measures.

6.3 RESULTS

Twenty min of passive exposure to 3°C and 40°C resulted in T_m being reduced ($P<0.01$) in the CT and increased ($P<0.01$) in the HT (Fig 6.1). The cold exposure also resulted in an increase ($P<0.01$) in plasma noradrenaline concentration and a decrease ($P<0.05$) in plasma NH_3 concentration (Table 6.1). No other measured physiological or metabolic parameter was affected by the respective passive exposures (Table 6.1).

Mean $\dot{V}O_2$ during exercise was not different between the three trials (Table 6.2). Although mean RER during exercise was not different between the HT and the CT both of these values were higher ($P<0.05$) compared with the RT. T_m was not different when comparing the PRE-EXP values in the HT with the PRE-EXP values in the CT or the pre-exercise values in the RT. Passive exposure to the respective environmental conditions, however, lead to differences ($P<0.01$) in pre-exercise measurements between the three trials. These differences were augmented ($P<0.01$) following exercise (Fig. 6.1). Although HR rose ($P<0.01$) in each trial, it reached a plateau in the CT at 15 min and was lower ($P<0.01$) from this point until the end of exercise when compared with the other trials. In contrast, HR continued to rise beyond this time in the HT and the RT. The rise, however, was more rapid in the HT such that HR was higher ($P<0.01$) in this trial compared with the RT at 15 min of exercise and beyond (Fig 6.1). Although T_r rose ($P<0.01$) in all trials, it did not rise until 20 min into the exercise in the CT and reached a plateau at 30 min. In contrast, T_r rose throughout exercise in the HT and the RT. T_r was higher ($P<0.01$) in the HT at 30 min of exercise and thereafter compared with the RT and the CT. Furthermore, T_r was higher ($P<0.01$) at these times during the RT compared with the CT (Fig. 6.1).

Blood glucose concentrations were not different at rest between the three trials. During exercise, blood glucose was higher ($P<0.01$) at 30 and 40 min of exercise in the HT compared with the CT and the RT. Blood glucose concentration did not differ at any points in these latter trials (Fig. 6.2). Blood lactate concentration was not different at rest between the three trials but was higher ($P<0.05$) at all points during exercise in the HT compared with the other trials. Blood lactate during exercise was not different between the CT and RT (Fig 6.2). Plasma NH_3 concentrations were lower ($P<0.05$) at rest in the CT compared with the HT and the RT. Although no differences were observed during exercise between the HT and the RT, plasma NH_3 concentration was lower at 10 ($P<0.01$), 20 and 40 min ($P<0.05$) of exercise in the CT when compared with the RT and HT (Fig. 6.2). Plasma adrenaline concentration was not different between the three trials at rest but was higher ($P<0.01$) during exercise in the HT compared with the CT and the RT (Fig. 6.2). Resting plasma noradrenaline was lower ($P<0.01$) in the HT compared with the CT and the RT. Concentrations of this hormone were higher ($P<0.01$) in the HT compared with the CT and the RT at 40 min of exercise. Furthermore, the concentration of noradrenaline at this time was higher RT compared with the CT (Fig. 6.3).

Pre-exercise muscle lactate concentrations were not different between the three trials. The post-exercise value was higher ($P<0.01$) in the HT, and tended to be higher ($P=0.05$) in the RT compared with the resting value. No difference, however, was observed between pre and post-exercise concentrations of muscle lactate in the CT. The post-exercise muscle lactate concentrations were higher ($P<0.01$) in the HT compared with the CT and the RT. The post-exercise muscle lactate concentrations in these latter trials were not statistically different (Fig. 6.4). There was no difference in pre-exercise muscle glycogen concentration between the three trials. The post-exercise concentration was lower ($P<0.01$) in all trials when compared with pre-exercise levels.

Furthermore, the post-exercise muscle glycogen concentration was lower ($P<0.05$) in the HT compared with the CT and RT. Muscle glycogenolysis was greater ($P<0.01$) in the HT compared with the CT and tended ($P=0.05$) to be greater when compared with the RT. No difference in muscle glycogenolysis or post-exercise muscle glycogen content was observed between the CT and the RT (Table 6.3).

Concentrations of intramuscular ATP, IMP and AMP were unaffected by exercise or temperature (Table 6.4). Although there was an overall accumulation ($P<0.05$) of ADP (Table 6.4) and NH_3 (Fig. 6.4)in the muscle in the three trials, no differences in these metabolites were observed at rest or following exercise between the trials (Table 6.4). Muscle CP and C concentrations were not different between the three trials prior to exercise. Exercise resulted in a lower ($P<0.01$) CP and higher ($P<0.05$) C concentration within the muscle. Post-exercise muscle CP degradation and C concentration were higher ($P<0.05$) in the HT compared with the CT and RT. Muscle CP and C concentrations were not different between the RT and the CT (Fig. 6.5).

A significant correlation ($R=0.56, P<0.05$) was observed between the exercise induced change in rectal temperature and muscle glycogenolysis (Fig. 6.6).

	HT		CT	
	PRE-EXP	POST-EXP	PRE-EXP	POST-EXP
METABOLITES				
Muscle				
ATP	25.6±0.9	25.7±0.9	25.0±0.5	26.0±0.6
ADP	2.56±0.09	2.67±0.10	2.64±0.18	2.92±0.15
AMP	0.08±0.02	0.09±0.02	0.11±0.02	0.12±0.02
IMP	0.07±0.01	0.08±0.02	0.10±0.02	0.13±0.04
Ammonia	0.32±0.07	0.32±0.07	0.43±0.06	0.28±0.06
Lactate	5.5±0.5	6.4±1.4	5.9±0.9	7.2±1.3
Glycogen #	604±53	588±45	605±47	592±41
CP	92.4±2.0	91.8±2.0	94.9±4.7	85.2±5.7
C	41.1±2.3	41.6±2.8	38.6±3.7	48.3±6.1
Whole Blood Metabolites				
Glucose ~	4.3±0.1	4.8±0.3	4.3±0.2	4.4±0.1
Lactate ~	1.1±0.1	1.1±0.1	1.2±0.1	1.2±0.1
Plasma Metabolites and Hormones				
Ammonia @	19.4±3.1	27.0±4.0	20.8±3.6 *	12.8±1.4
Adrenaline +	29.9±5.7	40.2±4.3	27.9±5.2	28.6±4.4
Norad'line -	0.26±0.03	0.17±0.05	0.28±0.03 **	0.50±0.03

Table 6.1. *Intramuscular concentrations of muscle, whole blood and plasma metabolites and hormones prior to (PRE-EXP) and following (POST-EXP) 20 min of passive exposure to 40°C RH20% (HT) and 3°C RH~50%.* Values are expressed in mmol. kg^{-1} dry weight unless otherwise stated. # indicates value expressed in mmol. kg glucosyl $units^{-1}$ dry weight, ~ indicates values expressed in mmol. l^{-1}, @ indicates value expressed in umol. l^{-1}, + indicates value expressed in pg. ml^{-1} and - indicates value expressed in ng. ml^{-1}. * indicates difference ($P<0.05$) in PRE-EXP CT value compared with the value POST-EXP CT, ** indicates difference ($P<0.01$) in PRE-EXP CT value compared with the value POST-EXP CT. Values are expressed as means ± SE (n=7).

	TRIAL		
	HT	RT	CT
O_2 Uptake ($ml.kg^{-1}.min^{-1}$)	42.2±0.8	42.3±0.6	42.3±0.6
RER	0.89±0.00	0.87±0.01 *	0.89±0.00

Table 6.2. *Mean Oxygen (O_2) uptake and mean respiratory exchange ratio (RER) during exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH ~ 50% (CT).* * indicates difference ($P<0.05$) in the RT compared with the CT and the HT. Values are means ± SE (n=13).

TRIAL	PRE-EX	POST-EX	Δ
HT	588±45 a	340±40 *	248±15
RT	615±68 a	421±62	194±19 +
CT	592±41 a	447±57	145±27 **

Table 6.3 *Muscle glycogen concentrations before (PRE-EX) and after (POST-EX) exercise and muscle glycogenolysis (Δ) in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH ~ 50% (CT).*

Values are means ± SE (n=8) expressed in mmol glucosyl units. kg^{-1} dry weight. a indicates difference ($P<0.01$) POST-EX compared with PRE-EX, * indicates difference ($P<0.05$) HT compared with other trials, ** indicates difference ($P<0.01$) CT compared with other trials + indicates $P=0.05$ when comparing muscle glycogenolysis in the HT with that in the RT.

	HT		RT		CT	
	PRE-EX	POST-EX	PRE-EX	POST-EX	PRE-EX	POST-EX
ATP	25.7±0.9	26.7±1.6	26.1±0.4	26.1±0.3	26.0±0.6	26.5±0.8
ADP	+2.67±0.10	3.30±0.24	2.83±0.10	3.11±0.26	2.92±0.15	3.22±0.13
AMP	0.09±0.02	0.16±0.03	0.12±0.02	0.15±0.03	0.12±0.02	0.13±0.02
IMP	0.08±0.02	0.23±0.04	0.05±0.01	0.14±0.03	0.13±0.04	0.12±0.02

Table 6.4. *Intramuscular concentrations of adenosine 5'triphosphate (ATP), adenosine 5'diphosphate (ADP), adenosine 5'monophosphate (AMP) and inosine 5'monophosphate (IMP) before (PRE-EX) and after (POST-EX) exercise in 40^{o}C RH20% (HT), 20^{o}C RH20% (RT) and 3^{o}C RH ~ 50% (CT).* Values are means ± SE (n=7) and expressed in mmol. kg^{-1} dry weight. + indicates higher ($P<0.05$) mean exercise concentration compared with rest.

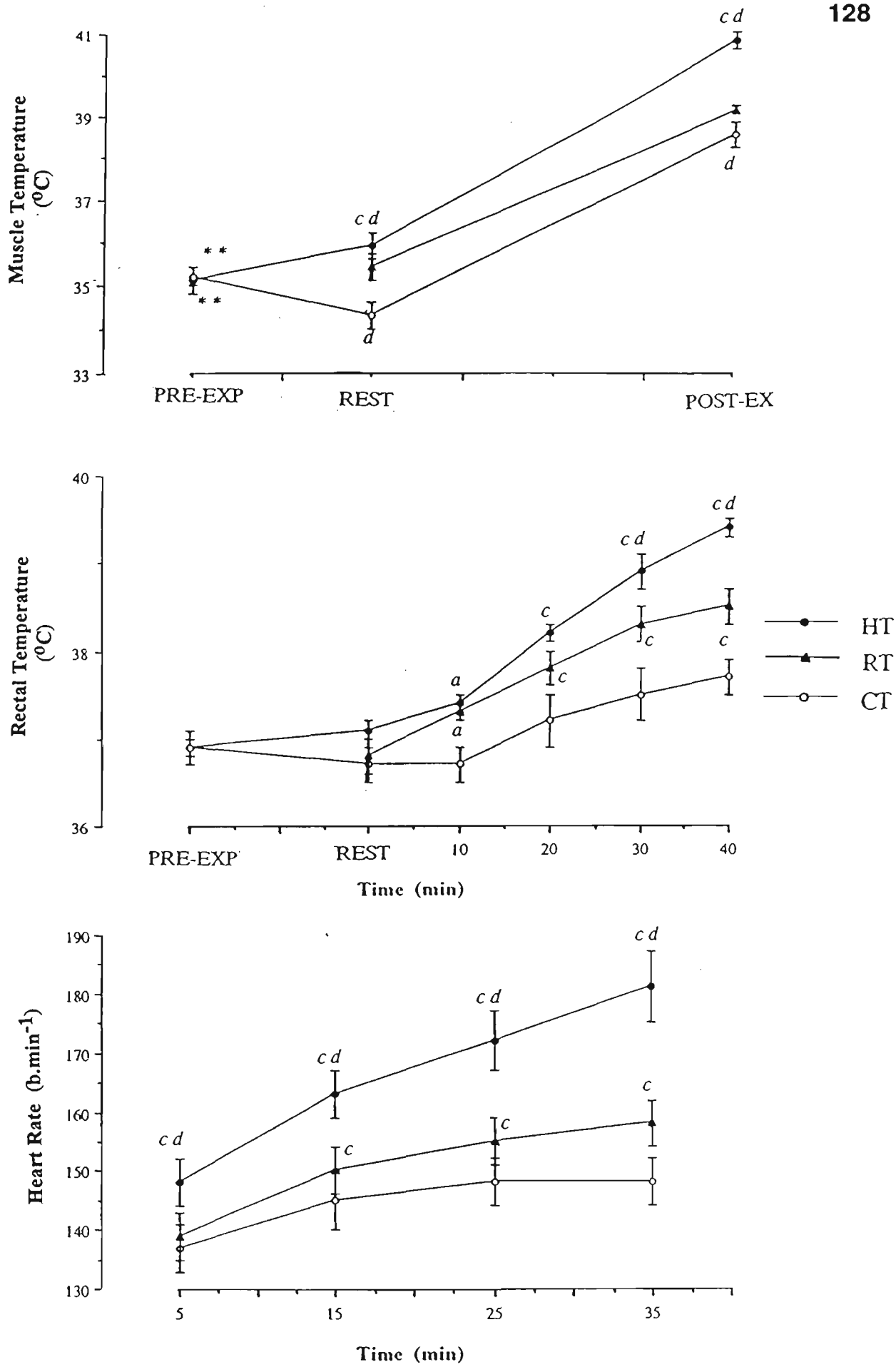


Fig. 6.1. Muscle and rectal temperature and heart rate prior to 20 minutes of passive exposure (PRE-EXP) prior to (REST) during and following (POST-EX) exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH~50% (CT). Values are means ± SE (n=7). ** indicates difference ($P<0.01$) PRE-EXP compared with REST. ^{c} indicated difference ($P<0.01$) from CT, ^{d} indicates difference ($P<0.01$) from RT, ^{a} indicated difference ($P<0.05$) from CT

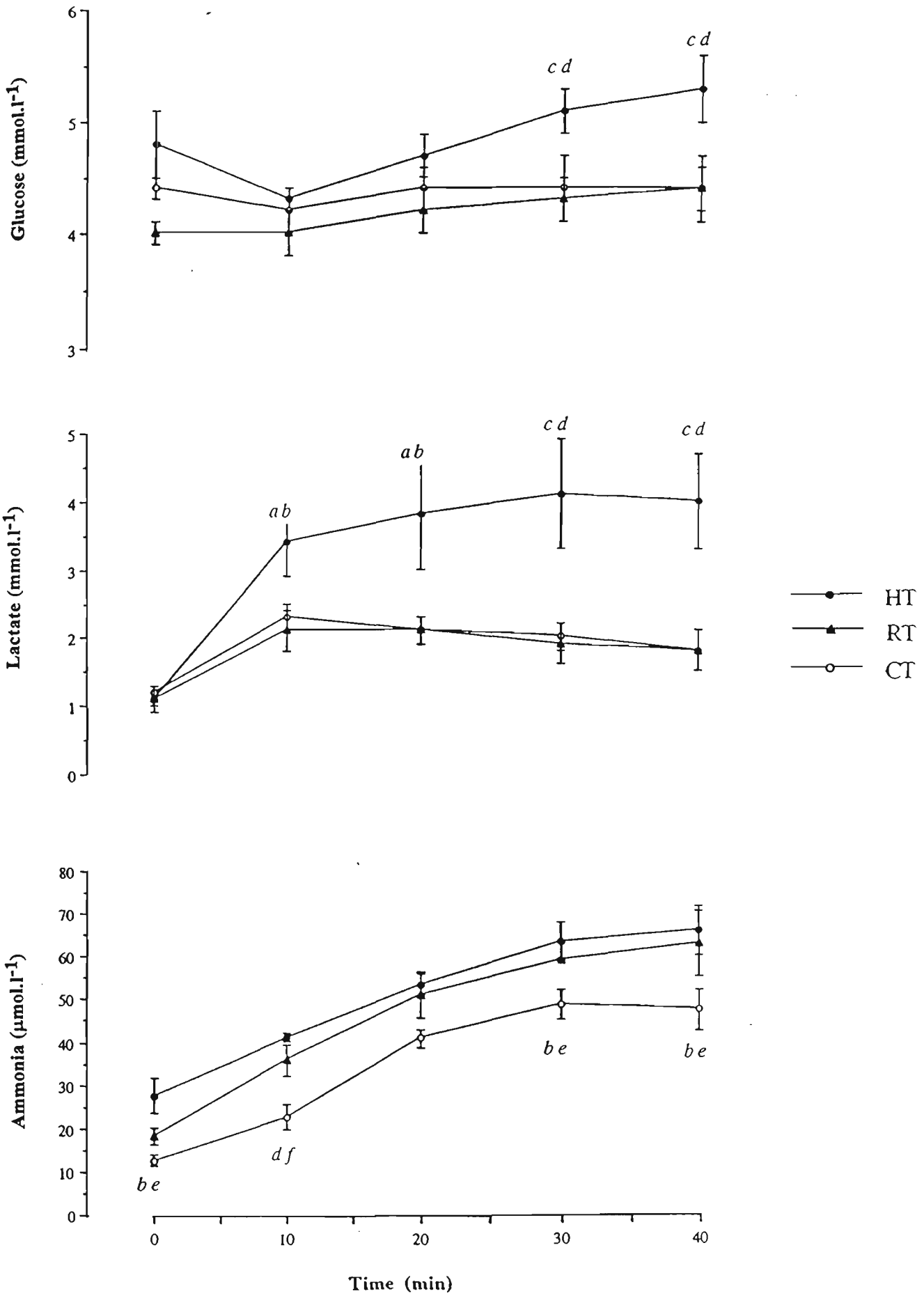


Fig. 6.2. Blood glucose, lactate and plasma ammonia accumulation during exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH ~ 50% (CT). Values are means ± SE (n=7). *c* indicated difference ($P<0.01$) from CT, *d* indicates difference ($P<0.01$) from RT, *a* indicated difference ($P<0.05$) from CT, *b* indicates difference ($P<0.05$) from RT, *f* indicates difference ($P<0.01$) from HT, *e* indicates dif

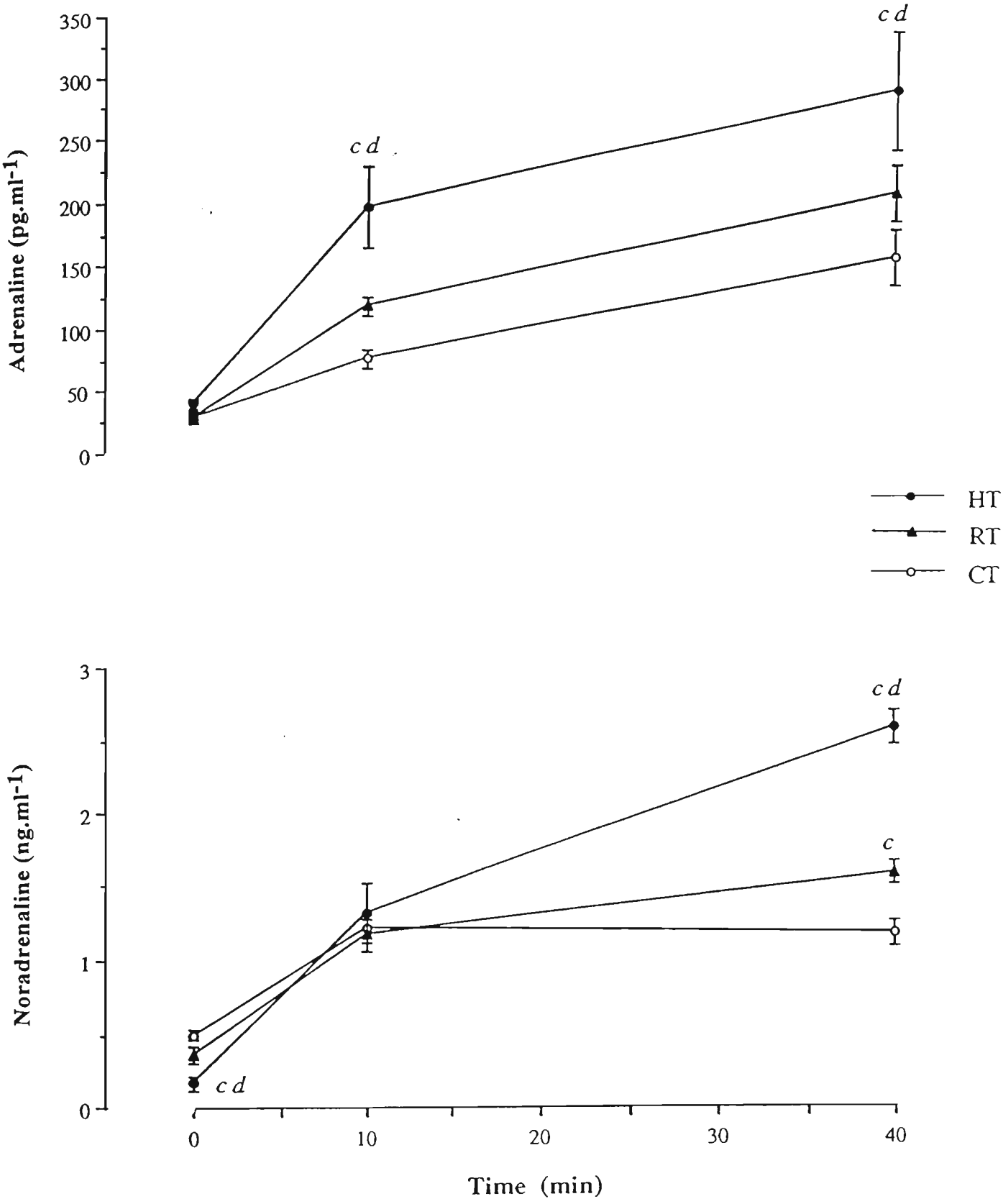


Fig. 6.3. Plasma adrenaline and noradrenaline concentrations during exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH ~ 50% (CT). Values are means ± SE (n=7). ^{c} indicated difference ($P<0.01$) from CT, ^{d} indicates difference ($P<0.01$) from RT.

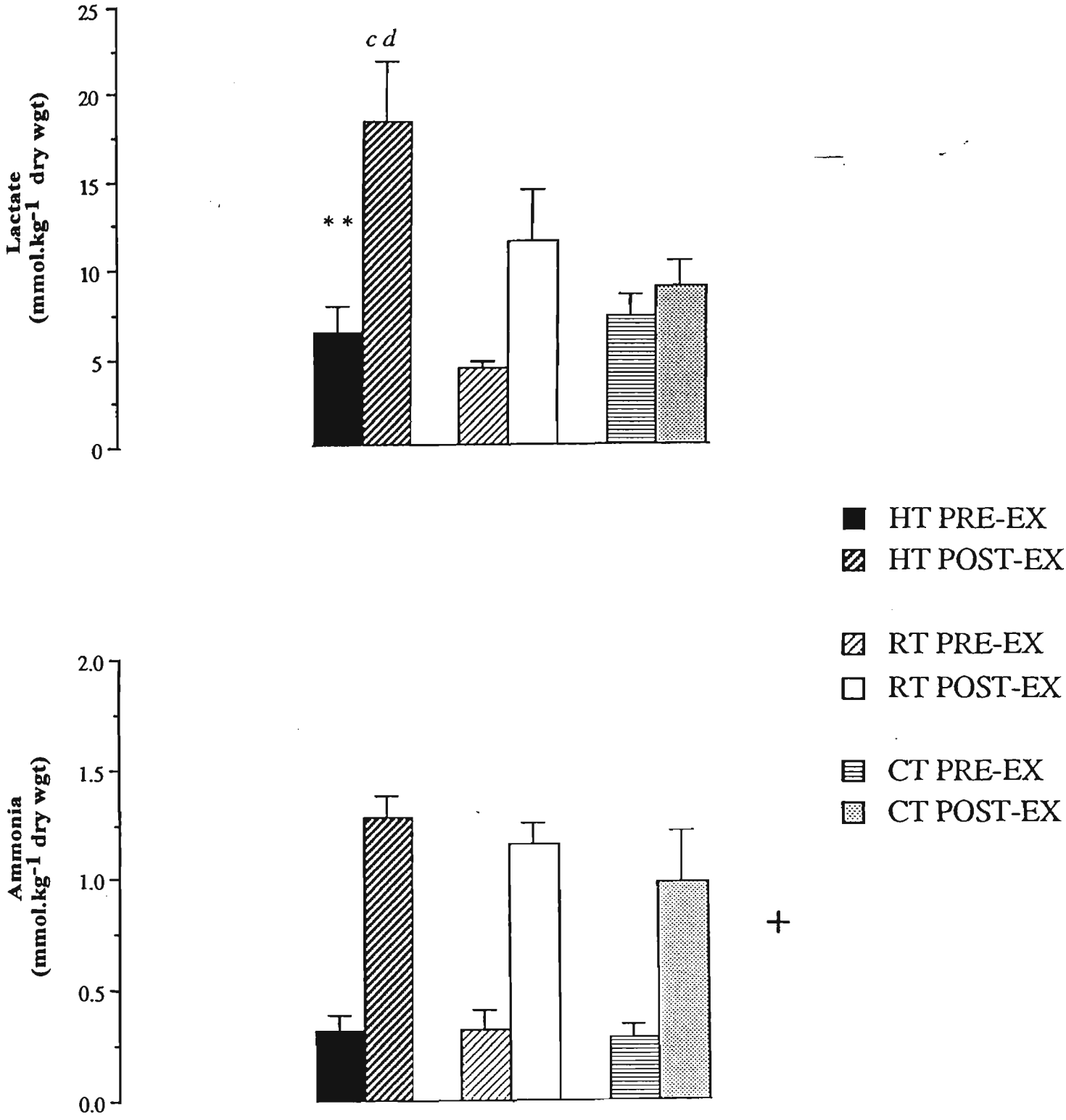


Fig. 6.4. Muscle lactate and muscle ammonia concentration prior to (PRE-EX) and following (POST-EX) exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH~50% (CT). Values are means ± SE (n=7). ^{c} indicated difference ($P<0.01$) from CT, ^{d} indicates difference ($P<0.01$) from RT, ** indicates difference ($P<0.01$) POST-EX compared with PRE-EX. $^{+}$ indicates higher ($P<0.05$) mean exercise concentration compared with rest.

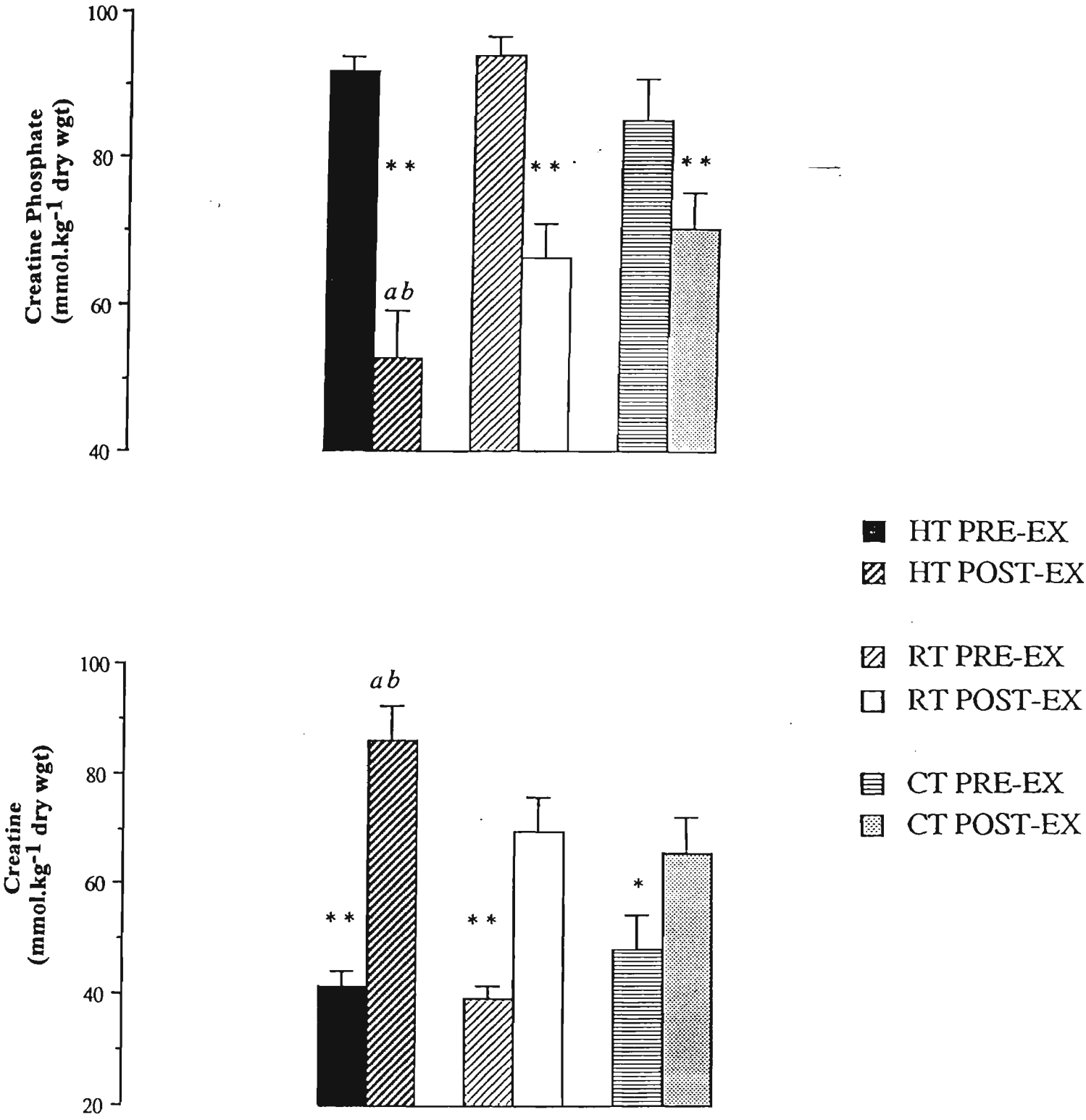


Fig. 6.5. Muscle creatine phosphate (CP) and creatine (C) prior to (PRE-EX) and following (POST-EX) exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH ~ 50% (CT). Values are means ± SE (n=7). ^{a} indicated difference ($P<0.05$) from CT, ^{b} indicates difference ($P<0.05$) from RT, ** indicates difference ($P<0.01$) POST-EX compared with PRE-EX, * indicates difference ($P<0.05$) POST-EX compared with PRE-EX.

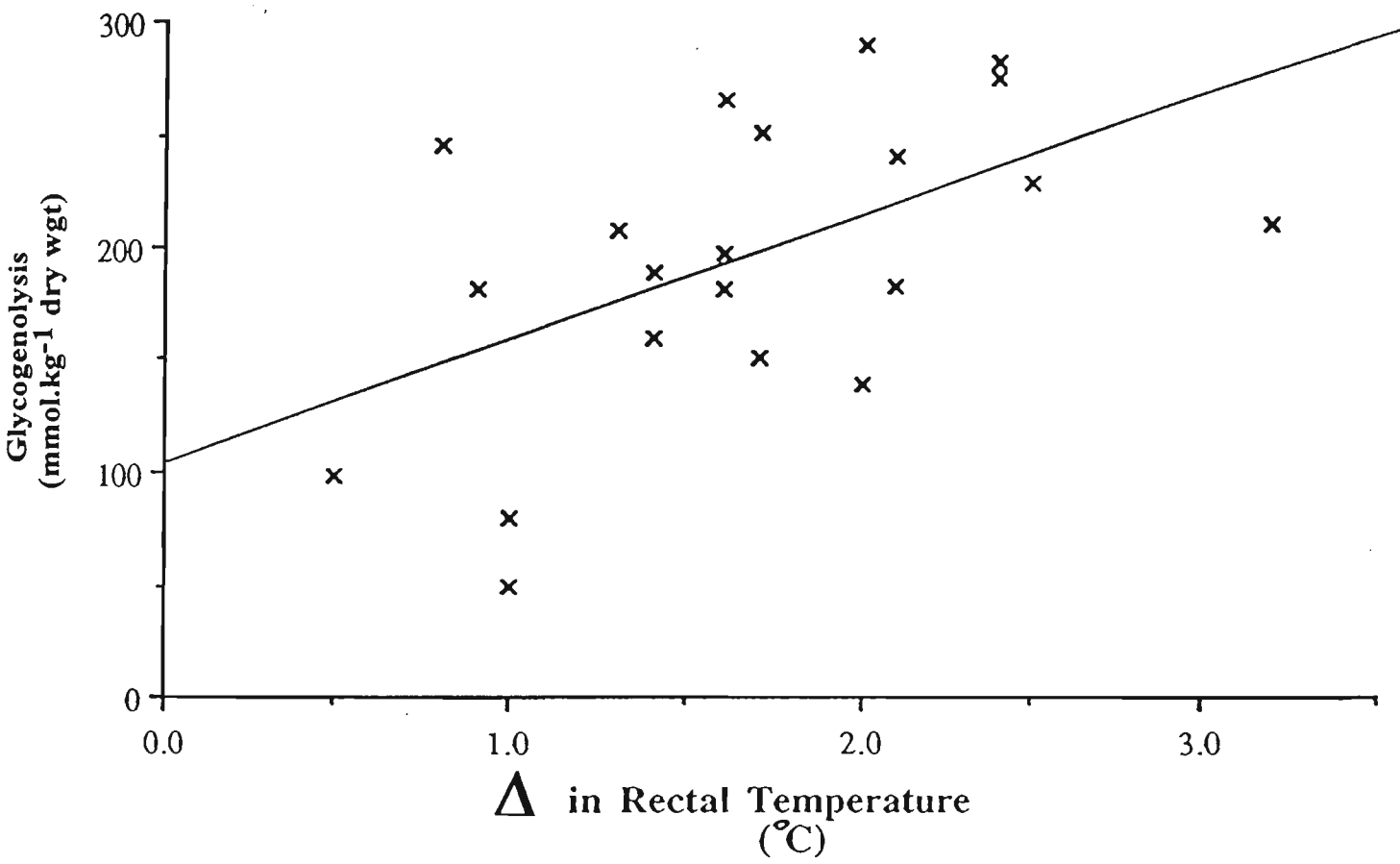


Fig. 6.6. Correlation (r=0.56, P<0.01) between exercise induced change in rectal temperature and muscle glycogen utilisation during exercise in 40°C RH20% (HT), 20°C RH20% (RT) and 3°C RH ~ 50% (CT) (n=7).

6.4 DISCUSSION

The results from this study demonstrate a relationship between body temperature and carbohydrate metabolism during exercise. Although the temperature difference between the CT and the RT was not sufficient to produce significant differences in muscle glycogenolysis (Table 6.3), muscle lactate accumulation (Fig. 6.4) or CP degradation (Fig. 6.5), mean values for these metabolites were less in the CT compared with the RT. Furthermore, a significant correlation was observed between the exercise induced change in rectal temperature and rate of muscle glycogenolysis (Fig. 6.6). The higher ($P<0.01$) muscle lactate accumulation (Fig. 6.4), greater ($P<0.05$) CP degradation (Fig. 6.5), higher ($P<0.05$) RER (Table 6.2) and tendency ($P=0.05$) for greater glycogenolysis (Table. 6.3) in the HT compared to the RT support the conclusion presented in Chapter 4 that muscle carbohydrate utilisation is increased when exercise in the heat is compared with exercise in a thermoneutral environment.

Although passive exposure to 40^{o}C resulted in an elevated muscle temperature (Fig. 6.1), this increase had no effect on the circulating catecholamines or on measured metabolism (Table 6.1). In contrast, the exposure to 3^{o}C produced a lower muscle temperature (Fig. 6.1), an increase in plasma noradrenaline and decrease in plasma NH_3. The observed rise in noradrenaline during passive, cold exposure despite no change in core temperature is in agreement with previous findings (Bergh et al., 1979; Johnson et al., 1977). Since noradrenaline is released by the both adrenal medulla and peripheral nerve endings whilst adrenaline is secreted by the adrenal medulla alone (Tepperman, 1980), the lower environmental temperature is likely to have resulted in an increase in sympathetic nervous activation during inactivity. The lower NH_3 accumulation following cold exposure may be related to

vasoconstriction at the sampling site. During acute cold exposure superficial blood flow decreases as skin temperature declines below 34°C (Viecsteinas et al., 1982). Since the gastrointestinal tract (Onstad & Zieve, 1979) and the kidneys (Owen et al., 1960) are the major contributors to plasma NH_3 concentration at rest, it is possible that NH_3 released from these organs reached the forearm vein to a lesser extent as peripheral temperature decreased. Another possibility for the lower plasma NH_3 accumulation observed after passive cold exposure is that enhanced clearance may have occurred due to a possible increase in ventilation. Expired air contains NH_3 (Jacques et al., 1959) and it has been speculated that the lung is a major clearance organ for this metabolite (Graham & MacLean, 1992). Oxygen uptake has been found to be higher during exposure to the cold (Holmer & Bergh, 1974; Hong & Nadel, 1979), attributed to the added oxygen requirements for shivering (Young, 1990). Since many subjects experienced shivering in the 20 min of cold exposure, an increased $\dot{V}O_2$ and possibly an increased ventilation may have occurred. Unfortunately ventilatory data were not obtained during this period.

In a recent publication (Yaspelkis et al. 1993) the authors hypothesised that the rate of muscle glycogen utilisation may be slowed by a cold environment, but may not be substantially affected by a hot environment. The data in the present study do not support this hypothesis. The thermoregulatory load, reflected in heart rate, rectal and muscle temperatures during exercise (Fig. 6.1), was lower in the CT compared with the RT, but was also higher in the HT compared with the RT. In addition, (Table 6.3), muscle lactate accumulation (Fig.6.4) muscle CP degradation (Fig. 6.5) and mean RER (Table 6.2) were higher in the HT compared with RT and CT. Furthermore muscle glycogenolysis was higher in the HT compared with the CT, and tended ($P=0.05$) to be higher in the HT compared with the RT (Table 6.3) indicating that the rate of muscle glycogen utilisation and anaerobic metabolism is indeed affected

during exercise in a hot environment. Although mean values for muscle glycogen utilisation (Fig. 6.3), muscle lactate accumulation (Fig 6.4) and muscle CP degradation (Fig. 6.5) were higher in the RT compared with the CT, the differences were not significant. From this it may be inferred that the temperature differential between the RT and the CT was not sufficient to produce statistical differences in metabolites, rather than implying that cooling has no substantial effect on muscle metabolism. This inference is supported by the fact that the magnitude of difference between rectal and muscle temperature and heart rate in the RT and the CT at the end of exercise was lower than that which was observed between the HT and the RT (Fig. 6.1). This would indicate that the difference in thermal stress between exercise in different ambient conditions must be of a sufficient magnitude to produce differences in metabolites. Furthermore, the significant correlation between the exercise induced change in rectal temperature and rate of glycogenolysis (Fig 6.6) indicates a graded response between body temperature and glycogenolysis during exercise.

The high mean RER in the CT (Table 6.2) compared with the RT and the HT was an unexpected finding. Since muscle glycogenolysis (Table 6.3), muscle lactate accumulation (Fig. 6.2) and plasma catecholamines (Fig. 6.3) were lowest in this trial, one would have expected carbohydrate oxidation to be lowest also. Of note, however, is that RER is a measure of whole body substrate utilisation. Although a measure of glycogen utilisation in the active muscle was obtained, no measure of energy turnover in the inactive muscle is available. It is possible that involuntary muscle activity associated with shivering in non active muscles contributed to the higher RER observed in the CT. Carbohydrate is the major fuel for the increased energy demand caused by shivering (Vallerand et al., 1988; Vallerand & Jacobs, 1989). Although observable shivering ceased at the onset or very early into exercise, Pozos (1981) observed increased EMG activity in individuals without observable shivering and

attributed this to an increased muscle tone. Although rectal temperature did not fall, muscle temperatures in the non-active muscle may have done so. Webb (1992) observed the onset of shivering to occur in muscles at temperatures ranging between 33 and 35^{o}C. Furthermore, Jacobs et al. (1985) found muscle glycogenolysis to be greater during low intensity activity at 9^{o}C compared with 21^{o}C even though rectal temperature was similar between the two trials. The hypothesis that shivering may have influenced the RER in the CT is speculative, however, since $\dot{V}O_2$ was not different during exercise in this trial.

The lower ($P<0.05$) plasma NH_3 concentration observed during exercise in the CT compared with the HT and the RT (Fig 6.2) is in contrast with data presented in previous chapters of this dissertation which suggest that in the trained individual, NH_3 production is not related to environmental temperature. The observation in this study, however, is likely to be influenced by the lower resting NH_3 level observed in the CT, mechanisms for which have been previously discussed. If NH_3 efflux was similar between the three trials, production during exercise would not have differed between the trials, since the exercise induced change in plasma NH_3 accumulation was not different between the trials, with the values being 39.0±6.2 vs 43.7±7.1 and 36.7±3.6 ($P=0.5$) for the HT, RT and CT respectively. The similar ($P=0.31$) muscle NH_3 concentrations between the three trials following exercise (Fig. 6.4) supports the hypothesis that muscle NH_3 production during exercise did not differ between the trials. Adenine nucleotide degradation and IMP accumulation did not differ between the three trials (Table 6.4). This observation supports those presented in previous chapters and indicates that adenine nucleotide degradation in the trained individual is not influenced by environmental temperature during 40 min of submaximal exercise.

The mechanisms for alterations in glycogenolytic rate during exercise in different ambient conditions have been discussed in previous chapters. It is likely that muscle glycogenolysis (Table 6.3) is influenced by changes in catecholamines (Fig 6.3) and muscle temperature (Fig. 6.1), both of which are influenced by body temperature changes. A significant correlation (Fig 6.6) was observed between glycogenolysis and the exercise-induced change in rectal temperature. Of note, however, is the observation that correlations between glycogenolysis and the exercise-induced rise in adrenaline or glycogenolysis and the rise in muscle temperature did not produce significance (data not shown). It is likely, therefore, that an increase in thermal stress, reflected in a rise in core temperature, results in a synergistic effect on the mechanisms associated with metabolic adaptations.

In summary, this study indicates that exercise in the heat in unacclimated individuals places greater reliance upon carbohydrate as a fuel source when compared with exercise in a 'normal' or 'thermoneutral environment'. Furthermore, exercise in a cool environment, does not promote accelerated glycogenolysis, rather, it may attenuate carbohydrate utilisation in active muscle. Muscle glycogenolysis and anaerobic glycolysis during submaximal exercise in different environmental temperatures appears to be related to body temperature changes which, in turn, influences muscle temperature and the sympatho-adrenal response. Further research examining the effect of exercise in cool conditions compared with that in a so called 'thermoneutral' environment on muscle metabolism using a larger sample size and/or a larger temperature difference is warranted.

CHAPTER 7

THE EFFECT OF INCREASED MUSCLE TEMPERATURE ON MUSCLE METABOLISM DURING EXERCISE

7.1 INTRODUCTION

The effect of temperature on contractile processes in isolated muscle preparations has been well established (Lannergren & Westerblad, 1987; Lannergren & Westerblad, 1988). The optimal temperature for force production in mammalian muscle is reported to be between 20 and 30^{o}C *in vitro* (Lannergren and Westerblad, 1987), although the optimal muscle temperature during isotonic contractions ranges between 30 and 37^{o}C (Faulkner, 1980). The effect of temperature on both tension development and metabolic processes within the intact muscle is more complex, however, because of additional factors such as temperature effects on neuromuscular transmission, oxygen and substrate supply to the muscle and circulating hormone levels. In human muscles examined *in vivo*, endurance time increases with an increase in temperature up to 30^{o}C (Faulkner, 1980), consistent with the effect of temperature on the enzymatic processes which would enhance skeletal muscle function (Lehninger, 1982). A decline in endurance time during sustained isometric contractions, however, has been found at muscle temperatures above 32^{o}C (Clarke et al., 1958). The increase in fatiguability as muscle temperature is increased above this temperature has been related to the effect of temperature on mitochondrial ATPase activity (Brooks et al., 1971), and to the disturbance of the excitation/contraction coupling process (Edwards et. al., 1972).

Whole body studies examining the effect of increased muscle temperature on endurance time and metabolism have found prolonged endurance, lower lactate concentrations and a decrease in the degradation of high energy phosphates when comparing exercise at lower muscle temperatures with that at higher muscle temperatures (Edwards et al., 1972; Kruk et al., 1985; Kozlowski et al., 1985). In these experiments, however, rectal temperature followed the same pattern as muscle temperature. As discussed in the previous chapters, increased body temperature increases circulating catecholamine levels which are associated with alterations in metabolism. It is not possible, therefore, to attribute changes in metabolism *in vivo* to an increased muscle temperature when core temperature also rises. This study was undertaken to examine the effect of an increased muscle temperature, in the absence of any rise in core temperature, on muscle metabolism during exercise in humans.

7.2 METHODS

Eight active but not specifically trained males (29.4 ± 2.4 years; 175 ± 2cm; 70.9 ± 3.3 kg; $\dot{V}O_2max$ = 52.5 ± 2.3 ml. kg^{-1}. min^{-1}) volunteered as subjects for this study. $\dot{V}O_2max$ was determined in a thermoneutral environment and a workload estimated to require 115% of $\dot{V}O_2max$ was calculated (see Chapter 3 for respective protocols). The subjects were required to cycle on a friction braked ergometer at this workload on two occasions for two minutes. On one occasion, exercise was performed without any pre-treatment (CT); on the other, the quadriceps femoris of one limb was wrapped in a heating blanket for 60 minutes prior to exercise (HT). Four subjects performed the CT first and completed the HT at least one week later. The order was reversed for the remaining subjects.

Subjects arrived at the laboratory, were weighed nude and inserted a rectal thermistor probe. Rectal and muscle temperatures were recorded prior to heating (pre-heat), prior to exercise (pre-ex) and immediately following exercise (post-ex) in the HT and pre-ex and post-ex in the CT. Blood samples were obtained pre-ex, post-ex and after 5, 10 and 15 min of passive recovery in both trials. In addition, a pre-heat sample was collected in the HT. Blood samples were analysed for glucose, lactate and pH. Plasma samples were analysed for NH_3, adrenaline and noradrenaline. Muscle samples were obtained pre-ex and post-ex in both trials and pre-heat in the HT. These samples were analysed for ATP, ADP, IMP, AMP, inosine, hypoxanthine, NH_3, CP, C, glycogen and lactate.

The data from the HT and the CT were analysed using ANOVA with repeated measures. One analysis was conducted to compare non-exercising measurements (pre-heat, pre-ex in the HT and pre-ex in the CT). A further analysis was conducted to compare treatment (HT vs CT) and exercise (pre-ex vs post-ex). A Student t-test was used to compare muscle glycogenolysis between the trials.

7.3 RESULTS

The warming of the limb increased ($P<0.01$) muscle temperature by $2.2^{o}C$. Although muscle temperature was increased by exercise in both trials, post-ex values in the HT were higher ($P<0.05$) when compared with the CT (Fig. 7.1). In contrast, rectal temperature was not different between the trials at rest, and was not changed by exercise (Fig. 7.1). The increase in muscle temperature did not affect resting concentrations of blood and muscle metabolites (Table 7.1). Exercise increased ($P<0.05$) concentrations of blood glucose, lactate and plasma NH_3 and decreased ($P<0.05$) blood pH, but no differences were observed between the two trials (Fig. 7.2). Although exercise increased ($P<0.01$) concentrations of both adrenaline and noradrenaline, no differences were observed between the trials at any point (Fig. 7.4).

No differences in ADP, AMP or hypoxanthine were observed at rest or following exercise in the two trials (Table 7.2). ATP was reduced ($P<0.01$), inosine increased ($P<0.05$) and IMP increased ($P<0.01$) following exercise in both trials. Post-ex concentrations of ATP were lower ($P<0.01$) and IMP concentration was higher ($P<0.01$) in the HT compared with the CT (Table 7.2). Post-exercise inosine levels were not different when comparing the two trials. Muscle NH_3 (Table 7.2) and muscle lactate concentrations (Fig 7.4) were higher ($P<0.01$) following exercise in both trials when compared with pre-ex values. Furthermore, post-exercise concentrations of both lactate and NH_3 were higher ($P<0.05$) in the HT when compared with the CT. Muscle CP was reduced ($P<0.01$) and muscle C increased ($P<0.01$) when post-ex values were compared with those pre-ex in both trials. Post-ex concentrations were not significantly different in the HT compared with the CT, with the P value = 0.06 and 0.09 for CP and C, respectively (Fig. 7.5). Muscle glycogen concentration was reduced ($P<0.05$) when comparing post-ex with pre-ex values in both trials. No difference in muscle glycogenolysis between the HT and the CT (Fig. 7.4).

	PRE-HEAT	PRE-EX
Muscle Metabolites		
ATP *	25.6 ± 1.3	25.1 ± 1.1
ADP *	2.17 ± 0.26	2.21 ± 0.20
AMP *	0.05 ± 0.02	0.08 ± 0.02
IMP *	0.05 ± 0.01	0.08 ± 0.02
Inosine *	<0.01	<0.01
Hypoxanthine *	<0.01	<0.01
Ammonia *	0.59 ± 0.12	0.62 ± 0.12
Lactate *	5.2 ± 0.7	6.9 ± 0.6
Glycogen #	419 ± 30	393 ± 30
CP *	83.7 ± 2.6	84.9 ± 3.1
C *	43.3 ± 3.5	41.4 ± 5.4
Whole Blood Metabolites		
Glucose ~	4.4 ± 0.1	4.6 ± 0.1
Lactate ~	1.3 ± 0.2	1.0 ± 0.1
pH	7.32 ± 0.01	7.35 ± 0.01
Plasma Metabolites & Hormones		
Ammonia @	13.8 ± 1.1	12.2 ± 1.5
Adrenaline +	31.2 ± 6.6	36.7 ± 4.7
Noradrenaline -	0.31 ± 0.04	0.26 ± 0.04

Table 7.1. *Concentrations of muscle, whole blood and plasma metabolites and hormones prior to (PRE-HEAT) and following (PRE-EX) heating of the quadriceps femoris muscle for 60 min.*

* indicates values expressed in mmol. kg^{-1} dry weight, # indicates value expressed in mmol. kg glucosyl $units^{-1}$ dry weight, ~ indicates values expressed in mmol. l^{-1}, @ indicates value expressed in umol. l^{-1}, + indicates value expressed in pg. ml^{-1} and - indicates value expressed in ng. ml^{-1}. Values are expressed as means ± SE (n=8).

	HT		CT	
	PRE-EX	POST-EX	PRE-EX	POST-EX
ATP	25.1 ± 1.1	17.2 ± 0.9 **	24.8 ± 0.8	19.4 ± 0.9 ** *a*
ADP	2.21 ± 0.20	2.15 ± 0.17	2.15 ± 0.18	2.05 ± 0.18
AMP	0.08 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.02
IMP	0.08 ± 0.02	3.52 ± 0.67 **	0.05 ± 0.01	2.43 ± 0.63 ** *a*
Ammonia	0.62 ± 0.12	4.54 ± 0.47 **	0.60 ± 0.11	3.26 ± 0.79 ** *b*
Inosine	<0.01	0.08 ± 0.02 *	0.01 ± 0.01	0.05 ± 0.01 *
Hyp'thine	<0.01	<0.01	<0.01	<0.01

Table 7.2. *Intramuscular concentrations of adenosine 5'triphosphate (ATP), adenosine 5'diphosphate (ADP), adenosine 5'monophosphate (AMP), inosine 5'monophosphate (IMP), ammonia, inosine and hypoxanthine (hyp'thine) before (PRE-EX) and after (POST-EX) exercise with (HT) and without (CT) 60 min of pre-exercise heating of the quadriceps femoris muscle.* Values expressed in mmol. kg^{-1} dry weight (n=8).

** indicates difference ($P<0.01$) PRE-EX compared with POST-EX, * indicates difference ($P<0.05$) PRE-EX compared with POST-EX, *a* indicates difference ($P<0.01$) in POST-EX HT when compared with POST-EX CT and *b* indicates difference ($P<0.05$) in POST-EX HT when compared with POST-EX CT.

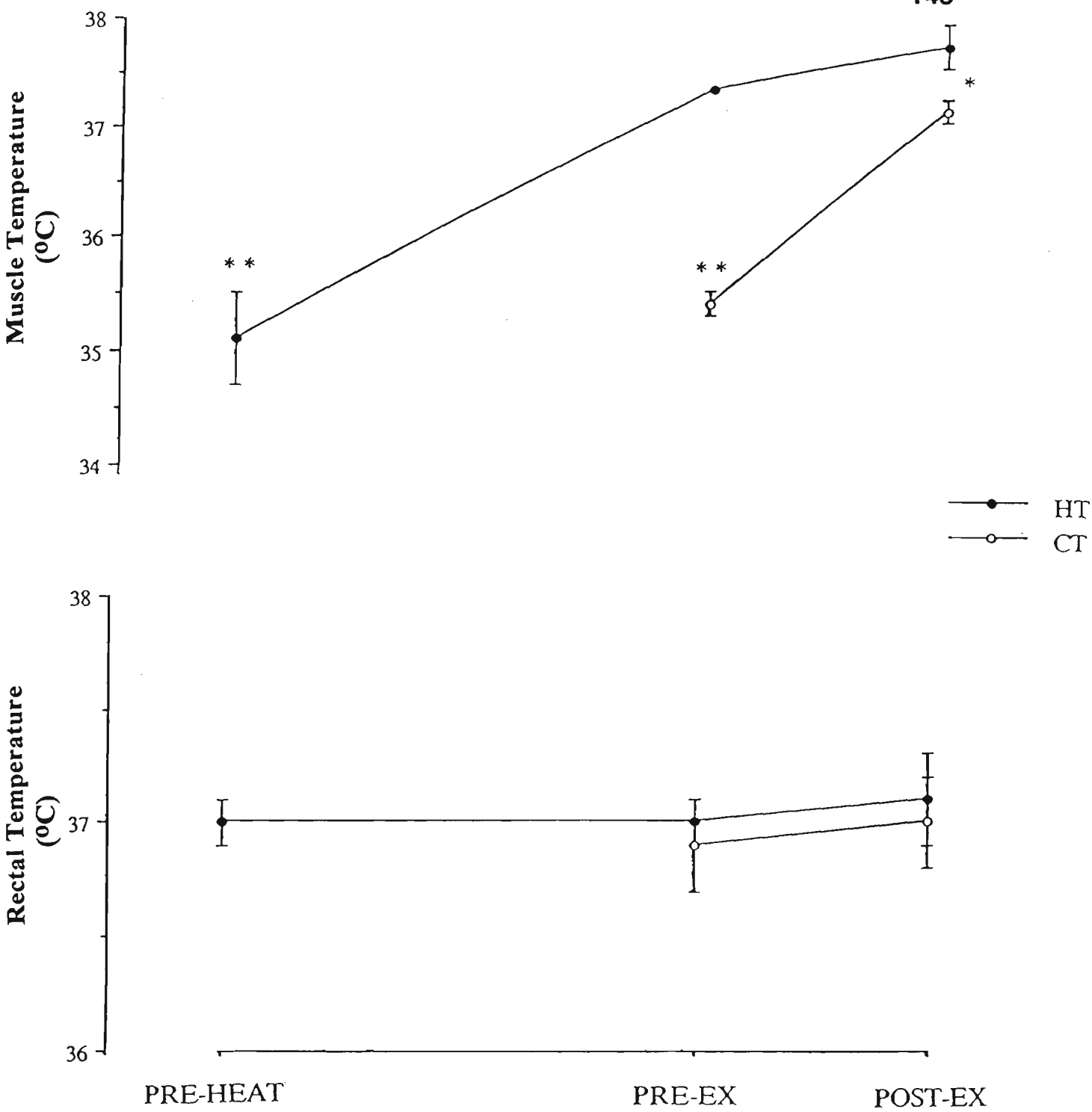


Fig. 7.1. Muscle and rectal temperature before heating (PRE-HEAT), before (PRE-EX) and following (POST-EX) exercise with (HT) and without (CT) 60 min of pre-exercise heating of the quadriceps femoris muscle. Values are means ± SE (n=8). ** indicates difference ($P<0.01$) from PRE-EX HT, * indicates difference ($P<0.05$) POST-EX HT compared with POST-EX CT.

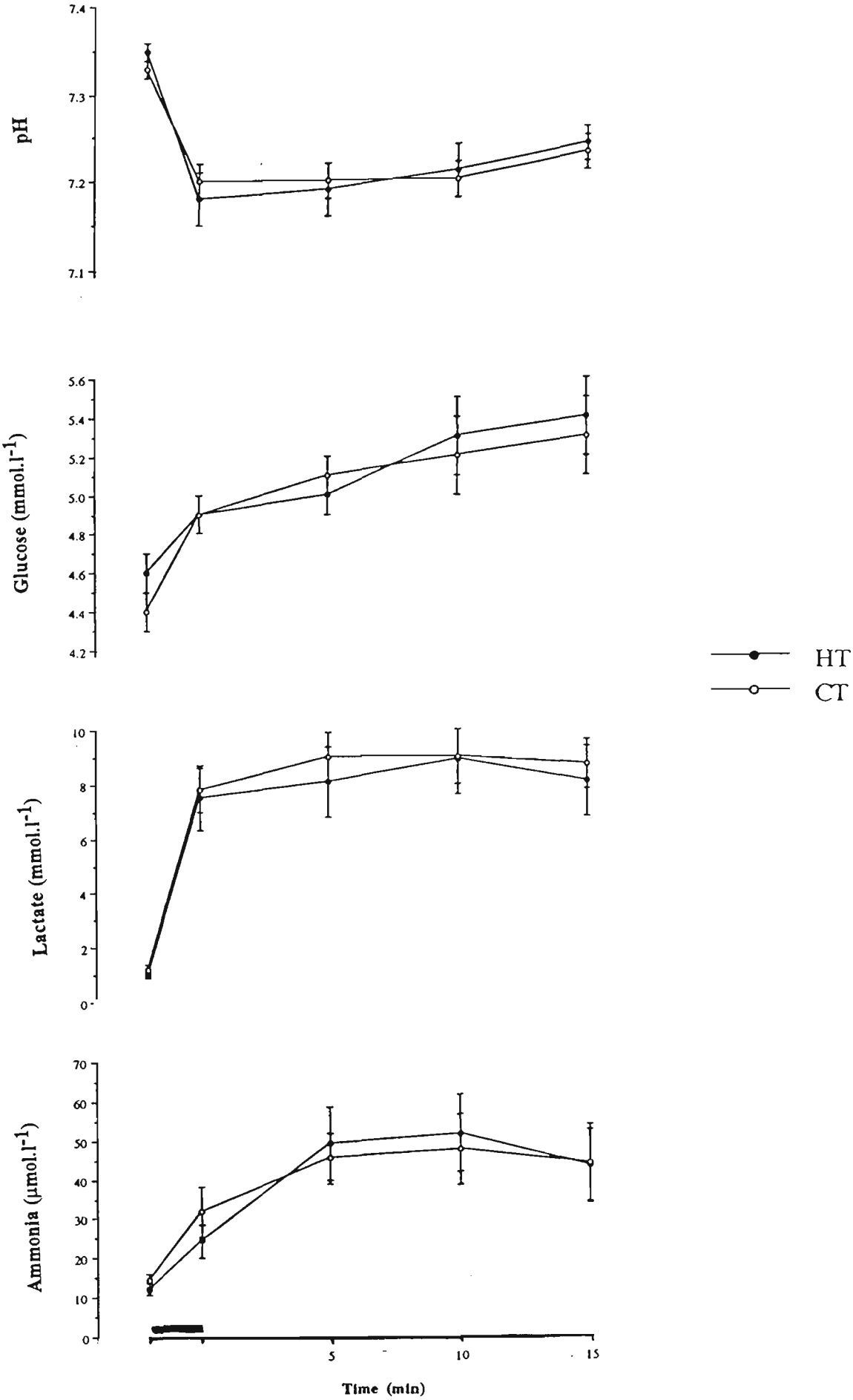


Fig. 7.2. Concentrations of blood lactate, glucose, pH and plasma ammonia at rest, immedietely following during recovery from exercise with (HT) and without (CT) 60 min of pre-exercise heating of the quadriceps femoris muscle. Values are means ± SE (n=8). ▬ indicates exercise.

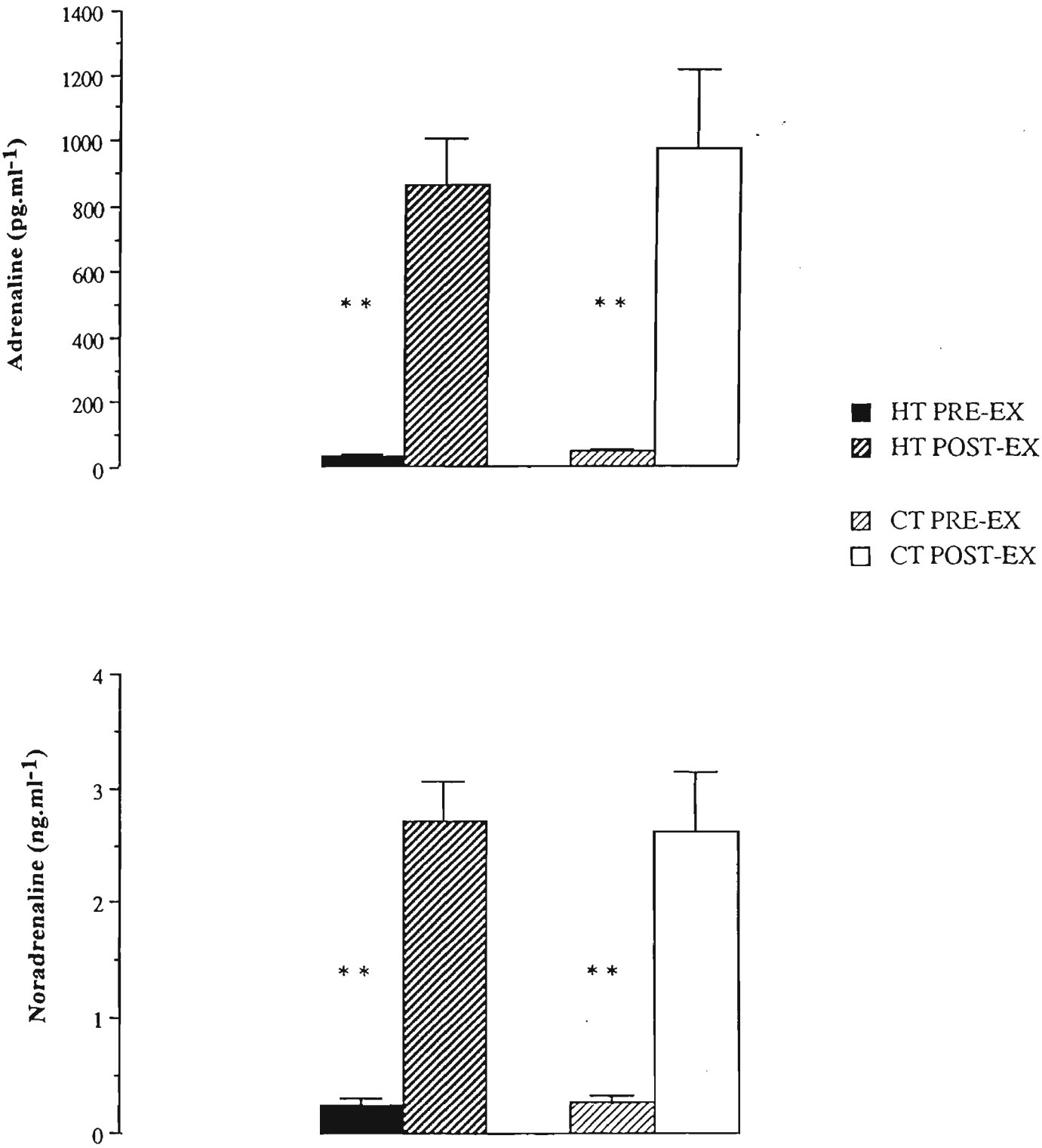


Fig. 7.3. Plasma adrenaline and noradrenaline concentrations before (PRE-EX) and following (POST-EX) exercise with (HT) and without (CT) 60 min of pre-exercise heating of the quadriceps femoris muscle. Values are means ± SE (n=8). ** indicates difference ($P<0.01$) POST-EX compared with PRE-EX.

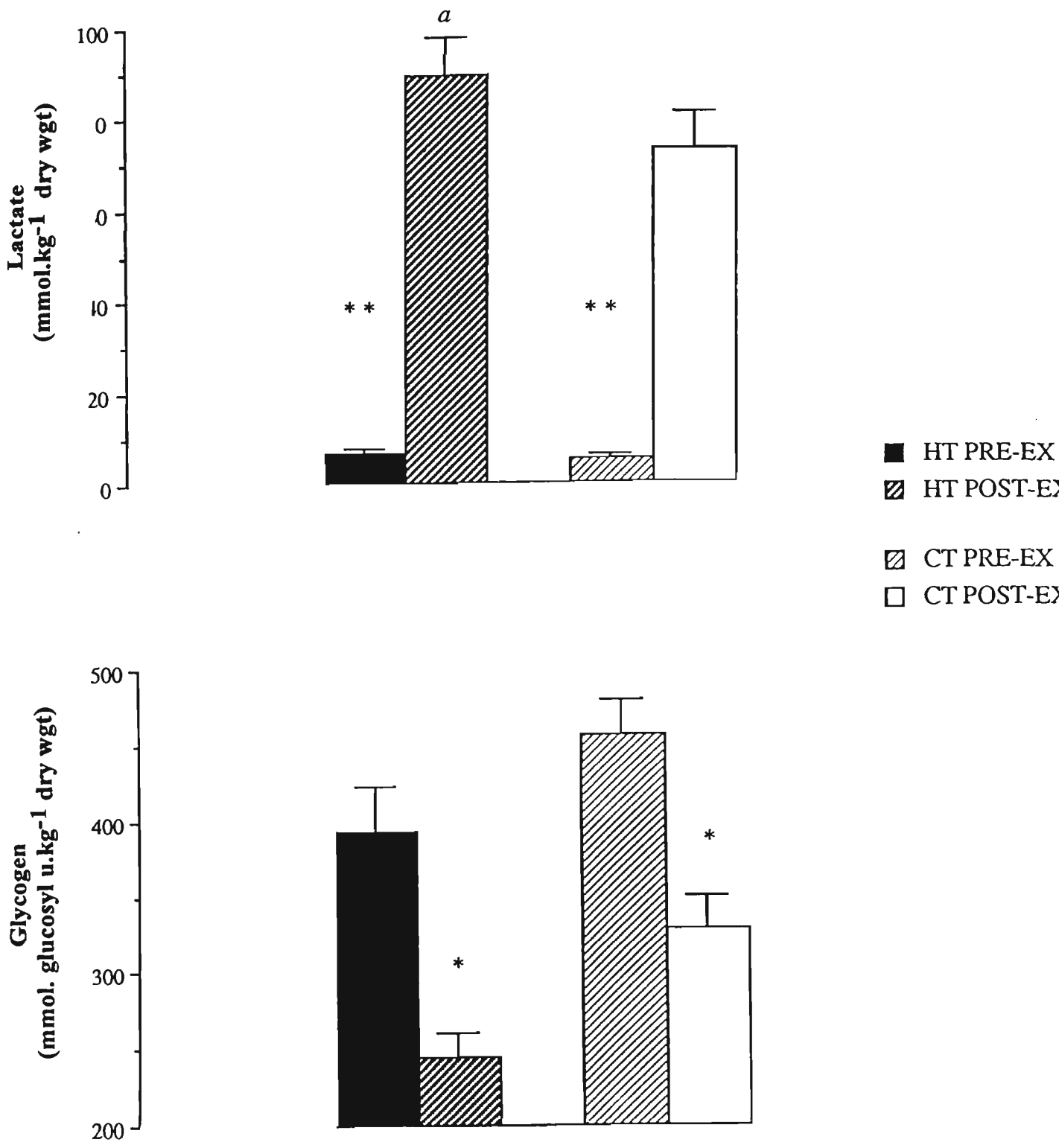


Fig. 7.4. Muscle lactate and glycogen concentrations before (PRE-EX) and following (POST-EX) exercise with (HT) and without (CT) 60 min of pre-exercise heating of the quadriceps femoris muscle. Values are means ± SE (n=8). ** indicates difference ($P<0.01$) POST-EX compared with PRE-EX, * indicates difference ($P<0.05$) POST-EX compared with PRE-EX, ^{a} indicates difference ($P<0.05$) POST-EX HT compared with POST-EX CT.

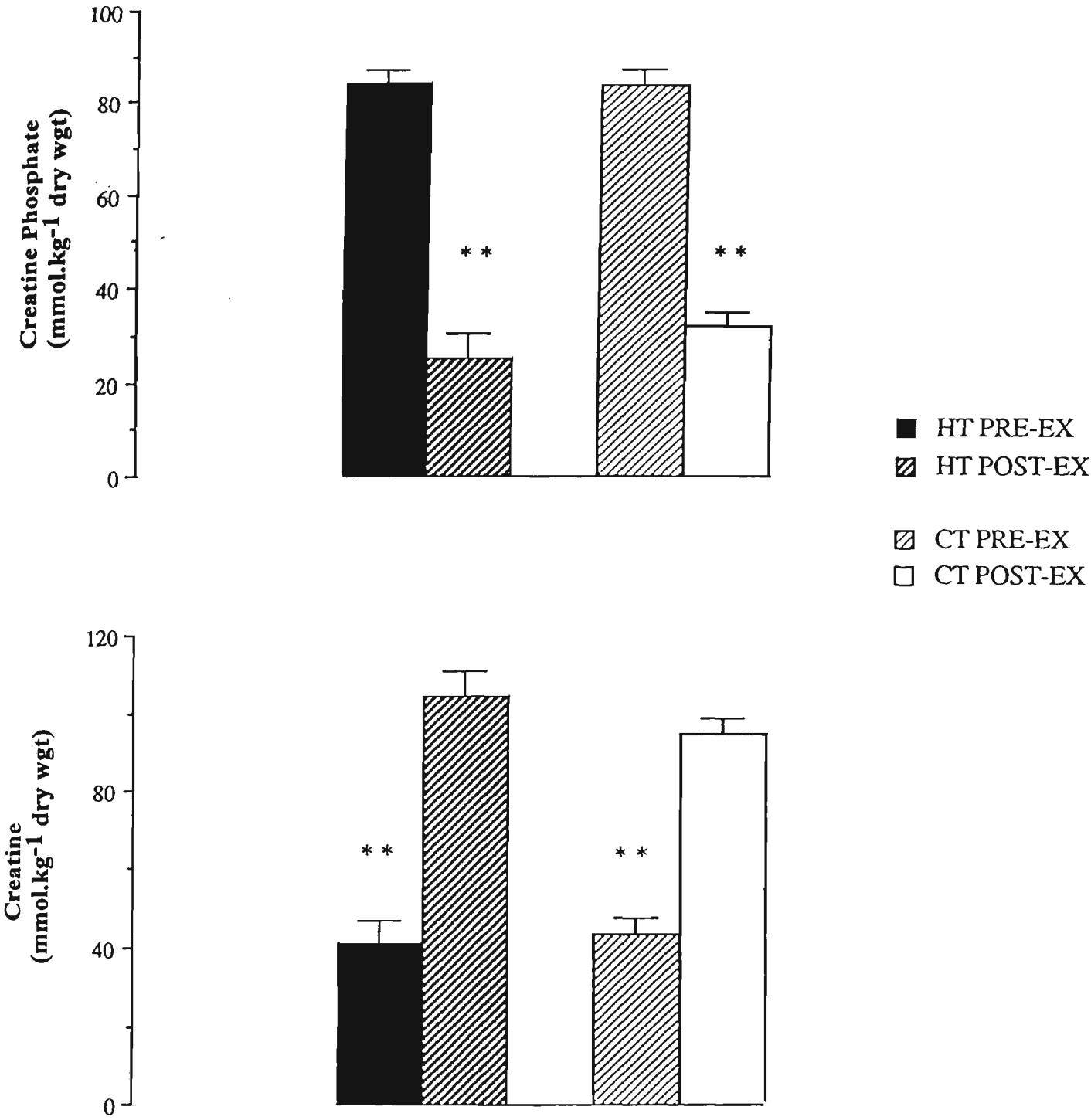


Fig. 7.5. Muscle creatine phosphate (CP) and Creatine (C) concentrations before (PRE-EX) and following (POST-EX) exercise with (HT) and without (CT) 60 min of pre-exercise heating of the quadriceps femoris muscle. Values are means ± SE (n=8). ** indicates difference ($P<0.01$) POST-EX compared with PRE-EX.

7.4 DISCUSSION

The results from this study demonstrate that an increased muscle temperature, whilst having little effect on metabolism at rest, causes a change in muscle metabolism during exercise. The observed changes in lactate (Fig. 7.4) and NH_3 accumulation, IMP formation, adenine nucleotide degradation (Table 7.2) and the tendency ($P=0.06$) for greater CP degradation (Fig. 7.5) suggest that an increased muscle temperature increases glycolysis and ATP degradation during exercise. It is likely that an the higher muscle temperature, reported in previous chapters of this dissertation contributes, in part, to the alterations in muscle metabolism observed during prolonged exercise in the heat compared with similar exercise in a cooler environment.

The plasma catecholamine levels were increased as a result of exercise (Fig. 7.3) supporting previous findings (Bloom et al., 1976; Christensen & Brandsborg, 1973). Post-exercise concentrations, however, were not different between the trials. Likewise, the post-exercise rectal temperatures were not different between the trials (Fig 7.1). These data indicate that although plasma catecholamines rise as a result of exercise, an increase in muscle temperature does not augment the exercise-induced increase in these hormones. The increase in circulated adrenaline during exercise in the heat compared with that in a cooler environment, reported in previous chapters, is likely therefore, to be influenced by core, rather than peripheral, temperature. The influence of the peripheral thermoreceptors on the sympatho-adrenal response during whole body heating cannot, however, be totally discounted since whole body heating is likely to stimulate all the cutaneous receptors. In contrast, only a small area of the periphery was heated in this experiment.

The greater adenine nucleotide degradation (Table 7.2), higher CP

degradation (Fig. 7.5) and higher concentrations of both IMP and NH_3 observed post-exercise in the HT (Table 7.2) suggest that the imbalance between energy supply and utilisation during short term, maximal exercise (Boobis, 1987) is augmented by an increase in muscle temperature. As discussed in Chapter 2, during short term maximal exercise the rate of ATP hydrolysis exceeds the rate of ADP phosphorylation (Tullson & Terjung, 1991) and leads to the formation of AMP via the myokinase reaction. The activation of AMP deaminase leads to the formation of IMP and NH_3.

The increased ATP depletion during contractions at higher temperatures is in agreement with previous findings (Edwards et al., 1972, Kozlowski et al., 1985). The precise mechanism/s responsible for this phenomenon is unclear, although may be related to mitochondrial ATPase activity (Brooks et al., 1971) and/or an increased energy cost associated with cross bridge cycling (Edwards et al., 1972). Brooks et al. (1971) have demonstrated that an increase in muscle temperature results in a decreased phosphorylative efficiency in the mitochondria. If the changes observed in mitochondrial function *in vitro* occur *in vivo*, the rate of ATP rephosphorylation in the mitochondria during muscle contraction at higher muscle temperatures may diminish. Although the exercise protocol in this study was supramaximal and of short duration, this type of exercise results in approximately 75% of the energy demand being met by aerobic metabolism (Gastin, 1992). If the rate of ATP supply from the mitochondria is diminished via an increase in temperature, it is likely that the reduced ATP rephosphorylation will result in transient increases in ADP and AMP. Hence activation of the myokinase reaction could account for the observed decrease in ATP concentration and increase in IMP and NH_3 accumulation observed in this study. Furthermore, an increased muscle temperature may increase the activation of AMP deaminase, 5'- nucleotidase and nucleoside phosphorylase, through a temperature (Q_{10}) effect on enzyme

activity. Hence, adenine nucleotide depletion would increase (Tullson & Terjung, 1990; Tullson & Terjung, 1991). An increase in muscle temperature may also increase glycolysis and consequently muscle lactate accumulation with increased PFK and lactate dehydrogenase (LDH) activity and decreased phosphorylative efficiency. An increased rate of glycolysis will increase anaerobic ATP supply, but this increase will not compensate for a decrease in mitochondrial ATP rephosphorylation. A net reduction of ATP rephosphorylation is likely, since the ATP yield from oxidative phosphorylation is higher compared with that derived from anaerobic glycolysis. The postulation of an increased muscle temperature resulting in a tendency for actin-myosin head slippage leading to an increased energy cost in the maintenance of tension at higher temperatures is speculative. These data cannot shed any light on this hypothesis since it was impossible to examine the excitation/contraction coupling processes. The present results reflect a greater anaerobic glycolysis and ATP turnover in the warmed muscle. It is possible that these phenomena are related to a temperature effect on enzyme activity and/or an decrease in mitochondrial phosphorylative efficiency.

Of note, is the lack of 1:1 stoichiometry between ATP degradation and IMP formation or ATP degradation and NH_3 accumulation (Table 7.2). Previous research indicates that during dynamic exercise the formation of IMP and NH_3 is usually matched by the fall in ATP concentration (Eriksson et al., 1985; Katz et al., 1986a). In contrast, recent studies in both rats (Tullson et al., 1990; Tullson and Terjung, 1992) and humans (Bangsbo et al., 1992, Green et al., 1993) have found ATP degradation to exceed IMP formation and NH_3 accumulation. It has been suggested that this lack of stoichiometry may be accounted for, in part, by purine nucleotide cycling resulting in some accumulation of adenylosuccinate (Bangsbo et al., 1992). Adenylosuccinate formation is unlikely, however, to totally account for the lack of

stoichiometry observed in this study, since the exercise resulted in marked IMP accumulation in both trials (Table 7.2). Adenylosuccinate formation in electrically stimulated rat muscle has been found to be minor (Goodman & Lowenstein, 1977), since adenylosuccinate synthetase is inhibited by IMP accumulation. Since IMP can be further degraded to inosine and hypoxanthine (Tullson & Terjung, 1991), which can diffuse into the blood, it is possible that the accumulation of these metabolites in the blood may account for the lack of stoichiometry. Although plasma inosine or hypoxanthine was not measured in this study, the inability to detect muscle hypoxanthine and the low concentrations of muscle inosine post-exercise (Table 7.2) indicate that purine loss is unlikely to account for the lack of stoichiometry.

Recent evidence suggests the existence of an acid insoluble purine derivative which is an oligomer of ATP and phosphoglycerate (PGA) (Tullson & Terjung, 1992). Since the muscle samples in this study were extracted in acid, this ATP-PGA oligomer, were it to be present in the samples, would have been remained in the acid insoluble portion. Although the presence of this purine substance in the samples is speculative, since, it has been found only in slow-twitch rat muscle, it may explain the lack of stoichiometry observed in this study.

The similar values observed post-exercise and during recovery in the blood and plasma metabolites were expected since only a small area of one limb was heated and the experimental model involved two legged cycling. The blood glucose data (Fig. 7.2) suggest a similar rate of hepatic glycogenolysis and glucose uptake by the tissues. Since catecholamine secretion during exercise has been found to be a major stimulus for hepatic glucose production (Galbo et al., 1978), the catecholamine data (Fig. 7.3) suggest that hepatic glycogenolysis was unaffected by passive warming. Blood lactate and plasma

NH_3 levels (Fig. 7.3) did not reflect concentrations of these metabolites within the heated muscle. Since only one quadriceps muscle group was heated and exercise involved two legged cycling, it is likely, however, that once these metabolites diffused from the muscle into the circulation, they were somewhat diluted, since production and subsequent diffusion would have occurred in other contracting muscles which were not heated. Although blood pH was not different post-exercise or during recovery between the two trials (Fig. 7.2), it is possible that the pH in the pre-heated muscle following exercise was lower since post-exercise muscle lactate concentration in the HT (Fig. 7.5) was higher when compared with the CT. As with blood lactate and plasma NH_3, blood pH levels post-exercise and in recovery may have been influenced by the pH of non-heated contracting muscle.

The higher post-exercise lactate concentration in the HT when compared with the CT indicates an increased glycolytic rate during exercise at higher muscle temperatures. This observation supports the findings of Edwards et al. (1972) who found an increase in lactate, pyruvate and hexose monophosphate levels during isometric contractions at higher muscle temperatures. Although the post-exercise muscle lactate levels were higher in the HT compared with the CT, muscle glycogenolysis was not different between the two trials (Fig. 7.5). It is likely that these results were influenced by resting glycogen. Of the eight subjects tested in this study, six had accelerated glycogen depletion in the HT compared with the CT. The other two, however, commenced exercise in the CT with a much higher glycogen level compared with the HT and subsequently utilised more glycogen in this trial. A mass action effect on glycogenolytic rate, is likely. This hypothesis is supported by the observations of Robergs et al. (1991) who found a decreased muscle lactate accumulation following a maximal cycling bout when preceded by an active warm-up. These authors attributed this difference to a transient increase in the aerobic contribution to muscle energy

metabolism, secondary to an improved muscle blood flow associated with the warm-up. Interestingly, however, no difference was observed in muscle glycogenolysis, but subjects commenced the warm up trial with slightly lower pre-trial glycogen concentrations. It is possible that this may have influenced glycogenolysis through a mass action effect (Richter et al., 1986) in a similar manner to the data reported in this study.

In summary, an increase in muscle temperature *per se* increases muscle glycolysis, reflected in muscle lactate accumulation and adenine nucleotide degradation during exercise. Circulating catecholamine levels were not different between the two trials and cannot account for the increase in metabolism observed when exercise is preceded with passive warming. Although the mechanisms for the changes observed in muscle metabolism are speculative, it is possible that a reduced mitochondrial phosphorylative efficiency and a Q_{10} effect on enzyme activity are responsible for the observed metabolic differences. The increased muscle temperature observed during exercise in the heat, reported in previous chapters, may be a factor contributing to the observed metabolic alterations.

CHAPTER 8

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

8.1 SUMMARY

It has long been established that an individual's endurance capacity is affected by both the environmental temperature and the degree to which the individual has adapted to the particular environment. It is also established that exercise tolerance under different environmental conditions and/or acclimation states is influenced, in part, by different levels of thermoregulatory stress and subsequent alterations in the cardiovascular response. What is less well understood, however, is the extent to which thermoregulatory stress affects muscle metabolism which, in itself, could directly influence endurance capacity. The present series of studies that comprise this dissertation was undertaken to examine the effect of different environmental temperatures and changes in acclimation status on muscle metabolism during exercise.

8.1.1 Muscle Metabolism During Exercise in the Heat: Effect of Acclimation

Previous research has produced conflicting results with respect to the metabolic response to prolonged submaximal exercise in the heat, with particular reference to glycolysis and glycogenolytic rate. Some authors (Fink et al., 1975; King et al., 1985; Kirwan et al., 1987; Kozlowski et al., 1985;

Young et al., 1985) observed glycolysis and/or glycogenolysis to increase by the combination of heat stress and exercise, whilst others (Nielsen et al., 1990; Yaspelkis et al., 1993) have found no change. The first two studies described in this dissertation examined the metabolic process during acute, and after chronic exposure to, exercise in the heat.

The observed increase in muscle glycogenolysis (Table 4.2; Fig. 4.7), muscle lactate accumulation (Fig. 4.5), RER (Table 4.1) and CHO oxidation (Table 4.1) indicate that exercise in the heat results in a substrate shift towards greater carbohydrate utilisation. These results are supported by the data obtained in study three. Indeed, when the $20^{o}C$ vs $40^{o}C$ data from Chapter 4 is pooled with the same data from Chapter 6, increasing the sample size to twelve (Table 8.1; Table 8.2; Table 8.3), the differences in muscle glycogenolysis and muscle lactate accumulation are highly significant ($P<0.01$). It is acknowledged that the target workloads were slightly different between the two studies. Nevertheless, in reality the workloads achieved were very similar and indeed not significantly different. It appears that differences in protocol can explain the discrepancies in the literature with respect to muscle carbohydrate utilisation. On one hand, Nielsen et al. (1990) employed a non-counterbalanced protocol whereby the subjects commenced exercise in the heat after completing 30 minutes of exercise in a thermoneutral environment. Consequently, at the onset of exercise, glycogen content was 56 mmol. kg^{-1} (dry weight) lower in the heat. This may have affected utilisation rates. On the other hand, Yaspelkis et al. (1993) used a protocol whereby the temperature difference between the two trials was, a relatively small $10^{o}C$, albeit in conjunction with a relative humidity of 49%, which is considerably higher than that which has been employed in the studies reported in this dissertation and in others. Nevertheless, the difference in rectal temperatures at the end of exercise between the hot and cooler trial was $0.4^{o}C$ compared with an average

		TRIAL	
		20°C	40°C
O_2 Uptake ($l.min^{-1}$)		2.94±0.5	2.94±0.6
Heart Rate ($b.min^{-1}$)		150±2	168±2**
RER		0.88±0.01	0.91±0.01*
Rectal Temp. (°C)	PRE-EX	37.0±0.1	37.2±0.1
	POST-EX	38.6±0.1	39.6±0.1**
Muscle Temp. (°C)	PRE-EX	35.6±0.2	36.0±0.4
	POST-EX	39.0±0.1	40.7±0.1**

Table 8.1. *Mean O_2 uptake, mean heart rate, mean respiratory exchange ratio (RER) and rectal and muscle temperature before (PRE-EX) and after (POST-EX) exercise in 20°C RH 20% and 40°C RH 20%.*
Values are means ±SE, (n=12). * indicates difference ($P<0.05$) 40°C compared with 20°C, ** indicates difference ($P<0.05$) 40°C compared with 20°C. Data from the six subjects who were biopsied in Chapter 4 has been combined with the data from the seven subjects in Chapter 6 (one subject was common and the data from the two studies for this subject has been averaged).

		TRIAL	
		20°C	40°C
Glycogen	PRE-EX	567±46	545±34
	POST-EX	401±45	326±33**
		166±20	218±18**
Lactate	PRE-EX	5.5±0.6	7.2±0.9
	POST-EX	12.0±2.0	20.7±2.2**
Ammonia	PRE-EX	0.32±0.06	0.34±0.05
	POST-EX	0.98±0.09	1.25±0.13*
ATP	PRE-EX	25.9±1.0	25.8±1.1
	POST-EX	25.4±0.9	24.1±1.0
IMP	PRE-EX	0.06±0.01	0.14±0.03
	POST-EX	0.14±0.05	0.29±0.06
CP	PRE-EX	84.6±3.8	84.4±3.7
	POST-EX	67.2±3.6	50.9±4.0**

Table 8.2. *Intramuscular concentrations of glycogen, lactate, ammonia, adenosine 5'diphosphate (ATP), inosine 5'monophosphate (IMP) and creatine phosphate (CP) before (PRE-EX) and after (POST-EX) exercise in 20°C RH 20% and 40°C RH 20%.*

Values are means ±SE, expressed in mmol. kg^{-1} dry wt, (n=12). * indicates difference ($P<0.05$) 40°C compared with 20°C, ** indicates difference ($P<0.05$) 40°C compared with 20°C. Data from the six subjects who were biopsied in Chapter 4 has been combined with the data from the seven subjects in Chapter 6 (one subject was common and the data from the two studies for this subject has been averaged).

		TRIAL	
		20°C	40°C
Plasma Hormones & Metabolites			
Adrenaline (pg. ml^{-1})	Rest	74.8±16.0	125.2±27.6*
	Min 10	238.6±42.5	418.8±70.1**
	Min 40	322.6±43.8	489.0±71.6**
Ammonia (umol. l^{-1})	Rest	20.1±1.6	23.3±2.9
	Min 10	38.1±3.4	37.7±2.4
	Min 20	50.0±4.3	49.5±3.8
	Min 30	56.0±5.7	56.4±5.3
	Min 40	56.9±6.1	59.8±6.1
Whole Blood Metabolites			
Glucose (mmol. l^{-1})	Rest	4.2±0.1	4.5±0.1
	Min 10	4.2±0.1	4.6±0.1**
	Min 20	4.4±0.2	5.0±0.1**
	Min 30	4.6±0.1	5.3±0.2**
	Min 40	4.6±0.1	5.5±0.2**
Lactate (mmol. l^{-1})	Rest	1.2±0.1	1.1±0.1
	Min 10	2.2±0.4	3.2±0.4**
	Min 20	2.2±0.4	3.5±0.6**
	Min 30	1.9±0.3	3.6±0.6**
	Min 40	1.8±0.3	3.7±0.5**

Table 8.3. *Concentrations of plasma adrenaline, plasma ammonia, blood glucose and blood lactate before (PRE-EX) and after (POST-EX) exercise in 20°C RH 20% and 40°C RH 20%.*

Values are means ±SE, expressed in mmol. kg^{-1} dry wt, (n=12). * indicates difference ($P<0.05$) 40°C compared with 20°C, ** indicates difference ($P<0.05$) 40°C compared with 20°C. Data from the six subjects who were biopsied in Chapter 4 has been combined with the data from the seven subjects in Chapter 6 (one subject was common and the data from the two studies for this subject has been averaged).

difference of 1°C in the studies reported here (Table 8.1). In addition, their subjects were likely to be heat acclimated as acknowledged by the authors since the heat trial temperature (approximately 34°C) was actually lower than the mean ambient temperature for the testing period. Since these potentially confounding methodological issues were avoided in the series of experiments described in this dissertation, the results indicate that muscle carbohydrate utilisation is augmented during submaximal exercise in a hot environment.

The effect of temperature on the rate of carbohydrate utilisation during exercise in the heat appears to be attenuated after chronic exercise in a hot environment (i.e. heat acclimation). This hypothesis is supported by the lower glycogenolysis in type I fibres (Fig. 5.7), RER (Table 5.1) and muscle lactate accumulation (Fig. 5.5) observed in studies reported in this dissertation. It is not supported, however, by the observation that muscle glycogenolysis was not significantly lower (Table 5.2) following acclimation. As discussed in Chapter 5, the conflict may be due to the problem of different resting glycogen levels affecting glycogenolytic rate due to a mass action effect. Both King et al. (1985) and Kirwan et al. (1987) observed a large attenuation in muscle glycogenolysis during exercise in the heat following acclimation. No previous studies have, to this time, observed a similar rate of muscle glycogen utilisation during exercise in the heat before and after acclimation.

Although carbohydrate utilisation appears to be altered during exercise in the heat before and after acclimation, high energy phosphate and adenine nucleotide data suggest that the rate of ATP degradation did not exceed its rate of rephosphorylation during 40 min of exercise in a hot environment. Neither ATP nor IMP levels were significantly affected by exercise in a thermoneutral environment, or in the heat prior to, or following, acclimation

(Table 4.3; Table 5.3). This result is not unexpected since post exercise glycogen levels were approximately 300 mmol glucosyl u. kg^{-1} (Table 4.2; Table 5.2), a concentration well in excess of that found in glycogen depleted muscle. In contrast, post-exercise muscle NH_3 concentration was significantly elevated when compared with rest in the three trials (Fig. 4.5; Fig 5.5). During studies reported in Chapters 4 and 6 muscle NH_3 accumulation did not differ when comparing exercise in a thermoneutral environment with that in the heat. A higher ($P<0.05$) post-exercise muscle NH_3 concentration was observed, however, at $40^{o}C$ compared with $20^{o}C$ when the data from the respective studies were pooled (Table 8.2). This result indicates that muscle NH_3 concentration is elevated during exercise in the heat, since a difference was observed when the sample size was increased. Interestingly, plasma NH_3 concentration (Table 8.3) did not reflect muscle NH_3 accumulation. These data do not support the work of Snow et al. (1993a) who found a higher post-exercise plasma NH_3 concentration in $40^{o}C$ compared with $20^{o}C$. Of note, however, the subjects in the study by Snow et al. (1993a) were untrained whilst the subjects used in these studies were well trained. As discussed in Chapter 4, it is likely that training status could best explain the difference in plasma NH_3 concentration between the two studies.

The increase in muscle NH_3 was found despite no difference in muscle IMP accumulation (Table 8.2). The pooled data, along with those from Chapters 4 and 6, which show an exercise-induced rise in NH_3 but no such increase in IMP in each trial, indicate that NH_3 is coming from sources other than AMP deamination. The mechanism for NH_3 accumulation will be discussed subsequently.

Creatine phosphate degradation was higher in the heat compared with a thermoneutral environment in studies reported in Chapter 4 (Fig 4.6), Chapter 6

(Fig 6.5) and when the data from these studies were pooled (Table 8.2). These data are in agreement with those of Kozlowski et al. (1985), who observed a greater CP degradation in exercise-induced hyperthermic dogs. Interestingly, CP degradation (Fig. 5.6), unlike lactate and glycogenolysis in type I fibres (and possibly overall glycogen utilisation) was not attenuated by the process of acclimation. CP levels have never been previously examined during exercise in the heat before and following acclimation, and further investigation is warranted. It appears that the thermoregulatory stress, whilst attenuated by the process of acclimation, may not be sufficiently reduced to result in changes to CP degradation.

In summary, the series of studies reported in this dissertation indicate that muscle metabolism, in trained endurance athletes, is indeed altered through the combination of exercise and heat stress. Furthermore, many of these changes are reversed after chronic exposure to exercise in the heat.

8.1.2 Muscle Metabolism During Exercise in a Cool Environment

A cool environment results in a detrimental or favourable effect on exercise performance depending on the severity of the environment and/or the exercise. Data reported in Chapter 6, indicate that an environment-induced attenuation in the increase in core temperature stress during submaximal exercise, may result in a reduced reliance upon carbohydrate in the contracting muscle. Muscle glycogen utilisation (Fig 6.3), muscle lactate accumulation (Fig 6.4) and muscle CP degradation (Fig 6.5) were, however, not significantly lower during exercise in $3^{o}C$ compared with exercise in $20^{o}C$. As discussed in Chapter 6, these data may indicate that the temperature difference between the two trials was not large enough to produce a sufficient difference in the thermal stress reflected in heart rate, rectal and muscle temperature

measurement. Alternatively, since there was a decrease in glycogenolysis, CP degradation and a lower post-exercise lactate concentration when comparing exercise in a cool environment with that in a so called 'thermoneutral environment', it is possible that a larger sample size may result in statistical differences. Further investigation in this area is warranted. The similar post-exercise concentrations of ATP, ADP, AMP and IMP (Table 6.4) when comparing exercise in a thermoneutral environment with that in a cool environment support findings presented in Chapters 4 and 5 which indicate that adenine nucleotide metabolism during 40 min of submaximal exercise in trained individuals is not temperature dependent.

In summary, results from the three studies reported in this dissertation indicate that, in the trained individual, muscle carbohydrate utilisation is increased as thermoregulatory stress, reflected in rectal temperature and heart rate data, is increased. This is supported by the significant correlation ($P<0.01$) between the exercise-induced change in rectal temperature and the rate of muscle glycogenolysis reported in Chapter 6.

8.1.3 Mechanisms for Alterations to Muscle Metabolism During Exercise at Different Environmental Temperatures

There is little research which has attempted to identify mechanisms for metabolic changes observed with exercise at different ambient temperatures, although a number of mechanisms have been postulated to account for such changes. These have included; a change in O_2 delivery secondary to an alteration in muscle blood flow (Fink et al., 1975; Rowell, 1974), a change in circulating catecholamines (King et al., 1985; Kirwan et al., 1987; Yaspelkis et al., 1993), a Q_{10} effect on metabolism (Young et al., 1985; Kozlowski et al., 1985) and an alteration in the neuromuscular recruitment pattern of

contracting muscle (Young et al., 1985). The studies reported in this dissertation have examined three of the four proposed mechanisms. The data indicate that both circulating catecholamines and a Q_{10} effect on metabolism are mechanisms for observed changes in muscle metabolism. Since no change in specific fibre type recruitment patterns were found, such a mechanism cannot explain the metabolic differences. Changes in muscle blood flow could be inferred from some of the data reported in this dissertation, e.g. CP degradation and $\dot{V}O_2$ measurement. Since blood flow was not measured in any study, the inferred changes are speculative.

The schema proposed for the effect of environmental temperature on muscle metabolism is summarised in Fig. 8.1. This hypothesis has been derived from previous observations and from the work conducted in the present study. When humans exercise in a thermoneutral environment, both their rectal and muscle temperature increase (Table 4.1; Fig. 6.1) as a result of the transfer of chemical energy into mechanical work and also resulting in the production of heat. Ambient temperature changes of sufficient magnitude during exercise, result in changes in rectal and muscle temperature in the same direction (Table 4.1; Fig. 6.1). The change in core and muscle temperature has two major effects which appear to influence muscle metabolism. As reported in Chapter 7, the grater the muscle temperature during exercise, the greater the rate of muscle glycolysis, and perhaps, muscle glycogenolysis. Since muscle temperature was the only variable manipulated and core temperature was not altered in either trial, it can be concluded that a Q_{10} effect must, at least in part, play a role in regulating muscle metabolism during exercise in different environmental conditions. When the exercise-induced increase in muscle temperature is attenuated by either reducing the ambient temperature (Fig. 6.1), or by acclimation (Fig.5.1), muscle glycolysis is reduced and glycogenolysis appears to follow a similar pattern. Muscle temperature, therefore, appears to be an

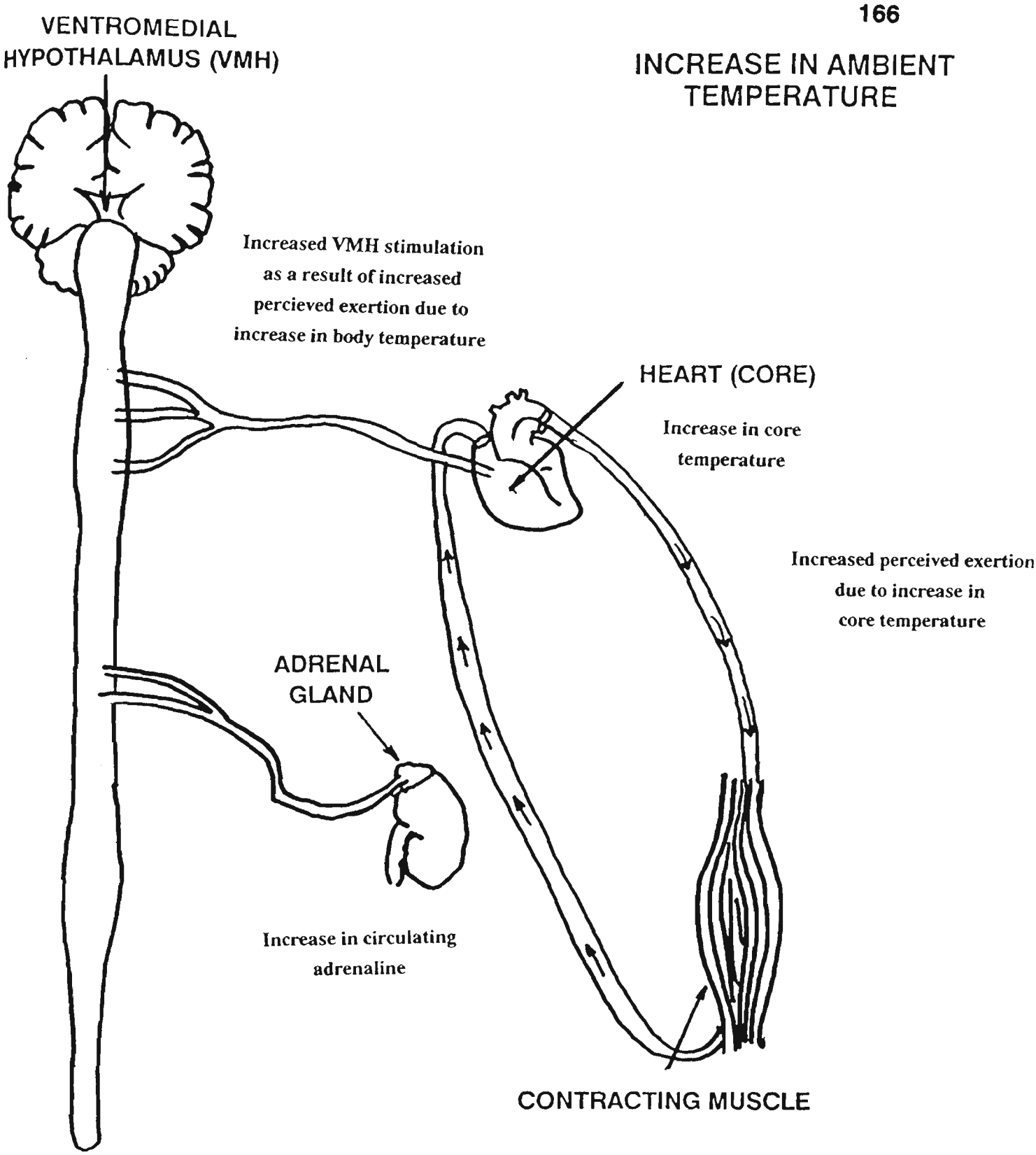


Fig. 8.1. The effect of an increase in ambient temperature on muscle

important mechanism for regulating muscle metabolism during prolonged submaximal exercise.

The adrenal medullary response also appears to affect muscle glycolysis and glycogenolysis during submaximal exercise in different environmental temperatures. It has been suggested (Galbo, 1983) that during prolonged exercise adrenaline is secreted in response to a fall in blood glucose. The resultant rise in hepatic glycogenolysis represents a classical negative feedback system related to blood glucose control and possibly carbohydrate demand by the muscles. Data from the studies reported in this dissertation, however, do not support the existence of such a control system. Rather than fall, blood glucose increased throughout exercise in all conditions. Adrenaline levels also rose throughout exercise. The mechanisms for sympatho-adrenal stimulation appear to come from a signal which is not metabolic in nature but rather is a function of the workload requirements of the activity and body temperature. The data reported in this dissertation tend to support the theory postulated by Shimazu (1992) and Kjaer (1992) that catecholamine secretion is controlled by a 'central command' and serves as a feed-forward control. In all studies adrenaline rose most rapidly in the first 10 min of exercise (Fig 4.3; Fig 5.3; Fig 6.3). Furthermore, adrenaline levels were significantly higher 10 min into exercise in the heat compared with that in a thermoneutral environment. It is more likely, that exercise in the heat, by increasing body temperature, increased perceived exertion and resulted in greater sympathetic activation via increased stimulus to the central neuroendocrine control centres in the ventro-medial hypothalamus (VMH). Hence, the workload and the body temperature have additive effects in terms of stimulation of these centres. In this schema, the sympathetic response to exercise in the heat would be similar to that observed by Kjaer et al. (1987) with the administration of curare during submaximal exercise. That is, as perceived exertion increases so to does the sympatho-adrenal response.

The combined effects of both muscle temperature and circulating adrenaline appear to influence muscle metabolism. As previously discussed, a significant correlation was found in the study reported in Chapter 6 when comparing the exercise-induced change in rectal temperature with that in muscle glycogenolysis. During exercise, rectal temperature would appear to influence both adrenaline secretion and muscle temperature to some extent. Interestingly, when correlations were performed between the exercise-induced change in glycogen utilisation and the exercise-induced change in either muscle temperature or adrenaline secretion, the results were not significant. Furthermore, when Q_{10} calculations were conducted on the data presented in Chapters 4 and 5, the difference in muscle temperatures between the trials were unable to account for the magnitude of change in the muscle metabolites. Hence, neither a Q_{10} effect nor a change in catecholamines appear to be totally responsible for the observed changes in muscle metabolism during submaximal exercise reported in this series of experiments. Rather, a synergism between adrenaline levels and muscle temperature is likely.

The observation that CP degradation is increased during exercise in the heat is most interesting. The mechanism/s for such an observation is/are, however, not readily apparent. There are a number of possible mechanisms which could account for such a metabolic response. The breakdown of CP may be a direct effect of an increase in muscle temperature. As reported in Chapter 7, CP degradation tended ($P=0.06$) to be higher during exercise which followed limb heating compared with similar exercise under control conditions. These results tend to support those of Kozlowski et al. (1985) who found higher CP degradation in hyperthermic exercising dogs. The lower CP during exercise in the heat may result from an increase in the creatine kinase reaction due to the possible lowering of muscle pH. Although a direct measure of muscle pH was not made, the higher muscle lactate accumulation in the heat in studies reported in both Chapters 4 and 6, may have resulted in lower muscle pH. Such a change

would tend to drive the creatine kinase reaction resulting in CP degradation and C formation (Harris et al., 1977). An increase in CP degradation during submaximal exercise in the heat may result if blood flow to the active muscle is compromised. Depending upon the mechanism involved, the increase in CP degradation may have occurred early or late in the exercise. Subjects in the present experiments were exposed to the hot environment for 20 min prior to exercise. The resultant cutaneous vasodilation may have produced a reduction in blood flow to the contracting muscle in the transition from rest to exercise. In a recent publication by Yaspelkis et al. (1993), the subjects also sat on a cycle ergometer in the appropriate environmental condition for some time before exercise. The authors reported plasma volume to be lower at the onset of exercise in the heat compared with a thermoneutral environment, but the magnitude of difference did not alter as exercise progressed. Hence, it is possible that as exercise commenced it did so with a slightly reduced contracting muscle blood flow. Unfortunately, CP was not measured in the study by Yaspelkis et al. (1993), however their data support the hypothesis that blood flow may be compromised in the transition from rest to work during exercise in the heat. Alternatively, CP degradation may have increased late in exercise in the heat as blood flow to the contracting muscle was compromised by the need to send blood to the skin for evaporative cooling. From the data presented in this dissertation, it is only possible to speculate on the mechanisms responsible for increased CP degradation during exercise in the heat, since blood flow was not measured during these studies and CP was only measured prior to and following exercise in each trial. Further research examining muscle blood flow and muscle CP degradation during exercise in the heat is warranted.

Other explanations for alteration to CP depletion during exercise in the heat are possible. CP resynthesis may be impaired during exercise in the heat due to a lack of substrate supply. Hultman et al. (1967) reported that a lack

of glycogen inhibited the rate of high energy phosphate resynthesis. Although whole muscle glycogen availability was not compromised, thus not resulting in measurable adenine nucleotide degradation across the whole muscle, during 40 min of submaximal exercise in the heat, PAS staining revealed that approximately 19% of type I fibres were rated as containing either very little (rating 1) or no (rating 0) glycogen compared with 5% and 2% during exercise in a thermoneutral environment and in the heat following acclimation, respectively (Fig 4.7; Fig 5.7). It is possible that the differences in CP degradation are due to the compromised metabolic capacity in these fibres. The CP data may also have been influenced by the circulating adrenaline levels although the literature examining this phenomenon has produced conflicting results (Chasiotis et al., 1983b; Raz et al., 1991). The research reported in this dissertation is unable to provide a definitive mechanism for the observed differences in CP degradation.

The mechanisms responsible for the observations regarding NH_3 metabolism need further investigation. As previously discussed, although muscle NH_3 accumulation following submaximal exercise was not different in any of the first three studies, pooled data from Chapters 4 and 6 resulted in a higher ($P<0.05$) muscle NH_3 accumulation during exercise in the heat (Table 8.2). This occurred despite four of the 12 subjects having a marginally higher muscle NH_3 accumulation in the thermoneutral environment (see Appendices D-F). Further clarification of the effect of exercise in the heat on the muscle NH_3 accumulation of trained individuals is warranted. Although there was a higher muscle NH_3 accumulation with a sample size of 12, plasma NH_3 accumulation was almost identical (Table 8.3). There are a number of possibilities which could explain this finding. Although it remains to be clearly established, a reduction in muscle blood flow during exercise in the heat may reduce NH_3 efflux, thereby resulting in an increased intramuscular NH_3 accumulation. If

NH_3 production is increased and removal from the muscle is not compromised, it is possible that clearance from the plasma is increased during exercise in the heat. Although vasoconstriction occurs in the splanchnic region (Rowell et al., 1969) and in the inactive muscle (Rowell, 1986) during exercise in the heat, potential sources for NH_3 clearance, splanchnic NH_3 uptake is not altered during exercise (Eriksson et al., 1985). The lung and the sweat are two sites for NH_3 clearance. Since body weight loss during 40 min of exercise are higher in the heat compared with a thermoneutral environment (Table 4.1), it is likely that sweat output is higher. Sweat contains significant amounts of NH_3 (Czarnowski & Gorski, 1991). Although it was previously speculated that sweat NH_3 was produced ny the sweat glands (Graham & MacLean, 1992), Czarnowski et al. (1992) clearly observed that the source of sweat NH_3 originates from muscle NH_3 produced diffusing into the plasma and appearing in the sweat. NH_3 is also removed in the lung (Jacquez et al., 1959) and it is possible that NH_3 clearance via this organ may have influenced the results.

Post-exercise muscle IMP accumulation was not increased compared with resting levels in any submaximal exercise trials. Furthermore, post-exercise IMP concentration was not different between trials at different temperatures or acclimation states (Table 4.3; Table 5.3; Table 6.4). NH_3 production, therefore, may be associated with amino acid catabolism. This hypothesis, however, is not supported by Dolny & Lemon (1988) who suggest that protein metabolism, as measured by urea excretion, is reduced during exercise in the heat. The lack of stiochiometry between post-exercise IMP and NH_3 accumulation could result from the activation of the PNC, leading to IMP reamination. Aragon et al. (1981) present evidence that PNC activity occurs during exercise. If PNC cycling were to occur, however, the amino nitrogen group would be donated from aspartate (Goodman & Ruderman, 1982), resulting in an overall increase in amino acid catabolism during exercise. Further research examining amino acid catabolism during exercise in the heat is warranted.

8.1.4 Muscle Metabolism and Fatigue during Exercise at Different Environmental Temperatures

In a recent review, Nielsen (1992) speculates that altered muscle metabolism may contribute to the early fatigue observed during exercise in a hot environment. The data reported in this dissertation, although not entirely conclusive, indicate that fatigue during exercise in the heat is non-metabolic. As reported in Chapters 4 and 6, of the subjects from whom muscle samples were obtained, four subjects were unable to finish the 40 min of exercise in the heat. The reasons for this are not readily apparent, however, the metabolic parameters of both groups of subjects show no differences when compared (Table 8.4). Although data from only 4 subjects are reported, ATP, IMP and NH_3 were not elevated above that of the 'non-fatigue' group, indicating that muscle energy turnover was not compromised in those individuals who could not continue to exercise in the heat. Furthermore, muscle glycogen concentrations were not different between the two groups, nor were they particularly low at the end of exercise (Table 8.4). Fatigue, therefore, appears to be controlled by other factors which could include central fatigue or peripheral factors which could include cellular dehydration or a failure of excitation/contraction coupling, as suggested by Green (1992). This hypothesis is supported by two previous studies which examine metabolism during exercise to exhaustion in the heat. Nielsen et al. (1990) observed fatigue during exercise in the heat despite the fact that muscle circulation was maintained and substrate availability and turnover were adequate. Hence, it was postulated that at core temperatures $>39^{o}C$ the central nervous system may diminish the drive for motor performance. This hypothesis was further supported by a second study from the same laboratory which examined exercise to exhaustion during heat acclimation. Nielsen et al. (1993) reported that although time to exhaustion increased by 60% over the acclimation period, core temperature at exhaustion was 39.7 ±

		SUBJECTS	
		FATIGUE	NON-FATIGUE
ATP	PRE-EX	24.8±0.8	25.1±1.0
	POST-EX	26.6±3.0	24.5±1.1
IMP +	PRE-EX	0.14±0.03	0.14±0.07
	POST-EX	0.19±0.07	0.34±0.07
AMP +	PRE-EX	0.06±0.02	0.07±0.01
	POST-EX	0.10±0.02	0.14±0.02
ADP +	PRE-EX	2.23±0.21	2.49±0.13
	POST-EX	2.85±0.56	2.96±0.17
Ammonia +	PRE-EX	0.38±0.07	0.32±0.07
	POST-EX	1.35±0.24	1.20±0.17
Glycogen +	PRE-EX	557±85	545±37
	POST-EX	328±90	327±27
Lactate +	PRE-EX	7.1±1.1	6.1±1.1
	POST-EX	21.1±5.1	19.9±1.9
CP +	PRE-EX	88.5±3.5	82.3±5.3
	POST-EX	58.7±10.2	47.0±2.8

Table 8.4. *Intramuscular concentrations of adenosine 5'triphosphate (ATP), inosine 5'monophosphate (IMP), adenosine 5'monophosphate (AMP), adenosine 5'diphosphate (ADP), ammonia, glycogen, lactate and creatine phosphate (CP) before (PRE-EX) and after (POST-EX) exercise in 40^{o}C RH 20% in subjects (n=8) who completed (NON-FATIGUE) and subjects (n=4) who fatigued before completing (FATIGUE) 40 minutes of submaximal exercise.*
Values are means ±SE, expressed in mmol. kg^{-1} dry wt, (n=12). $^{+}$ indicates main effect ($P<0.05$) for time. Data from the six subjects who were biopsied in Chapter 4 has been combined with the data from the seven subjects in Chapter 6 (one subject was common and the data from the two studies for this subject has been averaged).

0.15^{o}C on each of the nine days of exercise. Interestingly, metabolites such as blood glucose and lactate and hormones such as adrenaline were reduced over the acclimation period. The data of Nielsen et al. (1990) and Nielsen et al. (1993), along with those reported in this dissertation suggest that although muscle metabolism is influenced by environmental temperature, the ability to prolong exercise in the heat is dependent on factors other than metabolism.

8.2 RECOMMENDATIONS FOR FUTURE RESEARCH

Although the work presented in this dissertation provides further insight into muscle metabolism during exercise at different environmental temperatures, it has also raised some questions which need further clarification. As discussed throughout the dissertation, the role of muscle blood flow during exercise in the heat is still equivocal. Although the human studies (Nielsen et al., 1990; Savard et al., 1988) which have measured muscle blood flow during exercise in the heat have found it to be unchanged, the previously discussed methodological problems associated with these studies may have influenced the blood flow observations. Of note, these studies observed very little change in metabolism with no change in blood flow. Other studies (Fink et al., 1975; Young et al., 1985), along with the work reported here, found metabolism to be influenced by environmental temperature but, did not measure blood flow. Interestingly, Nielsen (1992) reports that in preliminary investigations a tendency for increased muscle blood flow following acclimation was observed. A further study examining muscle blood flow and muscle metabolism during exercise at different environmental temperature which is both counter-balanced (i.e trials on different days) and performed so that heat dissipatory mechanisms are unimpaired (i.e not using water-perfused suits to control thermal response) is needed to shed further light on muscle perfusion during submaximal exercise.

Evidence from this dissertation suggests that fatigue during exercise in the heat is not related to metabolism. Further investigation examining muscle metabolism during exercise to fatigue in the heat is warranted. The data presented here suggest that the substrate availability at fatigue during exercise in the heat is adequate and there no accumulation of metabolic factors associated with fatigue. In contrast, exercise to exhaustion in a cool environment whereby body temperature is maintained $<39^{o}C$ results in elevated IMP and reduced ATP levels along with glycogen depletion (Constantin-Teodosiu et al., 1992; Sahlin et al., 1990; Spencer et al., 1991b). A comparative study examining exercise to exhaustion in hot and cool conditions would clarify the role of metabolism in fatigue processes. A tracer (^{3}H labelled glucose) study may provide insight into the rate of hepatic glycogenolysis (Ra) and rate of muscle glucose uptake (Rd) during exercise in the heat. Since a study of this nature has not been conducted, it is not currently possible to asses glucose metabolism in the heat. Furthermore, if Ra is greater than Rd resulting in hyperglycaemia, then the in the presence of increased sympatho-adrenal activity would provide further evidence of 'feed-forward' response from the neuroendocrine control centres in the CNS, as proposed by Shimazu (1992) and Kjaer (1992). The role of adrenaline on glycogenolysis during exercise has been well investigated (Greenhaff et al., 1991; Jansson et al. 1986). The study reported in Chapter 7, indicated that an increase in muscle temperature, alone, can be responsible for altering muscle metabolism. This study, however, utilised an exercise intensity much higher than others reported in this dissertation, which was necessitated by the requirement to maintain core temperature during exercise. For this reason, there remains some doubt as the relative contribution of the increase in muscle temperature and the increase in sympatho-adrenal activity to the altered exercise metabolism during prolonged exercise. What remains to be determined, therefore, is the extent of the alteration to muscle metabolism during prolonged exercise in the absence of

any change in muscle temperature. A study which examines this phenomenon involving a maintenance of muscle temperature with an increase in core temperature may answer this question. Finally, the precise mechanisms for the improved thermoregulatory response observed after heat acclimation require further research. As discussed in Chapter 5, it is unclear whether plasma volume expansion alone, or in conjunction with other thermoregulatory adaptations to acclimation, is responsible for the observed changes in temperature and sympatho-adrenal response. Further research examining the effect of acute plasma volume expansion, on muscle metabolism during exercise in the heat could clarify the answer to this question.

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Appendix A

Subject Correspondence

VICTORIA UNIVERSITY OF TECHNOLOGY

STANDARD CONSENT FORM FOR SUBJECTS
INVOLVED IN EXPERIMENTS

CERTIFICATION BY SUBJECT

I, ...
of .....
certify that I have the legal ability to give valid consent and that I am voluntarily giving my consent to participate in the experiment entitled:

"..... .....
....."

being conducted at Victoria University of Technology by:

.....

I certify that the objectives of the experiment, together with any risks to me associated with the procedures listed hereunder to be carried out in the experiment, have been fully explained to me by:

.....

and that I freely consent to participation involving the use on me of these procedures.

Procedures

.....
.....
.....
.....
.....

I certify that I have had the opportunity to have my questions answered and that I understand that I can withdraw from this experiment at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the confidentiality of the information I provide will be safeguarded.

Signed: .....)
)
Witness other than the experimenter:) Date: .....
)
.....)

MUSCLE BIOPSY QUESTIONNAIRE

NAME: _____

AGE: _____ **years**

1. Have you or your family suffered from any tendency to excessively bleed? (eg. haemophilia)

   Yes No Don't know

   If yes, please elaborate.... _____

   _____

2. Are you allergic to local anaesthetic?

   Yes No Don't know

   If yes, please elaborate.... _____

   _____

3. Do you have any skin allergies?

   Yes No Don't know

   If yes, please elaborate.... _____

   _____

4. Have you any allergies that should be made known?

   Yes No Don't know

   If yes, please elaborate.... _____

   _____

5. Are you currently on any medication?

   Yes No Don't know

   If yes, what is the medication? _____

   _____

6. Do you have any other medical problem that should be made known?

   Yes No

   If yes, please elaborate.... _____

   _____

7. Have you ever fainted when you have had an injection or blood sample taken?

   Yes No Never had it done

   If yes, please elaborate.... _____

To the best of my knowledge, the above questionnaire has been completed accurately and truthfully.

Signature _____

Date _____

CARDIOVASCULAR RISK FACTOR QUESTIONNAIRE

In order to be eligible to participate in the experiment investigating

you are required to complete the following questionnaire which is designed to assess the risk of you having a cardiovascular event occurring during an exhaustive exercise bout.

Name: _____ **Date:** _____

Age: _____ years

Weight: _____ kg **Height:** _____ cms

Give a brief description of your average activity pattern in the past 2 months:

_____

_____

_____

Circle the appropriate response to the following questions.

1. Are you overweight? Yes No Don't know
2. Do you smoke? Yes No Social
3. Does your family have a history of premature cardiovascular problems (eg. heart attack, stroke)? Yes No Don't know
4. Are you an asthmatic? Yes No Don't know
5. Are you a diabetic? Yes No Don't know
6. Do you have a high blood cholesterol level? Yes No Don't know
7. Do you have high blood pressure? Yes No Don't know
8. Are you on any medication? Yes No
   If so, what is the medication? _____
9. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in strenuous exercise? No
   Yes, please elaborate _____

I, _____, believe that the answers to these questions are true and correct.

Signed: _____

Date: _____

INFORMATION FOR THE SUBJECT

Exercise performance is affected by both hot and cold environmental conditions due to a number of factors. It has been postulated that the rate of glycogen breakdown within the exercising muscle is accelerated in the heat and somewhat reduced in cold conditions, due to body and muscle temperature changes. In this study we hope to obtain new insight into the processes which are responsible for changes in skeletal muscle metabolism during exercise performance in both hot and cold conditions.

As a volunteer, you will be asked to undergo a series of closely supervised tests which will take place from May until September. These tests should not interfere with your training too greatly and in fact you will gain valuable information into your personal adaptations to exercise in varying environmental conditions. Fistly, you will undergo a Vo_2 max test in thermoneutral conditions (20^{o}C with a reletive humidity level of 20%). You will be required to exercise on a bicycle ergometer for a period of about 20 minutes. During this time the resistance on the ergometer will be incrementally increased at two minute intervals for the first six minutes then at minute intervals until exhaustion. This will tell us your maximal aerobic capacity, how aerobically fit you actually are. You will then be asked to exercise on a bicycle ergometer for 40 minutes at 70% of your maximal capacity on three seperate occasions, once in the heat (40^{o}C RH 20%), once in thermoneutral conditions (20^{o}C RH 20%) and once in the cold (5^{o}C RH20%). These tests will be seperated by one week. Before and during exercise we will take small blood samples from a small plastic tube that will be positioned in a forearm vein. The total volume of blood taken at each test is about 75 ml, or 1/6th of that taken during a blood transfusion. Your body will replace this lost blood very quickly. The risks are very minimal but are nevertheless explained in the document entitled "Catherization".

In addition, 20 minutes prior to, immediately before and after exercise we will obtain small samples of muscle from your thigh using a special needle. These biopsies are performed using local anaesthesia and so the small incision is painless. The muscle is usually a little discomforting for about a day and a small scar will remain. All biopsies will be performed by a qualified doctor, experienced in performing muscle biopsies, and therfore pain and the possible risks are minimal. However, these are explained in the document entitled "Muscle Biopsy Procedure". In this experiment we will monitor your body temperature by means of a small, flexible thermometer inserted into the rectum. There is no pain or discomfort associated with this however uninviting it may seem. The rectal probes will be thoroughly sterilised. We will also measure your muscle temperature with a small thermometer needle inserted into your muscle prior to exercise and after 40 minutes. These temperatures will be taken immediatedly after the biopsy and thus will be painless as a result of the local anaesthetic. These procedures will be followed in all three trials.

As a participant, you will help us better understand the responses to exercise under heat stressed conditions. Apart from this, you will gain valuable insight into your personal responses to exercise. You will recieve information on your muscle fibre type, maximal aerobic capacity and anaerobic threshold which can be an invaluable tool to use during training. Please do not hesitate to ask me how to maximise your performance from the data received, and if you so wish I will design a training regimen using this information.

If you have any questions please ask.

By signing the consent form you are indicating that the tests and procedures have been explained to, and understood by you and that you are voluntarily participating in this study. You are free to call me 0n (03) 688 4603 (B) or (03) 432 0084 at any time if you have questions on any aspects of the study. You are also free to withdraw from the study at any time.

Yours Sincerely,

Mark Febbraio

B.App. Sci.

Dip. Ed.

30th April, 1992

Dear ----,

I have finally finished all the analysis and am pleased to let you know that all the analyses were successful. I hope the data that I present here can be of some benefit to your training and racing. If you have any queries regarding interpretation of the data, do not hesitate to call me. In order to help you better understand your results, I will present your data along with the trained group's mean score, and where possible include results found in untrained population. Finally, I will interprete those data which I feel you could base some training around.

PARAMETER	YOUR RESULT	GROUP MEAN	UNTRAINED
	% TYPE	% TYPE	% TYPE
MUSCLE FIBRE	I IIA IIB	I IIA IIB	I IIA IIB
TYPE	60 37 3	61 36 3	31 36 33
VO_2 max	ml/kg/min	ml/kg/min	ml/kg/min
	64.3	64.7	40-50
ANAEROBIC	$\%VO_2$ H/R	$\%VO_2$ H/R	$\%VO_2$ H/R
THRESHOLD	94 194	83 177	75 --
MUSCLE LACTATE			
ACCUMULATION	RTT PRE PO	RTT PRE PO	
(AT 40 MIN EX.)	10.7 15.4 14.6	12.1 23.5 15.7	
MMOLES/KG dw.			
MUSCLEE GLYCOGEN			
UTILISATION	60 139 112	129 183 157	
MMOLES/KG dW.			

From these data I would suggest that you have made the right decision in persuing an endurance type sport. Your muscle fibre type suggests that genetically, you are suited to endurance as you have a high number of type I (slow twitch) fibres. In addition, you have trained your type II (fast twitch) fibres to exhibit fatigue resistant characteristics, as nearly all your type II fibres are IIa (fatigue resistant fast twitch). Your VO_2 max is quite high, however your anaerobic threshold is remarkably high, placing you in the elite category. If you wish to encorporate interval training into your program, attempt to elevate your heart rate to slightly above 194 bpm per interval. However, at 94% there is not much room for improvement! Perhaps you should concentrate on some base miles to increase your aerobic fitness thus elevating your maximal oxygen capacity. If you have some questions call me.

Enclosed is a photo of your muscle cross section. The dark fibres are type I and the light ones type two. Once again let me extend my gratitude for your participation. Without my subjects my forthcoming Ph D. would be impossible.

Mark Febbraio

B.App.Sci.
Dip.Ed.

DIET # 1

(80% CHO)
(~ 13000kJ)

BREAKFAST

6 Wheetbix + 500ml skim milk
1 large banana
500ml orange juice

LUNCH

Lean cuisine meal
2 slices wholemeal bread
1 large banana
375ml soft drink

DINNER

250g (cooked) pasta
1/2 jar Weight Watchers pasta sauce
425g can Goulburn Valley tinned fruit
250ml orange juice

SNACK

2 crumpets
1 tablespoon jam on each
250ml orange juice

*** For 70kg human**
*delete snack for 60 kg human
* add snack for 80 kg human

Appendix B

Analytical Methods

MUSCLE LACTATE DETERMINATION

Principle

Lactate + NAD^+ ----------> pyruvate + NADH + H^+
lactate dehydrogenase

Pyruvate + Hydrazine ----------> pyruvate hydrazone

MUSCLE GLYCOGEN DETERMINATION

Principle

Glucose + ATP----------> ADP + G-6-P
hexokinase

G-6-P + NADP----------> 6-P-Gluconolactone + NADPH
G-6-P dehydrogenase

* 500 and 50μM lactate standards and 500 and 250 μM glucose standards are used in the assay

* assays are run with a set of NADH standards (50, 100, 200, 400μM)
- read absorbance of these on spectrophotometer at 340 nm and adjust concentration according to the MEC (x 10^{-3}) (6220)
-record actual standard concentration and construct a standard curve (NADH concentration and fluorescence).

* if the metabolite standard fluorescences are within 10% error of predicted standard fluorescence, the assay is successful.

MUSCLE ATP & CP DETERMINATION

Principle

creatine phosphate + ADP ----------> creatine + ATP
creatine kinase

ATP + glucose ----------> ADP + G-6-P
hexokinase

G-6-P + NADP----------> 6-P-Gluconolactone + NADPH
glucose dehydrogenase

* 500 and 250μM ATP & CP standards are used in the assay

* assay is run with a set of NADH standards (50, 100, 200, 400μM)
- read absorbance of these on spectrophotometer at 340 nm and adjust concentration according to the MEC (x 10^{-3}) (6220)
-record actual standard concentration and construct a standard curve (NADH concentration and fluorescence).

* if the metabolite standard fluorescences are within 10% error of predicted standard fluorescence, the assay is successful.

MUSCLE CREATINE DETERMINATION

Principle

creatine + ATP ----------> ADP + P-creatine
creatine kinase

ADP + P-pyruvate ----------> ATP + pyruvate
pyruvate kinase

pyruvate + NADH + H^+----------> lactate + NAD^+
lactate dehydrogenase

* 200μl of a 15mM NADH stock is added to the cocktail. Before addition of the NADH, measure NADH stock concentration on a spectrophotometer to calculate actual concentration. This will allow you to calculate the exact concentration of NADH in the cocktail to assess the performance of the assay.

Calculations for Determining Metabolite Concentrations

Calculation for the metabolites is as follows:

Formula:

$$\text{no. moles} = \frac{\text{sample fluorescence}}{\text{standard Fluores.}} \times \text{Std. Molarity(M)} \times \text{vol. of sample}$$

You must correct for the fact that you have 250 ul of extract one, which you then transferred 200ul to 50ul of $KHCO_3$ to make 250ul of extract two. You must also take into consideration the weight of the extract. Examples of a method for calculation is as follows:

CALCULATIONS TO DETERMINE MUSCLE GLYCOGEN CONCENTRATION

Extraction

A known weight of muscle was placed into 0.5 ml of 2M HCl. This was cooked for two hours. After cooking 1.5 ml of 0.667M NaOH was added. Therefore, a known weight of muscle was extracted in 2 ml of fluid. For glycogen assay the standard was 0.5 mM glucose.

Calculations

10 µl of standard contains $10 \times 10^{-6} \times 0.5 \times 10^{-3}$ moles of glucose and gives x fluorescence units (FU).

10 µl of sample extract contains y moles and produces z FU. Therefore

$y = z / x \times 10 \times 10^{-6} \times 500 \times 10^{-6}$ moles.

Muscle sample contains y x 200 moles / weight of muscle.

Convert units to $mmol.kg^{-1}$ dry weight.

CALCULATIONS TO DETERMINE MUSCLE ATP CONCENTRATION

Extraction

A known weight of muscle was extracted in 250 µl of 1.0M $HClO_4$ (extract 1).

200 µl of extract 1 was removed and added to 50 µl of 2.1M $KHCO_3$ to form extract 2.

For ATP the standard was 200 µM.

Calculations

10 µl of standard contains $10 \times 10^{-6} \times 200 \times 10^{-6}$ moles of ATP and gives x FU.

Therefore 2×10^{-9} moles gives x FU.

10 µl of extract contains y moles of ATP and produces z FU.

$y = (z / x)\ 2 \times 10^{-9}$ moles.

Extract 2 contains y x 25 moles of ATP.

Extract 1 contains the number of moles of ATP in extract 2 / 0.8.

Muscle ATP content = the number of moles of ATP in extract 1 (expressed in

[redacted] (expressed in kg).

Muscle Ammonia Analysis

WEIGHING SAMPLES

Muscle is cut into small pieces, dissected from obvious connective tissue and weighed into labelled eppendorf tubes if analysis will be completed that day or into cryules if weighed muscle is to be stored for later analysis. Muscle weights can vary from 5 to 12 mg wet weight. These procedures are to be carried out at temperatures below -20 C

FIA ANALYSIS OF MUSCLE SAMPLES

Prepare standards in 10 % methanol. Suggested concentrations are blank (10% MeOH), 0.25 mg/l, 0.5 mg/l, 1 mg/l, 2 mg/l, 4 mg/l. Store on ice during analysis. Keep in refrigerator (4C) for one week.

Prepare reagent streams for FIA. Carrier stream Milli Q water, filtered and degassed using Milli Q filtration system. O.1 M NaOH stream (filtered and degassed) and indicator stream (10 ml of stock made up to 500 ml with Milli Q water, filtered and degassed). Store reagent streams in special CO2 proof bottles and maintain constant temp at 30 C

Detector set up: wavelength 590 nm
gain factor 10
max absorbance 1.00
min absorbance -.02
seconds/cm 15

Pump Tubing: -Carrier stream (left hand pump) Red - red
-Indicator stream (right hand pump) Red - Red
-NaOH stream (rh pump) black - black

-Injection stream (rh pump) orange - yellow

Injection loop: 100 ul plus injector pieces

Sample size used to fill loop approx. 140 ul.

Indicator stream titrated to 480 - 490 absorbance units.

INITIAL PROCEDURE

(Test run with internal standards to varify procedure)

Before commencing with muscle extracts run a trial analysis of internal standards (known NH_4Cl concentration made up in water). these internal standards should range from 0.1 mM to 1.5 mM. Add 10μ of the internal standard to an eppendorf tube an extract as per method (treat as 10 mg sample) then analyse using standards ranging from 0.25mg/l up to 5.0-6.0 mg/l.

A 5-8% variation in the analysis of muscle NH_3 has been reported (Katz. et al.1986). If the values vary by more than 8-10% do not proceed with muscle analysis.

PROCEDURE FOR EXTRACTION

Initial extraction takes place in cryotome precooled to approx -20 C.
If muscle needs to be transferred from cryules to eppendorf tubes ensure eppendorf tubes are precooled. Also precool tweesers just in case any muscle is dropped into cryotome. The 0.6 M PCA - 30% methanol should also be precooled to -20 C.

Add 50 ul of 0.6 M PCA - 30% MeOH to muscle weighing less than 8 mg and 55 ul to the rest (calibrate all pipettes on scales). Allow to sit for 30 min. at approx -20 C. Also run a set of blanks for the two extraction volumes.

Add 100 ul of ice-cold 0.6 M PCA to muscle weighing less than 8 mg and 110 ul to the rest. Store on ice for 15 min. during which time periodically vortex tubes. (Precool eppendorf centrifuge to 2 C takes approximately 18 min at 8000 rpm).

After 15 min. add 58 ul of ice cold 1.8 M KOH to muscle weighing less than 8 mg (or 68 ul to other samples). Vortex and centrifuge for 2 min at 28,000 rpm, acceleration profile 9, at 2 C.

Immediately remove all the supernatant with glass pasteur pipettes and place it into appropriately labelled eppendorf tubes which have been sitting in ice. Store the supernatant on ice and analyse within an hour.

H.P.L.C. analysis of Adenine Nucleotides
(in muscle extracts)

Buffer A -(0.15 M Ammonium dihydrogen phosphate - pH 6.00)
made from 10mls of 85% Orthophosphoric acid (Aristar Grade BDH 45010) for every litre of buffer required into MilliQ water (about 80% of the total necessary volume), then pH to 6.00 with ammonium hydroxide (Univar Grade AJAX 2672) (approx. 10-12 mls of 28% NH_4OH per litre). Make up to required volume with MilliQ water.

-best used within 24-48 hours of making solution (refrigerate when not in use i.e. temporary storage)

-1 litre is enough for a 12 hour period, therefore is best made on a daily basis in 1 (at most 2) litre amounts.

Buffer B -1 litre of Acetonitrile and Methanol (50/50, v/v).

-this can be used for up to a week but must be degassed prior to reuse.

-all buffers except Milli-Q water are to be filtered and degassed and then connected to the Helium outlets for Purging at regular intervals.

H.P.L.C.

Column Hibar Lichrosphere 100 CH-18/2 (Merck) (250 x 4 mm)

Detector Biorad UV monitor model 1305 - wavelength 254nm

flush Flush pump A with H_2O 15-20 mins, flow rate 1ml/min

Run program

Time	%A	%B
0	100	0
5.5	100	0
25.5	85	15
30	100	0

Flush with 100% of Buffer A for ten minutes between injections.

Storage (overnight i.e. < 24 hours)
Flush column with water for 30 min (pump A), also rinse injection port and loop with water (5 times in each position).
Flush column intermittantly with Buffer B (every 25-35 injections) before the water flush for longevity of the column.

(long term storage i.e. >24 hours)
Flush 50% pump A with Acetonitrile/Methanol (50/50) and 50 % pump B with methanol. Also rinse injection port and loop with methanol (5 times in each position).

Setting up and using HPLC (From overnight storage with MilliQ)
-connect buffers to pumps, flush lines of air bubbles, then run "Run program" without injection to flush column. (See below).

Using HPLC/PC:

-check method is correct (press method key)
-press graph key.
-ensure injection port is in the "load" position then press start key (this will prime the system to begin when the injection port is turned to inject)
-load the sample with the injector syringe (60-65 microlitres) then turn the injector port to "inject".
-with this the system should begin run to run (i.e. clock on monitor begins to dial)

at termination of run;

-ensure that the run has been saved by pressing the save key (shift - save/load), entering filename and pressing enter.
-next step is to send it to channel A by pressing the A (there should then be a cue - file saved.)
-return injection loop back to load, then press start key to prepare another run. Time between injection begins immediately run is terminated
-to integrate data esc key activates

BLOOD LACTATE

Principle:- The enzyme lactate dehydrogenase (LDH) catalyses the the transfer of hydrogen from the cofactor NADH to pyruvic acid to produce lactic acid. However, the equilibrium highly favours the reduction of pyruvic acid:

$$\text{Lactic acid} + NAD^{+} \overset{LDH}{\Longleftrightarrow} \text{Pyruvic acid} + NADH$$

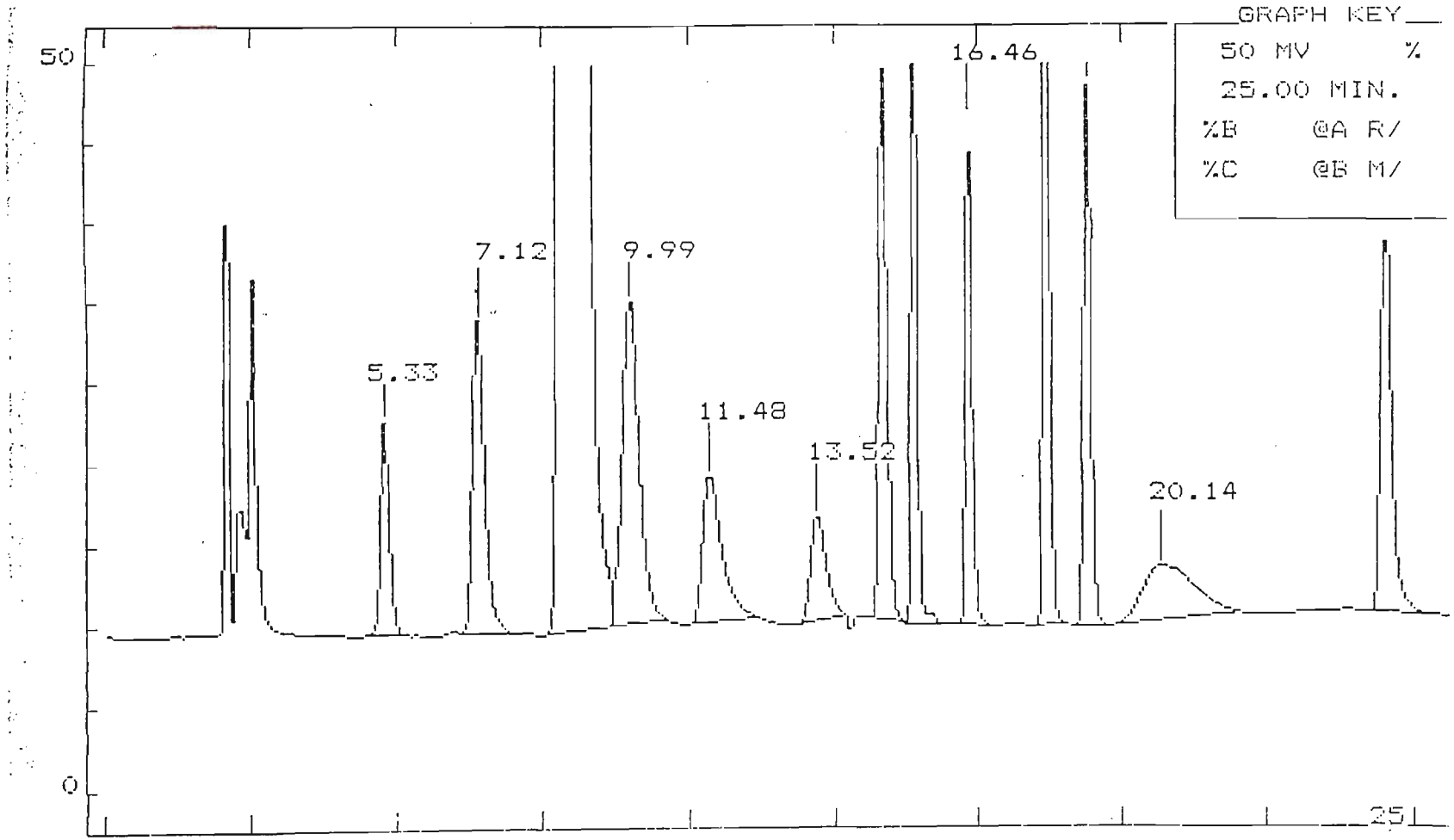
$$K_{eq} = 3.6 \times 10^{11}\ M^{-1}$$

In the presence of an excess of the cofactor NAD+ and lactate dehydrogenase activity, and at a pH of 9.6, nearly all of the lactate, in deproteinized whole blood, is converted to pyruvate. To ensure the reaction goes to completion, pyruvic acid, in the presence of hydrazine, is removed by converting it to pyruvatehydrazone. The appearance of NADH yields an observable method to quantify the concentration of lactate originally present. The concentration of NADH was measured spectrophotometrically at wavelength of 340 nm using a spectrophotometer (Shimadzu UV-120-02) in conjunction with a flow cell apparatus (Sigma Technical Bulletin, No 826 UV, Oct 1986).

A set of standards ranging in concentration from 1 mM to 10mM was run with each analysis in conjunction with a reagent blank.

Appendix C

Examples of HPLC Chromatographs


GRAPH KEY
50 MV %
25.00 MIN.
%B @A R/
%C @B M/
50
0
25
5.33
7.12
9.99
11.48
13.52
16.46
20.14
SEQ INJ 1/ 1 SYSTEM:
DATA FILE: TANS12611.
03:29 01/01/1980

Standard 1

==========
AREA/PERCENT REPORT
==========

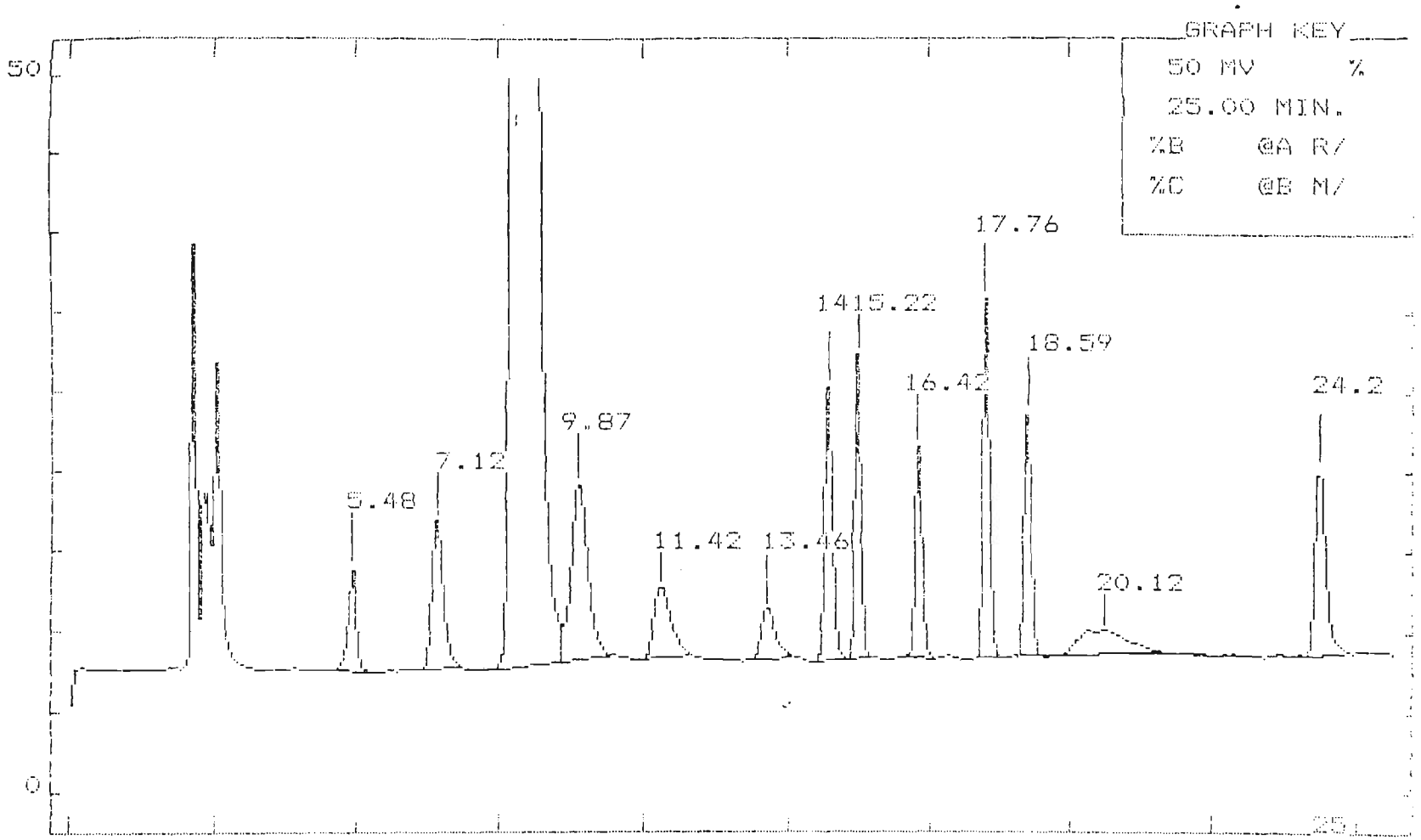
*****SAMPLE ID***** SOLVENT: [ ]
* * COLUMN: [ ]
* VIAL #: 0 * MEMO: [ ]
****

METHOD: [12] [staph file]
INTEGRATION: [ ] [ ]
SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [MIN] TO [35.00 MIN]

IS AMT: [1] WGT: [1] DIL FAC: [1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
5.33	uric acid	2741	0.86%	14532
7.12	IMP	5687	1.78%	21394
8.85	ATP	251385	78.98%	770824
9.99	ADP	8018	2.51%	22152
11.48	Hypoxanthine	4151	1.30%	10098
13.52	Xanthine	2413	0.75%	7178
14.79	AMP	7002	2.20%	38919
15.40	NADP	6896	2.16%	54263
16.46	Xanthosine	5121	1.60%	35282
17.97	NAD	8130	2.55%	57421
18.76	Inosine	6111	1.92%	37615
		4122	1.29%	3739
		6506	2.04%	28323
TOTALS:		318289	100.00%	


GRAPH KEY
50 MV %
25.00 MIN.
%B @A R/
%C @B M/
50
0
25
5.48
7.12
9.87
11.42
13.46
14
15.22
16.42
17.76
18.59
20.12
24.2

Standard 2

==========
AREA/PERCENT REPORT
==========

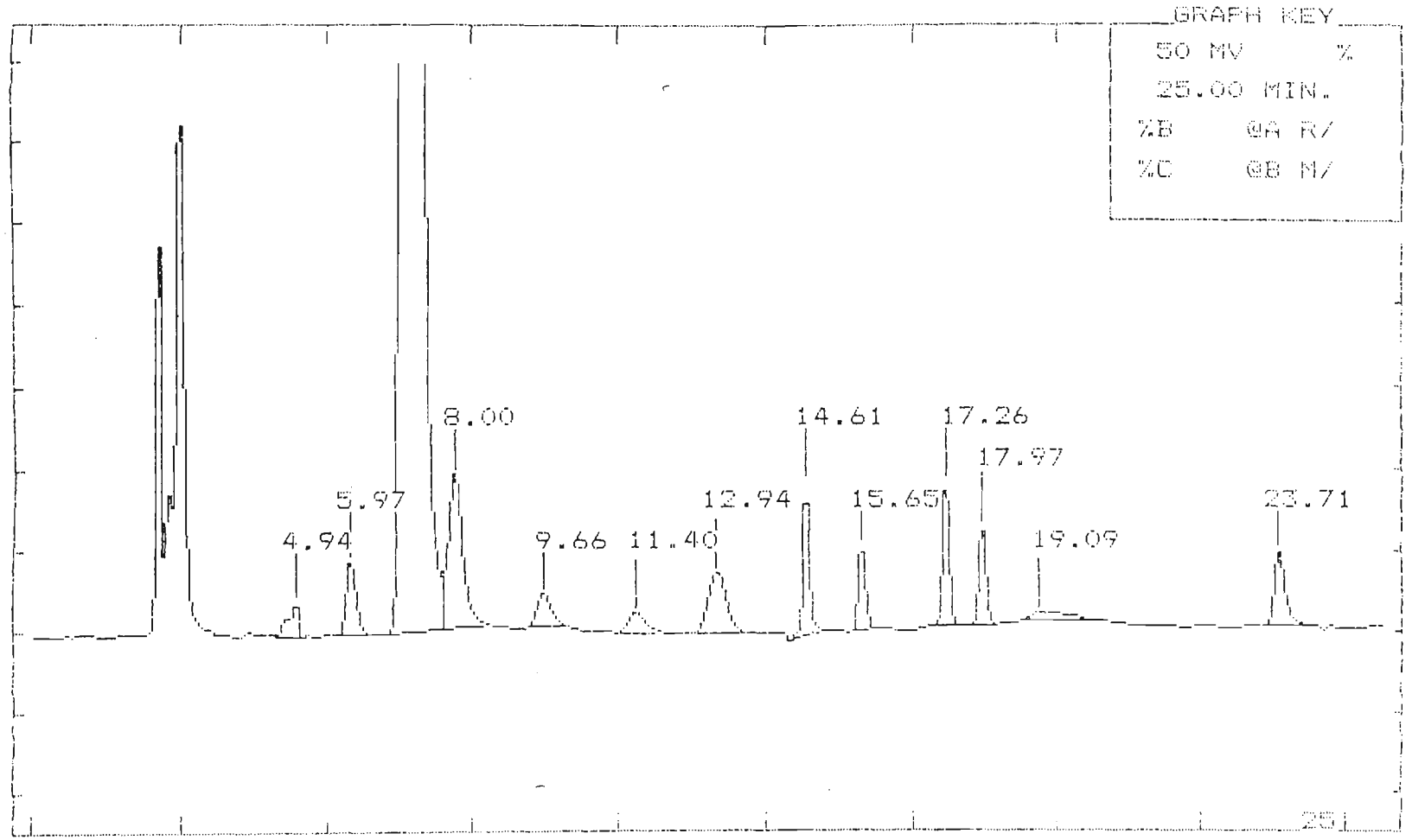
*****SAMPLE ID***** SOLVENT: [ ]
* * COLUMN: [ ]
* VIAL £: 0 * MEMO: [ ]
****

METHOD: [12] [staph file]
INTEGRATION: [ ] [ ]
SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [MIN] TO [35.00 MIN]

IS AMT: [1] WGT: [1] DIL FAC: [1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
5.48	uric acid	1229	0.57%	7268
7.12	IMP	2054	1.14%	10150
8.76	ATP	183203	85.58%	574632
9.87	ADP	4457	2.06%	12203
11.42	Hypoxanthine	1769	0.82%	4866
13.46	Xanthine	1064	0.49%	3454
14.64	AMP	3338	1.55%	19124
15.22	NADP	2918	1.36%	21250
16.42	Xanthosine	2319	1.08%	14829
17.76	NAD	3774	1.76%	25200
18.59	Inosine	2756	1.28%	17000
20.12	Adenine	1680	0.78%	1620
24.27	Adenosine	3025	1.41%	13164
TOTALS:		214062	100.00%	


GRAPH KEY
50 MV %
25.00 MIN.
%B @A R/
%C @B M/
4.94
5.97
8.00
9.66
11.40
12.94
14.61
15.65
17.26
17.97
19.09
23.71
25

Standard 3

==========
AREA/PERCENT REPORT
==========

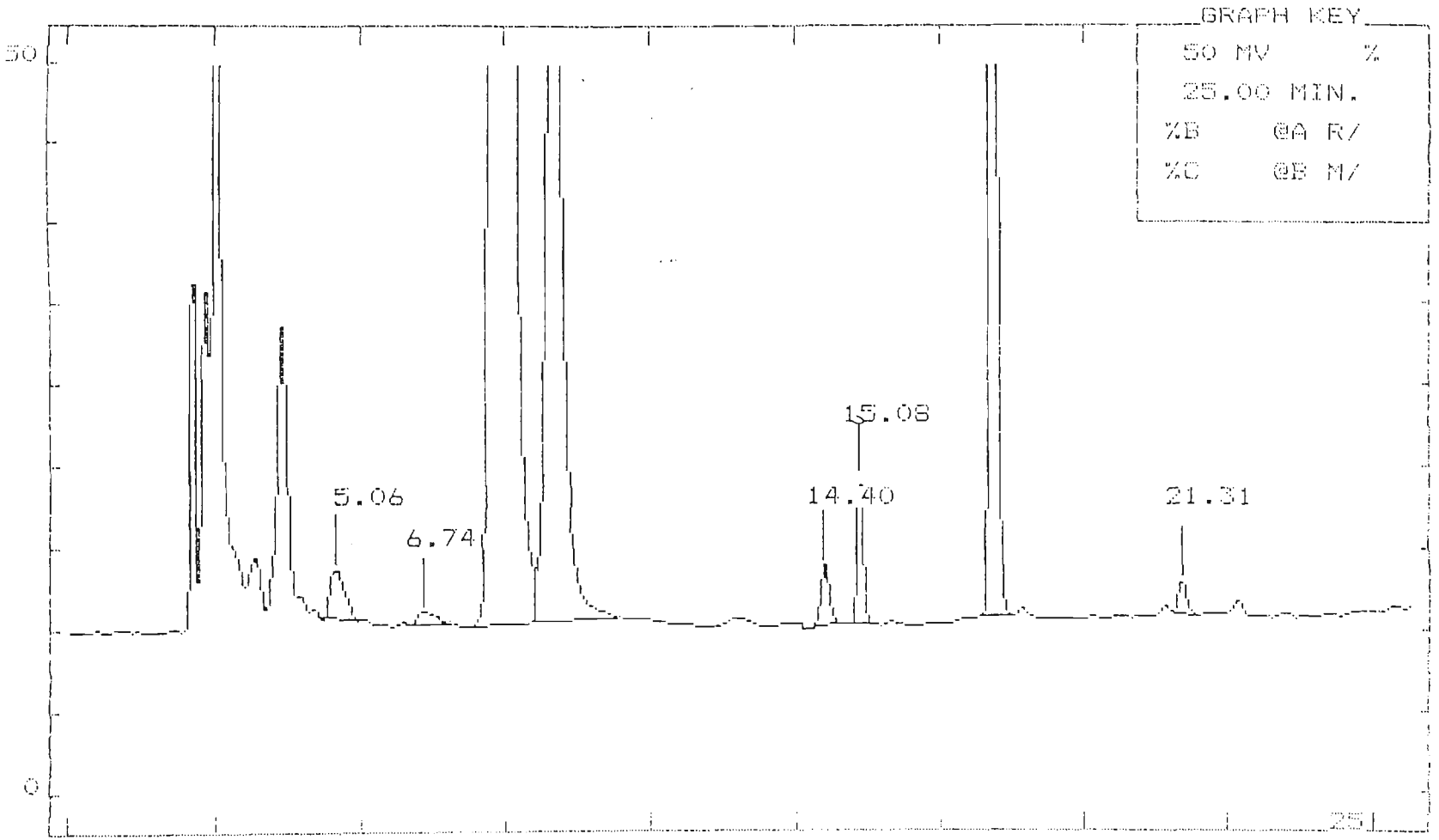
*****SAMPLE ID***** SOLVENT: [ ]
* * COLUMN: [ ]
* VIAL #: 0 * MEMO: [ ]
****

METHOD: [12] [staph file]
INTEGRATION: [ ] [ ]
SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [MIN] TO [35.00 MIN]

IS AMT: [1] WGT: [1] DIL FAC: [1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
4.94	uric acid	484	0.22%	2397
5.97	IMP	1064	0.48%	5033
7.11	ATP	205683	93.66%	766546
8.00	ADP	3384	1.02%	10573
9.66	Hypoxanthine	682	0.31%	2262
11.40	Xanthine	448	0.20%	1328
12.74	AMP	1524	0.69%	4183
14.61	NADP	1402	0.63%	10701
15.65	Xanthosine	916	0.41%	5432
17.20	NAD	1438	0.65%	9995
17.97	Inosine	1055	0.48%	6724
19.09	Adenine	390	0.17%	577
23.71	Adenosine	1145	0.52%	5147
TOTALS:		219589	100.00%	



Resting Sample

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AREA/PERCENT REPORT
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*****SAMPLE ID***** SOLVENT: [ ]
* * COLUMN: [ ]
* VIAL #: 0 * MEMO: [ ]
****

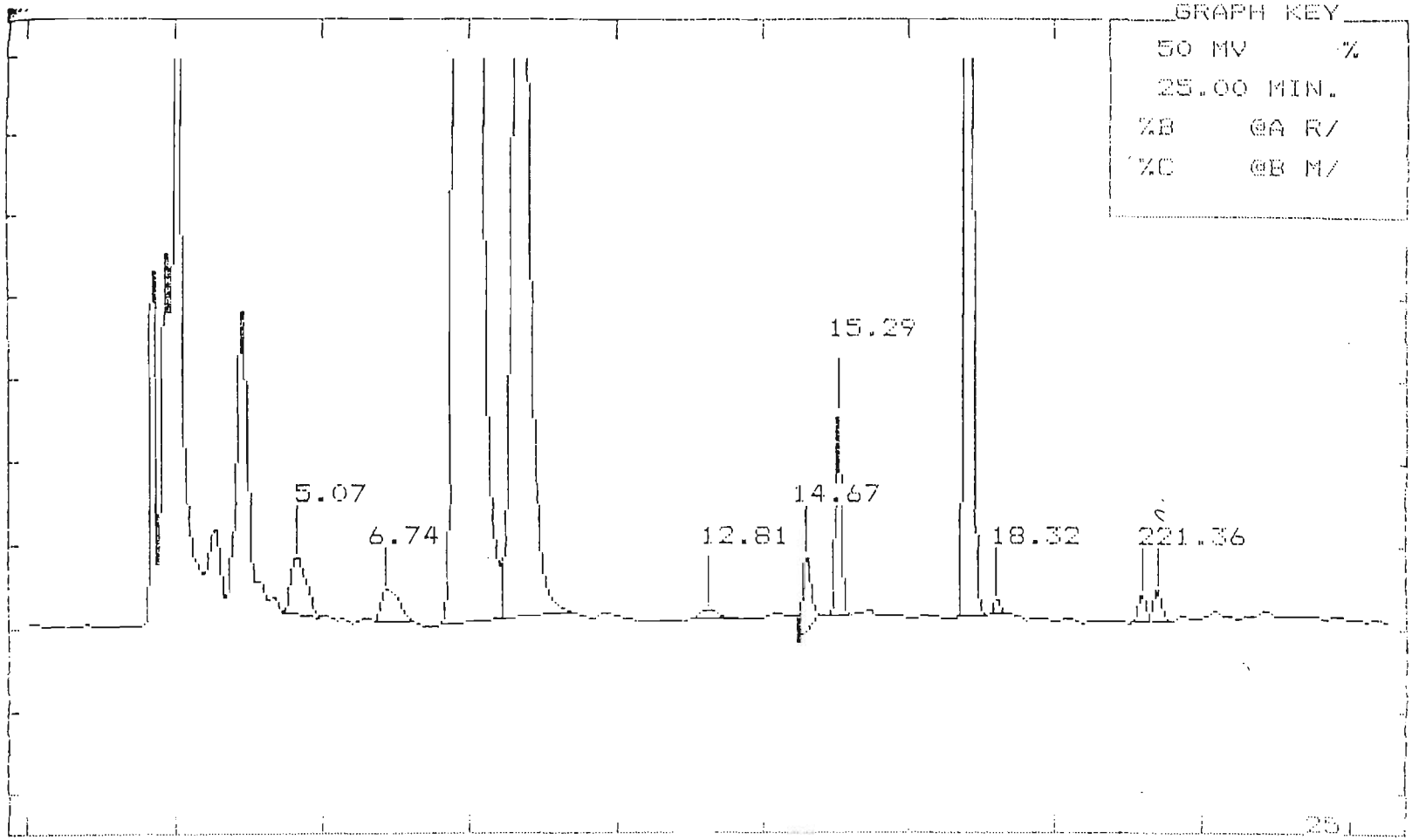
METHOD: [12] [staph file]
INTEGRATION: [ ] [ ]
SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [MIN] TO [35.00 MIN]

IS AMT: [1] WGT: [1] DIL FAC: [1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
5.06	uric acid	973	0.46%	3314
6.74	IMP	290	0.13%	905
8.22	ATP	169833	80.86%	570102
9.27	ADP	19941	9.51%	69380
14.40	AMP	750	0.35%	4180
15.08	NADP	1351	0.64%	10155
17.65	Adenine	16431	7.82%	109811
21.31	Adenosine	374	0.18%	2238
TOTALS:		210018	100.00%	

SEQ INJ 1/ 1 SYSTEM[ ]
DATA FILE: DT-H-R.
00:14 01/01/1980



Submaximal Exercise Sample

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AREA/PERCENT REPORT
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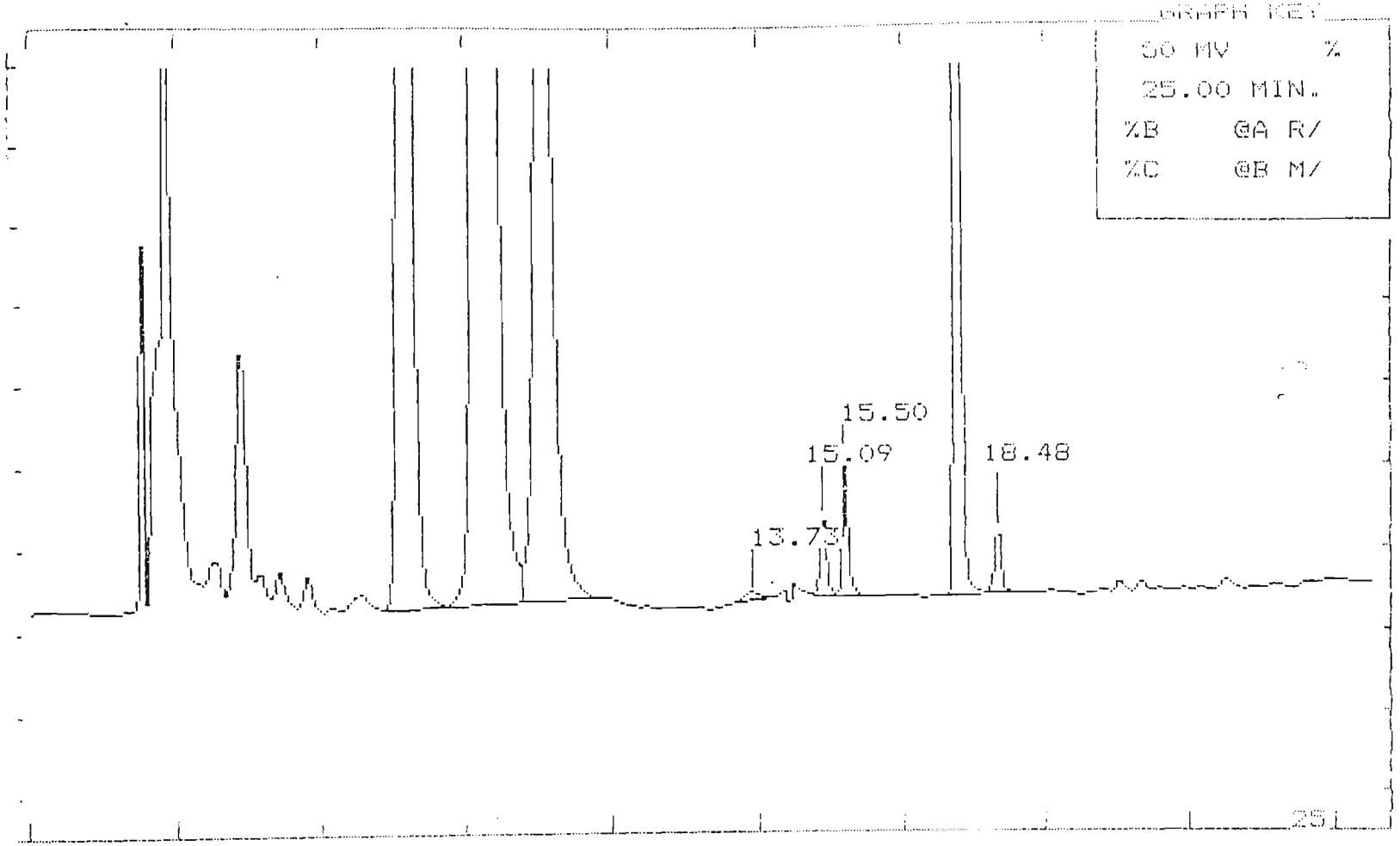
*****SAMPLE ID***** SOLVENT: [ ]
* * COLUMN: [ ]
* VIAL £: 0 * MEMO: [ ]
****

METHOD: [12] [staph file]
INTEGRATION: [ ] [ ]
SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [MIN] TO [35.00 MIN]

IS AMT: [1] WGT: [1] DIL FAC: [1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
5.07	uric acid	1240	0.54%	3773
6.74	IMP	773	0.33%	2126
8.22	ATP	182809	80.00%	601259
9.26	ADP	21384	9.38%	66331
12.81	Xanthine	199	0.08%	595
14.67	AMP	926	0.40%	5092
15.29	NADP	1749	0.76%	13541
17.77	Xanthosine	17115	7.49%	116956
18.32	NAD	76	0.04%	918
21.06	Inosine	317	0.13%	1865
21.36	Adenine	382	0.16%	2067
27.29	Adenosine	441	0.19%	2171
28.84		550	0.24%	992
TOTALS:		228501	100.00%	



Supramaximal Exercise Sample

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AREA/PERCENT REPORT

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*****SAMPLE ID***** SOLVENT: [ ]
* * COLUMN: [ ]
* VIAL #: 0 * MEMO: [ ]
****

METHOD: [12] [staph file]
INTEGRATION: [ ] [ ]
SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [MIN] TO [35.00 MIN]

IS AMT: [1] WGT: [1] DIL FAC: [1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
7.18	IMP	39440	18.82%	160779
8.68	ATP	12c81c	c0.03%	410148
9.83	ADP	24986	11.9%	73408
13.73	AMP	148	0.07%	551
15.09	NADP	753	0.36%	5310
15.50	Xanthosine	1181	0.54%	9040
17.70	NAD	1555c	7.42%	118714
18.48	Inosine	634	0.30%	4348
TOTALS:		209487	100.00%	

SEQ INJ 1/ 1 SYSTEM[1
DATA FILE: VC-N-0.
01:48 01/01/1980

Appendix D

Data from Study 1

							SUBJECT A						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.85						
Oxygen Uptake (l.min)						3.04							3.17
Heart Rate (b.min)						171							145
RER						0.93							0.89
Weight (kg)	77				75.7			76.2				76.5	
Rectal Temperature (C)	37.8	38.3	38.8	39.2	39.7			37.5	38	38.3	38.4	38.6	
Muscle Temperature (C)	37.4				40.9			36.5				38.9	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	522				383			515				455	
Lactate (mmol.kg dw)	9.3				15.4			7.5				10.7	
Creatine Phosphate (mmol.kg dw)	91.5				47.5			88.3				89.2	
Creatine (mmol.kg dw)	50.8				94.8			54				53.1	
ATP (mmol.kg dw)	24.8				25.5			30.2				30.8	
ADP (mmol.kg dw)	2.2				2.3			2.4				2.3	
AMP (mmol.kg dw)	0.05				0.08			0.08				0.14	
IMP (mmol.kg dw)	0.03				0.08			0.04				0.05	
Ammonia (mmol.kg dw)	0.4				0.66			0.38				0.67	
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.5	5.1	5.5	6.2	6.6			4.2	4.5	4.5	4.7	4.8	
Ammonia (umol.l)	18.7	21	22	22.4	23.3			27.8	31.8	31.4	31	31	
FFA (mmol.l)													
Lactate (mmol.l)	0.9	1.8	2	2.5	2.6			0.8	1	0.9	0.9	0.8	
HORMONES													
Adrenaline (pg.ml.)													
Noradrenaline (ng.ml.)													
Insulin (uU.ml)	8.9	7.9			7.4			5.5	6.2			6.1	
Glucagon (pg.ml)	266.4				297.8			266.4				286.6	
Cortisol (ug.dl)	31.9	41.1			31			43.1	41.2			20	
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		60%							40%				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres	10%	51%	35%	10%	4%			90%	9%	1%	0%	0%	
Type II Fibres	91%	9%	0%	0%	0%			100%	0%	0%	0%	0%	

							SUBJECT B						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.08						
Oxygen Uptake (l.min)						2.67							2.63
Heart Rate (b.min)						181							152
RER						1.01							0.898
Weight (kg)	68.8				67.7			70				67.8	
Rectal Temperature (C)	37.9	38.1	38.5	39	39.6			37.4	37.7	38.1	38.4	38.6	
Muscle Temperature (C)	37				40.5			35.8				38.9	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	627				456			655				560	
Lactate (mmol.kg dw)	7.3				23.4			6				7.6	
Creatine Phosphate (mmol.kg dw)	47.5				43.3			65.4				60.9	
Creatine (mmol.kg dw)	56.3				60.5			38.4				42.9	
ATP (mmol.kg dw)	20.4				19.5			19.7				19.5	
ADP (mmol.kg dw)	2				2.5			1.8				1.8	
AMP (mmol.kg dw)	0.07				0.05			0.04				0.03	
IMP (mmol.kg dw)	0.61				0.66			0.08				0.06	
Ammonia (mmol.kg dw)	0.76				2.17			0.16				1.15	
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.6	4.6	5.2	5.3	5.3			4.5	4.5	4.9	4.9	5	
Ammonia (umol.l)	8.7	35.3	54.9	64.9	69.4			22.9	44.9	59.8	67.3	61.7	
FFA (mmol.l)													
Lactate (mmol.l)	2.1	4.1	4.5	5.4	5.5			1.6	2.1	2.1	2.3	2	
HORMONES													
Adrenaline (pg.ml.)													
Noradrenaline (ng.ml.)													
Insulin (uU.ml)	12.4	17			7.4			7.4	6.1			5.6	
Glucagon (pg.ml)	225				215.3			155.8				234.1	
Cortisol (ug.dl)	27.7	32.7			36.6			22.6	29.4			25.7	
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		59%							41%				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres	100%	0%	0%	0%	0%			100%	0%	0%	0%	0%	
Type II Fibres	100%	0%	0%	0%	0%			100%	0%	0%	0%	0%	

							SUBJECT C						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							5.12						
Oxygen Uptake (l.min)						3.46							3.56
Heart Rate (b.min)						169							156
RER						0.915							0.865
Weight (kg)	75.3				74.4			74.8				74.2	
Rectal Temperature (C)	36.9	37.8	38.2	38.9	39.7			36.7	37.4	37.9	38.4	38.8	
Muscle Temperature (C)	36				40.6			35.2				39.3	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	477				243			467				330	
Lactate (mmol.kg dw)	8.1				28.3			9.1				12.2	
Creatine Phosphate (mmol.kg dw)	72.9				42			68.5				65.7	
Creatine (mmol.kg dw)	32.3				63.2			36.7				39.5	
ATP (mmol.kg dw)	23.4				27.8			23.5				23	
ADP (mmol.kg dw)	1.9				2.6			1.9				1.8	
AMP (mmol.kg dw)	0.05				0.13			0.02				0.03	
IMP (mmol.kg dw)	0.2				0.41			0.06				0.08	
Ammonia (mmol.kg dw)	0.11				0.65			0.12				0.6	
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.8	5.2	5.5	5.6	5.2			4.3	4.2	4.4	4.4	4.4	
Ammonia (umol.l)	11.7	44.4	55.2	52.8	62.6			12	28.8	40	42.6	50.4	
FFA (mmol.l)													
Lactate (mmol.l)	1.3	3.4	2.6	2.6	3.2			0.8	2.5	1.9	1.8	1.6	
HORMONES													
Adrenaline (pg.ml.)													
Noradrenaline (ng.ml.)													
Insulin (uU.ml)	4.1	3			4.3			5	4.8			4.2	
Glucagon (pg.ml)	160.7				186.1			131.1				160.4	
Cortisol (ug.dl)	14.5	29.3			25.9			23	28.1			19.8	
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		59%							41%				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres	12%	45%	26%	10%	7%			22%	26%	37%	12%	3%	
Type II Fibres	94%	6%	0	0	0			79%	19%	2%	0	0	

							SUBJECT D						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.35						
Oxygen Uptake (l.mln)						2.5							2.63
Heart Rate (b.min)						156							138
RER						0.878							0.843
Weight (kg)	73.8				72.7			74				73.5	
Rectal Temperature (C)	37.1	38	38.4	38.9	39.3			37.2	37.4	37.8	37.9	38	
Muscle Temperature (C)	37				40.5			35.6				38.8	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	373				248			382				240	
Lactate (mmol.kg dw)	10.7				19.9			6.8				8.8	
Creatine Phosphate (mmol.kg dw)	76.4				44.7			60.9				57.6	
Creatine (mmol.kg dw)	42.9				74.6			39.2				61.7	
ATP (mmol.kg dw)	26.1				25.6			25				26.8	
ADP (mmol.kg dw)	2.3				3			2.2				2.4	
AMP (mmol.kg dw)	0.08				0.2			0.06				0.06	
IMP (mmol.kg dw)	0.1				0.61			0.02				0.01	
Ammonia (mmol.kg dw)	0.27				0.77			0.35				0.51	
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.7	4.8	5	5	5			4.3	4.2	4.4	4.4	4.4	
Ammonia (umol.l)	20	24.1	30	32.7	30.4			18.7	23.5	38.8	36.6	32.9	
FFA (mmol.l)													
Lactate (mmol.l)	0.9	1.1	1.1	1.2	1.4			1.1	0.9	0.8	0.7	0.8	
HORMONES													
Adrenaline (pg.ml.)													
Noradrenaline (ng.ml.)													
Insulin (uU.ml)	6.3	5.7			9			8.2	4.8			6.1	
Glucagon (pg.ml)	203.6				223.5			177.5				411.9	
Cortisol (ug.dl)	32.8	21.7			19.7			23.3	23.1			19.5	
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		64%							36%				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres	15%	42%	26%	10%	7%			35%	22%	39%	4%	0%	
Type II Fibres	72%	20%	8%	0%	0%			92%	7%	1%	0%	0%	

							SUBJECT E						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.16						
Oxygen Uptake (l.min)						3.02							2.85
Heart Rate (b.min)						182							139
RER						0.893							0.85
Weight (kg)	56.6				55.8			57.1				56.5	
Rectal Temperature (C)	37.8	38.3	39	39.6	40			37.4	37.8	38.4	38.7	38.9	
Muscle Temperature (C)	37.3				41.2			35.4				39.1	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	477				342			510				435	
Lactate (mmol.kg dw)	6				19.8			3.5				9.3	
Creatine Phosphate (mmol.kg dw)	80.8				69.8			79.9				78.5	
Creatine (mmol.kg dw)	43.7				54.7			44.6				46	
ATP (mmol.kg dw)	27				25			24.5				27	
ADP (mmol.kg dw)	2.2				2.2			1.9				2.6	
AMP (mmol.kg dw)	0.08				0.2			0.06				0.06	
IMP (mmol.kg dw)	0.1				0.04			0.05				0.21	
Ammonia (mmol.kg dw)	0.25				0.66			0.31				0.59	
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.8	5.2	5.8	5.8	5.9			4.1	4.7	4.9	5.3	5.3	
Ammonia (umol.l)	23.6	33	43.2	39.5	40.9			23.4	48.6	44.9	45.3	40.2	
FFA (mmol.l)													
Lactate (mmol.l)	0.9	1.8	1.7	1.6	2.2			1.2	1.5	1.5	1.5	1.44	
HORMONES													
Adrenaline (pg.ml.)													
Noradrenaline (ng.ml.)													
Insulin (uU.ml)	7.8	6.2			2.7			3.9	5.1			7.5	
Glucagon (pg.ml)	154.6				186			105.5				107.5	
Cortisol (ug.dl)	11.4	12.3			19.3			10.3	9.1			14.1	
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		71%							29%				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres	30%	36%	30%	4%	0%			46%	34%	10%	9%	1%	
Type II Fibres	68%	13%	18%	1%	0%			60%	36%	4%	0%	0%	

							SUBJECT F						
											RT		
			HT										
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							3.98						2.31
Oxygen Uptake (l.min)						2.34							174
Heart Rate (b.min)						180							0.89
RER						0.99							
Weight (kg)	61				60			60.8				60.3	
Rectal Temperature (C)	37.8	38.3	39	39.6	40			37.4	37.8	38.4	38.7	38.9	
Muscle Temperature (C)	38				40.3			35.5				39.3	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	414				125			399				138	
Lactate (mmol.kg dw)	7.3				34.1			10.1				23.8	
Creatine Phosphate (mmol.kg dw)	86.7				44.7			75				56.8	
Creatine (mmol.kg dw)	35.1				77.1			46.8				65	
ATP (mmol.kg dw)	23				20.7			23.5				23.3	
ADP (mmol.kg dw)	1.7				2.1			1.7				2	
AMP (mmol.kg dw)	0.02				0.13			0.03				0.03	
IMP (mmol.kg dw)	0.18				0.38			0.12				0.36	
Ammonia (mmol.kg dw)	0.36				1.71			0.56				1.19	
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.2	4.9	5.1	5.7	5.7			4.5	4.8	5.2	5.2	5.2	
Ammonia (umol.l)	22	50.7	68.5	72.8	87.2			29.9	60.1	75.3	84.9	83.9	
FFA (mmol.l)													
Lactate (mmol.l)	1.1	5.3	6.1	4.2	4			1.1	5.6	6.2	4.2	4	
HORMONES													
Adrenaline (pg.ml.)													
Noradrenaline (ng.ml.)													
Insulin (uU.ml)	7.3	4.9			3.6			8.2	4.8			6.1	
Glucagon (pg.ml)	291.8				404.6			371.9				536.3	
Cortisol (ug.dl)	18.1	21.6			24.4			10.8	12.6			22.8	
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		55%							45%				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres	7%	14%	19%	38%	22%			62%	30%	8%	0%	0%	
Type II Fibres	68%	13%	18%	1%	0%			60%	36%	4%	0%	0%	

							SUBJECT G						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.01						
Oxygen Uptake (l.min)						2.86							3.4
Heart Rate (b.min)						180							177
RER						0.998							0.876
Weight (kg)	65.77				64.58			66.06				65.16	
Rectal Temperature (C)	37.7	38	38.6	39.4	39.7			37	37.5	38.2	38.9	39.2	
Muscle Temperature (C)	36.5				40.5			35.7				38.9	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.1	4.9	6.7	7.8	8.5			4.3	5.2	6.1	7.3	8.2	
Ammonia (umol.l)	18.7	27.1	32.6	37.1	40			8.9	23.2	30.9	36.8	41.9	
FFA (mmol.l)	0.71	0.22	0.25	0.33	0.5			0.21	0.13	0.12	0.1	0.09	
Lactate (mmol.l)	1.1	3	3.6	7.8	7.2			1.6	3.9	5.3	6.1	5.4	
HORMONES													
Adrenaline (pg.ml.)	176	453.2			582.1			167	285			507.8	
Noradrenaline (ng.ml.)	0.88	3.49			7.51			1.02	3.61			5.51	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

							SUBJECT H						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.45						
Oxygen Uptake (l.min)						3.13							3.38
Heart Rate (b.min)						186							155
RER						0.928							0.85
Weight (kg)	61.26				59.91			61.9				60.98	
Rectal Temperature (C)	36.6	37.4	38.3	39.1	39.5			36.8	37.2	37.9	38.3	38.7	
Muscle Temperature (C)	36				39.9			35.2				39.2	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.2	4.5	4.8	5.2	5.3			4.2	4.5	4.8	4.9	4.7	
Ammonia (umol.l)	18.32	34.6	45.8	49.56	50.11			22.62	42.21	44.6	49.6	53.3	
FFA (mmol.l)	0.86	0.44	0.54	0.61	0.67			0.43	0.29	0.38	0.41	0.44	
Lactate (mmol.l)	0.9	3.1	3.5	3	2.6			1.4	1.8	2.5	1.8	1.8	
HORMONES													
Adrenaline (pg.ml.)	269.4	828.6			452.6			81.9	381.6			423.5	
Noradrenaline (ng.ml.)	0.28	2.06			2.6			0.35	1.11			1.7	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

							SUBJECT I						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.09						
Oxygen Uptake (l.min)						3.21							3.25
Heart Rate (b.min)						163							145
RER						0.915							0.925
Weight (kg)	72.68				71.13			72.12				71.41	
Rectal Temperature (C)	36.7	37.9	38.5	39	39.6			36.9	37.1	37.8	38.2	38.6	
Muscle Temperature (C)	36.4				39.9			36				39.4	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.4	5.1	5.8	6.4	7			4.3	4.3	4.6	5.1	5	
Ammonia (umol.l)	13.09	20.57	33.19	42.08	57.78			6.92	20.57	36.47	43.38	46.28	
FFA (mmol.l)	0.59	0.41	0.56	0.66	0.75			0.44	0.44	0.46	0.59	0.67	
Lactate (mmol.l)	1.7	3.5	4.7	7.5	6.6			1.3	2.5	2.2	2.6	3	
HORMONES													
Adrenaline (pg.ml.)	158.8	579.1			768			60.9	341.6			402.5	
Noradrenaline (ng.ml.)	0.56	3.08			4.68			0.6	1.48			3.92	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

							SUBJECT J						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.43						
Oxygen Uptake (l.min)						3.12							3.19
Heart Rate (b.min)						175							165
RER						0.95							0.918
Weight (kg)	67.21				66.16			67.34				66.58	
Rectal Temperature (C)	37.4	37.9	38.8	39.4	39.8			37	37	37.5	38.1	38.2	
Muscle Temperature (C)	34.8				40			35.4				39	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.3	5.2	5.7	6.2	6.5			4.2	4.3	5.1	5.2	5.3	
Ammonia (umol.l)	19.87	28.42	35.06	42.08	42			10.02	30.29	43.38	42.09	40.02	
FFA (mmol.l)	0.51	0.56	0.51	0.47	0.39			0.81	0.47	0.69	0.7	0.81	
Lactate (mmol.l)	0.9	2.9	2.9	3.4	3.8			0.8	2	1.8	1.9	2	
HORMONES													
Adrenaline (pg.ml.)	203.8	695.3			729.2			87.2	382.4			285.9	
Noradrenaline (ng.ml.)	0.39	4.79			2.46			0.53	2.37			3.73	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

							SUBJECT K						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							5.27						
Oxygen Uptake (l.min)						3.82							3.81
Heart Rate (b.min)						175							183
RER						0.883							0.858
Weight (kg)	75				73.88			75.87				74.92	
Rectal Temperature (C)	37	37.9	38.6	39.2	39.7			37.3	37.8	38.3	38.4	38.8	
Muscle Temperature (C)	38.4				40.4			38.6				38.8	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.7	4.1	4.5	5.2	5			4.2	3.6	3.9	3.9	4.1	
Ammonia (umol.l)	17.2	36.47	46.75	43.01	41.14			7.01	32.78	42.08	45.44	41.14	
FFA (mmol.l)	1.09	0.54	0.6	0.75	0.78			0.61	0.37	0.43	0.46	0.61	
Lactate (mmol.l)	1	2.9	3.4	3.5	2.8			0.8	4	4.4	3.9	3.4	
HORMONES													
Adrenaline (pg.ml.)	132	511.3			468.5			134.8	253.2			390.1	
Noradrenaline (ng.ml.)	0.37	3.11			5.29			0.4	1.85			2.87	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

							SUBJECT L						
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.18						
Oxygen Uptake (l.min)						2.99							3.42
Heart Rate (b.min)						188							175
RER						0.928							0.888
Weight (kg)	67.66				66.78			68.04				67.35	
Rectal Temperature (C)	36.8	37.9	38.6	39.1	39.5			37	37.7	38.2	38.6	38.7	
Muscle Temperature (C)	36.5				40			36.5				39	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.9	6.1	7	8.1	8.5			5.7	5	5.4	5.5	5.6	
Ammonia (umol.l)	17.76	21.13	28.05	26.37	29.92			16.36	22.44	25.71	29.92	34.57	
FFA (mmol.l)	0.61	0.4	0.5	0.43	0.46			0.35	0.24	0.26	0.28	0.31	
Lactate (mmol.l)	1.9	4.2	5.1	4.9	4.6			1.3	2.6	2	1.7	1.4	
HORMONES													
Adrenaline (pg.ml.)	221.3	870.5			1070.2			200.6	642.1			714.5	
Noradrenaline (ng.ml.)	0.35	2.85			4.19			0.7	2.15			2.98	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

						SUBJECT M							
			HT							RT			
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)							4.08						
Oxygen Uptake (l.min)						2.99							3.2
Heart Rate (b.min)						163							159
RER						0.918							0.865
Weight (kg)	62.14				61.18			60.71				60.05	
Rectal Temperature (C)	36.7	37.8	38.3	39	39.4			36.9	37.1	37.6	38.2	38.7	
Muscle Temperature (C)	36.7				40.4			35.3				39.2	
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)													
Lactate (mmol.kg dw)													
Creatine Phosphate (mmol.kg dw)													
Creatine (mmol.kg dw)													
ATP (mmol.kg dw)													
ADP (mmol.kg dw)													
AMP (mmol.kg dw)													
IMP (mmol.kg dw)													
Ammonia (mmol.kg dw)													
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.8	4.4	4.8	5.1	4.9			3.8	3.5	4.3	4.6	4.2	
Ammonia (umol.l)	28.24	30.38	38.47	37.4	42.08			14.03	35.56	45.82	49.95	56.1	
FFA (mmol.l)	0.48	0.32	0.37	0.37	0.35			0.54	0.42	0.49	0.58	0.67	
Lactate (mmol.l)	0.7	3.3	3.5	2.7	2			1.1	1.9	1.8	1.5	1.3	
HORMONES													
Adrenaline (pg.ml.)	118.7	552.2			748.5			118.1	227.6			348.7	
Noradrenaline (ng.ml.)	0.42	2.04			3.01			0.57	1.24			1.73	
Insulin (uU.ml)													
Glucagon (pg.ml)													
Cortisol (ug.dl)													
HISTOCHEMISTRY		*TYPE I*							*TYPE II*				
		HT RATING							*RT RATING*				
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0	
Type I Fibres													
Type II Fibres													

Appendix E

Data from Study 2

							SUBJECT A							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.85							4.82
Oxygen Uptake (l.min)						3.04							3.13	
Heart Rate (b.min)						171							158	
RER						0.93							0.89	
Weight (kg)	77				75.7			76.2				74.9		
Rectal Temperature (C)	37.8	38.3	38.8	39.2	39.7			36.8	37.7	38.2	38.7	39.2		
Muscle Temperature (C)	37.4				40.9			36.2				40		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)	522				383			604				492		
Lactate (mmol.kg dw)	9.3				15.4			7.3				14.6		
Creatine Phosphate (mmol.kg dw)	91.5				47.5			92.9				82.7		
Creatine (mmol.kg dw)	50.8				94.8			49.4				59.6		
ATP (mmol.kg dw)	24.8				25.5			28.3				25.4		
ADP (mmol.kg dw)	2.2				2.3			2.3				4.7		
AMP (mmol.kg dw)	0.05				0.08			0.05				0.99		
IMP (mmol.kg dw)	0.03				0.08			0.01				0.11		
Ammonia (mmol.kg dw)	0.4				0.66			0.29				0.99		
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.5	5.1	5.5	6.2	6.6			4.4	4.7	5	5	5		
Ammonia (umol.l)	18.7	21	22	22.4	23.3			26.1	32.7	30.9	28.1	32		
FFA (mmol.l)														
Lactate (mmol.l)	0.9	1.8	2	2.5	2.6			0.8	1.6	1.6	1.8	2.1		
HORMONES														
Adrenaline (pg.ml.)														
Noradrenaline (ng.ml.)														
Insulin (uU.ml)	8.9	7.9			7.4			8.5	7.9			7.4		
Glucagon (pg.ml)	266.4				297.8			258.2				350.9		
Cortisol (ug.dl)	31.9	41.1			31			18.5	27			30.6		
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		60%							40%					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres	10%	51%	35%	10%	4%			77%	23%	0%	0%	0%		
Type II Fibres	91%	9%	0%	0%	0%			89%	9%	2%	0%	0%		

							SUBJECT B							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.08							4.12
Oxygen Uptake (l.min)						2.67							2.46	
Heart Rate (b.min)						181							156	
RER						1.01							0.88	
Weight (kg)	68.8				67.7			70				68.9		
Rectal Temperature (C)	37.9	38.1	38.5	39	39.6			37.1	37.3	37.8	38.2	38.7		
Muscle Temperature (C)	37				40.5			36.6				39.9		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)	627				456			565				423		
Lactate (mmol.kg dw)	7.3				23.4			5.6				8		
Creatine Phosphate (mmol.kg dw)	47.5				43.3			58.8				52.6		
Creatine (mmol.kg dw)	56.3				60.5			45				51.2		
ATP (mmol.kg dw)	20.4				19.5			21.2				19.8		
ADP (mmol.kg dw)	2				2.5			1.7				1.8		
AMP (mmol.kg dw)	0.07				0.05			0.04				0.03		
IMP (mmol.kg dw)	0.61				0.66			0.15				0.05		
Ammonia (mmol.kg dw)	0.76				2.17			0.32				1.11		
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.6	4.6	5.2	5.3	5.3			4.4	4.1	4.4	4.4	4.6		
Ammonia (umol.l)	8.7	35.3	54.9	64.9	69.4			28	46.6	59.8	68.3	67		
FFA (mmol.l)														
Lactate (mmol.l)	2.1	4.1	4.5	5.4	5.5			1.8	2	2.2	2.3	2.1		
HORMONES														
Adrenaline (pg.ml.)														
Noradrenaline (ng.ml.)														
Insulin (uU.ml)	12.4	17			7.4			7.4	4.1			7.1		
Glucagon (pg.ml)	225				215.3			197.9				300.7		
Cortisol (ug.dl)	27.7	32.7			36.6			15.1	17.9			17.4		
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		59%							41%					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres	100%	0%	0%	0%	0%			100%	0%	0%	0%	0%		
Type II Fibres	100%	0%	0%	0%	0%			100%	0%	0%	0%	0%		

							SUBJECT C							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							5.12							5.09
Oxygen Uptake (l.min)						3.46							3.36	
Heart Rate (b.min)						169							158	
RER						0.915							0.898	
Weight (kg)	75.3				74.4			76.1				75		
Rectal Temperature (C)	36.9	37.8	38.2	38.9	39.7			36.9	37.4	38	38.7	39.3		
Muscle Temperature (C)	36				40.6			36				41.2		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)	477				243			502				321		
Lactate (mmol.kg dw)	8.1				28.3			11.3				18.6		
Creatine Phosphate (mmol.kg dw)	72.9				42			83.9				42.3		
Creatine (mmol.kg dw)	32.3				63.2			21.3				62.9		
ATP (mmol.kg dw)	23.4				27.8			25.7				25.5		
ADP (mmol.kg dw)	1.9				2.6			2.3				3.9		
AMP (mmol.kg dw)	0.05				0.13			0.07				0.28		
IMP (mmol.kg dw)	0.2				0.41			0.05				0.63		
Ammonia (mmol.kg dw)	0.11				0.65			0.22				0.96		
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.8	5.2	5.5	5.6	5.2			4.5	4.6	4.8	5.1	4.8		
Ammonia (umol.l)	11.7	44.4	55.2	52.8	62.6			14	34.8	42.4	43.8	44.9		
FFA (mmol.l)														
Lactate (mmol.l)	1.3	3.4	2.6	2.6	3.2			0.9	2.6	1.9	1.9	1.9		
HORMONES														
Adrenaline (pg.ml.)														
Noradrenaline (ng.ml.)														
Insulin (uU.ml)	4.1	3			4.3			5.5	4.6			4.3		
Glucagon (pg.ml)	160.7				186.1			187.7				364.1		
Cortisol (ug.dl)	14.5	29.3			25.9			16.5	23.6			19.9		
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		59%							41%					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres	12%	45%	26%	10%	7%			35%	57%	8%	0%	0%		
Type II Fibres	94%	6%	0	0	0			94%	6%	0%	0	0		

							SUBJECT D							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.35							4.26
Oxygen Uptake (l.min)						2.5							2.67	
Heart Rate (b.min)						156							145	
RER						0.878							0.85	
Weight (kg)	73.8				72.7			74.5				73.4		
Rectal Temperature (C)	37.1	38	38.4	38.9	39.3			36.9	37.3	37.8	38.1	38.4		
Muscle Temperature (C)	37				40.5			35.4				38.9		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)	373				248			370				261		
Lactate (mmol.kg dw)	10.7				19.9			10.1				14.7		
Creatine Phosphate (mmol.kg dw)	76.4				44.7			71.1				57.5		
Creatine (mmol.kg dw)	42.9				74.6			48.2				61.8		
ATP (mmol.kg dw)	26.1				25.6			21.8				25.9		
ADP (mmol.kg dw)	2.3				3			2.3				2.4		
AMP (mmol.kg dw)	0.08				0.2			0.64				0.1		
IMP (mmol.kg dw)	0.1				0.61			0.51				0.18		
Ammonia (mmol.kg dw)	0.27				0.77			0.31				0.72		
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.7	4.8	5	5	5			4.5	4.4	4.7	4.7	4.7		
Ammonia (umol.l)	20	24.1	30	32.7	30.4			28.5	28.8	28	33.6	31.8		
FFA (mmol.l)														
Lactate (mmol.l)	0.9	1.1	1.1	1.2	1.4			0.9	1.1	1.2	1.1	1.1		
HORMONES														
Adrenaline (pg.ml.)														
Noradrenaline (ng.ml.)														
Insulin (uU.ml)	6.3	5.7			9			12.9	7.7			16.9		
Glucagon (pg.ml)	203.6				223.5			140.7				157.7		
Cortisol (ug.dl)	32.8	21.7			19.7			15.4	19.4			13.2		
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		64%							36%					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres	15%	42%	26%	10%	7%			64%	33%	1%	0%	0%		
Type II Fibres	72%	20%	8%	0%	0%			100%	0%	0%	0%	0%		

							SUBJECT E							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.16							4.05
Oxygen Uptake (l.min)						3.02							2.87	
Heart Rate (b.min)						162							158	
RER						0.893							0.89	
Weight (kg)	56.6				55.8			57.8				55.6		
Rectal Temperature (C)	37.8	38.3	39	39.6	40			36.5	37.6	38.4	39.2	39.7		
Muscle Temperature (C)	37.3				41.2			36				40.3		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)	477				342			686				403		
Lactate (mmol.kg dw)	6				19.8			4.4				11		
Creatine Phosphate (mmol.kg dw)	80.8				69.8			77.7				64.4		
Creatine (mmol.kg dw)	43.7				54.7			46.8				60.1		
ATP (mmol.kg dw)	27				25			26.5				26.6		
ADP (mmol.kg dw)	2.2				2.2			2.2				2.3		
AMP (mmol.kg dw)	0.08				0.2			0.04				0.1		
IMP (mmol.kg dw)	0.1				0.04			0.18				0.17		
Ammonia (mmol.kg dw)	0.25				0.66			0.35				0.73		
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.8	5.2	5.8	5.8	5.9			4.8	4.8	5.5	5.6	5.8		
Ammonia (umol.l)	23.6	33	43.2	39.5	40.9			34.6	43	46.8	45.8	53.2		
FFA (mmol.l)														
Lactate (mmol.l)	0.9	1.8	1.7	1.6	2.2			1.3	2	1.8	2.2	1.8		
HORMONES														
Adrenaline (pg.ml.)														
Noradrenaline (ng.ml.)														
Insulin (uU.ml)	7.8	6.2			2.7			9.5	5.5			5.8		
Glucagon (pg.ml)	154.6				186			154.6				186		
Cortisol (ug.dl)	11.4	12.3			19.3			14.6	12.7			17.4		
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		71%							29%					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres	30%	36%	30%	4%	0%			44%	44%	7%	5%	0%		
Type II Fibres	68%	13%	18%	1%	0%			80%	20%	0%	0%	0%		

							SUBJECT F							
			HT				Pre HT			AT				Post AT
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean		0	10	20	30	40	Mean	
Maximal Oxygen Uptake (l.min)							3.98							3.57
Oxygen Uptake (l.min)						2.34							2.38	
Heart Rate (b.min)						180							171	
RER						0.99							0.95	
Weight (kg)	61				60			62.4				61.2		
Rectal Temperature (C)	37.8	38.3	39	39.6	40			36.9	37.1	37.8	38.4	39		
Muscle Temperature (C)	38				40.3			35.9				39.3		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)	414				125			384				282		
Lactate (mmol.kg dw)	7.3				34.1			12.4				27.1		
Creatine Phosphate (mmol.kg dw)	86.7				44.7			68.9				33.3		
Creatine (mmol.kg dw)	35.1				77.1			52.9				88.5		
ATP (mmol.kg dw)	23				20.7			22.5				22.6		
ADP (mmol.kg dw)	1.7				2.1			1.5				1.8		
AMP (mmol.kg dw)	0.02				0.13			0.03				0.05		
IMP (mmol.kg dw)	0.18				0.38			0.55				0.25		
Ammonia (mmol.kg dw)	0.36				1.71			0.6				1.16		
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.2	4.9	5.1	5.7	5.7			3.9	4.2	4.6	5.2	5.6		
Ammonia (umol.l)	22	50.7	68.5	72.8	87.2			34.5	51.4	59.8	60.8	56.1		
FFA (mmol.l)														
Lactate (mmol.l)	1.1	5.3	6.1	4.2	4			1.2	4.9	4.8	3.7	3.9		
HORMONES														
Adrenaline (pg.ml.)														
Noradrenaline (ng.ml.)														
Insulin (uU.ml)	7.3	4.9			3.6			4.8	4.4			4.8		
Glucagon (pg.ml)	291.8				404.6			333.9				351.9		
Cortisol (ug.dl)	18.1	21.6			24.4			7	8.2			24.4		
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		55%							45%					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres	7%	14%	19%	38%	22%			79%	13%	5%	2%	1%		
Type II Fibres	68%	13%	18%	1%	0%			56%	36%	8%	0%	0%		

							SUBJECT G							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.01							4.08
Oxygen Uptake (l.min)						2.86							3.1	
Heart Rate (b.min)						180							177	
RER						0.998							0.888	
Weight (kg)	65.77				64.58			64.99				63.15		
Rectal Temperature (C)	37.7	38	38.6	39.4	39.7			37.3	39.7	38.5	39.1	39.8		
Muscle Temperature (C)	36.5				40.5			36.2				39.7		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.1	4.9	6.7	7.8	8.5			4.3	4.5	4.8	5.7	6.3		
Ammonia (umol.l)	18.7	27.1	32.6	37.1	40			25.6	23.4	28.7	31.3	39.3		
FFA (mmol.l)	0.71	0.22	0.25	0.33	0.5			0.64	0.38	0.42	0.33	0.32		
Lactate (mmol.l)	1.1	3	3.6	7.8	7.2			0.7	2.16	3.17	3.76	3.41		
HORMONES														
Adrenaline (pg.ml.)	176	453.2			582.1			142.4	362.8			257.7		
Noradrenaline (ng.ml.)	0.88	3.49			7.51			0.53	2.98			6.91		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

							SUBJECT H							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.45							4.37
Oxygen Uptake (l.min)						3.13							3.15	
Heart Rate (b.min)						186							175	
RER						0.928							0.848	
Weight (kg)	61.26				59.91			61.66				60.12		
Rectal Temperature (C)	36.6	37.4	38.3	39.1	39.5			36.5	37.2	37.9	38.5	39.2		
Muscle Temperature (C)	36				39.9			35.6				40.1		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.2	4.5	4.8	5.2	5.3			4.1	4.1	4.5	4.8	4.8		
Ammonia (umol.l)	18.32	34.6	45.8	49.56	50.11			22.44	36.09	42.08	45.44	48.15		
FFA (mmol.l)	0.86	0.44	0.54	0.61	0.67			0.69	0.4	0.55	0.57	0.47		
Lactate (mmol.l)	0.9	3.1	3.5	3	2.6			0.9	2	2.3	2.6	2.2		
HORMONES														
Adrenaline (pg.ml.)	269.4	828.6			452.6			229	502.2			452.4		
Noradrenaline (ng.ml.)	0.28	2.06			2.6			0.13	1.5			3		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

							SUBJECT I							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.09							4.15
Oxygen Uptake (l.min)						3.21							3.08	
Heart Rate (b.min)						163							157	
RER						0.915							0.893	
Weight (kg)	72.68				71.13			71.36				70		
Rectal Temperature (C)	36.7	37.9	38.5	39	39.6			36.6	37.7	38.3	38.9	39.4		
Muscle Temperature (C)	36.4				39.9			35.6				40.6		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.4	5.1	5.8	6.4	7			4.1	4.1	4.7	5.1	5.5		
Ammonia (umol.l)	13.09	20.57	33.19	42.08	57.78			22.07	23.84	32	43.38	48.1		
FFA (mmol.l)	0.59	0.41	0.55	0.66	0.75			0.64	0.45	0.44	0.52	0.6		
Lactate (mmol.l)	1.7	3.5	4.7	7.5	6.6			1.2	3.2	4.1	5.5	5.2		
HORMONES														
Adrenaline (pg.ml.)	158.8	579.1			768			66.6	352.4			341.1		
Noradrenaline (ng.ml.)	0.56	3.08			4.68			0.74	2.62			3.59		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

							SUBJECT J							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.43							4.44
Oxygen Uptake (l.min)						3.12							2.84	
Heart Rate (b.min)						175							161	
RER						0.95							0.943	
Weight (kg)	67.21				66.16			67.43				66.39		
Rectal Temperature (C)	37.4	37.9	38.8	39.4	39.8			36.6	37.4	38.2	38.8	39.3		
Muscle Temperature (C)	34.8				40			35.6				39.9		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.3	5.2	5.7	6.2	6.5			4.7	5.1	5.7	6.1	6		
Ammonia (umol.l)	19.87	28.42	35.06	42.08	42			22.44	34.6	41.14	40.76	41.33		
FFA (mmol.l)	0.51	0.56	0.51	0.47	0.39			0.6	0.44	0.52	0.56	0.62		
Lactate (mmol.l)	0.9	2.9	2.9	3.4	3.8			0.8	2.5	2.3	2.3	1.7		
HORMONES														
Adrenaline (pg.ml.)	203.8	695.3			729.2			250	444.1			353.8		
Noradrenaline (ng.ml.)	0.39	4.79			2.46			0.44	2.5			3.22		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

							SUBJECT K							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							5.27							4.85
Oxygen Uptake (l.min)						3.62							3.49	
Heart Rate (b.min)						175							169	
RER						0.883							0.835	
Weight (kg)	75				73.68			73.43				72.04		
Rectal Temperature (C)	37	37.9	38.6	39.2	39.7			37.2	37.8	38.6	39	39.5		
Muscle Temperature (C)	38.4				40.4			38.6				40.2		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.7	4.1	4.5	5.2	5			4.4	4.2	4.5	4.7	4.6		
Ammonia (umol.l)	17.2	36.47	46.75	43.01	41.14			24.77	40.21	43.01	35.53	37.4		
FFA (mmol.l)	1.09	0.54	0.6	0.75	0.78			1	0.92	1.28	1.08	1.27		
Lactate (mmol.l)	1	2.9	3.4	3.5	2.8			0.7	2	2.6	2.1	2.1		
HORMONES														
Adranaline (pg.ml.)	132	511.3			468.5			32.8	330.7			289.7		
Noradrenaline (ng.ml.)	0.37	3.11			5.29			0.22	3.14			4.56		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

							SUBJECT L							
			HT							AT				
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.18							4
Oxygen Uptake (l.min)						2.99							2.86	
Heart Rate (b.min)						188							180	
RER						0.928							0.928	
Weight (kg)	67.66				66.78			67.27				66.22		
Rectal Temperature (C)	36.8	37.9	38.6	39.1	39.5			37.7	37.9	38.5	39.1	39.5		
Muscle Temperature (C)	36.5				40			38.7				40.5		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.9	6.1	7	8.1	8.5			4.8	5.5	5.6	5.5	5.7		
Ammonia (umol.l)	17.76	21.13	28.05	26.37	29.92			18.05	25.24	28.98	31.79	32.73		
FFA (mmol.l)	0.61	0.4	0.5	0.43	0.46			0.35	0.28	0.31	0.35	0.38		
Lactate (mmol.l)	1.9	4.2	5.1	4.9	4.6			1	2.77	1.9	1.8	2		
HORMONES														
Adrenaline (pg.ml.)	221.3	870.5			1070.2			46.1	653			390		
Noradrenaline (ng.ml.)	0.35	2.85			4.19			0.142	2.31			2.37		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

						SUBJECT M								
										AT				
			HT											
PHYSIOLOGICAL PARAMETERS	0	10	20	30	40	Mean	Pre HT	0	10	20	30	40	Mean	Post AT
Maximal Oxygen Uptake (l.min)							4.08							4.15
Oxygen Uptake (l.min)						2.99							3.1	
Heart Rate (b.min)						163							154	
RER						0.918							0.858	
Weight (kg)	62.14				61.18			61.72				60.04		
Rectal Temperature (C)	36.7	37.8	38.3	39	39.4			36.8	37.3	37.8	38.3	38.7		
Muscle Temperature (C)	36.7				40.4			33.7				39.7		
MUSCLE METABOLITES														
Glycogen (mmol.kg dw)														
Lactate (mmol.kg dw)														
Creatine Phosphate (mmol.kg dw)														
Creatine (mmol.kg dw)														
ATP (mmol.kg dw)														
ADP (mmol.kg dw)														
AMP (mmol.kg dw)														
IMP (mmol.kg dw)														
Ammonia (mmol.kg dw)														
PLASMA/BLOOD METABOLITES														
Glucose (mmol.l)	4.8	4.4	4.8	5.1	4.9			4.8	5.2	5.1	5.1	4.8		
Ammonia (umol.l)	28.24	30.38	36.47	37.4	42.08			24.31	32.72	39.27	37.4	36.94		
FFA (mmol.l)	0.48	0.32	0.37	0.37	0.35			0.6	0.55	0.68	0.63	0.55		
Lactate (mmol.l)	0.7	3.3	3.5	2.7	2			1.3	2.9	2.7	2.4	1.8		
HORMONES														
Adrenaline (pg.ml.)	118.7	552.2			748.5			319	665.1			578		
Noradrenaline (ng.ml.)	0.42	2.04			3.01			0.23	1.71			3.17		
Insulin (uU.ml)														
Glucagon (pg.ml)														
Cortisol (ug.dl)														
HISTOCHEMISTRY		*TYPE I*							*TYPE II*					
		HT RATING							*AT RATING*					
Post-exercise PAS stain	4	3	2	1	0			4	3	2	1	0		
Type I Fibres														
Type II Fibres														

Appendix F

Data from Study 3

												SUBJECT A										
				HT								CT						RT				
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	20	30	40	Mean		Pre-Exp	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)								5.12														
Oxygen Uptake (l.min)							3.28								3.17							3.24
Heart Rate (b.min)							188								161							180
RER							0.883								0.878							0.853
Rectal Temperature (C)	36.4	36.6	36.9	36.6	39.1	39.8			36.5	36.3	36.4	37	37.5	37.9			37.2	37.9	38.6	39	39.2	
Muscle Temperature (C)	35.4	36.4				41			35	32.9				38.3			36.6				39.6	
MUSCLE METABOLITES																						
Glycogen (mmol.kg dw)	541	520				308			592	585				388			481				342	
Lactate (mmol.kg dw)	5.2	5.8				10.1			3.7	8.9				7.6			3				7.8	
Creatine Phosphate (mmol.kg dw)	85.7	87				60.7			85.2	83.8				73.8			85.6				89	
Creatine (mmol.kg dw)	38	34.7				61			38.5	37.9				47.6			38.1				52.7	
ATP (mmol.kg dw)	27.4	24.9				26.1			23.9	25.6				26.9			26.6				25.5	
ADP (mmol.kg dw)	3.01	2.59				3.6			2.6	2.84				3.24			2.78				3.02	
AMP (mmol.kg dw)	0.06	0.06				0.17			0.12	0.12				0.31			0.1				0.1	
IMP (mmol.kg dw)	0.07	0.04				0.06			0.09	0.21				0.1			0.05				0.2	
Ammonia (mmol.kg dw)	0.33	0.19				1.41			0.66	0.33				1.53			0.13				1.52	
PLASMA/BLOOD METABOLITES																						
Glucose (mmol.l)	4.6	4.1	4.2	4.7	5.3	5.7			4.6	4.3	4.5	4.7	5	4.8			4.2	4.1	4.4	4.6	4.6	
Ammonia (umol.l)	18.9	43	41.2	60.7	72	82.3			22.4	15	17.4	41.1	58.4	81.7			21.5	47.7	78.7	100	104.7	
Lactate (mmol.l)	1.2	1.4	1.9	2.2	1.8	2			1	1	1.8	2	1.6	1.3			0.8	1.3	1.3	1	1	
HORMONES																						
Adrenaline (pg.ml.)	31.8	54.8	172.8			312.7			23.7	25.7	47.4			184.8			20.3	184			228.8	
Noradrenaline (ng.ml.)	0.15	0.09	0.94			2.22			0.18	0.44	0.98			0.97			0.23	1.28			1.68	

												SUBJECT B										
																			RT			
				HT								CT										
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	20	30	40	Mean		Pre-Exp	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)								4.58														2.8
Oxygen Uptake (l.min)							2.92								2.98							145
Heart Rate (b.min)							182								137							0.83
RER							0.88								0.9							
Rectal Temperature (C)	38.8	38.8	37.4	38.2	38.8	39.2			37.2	37	37.3	37.7	37.8	38			38.5	37.1	37.8	38	38.2	
Muscle Temperature (C)	34	35.3				40.3			35.6	34.2				37			34.4				39.2	
MUSCLE METABOLITES																						
Glycogen (mmol.kg dw)	884	857				375			882	887				817			586				436	
Lactate (mmol.kg dw)	5.1	3.1				19.5			9.8	13.8				5.7			8.1				11.1	
Creatine Phosphate (mmol.kg dw)	92.1	91.4				52.8			90.8	88.3				88.9			94.3				88	
Creatine (mmol.kg dw)	38.2	38.9				77.7			39.7	42				43.4			38				84.3	
ATP (mmol.kg dw)	27.3	28.4				21.8			25.8	27.2				28.1			25.5				25.4	
ADP (mmol.kg dw)	2.49	3.1				2.92			2.98	2.87				3.22			3.06				3.18	
AMP (mmol.kg dw)	0.08	0.04				0.29			0.1	0.08				0.08			0.07				0.08	
IMP (mmol.kg dw)	0.07	0.05				0.36			0.13	0.38				0.04			0.04				0.13	
Ammonia (mmol.kg dw)	0.38	0.4				1.32			0.31	0.38				0.39			0.12				0.9	
PLASMA/BLOOD METABOLITES																						
Glucose (mmol.l)	4.4	4.7	4.4	5.2	5.8	5.7			4.5	4.5	4.1	4.8	4.8	4.8			4.5	4.2	4.8	4.8	4.7	
Ammonia (umol.l)	18.7	16	42.1	57	61.7	65.5			15	10.2	20.8	48.8	47.7	35.5			20.8	30.9	48.8	53.3	58.1	
Lactate (mmol.l)	0.8	0.8	5.9	7.7	7.5	8.7			1.8	1.8	1.8	1.7	1.5	1.3			2.1	1.4	1.8	1.3	1.2	
HORMONES																						
Adrenaline (pg.ml.)	23.2	30	131.1			183.1			34.8	31.4	92.2			117.2			58.7	77.7			148.1	
Noradreneline (ng.ml.)	0.32	0.18	0.97			2.37			0.25	0.49	1.25			0.97			0.64	1.02			1.46	

												SUBJECT C									
												CT							RT		
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	HT 20	30	32	Mean		Pre-Exp	0	10	20	30	32	Mean		0	10	20	30	32	Mean
Maximal Oxygen Uptake (l.min)								4.97													
Oxygen Uptake (l.min)							3.22								3.17						3.22
Heart Rate (b.min)							177								163						181
RER							0.91								0.89						0.88
Rectal Temperature (C)	37.4	37.5	37.7	38.5	39.8	39.8			37.4	37.2	37.2	37.9	38.4	38.4			37.6	37.7	38.1	38.7	38.8	
Muscle Temperature (C)	34.8	35.7				40			35.1	34.2				38.8			38				38.7	
MUSCLE METABOLITES																					
Glycogen (mmol.kg dw)	843	800				559			792	735				658			952				745	
Lactate (mmol.kg dw)	5.3	10.1				21.3			5.2	8.7				11.8			4.2				6.8	
Creatine Phosphate (mmol.kg dw)	98.3	97.8				38.7			87	88.2				70.4			97.7				89.9	
Creatine (mmol.kg dw)	38.4	37				98.1			47.8	46.6				64.4			37.1				44.9	
ATP (mmol.kg dw)	22.7	23.2				34.9			23.5	28				24.5			25.8				27.3	
ADP (mmol.kg dw)	2.48	2.88				4.48			2.09	3.49				2.99			2.51				3.38	
AMP (mmol.kg dw)	0.05	0.05				0.11			0.05	0.14				0.07			0.06				0.13	
IMP (mmol.kg dw)	0.08	0.2				0.2			0.01	0.07				0.09			0.03				0.02	
Ammonia (mmol.kg dw)	0.15	0.59				1.59			0.27	0.44				1.08			0.72				1.17	
PLASMA/BLOOD METABOLITES																					
Glucose (mmol.l)	4	4.8	4.3	5	5.8				4.9	4.8	4.9	5.4	5.5				4	4.7	4.8	4.8		
Ammonia (umol.l)	11.7	33.7	42	58.1	83.8				20	14	23.4	39.2	51.4				21.5	47.7	59.9	83.1		
Lactate (mmol.l)	1	1.2	4.2	4.4	5.4				1.2	1	2.4	2.3	1.7				1.2	3.3	2.4	2.1		
HORMONES																					
Adrenaline (pg.ml)	23.9	55.6	304.8			347.3			21.3	21.5	62			108.4			20.5	11.4			139.1	
Noradrenaline (ng.ml)	0.28	0.17	2.25			3			0.21	0.64	1.44			1,35			0.23	1.88			1.69	

												SUBJECT D									
				HT								CT							RT		
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	20	30	40	Mean		Pre-Exp	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)								6.00														
Oxygen Uptake (l.min)							3.26								3							3.28
Heart Rate (b.min)							157								139							148
RER							0.91								0.9							0.84
Rectal Temperature (C)	37.2	37.3	37.7	38.1	38.7	39.4			36.1	36.2	36.5	36.8	37.4	37.6			36.6	36.8	37.3	37.7	38	
Muscle Temperature (C)	36.4	36.6				41.2			34.7	34.4				39.4			35.4				39.4	
MUSCLE METABOLITES																						
Glycogen (mmol.kg dw)	484	463				270			395	409				250			399				210	
Lactate (mmol.kg dw)	8	8.8				8.9			4.1	5.5				8.3			4.3				5.7	
Creatine Phosphate (mmol.kg dw)	97.5	98.9				80			95.8	92.5				84.2			94.5				89.8	
Creatine (mmol.kg dw)	38.4	34				73.9			38.3	41.4				89.7			39.4				84.1	
ATP (mmol.kg dw)	27.4	26.4				26.2			27.2	26.1				27.2			26.3				27.6	
ADP (mmol.kg dw)	2.88	2.62				2.92			2.69	2.82				3.34			2.89				2	
AMP (mmol.kg dw)	0.19	0.21				0.18			0.21	0.18				0.19			0.18				0.21	
IMP (mmol.kg dw)	0.1	0.11				0.18			0.08	0.09				0.23			0.09				0.17	
Ammonia (mmol.kg dw)	0.18	0.48				0.91			0.41	0.38				0.7			0.5				1.35	
PLASMA/BLOOD METABOLITES																						
Glucose (mmol.l)	4.3	8.5	4.2	4	4.8	4.9			3.8	4.1	3.8	4.1	3.7	3.8			4.1	4.2	4.5	4.5	4.4	
Ammonia (umol.l)	24.3	25.2	44	50.5	60	49.5			13.1	9.4	18	31.8	35.5	30.9			18.7	33.7	54.2	54.2	60.8	
Lactate (mmol.l)	1.4	1.8	2.2	2.1	2.4	2.7			0.9	0.9	1.8	1.8	1.5	1.3			0.9	1.6	1.5	1.3	1	
HORMONES																						
Adrenaline (pg.ml.)	32.7	41.5	331.7			494			10.4	10.1	101.5			169.2			34.9	184			281	
Noradrenaline (ng.ml.)	0.39	0.45	1.87			2.87			0.37	0.42	1.06			1.21			0.34	1.09			1.3	

												SUBJECT E									
				HT								CT							RT		
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	20	30	39	Mean		Pre-Exp	0	10	20	30	39	Mean		0	10	20	30	39	Mean
Maximal Oxygen Uptake (l.min)								4.11														
Oxygen Uptake (l.min)							2.39								2,76							2.89
Heart Rate (b.min)							183								141							145
RER							0.88								0.87							0.87
Rectal Temperature (C)	37.2	37.5	37.9	38.5	38.9	39.2			37.3	37.2	37.1	37.8	37.8	37.7			38.7	37.2	37.7	38	38.3	
Muscle Temperature (C)	35.8	38.9				41.1			36.1	35.8				38.9			35.1				38.9	
MUSCLE METABOLITES																						
Glycogen (mmol.kg dw)	522	636				285			573	593				494			528				345	
Lactate (mmol.kg dw)	4.8	4.9				9.2			4.1	4.9				6.6			4.8				7.4	
Creatine Phosphate (mmol.kg dw)	90.9	88.7				81.8			89.8	88.7				78.1			86.4				81.1	
Creatine (mmol.kg dw)	45.8	48				88.2			48.9	47.9				80.6			50.3				75.6	
ATP (mmol.kg dw)	24.8	25.5				26.7			28.3	23.9				29.1			25.3				25.8	
ADP (mmol.kg dw)	2.18	2.34				2.58			2.58	2.27				3.25			2.42				2.9	
AMP (mmol.kg dw)	0.1	0.11				0.13			0.11	0.1				0.14			0.2				0.2	
IMP (mmol.kg dw)	0.09	0.07				0,14			0.07	0.06				0.09			0.07				0.14	
Ammonia (mmol.kg dw)	0.35	0.32				1.42			0.88	0.11				2.05			0.49				1,18	
PLASMA/BLOOD METABOLITES																						
Glucose (mmol.l)	4.2	4.3	4.3	4.8	5.5	6			4.3	4.8	3.9	4	4.3	4.7			3.5	3.2	3.8	4	4	
Ammonia (umol.l)	29	27.1	36.4	40.2	44.9	43.9			30	14	20.6	37.4	38.3	34.6			18.2	28.1	39.3	44.9	41.1	
Lactate (mmol.l)	1.2	0.9	2.4	2.7	2.9	3			0.9	1.1	2.1	1.7	1.8	1.9			0.8	1.8	2.2	1.8	1.8	
HORMONES																						
Adrenaline (pg.ml.)	81.8	33.4	178.8			380			18.7	29.7	84.8			288.8			11.9	103.2			338.3	
Noradrenaline (ng.ml.)	0.2	0.11	1.08			2.19			0.28	0.62	1.52			1.4			0.57	1.35			1.44	

												SUBJECT F										
				HT								CT								RT		
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	20	30	40	Mean		Pre-Exp	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)								4.98														
Oxygen Uptake (l.min)							2.88								3.18							3.28
Heart Rate (b.min)							186								181							183
RER							0.9								0.91							0.93
Rectal Temperature (C)	36.6	36.9	37.2	37.8	38.5	39.3			36.7	37	37.1	37.6	37.7	37.9			36.9	37.3	37.8	38.2	38.5	
Muscle Temperature (C)	35.9	36.2				41.1			35.7	34.7				36.4			36.1				36.6	
MUSCLE METABOLITES																						
Glycogen (mmol.kg dw)	480	509				237			537	499				318			674				409	
Lactate (mmol.kg dw)	3.7	2				24.4			8	2.9				8.9			3.9				14.4	
Creatine Phosphate (mmol.kg dw)	94.4	94.3				42			121.5	101.5				78.8			108.3				58.3	
Creatine (mmol.kg dw)	43.4	45.5				97.9			18.4	38.3				83			33.5				83.5	
ATP (mmol.kg dw)	27.9	25.9				28.2			28.1	27				29			28.3				25.5	
ADP (mmol.kg dw)	2.51	2.91				3.1			2.22	3.03				2.87			3.12				3	
AMP (mmol.kg dw)	0.06	0.06				0.09			0.09	0.06				0.08			0.08				0.06	
IMP (mmol.kg dw)	0.06	0.06				0.4			0.24	0.03				0.11			0.05				0.1	
Ammonia (mmol.kg dw)	0.68	0.04				1			0.28	0.04				0.65			0.12				1.18	
PLASMA/BLOOD METABOLITES																						
Glucose (mmol.l)	4.8	4.9	5	5	4.7	4.7			4.1	4	4	3.7	3.8	3.8			4.2	3.8	4.1	4	4	
Ammonia (umol.l)	7	14	39.3	56.1	82.3	84.1			8.9	8	21.5	41.1	59.8	57.9			7.8	22.9	35.5	44.9	51.9	
Lactate (mmol.l)	0.9	0.9	3.1	2.8	3.3	2.9			1.5	1.3	2.9	2.7	2.4	2			1	2.8	2.8	2.5	2.4	
HORMONES																						
Adrenaline (pg.ml.)	18.5	39.8	143.3			167.7			49.8	47.8	70.9			107.2			35.5	105.8			140.8	
Noradrenaline (ng.ml.)	0.32	0.1	0.93			2.6			0.28	0.41	1.08			0.79			0.23	1.28			1.86	

												SUBJECT G										
				HT								CT							RT			
PHYSIOLOGICAL PARAMETERS	Pre-Exp	0	10	20	30	40	Mean		Pre-Exp	0	10	20	30	40	Mean		0	10	20	30	40	Mean
Maximal Oxygen Uptake (l.min)								5.02														
Oxygen Uptake (l.min)							3.8								3.42							3.12
Heart Rate (b.min)							152								129							137
RER							0.92								0.88							0.9
Rectal Temperature (C)	38.8	37.1	37.3	37.8	38.4	39.1			38.8	35.7	35.8	35.6	36.1	38.6			38.2	37	38	38.3	38.7	
Muscle Temperature (C)	34.5	34.5				40.3			34.4	34.2				38.8			34.4				39.1		
MUSCLE METABOLITES																						
Glycogen (mmol.kg dw)	695	638				348			884	853				408			887				458	
Lactate (mmol.kg dw)	8.7	12.1				34.2			8.8	8.1				18.8			3.5				27.8	
Creatine Phosphate (mmol.kg dw)	85.8	84.7				33.9			84.2	44.8				94			52.9				68.3	
Creatine (mmol.kg dw)	51.8	52.9				103.7			53.4	92.8				43.8			84.7				83.5	
ATP (mmol.kg dw)	22	22.7				22.8			24.2	24.8				24.1			25.8				25.8	
ADP (mmol.kg dw)	2.59	2.58				3.58			3.48	3.33				3.81			3				4.32	
AMP (mmol.kg dw)	0.08	0.05				0.12			0.09	0.19				0.18			0.17				0.15	
IMP (mmol.kg dw)	0.03	0.05				0.24			0.11	0.07				0.15			0.04				0.23	
Ammonia (mmol.kg dw)	0.17	0.22				1.3			0.62	0.3				0.48			0.18				0.77	
PLASMA/BLOOD METABOLITES																						
Glucose (mmol.l)	4.1	4.4	4	4	4.1	4.3			4.2	4.2	3.9	4.2	4	3.8			3.7	3.6	3.4	3.7	4	
Ammonia (umol.l)	28.2	35.6	43	52.8	67.3	70			38.6	19	39.3	48.6	49.8	54.2			28.2	35.6	43	52.8	57.9	
Lactate (mmol.l)	1	1	3.8	4.9	5.1	5.2			1.3	1.6	3.2	2.8	3.2	2.9			1.2	2.5	3.1	3.2	3.2	
HORMONES																						
Adrenaline (pg.ml.)	17.9	28.4	110.7			172			38.8	34.1	81.9			133.1			17.1	77.8			159.5	
Noradrenaline (ng.ml.)	0.21	0.13	1.19			2.83			0.4	0.05	1.2			1.23			0.22	0.08			1.07	

Appendix G

Data from Study 4

							SUBJECT A						
				HT						CT			
					Recovery						Recovery		
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	10	15		Pre-Ex	Post-Ex	5	10	15	
Maximal Oxygen Uptake (l/min)							3.41						
Rectal Temperature (C)	36.8	36.8	36.9					36.8	36.9				
Muscle Temperature (C)	33.9	37.2	37.2					35.5	36.8				
MUSCLE METABOLITES													
Glycogen (mmol/kg dw)	452	440	249					370	254				
Lactate (mmol/kg dw)	3.2	7.1	130					3	104.8				
Creatine Phosphate (mmol/kg dw)	85.6	87.8	13.3					97	31.5				
Creatine (mmol/kg dw)	60	48.9	122.5					63.3	104.8				
ATP (mmol/kg dw)	21.7	24.2	11.8					26.6	16.7				
ADP (mmol/kg dw)	1.82	2.24	1.93					2.36	2.11				
AMP (mmol/kg dw)	0.06	0.17	0.01					0.09	0.09				
IMP (mmol/kg dw)	0.02	0.04	7.59					0.04	6.65				
Inosine (mmol/kg dw)	<0.01	<0.01	0.18					<0.01	0.1				
Hypoxanthine (mmol/kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol/kg dw)	0.59	0.7	7.04					1.09	7.61				
PLASMA/BLOOD METABOLITES													
Glucose (mmol/l)	4.3	4.6	4.6	4.5	4.7	5.5		4.4	4.8	5.3	5.5	5.8	
Ammonia (umol/l)	11.7	22	33.1	47.9	61.6	61.7		18.2	22.5	57	87.7	95.8	
Lactate (mmol/l)	1	1.2	8	11.7	14.1	14.3		1.7	9.1	11.2	12.5	12.6	
pH	7.33	7.35	7.1	7.2	7.19	7.19		7.36	7.22	7.21	7.17	7.18	
HORMONES													
Adrenaline (pg/ml.)	12.6	32.7	1415					32.7	2111				
Noradrenaline (ng/ml.)	0.29	0.34	2.09					0.2	3.09				

							SUBJECT B						
				HT						CT			
					Recovery						Recovery		
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	10	15		Pre-Ex	Post-Ex	5	10	15	
Maximal Oxygen Uptake (l/min)							4.87						
Rectal Temperature (C)	37	36.9	37					37.1	37.4				
Muscle Temperature (C)	35.6	37.3	37.3					35.3	37.3				
MUSCLE METABOLITES													
Glycogen (mmol/kg dw)	350	315	242					490	461				
Lactate (mmol/kg dw)	3.8	3.8	97.3					3.4	66.3				
Creatine Phosphate (mmol/kg dw)	76.4	81.1	4.9					80	19.8				
Creatine (mmol/kg dw)	36.1	31.5	133.9					29.7	93.6				
ATP (mmol/kg dw)	23.6	23.3	16.6					25.4	17.2				
ADP (mmol/kg dw)	2.2	2.3	2.33					2.45	2.03				
AMP (mmol/kg dw)	0.03	0.05	0.09					0.05	0.1				
IMP (mmol/kg dw)	0.03	0.02	3.38					0.05	1.8				
Inosine (mmol/kg dw)	<0.01	<0.01	0.11					0.02	0.07				
Hypoxanthine (mmol/kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol/kg dw)	0.49	0.27	4.5					0.42	1.1				
PLASMA/BLOOD METABOLITES													
Glucose (mmol/l)	4	4.1	4.6	4.1	4.1	4.3		4.1	4.5	4.5	4.4	4.5	
Ammonia (umol/l)	13.5	11.2	16.8	21	22.5	12		11.7	16.8	28.1	30	27	
Lactate (mmol/l)	1.1	0.7	7	6	5.9	5.3		1.2	5	7.7	7	6.6	
pH	7.36	7.18	7.21	7.24	7.26	7.3		7.3	7.25	7.24	7.25	7.26	
HORMONES													
Adrenaline (pg/ml.)	23.7	24.9	411.8					10.1	264.3				
Noradrenaline (ng/ml.)	0.28	0.25	2.14					0.14	1.13				

							SUBJECT C						
				HT						CT			
					Recovery						Recovery		
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	10	15		Pre-Ex	Post-Ex	5	10	15	
Maximal Oxygen Uptake (l.min)							4.53						
Rectal Temperature (C)	37.1	37.1	37.2					37	37.1				
Muscle Temperature (C)	35.1	37.3	37.4					35.5	37				
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	313	286	209					622	341				
Lactate (mmol.kg dw)	4.9	6.6	69.8					7.8	59.2				
Creatine Phosphate (mmol.kg dw)	83.6	87.3	42.1					84.6	37.3				
Creatine (mmol.kg dw)	65.7	51.9	97					64.6	101.9				
ATP (mmol.kg dw)	26.3	20.3	17.2					24.6	19				
ADP (mmol.kg dw)	2.79	2.38	2.64					2.59	2.16				
AMP (mmol.kg dw)	0.09	0.1	0.06					0.1	0.06				
IMP (mmol.kg dw)	0.12	0.07	3.1					0.05	1.61				
Inosine (mmol.kg dw)	<0.01	0.01	0.15					0.02	0.03				
Hypoxanthine (mmol.kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol.kg dw)	0.49	0.6	4.74					0.39	2.08				
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.2	4.7	5.1	4.8	5	5.1		4.5	5.9	5.4	5.4	5.5	
Ammonia (umol.l)	10.9	11.7	25.7	29.5	32.7	30		9.7	16	20	23.1	21.7	
Lactate (mmol.l)	1.6	0.8	8.8	7.2	7.9	8.4		1.6	7.2	7	7	6.8	
pH	7.33	7.36	7.13	7.16	7.18	7.2		7.36	7.16	7.21	7.21	7.23	
HORMONES													
Adrenaline (pg.ml.)	21.4	18.3	710.2					38.5	749.9				
Noradrenaline (ng.ml.)	0.29	0.1	3.12					0.27	3.06				

							SUBJECT D						
				HT						CT			
					Recovery						Recovery		
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	10	15		Pre-Ex	Post-Ex	5	10	15	
Maximal Oxygen Uptake (l.min)							3.8						
Rectal Temperature (C)	36.7	36.8	36.9					36	36.1				
Muscle Temperature (C)	33.2	37.2	36.1					35.3	36.7				
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	372	418	296					450	337				
Lactate (mmol.kg dw)	3	6.7	53.4					2.9	58				
Creatine Phosphate (mmol.kg dw)	77.3	90.5	21.2					72	28.8				
Creatine (mmol.kg dw)	38.1	19.6	89.1					38	81.4				
ATP (mmol.kg dw)	23.4	25.7	19.2					21.1	19.7				
ADP (mmol.kg dw)	1.88	2.11	1.86					1.69	2.08				
AMP (mmol.kg dw)	0.02	0.08	0.05					0.08	0.1				
IMP (mmol.kg dw)	0.03	0.02	0.86					0.04	1.43				
Inosine (mmol.kg dw)	<0.01	<0.01	0.02					<0.01	0.1				
Hypoxanthine (mmol.kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol.kg dw)	1.37	1.34	2.65					0.36	1.68				
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.5	4.3	5	5.8	6.1	6.2		4.5	5.1	5.8	6.1	6	
Ammonia (umol.l)	18.7	12.2	19	24.3	22.4	19.6		20.6	44.9	43	38.3	28.1	
Lactate (mmol.l)	0.7	0.7	7	8.9	6.9	5.5		0.7	8	8.7	8.9	8.7	
pH	7.29	7.36	7.21	7.19	7.22	7.28		7.32	7.21	7.15	7.1	7.11	
HORMONES													
Adrenaline (pg.ml.)	63.3	52.9	1084					71.8	1605.1				
Noradrenaline (ng.ml.)	0.53	0.38	4.95					0.54	5.45				

							SUBJECT E						
				HT						CT			
					Recovery						Recovery		
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	10	15		Pre-Ex	Post-Ex	5	10	15	
Maximal Oxygen Uptake (l.min)							3.44						
Rectal Temperature (C)	37.6	37.2	37.8					37.1	37.2				
Muscle Temperature (C)	38	37.5	37.8					35.4	37.4				
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	544	548	269					548	330				
Lactate (mmol.kg dw)	5.7	9	90.8					4.5	76.2				
Creatine Phosphate (mmol.kg dw)	90	94.8	21.3					86	37.2				
Creatine (mmol.kg dw)	28	21.2	94.6					30	78.8				
ATP (mmol.kg dw)	24.7	24.6	18.6					24	21				
ADP (mmol.kg dw)	1.43	1.61	1.93					1.97	2				
AMP (mmol.kg dw)	0.14	0.15	0.11					0.12	0.18				
IMP (mmol.kg dw)	0.07	0.21	4					0.12	1.97				
Inosine (mmol.kg dw)	<0.01	<0.01	0.03					<0.01	<0.01				
Hypoxanthine (mmol.kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol.kg dw)	0.32	0.4	3.9					0.2	2.69				
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.5	4.8	4.9	4.8	5.3	5.2		4.9	5.1	5	5.4	5.4	
Ammonia (umol.l)	13.1	13	16.4	36.9	46.8	37.4		7.5	11.7	17	16.6	17.2	
Lactate (mmol.l)	2.2	1.5	4.6	6.6	8.7	7		0.5	3.9	5.3	4.7	5.2	
pH	7.28	7.35	7.28	7.22	7.23	7.28		7.3	7.3	7.24	7.26	7.3	
HORMONES													
Adrenaline (pg.ml.)	20.2	38	292.2					32.6	129.5				
Noradrenaline (ng.ml.)	0.17	0.17	1.68					0.3	1.09				

							SUBJECT F						
				HT						CT			
					Recovery						Recovery		
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	10	15		Pre-Ex	Post-Ex	5	10	15	
Maximal Oxygen Uptake (l.min)							3.42						
Rectal Temperature (C)	37.2	37	37.1					37	37.1				
Muscle Temperature (C)	35.6	37.2	37.6					35.6	37.3				
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	528	432	311					396	326				
Lactate (mmol.kg dw)	7.9	8	94.8					6.9	49.8				
Creatine Phosphate (mmol.kg dw)	96.2	93.4	38.1					97.2	37.9				
Creatine (mmol.kg dw)	42.9	45.6	101.9					41.8	101.2				
ATP (mmol.kg dw)	24	24.7	19.3					22.7	19.8				
ADP (mmol.kg dw)	1.4	1.46	1.31					1.22	0.91				
AMP (mmol.kg dw)	0.01	0.02	0.05					0.02	0.05				
IMP (mmol.kg dw)	0.06	0.15	3.32					0.08	2				
Inosine (mmol.kg dw)	<0.01	<0.01	0.03					<0.01	0.01				
Hypoxanthine (mmol.kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol.kg dw)	0.58	0.41	5.68					0.79	5.21				
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.5	4.7	5.2	5.2	5.7	5.6		4.7	5	5.6	5.5	5.5	
Ammonia (umol.l)	11.2	8.4	46.3	87.4	93.5	72.9		17.7	33.2	49.1	67.8	68.4	
Lactate (mmol.l)	0.8	0.9	13	14.3	13.7	11.8		1	11.1	11.7	12	10.9	
pH	7.3	7.32	7.02	7.01	7.06	7.11		7.35	7.1	7.1	7.13	7.16	
HORMONES													
Adrenaline (pg.ml.)	23.6	48.1	1329.7					37.1	1422.5				
Noradrenaline (ng.ml.)	0.35	0.4	2.79					0.24	3.67				

							SUBJECT G						
				HT						CT			
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	Recovery 10	15		Pre-Ex	Post-Ex	5	Recovery 10	15	
Maximal Oxygen Uptake (l.min)							3.33						
Rectal Temperature (C)	36.5	36.4	36.6					36.6	36.6				
Muscle Temperature (C)	35.6	37.3	38.6					35.2	37.3				
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	437	381	191					459	306				
Lactate (mmol.kg dw)	4.8	7	108.8					8.3	112.2				
Creatine Phosphate (mmol.kg dw)	74.6	72.1	17.1					72.6	20.8				
Creatine (mmol.kg dw)	53.6	54.9	110.9					54.8	107				
ATP (mmol.kg dw)	33.4	30.7	16.3					29.1	18.3				
ADP (mmol.kg dw)	3.6	3.24	2.44					2.74	2.44				
AMP (mmol.kg dw)	0.01	0.08	0.01					0.01	0.01				
IMP (mmol.kg dw)	0.01	0.08	3.62					0.01	3.19				
Inosine (mmol.kg dw)	<0.01	0.08	0.08					0.05	0.05				
Hypoxanthine (mmol.kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol.kg dw)	0.54	0.82	4.19					0.78	4.18				
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.2	4.6	4.7	5.6	6.8	6.8		4.3	4.4	4.9	6	6.2	
Ammonia (umol.l)	18	9	23.4	62.4	49.6	30		17.8	35.5	86.4	45.8	37.4	
Lactate (mmol.l)	1.2	1.1	2.3	2.6	3.2	2.8		1.5	9.1	12.1	10.1	10	
pH	7.36	7.37	7.2	7.23	7.27	7.31		7.38	7.19	7.22	7.24	7.27	
HORMONES													
Adrenaline (pg.ml.)	26.4	26	821.1					52.7	810.9				
Noradrenaline (ng.ml.)	0.21	0.3	2.6					0.23	2.03				

							SUBJECT H						
				HT						CT			
PHYSIOLOGICAL PARAMETERS	Pre-Heat	Pre-Ex	Post-Ex	5	Recovery 10	15		Pre-Ex	Post-Ex	5	Recovery 10	15	
Maximal Oxygen Uptake (l.min)							3.24						
Rectal Temperature (C)	37.4	37.4	37.6					37.5	37.5				
Muscle Temperature (C)	36	37.6	38.1					35.6	37.5				
MUSCLE METABOLITES													
Glycogen (mmol.kg dw)	353	328	194					412	266				
Lactate (mmol.kg dw)	8	8	71.9					7.8	53.9				
Creatine Phosphate (mmol.kg dw)	65.5	72	43.3					83.3	42.5				
Creatine (mmol.kg dw)	44.6	57.9	87					46.9	88.1				
ATP (mmol.kg dw)	28	27.1	20					25.9	24.5				
ADP (mmol.kg dw)	2.3	2.5	2.76					2.21	2.67				
AMP (mmol.kg dw)	0.02	0.01	0.04					0.02	0.01				
IMP (mmol.kg dw)	0.04	0.02	2.27					0.02	0.93				
Inosine (mmol.kg dw)	<0.01	<0.01	0.04					0.05	0.05				
Hypoxanthine (mmol.kg dw)	<0.01	<0.01	<0.01					<0.01	<0.01				
Ammonia (mmol.kg dw)	0.35	0.54	3.63					0.78	1.56				
PLASMA/BLOOD METABOLITES													
Glucose (mmol.l)	4.7	4.8	5.2	5.5	5.8	5.4		4.1	4.7	4.4	4.6	4.5	
Ammonia (umol.l)	13.1	9.8	17.3	75.3	75.3	70.1		13.1	58.7	57	46.8	43.5	
Lactate (mmol.l)	1.8	1	8.4	9.8	10.4	9.6		1.4	8.7	8.3	9.9	8.6	
pH	7.37	7.4	7.25	7.24	7.24	7.26		7.31	7.14	7.22	7.24	7.28	
HORMONES													
Adrenaline (pg.ml.)	58.4	52.3	790.2					87.9	630				
Noradrenaline (ng.ml.)	0.32	0.29	2.23					0.3	1.24				

Appendix H

CHO oxidation & Energy Expenditure Method

CARBOHYDRATE OXIDATION RATE

The rate of carbohydrate oxidation during exercise has been calculated from the following formulae shown below. In the first instance, the proportion (Y) of carbohydrate which is oxidised at a given RER is calculated from the following regression;

$$Y = (3.36 \times X) - 2.37 \qquad \text{where } X = RER$$

The energy derived per litre of O_2 consumed (Z) at a given RER is calculated as follows;

$$Z = (6.66 \times X) + 14.94$$

From these two derived values and the known energy content per gram for carbohydrate (16 kJ/gm) the rate of carbohydrate consumption (C) can be calculated as follows;

$$C = (V \times Z \times Y)/16$$

ENERGY EXPENDITURE

The energy expenditure for exercise was calculated from the relationship between RER and the energy derived per litre of oxygen consumed as given in the table by Peronnet & Massicotte (1991).