Plasma Ammonia and Lactate Response To Anaerobic Exercise, and Their Relationship To Muscle Fiber Type

GRADUATE THESIS

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By

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Abstract

The aim of this study was to investigate the changes in the concentrations of plasma ammonia and lactate to an anaerobic exercise response, lasting 90 seconds (s) in duration. The secondary purpose was to plot this relationship versus muscle fibre type. Four muscle biopsy sample (7 mg each) were taken from each subject (n = 25). All muscle samples were stained using the myosin ATPase pH 10.0, 4.30 and 4.58 technique. This allowed for differentiation of the ST, FTa and FTb muscle fibres. Two weeks post surgery blood was sampled from an indwelling catheter in the antecubital vein before, immediately after, and four minutes after 90s of exercise. Plasma blood was assayed for both ammonia and lactate. A one way analysis of variance (ANOVA) with repeated measures was performed for each metabolite followed by a Tukey HSD post-hoc test. Correlations were calculated for exercise, blood, and muscle variables.

Muscle fibre profiles percentages were as follows ST - 53.48 (±8.82), FT - 46.52 (±8.82), FTb (percentage of FT population) - 8.73 (±4.63). The peak power was 6.54 W/kg (±0.83) and the relative capacity was 687.3 J/kg (±86.3). The capacity score was higher than scores reported previously for the similar protocol. Mean plasma ammonia values were 66.65 (±33.45), 130.0 (±37.15), and 251.8 (±61.82) μmol/l at rest, immediately post-exercise and at 4 minutes post-exercise respectively. The increase in ammonia concentration over time was significant ($p < 0.01$). Mean plasma lactate values were 1.45 (±.52), 8.30 (±3.11), and 14.08 (±2.62) mmol/l at rest, immediately post-exercise and 4 minutes post-exercise respectively. Lactate increase over time was significant. There was a significant positive relation between plasma ammonia and lactate over time ($r = .77$, $p < 0.05$). Regression analysis of percentage FT muscle fibre and ammonia increase demonstrated a correlation of $r = .58$ ($p < 0.05$, one-tailed) amongst those individuals with a 40 percent or greater FT profile.

It appears that in this study plasma ammonia values were higher at 4 min. post exercise than at immediately post exercise. Ammonia and lactate rise linearly with exercise and have been demonstrated to be related over time. A relation between ammonia and FT muscle fibre existed in this examination; the greater the percentage FT fibres the higher the ammonia concentration in blood plasma.

Chapter 1

INTRODUCTION

Purpose

The primary purpose of this study was to investigate the changes in the concentrations of plasma ammonia and lactate resulting from an anaerobic exercise response, lasting 90 seconds (s) in duration. The secondary purpose was to plot the relationship of the ammonia and lactate response to muscle fibre type.

Significance of Study

It has been shown that the muscle fibre type most suitable for the production of ammonia is the fast-twitch (FT) fibres (Dudley & Terjung, 1985a; Dudley & Terjung, 1985b; Graham, Pedersen, & Saltin, 1987; Meyer & Terjung, 1979). High intensity exercise (70%+ of VO_2 max) produces the greatest amount of blood ammonia (Banister, Rejendra & Mutch, 1985; Mutch & Banister, 1983a; Mutch & Banister, 1983b). If a relationship between blood ammonia and high intensity exercise exists, then it may be possible to estimate an individual's muscle fibre type by sampling his/her blood ammonia before and after short term exercise. A regression analysis of the ammonia data would be required to predict muscle fibre type. If this technique is proven to be valid then there would be no need for a scientist to use an invasive protocol such as a muscle biopsy in order to classify skeletal muscle fibres.

Delimitations

The investigator examined plasma ammonia and lactate taken from an indwelling catheter in a superficial forearm vein. The assay for blood ammonia was a revised technique outlined by Kun and Kearney (1974) using a modified plasma procedure (Sigma Diagnostics, St. Louis, Missouri). This technique (Kun & Kearney, 1974) is the standard blood analysis protocol and has been used previously (Banister, Allen, Mekjavic, Singh, Legge, & Mutch, 1983; Broberg & Sahlin, 1988; Dudley, Staron, Murray, Hagerman, & Luginbuhl, 1983; Meyer & Terjung, 1979). The modified plasma procedure was used because of its ease in execution (E.W. Banister, personal communication, August 1989). Lactate was assayed using a calibrated lactate analyzer (Model 23L - Lactate Analyzer, Yellow Springs Instrument Co., Yellow Springs, Ohio).

The exercise protocol used was a modified 90s test of anaerobic lactate capacity according to the procedures outlined previously (Simoneau, Lortie, Boulay, & Bouchard, 1983). This protocol consists of riding on a bicycle ergometer set at resistance of 0.05 kiloponds/kilogram bocy weight of each individual. The procedures outlined by Simoneau et al. (1983) require the subject to cycle all-out for 90s at this setting. However, in this study the first 30s of exercise is modified with a pacing interval.

Limitations

Genetic endowment on variables such as exercise tolerance and muscle fibre type cannot be controlled for in this study.

While verbal encouragement was given to all subjects, the only indicator used for assessing maximal work effort was the performance score of the individual subject.

Definitions

Acidosis - reduced alkalinity of the blood or body tissue, or increased acidity

Adenosine Diphosphate - ADP, the remaining molecule with two phosphate groups when adenosine triphosphate (ATP) is hydrolysed

Adenosine Monophosphate - AMP, the remaining molecule with a single phosphate group when ADP is hydrolysed, found in the Purine Nucleotide Cycle (PNC)

Adenosine Triphosphate - ATP, high energy phosphate

Adenylate Deaminase - also called AMP deaminase, enzyme responsible for the deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP), found in the PNC

Adenylosuccinase - enzyme which breaks down adenylosuccinate to fumarate, found in the PNC

Adenylosuccinate Synthetase - enzyme which breaks down aspartate and guanosine triphosphate (GTP) to guanosine diphosphate (GTP) and inorganic phosphate (Pi), found in the PNC

Aerobic - in the presence of oxygen

Ammonia - NH_3, metabolite produced in the muscle primarily

through the purine nucleotide cycle (PNC), may function as a fatigue agent

Anaerobic - in the absence of oxygen

Break Point - point where a metabolite increases rapidly

Catabolism - metabolism involving the release of energy and resulting in the breakdown of complex materials

Cell Ischemia - lack of blood influx to the cell

Correlation - relationship existing between mathematical or statistical variables which tend to vary, be associated, or occur together in a way not expected on the basis of chance alone

Deamination - the removal, usually by hydrolysis, of the NH_2 radical from an amino compound (also called deaminization)

Fumarate - Kreb's Cycle intermediate, product of adenylosuccinate in the presence of adenylosuccinase, found in the purine nucleotide cycle

Glycolysis - a series of enzymatically catalyzed reactions, occurring within cells, by which glucose and other sugars are broken down to yield lactic acid or pyruvic acid

GTP - guanosine triphosphate, high energy phosphate, similar to ATP; however contains an extra oxygen molecule in its chemical structure

H^+ - hydrogen ion concentration, denoting acidity

Hydrolysis - separation of a molecule by water or H^+

Hyperammonemia - excessive ammonia accumulation in the blood

Hyperoxia - excessive oxygen

Inosine Monophosphate - IMP, remaining molecule after which ATP

is hydrolysed, found in the PNC

Inorganic Phosphate - remaining phosphate molecule after the hydrolysis of ATP to ADP

Isometric - muscle contraction where tension is developed, but there is no change in the length of the muscle

Lactate Threshold - point where blood lactate concentration starts to increase non-linearly during continuous exercise, also referred to as lactate's 'Break point' or onset of blood lactate accumulation (OBLA)

Metabolic Clearance Rate - MCR, parameter describing the volume of blood and other body fluids (ml) from which the metabolite is cleared (removed) per unit time (min)

Muscle Biopsy - the extraction of small pieces of muscle tissue for chemical and/or histological analyses and study

Myokinase Reaction - also adenylate kinase reaction, 2ADP yields ATP + AMP

Noninvasive - not involving penetration of the skin of an intact organism

Orthophosphate - salt or ester of orthophosphoric acid (H_3PO_4), an example is inorganic phosphate (Pi)

Phosphofructokinase - PFK, rate limiting enzyme of glycolysis which changes fructose 6-phosphate to fructose 1, 6-diphosphate. It is speculated that PFK probably places a limit on glycolysis during all-out exercise

Purine Nucleotide Cycle - PNC, physiological cycle responsible for the major production of ammonia in skeletal muscle

Regression Analysis - mathematical relationship between two or more correlated variables

Regression Line - "line of best fit" whose equation is $\hat{y} = bx + a$ where: $\hat{y}$ = predicted value of y

b = slope to the regression line

a = intercept (value of y when x = 0)

x = value of the predictor (dependent variable)

Serial Point Sampling - blood sampling in successive intervals

Submaximal - exercise performed at continuous but low intensity

Supramaximal - exercise performed beyond maximum intensity (greater than 100% of VO2 max)

TAN - total adenine nucleotide pool, ATP + ADP + AMP

Ventilatory Threshold - noninvasive method for determination of lactate threshold, rate at which exercise/oxygen uptake leads to a nonlinear increase in pulmonary ventilation

VO_2 Max - maximal amount of oxygen consumed per unit time (minute), may be expressed as an absolute in Litres/minute (L/min) or relative to body weight in millilitres/Kilogram/minute (ml/kg/min).

Chapter 2

REVIEW OF LITERATURE

Introduction

Ammonia production in blood and skeletal muscle should be investigated at a biochemical level. The techniques for this inquiry have been well established (Kun & Kearney, 1974) and modified in order to provide a quick, quantitative enzymatic determination of ammonia (Sigma Diagnostics, 1989). Furthermore, lactate analyzers have given scientists the ability to measure blood and plasma lactate levels almost instantly (Yellow Springs Instruments, 1989).

Ammonia is produced in the muscle primarily through the activity of the Purine Nucleotide Cycle (PNC) (Lowenstein, 1972). In the PNC, adenosine monophosphate (AMP) is deaminated (hydrolytic removal of the NH_2 radical) to inosine monophosphate (IMP) and ammonia by the enzyme adenylate deaminase (Banister et al., 1985; Lowenstein, 1972; Manfredi & Holmes, 1984; Sutton, Toews, Ward, & Fox, 1980; and Terjung, Dudley, Meyer, Hood, & Gorski, 1986). In the literature it is generally agreed that adenylate deaminase (also referred to as AMP deaminase) catalyses the reaction responsible for the major production of ammonia in skeletal muscle (Banister et al., 1985; Broberg & Sahlin, 1988; Lo & Dudley, 1987; Meyer & Terjung, 1979; and Mutch & Banister, 1983a). Figure 1 gives an illustration of this relationship.

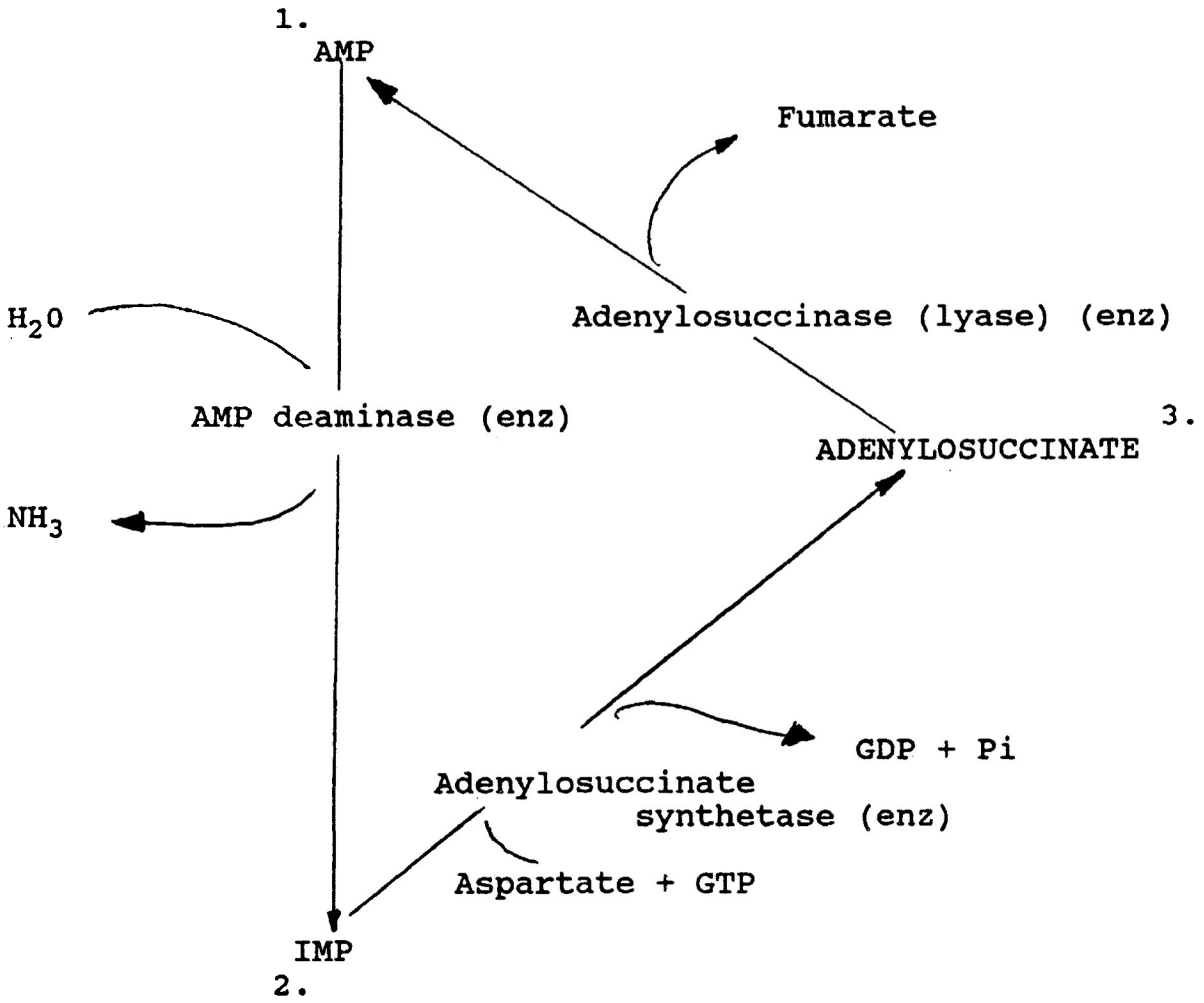


Figure 1. The Purine Nucleotide Cycle, (enz) denotes an enzyme (modified from: Lowenstein, 1972).

Contrary to the Adenine Nucleotide Cycle (which is made up of the molecules ATP, ADP, AMP), whose role involves extracelluar efflux, the functions of the PNC are internal to the cell (Terjung et al., 1986). Because the ATP hydrolysis/ADP rephosphorylation cycling process involves a major aspect of adenine nucleotide metabolism (Terjung et al., 1986), in contracting muscle, it is speculated that the removal of AMP by deamination may serve to maintain the ratios of ATP to ADP and AMP during periods of high ATP utilization (Meyer & Terjung, 1979). This process would drive the myokinase reaction (2 ADP -> AMP + ATP) toward ATP production via mass action effect (Meyer & Terjung, 1979), while forming ammonia and accelerating the rate of glycolysis by activation of phosphofructokinase (PFK) (Meyer & Terjung, 1979; Terjung et al., 1986). This reaction is irreversible (Meyer & Terjung, 1979), but the IMP formed may be reaminated during recovery by the reactions catalyzed by adenylosuccinate synthetase and adenylosuccinase, respectively (Figure 1) (Banister et al., 1985; Katz, Sahlin, & Henriksson, 1986b; Meyer & Terjung, 1979; and Mutch & Banister, 1983a).

Adenylosuccinate synthetase is inhibited by high levels of IMP and low levels of guanosine triphosphate (GTP); while AMP deaminase is effectively inhibited by orthophosphates (for example inorganic phosphate) at physiological levels (1.0 mM) (Terjung et al., 1986). At rest these mechanisms function to keep the production of the purine nucleotides at a minimal level; however, during exercise there is a relative depletion of high

energy phosphates increasing the activity of both enzymes (Banister et al., 1985). The activity of both adenylosuccinate synthetase and AMP deaminase appear to stabilize the energy charge during periods when the rate of ATP utilization exceeds its rate of supply (Meyer & Terjung, 1979). Should this be the case, then AMP removal will shift ADP to ATP via the myokinase reaction (Meyer & Terjung, 1979).

Research evidence, however, has demonstrated that ammonia may not influence glycolysis directly (Katz, Broberg, Sahlin, & Wahren, 1986a; Katz et al., 1986b). During dynamic exercise muscle ammonia increases while the total adenine nucleotide (TAN) pool (TAN = ATP + ADP + AMP) decreases demonstrating a high activity of AMP deaminase (deaminating AMP) (Katz et al., 1986b). Because the increase in muscle ammonia does not substantially increase compared to the TAN pool decrease it can be argued that ammonia may not have a direct influence on glycolysis (Katz et al., 1986a; Katz et al., 1986b). Rather it would be the substrate AMP (which will be deaminated releasing ammonia in the PNC) that directly influences PFK and hence glycolysis (Katz et al., 1986a; Katz et al., 1986b). Thus it would appear that ammonia production during exercise does not play an important role in the regulation of glycolysis per se (Katz et al., 1986a; Katz et al., 1986b).

Nonetheless, others argue (Mutch & Banister, 1983a) that ammonium ions appear to induce the activity of PFK without a change in pH. PFK itself is inhibited by physiological levels of

ATP at pH values below 7.1 (Lowenstein, 1972); therefore, it is conceivable that the overall rate of glycolysis and hence the development of lacticacidemia may be influenced by the PNC (Mutch & Banister, 1983a).

Several researchers (Banister et al., 1985; Broberg & Sahlin, 1988; Meyer and Terjung, 1979; Mutch & Banister, 1983a), have proposed that the PNC is a major pathway for the production of ammonia in skeletal muscle. In skeletal muscle adenylate deaminase activity is high, whereas the activities of glutamate dehydrogenase and glutaminase (important enzymes in ammonia production in the liver and kidney) are negligible confirming the role of the PNC (Lowenstein, 1972).

AMP and AMP Deaminase

The PNC undergoes three primary reactions which have been illustrated in figure 1. It has been documented that the muscle retains the bulk of IMP (Harris, Marlin, & Snow, 1987), and during recovery resynthesizes it to AMP via adenylosuccinate and consequently increasing the availability for ATP production by way of the myokinase reaction (Meyer & Terjung, 1979).

Monitoring the concentration of AMP and the activity of AMP deaminase is meaningful when evaluating the maintenance of the free energy of ATP hydrolysis; the ammonia formed may accelerate the rate of glycolysis and/or serve as an avenue for amino acid deamination (Dudley & Terjung, 1985a; Dudley & Terjung, 1985b; Harris et al., 1987; Meyer & Terjung, 1979).

Invariably, the consequences of anaerobic exercise leads to cellular acidosis. Also, it appears that AMP deaminase is abundant in fast-twitch (FT) muscle fibres (Dudley & Terjung, 1985b). These fibres are characteristically poorly oxidized (Dudley & Terjung, 1985b), thus, it may be important to investigate cellular acidosis and its contribution to AMP deaminase activity during intense exercise (the type of exercise which recruits primarily FT fibres).

Dudley and Terjung (1985b) investigated the influence of increasing the cellular H^+ concentration, which accompanies lactic acid accumulation, and have suggested that this acidosis is a major factor in activation of AMP deaminase activity. However, work by Graham et al. (1987) demonstrated that during hyperoxia induced exercise, ammonia levels continued to increase, whereas blood and muscle lactate levels declined. Rising concentrations of AMP, ADP, and H+ increase AMP deaminase activity, whereas increasing the orthophosphates concentrations reduces the enzyme's activity (Dudley & Terjung, 1985b). Because of conflicting evidence, it may be worthwhile to study the relationship which may exist between H^+ and AMP deaminase.

At rest the concentration of AMP in muscle is below the rate constant and thereby inhibits enzyme activity (Dudley & Terjung, 1985b). During muscular contraction, the activity of AMP deaminase is suddenly increased resulting in a high rate of AMP deamination occurring only when the estimated cellular pH is approximately 6.6 and below (Dudley & Terjung, 1985b). It is

significant to restate that the enzyme PFK is inhibited at a pH of 7.1 and below (Lowenstein, 1972). Based on these findings, it appears that muscle fibres require a state of acidosis to increase the activity of AMP deaminase, and consequently for the production of ammonia to persist or commence. PFK inhibition by cellular acidosis may be countered by the increasing reamination of AMP through IMP and adenylosuccinate; therefore, maintaining the rate of glycolysis. Other researchers (Gollnick, Bayly & Hodgson, 1986) argue that lactate is not the sole cause for the cell to be acidic, as is the case during cellular ischemia (Dudley & Terjung, 1985b). Furthermore, Dudley and Terjung (1985b) found it possible to activate AMP deaminase in high-oxidative FT red muscle (FTa) of the rat by ischemia-induced acidosis. It has been demonstrated that increases in AMP deaminase activity occurred in a cell environment characterized by acidosis (Dudley & Terjung, 1985b; Harris et al., 1987); however, cellular acidosis within the muscle itself does not ensure a high rate of AMP deamination (Dudley & Terjung, 1985b; Harris et al., 1987). Two factors appear to influence the enzymatic activity of AMP deaminase in the FT muscle group: 1) H^+ ion concentration (mentioned previously) and 2) AMP substrate accumulation (Dudley & Terjung, 1985b; Meyer & Terjung, 1980a; Meyer & Terjung, 1980b; and Meyer & Terjung, 1979).

Further investigation reveals that AMP deaminase activity in muscle is dependent on the type of FT fibre (Dudley & Terjung, 1985b; Meyer & Terjung, 1980a). FT fibres have been classified

into FTA (high oxidative fast-twitch) or FTB (high glycolytic fast-twitch) (Dudley & Terjung, 1985a; Dudley & Terjung, 1985b; Meyer & Terjung, 1980a). Dudley and Terjung (1985b) showed that elevating the AMP substrate concentration accelerated AMP deaminase activity in FTA fibres; conversely, the increase in H^+ ion concentration (acidotic) was more of an influential factor accelerating AMP deaminase in FTB fibres.

Ammonia's role as a buffer has not been well established because it appears that ammonia accumulation in skeletal muscle could only buffer between 1 to 3% of the hydrogen ions present; however, this role should not be totally disregarded (Katz et al., 1986a; Katz et al., 1986b; MacLaren, Gibson, Parry-Billings, Edwards, 1989).

Determining Exercise Intensity

Banister et al. (1985) have reviewed the literature regarding ammonia production during exercise and have found that intensities must exceed 70% of VO_2max for significant blood ammonia increases to occur. Conversely, intensities of 50% or less of VO_2 max have no effect on the concentration of blood ammonia (Babij, Matthews, & Rennie, 1983; Banister et al., 1985; Banister et al., 1983). To quantify training, coaches/scientists need a tool to measure exercise intensity. Some researchers speculate that ammonia can be used as a measure of exercise intensity because blood ammonia levels peak immediately after exercise (Mutch & Banister, 1983a; Mutch & Banister, 1983b).

This was substantiated by serial blood sampling every 30 seconds (Banister et al., 1983). However, Urhausen, Heckmann and Kinderman (1988) concluded that further studies are needed before ammonia can be used systematically for performance diagnostics or control of training intensities.

Presently, one method for measuring exercise intensity has been the anaerobic threshold obtained via either lactate or ventilatory thresholds (Brooks, 1985; Vandewalle, Péré, & Monod, 1987). Many authors have documented the response of lactate and ammonia during increasing exercise intensities (Buono, Clancy & Cook, 1984; Dudley et al., 1983; Graham et al., 1987; Sehling-Werle, Schnelder, Hageloch, & Weicker, 1988; Urhausen et al., 1988; and Witt, Schlicht, Rieckert, 1988). However, only a few have suggested that ammonia is a better indicator of exercise intensity, compared to employing either the lactate or ventilatory thresholds (Banister et al., 1985; Mutch & Banister, 1983a; and Sehling-Werle et al., 1988). The rationale for using ammonia, as opposed to lactate, appears to be that the assay for ammonia is simple, relatively inexpensive, and can be completed within 5 min (Mutch & Banister, 1983b). Because ammonia concentration is an indicator for the deaminase activity, this parameter seems to be more suitable for training control than lactate (Sehling-Werle et al., 1988).

Investigators have determined that ammonia accumulation in plasma blood during anaerobic exercise is a true reflection of ammonia production (Banister et al., 1983; Eriksson, Broberg,

Björkman, & Wahren, 1985; Katz et al., 1986a). Therefore the ammonia value at the cessation of anaerobic exercise would be the peak blood ammonia value (Banister et al., 1985; and Mutch & Banister, 1983a).

Lactate's use as an indicator of anaerobic exercise may be questioned for the following reasons: 1) while it has been shown that ammonia levels peak after exercise; lactate continues to rise some time after exercise completion (Buono et al., 1984; Graham et al., 1987; Harris et al., 1987; Mutch & Banister, 1983a and Urhausen et al., 1988), 2) it has been reported that blood lactate concentrations are lower than muscle lactate concentrations (Vandewalle et al., 1987), 3) lactate within the active muscle is not in equilibrium with that of extracelluar fluids probably because of an active transport of lactate across cell membranes (Vandewalle et al., 1987), 4) cardiac muscle has a high affinity for the oxidation of lactate, and 5) slow-twitch (ST) fibres and less active muscle fibres use lactate as a fuel (Brooks, 1988; Brooks & Fahey, 1985).

The precise demarcation of ammonia as the end-point of exercise avoids the uncertainty implicit in serial point sampling of post exercise to obtain peak blood lactate values (Banister et al., 1985). Mutch and Banister (1983a) suggest that a reduction in blood ammonia during exercise may increase an individual's capacity for exhaustive activity. Some researchers go as far as to insinuate that endurance training reduces the magnitude of exercise-induced hyperammonemia in humans (Lo & Dudley, 1987).

Ammonia and Muscle Fibre Type

Many researchers have investigated the relationship between muscle fibre type and ammonia production (Dudley & Terjung, 1985a; Dudley & Terjung, 1985b; Graham et al., 1987; Meyer & Terjung, 1979; Sehling-Werle et al., 1988; and Witt et al., 1988). It would appear that there is a strong relationship between FT muscle fibres and the production of ammonia during high intensity exercise (Banister et al., 1985; Dudley et al., 1983; Meyer & Terjung, 1979; and Sehling-Werle et al., 1988). In partial agreement, Graham et al. (1987) hypothesized that the FT (especially FTa) fibres are the major factor contributing to ammonia production during the early phase of activity, but may not be predominant in activities of longer duration. Ammonia production from the FT fibres at the onset of exercise occurs because the activity is highly anaerobic (greater than 70% VO_2 max) (Dudley & Terjung, 1985a). However, by using hyperoxia (breathing excessive oxygen) in exercise protocols, it has been demonstrated that during longer duration activity (more aerobic and less intense) the major source of blood ammonia comes from the ST (slow-twitch) muscle fibres (Graham et al., 1987) Other researchers (Dudley & Terjung, 1985a; Dudley & Terjung, 1985b; Meyer & Terjung, 1979; Witt et al., 1988) do not necessarily agree with the findings of Graham et al. (1987), but this may be the result of not investigating activities of a more prolonged, less intensive nature. Perhaps this is a consequence of the stress specific fibre recruitment pattern (i.e. anaerobic

activity calls upon the FT fibres for work performance, while aerobic activity calls upon the ST fibres for work performance) (Sehling-Werle et al., 1988); it may also be because of the biochemical make-up of the muscle fibre (Dudley et al., 1983; Meyer & Terjung, 1979). Further investigations considering ammonia production during extended exercise may reveal a better understanding of the ST fibre's role.

Recent evidence establishing a strong relationship between blood ammonia production, anaerobic exercise and FT fibres, may suggest a noninvasive method of fibre type prediction. Researchers have attempted noninvasive fibre type determination using nuclear magnetic resonance (NMR) (Boicelli, Baldassarri, Borsetto, & Conconi, 1989). This investigation measured creatine phosphate (CrP) to adenosine triphosphate (ATP) and CrP to inorganic phosphate (Pi) ratios via NMR; however, no measurement of muscle fibre type was made (Boicelli et al., 1989). A direct prediction of muscle fibre type using ammonia production during anaerobic high-intensity exercise has not been fully substantiated. It would, therefore appear that the literature does not reveal a systematic method for noninvasive fibre type determination.

It has been stated previously that the deamination of AMP produces IMP and ammonia respectively. Upon examining the deaminase activity of different muscle fibre types, it seems that the FT fibres contain the appropriate enzyme activity to activate the PNC, trigger the loss of ATP and ultimately lead to the

accumulation of ammonia and IMP (Dudley & Terjung, 1985a; Dudley & Terjung, 1985b; Dudley et al., 1983). Furthermore, one group of scientists (Meyer & Terjung, 1980a; Meyer & Terjung, 1980b; and Meyer & Terjung, 1979) have demonstrated that AMP deamination occurs readily in both the FTa and FTb fibres, but not in the ST fibres. In support of this previous research, Dudley et al. (1983) found that an inverse relationship existed between the increase in blood NH_3 and ST fibres in the vastus lateralis muscle. When exercise was equated at submaximal and supramaximal levels, it was established that the group having the high percentage of ST fibres had lesser increases in blood ammonia compared with the group with fewer ST fibres (Dudley et al., 1983). In other words, the greater the percentage of FT fibres the greater potential to yield an abundance of blood ammonia during high-intensity exercise. The results obtained by Dudley et al. (1983) are supported by previous research; in human skeletal muscle AMP deamination is confined primarily to the FT fibres (Meyer & Terjung, 1980a; Meyer & Terjung, 1980b; Meyer & Terjung, 1979). Likewise, Katz et al. (1986b) have demonstrated that during isometric muscle contraction AMP deaminase activity was limited to the FT fibres.

Ammonia/Lactate Relationship

A number of researchers have explored the exercise induced ammonia and lactate response in both skeletal muscle and plasma blood (Dudley et al., 1983; Katz et al., 1986b). Investigators have demonstrated that during incremental exercise blood ammonia and blood lactate rise linearly (Babij et al., 1983; Banister et al., 1983; Broberg & Sahlin, 1988; and Harris & Dudley, 1989). Moreover, correlations between blood ammonia and lactate were found to be as high as 0.82 (Buono et al., 1984) and 0.94 (Graham et al., 1987) during the first stages of exercise. However, during exercise of longer duration blood ammonia continues to increase, while blood lactate accumulation decreases (Broberg & Sahlin, 1988). Likewise, the correlation between blood ammonia and lactate decreases (0.09) as the exercise duration is extended (Graham et al., 1987). Nonetheless, it is important to note that exercise duration was approximately 10 minutes in the Buono et al. (1984) study before any significant increase in either ammonia or lactate transpired.

Buono et al. (1984) illustrated that the break points (non-linear increase) for blood lactate and ammonia were similar. However, Graham et al. (1987) demonstrated that during exercise of longer duration (40 minutes) the rise of ammonia and lactate differ; lactate accumulation decreases while ammonia continues to increase linearly. Likewise, during recovery, Harris et al. (1987) established that blood lactate concentrations approached its resting values after approximately 60 minutes, whereas

ammonia remained elevated. Albeit this work (Harris et al., 1987) has been illustrated in animals, at present there appears to be few studies which demonstrate a similar recovery phenomena in humans. These findings are contrary to Banister et al. (1983); ammonia peaked immediately post exercise and rapidly decreased in plasma during recovery.

Lactate accumulation in the blood does not necessarily represent production in muscle (i.e. metabolic clearance rate of lactate - lactate is being removed from the blood at the same time it is being infused); therefore, the amount of lactate in the blood is not the total amount of lactate that may be produced. On the other hand, the amount of ammonia in the blood (whole and plasma) is a good representation of production (Katz et al., 1986a), as well as revealing an increased rate of purine nucleotide cycling (Harris et al., 1987).

Banister et al. (1983) was the first group to undertake serial blood sampling every 30 seconds. Buono et al. (1984) employed serial blood sampling every minute of exercise and obtained ammonia and lactate breakpoints; however, minute sampling during extended exercise may in fact miss the actual break point for ammonia accumulation in the blood. The results of Banister et al. (1983) were very meaningful in that the break point for ammonia differed from that of lactate during anaerobic exercise. Nonetheless, it appears that no other investigator(s) has attempted serial blood sampling of less than one minute.

The results of the serial blood sampling every 30-60 seconds

demonstrated that while blood lactate and blood ammonia increase linearly, the two metabolites are independent of each other (Banister et al., 1983) and may have independent break points. Furthermore, based on the conclusions put forward by Harris et al. (1987), it may be speculated that the ammonia response during graded exercise is relatively independent of lactate metabolism (Urhausen et al., 1988). Notwithstanding the conclusions by Buono et al. (1984) (lactate and ammonia accumulation during graded exercise are linearly related, as are their respective break points), it may be reasonable to suggest that the linearity observed between lactate and ammonia early in exercise may very well be the result of the experimental methodology. This may be tested by sampling both metabolites at shorter intervals (i.e. every 30 seconds or more). Indeed it is recommended that further studies be undertaken before an established ammonia-lactate relationship or non-relationship can be validated (Urhausen et al., 1988). A schematic representation of the ammonia and lactate interaction can be seen in figure 2.

Implications to Physical Training

Sufficient evidence has demonstrated that ammonia accumulation during short term intense exercise originates from the FT muscle fibres (Banister et al., 1985; Dudley et al., 1983; Meyer & Terjung, 1979; and Sehling-Werle et al., 1988). Buono et al. (1984), Graham et al. (1987), and Harris et al. (1987) have reported that during incremental short term exercise blood

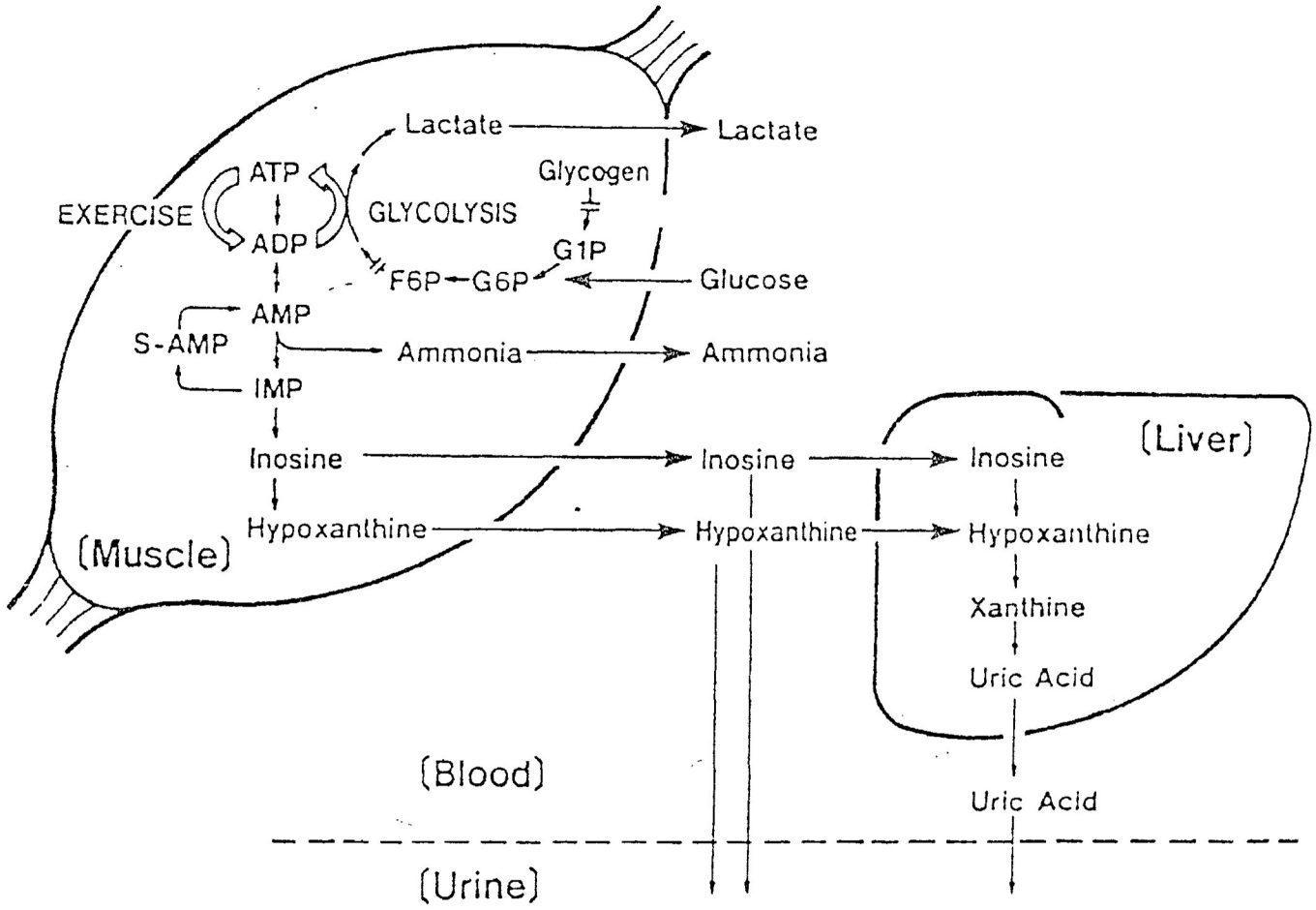


Figure 2: Ammonia/Lactate interaction (Modified from: Mino, Kono, Hara, Shimizu, Yamada, Kawachi, Kiyokawa, & Tarui, 1987).

ammonia rises linearly; however, it seems that no author has attempted to predict muscle fibre type from changes in blood ammonia accumulation during exercise. Although Sehling-Werle et al. (1988) have concluded that ammonia may be used to determine the participation of different fibre types, the use of ammonia as a fibre predictor was not clearly substantiated.

Anaerobic Exercise Protocol

A 90 second test on a bicycle ergometer has been developed to assess anaerobic lactic acid capacity (Serresse, Ama, Simoneau, Lortie, Bouchard and Boulay, 1989; Serresse, Lortie, Bouchard, & Boulay, 1988; Simoneau et al., 1983). The test consists of an all-out effort of cycling on a modified bicycle ergometer against a known resistance (Simoneau et al., 1983). The mean watts of the 90 s test is lower than that of the 30 s Wingate test (Bar-Or, Dotan, & Inbar, 1977; Inbar & Bar-Or, 1979; LaVoie, Dallaire, Brayne, & Barrett, 1984) or tests of 10 s in duration (Serresse et al., 1988). However, the average capacity scores reported for the 90 s protocol are generally higher (Serresse et al., 1989; Serresse et al., 1988; and Simoneau et al., 1983) than previous tests. The longer test duration may contribute to this result.

During 90 s of exercise the relative energy contribution is 12%, 42%, and 46% phosphagenic, glycolytic and oxidative respectively (Serresse et al., 1988). The nature of the 90 s protocol is to utilize the capacity of the glycolytic energy

system for work. It has been shown that during exercise of lesser durations (50s), glycolysis and glycogenolysis are activated (Hultman & Sjoholm, 1983): as well, glycolysis is thought to be activated as early as 5 seconds after the initiation of muscular contraction resulting in pronounced lactate accumulation (Hultman & Sjoholm, 1983; Jacobs, Tesch, Bar-Or, Karlsson, & Dotan, 1983; Serresse et al., 1988). This is in disagreement with Jacobs et al. (1983) who reported that significant glycolysis does not occur until endogenous phosphagen levels reach some critically low level.

Previously, attempts have been made to determine a method for anaerobic energy expenditure (Hultman & Sjoholm, 1983; Jacobs, Bar-Or, Karlsson, Dotan, Tesch, Kaiser, & Inbar, 1982; Thomson & Garvie, 1981); however, because a true refection of glycolytic capacity would be a test longer than 60 s but shorter than 120 s in duration (Serresse et al., 1988; Simoneau et al., 1983), such protocols were either too short (Jacobs et al., 1982) or too long. Furthermore, Serresse et al. (1988) have shown that from 30 to 90 s of anaerobic exercise, glycolysis still makes a significant contribution to energy metabolism.

Since it has been illustrated that blood lactate peaks at 4 minutes post exercise (Thomson & Garvie, 1981) and that the ideal anaerobic test should last approximately 90 s (Serresse et al., 1988), then it would be logical to blood sample at 4 minutes after the protocol.

Characteristics of Skeletal Muscle Fibre Types

Human skeletal muscle can be classified into three types of fibres. As previously mentioned these are FTa (IIa), FTb (IIb), and ST (I) fibres (Barnard, Egerton, Furukawa, & Peter, 1971; Brook & Kaiser, 1970; Essén, Jansson, Henriksson, Taylor, & Saltin, 1975; Gollnick, Armstrong, Saubert, Piehl, & Saltin, 1972; Saltin, Henriksson, Nygaard, & Andersen, 1977). Using fibre staining techniques (Gollnick, & Matoba, 1984; Gollnick, Parsons, & Oakley, 1983; Guth & Samatha, 1969; Matoba & Gollnick, 1984) fibres can be differentiated based on the skeletal muscle fibre's pH liability, sulfhydryl dependence (Brooke & Kaiser, 1970), and adenosine triphosphatase (ATPase) activity (Brooke & Kaiser, 1969) (see table 1).

The chemical processes which allow for differentiation of muscle fibre are controlled by the particular fibre's biochemical composition. Therefore differences would be expected between the three types of muscle fibres. Such distinctions of fibre type are based on the ATPase activity of the fibres after pre-incubations in either alkaline or acidic solutions (see table 1). ST fibres incubated at an alkaline pH, such as 10.3 (Guth & Samaha, 1969), have little or no ATPase activity while the FT fibres have an increased activity (Newsholme & Leech, 1988a; Saltin et al., 1977). If the incubation medium was acidic, the ST fibres would be activated (Gollnick & Matoba, 1984; Gollnick et al., 1983), and the FTb fibres would be only partially

Table 1: Properties of different skeletal muscle fibres in humans

Property	ST	FTa	FTb
ATPase activity at pH 10.3 (colour)	light	dark	dark
ATPase activity at pH 4.50 (colour)	dark	light	medium
ATPase activity at pH 4.3 (colour)	dark	light	light
Speed of Contraction	slow	fast	fast
Resistance to fatigue	high	moderate	low
Glycolytic capacity	low	moderate	high
Oxidative capacity	high	moderate	low
Glycogen store	moderate-high	moderate-high	moderate-high
Triaclglyerol store	high	moderate	low
Capillary supply	good	moderate	poor
Myoglobin content	high	low	low

(Modified from: Clarkson, Kroll, & McBride, 1980; Essén et al., 1977; Essén-Gustavsson & Henriksson, 1984; Newsholme & Leech, 1988a, p. 213; and Saltin et al., 1977).

activated (Newsholme & Leech, 1988a; Saltin et al., 1977). A comprehensive list of these properties can be seen in table 1.

In terms of glycolytic capacity the FT fibres, more specifically the FTb fibres, have the highest glycolytic capacity (Newsholme & Leech, 1988a; Saltin et al., 1977). The oxidative capacity, however, of the FTb fibre is low; unlike that of the ST muscle fibre (Newsholme & Leech, 1988a; Saltin et al., 1977). While it is evident that the FTa fibres are oxidative (Newsholme & Leech, 1988a; Saltin et al., 1977) (table 1), human skeletal muscle does not contain a fast twitching fibre which has as high or higher oxidative capacity as the ST fibres of the same muscle (Essén et al., 1975).

Enzymes, which will effect the oxidative or glycolytic capacity, are distributed differently amongst the three muscle fibres (see table 2). Phosphofructokinase (PFK) is more abundant in the FT fibres than the ST, and more abundant in the FTb than in the FTa (Essén et al., 1975; Essén-Gustavsson & Henriksson, 1984). Generally, the glycolytic enzymes (adenylate kinase, PFK, lactate dehydrogenase-LDH, and glyceraldehydephosphate dehydrogenase-GAPDH) are approximately twice as high in the FT fibres (Chi, Hintz, Coyle, Martine, Ivy, Nemeth, Holloszy, & Lowry, 1983; Essén-Gustavsson & Henriksson, 1984). Conversely the mitochondrial enzymes (beta-hydroxyacl-CoA dehydrogenase, citrate synthase, malate dehydrogenase-MDH, and succinate dehydrogenase-SDH) are more abundant in the ST muscle fibres

Table 2: Enzyme distribution in different skeletal muscle fibres

Enzyme	ST	FTA	FTB
PFK	low	high	highest
LDH	low	high	high
adenylate kinase	low	high	high
GAPDH	low	high	high
Beta-hydroxyl-CoA dehydrogenase	high	low	low
Citrate synthase	high	low	low
MDH	high	low	low
SDH	high	low	low

(Modified from: Chi et al., 1983; Essén et al., 1975; Essén-Gustavsson & Henriksson, 1984).

(Essén et al., 1975; Chi et al., 1983). The enzymes and their distribution can be seen in table 2.

Chapter 3

METHODS AND PROCEDURES

Purpose

The primary purpose of this study was to investigate the response of plasma ammonia and lactate to anaerobic exercise. The secondary purpose was to plot the response of plasma ammonia and lactate with muscle fibre type.

Subjects

Twenty-five male subjects volunteered, representing a heterogeneity of athletic backgrounds, from Lakehead University, Thunder Bay, Ontario. The age, height, and weight were recorded for all subjects (see chapter 4). The subjects were informed of the purpose and possible risks involved in the study before giving their voluntary signed consent.

Measures

Muscle Fibre Profile:

Muscle biopsies were taken from the lateral aspect of the vastus lateralis, fifteen (15) centimetres above the upper margin of the patella following the technique described by Bergstrom (1962).

For histochemical analysis, 10μm sections were cut in an ultra-low temperature microtome (-20^{o}C), adhered to microscope slide cover slips, and placed in muscle staining jars. The tissue samples were assayed for myofibrillar ATPase activity at pH 10.0, 4.58, and 4.3 employing the histochemical method

outlined by Padykula and Herman (1955) (Appendix C).

Histochemical staining allowed for the differentiation of the three fibre types. At preincubation of pH 10.0 the FT muscle fibres are alkaline-stable (dark stain) and the ST muscle fibres are alkaline-labile (light stain) (figure 3a). By contrast, at an acidic pH preincubation (4.30 and 4.58) the ST fibres are acid-stable and exhibit a dark stain (figure 3b). However at an acid preincubation of pH 4.58, the FTa fibres are acid-labile and exhibit a light stain, whereas the FTb fibres are intermediate in their reaction to acid preincubation (figure 3c).

When three or more biopsies are extracted, it has been shown that it is sufficient to count only 25 fibres (Lexell & Taylor, 1989), however, in this study all cell-fibres were counted to increase accuracy.

Blood Ammonia:

Blood was collected in heparinized vacutubes and kept in an ice bath (-2°C) for no more than 30 minutes, after which the vacutubes were centrifuged (2000 X gravity) for 10 minutes to separate whole blood from plasma. The plasma was assayed for ammonia using the enzymatic determination kit by Sigma (Sigma Diagnostics, St. Louis, Missouri) (Appendix A). This procedure has been modified from the standard blood assay (Kun & Kearny, 1974). The ammonia diagnositic technique has been used previously with success (E.W. Banister, personal communication August 1989). However, when the ammonia assay procedure was

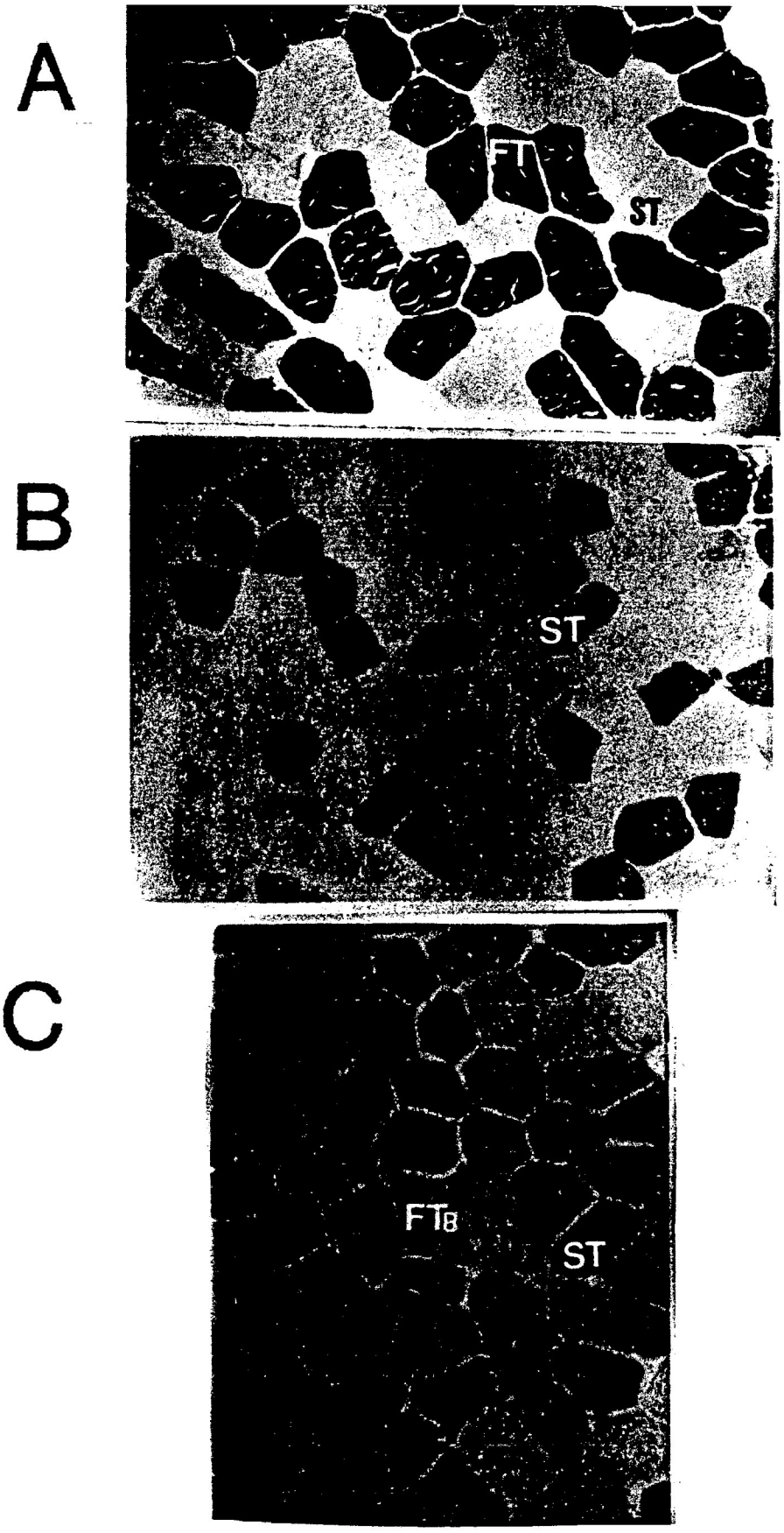


Figure 3: Serial sections of the same muscle,
a) muscle stain pH 10.0, b) muscle stain pH 4.30,
c) muscle stain pH 4.58

performed on the first fifteen subjects, there were some problems with the results. In all the ammonia diagnostic procedure was performed on twelve subjects.

Sterility of the cuvets was the factor which contributed to the observed discrepancies. Furthermore, double distilled deionized water had to be used in the chemical mixtures as a precaution, this was not outlined in the procedures from the manufacturer. Assays were also done in triplicate in order to minimize error due to chance. Because of the nature of the ammonia diagnostic protocol, analysis was performed on only twelve of the twenty-five subjects; however, lactate analysis was performed on all twenty-five. Technical difficulties did not allow ammonia scores to be reported during the exercise treatment because of the missing data on many subjects.

Blood Lactate:

Blood was collected in vacutubes containing sodium fluoride and potassium oxalate and was centrifuged immediately to separate whole blood. Both whole blood and plasma were analyzed by injecting 5.0 mmol/l of the sample into a lactate analyzer (Model 23L- Yellow Springs Instrument Co., Yellow Springs, Ohio) by the methods outlined by the manufacturer (Appendix B).

Cycle Ergometer Test:

Cycle revolutions were counted by a Radio Shack TRS-80 micro-colour computer (model MC-10). Power was determined by taking the highest number of revolutions in a 5 second interval, multiplying it by the resistance (kiloponds, Kp), multiplying by

12 (amount of 5 second intervals in a minute) and multiplying by 6 meters (distance the ergocycle's fly wheel covers in one revolution). This figure was divided by the subject's body weight (kg) to give a measure in Kpm/min/kg, and then was divided by 6.12 to convert to watts, the standard power measure.

Capacity was determined by taking the total number of revolutions in 90s and multiplying it by the resistance (Kp), and by 1.5 (to express per minute) and by 6 meters. This figure was divided by the subject's body weight (kg) to give a measure in Kpm/min/kg, and then was divided by 0.10197 to convert to joules, the standard capacity measure.

Equipment

For analysis of ammonia, an Ammonia Diagnostic Kit (Sigma Diagnostics, St. Louis, Missouri), and a Bausch and Lomb spectrophotometer, model Spectronic 20, calibrated with potassium dichromate versus double distilled deionized water were employed. A calibrated lactate analyzer, YSI Model 23L (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) was used to anlyze for lactate. The exercise test was performed on a calibrated Monarch Bicycle Ergometer, model 868 (Monarch, Stockholm, Sweeden). Cycle revolutions were counted by a Radio Shack TRS-80 micro-colour computer, model MC-10.

Procedures

All subjects refrained from athletic activities 24 hours prior to the muscle biopsy in order to minimize muscle cell damage that may occur as a result of athletic participation. The

muscle biopsies were approved by the Ethics Committee on Human Testing at Lakehead University. One muscle biopsy was taken from the lateral aspect of the vastus lateralis, fifteen (15) centimetres above the upper margin of the patella by a trained physician. All samples were embedded in OCT (Ames Tissue-Tek) mounting medium which provided cryoprotectant and support, and frozen in 2-methylbutane cooled by liquid nitrogen. Four muscle samples were taken from the same incision, two shallow and two deep.

Two weeks after the muscle biopsy, the same subjects reported back to the Human Performance Laboratory at Lakehead University to receive the exercise treatment. Subjects refrained from physical activity 24 hours prior to reporting to the laboratory and were post absorptive, not ingesting any food, for a minimum of 4 hours prior to testing. The exercise treatment is a measure of the capacity of the anaerobic lactate system. The test protocol was the modified ergocycle test outlined by Simoneau et al. (1983), which has been shown to have a test-retest reliability of 0.99 (Simoneau et al., 1983). This exercise treatment has been used previously (Serresse et al., 1989; Serresse et al., 1988; Simoneau et al., 1983).

The protocol consists of riding on a bicycle ergometer set at a resistance of 0.05 kiloponds/kilogram (kp/kg) body weight of each individual. Subjects received a 10 minute warm up at no resistance prior to the exercise treatment. After the warm up, an indwelling catheter (Desert Medical Inc., Sandy, Utah) with

extension tubing (Abbott Hospital Supplies, North Chicago, Illinois) was inserted in the antecubital forearm vein of each subject by a registered nurse and a trained technician.

The procedures outlined by Serresse et al. (1989), Serresse et al. (1988), and Simoneau et al. (1983) have the subjects perform 90 seconds (s) of all-out exercise. However, in this experiment the procedures were modified so that during the first 30s of exercise all subjects paced their pedalling rate at 110 rotations/minute (rpm). After 30s of pacing, the subjects cycled all-out in a seated position for the remainder of the 90s period. This modification was based on the recommendations of J.A. Simoneau (personal communication, February 17, 1990).

Blood was drawn from the catheter at rest, 30s, and 60s during exercise, immediately post exercise (90s) and 4 minutes post exercise. Blood was slowly injected into two different vacutubes for subsequent analysis. One vacutube contained sodium heparin (for ammonia analysis) and the other vacutube contained sodium fluoride and potassium oxalate (for lactate analysis). The sodium heparin vacutubes were kept in an ice bath and were centrifuged within 15 minutes post drawing blood. While the blood drawn in the sodium fluoride and potassium oxalate vacutubes was centrifuged and analyzed for lactate immediately.

Statistical Analysis

A one way analysis of variance (ANOVA) with repeated measures on time was executed for each metabolite (ammonia and lactate). A Tukey Honestly Significant Difference (HSD) post hoc

test was also administered, where applicable, to assess significance between all possible null hypotheses.

A Pearson Product Moment Correlation with a one tailed (one direction assumed) coefficient test of significance ($p > 0.05$, 0.01) was performed on the following variables of the anaerobic 90s test:

1. peak power (W/kg) versus total capacity (in joules/kg),
2. power scores for each time interval (30, 60 and 90s) versus total capacity,
3. fibre profile versus peak power,
4. fibre profile versus total capacity.

A Pearson Product Moment Correlation with a one tailed coefficient test of significance ($p > 0.05$, 0.01) was also executed for the following plasma variables:

1. ammonia at rest, immediately post-exercise, and 4 minutes post-exercise versus fibre profile;
2. lactate at rest, immediately post-exercise, and 4 minutes post-exercise versus fibre profile,
3. ammonia change (delta) versus fibre profile,
4. lactate change (delta) versus fibre profile,
5. ammonia versus lactate over time (rest, immediately post-exercise and 4 minutes post-exercise),
6. ammonia at 4 minutes versus peak power,
7. ammonia at 4 minutes versus total capacity,
8. lactate at 4 minutes versus peak power, and
9. lactate at 4 minutes versus total capacity.

Chapter 4

RESULTS

Physical Characteristics of Subjects

The mean age (22.6 years, ±3.03), height (177 cm, ±5.5), and weight (77.98 kg, ±8.6) are listed in Table 3.

Muscle Fibre Profile

The mean percentage ST fibre distribution was 53.48 (±8.82), and the mean percentage of FT fibre distribution was 46.52 (±8.82). The range of ST fibre percentage was from 39 to 71, conversely the range of FT fibre percentage was from 29 to 61.

With respect to FT fibres, 24.5% (±11.26) were FTb fibres, with a range from 5 to 49 percent. FTb fibres represented 8.73% (±4.63) of the total fibre profile and had a range from 1 to 20 percent. The fibre profile of all 25 subjects is listed in Table 4.

Exercise Protocol

The power outputs in watts/kilogram (W/kg) for the 90s test can be seen in Table 5. The mean peak power for the 25 subjects was 6.54 W/kg, and occurred between 30 and 50s of exercise (see Table 6). The total capacity of the group was 53,960 joules and 687.3 joules/kilogram body weight (see Table 6).

Table 3: Descriptive Statistics of Physical Characteristics (n = 25)

Variable	Mean		S.D.	Minimum	Maximum
Age	22.6	yrs.	±3.03	19.0	31.0
Height	177.0	cm.	±5.50	165.0	188.3
Weight	77.98	kg.	±8.6	64.2	95.0

Table 4: Descriptive Statistics of Muscle Fibre Profile (n = 25)

Fibre	Mean	S.D.	Minimum	Maximum
%ST	53.48	±8.82	39	71
%FT	46.52	±8.82	29	61
%FTb of FT	24.50	±11.26	5	49
%FTb of All Fibres	8.73	±4.63	1	20

* note FTb/FT is the percentage of FTb fibres of the FT population, FTb/all fibres is the percentage of FTb fibres of the total amount of fibres - both FT and ST.

Table 5: Descriptive Statistics of Power Output Over Ninety Seconds (n = 25)

Time	Watts/Kg	S.D.	Range
30s	5.383	±0.57	4.5 - 6.5
60s	4.913	±0.94	3.5 - 6.5
90s	4.017	±0.60	3.0 - 5.3

Table 6: Descriptive Statistics of Peak Power and Capacity (n = 25)

Variable	Mean	S.D.	Range
Peak Power (PP) (post 30 sec.)	6.54 W/kg	±0.83	4.7 - 8.2
Time of PP (post 30 sec.)	38.48 s	±5.32	30 - 50
Capacity	53960 J	±8348	38410 - 75310
Relative Capacity (per kg body weight)	687.3 J/kg	±86.3	575.6 - 991.0

Blood

Plasma ammonia values increased from rest to immediately post-exercise and were highest at 4 minutes post-exercise (see table 7). Mean ammonia values were 66.65 (±33.45), 130.0 (±37.15), and 251.8 (±61.82) μmol/l at rest, immediately post-exercise and at 4 minutes post-exercise respectively. A one-way ANOVA with repeated measures revealed a significant main effect, $F(3,22) = 46.37$, $p < .01$. Post-hoc analysis using Tukey HSD found that ammonia increased significantly from rest to immediately post-exercise, from rest to 4 minutes post-exercise, and from immediately post-exercise to 4 minutes post-exercise (see Figure 4a).

Plasma lactate values increased from rest to immediately post-exercise and were highest at 4 minutes post-exercise (see table 8). Mean plasma lactate was 1.45 (±.52), 8.30 (±3.11), and 14.08 (±2.62) mmol/l at rest, immediately post-exercise and 4 minutes post-exercise respectively (see Table 8). A one-way ANOVA with repeated measures revealed a significant main effect, $F(3,44) = 221.33$, $p < .01$. Post-hoc analysis using Tukey HSD found that plasma lactate increased significantly from rest to immediately post-exercise, from rest to 4 minutes post-exercise, and from immediately post-exercise to 4 minutes post-exercise (see Figure 4b).

The relation between ammonia and lactate in 12 cases was also investigated in order to observe similarities in response to

Table 7: Plasma ammonia response to 90s of anaerobic exercise (n = 12)

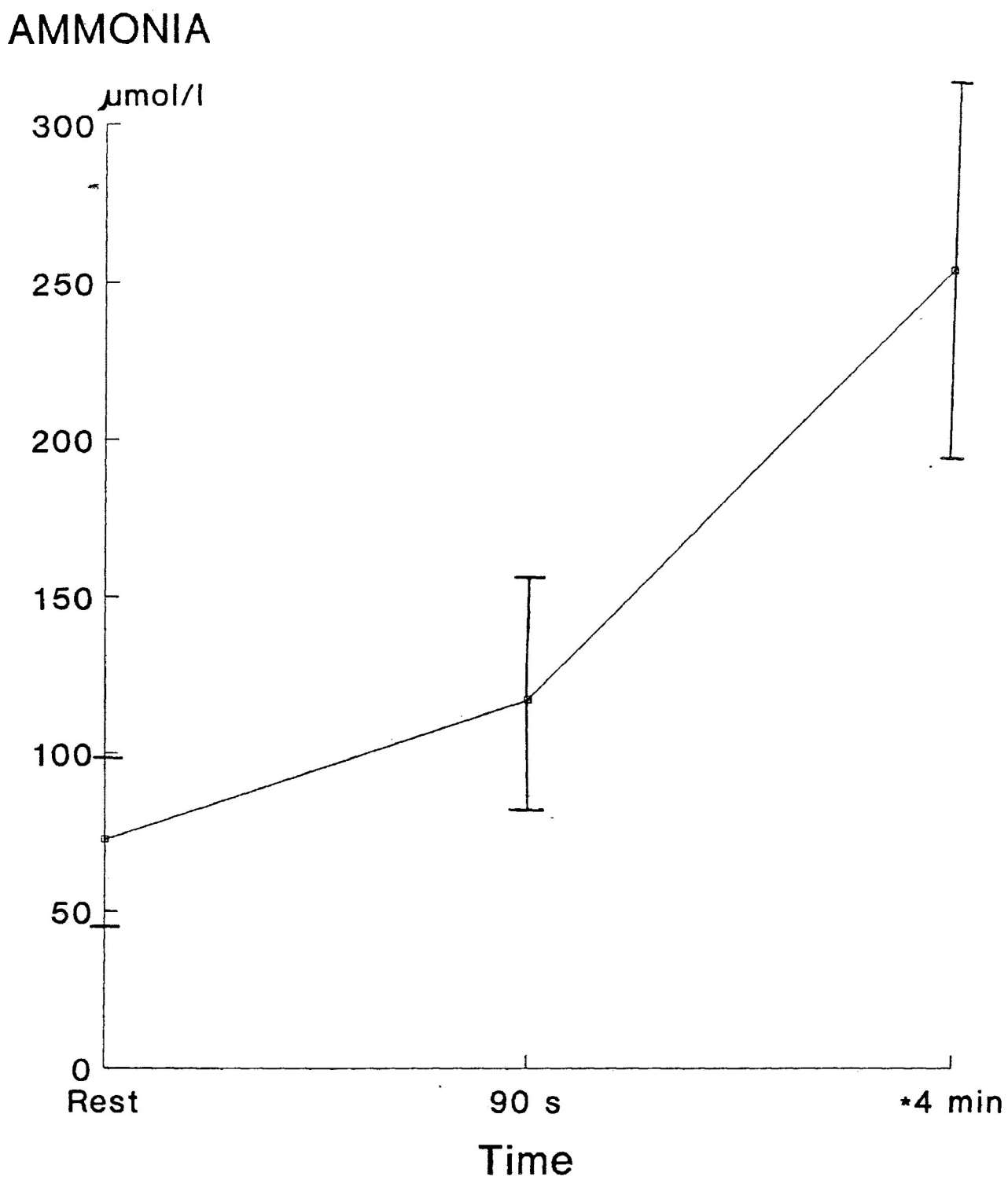
Time	Mean	S.D.	Range
Rest	66.65 μmol/l	±33.45	10.4 - 116.8
Immediately post-exercise	130.00 μmol/l	±37.15	64.9 - 194.7
4 min. post	251.80 μmol/l	±61.82	166.1 - 363.4

*note: plasma ammonia values increased significantly ($p < .01$) from rest to immediately post-exercise, rest to 4 minutes post-exercise, and from immediately post exercise to 4 minutes post-exercise.

Table 8: Plasma lactate response to 90s of anaerobic exercise (n = 25)

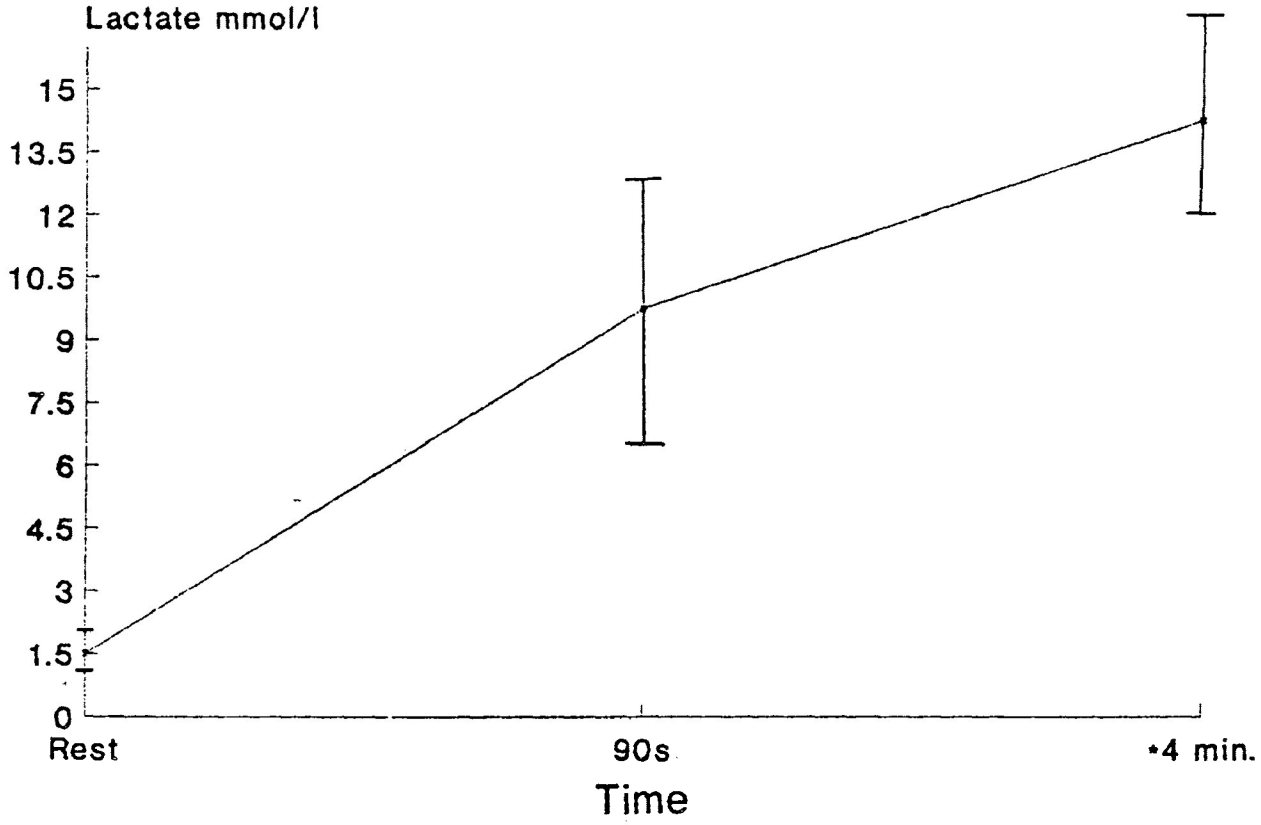
Time	Mean	S.D.	Range
Rest	1.45 mmol/l	±0.52	0.70 - 2.50
Immediately post-exercise	8.30 mmol/l	±3.11	2.40 - 14.50
4 minutes post	14.08 mmol/l	±2.62	8.60 - 19.60

*note: plasma lactate values increased significantly ($p < .01$) from rest to immediately post-exercise, rest to 4 minutes post-exercise, and from immediately post exercise to 4 minutes post-exercise.



*4 min. post exercise

Figure 4: a) Ammonia increase over time



* 4 min. post exercise

Figure 4: b) Lactate increase over time

the 90s of anaerobic exercise. There was a significant positive relation between plasma ammonia and lactate at 4 minutes post-exercise ($r = .62$, $p < .05$). Additionally, ammonia and lactate were correlated over time ($r = .77$, $p < .05$).

Muscle Fibre Type

The secondary purpose of this investigation was to determine if there was a significant relationship between the blood metabolite(s) response and muscle fibre profile. Therefore, correlations were calculated between different time intervals and muscle fibre type. Regression analysis was employed when a significant correlation was found between ammonia and muscle fibre profile. The muscle fibre profile of these 12 subjects can be seen in Table 9.

There was no significant correlation between the percentage of fast-twitch (type a or b) or slow-twitch fibres and plasma ammonia. No significant correlation was found between ammonia and muscle fibre type. However, when the ammonia levels immediately post-exercise were plotted versus percentage FT muscle fibre there was a slight linear trend. This trend had two outliers with a FT fibre profile below 40%. When the two subjects were removed from the analysis a more linear trend was found. Therefore, ammonia values were compared amongst only those individuals with a 40% or greater FT profile. With this subgroup of subjects ($n = 10$) there was a significant positive correlation ($r = .58$, $p < .05$, one-tailed) between ammonia levels

Table 9: Muscle fibre profile of subjects (n = 12)

Fibre	Mean	S.D.	Range
ST	51.17	±10.14	29.0 - 61.0
FT	48.83	±10.14	39.0 - 71.0
FTb/FT	26.10	±13.90	5.0 - 49.0
FTb/all fibres	8.80	±7.50	1.0 - 18.0

* note FTb/FT is the percentage of FTb fibres of the FT population, FTb/all fibres is the percentage of FTb fibres of the total amount of fibres - both FT and ST.

at 90s and FT muscle fibre. The regression equation for this was $\hat{Y} = 0.0614x + 44.27$ (see Figure 5).

It was also of interest to examine if a possible relation existed between ammonia change (ammonia levels at 90s minus ammonia levels at 4 minutes post-exercise) and muscle fibre type. The correlation coefficient for ammonia change versus %FT muscle fibre was positive, $r = .015$. This was not found to be significant ($p > 0.05$, one-tailed). Furthermore, when ammonia change was compared with %FTb fibres, the correlation coefficient was not significant ($r = -.14$, $p > .05$, one-tailed).

Plasma lactate at four minutes post-exercise showed a significant positive correlation ($r = .55$, $p < .05$, one-tailed) with the fast-twitch b fibre. When lactate change scores (lactate immediately post-exercise minus lactate 4 minutes post-exercise) were considered, no significant correlations ($p > .05$, one-tailed) were found versus fibre profile, nor versus power and capacity measures of the anaerobic 90s test.

Peak power (W/kg) was significantly correlated ($r = 0.66$, $p < 0.01$, 1-tailed) with the total relative capacity (joules/kg). Table 10 lists the correlations of other selected variables which were not statistically significant ($p > 0.01$).

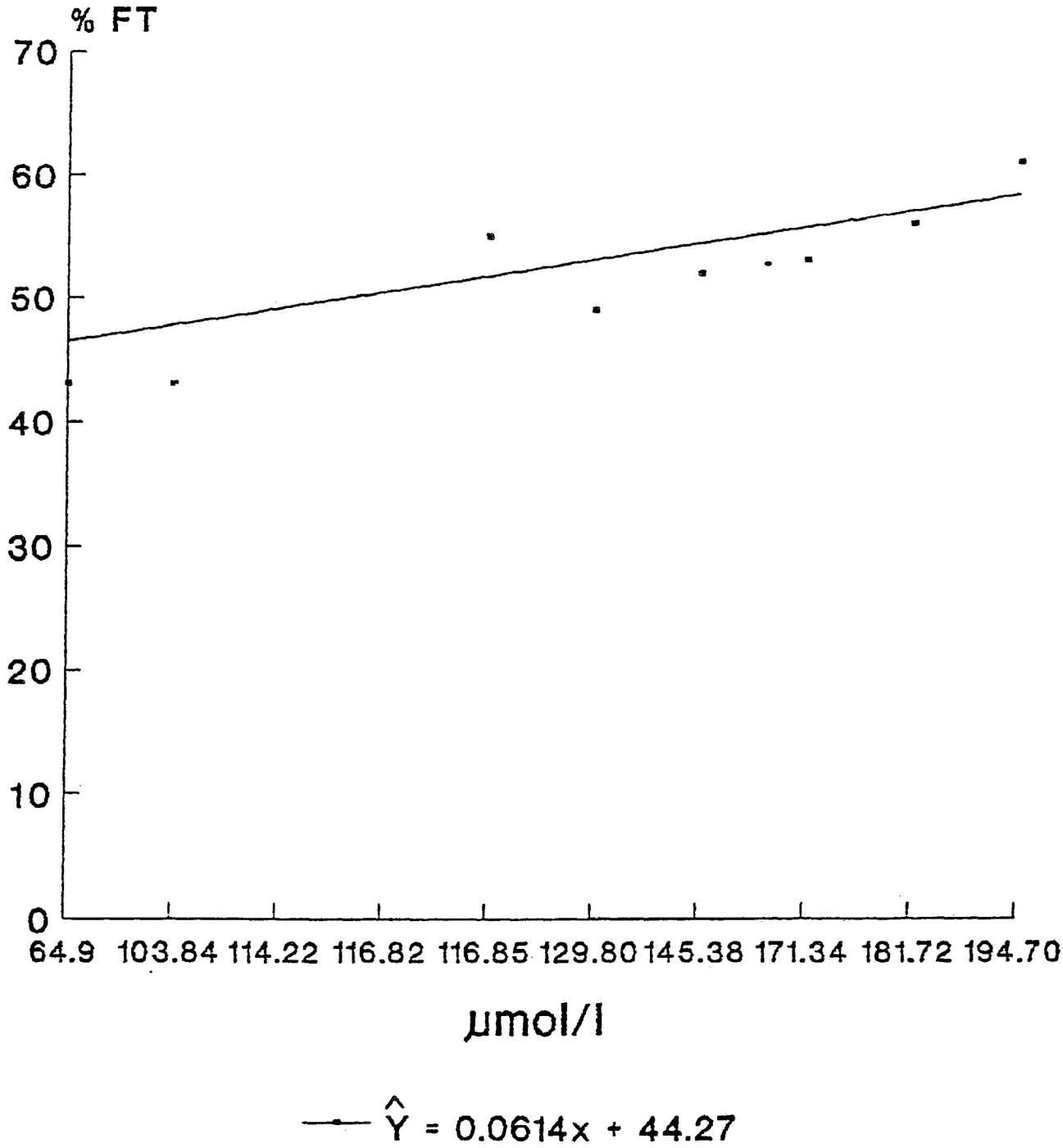


Figure 5: Ammonia vs percentage of FT fibres

Table 10: Correlations of selected variables

	Capacity (J/kg)	%FT	%ST
%FT	-0.1320	-	-
%ST	0.1320	-	-
Watts at 60s	-0.0939	-0.0721	-0.0754
Watts at 90s	-0.0926	-0.0715	-0.0748

Chapter 5

DISCUSSION

Ammonia and Anaerobic Exercise

Many researchers have demonstrated that plasma ammonia accumulation peaks immediately following intense anaerobic exercise (Babij et al., 1983; Banister et al., 1985; Banister et al., 1983; Harris et al., 1987). However, results from the present study demonstrated that plasma ammonia levels were consistently (diagnostic assays done in triplicate) higher at 4 minutes post-exercise (253 μmol/l) than immediately post-exercise (116 μmol/l), which is in agreement with the results of previous studies (Buono et al., 1984; Harris & Dudley 1989). Nonetheless, the relative intensities of the exercise protocol employed by Buono et al. (1984) and Harris & Dudley (1989) were somewhat different from the present investigation.

The anaerobic exercise protocol used by Buono et al. (1984) involved 1 minute of pedalling (70 rpm) at 25 W with a workload increase of 25 W every minute until exhaustion. The approximate duration of exercise time reported in their study was 10 minutes (Buono et al., 1984). Similarly, Harris & Dudley (1989) employed an exercise protocol with a pedalling rate of 70 or 80 rpm for the first minute at 100-120 W, and increased the workrate by 35-40 W at the end of each minute. Total duration of the test was 5-8.5 minutes. However these forms of exercise were not as

intense as the 90s bicycle test outlined in this study.

The mean plasma ammonia value of 251.8 μmol/l observed at 4 minutes post-exercise is higher than the post-exercise recovery values 153.0 and 122.0 μmol/l reported by Harris & Dudley (1989), and Buono et al. (1984) respectively. It is possible that the severe exercise stress imposed by the 90s protocol contributes to the accumulation of ammonia in blood during the recovery period. Perhaps the physiological demand, on the leg musculature, in such a short time frame, is such that the complete diffusion of ammonia out of skeletal muscle may not occur until the cessation of exercise (Graham, Richter, & Saltin, 1989), and thus would account for the rise in ammonia observed at 4 minutes post-exercise. Furthermore, Harris and Dudley (1989) state that ammonia is a weak base and hence a higher concentration of ammonia will exist in the biological compartment with the lower pH (Lawrence, Jacques, Dienst, Poppell, Randall, & Roberts, 1957).

Muscle pH post anaerobic exercise is more acidic than blood pH at the same time interval (Mutch & Banister, 1983a; Newsholme & Leech, 1988b). Therefore, there is justification for stating that immediately post-exercise large quantities of ammonia may still be in the muscular compartment due to the relatively lower pH existing in muscle (Harris & Dudley, 1989).

It has been suggested that the major processes for removing ammonia from the plasma are very rapid (Graham, Bangsbo, Gollnick, Juel, & Saltin, 1990). Graham and colleagues (1990)

suggest that the purine nucleotide cycle works in series, with the resynthesis portion of the cycle operating predominantly in recovery. Assuming that active resynthesis is occuring, then the ammonia present in blood plasma may not be a true representation of ammonia production. The actual ammonia value would be higher.

High intensity exercise of short duration places a heavy emphasis on anaerobic glycolysis and results in the production of protons as well as lactate (Newsholme & Leech, 1988b). The plasma lactate concentration was higher at 4 minutes post-exercise (14.08 mmol/l) than immediately post-exercise (8.30 mmol/l). This may indicate that the blood compartment becomes more acidic during recovery while lactate and H+ diffuse into the blood (Buono et al., 1984; Sahlin, Alvestrand, Brandt, & Hultman, 1978). Should the blood compartment become more acidic, ammonium ions may diffuse into blood (Harris & Dudley, 1989) during the 4 minutes post-exercise. Furthermore, investigations by Lawrence et al. (1957) have established that a lowering of blood pH (acidosis) increases plasma ammonia levels; conversely, increasing blood pH (alkalosis) decreases plasma ammonia levels. It was observed in this study that plasma ammonia values were higher when corresponding plasma lactate values were higher and that the two metabolites were correlated at 4 minutes post-exercise and over time.

Graham et al. (1990) were unable to find concomitant increases in the muscle-to-venous H+ and muscle-to-venous ammonia ratios suggesting that the ammonia exchange across the sarcolemma

may be regulated by factors in addition to H+. The lungs may help regulate ammonia equilibrium (Graham et al., 1990). Moreover, the lungs are the site which receive the entire cardiac output, and coupled with the increased concentration gradient and the high permeability of ammonia, support the suggestion that ammonia may be released from the blood and expired (Graham et al., 1990).

The FT muscle fibres are primarily recruited for work during anaerobic-type exercise (Gerard, Caiozzo, Rubin, Prietto, & Davidson, 1986; Meyer et al., 1980b; Schmidtbleicher & Haralambie, 1981). Therefore, the ammonia present in blood plasma may be a reflection of the stress specific muscle fibre recruitment pattern; intensive exercise recruits the FT fibres (more abundant AMP deaminase and glycolytic enzymes), while less intensive exercise recruits the ST Fibres (more abundant oxidative enzymes). Additionally Katz et al., (1986b) argued that the AMP deaminase activity increases with a greater percentage of FT fibres. If the majority of fibres recruited were of the FT type, then there is a greater probably to produce ammonia. This phenomenon may have occurred during the 90s protocol because the subjects with a higher percentage of FT appeared to release more ammonia in blood plasma (see figure 5). The muscle fibre recruitment nature of exercise has been explained previously (Dudley et al., 1983; Meyer et al., 1980b; Meyer et al., 1979).

Ammonia, Lactate and Anaerobic Exercise

While it was demonstrated that both plasma ammonia and lactate increased significantly over time, ammonia and lactate appeared to be correlated at 4 minutes post exercise. Delayed ammonia diffusion out of the muscle may explain why the values are more correlated post-exercise. Some researchers (Babij et al., 1983; Buono et al., 1984) have confirmed ammonia and lactate to be linearly related during exercise. Although this investigation was unable to sample blood every 30 seconds, ammonia and lactate were linearly related ($r = .77$) over time (pooled data before and after the exercise period, and during recovery) (see Figure 6).

It has been demonstrated that in humans maximal blood lactate concentrations are found 3-5 minutes post exercise (Babij et al., 1983; Buono et al., 1984). This is in agreement with the observations found in this study. The relation between 4 minute ammonia and 4 minute lactate ($r = 0.62$), and between ammonia and lactate over time ($r = .77$) may suggest an influence on active glycolysis, primarily on PFK. Previous investigators concluded that ammonia relieves the inhibitory effect of ATP on PFK (Newsholme & Leech, 1988c; Sugden & Newsholme, 1975). However, Katz et al. (1986b) argued that ammonia has no direct stimulation on PFK, but rather it is the AMP recycling which de-inhibits the enzyme. Additionally, when the PNC is fully operative, there is a net conversion of aspartate to fumarate and ammonia (Meyer et

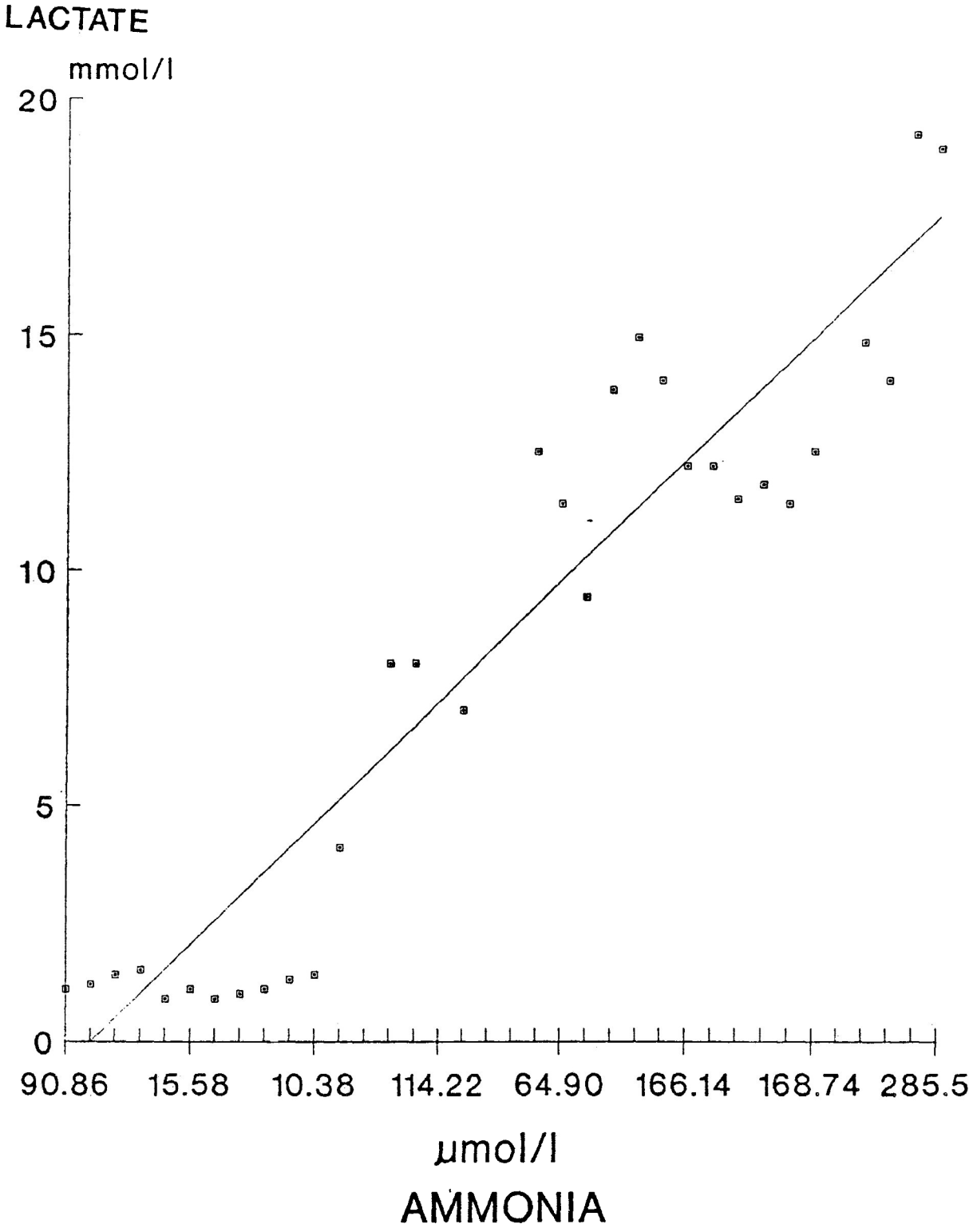


Figure 6: Ammonia vs lactate over time

al., 1980b). The contribution of a citric acid cycle intermediate may support accelerated aerobic metabolism during muscular contraction (Meyer et al., 1980b). In theory this could occur because aspartate produced from other amino acids can undergo transamination with oxaloacetate (regenerated from fumarate by action of the enzymes fumarate dehydratase and malate dehydrogenase) (Newsholme and Leech, 1988c). Furthermore, aspartate may enter the mitochondrion as the carbon skeleton of oxaloacetate (there is no transporter for oxaloacetate) in the process known as the malate/aspartate shuttle; providing reducing equivalents from the cytosol into the mitochondrion (Newsholme and Leech, 1988a).

Regardless of the mechanisms, the findings of these researchers (Katz et al., 1986b; Meyer et al., 1980b; Newsholme & Leech, 1988c; and Sugden & Newsholme, 1975) establish that a glycolytic process (primarily the production of lactate) and a purine nucleotide process (primarily the production of ammonia) are occurring simultaneously. This would lend support to the observed correlation between ammonia and lactate over time.

Ammonia, Lactate and Muscle Fibre Type

Research has shown that the FT muscle fibres, in particular the type FTb or FT glycolytic, are the source for ammonia production during strenuous exercise (Dudley et al., 1983; Meyer et al., 1980b; Meyer et al., 1979). However, Graham et al. (1987) have demonstrated that the type I fibres are a major

source of ammonia during long duration exercise and that the FTa fibres contribute ammonia in the early phase of activity. During longer duration activities skeletal muscle amino acid metabolism can interact with AMP deaminase activity to produce ammonia (Graham et al., 1989; Graham et al., 1987). However as in the present study it is unlikely that during short duration exercise amino acid metabolism can contribute to the plasma ammonia exercise response (MacLean, Spriet, Hultman & Graham, 1989).

In a subgroup of the study (n=10), a relation was found between fibre profile and ammonia in blood plasma; those subjects with a greater percentage of FT muscle yielded higher amounts of ammonia. This association as been demonstrated previously (Banister et al., 1985; Dudley et al., 1983; Meyer & Terjung, 1979; and Sehling-Werle et al., 1988). However, it was difficult to ascertain whether there was any true ammonia-fibre type relationship. Therefore, caution must be employed when interpretating this association because of other components such has individual training regimes and potential utilization of the subjects' fibre type (Meyer & Terjung, 1979). Nonetheless, it is important to note that there was a significant correlation (0.69) between the FT fibre and ammonia immediately post-exercise.

It has been shown previously that there is a strong correlation between lactate and the FTb muscle fibre profile (Chi et al., 1983; Essén-Gustavsson & Henriksson, 1984; Newsholme & Leech, 1988a; Saltin et al., 1977). There was a correlation of r = .55 between lactate and the FTb fibre type in this study. Care

must be taken in interpreting this relation as the measurement of lactate production may be complicated by the fibre type heterogeneity of human muscle, i.e. lactate produced in one fibre may diffuse into an adjacent fibre and be oxidized (Katz & Sahlin, 1990).

Anaerobic Exercise

The 90s protocol outlined previously (Serresse et al., 1988; Simoneau et al., 1983) was used because it represents a true reflection of glycolytic capacity (Serresse et al., 1988; Simoneau et al., 1983). The exercise design was within the guidelines defining glycolytic capacity; a test greater than 60 s but shorter than 120 s in duration (Serresse et al., 1988; Simoneau et al., 1983).

The mean total work capacity (687.3 J/kg) of the 90s protocol in this study is higher than reported previously (Serresse et al., 1989; Serresse et al., 1988; Simoneau et al., 1983). Mean values reported by other authors were 558 (Serresse et al., 1989), 493 (Serresse et al., 1988), and 486 J/kg (Simoneau et al., 1983). The lowest value reported in our laboratory was 575.6 J/kg, while the highest was 991 J/kg. It would appear that inclusion of the pacing interval allowed the subjects to produce more total work than if there had been no pacing interval. The subjects in the present study represented a mixture of both aerobically and anaerobically trained athletes. Therefore, it is unlikely that the greater total work capacity demonstrated in this study was a reflection of the muscle fibre

profile of the group.

The mean peak power score (6.54 W/kg) was lower than the scores reported previously for the same 90s protocol (Serresse et al., 1989; Serresse et al., 1988; Simoneau et al., 1983). The 30s pacing interval does not truly allow peak power to be attained because the FT fibres are recruited earlier in exercise and perhaps are partially fatigued. Therefore, the pacing interval may effect peak power scores more than the physiological characteristics of the subjects.

Anaerobic capacity has been defined as the maximal amount of ATP formed by anaerobic processes during exercise (Medbo, Mohn, Tabata, Bahr, Vaage, & Sejersted, 1988). Glycolysis is primarily responsible for the formation of ATP anaerobically (Medbo et al., 1988; Newsholme & Leech, 1988a). It has been suggested that the 90s bicycle test is a more reliable estimate of total glycolytic capacity (Serresse et al., 1988); furthermore, the presence of plasma lactate indicates that anaerobic glycolysis has occurred (Juel, Bangsbo, Graham, & Saltin, 1990; Newsholme & Leech, 1988). Using plasma lactate values as an indicator of anaerobic capacity, the 90s protocol employed in this study was as intense as other anaerobic protocols (Juel et al., 1990; and Medbo et al., 1988). The mean plasma lactate concentration was 14.08 mmol/l (S.D. = ±2.62); in one individual the peak lactate score was 19.6 mmol/l. These lactate values were similar to the results observed by Juel and co-workers (1990), who used a leg extensor anaerobic protocol.

Chapter 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to investigate the plasma ammonia and lactate response to anaerobic exercise, lasting 90 seconds (s) in duration. The secondary purpose was to plot the relationship of the ammonia and lactate response to muscle fibre profile. Twenty-five subjects were students from various faculties at Lakehead University, Thunder Bay, Ontario.

Four muscle biopsies were taken from one site on each individual. The muscle was stained to obtain individual fibre profiles. All subjects performed 90s of all-out exercise, and blood was sampled in order to determine plasma ammonia and lactate values. These metabolite values were plotted against the muscle fibre profile in order to determine if there was a linear relationship amongst the variables.

Ammonia increase was tested for significance, as was lactate increase. Lactate was also plotted versus muscle fibre profile and was tested for correlation.

It was established that in this study ammonia in blood plasma was higher at 4 minutes post-exercise versus immediately post-exercise, and is in disagreement with some previous work (Babij et al., 1983; Banister et al., 1983). Furthermore, lactate and ammonia were related over time and more so at 4 minutes post-exercise (0.62) and over time (0.77). There was a

correlation (0.58) between plasma ammonia immediately post-exercise and the FT muscle fibre only when those subjects with a 40% or greater FT fibre profile were plotted.

The mean work capacity (687.3 J/kg) in this study was higher than values reported previously (Serresse et al., 1989; Serresse et al., 1988; Simoneau et al., 1983).

Conclusions

It was demonstrated that plasma ammonia and lactate respond similarly to a 90s test of anaerobic exercise; nonetheless, the two metabolites appear to be independent of each other. Fibre profile may explain the differences in ammonia levels immediately post-exercise. However, care must be taken in the interpretation of these results. The limited number of subjects in this subgroup does not allow one to confidently explain this phenomena. The ammonia diagnostic assay is far from being simple with respect to its procedures, and does require more than five minutes to obtain a value.

Recommendations

Although there was a relation observed between ammonia immediately post-exercise and the percentage of FT fibres, the number of examinations observed was not strong enough to confidently infer the phenomena consistently. Therefore, a higher number of subjects would certainly increase one's reliability of prediction.

The diagnostic assay used for ammonia analysis (Sigma Diagnostics, St. Louis, Missouri) was not as simple as published. The length of time to perform the procedure was longer than advertized and furthermore, the need to use deionized double distilled water is more fastidious than suggested.

The anaerobic protocol used in this study was highly intense. To determine the amount of ammonia still present in skeletal muscle after such exercise, it is recommended that a muscle biopsy should be performed before, after and 4 minutes after exercise in conjunction with blood sampling. The muscle should be analyzed for ammonia and compared with the blood metabolite values. This would give a good reflection of ammonia production in skeletal muscle, as well as to allow for comparison to blood plasma. Blood pH measured at similar intervals would further establish the relation between high levels of plasma ammonia and acidity.

Graham and colleagues (1990) suggest that the lungs are a major site of ammonia removal during exercise. Should this avenue be investigated than a better understanding of ammonia displacement would be found.

If muscle biopsies are performed in conjunction with blood assays, an analysis of the TAN pool would also be suggested. Comparing the TAN pool response not only to ammonia response, but also to lactate response, may give a better understanding of the glycolytic system.

With respect to the use of the 90s protocol, it would be

advised to manipulate the kilopond resistance according to the individual's fat-free weight of the individual. For example a 70 kg individual with 15% body fat would have a total of 59.5 kg fat free weight and the ratio, 0.05 kiloponds per kilogram fat free weight, would have a resistance of 3.0 kp. This would be particularly important to investigate with respect to anaerobic capacity because it is believed that anaerobic capacity is highly dependent on the mass of exercising muscle (Medbo et al., 1988).

APPENDIX A

Ammonia: Quantitative, Enzymatic Determination In Plasma at 340 nm (Sigma Diagnostics, St. Louis, Missouri)

Instrument and Materials Required

Instrument: A narrow- bandwidth spectrophotometer (Gilford, Beckman or Perkin-Elmer, etc.) is needed to provide accurate 340nm readings.

Materials: For most narrow-bandwidth spectrophotometers, 1-cm cuvetts capable of holding 3-ml volumes are used. Semimicro cuvetts may also be employed. Conventional or automatic pipets are needed to reliably deliver 0.02, 0.2, 5.0 and 10.0 ml. Water should be deionized and ammonia-free.

Manual Procedure:

A BLANK and CONTROL are included with each series of assays, An Ammonia Reagent vial is sufficient for 5-6 assays. For more than 1 assay, reconstitute and combine the appropriate number of vials. Use this solution as described in Step 1. Ammonia Reagent Multi-Assay vial is sufficient for a BLANK and CONTROL as well as 8 tests.

1. To BLANK cuvett, add 3.0 ml Ammonia Assay Solution and 0.2 ml water.
   To CONTROL cuvett, add 3.0 ml Ammonia Assay Solution and 0.2 ml of Ammonia Control Solution.
   To TEST cuvett, add 3.0 ml Ammonia Assay Solution and 0.2 ml Plasma.
   Mix and wait approximately 5 minutes for equilibration.

2. Read and record INITIAL absorbance of each cuvett at 340 nm vs water or Potassium Dichromate Solution as reference.

3. Add 0.02 ml L-GLDH Solution to all cuvetts.
   Mix by gentle inversion and wait approximately 5 minutes.

4. Read and record FINAL absorbance of each cuvett vs water or Potassium Dichromate Solution.

APPENDIX B

Lactate: Routine Sample Measurement Procedure Using A YSI Model 23L Lactate Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio)

1. Set to RUN, press and release the CLEAR button, zero as needed. (-0.1 to 0.2 are acceptable zero values.)

2. Rinse Syringepet twice with water, twice with 5.0 mmol/l standard.

3. Fill Syringepet with 5.0 mmol/l standard and inject.

4. Press and release CLEAR button.

6. Rinse the Syringepet twice with water and twice with the sample.

7. Fill Syringepet with sample and inject.

8. When the word READ is illuminated, record the sample value.

9. Press and release the CLEAR button.

10. Start again at step 2 if you have more samples to measure; if not, set the RUN/STANDBY switch to STANDBY.

APPENDIX C

Method For The Myosin ATPase Stain

From: Padykula, H.A., & Herman, E. (1955). The specificity of the histochemical method for adenosine triphosphatase. Journal of Histochemistry Cytochemistry, 3, 170-195.

Stains and solutions:

1. Acetate buffer (for acidic preincubations)
2. Pre-incubation medium (for alkaline preincubations)
3. Incubation medium (for both acidic and alkaline preincubations)
4. Calcium chloride, 1% $CaCl_2$
5. Cobalt chloride, 2% $CoCl_2$
6. Ammonium sulphide, 1% $(NH_4)_2S$

* All solutions can be pre-made and stored for 4-6 weeks except numbers 6 and 3 (for #3 add ATP to the pre-incubation medium, which itself can be stored)

** Store solutions #1, 2, (and 3 without ATP) at 4°C
*** Store solutions # 4 and 5 at room temperature

Preparation of Solutions:

1. Acetate Buffer	Amount	Purpose
Na-Acetate (anhydrous)	3.9g	Buffer
KCL	3.7g	Buffer
Distilled H_2O	500ml	Solvent

Adjust pH to 4.3 and 4.58 with concentrated glacial acetic acid (the exact pH's will depend upon the pH optima of the tissues) and bring up to volume of 500ml with H_2O.
The pH of these solutions must be checked each time they are used - pH at room temperature.

2. Pre-incubation Medium	Amount	Purpose
Glycine	3.04 g	Stabilizer
NaCl	2.34 g	Buffer salts
CaCl (Dihydrate)	3.20 g	Ca to remove excess PO_4
D. Distilled H_2O	400ml	Solvent
NaOH (0.1N)	1.44 g to 360ml H_2O	

Combine all solutions and pH to 10.0 with concentrated HCl.
Allow to come to room temperature prior to using!

3. Incubation Medium

This is made from the pre-incubation medium and fresh ATP. This should be pHed at 37°C

ATP	34 mg	68 mg	102 mg	136 mg
Preincubation med	20 ml	40 ml	60 ml	80 ml

* 17 mg/10 ml of preincubation medium

Procedure:

1. Tissue sections must be dry. Leave at room temperature for at least 2 hours if fresh sections, if stored sections then 30 minutes.

2.a) Preincubate at pH 10.0 for 10 min at 37°C in preincubation medium
  b) Preincubate at pH 4.58 for 1.5 min at 25°C in acetate buffer pH 4.58
  c) Preincubate at pH 4.30 for 1.5 min at 25°C in acetate buffer pH 4.30

* Each preincubation on a different section

3. Rinse sections well in distilled H_2O 5 times

4. Incubate for 30 min at 37°C in incubation medium (*note all samples incubated in this medium)

5. Repeat step # 3

6. Incubate in 1.0% $CaCl_2$ for 3 min at room temp.

7. Repeat step # 3

8. Incubate in 2.0% $CoCl_2$ for 3 min at room temp

9. Repeat step # 3

10. Incubate in 1% ammonium sulphide $(NH_4)_2S$ for 1 min at room temp

11. Repeat step # 3

12. Dehydrate in 70% EtOH for 2 min at room temp

13. Dehydrate in 80% EtOH for 2 min at room temp

14. Dehydrate in 100% EtOH for 2 min at room temp

15. Rinse twice in histoclear

16. Mount with permount

APPENDIX D

Consent Form For Participants

SUBJECT..... DATE..... TIME

1. I agree to participate in an investigation by Drs.....

..... and/or such assistants as may be selected by them, to perform the following procedure(s):

two muscle samples (5 to 7 mg) will be taken from a small 1 cm long incision from the vastus lateralis using a biopsy needle under local anaesthetic. Slight discomfort will persist for 1 to 2 hours following the surgery.

2. I also understand that slight muscle soreness may persist for a few days following surgery and that I may, at any time refuse to continue and/or begin the experiment and withdraw from participation. The Drs..... have assured me of confidentiality and will discuss the results personally with me in the future.

.....	.....	.....
Witness	Signature of Subject	Signature of Doctor(s)

APPENDIX E

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

SUBJECT..... DATE.....

TIME.....

1. I agree to participate in an investigation by..... ..... and/or such assistants as may be selected by them, to perform the following procedure (s):

A) a 90 second all-out bicycle ride against a fixed resistance (0.05 kiloponds/kilogram body weight).

B) prior to exercise a blood catheter will be inserted into the antecubital vein, following which blood shall be sampled at rest, at 30 seconds, 60 seconds, and 90 seconds during exercise. One last sample will be taken at 4 minutes post exercise.

2. I also understand that at any time I can refuse to continue and/or begin the experiment and withdraw from participation. ..... has assured me of confidentiality and will discuss the results personally with me in the future.

.....	.....	.....
Witness	Signature of Subject	Signature of Researcher

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