THE EFFECT OF ENDURANCE TRAINING ON HUMAN AMMONIA METABOLISM

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ABSTRACT

This thesis examines human ammonia (NH_3) metabolism in trained and untrained individuals. Specifically, these aspects include: 1) plasma NH_3 accumulation during exercise at similar absolute and relative work intensities; 2) plasma NH_3 accumulation during recovery from maximal exercise; 3) the absolute and relative work intensities at which blood lactate (LA) and plasma NH_3 concentrations begin to rapidly accumulate (i.e the LA breakpoint - LABP, and NH_3 breakpoint - NH_3BP); 4) the relationship between the NH_3BP and LABP, and 5) the relationship between plasma NH_3, blood (LA), and muscle fibre type.

Seven untrained and six endurance trained males were tested on two occasions. Initially, each subject performed an incremental, maximal exercise test on a bicycle ergometer. On the second occasion each individual rode for up to 15 minutes at an exercise intensity equivalent to 85% of maximal oxygen uptake ($\dot{V}O_2$max). Venous blood was sampled throughout each exercise test and during recovery from the maximal exercise bout. Muscle biopsies were performed on 11 of the original 13 subjects (5 trained and 6 untrained) in order to determine the percentage of slow twitch fibres (%ST).

Based on the results the following comparisons between trained and untrained individuals were determined.

1. Trained athletes have a reduced plasma NH_3 accumulation during intense submaximal exercise at the same absolute workload.
2. Trained athletes have a similar plasma NH_3 accumulation during exercise at the same relative intensity.
3. Trained athletes have a reduced plasma NH_3 accumulation during recovery from maximal exercise.
4. The NH_3 BP occurs at similar absolute and relative exercise intensities in both groups.
5. The groups had significantly different relationships between: a) NH_3 BP and LABP,
   b) plasma NH_3 and blood LA accumulation during submaximal exercise.
   c) peak NH_3 accumulation and %ST fibres.

These findings suggest that endurance training alters NH_3 metabolism in exercising humans.

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CHAPTER 1

GENERAL INTRODUCTION

The production of ammonia (NH_3) by active skeletal muscle was first documented about sixty years ago (Parnas, 1929). Potentially, NH_3 can be produced in skeletal muscle via three biochemical pathways. These include the adenosine monophosphate (AMP) deaminase pathway (Lowenstein, 1972), the 5'-nucleotidase / adenosine deaminase pathway (Whitlock and Terjung, 1987), and amino acid deamination (Graham et al. 1987, Haralambie and Mössinger, 1980; Lowenstein and Goodman, 1978). A large number of investigations have established the importance of the AMP deaminase pathway, especially during intense exercise (Dudley and Terjung, 1985a; Flanagan et al. 1986; Jansson et al. 1987; Meyer and Terjung, 1979; Meyer and Terjung, 1980; Sahlin et al. 1978). Controversy still exists over the activation of this pathway during moderate work intensities (Flanagan et al. 1986; Meyer and Terjung, 1980), while it is generally believed that the AMP deaminase pathway is inactive during light work (Katz et al. 1986a; Meyer and Terjung, 1980). Very little information exists about the significance of the other potential NH_3 producing pathways. In fact, there is a lack of definitive evidence that intramuscular amino acid

catabolism can produce free NH_3, although indirect evidence suggests that it may occur (Haralambie and Mössinger, 1980; Lowenstein and Goodman, 1978).

A number of researchers have observed a significant increase in muscle NH_3 concentration in exercising animals (Constable et al. 1987; Dudley and Terjung, 1985a, Goodman and Lowenstein, 1978; Meyer and Terjung, 1979; Meyer and Terjung, 1980). Similarly, increases in NH_3 concentration in exercising human muscle have been observed (Graham et al. 1987; Katz et al. 1986a,b). Changes in human mixed muscle NH_3 concentration appear to be influenced by both exercise intensity and duration. During short duration moderate exercise (i.e., 50% $\dot{V}O_2$max) no increase in NH_3 is found (Katz et al. 1986a). At higher exercise intensities mixed muscle NH_3 concentration increases significantly (Graham et al. 1987; Katz et al. 1986a) and with a continuation of work further increases have been reported (Graham et al. 1987).

Plasma NH_3 concentration, like muscle NH_3 concentration, increases in response to increasing exercise intensity and duration (Babij et al. 1983; Buono et al. 1984; Eriksson et al. 1985; Graham et al. 1987; Maughan and Sadler, 1983; Wilkerson et al. 1977). The reported point at which a significant NH_3 elevation occurs in humans ranges between 35 and 70% $\dot{V}O_2$max (Babij et al. 1983; Buono et al. 1984; Eriksson et al. 1985; Wilkerson et al. 1977). This increase has been attributed to an increased NH_3 efflux from contracting skeletal muscle rather than a

decrease in plasma NH_3 removal rate (Eriksson et al. 1985; Graham, personal communication). NH_3 uptake by the liver is not reduced during exercise despite a reduction in splanchnic blood flow (Eriksson et al. 1985). As well, other major organs involved with plasma NH_3 removal (i.e., resting skeletal muscle and brain) probably increase their uptake rates during exercise (Lockwood et al. 1979; Meyer et al. 1980).

A number of studies using animals have investigated the effects of endurance training on muscle or plasma NH_3 accumulation during exercise (Barnes et al. 1964; Constable et al. 1987; Dudley and Terjung, 1985a; Miller and Lawrence, 1986). These studies have consistently reported that both muscle (Constable et al. 1987; Dudley and Terjung, 1985a) and blood NH_3 accumulation (Barnes et al. 1964; Miller and Lawrence, 1986) are reduced by training at a given absolute submaximal workload. Experiments investigating the effects of training on human muscle NH_3 concentration have not been conducted. However, the effects of training on blood NH_3 accumulation have been studied with mixed results. Whilst there is general agreement that blood NH_3 accumulation is unaltered at the same relative intensity after training there is disagreement as to the effect of training on NH_3 accumulation at the same absolute intensity (Hurley et al. 1984; Lo and Dudley, 1987). Clearly, human experimentation has not unequivocally supported the proposition that plasma NH3 accumulation is reduced by training.

There are many other aspects of NH_3 metabolism that may be influenced by endurance training that have not been investigated in either animals or humans. As previously mentioned the relative work intensity at which plasma NH_3 begins to rise above resting levels has been found to vary between 35 to 70% $\dot{V}O_2$max. This variation has been attributed to either methodological or individual differences (Banister et al. 1985). Some authors attribute the initial rise in plasma NH_3 concentration to AMP deaminase activity (Buono et al. 1984). Given that training may reduce flux through the AMP deaminase pathway (Constable et al. 1987; Dudley and Terjung, 1985a) some of the individual variability observed for the plasma NH_3 breakpoint may be attributed to variations in training status.

Another aspect of NH_3 metabolism and endurance training that is worthy of research is the effect endurance training may have on the blood lactate - plasma NH_3 relationship. The absence of the strong positive relationship normally found between blood lactate and plasma NH_3 accumulation has been used to diagnose various metabolic disorders (Fishbein et al. 1978; Haller et al. 1985; Hara et al. 1987; Sinkeler et al. 1985). Unfortunately, certain non-clinical factors (i.e., hyperoxia, muscle fibre type and exercise duration) have been found to affect the NH_3-lactate relationship and may interfere with the diagnostic ability of such tests (Dudley et al. 1983; Graham et al. 1987). One factor which has not

been considered and which could, potentially, effect the relationship between two variables is training status.

Another relationship that may be effected by training is the relationship between plasma NH_3 accumulation and muscle fibre type composition. Dudley et al. (1983) observed that plasma NH_3 accumulation measured immediately after a maximal exercise test was inversely related to the percentage of slow twitch fibres. Based on these results, Banister et al. (1985) suggested that peak plasma NH_3 accumulation may provide a relatively non-invasive estimate of human muscle fibre type. Before plasma NH_3 values can be used to estimate fibre type, it is necessary to know what factors can effect the relationship. Endurance training may be one such factor.

There is a large body of knowledge focusing on the effects of exercise on muscle NH_3 metabolism. However, this introduction has highlighted the fact that the effects of endurance training on NH_3 metabolism require further research. This thesis examines human NH_3 metabolism in trained and untrained individuals and specifically compares:

1. plasma NH_3 accumulation during submaximal work at the same absolute and relative workload;
2. plasma NH_3 accumulation during recovery from maximal exercise;
3. the relationship between plasma NH_3 and blood lactate during submaximal work and after maximal exercise;

4. the relationship between peak plasma NH_3 accumulation and muscle fibre type composition;

5. the absolute and relative work intensity at which NH_3 begins to rapidly accumulate in plasma;

6. the relationship between the work intensity at which NH_3 and lactate begin to rapidly accumulate in plasma.

CHAPTER 2

REVIEW OF LITERATURE

Literature relevant to the examination of the effects of endurance training on human ammonia (NH_3) metabolism during maximal and submaximal exercise will be reviewed. Specifically, this review focuses on the following areas:

1. Body organs and mechanisms involved with determining ammonia concentration in blood or plasma during rest, exercise and recovery from exercise,
2. Factors that influence the hyperammonaemia observed during exercise,
3. Skeletal muscle ammonia production,
4. The removal of ammonia from skeletal muscle,
5. The relationship between muscle ammonia accumulation and muscular fatigue.

2.1 Body Organs and Mechanisms Involved With Determining Ammonia Concentration In Blood Or Plasma During Rest, Exercise And Recovery From Exercise

At any point in time plasma NH_3 concentration reflects the net flux of NH_3 into and out of the circulation. As plasma NH_3 concentration is determined by the constant movement of NH_3 into and out of the circulation it is necessary to discuss the literature that identifies the factors and mechanisms that influence the level of circulating NH_3. Specifically, this section of the review will discuss the contribution of skeletal muscle and other tissue in determining plasma NH_3 concentration during rest, exercise and recovery.

2.1.1. Non-skeletal Muscle Tissue

2.1.1.1 Rest

Resting NH_3 concentration in normal human venous plasma ranges between 5 to 45 μM (mean approximately 25 μM) (Svensson and Anfält, 1982). Resting venous blood NH_3 values normally range between 40 to 85 μM (mean approximately 65 μM) (Banister et al. 1985; Dudley et al. 1983). Resting circulating NH_3 concentration is influenced by a number of organs (Eriksson et al. 1985; Lockwood et al. 1979; Newsholme and Leech, 1983; Onstad and Zieve, 1979; Owen et al. 1960). Published research generally concludes that the gastrointestinal tract (Lawrence et al. 1958; Onstad and Zieve, 1979) and the kidneys (Owen et al. 1960) are the major contributors to

plasma NH_3 concentration at rest. Potentially, NH_3 can be released into the plasma by erythrocytes (Lowenstein, 1972) and the catabolism of monoamines (Mutch and Banister, 1983), however, these mechanisms are thought to be minor sources of circulating NH_3 (Newsholme and Leech, 1983). To maintain resting plasma NH_3 levels at non-toxic concentrations the liver, brain and skeletal muscle remove NH_3 from the circulation (Bessman and Bradley, 1955; Eriksson et al. 1985; Lockwood et al. 1979; Rosado et al. 1962). Under normal conditions the liver is the most important NH_3 removing and detoxifying organ (Banister et al. 1985).

2.1.1.2 Exercise And Recovery From Exercise

Exercise produces a significant increase in plasma NH_3 concentration (Eriksson et al. 1985). Results from a number of studies suggest that this increase is primarily brought about by a large increase in the production and release of NH_3 from skeletal muscle (Eriksson et al. 1985; Fishbein et al. 1978; Katz et al. 1986a). Evidence that the liver does not reduce its uptake of circulating NH_3 (Eriksson et al. 1985) and the probabilty that inactive muscle and brain increase their NH_3 uptake during exercise (Lockwood et al. 1979; Meyer et al. 1980) strongly indicates that exercise induced hyperammonaemia is not caused by a decreased removal rate of plasma NH_3. It is possible that the gastrointestinal tract, kidneys, erythrocytes and catabolism of monoamines may play a part

in increasing systemic NH_3 levels during exercise. However, indirect evidence from research investigating muscle adenosine monophosphate deaminase deficiency (Fishbein et al. 1978), indicates that any role extra-muscular mechanisms may play (at least during short duration, high intensity activity) in NH_3 production is small and probably insignificant.

Recovery from exercise brings about a return of plasma NH_3 to pre-exercise concentrations (Eriksson et al. 1985). The return of exercise NH_3 levels to resting values is brought about by skeletal muscle (Eriksson et al. 1985; Katz et al. 1986a) and other organs. The non-skeletal muscle mechanisms include a transient increase in NH_3 uptake by the liver (Eriksson et al. 1985) and removal of NH_3 by the brain (Lockwood et al. 1979).

2.1.2 Skeletal Muscle Tissue

2.1.2.1 Resting Muscle

It has been mentioned previously that under normal conditions the liver is the most important organ in the removal and detoxification of circulating NH_3 (Banister et al. 1985). Evidence has been produced, however, which suggests that resting skeletal muscle may also contribute significantly to the removal of arterial NH_3 especially in response to hyperammonaemia caused by liver disease or exogenous infusion of NH_3 (Bessman and Bradley, 1955; Lockwood et al. 1979; Rosado et al. 1962). Resting skeletal muscle is also believed to remove NH_3 from the

circulation when plasma NH_3 concentration is elevated during exercise and immediately following exercise (Meyer et al. 1980). In resting individuals, not suffering from hyperammonaemia, skeletal muscle has been reported to remove 22% of the NH_3 that flows through muscle (Eriksson et al. 1985; Katz et al. 1986a). The biochemical pathways utilised by skeletal muscle to remove NH_3 are discussed in greater detail in a later section of this chapter (page 44).

2.1.2.2 Active Muscle

Studies by Eriksson et al. (1985) and Katz et al. (1986a) have both observed a net release of NH_3 from active muscle. Eriksson and his collegues found that 15 minutes of cycling exercise at 35, 55 and 80% $\dot{V}O_2$ max produced a mean NH_3 efflux rate of 3.6, 14.4 and 45.7 $\mu mol.min^{-1}$, respectively. Katz et al. (1986a) determined the NH_3 efflux rate from active skeletal muscle to be 2 and 89 $\mu mol.min^{-1}$ after riding for a short period of time at 50 and 100% $\dot{V}O_2$ max, respectively. On the basis of these results it has been concluded that exercise induced hyperammonaemia is caused by an accelerated NH_3 formation and release by exercising muscle (Eriksson et al. 1985). Increased skeletal muscle NH_3 production could result from an increased catabolism of either or both AMP (Lowenstein, 1972) and amino acids (Graham et al. 1987). A discussion on the biochemical mechanisms related to skeletal muscle NH_3 production and

factors which affect them are presented in another section of this review (page 22).

2.1.2.3 Recovery

Efflux of NH_3 from active muscle does not cease immediately exercise is stopped but returns to resting values over a period of time. Katz et al. (1986) reported that muscle NH_3 efflux rates fell from 89 ± 21 $\mu mol.min^{-1}$ immediately after maximal exercise to 52 ± 10 $\mu mol.min^{-1}$ after 10 minutes of recovery. The NH_3 efflux rate recorded 10 minutes after exercise was significantly greater than that recorded during basal conditions. Eriksson et al. (1985) reported that a net release of NH_3 from the recovering musculature was not detectable 30 minutes after intense submaximal exercise. In fact, the rate of muscle NH_3 release 30 minutes after exercise was not significantly different to the basal state. After 60 minutes of recovery, muscle was observed to remove NH_3 from, rather than release it into, the circulation.

2.2 Factors That Influence The Hyperammonaemia Observed During Exercise.

This section of the review will examine the relationship between plasma NH_3 accumulation and:

1) exercise intensity,

2) exercise duration,

3) exercise mode,

4) muscle fibre type,

5) endurance training.

To conclude this part of the review, a brief discussion concerning the return of NH_3 to pre-exercise levels will also be presented.

2.2.1 Exercise Intensity

Plasma NH_3 concentration, like blood lactate, increases in a curvilinear or exponential fashion in response to increasing exercise intensity (Babij et al. 1983; Buono et al. 1984; Eriksson et al. 1985; Wilkerson et al. 1977). Plasma NH_3 concentration measured immediately after exhaustive exercise reaches values that are 3 to 5 times greater than at rest (i.e., 80-120 μM)(Buono et al. 1984; Eriksson et al. 1985; Katz et al. 1986a). The relative work rate (as a proportion of maximum) required to produce a significant elevation of plasma NH_3 remains equivocal. The reported point at which a significant NH_3 elevation occurs in humans ranges between 35 to 70% $\dot{V}O_2$ max (Babij et al. 1983; Buono et al. 1984; Eriksson et al. 1985; Katz et al. 1986a; Wilkerson et al. 1977). These discrepancies in the critical exercise intensity required to elevate plasma NH_3 may be accounted for by a number of factors. Variations in exercise protocol may provide one explanation. For example, Buono et al. (1984) used an exercise protocol requiring 25 watt increments every minute and found significant NH_3 accumulation occurring at 55 to

60% $\dot{V}O_2$max. On the other hand, Eriksson et al. (1985) tested subjects at three different exercise intensities (35, 55, and 80% $\dot{V}O_2$max) for a period of 15 minutes at each workload, and found circulating NH_3 to rise significantly at 35% $\dot{V}O_2$max. The site of blood sampling may also contribute to the variation in the critical exercise intensity required to significantly elevate plasma NH_3 concentration. Arterial NH_3 concentration is likely to increase before changes in venous blood are observed because inactive muscle removes NH_3 (Eriksson et al. 1985). Another factor which may explain why plasma NH_3 accumulation has been observed at such a wide range of exercise intensities is muscle fibre type composition. At least two studies (Dudley et al. 1983; Graham et al. 1987) have found a significant inverse relationship between plasma NH_3 accumulation and the percentage of slow twitch fibres. Exercise training may also alter the relative exercise intensity at which NH_3 accumulates in the circulation, however, studies investigating this possibility have yet to be published.

2.2.2 Exercise Duration

Blood NH_3 values have been observed to continually increase throughout prolonged exercise bouts (Graham et al. 1987; Maughan and Sadler, 1983). In 1983, Maughan and Sadler determined blood NH_3 concentration in human subjects who rode to exhaustion at an exercise intensity equivalent to 75% $\dot{V}O_2$max. Blood NH_3 concentration

increased throughout the trial. Graham et al. (1987) determined both muscle and blood NH_3 concentration during exercise of 30 minutes duration at 75-80% $\dot{V}O_2$max. Muscle and blood NH_3 concentrations increased throughout the exercise bout. In preliminary trials, Graham (personal communication) has concluded that blood NH_3 concentration increases throughout prolonged exercise as a result of an increased NH_3 efflux from active muscle. Graham reached this conclusion after measuring NH_3 efflux from human quadricep muscles performing one-legged kicking exercise for 60 minutes at 80% of the maximum O_2 consumption for this muscle group.

2.2.3 Exercise Mode

Wilkerson et al. (1975) compared the venous blood NH_3 concentration taken 4 minutes after exhaustive exercise on either a treadmill or bicycle ergometer. The NH_3 levels measured after cycling (90.6 ± 8.2 μM) were significantly greater than those measured after treadmill exercise (65.9 ± 7.1 μM; $p<0.05$). They attributed the difference in blood NH_3 levels between the two exercise modes to either a difference in the severity of local muscle fatigue or to an alteration in plasma NH_3 clearance rate caused by changes in splanchnic blood flow. These authors argued that differences in local muscle fatigue was a questionable explanation for the difference in NH_3 concentration because other indices of muscle fatigue, such as blood lactate, were similar between the

two exercise modes. Future research needs to examine why NH_3 concentrations are higher after cycling exercise when compared to running. Conclusions cannot be made on the available information.

2.2.4 Muscle Fibre Type

Evidence suggests that blood NH_3 accumulation, determined immediately after intense, short duration exercise, is inversely related to the percentage of slow twitch fibres. Dudley et al. (1983) found a significant inverse relationship between the percentage of slow twitch fibres in the vastus lateralis muscle and increases in venous blood NH_3 levels after maximal exercise ($r = -0.75$, $n = 19$, $p < 0.01$). In the same study, Dudley et al. (1983) also investigated the effects of muscle fibre type on blood NH_3 accumulation after a 3 minute bout of cycling at 85 or 110% $\dot{V}O_2$ max. Once again the results indicated that less NH_3 accumulated in subjects who had the greatest percentage of slow twitch fibres.

Graham et al. (1987) recently determined the relationship between NH_3 accumulation in muscle and blood with muscle fibre type composition of the vastus lateralis muscle. Graham et al. (1987) determined these relationships during 30 minutes of cycling exercise at 75-80% $\dot{V}O_2$ max. Blood NH_3 concentration was measured at rest, and after 5, 15, and 30 minutes of exercise while muscle NH_3 values were determined at rest, and at 5 and 30 minutes of exercise. Blood NH_3 concentration was only related to

type I fibres (r=-0.77) at 15 minutes, while muscle NH_3 concentration was related to type IIa fibres after 5 (r=0.74) but not 30 minutes of exercise (r=0.09). Muscle NH_3 concentration was not related to type I fibre composition at any time. Graham et al. (1987) concluded that type IIa fibres were only a major factor in producing NH_3 during the early stages of exercise. These authors suggested that type I fibres may contribute significantly to NH_3 production and blood NH_3 accumulation when exercise duration was prolonged.

2.2.5 Endurance Training

The effects of endurance training on exercise induced hyperammonaemia has been studied by a number of researchers with mixed results. Barnes et al. (1964) compared the arterial NH_3 concentration of trained and untrained rats after 15 minutes of swimming. Training consisted of a 5 minute swim, once a day, for 1 week and a 15 minute swim, once a day for a further two weeks. Trained rats were reported to have a significantly lower ($p<0.05$) blood NH_3 level (28.2 ± 1.6 μM) than the untrained controls (66.5 ± 7.6 μM). The study by Barnes and his colleagues can be criticised for an inadequate documentation of the effectiveness of the training program. They reported that swim time to exhaustion was greater in trained rats implying that the training program was sufficient to enhance endurance performance. The authors did not apply any statistical tests to their swim time to

exhaustion data. When Student's T-test for two sample means was applied, no statistical difference ($p>0.05$) was found for swim time to exhaustion between the trained and untrained rats, a finding which seriously undermines the basis of this study. It is more likely that the reduced blood NH_3 levels reported by Barnes and his colleagues is due to differences in exercise intensity during the swim rather than any effects of training. The so called trained rats were familiarised with the mode of exercise unlike their untrained counterparts. Therefore it might be expected that the untrained rats would thrash about in the water, and thereby work at a greater intensity and accumulate more NH_3 in the 15 minute swim than the trained animals.

In a recently published abstract Miller and Lawrence (1986) compared the plasma NH_3 concentration of seven horses after the performance of the same absolute work bout before and after training. Details of the training program were not reported. The absolute work bout consisted of a 12 minute treadmill run at 4.5 metres per second. Miller and Lawrence (1986) reported that training significantly reduced immediate post-exercise plasma NH_3 levels (pre-training, 113.3 ± 11.0 μM; post-training, 82.1 ± 3.3 μM, $p<0.05$).

Lo and Dudley (1987) examined blood NH_3 content in human subjects at both the same absolute and relative work intensity pre and post-training. Eleven subjects trained 6 days per week for seven weeks alternating days of

continuous cycling (40 minutes/session) and interval running (5 repetitions, 5 minute work intervals). The NH_3 content of blood was determined immediately after 4 minutes of cycle ergometer exercise at power outputs of 120, 170 and 235 watt pre-training and at 170, 235 and 270 watt post-training. According to the authors these power outputs represented for each occasion relative workloads of approximately 65, 90 and 115% $\dot{V}O_2$max. In agreement with Miller and Lawrence (1986), Lo and Dudley found that training results in a significantly lower NH_3 concentration ($p<0.05$) during exercise of the same power output (i.e., 170 and 235 watt). They also reported that training did not effect plasma NH_3 accumulation during exercise of the same relative intensity.

In direct contrast to the previous studies, Hurley et al. (1984) failed to observe any training induced reduction in plasma NH_3 concentrations during the same absolute work bout. Like Lo and Dudley (1987), Hurley et al. (1984) reported that training did not alter plasma NH_3 levels during exercise of the same relative intensity. The training program used in the latter study included 12 weeks of high intensity interval work on a bicycle ergometer 3 days/week and continuous running for 40 minutes 3 days/week at 75% $\dot{V}O_2$max. Venous blood samples were drawn immediately before and after a 10 minute treadmill run ranging in intensity from 50 to 90% $\dot{V}O_2$max. Unfortunately, Hurley and his colleagues failed to publish all the NH_3 data that they reportedly collected. As a

result, a detailed critical analysis of their data is not possible.

In summary, conflicting data indicates that further research is required to resolve whether or not training influences plasma NH_3 accumulation during the same absolute work bout. It is possible that the results of Hurley et al. (1984) and Lo and Dudley (1987) conflict due to methodological differences. Factors such as exercise duration and exercise mode differed between the studies, both of which could have a major effect on the results (Graham et al. 1987; Wilkerson et al. 1975). At least two studies (Hurley et al. 1984; Lo and Dudley, 1987) have failed to observe a training induced change in plasma NH_3 concentration after exercise at the same relative intensity. The evidence presented in these papers, however, does not exclude the possibility that training can alter such NH_3 levels. Training effects may be elicited if exercise tests are longer in duration. The duration of the longest exercise test studied to date has been 10 minutes (Hurley et al. 1984). At least two studies have shown (Graham et al. 1987; Maughan and Sadler, 1983) that blood NH_3 levels continually rise during exercise. Therefore, it is quite possible that training may effect blood NH_3 levels during prolonged, but not short, duration exercise at the same relative intensity.

2.2.6 Recovery From Exercise

The rate and pattern of plasma NH_3 recovery after maximal exercise has been the subject of a number of investigations. Results from the literature suggest that basal levels of NH_3 are achieved between 15 and 30 minutes after intense exercise (Babij et al. 1983; Eriksson et al. 1985; Schwartz et al. 1958; Sinniah et al. 1970; Wilkerson et al. 1977). Despite this agreement, there has been considerable discrepancy about the way in which NH_3 returns to basal levels. A number of authors have reported that, at the cessation of maximal exercise, plasma NH_3 levels immediately fall (Babij et al. 1983; Banister et al. 1983; Mutch, 1983). In direct contrast, other authors have indicated that plasma NH_3 concentration either increases or remains stable for a period of time after the cessation of exercise (Buono et al. 1984; Katz et al. 1986a; Schwartz et al. 1958; Wilkerson et al. 1977). Buono et al. (1984) argued that inconsistencies in the way in which plasma NH_3 returned to basal values may be explained by the use of active or passive recovery periods. Unfortunately, researchers have not normally reported whether recovery periods were passive or active so the argument put forward by Buono et al. (1984) remains untested. Factors which may also influence the return of NH_3 to pre-exercise levels, other than the type of recovery, include muscle fibre type and state of training.

2.3 Skeletal Muscle Ammonia Production And Accumulation

This section of the review will discuss:

1. Ammonia accumulation in skeletal muscle during exercise,
2. The biochemical pathways used to produce ammonia in skeletal muscle,
3. Factors that may influence muscle ammonia production.

2.3.1 Ammonia Accumulation In Skeletal Muscle During Exercise

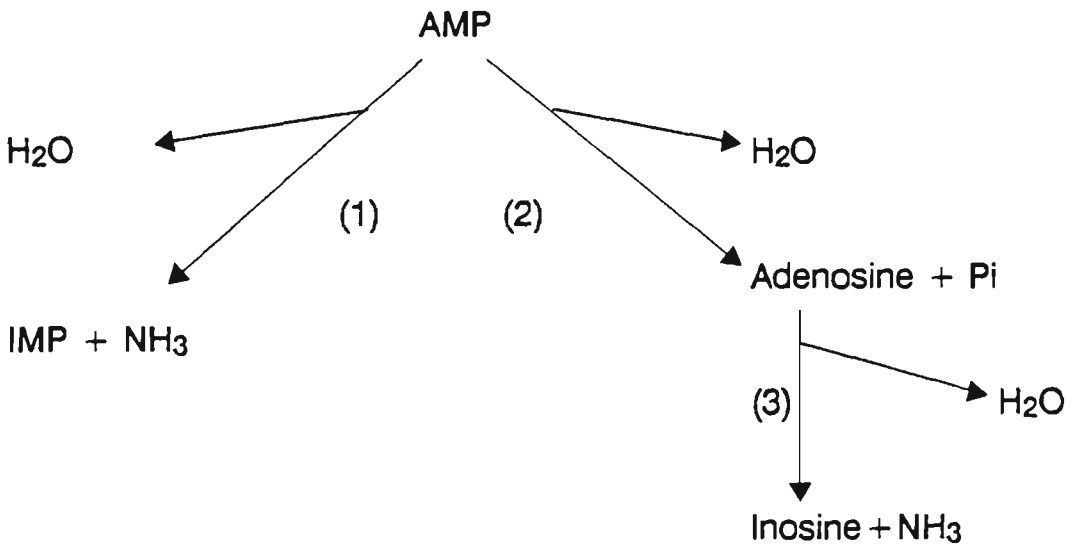
Accumulation of NH_3 has been reported to occur during exercise in both rat and human muscle (Constable et al. 1987; Dudley and Terjung, 1985a; Graham et al. 1987; Katz et al. 1986a,b; Meyer and Terjung, 1979; Meyer and Terjung, 1980; Meyer et al. 1980). After maximal dynamic exercise and exhaustive isometric exercise, human muscle NH_3 concentration has been reported to increase from resting values of 0.5 ± 0.1 and 1.3 ± 0.3 mmol.kg dry weight^{-1} (mmol.kg dw^{-1}), respectively, to 4.1 ± 0.5 and 3.6 ± 0.6, respectively (Katz et al. 1986a,b). Katz et al. (1986a) noted that muscle NH_3 concentrations were similar to resting values after 10 minutes cycling at 50% $\dot{V}O_2$max. Human muscle NH_3 concentration has also been determined at rest and during 30 minutes of cycling exercise at 75-80% $\dot{V}O_2$max (Graham et al. 1987). Resting muscle NH_3 concentration was found to be 0.32 mmol.kg wet weight^{-1} (≈1.39 mmol.kg dw^{-1}). After 5 and 30 minutes of

exercise muscle NH_3 concentration increased to approximately 0.38 (≈1.65 mmol.kg dw^{-1}) and 0.53 mmol.kg wet $weight^{-1}$ (≈2.3 mmol.kg dw^{-1}), respectively (Graham et al. 1987).

2.3.2 Biochemical Pathways For Ammonia Production In Active Muscle

Production of NH_3 by active skeletal muscle may arise from the catabolism of both adenosine monophosphate (AMP) (Lowenstein, 1972) and amino acids (Graham et al. 1987). Catabolism of AMP can occur in active skeletal muscle via two pathways (see Figure 2.1). First, AMP can be deaminated by the myosin bound enzyme AMP deaminase to produce inosine monophosphate (IMP) and NH_3 (Coffee and Solando, 1977; Lowenstein, 1972). This reaction is the first in a series of reactions that make up the purine nucleotide cycle (Figure 2.2) (Lowenstein, 1972). The second method by which AMP can be catabolized to yield NH_3 involves two reactions. AMP is initially dephosphorylated by 5'-nucleotidase to form adenosine and orthophosphate (Rubio et al. 1973). The adenosine formed in the first reaction is subsequently deaminated by adenosine deaminase to produce inosine and NH_3 (Akedo et al. 1972; Camici et al. 1986; Rubio et al. 1973).

Amino acid catabolism is another potential NH_3 producing source in active skeletal muscle (Graham et al. 1987). Definitive proof that the catabolism of amino acids produces free NH_3 during exercise is lacking, however,



(1) Reaction catalysed by AMP deaminase

(2) Reaction catalysed by 5'-nucleotidase

(3) Reaction catlaysed by Adenosine deaminase

Figure 2.1 Biochemical pathways for the production of ammonia from muscle AMP catabolism.

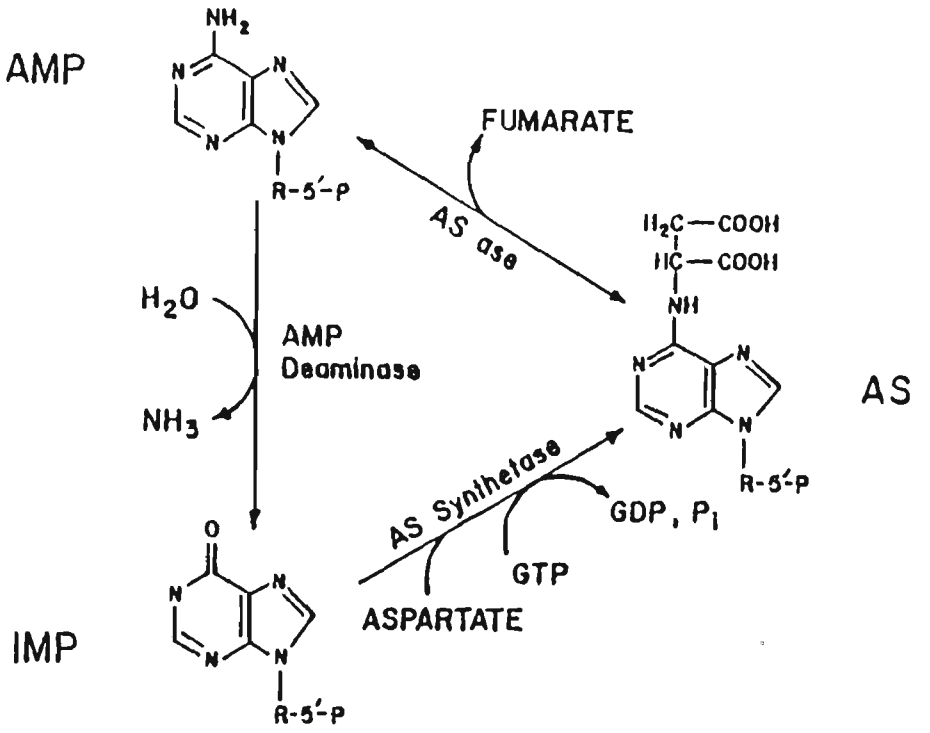
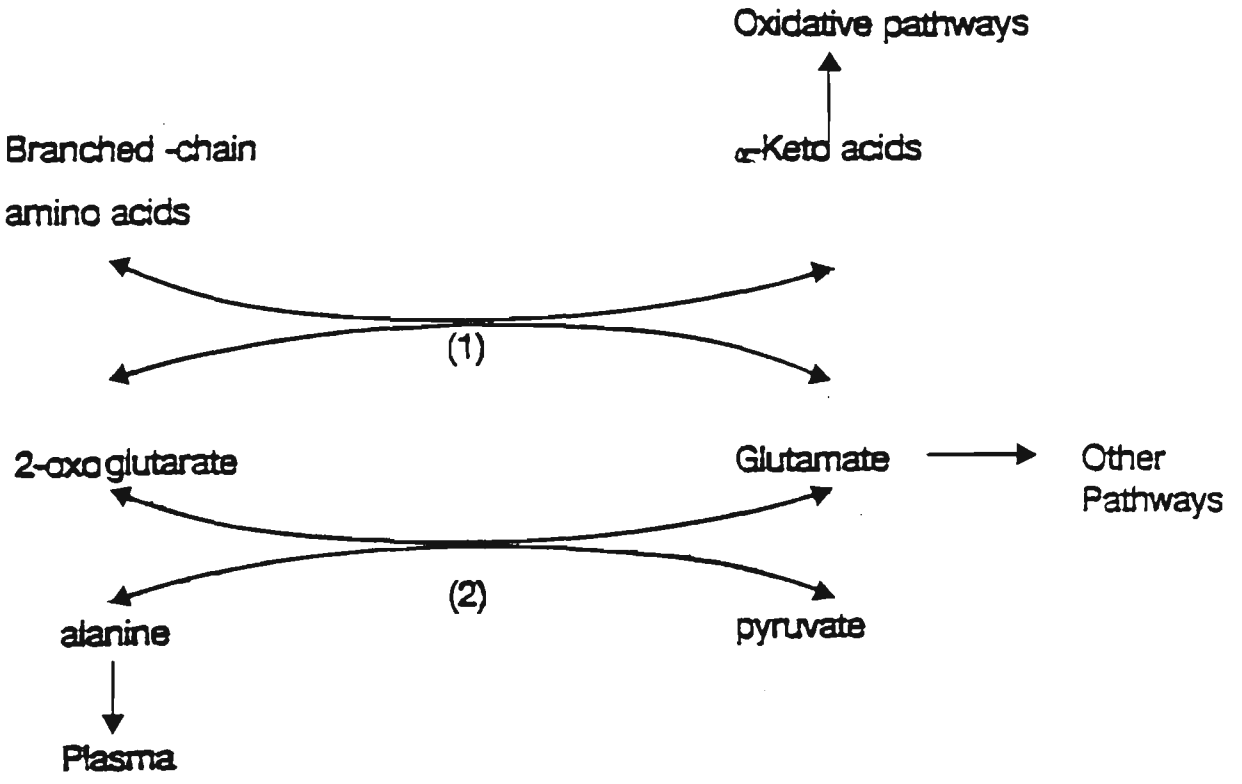


Figure 2.2 Reactions of the purine nucleotide cycle

Abbreviations: Adenylosuccinate Synthetase (AS synthetase), Adenylosuccinate lyase (AS ase), Adenylosuccinate (AS).

Reproduced from Terjung et al. (1986)

indirect evidence suggests that it may occur. The most likely NH_3 producing amino acids are those which may be oxidised by muscle. These include alanine, glutamate, glutamine, isoleucine, valine, leucine, aspartate, asparagine, threonine and glycine (Goodman and Ruderman, 1982). Branched-chain amino acids (leucine, isoleucine, valine) do not contribute directly to free NH_3 production because these amino acids undergo transamination before their carbon chain is oxidised (Goodman and Ruderman, 1982; Randle et al. 1984) (see Figure 2.3). The nitrogen from these amino acids is transferred initially to glutamate (Randle et al. 1984). From glutamate most, but not all, the nitrogen is transferred to form alanine (Wolfe et al. 1982; Wolfe et al. 1984). A small proportion of the nitrogen passed on from branched-chained amino acids to glutamate is involved in reactions not connected to alanine formation (Wolfe et al. 1984). Although, alanine may be oxidised by active skeletal muscle this is not regarded to be its major fate. Most evidence indicates that alanine is released from active muscle to be metabolised by the liver (Felig, 1975; Wolfe et al. 1982; Wolfe et al. 1984). If alanine is oxidised in skeletal muscle to any significant extent free NH_3 would not be produced directly because alanine's amino group is transferred to 2-oxoglutarate to form glutamate (Goodman and Ruderman, 1982). Similarly, oxidation of aspartate initially involves the transfer of the amino group to glutamate (Goodman and Ruderman, 1982).



(1) aminotransferases

(2) Alanine aminotransferase

Figure 2.3 Biochemical pathways involved with branched-chain amino acid catabolism in skeletal muscle.

Alternatively, the amino group of aspartate can be transferred to the purine nucleotide cycle where it may finish as free NH_3 (Lowenstein, 1972). This process could lead to the deamination of other amino acids within muscle, since aspartate can be formed by a transamination reaction with the amine originating from other amino acids (Terjung et al. 1986). Some authors argue that a major function of the purine nucleotide cycle during exercise is in effect to deaminate amino acids in order to increase citric acid cycle intermediates (Aragòn et al. 1980; Flanagan et al. 1986). In contrast, Meyer and Terjung (1980) argue that purine nucleotide cycling does not occur in contracting muscle fibres. By implication, it can be argued that deamination of aspartate via purine nucleotide cycling is not a source of NH_3 production during exercise. Operation of the purine nucleotide cycle during exercise is controversial (Flanagan et al. 1986; Meyer and Terjung, 1980) and is an issue which will be addressed in a later section of this review (page 35). As a result of this controversy, the potential for amino acid deamination via this pathway is uncertain.

Both threonine and glycine can be catabolized via the aminoacetone pathway (Figure 2.4) to produce, amongst other products, D-lactate and NH_3 (Haralambie and Mössinger, 1980). Haralambie and Mössinger (1980) have established the existence and activation of the aminoacetone pathway in human skeletal muscle during exercise.

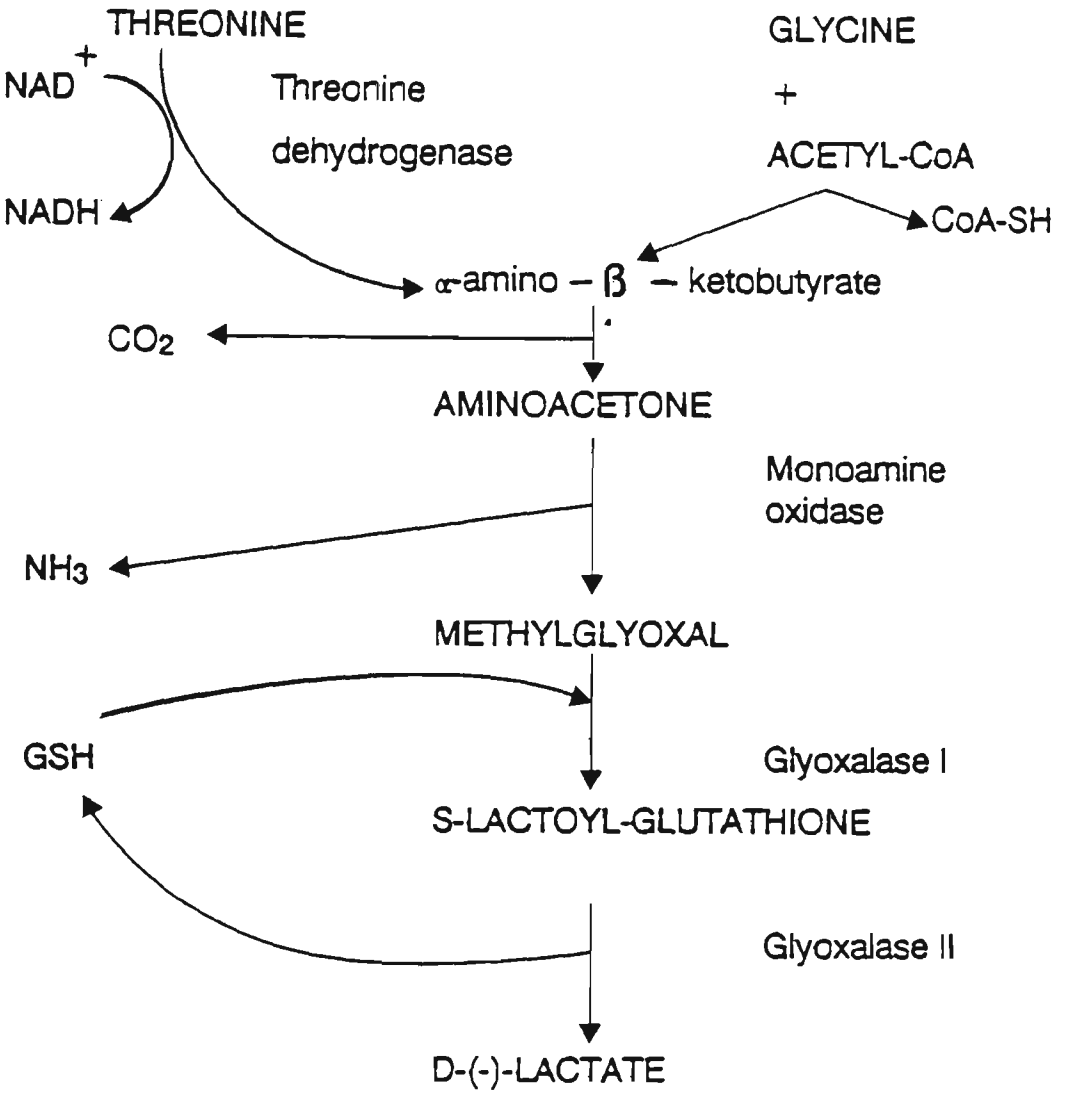


Figure 2.4 The aminoacetone pathway
Reproduced from Haralambie and Mossinger, 1980

The presence of glutaminase in mammalian skeletal muscle (Durschlag and Smith, 1985) suggests that glutamine may be converted to glutamate with a corresponding production of NH_3. Existing evidence for the use of this pathway is not strong since most studies have found muscle glutamine concentrations to either remain unchanged or increase despite an efflux of glutamine from active muscle (Bergström et al. 1985; Ericksson et al. 1985; Katz et al. 1986a). Further evidence that glutamine may not be catabolized is the finding that intramuscular glutaminase activity is unaltered even after 2 hours of exercise (Askew et al. 1979). The biochemical pathway for asparagine catabolism involves an enzymatic step which liberates free NH_3. Specifically, asparagine is hydrolysed to form aspartate and NH_3 via the action of asparaginase. Very little is known about the presence of this pathway in muscle, consequently the significance of NH_3 production via this mechanism remains unknown.

The fate of glutamate in skeletal muscle is very important considering its central role in the catabolism of alanine, aspartate, asparagine, glutamine and branched-chain amino acids. In the case of alanine, aspartate, and the branched-chain amino acids the fate of glutamate may determine whether catabolism of these amino acids can produce NH_3. Glutamate is oxidised in skeletal muscle as proven by carbon labelling studies (Askew et al. 1979). This oxidation, however, may occur with or without the production of free NH_3 depending upon which pathways

are followed. For example, labelled carbon dioxide from isotopically labelled glutamate may occur without free NH_3 production if glutamate is transferred to either pyruvate or oxaloacetate in transamination reactions (Goodman and Ruderman, 1982) (Figure 2.5). On the other hand, labelled carbon dioxide will be produced in conjunction with free NH_3 if glutamate is oxidatively deaminated via the glutamate dehydrogenase reaction. Whether significant deamination of glutamate occurs in human skeletal muscle is not yet known. The evidence collected to date is circumstantial and conflicting. Glutamate deamination in exercising muscle may not be significant for two reasons. First, the activity of rodent glutamate dehydrogenase in the direction of 2-oxoglutarate formation is quite low in comparison to its activity in the direction of glutamate formation (Lowenstein and Goodman, 1978). Secondly, during exercise, a large amount of glutamate is involved in a transamination reaction with pyruvate to form alanine and 2-oxoglutarate (Felig, 1977; Snell, 1980). On the other hand, there is evidence which indicates that deamination of glutamate may occur in contracting muscle. 2-oxoglutarate is a competitive inhibitor of glutamate dehydrogenase (Schoolwerth et al. 1980) and glutamate deamination is known to be inversely related to mitochondrial 2-oxoglutarate concentration in kidney and heart (McDaniel, 1987; Schoolwerth et al. 1980). Skeletal muscle 2-oxoglutarate concentration is reduced during exercise (Graham and Saltin, 1988). This reduction

C $\quad Glutamate + NAD^+ + H_2O \longleftrightarrow 2\text{-oxoglutarate} + NADH + NH_4^+ + H^+$

Glutamate
dehydrogenase

Figure 2.5 Some of the biochemical reactions involving glutamate.

may lead to increased glutamate deamination. Further investigation into the possible NH_3 producing role of intramuscular glutamate dehydrogenase in humans is warranted.

2.3.3 Factors That May Influence Muscle Ammonia Production

2.3.3.1 Muscle Fibre Type

2.3.3.1.1 AMP Deaminase Pathway

Dudley et al. (1985a) observed that NH_3 and IMP accumulation occurs to a significantly greater extent in rodent fast twitch white muscle fibres (FTW) than fast twitch red muscles (FTR) during moderately intense *in situ* stimulation (6 volts, 0.1 ms duration, 5 Hz). However, during intense *in situ* stimulation (6 Volts, 0.1 ms duration, 100 Hz) both FTR and FTW muscle fibre types of the rat deplete their ATP content and produce IMP and NH_3 to a similar extent (Meyer and Terjung, 1979). In contrast, slow twitch red fibres (STR) of the rat do not normally deplete their ATP content (Hintz et al. 1982; Meyer and Terjung, 1979; Whitlock and Terjung, 1987), nor develop a significant increase in IMP or NH_3 during intense stimulation conditions. Meyer and Terjung (1979) argue that there is a fundamental difference between fast twitch and slow twitch rat muscle in the activation of AMP deaminase, and consequently a large fibre type difference in NH_3

production via this pathway. Measurements of maximal AMP deaminase activity also supports the proposition that AMP deamination occurs predominately in FT fibres. Winder et al. (1974) determined maximal AMP deaminase activity in rat FT and ST muscle and reported that the deaminase activity was twofold greater in FT muscle. Similar fibre type differences in deaminase activity have also been found in cat muscle (Bockman and McKenzie, 1983).

In contrast to rat ST muscle, significant ATP depletion can occur in human ST fibres. Recently, Jansson et al (1987) determined ATP and IMP concentrations in single human muscle fibres biopsied before and after intense knee extension exercise. IMP content increased significantly in both type I and II fibres following exercise, however, IMP concentration was significantly greater in type II when compared to type I fibres. Decreases in ATP content were significant for both fibre types with somewhat larger responses for type II than type I. These results clearly indicate that NH_3 production, via the AMP deaminase reaction, can occur in all human fibre types after high intensity exercise.

2.3.3.1.2 5'-nucleotidase / Adenosine Deaminase Pathway

It is presently unknown whether NH_3 produced via the 5'-nucleotidase and adenosine deaminase pathway occurs preferentially in any one muscle fibre type (Whitlock and Terjung, 1987). Comparisons of enzymatic activity have shown that 5'-nucleotidase is relatively high in cat ST

muscle (Bockman and McKenzie, 1983) suggesting that this pathway is activated more readily in these fibres. Future investigations into the existence of fibre type differences in the activity of the 5'-nucleotidase / adenosine deaminase pathway is warranted.

2.3.3.1.3 Amino Acid Catabolism

Indirect evidence indicates that amino acid catabolism may occur more readily in ST fibres. Keul et al. (1972) reported that ST muscle in man has greater transaminase activity than FT muscle. Isotope labelling studies (Wolfe et al. 1982; Wolfe et al. 1984) have shown leucine to be oxidised at exercise intensities during which only ST fibres would normally be recruited (i.e., 30% $\dot{V}O_2$ max) (Sale, 1987). Unfortunately, evidence of branched-chain amino acid oxidation by ST fibres does not necessarily mean that these fibres produce NH_3 via this pathway (see pages 30-31).

2.3.3.2 Exercise Intensity

2.3.3.2.1 AMP Deaminase Pathway

It is unlikely that NH_3 is produced via the AMP deaminase pathway during light or moderate exercise (i.e., at least up to work intensities equal to 50% $\dot{V}O_2$ max). AMP deaminase activity is inhibited at rest by adenosine triphosphate (ATP) and gaunosine triphosphate (GTP) (Ronca-Testoni et al. 1970; Wheeler and Lowenstein, 1979).

Although inorganic phosphate (Pi) is also an inhibitor, its concentration in the resting state is probably too low to be effective (Snow et al. 1985). However, the increase in Pi concentration during activity will help maintain the enzyme in its inhibited state. Activation of the enzyme occurs when free ADP (Snow et al. 1985), and AMP concentrations increase (Ronca-Testoni et al. 1970) and intracellular pH falls (Dudley and Terjung, 1985b; Setlow and Lowenstein, 1967; Wheeler and Lowenstein, 1979). These activation conditions occur more readily during intense muscular contractions (Terjung et al. 1986). Further indirect evidence that AMP deaminase activity is low during light exercise intensities is the observation that the total adenine nucleotide pool (i.e., ATP + ADP + AMP) is not altered during exercise at 50% $\dot{V}O_2$ max (Katz et al. 1986a). If the AMP deaminase reaction was functioning at this exercise intensity a decrease in the total adenine nucleotide pool would be expected (Terjung et al. 1986) provided the purine nucleotide cycle was inoperative (see below).

During very intense exercise AMP deaminase appears to produce almost all the NH_3 in exercising muscle. Katz et al. (1986a) reported that total accumulation of NH_3 in muscle and blood (corresponds to 3.95 mmol.kg dw^{-1}) virtually matched the decrease in the total adenine nucleotide pool (3.90 mmol.kg dw^{-1}) after cycling exercise at 100% $\dot{V}O_2$ max. Unfortunately, Katz et al (1986a) did not determine intramuscular IMP concentration

to ascertain whether decreases in the total adenine nucleotide pool were matched by a similar increase in IMP. Because Katz et al. (1986a) failed to measure IMP, definitive conclusions about AMP deaminase activity cannot be reached. A number of animal studies have reported a stoichiometric link between IMP accumulation and a decrease in the total adenine nucloetide pool during short duration, intense exercise (Meyer and Terjung, 1979; Meyer et al. 1980) suggesting that the results of Katz et al. (1986a) probably reflect AMP deaminase activity. Further evidence of the dominant NH_3 producing role of AMP deaminase during intense exercise can be gleaned from studies of individuals who lack the enzyme. In these studies both intramuscular IMP (Sabina et al. 1984) and blood NH_3 concentration (Fishbein et al. 1978; Sabina et al. 1980; Sinkeler et al. 1985) are significantly reduced after short term exhaustive exercise in comparison to normal individuals.

The extent to which AMP deaminase activity produces NH_3 during exercise intensities in the range 50 - 90% $\dot{V}O_2$max remains controversial (Graham et al. 1987). Within this range NH_3 production via AMP deaminase activity may not be stoichiometrically linked to a decrease in the total adenine nucleotide pool because the purine nucleotide cycle may be operative (Flanagan et al. 1986). Outside this intensity range the purine nucleotide cycle is believed to be inoperative (Katz et al. 1986a,b; Meyer and Terjung, 1980). During low intensity exercise the purine

nucleotide cycle probably does not function because intracellular conditions inhibit AMP deaminase activity. Similarly, the purine nucleotide cycle probably does not operate during high intensity exercise because IMP and guanosine diphosphate (GDP) accumulation substantially inhibits the activity of adenylosuccinase (Aragòn et al. 1981; Goodman and Lowenstein, 1977). If the purine nucleotide cycle operates during exercise intensities in the range of 50-90% $\dot{V}O_2$max conventional methods of estimating AMP deaminase activity (i.e., depletion of total adenine nucleotide pool or IMP accumulation) cannot be used. In these circumstances NH_3 production via AMP deaminase activity may be underestimated.

2.3.3.2.2 5'-nucleotidase / Adenosine Deaminase Pathway

Indirect evidence suggests that the 5'-nucleotidase / adenosine deaminase pathway is a minor producer of NH_3. The activity of 5'-nucleotidase is less than 1% of the AMP deaminase activity in skeletal muscle (Bockman and McKenzie, 1983), while AMP deaminase activity is 500 fold greater than adenosine deaminase (Fishbein et al. 1978). Exercise intensity and its effect on 5'-nucleotidase / AMP deaminase pathway has not been established. 5'-nucleotidase and adenosine deaminase each exist as two isoenzymes in skeletal muscle (Camici et al. 1986; Ma and Magers, 1975). In the case of the nucleotidase it exists in either the muscle membrane (ecto form) or in the muscle cytoplasm (endo form) (Camici et al. 1986). Enzyme kinetic data

suggests that the ecto form is inhibited by ATP, ADP and CP (Camici et al. 1986). On the basis of this evidence the ecto form is probably activated at high work intensities. To complicate matters the endo form is thought to be free of ADP and ATP inhibition (Camici et al. 1986) indicating that this isoenzyme may be active at low exercise levels. The isoenzymes of adenosine deaminase (A and C) both display similar kinetic properties. Neither form of the deaminase is inhibited or stimulated by AMP, ATP, GMP or GTP (Akedo et al. 1972). Both isozymes are partially inhibited by inosine and both display an optimum pH near 7.0 (Akedo et al. 1972; Ma and Magers, 1975).

Several indirect pieces of evidence suggest that 5'-nucleotidase / adenosine deaminase pathway may not be involved in producing NH_3 during either light or heavy exercise intensities. As previously mentioned, individuals with AMP deaminase deficiency accumulate significantly less NH_3 in blood after maximal exercise (Fishbein et al. 1978; Sinkeler et al. 1985) than normal controls. Even with AMP deficiency most subjects display small increases in blood NH_3 concentration which may indicate that another NH_3 producing pathway exists in skeletal muscle. Sinkeler et al. (1986) determined plasma adenosine and inosine concentrations in normal and AMP deaminase deficient individuals after an exhaustive isometric forearm test. These authors reported that plasma adenosine marginally increased while plasma inosine was significantly reduced in AMP deficient individuals when compared to normal controls.

These results indicate that 5'-nucleotidase / adenosine deaminase pathway was not activated to any great extent during intense isometric forearm exercise.

During cycling exercise below 50% $\dot{V}O_2$ max no significant change has been observed for intramuscular ATP, ADP or AMP concentrations (Katz, 1986; Katz et al. 1986a). This data suggests that AMP catabolism via the 5'-nucleotidase / adenosine deaminase pathway does not occur at low work intensities. Definitive proof requires isotopic labelling of ATP and the inability to detect labelled adenosine and inosine during low intensity work.

2.3.3.2.3 Amino Acid Catabolism

As mentioned previously, there is a lack of definitive proof linking amino acid catabolism to NH_3 production. Nevertheless, indirect evidence has linked the breakdown of some amino acids to NH_3 production (see pages 23-33). If the catabolism of branched-chain amino acids results in the amino group ending up as free NH_3; then this could occur at low exercise intensities as branched-chain amino acids are oxidised in human skeletal muscle at 30% $\dot{V}O_2$ max (Wolfe et al. 1982; Wolfe et al. 1984). Oxidation rates are known to increase with increasing work intensity in rodent muscle (Henderson et al. 1985; White and Brooks, 1981), indicating that NH_3 production from this source may also increase.

The exercise intensity at which NH_3 production via the aminoacetone pathway begins to occur has not been established. Measurements of glycine and threonine concentration in muscle and plasma suggest that this pathway may be active during moderately intense exercise (i.e., 70% $\dot{V}O_2$max). Bergström et al. (1985) noted that both glycine and threonine concentrations decreased significantly in human plasma while muscle concentrations of glycine decreased and threonine remained unchanged. Felig and Wahren (1971) observed a significant uptake of threonine after 40 minutes of moderate exercise while noting no significant uptake for glycine. Metabolites of the aminoacetone pathway have been detected after maximal exercise intensities thus providing strong evidence that this pathway functions in human muscle (Haralambie and Mössinger, 1980).

2.3.3.3 Endurance Training

2.3.3.3.1 AMP Deaminase Pathway

Very few studies have investigated the effect of endurance training on the biochemical pathways that produce NH_3. There is evidence which suggests that NH_3 production via AMP deaminase is reduced with training. Winder et al. (1974) reported that AMP deaminase activity was significantly reduced in trained FTR fibres of the rat, while no changes were observed in the deaminase activity of FTW and STR muscle fibres. Winder et al. (1974) argued that

AMP deaminase activity was only reduced in FTR fibres because the training program (treadmill running 5 days per week for 12 weeks) predominately recruited this type of fibre. Dudley and Terjung (1985a) trained rats in order to increase the mitochondrial content of both FTW and FTR muscle fibres. These authors found that endurance training significantly decreased both the IMP and NH_3 concentration in FTW fibres after 3 minutes of in situ stimulation at 5 Hz (supramaximal stimuli 6V, 0.1 ms duration). Training, however, produced a nonsignificant decrease in the NH_3 and IMP content of FTR muscle fibres. Trained FTR fibres were not expected to reduce their accumulation of NH_3 as untrained FTR fibres did not accumulate significant quantities at the exercise intensity used in the investigation. Using a more intense exercise regime (in situ tetanic stimulation for 3 or 8 minutes, 50 Hz, 60 trains per minute, 100 ms per train), Constable et al. (1987) reported that both trained FTR and FTW rat muscle accumulated significantly smaller quantities of NH_3 than untrained muscles. Constable et al. (1987) inferred from their results that NH_3 production via AMP deaminase was reduced by training although no measures of intramuscular IMP concentrations were made.

2.3.3.3.2 5'-nucleotidase / Adenosine Deaminase Pathway

Limited information on the effects of endurance training on the 5'-nucleotidase / adenosine deaminase pathway is available. Knochel et al. (1985) determined the

activity of muscle 5'-nucleotidase in dogs before and after 6 weeks of training and found no change in activity. Although subject to other interpretations, this data indicates that the 5'-nucleotidase / adenosine deaminase pathway is unaffected by training. Researchers have not yet investigated the effects of training on adenosine deaminase activity in muscle.

2.3.3.3.3 Amino Acid Catabolism

Isotope studies have established that endurance training increases branched-chain amino acid oxidation rates in rodent muscle (Askew et al. 1979; Dohm et al. 1977; Henderson et al. 1985). Interestingly, both glutamate dehydrogenase activity (assayed in the direction of 2-oxoglutarate formation) and glutamate carbon chain oxidation also increase with training (Askew et al. 1979; Holloszy et al. 1970), indicating that training may enhance NH_3 production via amino acid catabolism, especially branched-chain amino acid catabolism. It must be stressed, however, that there is no direct evidence supporting the utilisation of this pathway in contracting muscle (see pages 30-33).

The activity of glyoxalase I, an enzyme in the aminoacetone pathway, has been measured in untrained and endurance trained individuals (Haralambie and Mössinger, 1980). Nonsignificant increases in glyoloxase I activity were observed in the endurance trained subjects indicating that the aminoacetone pathway may be more active in

conditioned muscle (trained vs untrained: 235 ± 64 vs 191.1 ± 37.9 μmoles of product.g wet weight^{-1}.min^{-1}; $p<0.15$). Endurance training does not change glutaminase activity (Askew et al. 1979), thus providing further evidence that this pathway is not utilised in exercising skeletal muscle.

2.4 Removal Of Skeletal Muscle Ammonia

This section of the review of literature will present the major biochemical pathways used to remove NH_3 from active muscle and discuss where possible their physiological significance. A discussion of the possible effects of endurance training on NH_3 removal will also be presented.

2.4.1 Muscle Ammonia Removed By Glutamate

Muscle glutamate (GLU) can be formed in two distinct ways. Firstly, GLU is formed from the transamination of various amino acids (predominately branched-chain amino acids) with 2-oxoglutarate (Goldberg and Chang, 1978). During these transamination reactions no net removal of free NH_3 occurs. Secondly, muscle GLU can be synthesised by combining free NH_3 (in the form NH_4^+) with 2-oxoglutarate, a reaction that is catalysed by glutamate dehydrogenase (Figure 2.6) (Palaiologos and Felig, 1976; Felig, 1977). As previously discussed (pages 31-33), doubt still exists about the direction in which the glutamate dehydrogenase reaction works during exercise. Despite these

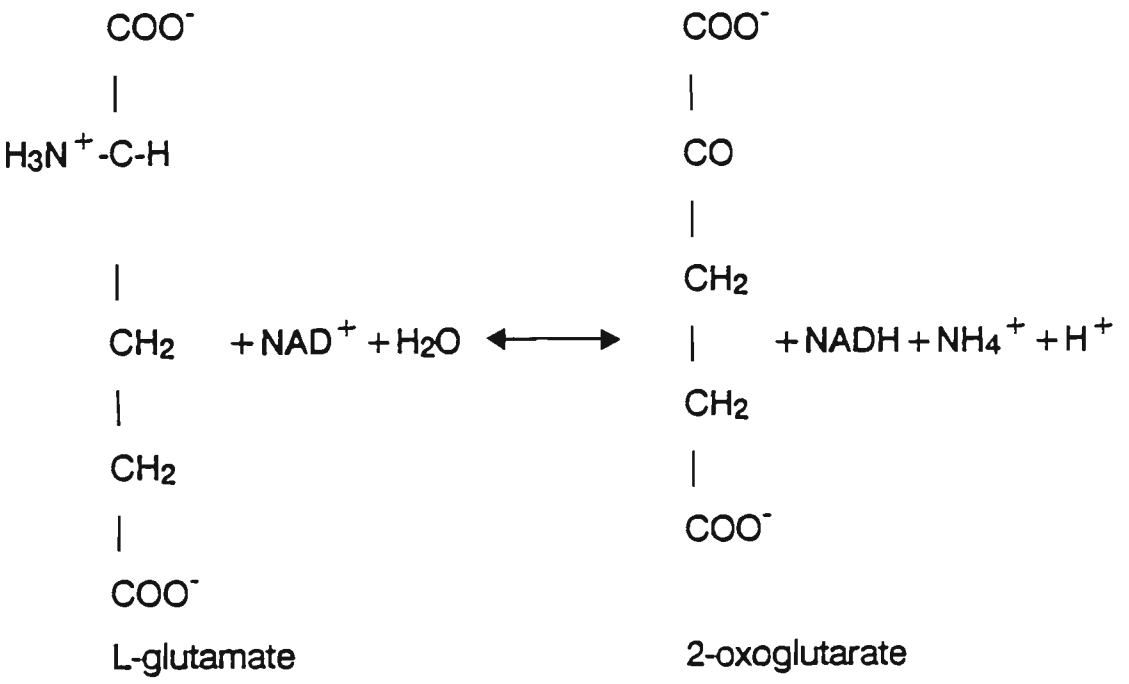


Figure 2.6 The glutamate dehydrogenase reaction

doubts, it is known that the final removal of NH_3 from the cell is not via GLU release as arteriovenous studies consistently show that exercising muscle does not release GLU into the circulation (Eriksson et al. 1985; Bergström et al. 1985; Katz et al. 1986a). Free NH_3, if removed by the glutamate dehydrogenase reaction probably leaves the muscle fibre in the form of alanine (Eriksson et al. 1985; Palaiologos and Felig, 1976; Snell, 1980).

2.4.2 Muscle Ammonia Removed By Glutamine

Glutamine (GLN) synthesis by skeletal muscle provides another mechanism by which NH_3 may be removed from muscle. GLN is produced by combining GLU and free NH_3 (in the form NH_4^+) and is catalysed by glutamine synthetase (Figure 2.7). In theory, glutamine production may rid muscle of two free NH_3 molecules if the glutamate used in the synthesis of glutamine originated from the glutamate dehydrogenase reaction, however, if the glutamate originated from transamination with other amino acids then only one free NH_3 molecule is utilised.

The ability of muscle to produce and release GLN during rest has been established for some time (Ruderman and Lund, 1972; Chang and Goldberg, 1978; Goodman and Lowenstein, 1977), however, muscle synthesis of GLN during intense exercise has been seriously questioned (Snell and Duff, 1980). Glutamine synthetase activity is substantially inhibited by increasing concentrations of inorganic phosphate, various amino acids and ADP (Rowe, 1985). The pH

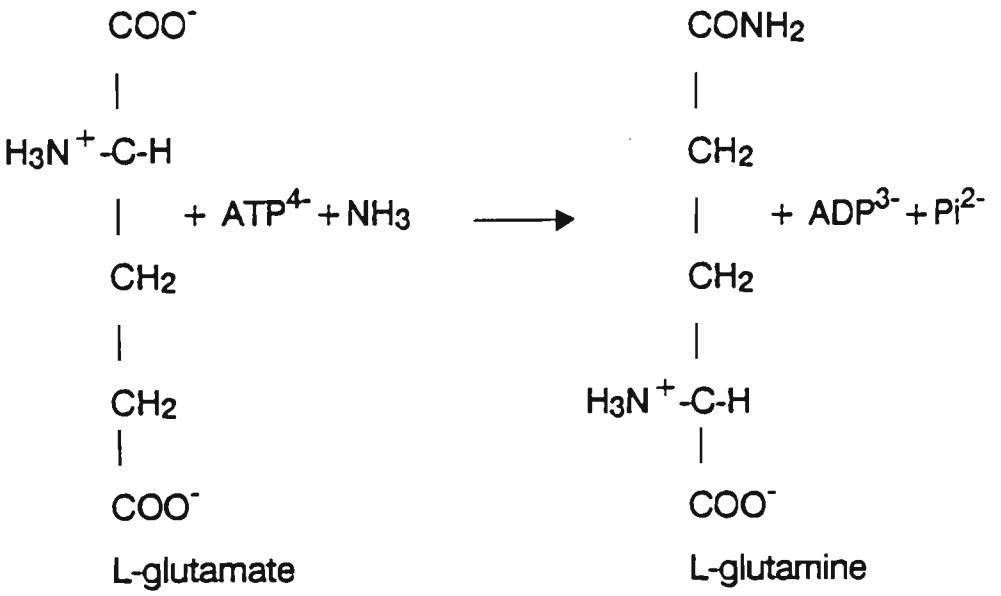


Figure 2.7 The glutamine synthetase reaction

optimum for the enzyme is 7.2 (Rowe, 1985). Intense exercise produces changes within active muscle (i.e., decreased pH and GLU concentration, increased inorganic phosphate and ADP concentrations) which would logically inhibit the activity of glutamine synthetase.

Evidence supporting the contention that glutamine synthetase is inactivated during intense exercise has been reported in both rat and human muscle (Meyer and Terjung, 1979; Goodman and Lowenstein, 1977; Katz et al. 1986a,b). In these studies intramuscular GLN concentration remained unaltered from resting values despite a significant increase in muscle NH_3 concentration. Further evidence which indicates that GLN is not involved in removing free NH_3 is the finding that the rate of GLN release from muscle remains unaltered from resting values until heavy exercise intensities are achieved (Eriksson et al. 1985; Katz et al. 1986a). This lack of an increase in GLN occurs despite the fact that active muscle releases NH_3 at moderate exercise intensities (Eriksson et al. 1985).

Katz et al. (1986a) calculated that only 8% of the NH_3 produced by adenine nucleotide catabolism could be removed from muscle by GLN release. Further arguments presented by these authors suggest that NH_3 removal by GLN synthesis during acute exhausting exercise may in fact be zero. GLU transport across muscle fibres is enhanced in the presence of insulin (Rennie and Watt, 1985). Katz et al. (1986a) hypothesized that the observed GLN release from muscle was not due to an increased intramuscular formation

of GLN but was the result of an increase in GLN transport out of the fibres stimulated by an increase in insulin delivered to the exercising muscle.

Bergström et al. (1985) reported that glutamine synthetase may be involved in removing intramuscular NH_3 during less intense exercise (i.e., 70% $\dot{V}O_2$max) unlike that observed during more strenuous exercise (> 90% $\dot{V}O_2$max). Bergström and his colleagues found that intramuscular GLN concentrations increased significantly ($p<0.05$) while GLU concentration decreased ($p<0.05$) after 10 minutes of cycling at 70% $\dot{V}O_2$max. The inverse relationship between GLU and GLN was interpreted to be evidence that glutamine synthetase was functioning during the exercise bout. Katz et al. (1986a) have seriously questioned the accuracy of the data published by Bergström et al. (1985). They calculated that the increase in muscle GLN concentration observed by Bergström et al. meant that glutamine synthetase must have been synthesising GLN at a rate of 1.4 mmol.kg $dw^{-1}.min^{-1}$. Further, they point out, that rat enyzme kinetic data suggests that, at the GLU concentration reported by Bergström et al. (1985), the maximum rate of GLN synthesis would be no more than 0.37 mmol.kg $dw^{-1}.min^{-1}$. From their own data, Katz et al. (1986a) point out that the rate of GLN synthesis in rats and humans during exercise is similar. Hence it seems reasonable to assume similar enzyme activities in both species. Hence they conclude that the data of Bergström et al. (1985) can be interpreted in one of two ways. Either

GLN was synthesised in a way which does not involve glutamine synthetase or the reported muscle GLU or GLN concentrations (or both) are incorrect. Until further research is conducted it remains unclear as to whether glutamine synthetase may or may not remove NH_3 during moderately intense exercise.

The effect of endurance training on GLN production during exercise has yet to be investigated. Consequently, it is not known whether training may alter muscle's ability to remove NH_3 via GLN production. Surprisingly, information regarding the effects of training on plasma GLN concentration does not seem to be available either. It is known, however, that plasma GLN concentration increases in proportion to exercise intensity (Babij et al. 1983). In view of the finding that muscle GLN release is not altered until intense exercise (Eriksson et al. 1985; Katz et al. 1986a), the increase in plasma GLN levels observed during mild exercise must result from a decreased removal of GLN from the circulation and/or from an increased release from tissue other than active muscle.

2.4.3 Muscle Ammonia Removed By Alanine

Alanine (ALA) is produced in skeletal muscle from glutamate in a transamination reaction shown in Figure 2.8. Muscle does not possess an enzyme capable of producing ALA directly from the combination of free NH_3 and pyruvate (Ruderman and Berger, 1974). ALA production can only be involved in removing free NH_3 if ALA is synthesised from

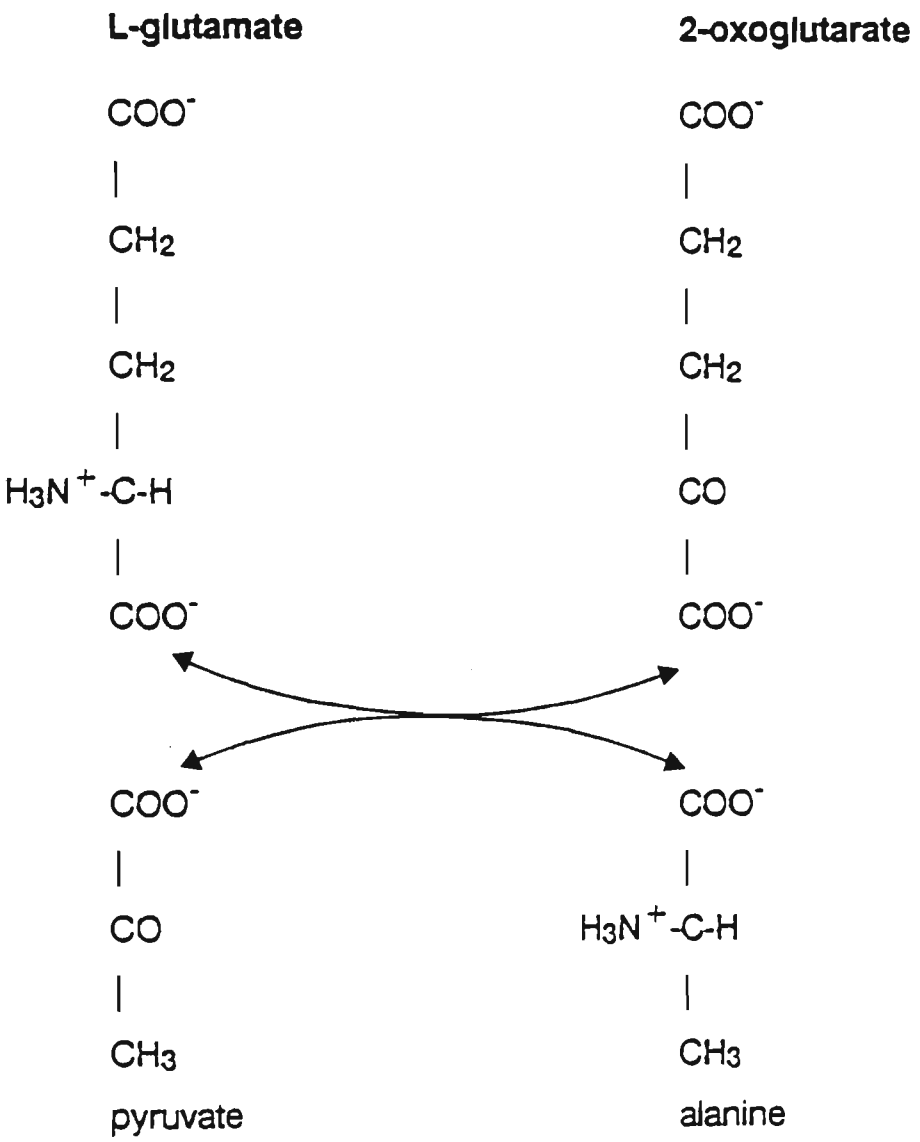


Figure 2.8 The alanine aminotransferase reaction

glutamate which was previously formed by utilizing free NH_3, for example, GLU formed via the glutamate dehydrogenase reaction. As has been mentioned previously, there is some evidence to suggest that GLU is formed by the glutamate dehyrogenase reaction during exercise (Palaiologos and Felig, 1976; Felig, 1977), however, the significance of this mechanism is not known. Consequently, the significance of ALA's role in removing free NH_3 is also unknown.

Human intramuscular ALA concentration increases significantly at moderate work intensities (i.e., 50% $\dot{V}O_2$max)(Bergström et al. 1985; Katz et al. 1986a). This suggests that ALA production may contribute to muscle NH_3 removal at these work levels. Significant increases in ALA release from active muscle have been observed at 55% $\dot{V}O_2$max with further increases in exercise intensity eliciting greater ALA release (Eriksson et al. 1985; Katz et al. 1986a). In contrast, significant increases in plasma ALA concentration occur below the exercise intensity required to elicit increases in muscle ALA release (Eriksson et al. 1985; Katz et al. 1986a). This finding indicates that at least in mild to moderate exercise, the increase in plasma ALA concentration may be the result of a reduced removal rate and/or an increased release of ALA into the circulation by tissue other than active skeletal muscle. Data published by Felig and Wahren (1971) indicates that the liver (the major organ involved in removing ALA from the circulation (Felig, 1975)) increases, rather than

decreases, its rate of ALA extraction during exercise. Therefore, the plasma ALA increase observed before changes in muscle ALA release is most likely due to an increased ALA release from non-skeletal muscle sources (e.g., heart, small intestine, kidney, adipose tissue and brain) (Snell, 1980).

Studies investigating training induced changes in ALA metabolism have measured either alanine amino-transferase activity or plasma ALA concentrations. Alanine aminotransferase activity is reported to increase in trained rat muscle indicating that ALA production may increase with training (Green et al. 1983; Molé et al., 1973). Molé et al. (1974) measured blood ALA concentration in trained and untrained men after 20 minutes of treadmill running at exercise intensities ranging between 54 to 93% $\dot{V}O_2$max. For a given oxygen consumption increases in ALA concentration did not differ significantly between the groups, however, for the same relative $\dot{V}O_2$, changes in blood ALA concentrations were significantly greater in the trained subjects. Einspar and Tharp (1985) determined plasma ALA concentrations in trained and untrained subjects after treadmill running for 10 minutes at a workload that was 15% above their anaerobic threshold. In agreement with Molé et al (1974), Einspar and Tharp (1985) found that the ALA concentration was significantly higher in the trained subjects. In contrast to the two studies previously cited, Bloom et al. (1976) reported that trained subjects accumulated significantly smaller quantities of ALA

compared to untrained subjects during cycling at relative work intensities between 45 to 75% $\dot{V}O_2$ max.

One possible explanation for the conflicting results is the influence of muscle fibre type. Baldwin et al. (1977) reported that exercising rodent fast glycolytic fibres and slow oxidative fibres accumulated greater quantities of alanine than fast oxidative glycolytic muscle fibres. All human studies investigating the effects of training on plasma ALA accumulation have used a cross-sectional experimental design. Unfortunately, no study has determined muscle fibre type and as a consequence there is a possibility that the results reported reflect fibre type differences rather than training. Obviously further research is required to ascertain the effects of endurance training on plasma ALA concentration during exercise of short duration.

2.4.4 Diffusion Of Ammonia From Muscle

The pH gradient between tissues determines their relative NH_3 concentrations and the direction of NH_3 movement between intra- and extracellular compartments (Mutch and Banister, 1983). Ammonium ions (NH_4^+) are trapped in tissues with a lower pH than plasma because only the non-protonated form of NH_3 freely diffuses across cell membranes (Visek, 1968). Meyer et al. (1980) used the Henderson-Hasselbach equation to calculate that resting skeletal muscle should have a total NH_3 (NH_3 + NH_4^+) concentration 2.5 times greater than plasma

(assuming pKa for NH_3 is 9.3 and the pH in plasma and muscle is 7.4 and 7.0, respectively). Continuous intense exercise to fatigue lowers the muscle-blood pH ratio (resting 7.0/7.4, fatigue 6.4/7.11) (Hermansen and Osnes, 1972). Based on the observed decrease in the muscle-blood pH ratio, it can be calculated that fatigued muscle will have an NH_3 concentration which is more than 5 times greater than plasma. These calculations highlight the fact that a small change in the muscle-blood pH ratio (resting ratio = 0.95, fatigue ratio = 0.90) can dramatically alter the concentration of total NH_3 within the two body compartments. These calculations also indicate that an increasing percentage of the accumulated NH_3 is trapped (in the form of ammonium ions) within fatigued muscle. Katz et al. (1986a) calculated that only 10% of NH_3 accumulated in fatigued human muscle was released into the circulation. This observation strongly suggests that diffusion plays a small role in removing NH_3 from active muscle during acute intense exercise.

Researchers have yet to investigate the effects of endurance training on the muscle-blood pH ratio during exercise. However, there is evidence which suggests that pH decreases observed in blood (Rasmussen et al. 1975) and muscle (Sahlin and Henriksson, 1984; Sharp et al. 1986; Troup et al. 1986) during exercise are less marked in trained individuals. Rasmussen et al. (1975) reported that venous blood pH was slightly higher during 15 minutes of heavy exercise after a five week interval training program

(pre-training pH = 7.2, post-training pH = 7.25). Sahlin and Henriksson (1984) reported that during isometric contraction to fatigue trained subjects had a smaller decrease in muscle pH (trained pH = 6.8, sedentary pH = 6.61, $p<0.01$) than sedentary subjects. These results indicate that training influences the muscle-blood pH ratio so that a greater percentage of the free NH_3 produced during exercise may diffuse from trained muscle into the plasma more readily than untrained muscle.

2.5 The Relationship Between Muscle Ammonia Accumulation And Muscular Fatigue

This section of the review will discuss the possible role of NH_3 in causing fatigue during exercise. Mutch and Banister (1983) linked the accumulation of NH_3 in muscle and plasma to peripheral and central fatigue, respectively. It will be the intention of this section to review the literature relevant to NH_3 and muscular fatigue by concentrating specifically on muscle lactate production. The literature related to NH_3 and central fatigue will not be discussed.

2.5.1 Ammonia and Muscular Fatigue

In the past, research has linked the accumulation of NH_3 in muscle and blood with increased muscle lactate production and peripheral fatigue caused by metabolic acidosis (Babij et al. 1983; Banister et al. 1983; Buono et al. 1984; Meyer and Terjung, 1980; Mutch and Banister,

1983). NH_3 was thought to increase the rate of glycolysis by activating phosphofructokinase (PFK), a key enzyme involved in glycolytic regulation (Abrahams and Younathan, 1971; Lowry and Passonneau, 1966; Tejawani et al. 1973; Uyeda and Racker, 1965). Unfortunately, this early research did not determine the effect of NH_3 on PFK activity in the presence of physiological concentrations of pottasium (K^+). In 1975, Sugden and Newsholme demonstrated that in mammalian skeletal muscle NH_3 has no direct stimulating effect on PFK in the presence of K^+ (100 mM). Since 1975, results from a number of studies have indicated that NH_3 is not a significant regulator of PFK (Dudley and Terjung, 1985a; Katz et al. 1986b; Terjung et al. 1986).

It has been suggested that NH_3 may stimulate PFK indirectly by buffering hydrogen ions, thereby raising pH and, consequently, stimulating glycolysis (Lowenstein, 1972; Mutch and Banister, 1983). Data presented by both Katz et al. (1986b) and Dudley and Terjung (1985a) show that NH_3 accumulation in muscle can buffer between 3-7% of the hydrogen ions produced from lactate and pyruvate accumulated during exercise. On the basis of this evidence it can be concluded that NH_3 accumulation during exercise is of minor importance for the muscle buffering process and it, therefore, appears unlikely that NH_3 accumulation is of importance for PFK activation via its ability to buffer hydrogen ions.

Potentially, the accumulation of NH_3 in muscle may decrease the rate of mitochondrial respiration thereby causing a decrease in pyruvate oxidation and an increase in muscle lactate production. A number of studies have reported that mitochondrial respiration in brain and liver cells is decreased when these cells are exposed to varying concentrations of NH_3 (range 20 μM - 10,000μM) (Hindfeldt and Siesjo; 1970; Katunuma et al. 1966; McKann and Tower, 1961; Worcel and Erecinska, 1962). A number of mechanisms have been postulated to explain how NH_3 may attenuate oxygen consumption. These mechanisms include the inhibition of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase (McKann and Tower, 1961), a decrease in reducing equivalents (NADPH or NADH) via the stimulation of metabolic pathways not involved in the production of ATP (Katunuma et al. 1966; Worcel and Erecinska, 1962), an inhibition of the electron transport system (Hindfeldt and Siesjo, 1970) or a reduction in the ability of reducing equivalents to be transported from the cytoplasm into the mitochondria (Hindfeldt and Siesjo, 1970). At present there appears to be no broad agreement as to which of the above mechanisms, if any, are responsible for inhibiting mitochondrial respiration. Furthermore it appears that no studies to date, have investigated the effect of NH_3 accumulation on muscle fibre mitochondrial function. It is not known therefore whether the inhibition of mitochondrial respiration observed in brain or liver cells occurs to the same extent, if at all, in muscle fibres.

The accumulation of NH_3 in muscle also has the potential to increase muscle lactate production by reducing the ability of muscle to remove pyruvate via the glyconeogenic pathway (Mutch and Banister, 1983). Bryla and Niedzwieka (1979) reported that 1 mM ammonium chloride reduced the activity of pyruvate carboxylase in liver cells. Pyruvate carboxylase is known to be present in human muscle and is involved in the first step in the conversion of pyruvate to muscle glycogen (Hermansen and Vaage, 1977; Newsholme and Leech, 1983). It is doubtful that NH_3's inhibition of pyruvate carboxylase contributes significantly to muscle lactate because it is unlikely that the glyconeogenic pathway operates to any significant extent during exercise. These doubts are based on the belief that the main fates for pyruvate during exercise are lactate formation or oxidation not muscle glycogen synthesis (Brooks, 1985). Obviously further research is required to ascertain the importance of the pyruvate carboxylase reaction during exercise before any definitive conclusions can be made about the importance or otherwise of NH_3's inhibition of pyruvate carboxylase.

From the evidence outlined above it appears that muscle NH_3 accumulation has very little to do with the increased production of muscle lactate and the subsequent peripheral fatigue observed after acute intense exercise. Over the past few years the evidence is becoming increasingly more convincing that NH_3 has no significant regulatory role to play in muscle metabolism but is simply

a byproduct of either, or both, muscle adenine nucleotide catabolism or amino acid catabolism.

2.6 Summary

Skeletal muscle NH_3 production may occur via three biochemical pathways which include; the AMP deaminase pathway (Lowenstein, 1972), the 5'-nucleotidase / adenosine deaminase pathway (Rubio et al. 1972; Whitlock and Terjung, 1987) and catabolic pathways for amino acids (Graham et al. 1987; Haralambie and Mössinger, 1980). During short duration, high intensity exercise the AMP deaminase pathway appears to be the major method by which NH_3 is produced (Katz et al. 1986a; Meyer and Terjung, 1979; Meyer et al. 1980). In contrast, during low intensity exercise AMP deaminase activity is low (Terjung et al. 1986) indicating that any NH_3 produced may comes from other NH_3 producing pathways. Muscle fibre type distribution of enyzme activities support the argument that the various NH_3 producing pathways may be recruited at different exercise intensities. AMP deaminase activity is more prevalent in fast twitch fibres (Bockman and Mckenzie, 1983; Winder et al. 1974) while the metabolic apparatus of the other NH_3 producing pathways occur substantially in slow twitch fibres (Bockman and Mckenzie, 1983; Kuel et al. 1972).

Both muscle and plasma NH_3 concentration increase with increasing exercise intensity and duration (Eriksson et al. 1985; Katz et al. 1986a). The return of plasma NH_3 concentration to the resting state normally occurs within 30 minutes of recovery (Babij et al. 1983; Eriksson et al. 1985). There is, however, a discrepancy within the literature about the way in which NH_3 returns to basal levels (Banister et al. 1983; Mutch, 1983, Buono et al. 1984). The critical exercise intensity at which plasma NH_3 concentration begins to rise above resting levels also remains equivocal (Babij et al. 1983; Buono et al. 1984; Eriksson et al. 1985; Katz et al. 1986a). Despite this uncertainty, there is strong evidence to suggest that the rise in plasma NH_3 concentration observed during exercise is the result of an increased NH_3 efflux from active muscle and not the result of a reduced removal rate by the various NH_3 removing organs (i.e., inactive muscle, liver, brain)(Eriksson et al. 1985). The rate of NH_3 efflux from muscle is not only dependent upon NH_3 production rates but is also influenced by the pH gradient between the muscle and blood (Mutch and Banister, 1983), and possibly, by the rate of NH_3 incorporation into amino acid synthesis, in particular glutamate and alanine (Palaiologos and Felig, 1976).

Endurance training reduces AMP deaminase activity and NH_3 production via this pathway in stimulated rodent muscle (Constable et al. 1987; Dudley and Terjung, 1985a; Winder et al. 1974). Whether this occurs in human muscle is

not known. Endurance training may not alter NH_3 production from the 5'-nucleotidase pathway (Knochel et al. 1985), however, there is evidence to suggest that training may increase NH_3 production via amino acid catabolism (Askew et al. 1979; Dohm et al. 1977; Henderson et al. 1985;; Holloszy et al. 1970). Plasma NH_3 accumulation is reduced in animals during exercise at the same absolute workload after training (Miller and Lawrence, 1986). Similar studies using human subjects have produced equivocal results (Hurley et al. 1984; Lo and Dudley, 1987). Several researchers have reported that training does not alter human plasma NH_3 accumulation when exercise at the same relative intensity is performed (Hurley et al. 1984; Lo and Dudley, 1987). Suprisingly, research investigating the effects of endurance training on muscle or plasma NH_3 concentration during recovery from exercise has not been conducted.

CHAPTER 3

METHODS AND PROCEDURES

To investigate the effects of endurance training on human ammonia metabolism, six endurance trained cyclists and seven active untrained males were studied during maximal and submaximal exercise on an electronically braked cycle ergometer (Mijnhardt KEM 2).

3.1 Subjects

Six endurance trained cyclists volunteered to participate in this investigation. One of the trained subjects was a professional road racer, while another three were elite Victorian triathletes. Six of the seven untrained subjects were recruited from the Physical Education and Recreation Department at Footscray Institute of Technology. All subjects were informed of the procedures and were required to sign an informed consent statement (Appendix A). Experimental procedures were approved by the Human Ethics Committee of Footscray Institute of Technology.

3.2 Maximal and Submaximal Exercise Tests

Subjects were required to attend the Footscray Institute of Technology Human Performance Laboratory for two exercise tests. On the first occasion, the subjects performed an incremental exercise bout to volitional exhaustion and on the second occasion they cycled for 15 minutes at an intensity which was approximately 85% of their maximum oxygen consumption ($\dot{V}O_2$max). The submaximal exercise test was conducted at least two days, but no more than seven days after the incremental exhaustive exercise bout. All subjects fasted for at least 12 hours before each work bout and all were asked to refrain from strenuous exercise for 24 hours prior to each test.

The $\dot{V}O_2$max test protocol consisted of 4 minutes of upright rested sitting, 4 minutes of unloaded pedalling, and there after, workload increments of 50 watt every 4 minutes until volitional exhaustion was attained. Subjects were encouraged to maintain a pedalling frequency between 80-90 revolutions per minute. During the final minute of each 4 minute period and during the final minute of exercise, oxygen consumption ($\dot{V}O_2$) and heart rate (HR) were determined. During the final 15 seconds of each workload, 3 ml of venous blood was sampled from an indwelling venous catheter (21 gauge) positioned in the antecubital space. Immediately prior to blood sampling, approximately 2 ml of blood was drawn and discarded in order to flush the catheter. Each sample was later analysed for blood lactate and plasma ammonia. Metabolic data

collection and blood sampling continued during the second, fifth, tenth, and twentieth minute of recovery.

The submaximal exercise test required each subject to rest in a seated position for three minutes on the bicycle and then ride at a pedalling frequency of 80-90 revolutions per minute for up to 15 minutes. Each subject was required to ride at a workload that would elicit 85% of each individuals $\dot{V}O_2$ max. The workload required to elicit 85% $\dot{V}O_2$ max was determined from the results of the previous maximal exercise test using linear regression analysis. Collection of $\dot{V}O_2$, HR, and venous blood, occurred after 3 minutes of rest and during every third minute of exercise. Five ml of blood was collected every sampling period and was analysed for blood lactate, plasma ammonia and plasma alanine.

3.3 Oxygen Consumption

During both exercise tests, air was inspired and expired through a Hans-Rudolf valve (dead space = 80 cm^3) and large diameter (internal diameter= 3.5 cm) plastic tubing. Expired air was directed through a spirometer (Pneumoscan S30) and into a mixing chamber. Samples of gas were continuously pumped from the mixing chamber into gas analysers which measured the oxygen (Applied Electrochemistry O_2 S-3A) and carbon dioxide concentration of the expired gases (Applied Electrochemistry CO_2 CD-3A). Before each test the gas analysers were calibrated with commercially prepared gas

mixtures whose composition had been verified by the Lloyd-Haldane apparatus. Data collected by various metabolic instruments were analysed on line by a microprocessor (Radio Shack TRS-80) which was programmed to calculate $\dot{V}O_2$ using standard techniques for open circuit indirect calorimetry (Consolazio et al. 1963). The main criterion used to judge the attainment of $\dot{V}O_2$ max is normally a levelling or decrease in $\dot{V}O_2$ with increasing workload (Mathews and Fox, 1976). This criterion was not met by most of the subjects in the present study. Therefore, the highest oxygen consumption recorded by each individual during the incremental exercise test was determined to be $\dot{V}O_2$ max. This measure may be best defined as peak $\dot{V}O_2$ rather than $\dot{V}O_2$ max, however the conventionally used terminology of $\dot{V}O_2$ max will be used throughout this thesis.

3.4 Heart Rate

An electrocardiogram was recorded during all exercise bouts using a modified standard lead 1 (CM5) and recorded on an Electrocardiogram Data Computer (Quinton Model 740). The HR ($b.min^{-1}$) was calculated by measuring the time taken for 6 consecutive R waves. HR was determined in the last 15 seconds of a given minute.

3.5 Blood Lactate

Blood drawn from the subjects during each test was analysed for blood lactate (LA). Immediately following

blood collection, 0.5 ml of blood was pipetted into a test tube containing 1 ml of cold 8% perchloric acid and vortexed. The denatured sample was centrifuged at 4°C for 10 minutes and the supernatant was decanted and refrigerated for no more than 48 hours before analysis. Blood lactate levels were determined in duplicate using an enzymatic technique (Sigma Chemical Co., Technical Bulletin, No. 826 UV, 1968). A set of lactate standards ranging in concentration from 1 mM to 20 mM was run with each set of samples. Details of the analytical precision of this technique are reported in Appendix B.

3.6 Plasma Ammonia

Blood drawn from the subjects during each test was analysed for plasma ammonia. For each plasma ammonia determination, 2 ml of blood was centrifuged immediately after sampling at 15000 G for 3 minutes. The resultant plasma was transferred to cryules and frozen in liquid nitrogen (LN_2) before being stored at -80°C for later analysis. Howanitz et al. (1984) reported that plasma ammonia concentration rises with prolonged storage. To avoid this increase in ammonia concentration, all samples were analysed in duplicate within 72 hours of collection. Plasma ammonia (NH_3) (in this thesis NH_3 has been used to denote the sum of NH_3 plus NH_4^+ content in plasma) was analysed by flow injection analysis (FIA), details of which are reported by Cardwell et al. (1987) (Appendix C). The analysis was carried out using a Tecator (5020) FIA

analyser and a gas diffusion cell (Chemifold V). Samples, 150 µl in volume, were manually injected into a carrier stream of 0.9% NaCl which subsequently mixed with a stream of 0.1 M NaOH / 0.9% NaCl. The resulting alkaline stream was pumped into the gas diffusion cell where ammonia diffused across a teflon membrane into an acid-base indicator stream (cresol red and thymol blue). The colour change in the indicator stream was measured spectrophotometrically (590 nm) and was proportional to the NH_3 concentration of the sample.

3.7 Plasma Alanine

Plasma alanine (ALA) was determined using high perfomance liquid chromatography following the methods outlined by Deyl et al. (1986). For each amino acid determination, approximately 1 ml of venous blood was centrifuged immediately upon collection and the plasma decanted. Two hundred and fifty µl of plasma was placed into Eppendorf centrifuge tubes containing 150 µl of 20% sulphosalicylic acid. The tubes were vortexed, centrifuged (15000 G for 3 minutes) and the supernatant decanted into cryules which were immediately frozen in LN_2. After each exercise test the samples were removed from the LN_2 and stored at -80°C. Analysis of the supernatant was completed within 2 months of collection. The amino acid profile of blood treated and stored in this manner is known not to change even after several months of storage (Deyl et al. 1986).

Fifty µl of the deproteinised supernatant was injected into a 0.2 M lithium citrate mobile phase (pH 3.0) which was pumped at a rate of 0.4 $ml.min^{-1}$ (Biorad HPLC pump 1330) to an ion exchange column (Interaction AA511). After separation on and elution from the column, post column flourescence derivatives of the amino acids were produced by reaction (at 95-100°C) with a solution of o-Phthalaldehyde (6 mM), boric acid (1 mM) and mercaptopropionic acid (5 $ml.l^{-1}$) (pH = 10.4). The amino acid derivatives were detected by a flourescence detector (Perkin Elmer LC 1000) and peaks recorded by a chart recorder (Cole Palmer 8376-20). Area under the peak was calculated using a Shimadzu integrator (Chromatopac C-EIB). The column was heated (Biorad Column Heater) at 36°C until glutamine was detected at which stage the temperature was raised to 60°C. The detection of amino acids ceased when alanine was detected. In between sample injections a 0.2 M lithium hydroxide solution was used to clean the column. Re-equilibration with the 0.2 M lithium citrate mobile phase and of the column temperature was always completed before sample injections. A standard amino acid mixture was run before the analysis of each subject's set of submaximal plasma samples (i.e. every sixth or seventh injection). Further details of the analytical technique are reported in Appendix B.

3.8 Histochemistry

Muscle biopsies were taken from 11 of the original

13 subjects (6 untrained, 5 trained) approximately 3 months after the last exercise test. Two muscle biopsies were taken through the same incision approximately one third of the length from the proximal edge of the patella to the spina iliaca anterior superior. One sample was removed from the superficial and the other taken from the deep portion of the vastus lateralis. Samples of muscle were mounted in an embedding medium (Lipshaw M1 embedding matrix) and immediately frozen in isopentane cooled by LN_2. The muscle samples were stored at -80°C until analysis. For histochemical analysis serial cross sections (10 µm thick) were cut on a cryotome (Lipshaw Cryotome 1500A) at -20°C, mounted on cover glasses, and stained for myofibrillar adenosine triphosphatase (ATPase) after preincubation of serial sections at pH 4.3 and 10.3 (Brooke and Kaiser, 1970). The use of two preincubation solutions was used to verify the correct identification of slow and fast twitch fibres. The percentage of slow twitch fibres (%ST) was determined by averaging the results of the superficial and deep muscle samples as suggested by Blomstrand and Ekblom (1982). The %ST fibres was determined to allow for statistical control of differences in fibre type between trained and untrained subjects. The %ST fibres was also determined in order to study the relationship between fibre type and other variables of interest.

3.9 Onset of Blood Lactate Accumulation

The onset of blood lactate accumulation (OBLA) was determined for each subject using the $\dot{V}O_2$ and LA data obtained during the maximal exercise test. The interpolated $\dot{V}O_2$ that corresponded to a venous LA value of 4 mM was defined to be the OBLA (McLellan, 1985). The incremental exercise protocol used to determine the OBLA was recommended by Karlsson and Jacobs (1982).

3.10 Plasma Ammonia and Blood Lactate Break Point

The transition point from a phase of slow increase in plasma NH_3 and LA to a phase of rapid increase during incremental exercise were termed the NH_3 break point and the LA break point, respectively (NH_3 BP and LABP). Both break points were determined using the methods described by Beaver et al. (1985). Log of LA (absolute concentration) and Log of plasma NH_3 (absolute concentration) were plotted against Log of $\dot{V}O_2$ ($l.min^{-1}$). From each of these plots three people were asked to determine independently the data point which separated the data into two segments (i.e., a flat and steep segment). Each segment was then fitted with a straight line, using linear regression analysis. The NH_3 BP and the LABP was determined to be the $\dot{V}O_2$ at which the two lines intersected. An NH_3 BP or LABP could not be determined for one of the trained subjects due to technical difficulties (i.e., non-patent catheter during certain periods of the exercise). The log-log transformations for each individual are displayed in Appendix D.

3.11 Statistical Analysis

BMDP computer programs were used to compute most of the statistics. Where appropiate, data were analysed using two-factor (Time and Training) repeated measures ANOVA or two-factor repeated measures ANCOVA, with %ST muscle fibres as the covariate. Whenever ANOVA or ANCOVA resulted in a significant interaction effect, simple main effects analysis was used to determine differences between the trained and untrained groups. ANCOVA was calculated using the data obtained on the 11 subjects who underwent the muscle biopsy procedure. ANOVA calculations were based on 13 subjects, 11 of whom consented to the biopsy.

In the submaximal exercise test all trained subjects and only two untrained subjects completed the designated time period. Of the remaining 5 untrained subjects, 1, 3, and 1 subjects completed 14, 11 and 9 minutes of exercise, respectively. In order to perform statistical procedures, the data collected from 3 subjects who were exhausted at minute 11 were included as data points in minute 12. Missing data points were calculated at minute 12 for the subject who was exhausted at minute 9. Missing data points were estimated with the BMDP description and estimation of missing data program using regression analysis.

To uncover the effect of exercise duration within each group ONE-WAY ANOVA was calculated. Whenever the ONE-WAY ANOVA disclosed a difference, a posteriori pairwise comparisons were made using the Newman-Keuls test. All ANOVA, simple main effects and Newman-Keuls analyses are

summarised in tables contained in Appendix E. Where appropriate, certain data were analysed using Student's T-test for testing means of two independent groups or paired data. Significance was determined using two-tailed T-tests. To examine the relationship between data, Pearson product-moment correlation coefficients and linear regression analysis using the least squares method (Winer, 1971) were computed. Differences between the slope of regression lines were calculated using the methods described by Goldstein (1964). The level of probability considered to reject the null hypothesis was set at $p<0.05$. Throughout this thesis reference to statistical significance assumes $p<0.05$, unless specified otherwise. Statistical analysis of chemical techniques involved calculating relative standard deviations (RSD = SD/mean x 100).

CHAPTER 4

TRAINING STATUS

4.1 Introduction

Cross-sectional studies, designed to investigate the effects of training, inevitably suffer from the problem of being unable to identify effects which are solely attributable to training. Existing differences between so called trained and untrained groups may be caused by training status or they may be caused by muscle fibre type composition, diet, lifestyle, genetic endowment etc. (Astrand and Rodahl, 1977). Although it is impossible to attribute all differences between trained and untrained groups to training per se, there are a number of factors which can be used to illustrate the existence of training effects. It is the intention of this chapter to highlight these factors.

4.2 Results

4.2.1 Activity Pattern of Subjects

For at least two months prior to this investigation the six endurance trained cyclists rode on average 270 kilometres per week (range 150-500 Km per week). The

untrained subjects had not participated regularly in an endurance type activity for at least six months prior to this study.

4.2.2 Muscle Fibre Type

The trained group (58.1 ± 5.96%, n=5) had significantly greater %ST fibres than the untrained group (41.4 ± 4.29%, n=6). The mean number of fibres counted per trained and untrained subjects was 409 ± 83.5 and 452 ± 54.7, respectively.

4.2.3 Maximal Exercise

The characteristics of both the trained and untrained groups are presented in Table 4.1. The trained subjects exhibited a significantly higher $\dot{V}O_2$max, OBLA (%$\dot{V}O_2$max) and LABP ($\dot{V}O_2$ l.min^{-1}) than the untrained subjects. When LABP was expressed as %$\dot{V}O_2$max no difference between the groups was found ($0.1<p<0.2$).

Significantly (ANOVA, n=13) lower heart rates (HR) were observed for the trained subjects during all the workloads performed between 50 and 300 watt (Figure 4.1). Resting heart rates tended to be lower in the trained group, but this difference did not reach significance (ANOVA, n=13, p=0.15). The trained subjects also exhibited a significantly (ANOVA, n=13) lower accumulation of blood LA at workloads of 200 watt or more, and after 5, 10 and

Table 4.1. Subject characteristics of trained and untrained groups. (All values means ± S.E. of mean).

	Untrained (n=7)	Trained (n=6)
Age (year)	23.9 ± 1.38	23.2 ± 2.19
Height (cm)	177.4 ± 0.96	186.4 ± 3.06 *
Weight (Kg)	73.4 ± 2.47	79.1 ± 3.52
$\dot{V}O_2$ max ($l.min^{-1}$)	3.93 ± 0.18	5.00 ± 0.14 *
$\dot{V}O_2$ max ($ml.kg^{-1}.min^{-1}$)	53.5 ± 1.33	64.3 ± 3.02 *
LABP ($\dot{V}O_2$ $l.min^{-1}$)	1.79 ± 0.11	2.84 ± 0.23 *
LABP (%$\dot{V}O_2$ max)	46.3 ± 3.95	55.3 ± 3.52
OBLA (%$\dot{V}O_2$ max)	74.0 ± 1.50	85.0 ± 0.91 *
%ST fibres	41.4 ± 4.29	58.1 ± 5.96 *

* Denotes significant difference between groups ($p<0.05$).

20 minutes of recovery (Figure 4.2). Except for minutes 5 and 10 of recovery, blood LA differences between the groups remained significant even when group differences in %ST muscle fibre type were statistically controlled (ANCOVA, n=11).

4.2.4 Submaximal Exercise

Both trained and untrained subjects were required to ride at approximately 85% $\dot{V}O_2$ max for up to 15 minutes. The workload performed during the submaximal exercise bout by the trained group (324.5 ± 10.9 watt) was significantly

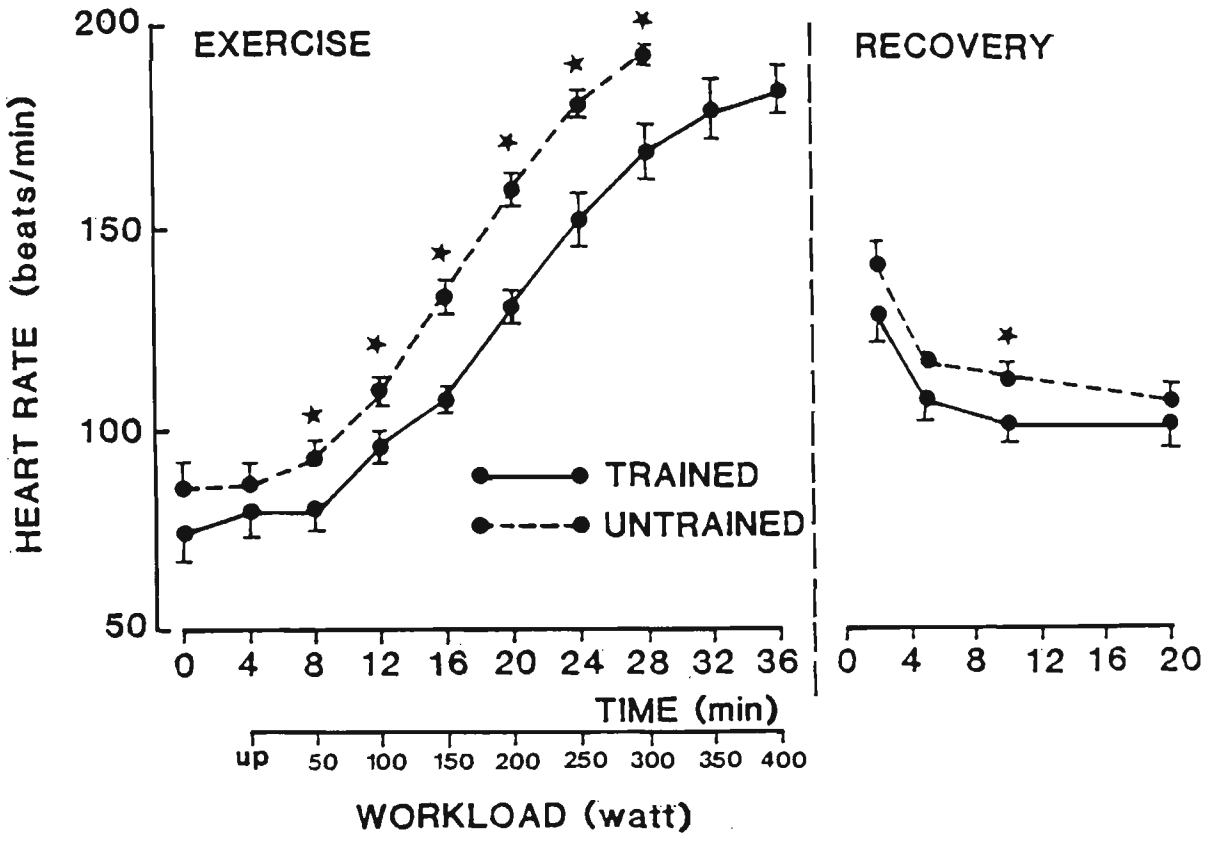


Figure 4.1 - Heart rates for trained and untrained individuals during exhausting, incremental exercise and passive recovery. (up - denotes unloaded pedalling; ★ $p < 0.05$ ANOVA, n = 13).

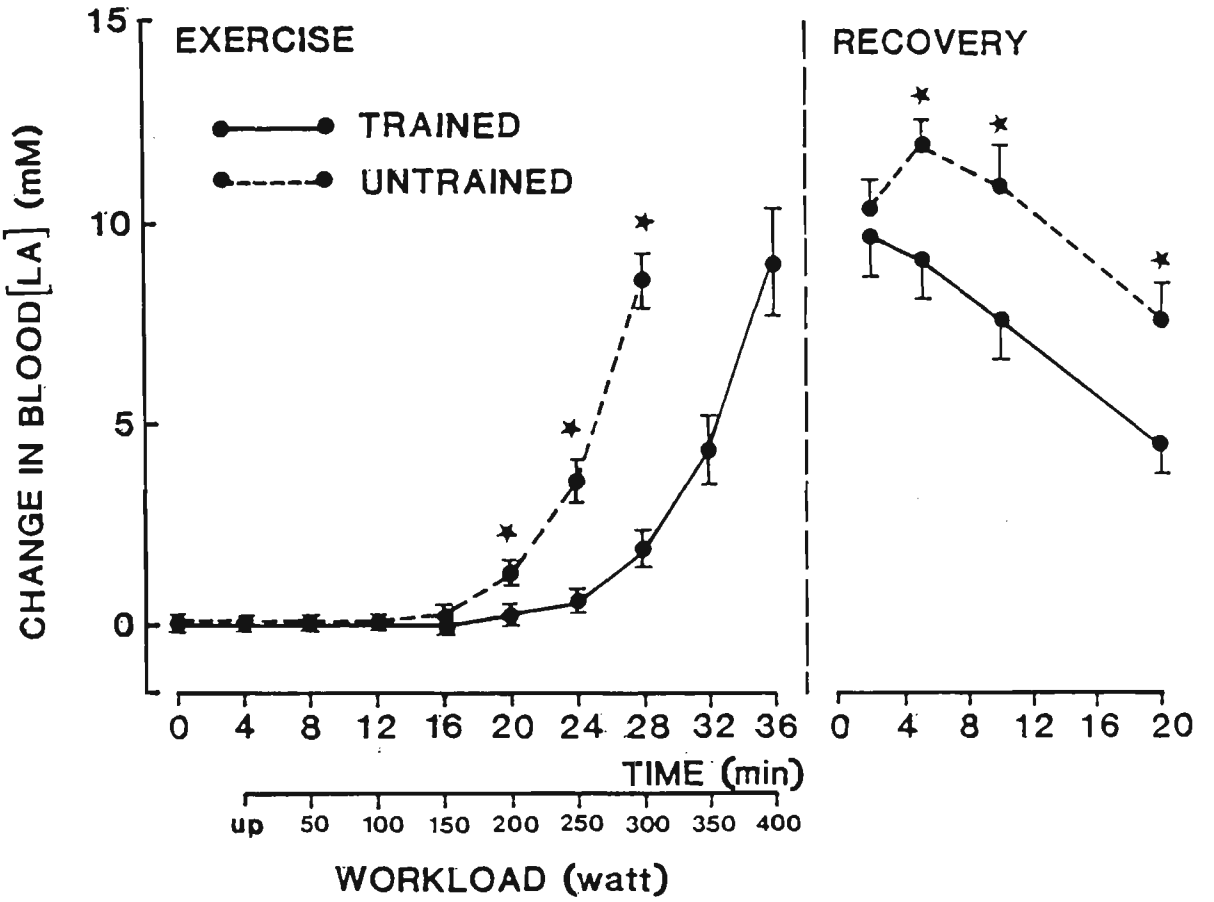


Figure 4.2 - The change in blood lactate concentration [LA] in trained and untrained individuals during exhausting, incremental exercise and passive recovery.

(up - denotes unloaded pedalling; ★ $p < 0.05$, ANOVA, $n = 13$).

greater than the untrained subjects (260.4 ± 9.2 watt). Every trained subject, but only two untrained subjects completed the 15 minute exercise bout.

The relative $\dot{V}O_2$ at which both groups worked during the submaximal exercise bout is shown in Figure 4.3. During the first 6 minutes of exercise there was no significant difference in the $\%\dot{V}O_2$ max between the groups. However, after 9 minutes the untrained subjects exercised at a significantly higher $\%\dot{V}O_2$ max compared to the trained (untrained= 90.2 ± 1.9; trained= 82.3 ± 1.3% $\dot{V}O_2$ max). Relative O_2 consumption did not change significantly after 6 or 9 minutes of exercise for the trained and untrained groups, respectively (ONEWAY-ANOVA).

The mean heart rate (HR) recorded throughout the submaximal exercise test for both groups is shown in Figure 4.4. Statistical analysis revealed a nonsignificant interaction between HR and time, therefore simple main effects analysis was inappropriate.

Resting blood LA concentration for the trained and untrained groups were 0.87 ± 0.17 and 1.21 ± 0.13 mM, respectively. The difference in resting blood LA was not significant (ANOVA and ANCOVA). The change in blood LA concentration (i.e., exercise value minus resting value) during the submaximal exercise bout is presented in Figure 4.5. The untrained subjects accumulated significantly more blood LA than the trained group after 9 minutes of exercise (ANOVA, n=13). This difference in LA accummulation between the groups remained significant even when muscle fibre type was used as a covariate (ANCOVA, n=11).

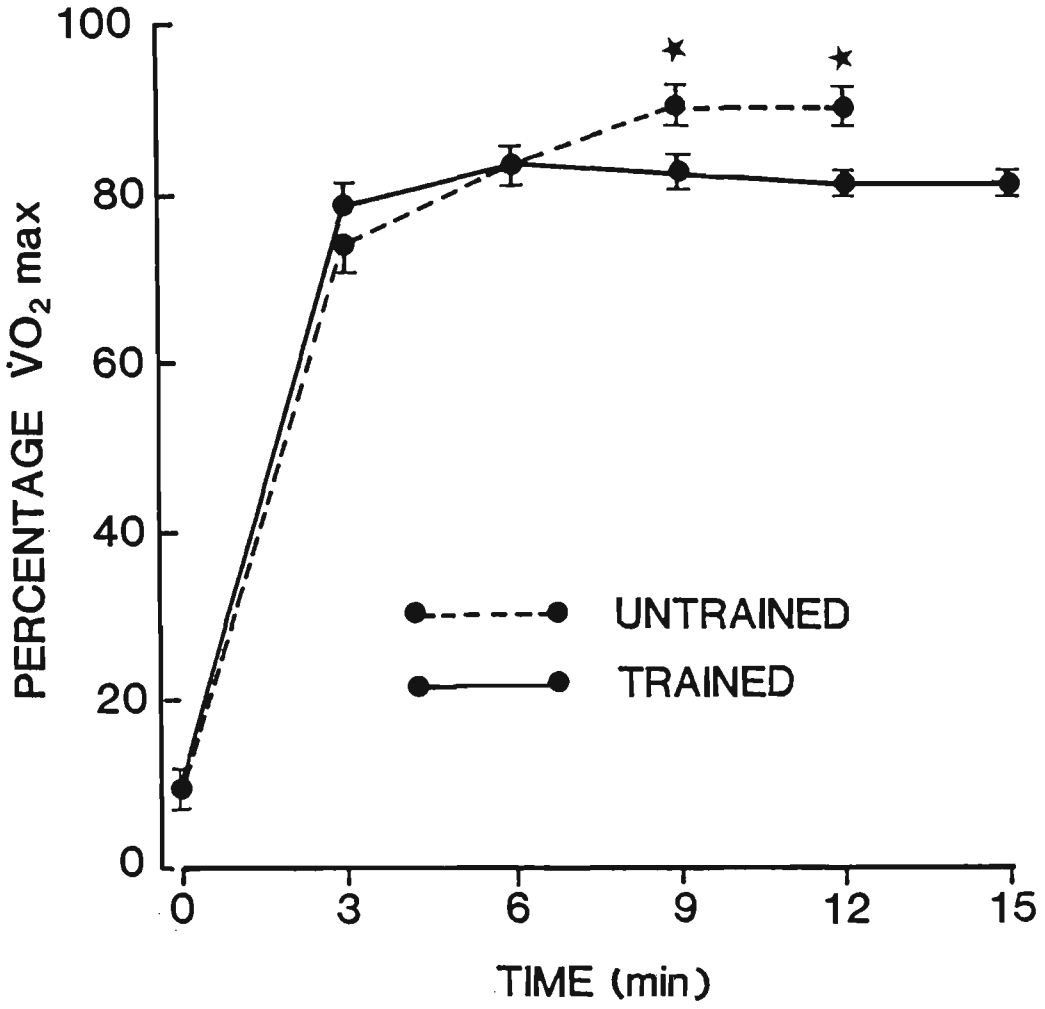


Figure 4.3 - Comparison of the percentage of maximal oxygen consumption ($\dot{V}O_2$ max) at which the trained and untrained subjects exercised during intense cycling.
(★ $p < 0.05$, ANOVA, $n = 13$).

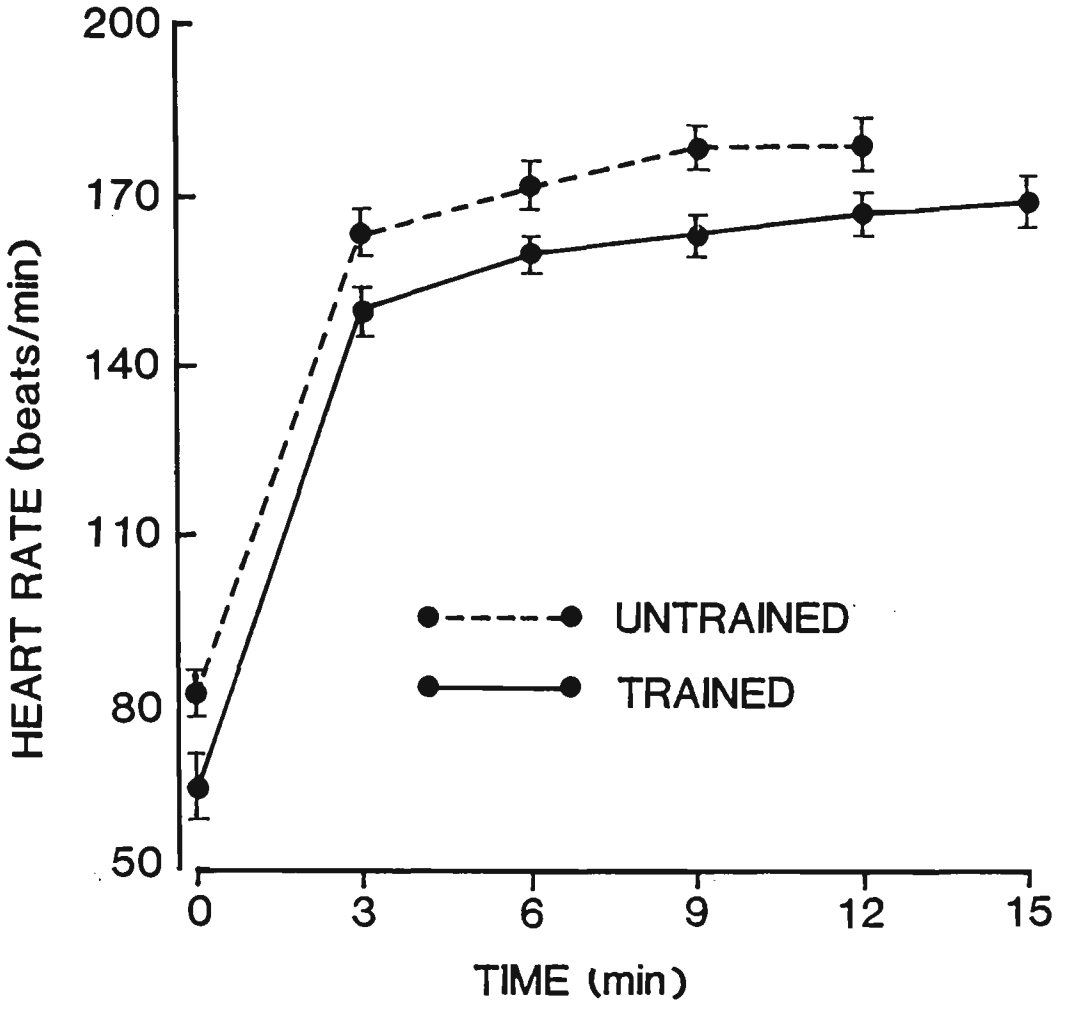


Figure 4.4 - Heart rates of trained and untrained individuals during intense cycling (≈85% $\dot{V}O_2$ max.)

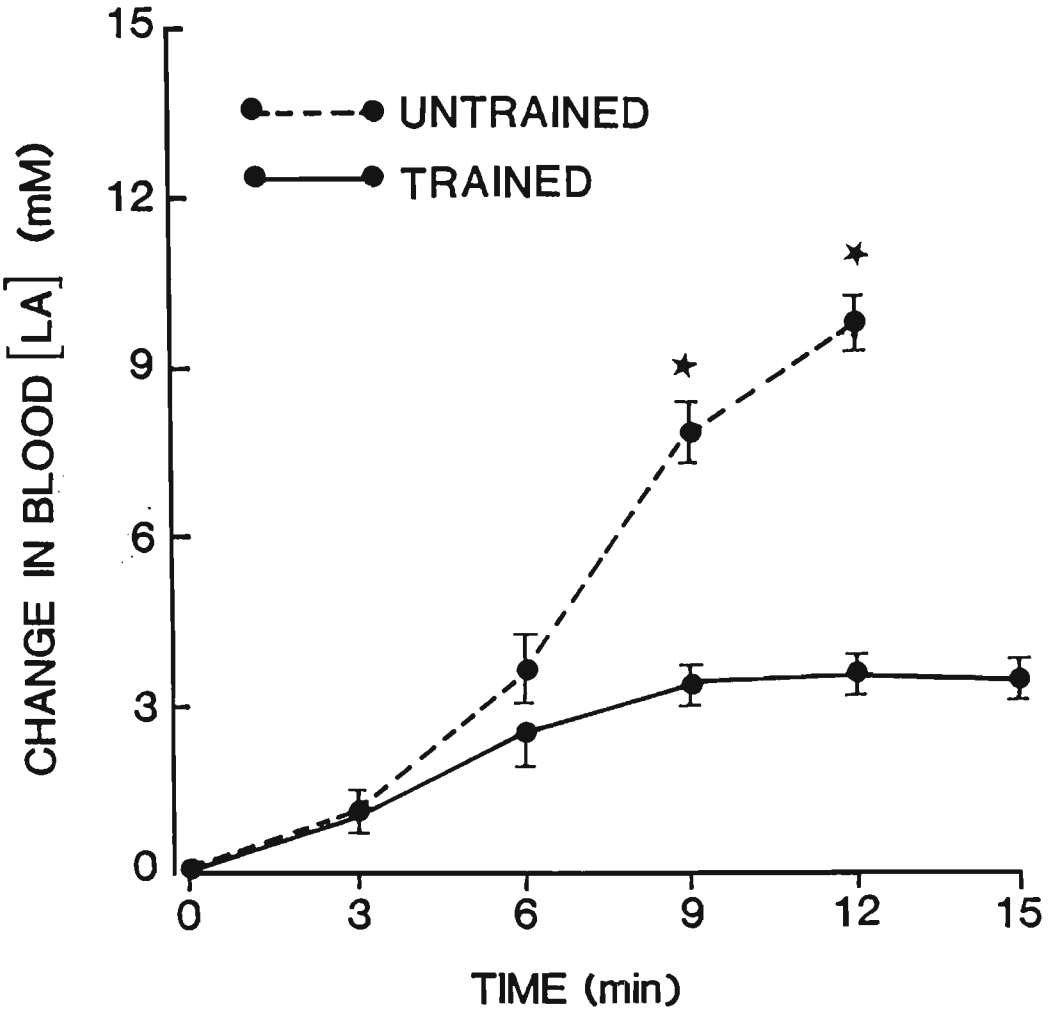


Figure 4.5 - The change in blood lactate concentration [LA] in trained and untrained individuals during intense cycling (≈85% $\dot{V}O_2$ max) ★ $p < 0.05$, ANOVA, $n = 13$.

4.3 Discussion

4.3.1 Muscle Fibre Type

The mean %ST fibres reported for the trained cyclists and untrained subjects used in the present study are consistent with previously reported values (Gollnick et al. 1972; Mackovà et al. 1986). Endurance trained individuals are known to have a significantly greater %ST fibres than untrained individuals (Baumann et al. 1987; Saltin and Gollnick, 1983), the results of the present study are, therefore, consistent with past research.

As muscle biopsies were taken 3 months after the exercise tests there is a possibility that the %ST fibres may have altered during the intervening period. If this occurred the use of %ST muscle fibres as a covariate in the analysis of the exercise data would be questionable. There is evidence, however, to suggest that muscle fibre type would not change during the intervening period. First, subjective activity analysis revealed that the activity pattern of the 11 subjects did not change during the period between the last exercise test and muscle sampling. Secondly, most studies have reported that muscle fibre type conversion between FT and ST fibres does not occur with endurance training or detraining in the vastus lateralis muscle (Andersen and Henriksson, 1977; Baumann et al. 1987; Gollnick et al. 1973, Ingjer, 1979; Schantz, 1986; Schantz et al. 1982).

4.3.2 Maximal Oxygen Uptake

In the present study, the mean $\dot{V}O_2$max achieved by the trained subjects (5.0 $l.min^{-1}$) was similar to that previously reported for elite cyclists (i.e., 4.9-5.2 $l.min^{-1}$) (Burke et al. 1977; Saltin and Åstrand, 1967). In contrast, the mean $\dot{V}O_2$max of the untrained group (3.93 $l.min^{-1}$) was higher than that reported for untrained physical education students (3.14 $l.min^{-1}$) (Burke et al. 1977). $\dot{V}O_2$max values are used extensively to document training status. Training is known to increase $\dot{V}O_2$max anywhere between 5 to 100%, depending on the training program and pre-training aerobic fitness level (Saltin et al. 1968). A significant difference in $\dot{V}O_2$max was found between the groups in the present study with the mean $\dot{V}O_2$max of the trained group being 27% greater than mean untrained value. This finding is supportive evidence of a difference in training status.

4.3.3 Drift in Oxygen Consumption

The submaximal exercise bout was designed to elicit a $\dot{V}O_2$ which was 85% of each individuals $\dot{V}O_2$max. After 6 minutes of exercise the relative $\dot{V}O_2$ was approximately 85% for both groups (Figure 4.3). Unfortunately, the relative $\dot{V}O_2$ for the untrained group continued to rise and by 9 minutes was significantly greater than the trained group. $\dot{V}O_2$ drift during heavy exercise has been noted previously and is known to be larger in untrained individuals (Casaburi et al. 1987). The cause of $\dot{V}O_2$

drift is unknown, however, it has been speculated that a number of mechanisms, which include body temperature, catecholamines, ventilation rate or LA metabolism, may be involved (Casaburi et al. 1987). As a consequence of the $\dot{V}O_2$ drift observed in this study, between group comparisons are only valid during the first 6 minutes of submaximal exercise.

4.3.4 Heart Rate

Reductions in resting HR after training have been well documented (Frick et al. 1967; Hurley et al. 1984). Resting HR's of the trained subjects tended to be lower than the untrained before the incremental exercise test but this difference did not reach significance ($p=0.15$). The trained subjects may have displayed an elevated HR prior to the first exercise test due to unfamiliarity with the testing apparatus. In contrast, the untrained group, (in the main physical education students), had considerable experience in being tested with this apparatus.

Trained individuals are expected to display a lower HR during exercise at the same absolute, but not the same relative intensity, when compared to sedentary people (Blomqvist and Saltin, 1983). In the present study, the trained group consistently recorded a significantly lower HR during submaximal exercise at the same absolute intensity than their untrained counterparts (Figure 4.1). This result provides further evidence of training status differences.

4.3.5 Blood lactate

Training is known to reduce blood LA concentrations during exercise at the same absolute submaximal intensity (Hurley et al. 1984; Robinson and Harmon, 1941). Importantly, LA accumulation was lower in the trained group at various submaximal intensities even when muscle fibre type differences between the groups were statistically controlled. This result provides strong evidence that the two groups differed in training status.

In the present study, no difference in blood LA accumulation was found between the groups (ANOVA or ANCOVA) during the first six minutes of the submaximal exercise bout, when the two groups were working at approximately the same relative intensity. This result is consistent with previous research as relative workloads in excess of 75% $\dot{V}O_2$max are not normally associated with a LA reduction caused by training (Hurley et al. 1984). LA accumulation at minutes 9 and 12 could not be compared across the groups because different relative exercise intensities were performed during this time period.

4.3.6 Lactate Break Point

The LABP ($\dot{V}O2$ $l.min^{-1}$) of the trained group was found to be higher than the untrained group. This is consistent with previous results (Henritze et al. 1985; Poole and Gaesser, 1985, Senay and Kok, 1977) and provides evidence that training status differed between the groups. The LABP expressed as %$\dot{V}O_2$max is known to increase after

training (Henritze et al. 1985; Poole and Gaesser, 1985; Senay and Kok, 1977), however, cross-sectional studies comparing the LABP (%$\dot{V}O_2$max) responses of trained and untrained individuals have produced equivocal results (Simon et al. 1986; MacDougall, 1977). There was a tendency for the trained subjects to display a higher LABP (%$\dot{V}O_2$max) than the untrained, however this difference did not reach statistical significance ($0.1>p<0.2$).

4.3.7 Conclusions

It is impossible to know with certainty whether the training status of the groups in the present study are different. However, differences in activity patterns and physiological responses indicate that differences in training status do exist. Importantly, conventional physiological methods for documenting training effects (i.e., $\dot{V}O_2$max, HR, LA accumulation, LABP) all support the belief that training status differed between the groups.

CHAPTER 5

TRAINING STATUS AND ITS EFFECT ON PLASMA AMMONIA ACCUMULATION DURING EXERCISE AT THE SAME ABSOLUTE AND RELATIVE INTENSITY.

5.1 Introduction

Studies using human subjects have yet to investigate the effects of endurance training on muscle NH_3 accumulation during exercise. The effects of training on blood NH_3 have been examined, however, with equivocal results for exercise at the same absolute intensity (Hurley et al. 1984, Lo and Dudley, 1987). Training has no reported effect on blood NH_3 accumulation during exercise performed at the same relative intensity (Hurley et al. 1984; Lo and Dudley, 1987). This chapter aims to re-examine the effects of training on plasma NH_3 concentration during exercise at the same absolute intensity in an attempt to resolve the existing conflict. As mentioned previously, training has no effect on plasma NH_3 concentration when exercise at the same relative intensity is performed. Despite an unaltered plasma NH_3 concentration training may effect NH_3 metabolism. One aspect of NH_3 metabolism that may change is the amount of free NH_3 incorporated into alanine (ALA). A more detailed picture of the effects of training on NH_3 metabolism may

be found if both plasma NH_3 and ALA are measured. This is the second aim of this chapter. The final aim of this chapter is to investigate the effects of training status on the plasma NH_3 accumulation during recovery from maximal exercise. To date, studies have only examined training effects on plasma NH_3 accumulation either during or immediately after exercise (Hurley et al. 1984; Lo and Dudley, 1987).

5.2 Results

5.2.1 Exercise at the Same Absolute Intensity

Resting plasma NH_3 levels were 11.7 ± 1.5 and 15.8 ± 2.1 μM for trained and untrained subjects, respectively. These NH_3 values were not significantly different (ANOVA and ANCOVA). The effects of endurance training on plasma NH_3 accumulation during incremental exercise is illustrated in Figure 5.1. Trained subjects exhibited a significantly smaller increase in plasma NH_3 concentration at 300 watt when compared to the untrained group (ANOVA, n=13). When muscle fibre type was used as a covariate, significantly smaller increases in NH_3 were found for the trained group at 250 and 300 watt (ANCOVA, n=11).

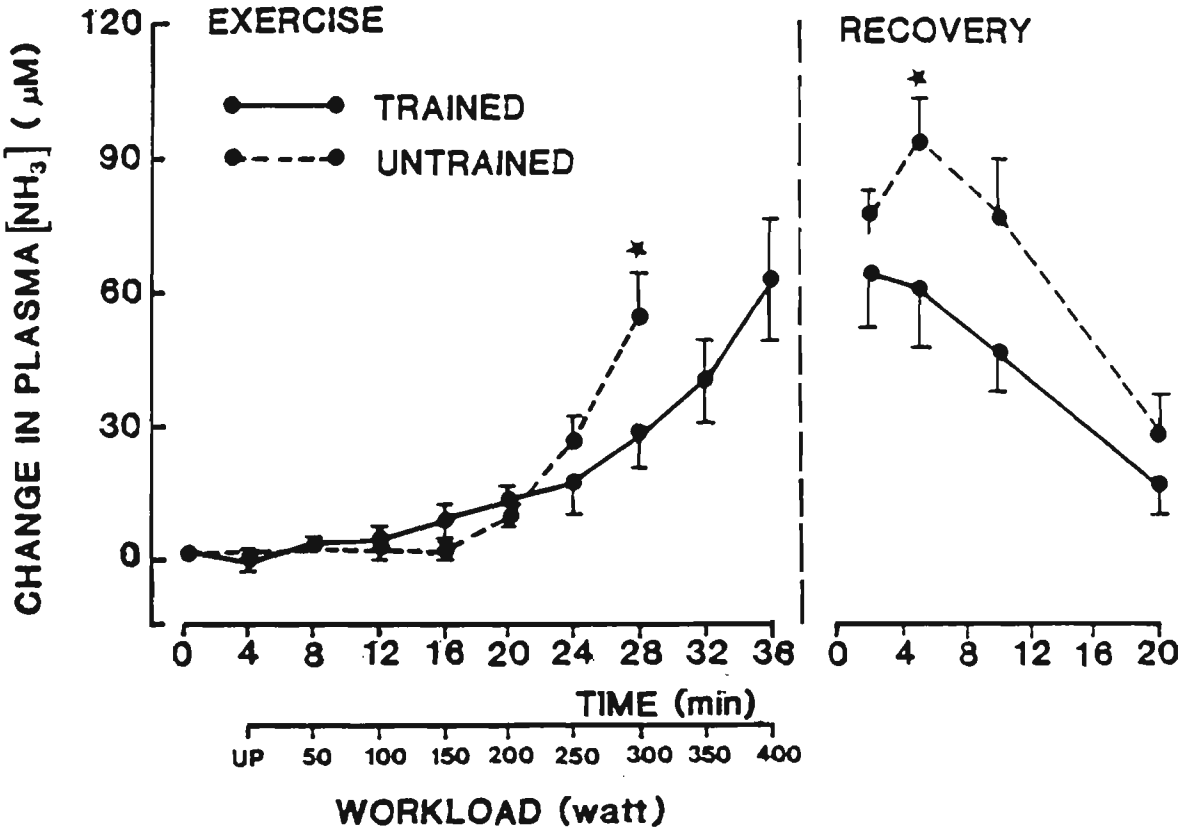


Figure 5.1 - The change in plasma ammonia concentration [NH_3] in trained and untrained individuals during exhausting, incremental exercise and passive recovery.

(up - denotes unloaded pedlaling; ★ $p < 0.05$, ANOVA, $n = 13$).

5.2.2 Plasma Ammonia During Recovery

Plasma NH_3 accumulation was significantly larger in the untrained compared to the trained subjects 5 minutes post-exercise (ANOVA, n=13) (Figure 5.1). Although there was a tendency for differences to be found at some periods of recovery (i.e., min 5, 10, and 20, $p<0.1$) plasma NH_3 accumulation across the groups was not different at any time when %ST muscle fibres was used as a covariate (ANCOVA, n=11).

The pattern of plasma NH_3 accumulation during recovery differed between the groups. After the cessation of exhausting exercise plasma NH_3 concentration continued to rise significantly (ONEWAY-ANOVA, n=7) during the first 5 minutes of recovery in the untrained group (Figure 5.2). In contrast, no significant increase in plasma NH_3 was observed in the trained group during the same recovery period (ONEWAY-ANOVA, n=6).

5.2.3 Exercise at the Same Relative Intensity

Resting plasma NH_3 values for the trained and untrained subjects were 15.5 ± 3.7 and 13.11 ± 2.1 μM, respectively. These NH_3 values were not significantly different (ANOVA or ANCOVA). The trained subjects tended to have a lower accumulation of NH_3 than the untrained at minute 3 ($p=0.07$) and minute 12 ($p=0.06$), however, this difference did not obtain statistical significance (Figure

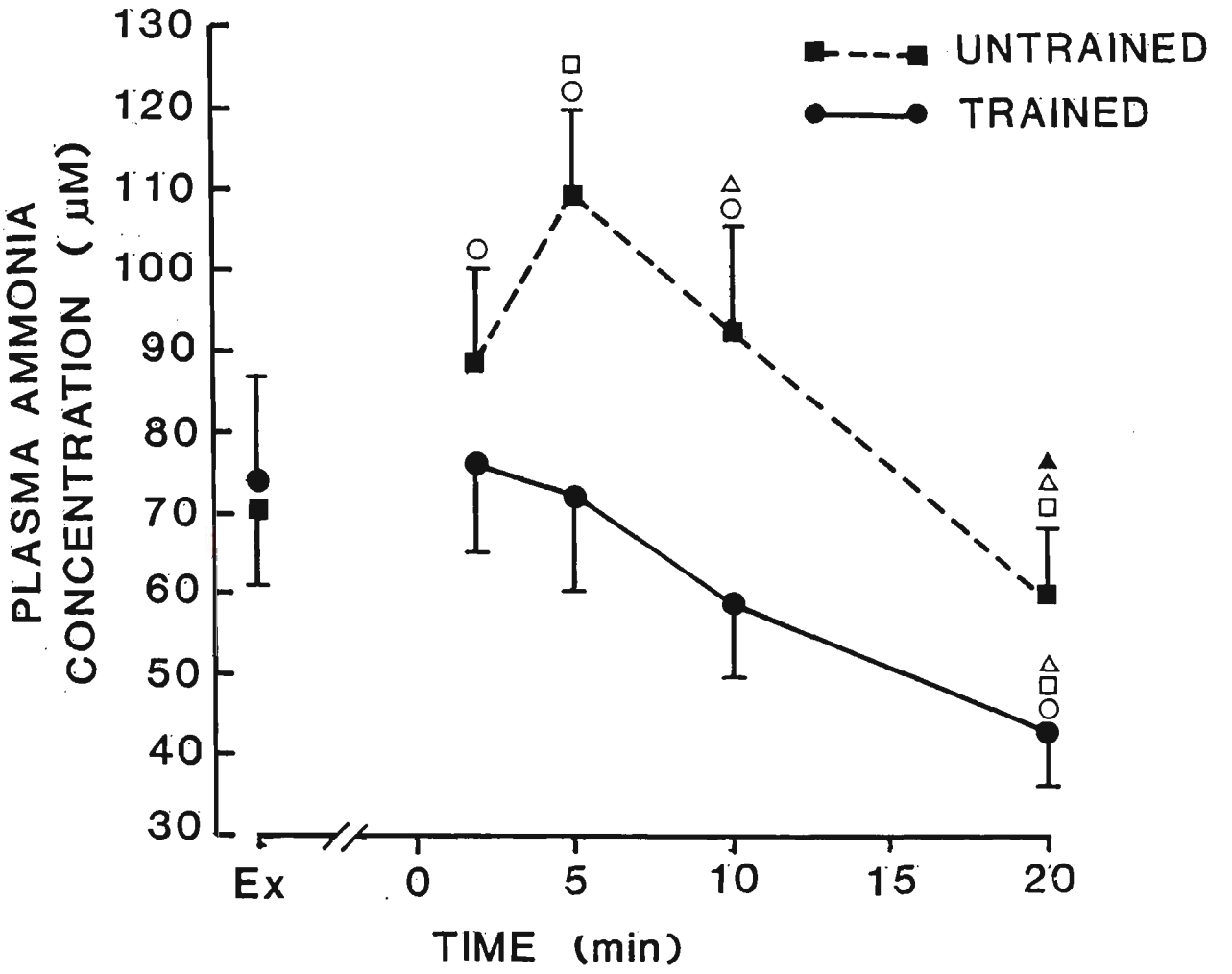


Figure 5.2 - Plasma ammonia concentration of trained and untrained individuals during the final minute of exercise (Ex) and during recovery from exhaustive, incremental exercise tests.

O different to Ex, □ different to Min 2 Recovery, Δ different to Min 5 Recovery, ▲ different to Min 10 Recovery; $p < 0.05$.

5.3). When muscle fibre type was used as a covariate no significant difference in plasma NH_3 accumulation was found between the groups at any time (ANCOVA, n=11). In both groups, the plasma NH_3 concentration rose above resting values after 6 minutes of exercise (Figure 5.4) and continued to rise significantly throughout the exercise period (ONEWAY-ANOVA).

Resting plasma ALA concentrations were similar across the groups (ANOVA or ANCOVA). Mean resting values were 360 ± 12.7 and 367.5 ± 25.67 μM for the trained and untrained, respectively. Training status appeared to have no effect on plasma ALA accumulation during exercise at the same relative intensity as no significant difference was found at any time, even when muscle fibre type was used as a covariate (Figure 5.5). The absolute ALA concentration rose above resting values after 6 minutes of exercise (ONEWAY-ANOVA) in both the trained and untrained groups (Figure 5.6). The pattern of ALA increase was similar in both groups.

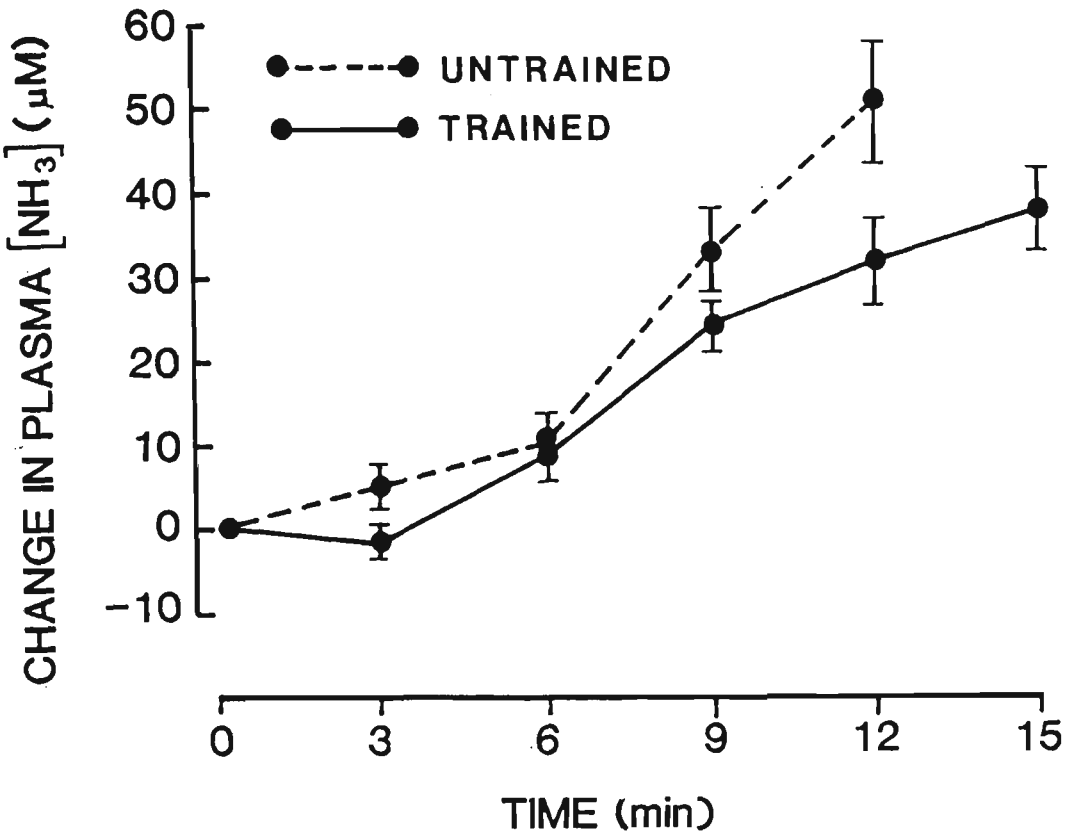


Figure 5.3 - The change in plasma ammonia concentration [NH_3] in trained and untrained individuals during intense cycling (≈85% $\dot{V}O_2$ max).

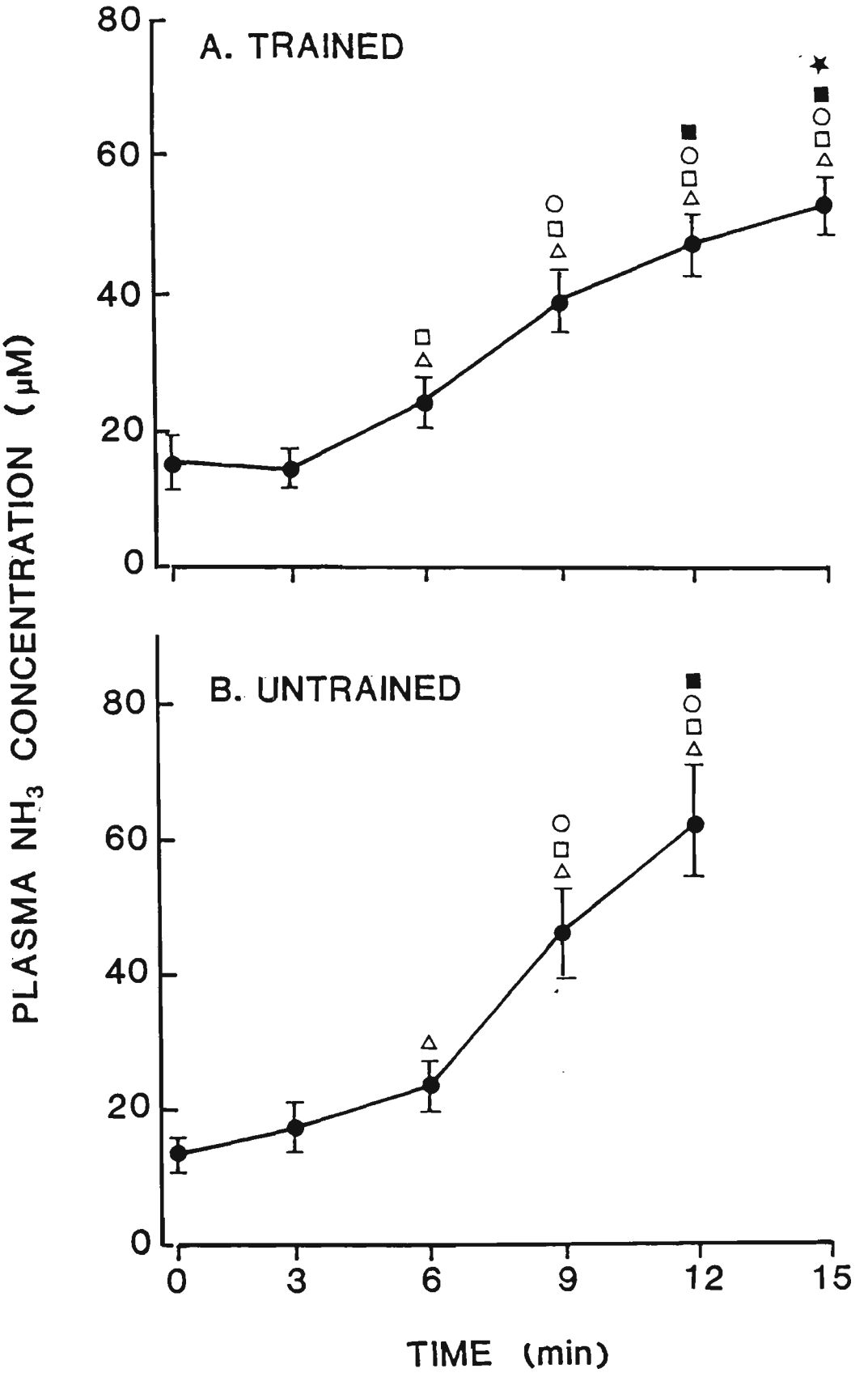


Figure 5.4 - Plasma ammonia concentration [NH_3] in trained and untrained individuals during intense cycling (≈85% $\dot{V}O_2$ max).

Δ different from rest, □ different from min. 3, O different from min. 6, ■ different from min. 9, ★ different from min. 12, $p < 0.05$.

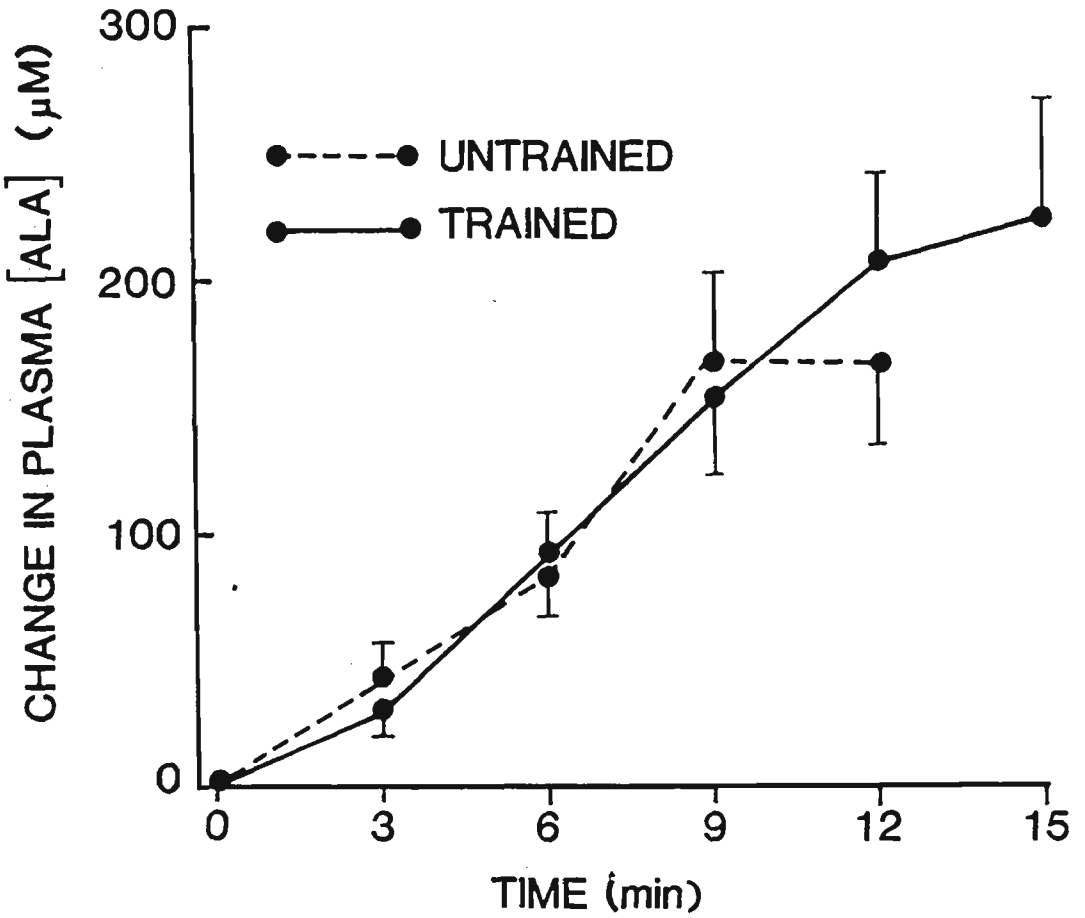


Figure 5.5 - The change in plasma alanine concentration [ALA] in trained and untrained individuals during intense cycling. (≈85% $\dot{V}O_2$ max).

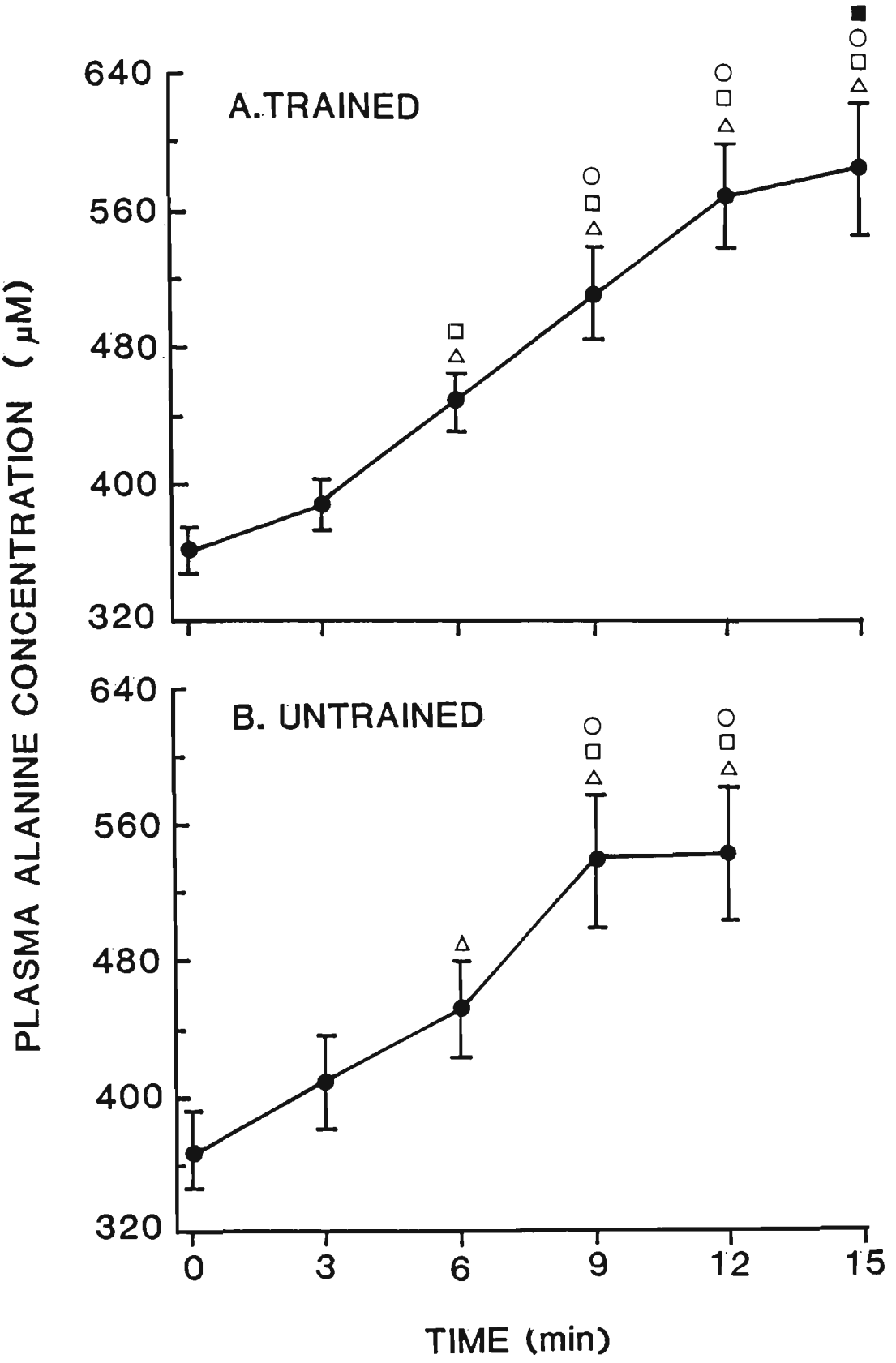


Figure 5.6 - Plasma alanine concentration [ALA] in trained and untrained individuals during intense cycling exercise (≈85% $\dot{V}O_2$ max). Δ different from rest, □ different from min. 3, O different from min. 6, ■ different from min. 9, $p < 0.05$.

5.3 Discussion

5.3.1 Resting Plasma Ammonia

Resting plasma NH_3 concentrations determined in the present study were similar to those reported by Katz et al. (1986a). Resting NH_3 values were not altered with training status. These data are in agreement with other authors (Hurley et al. 1984; Lo and Dudley, 1987). On the other hand, Berg et al. (1987) compared resting NH_3 values of 62 athletes with 25 untrained controls and found a significantly lower resting NH_3 level in the trained group. A difference in group size could explain the discrepancy between the results of the present study and the findings of Berg et al. (1987).

5.3.2 Exercise at the Same Absolute Workload

5.3.2.1 Plasma Ammonia Accumulation

As indicated earlier, the two groups in the present study differ in training status (see Chapter 4). This difference in training status should indicate differences in skeletal muscle oxidative capacity. Endurance training is known to increase the number of mitochondria in conditioned muscle (Holloszy and Coyle, 1984) and as a consequence, trained muscle has an enhanced ability to rephosphorylate ADP via mitochondrial respiration (Constable et al. 1987; Holloszy and Coyle, 1984). Dudley

and Terjung (1985a) argue that an increased ability to rephosphorylate ADP leads to a reduced production of NH_3 by a reduction in AMP catabolism via the AMP deaminase pathway. At least two studies (Hurley et al. 1984; Lo and Dudley, 1987) have attempted to ascertain whether a training induced decrease in muscle NH_3 production is reflected in a reduction in the accumulation of NH_3 in blood or plasma at a given submaximal workload. Lo and Dudley (1987) found that training reduced blood NH_3 levels while Hurley et al. (1984) reported that training had no effect. The results of the present study support the concept that plasma NH_3 accumulation is lowered with training. Plasma NH_3 levels were lower in the trained individuals at 250 and 300 watt when %ST fibres were used as a covariate. The effect of training on plasma NH_3 was not observed at low work levels because large increases in plasma NH_3 were not observed in either group.

5.3.2.2 Muscle Ammonia Accumulation

A training induced decrease in plasma NH_3 accumulation is best explained by reduced skeletal muscle production and accumulation, although the experimental data from the present study cannot exclude the possibility that plasma NH_3 accumulation is primarily reduced by changes in plasma NH_3 removal (see page 103). Interestingly, a theoretical argument, using data from this and other studies, can be mounted to support the hypothesis that human muscle NH_3 concentration is reduced by training.

The muscle-blood pH ratio determines the total protonated and non-protonated NH_3 concentration gradient across the muscle membrane (Mutch and Banister, 1983). Both trained and untrained individuals normally have a resting pH in muscle and blood which is 7.1 and 7.4, respectively (Henriksson and Sahlin, 1984; Hermensen and Osnes, 1972; Sharp et al. 1986; Sahlin et al. 1976). The pKa of NH_3 is 9.3 (Weast, 1975-76).

From the Henderson-Hasselbach equation

$$\left(\text{i.e., } pH = pKa + \text{Log } \frac{\text{Base}}{\text{Acid}}\right):$$

$$\text{Log } \frac{[NH_4^+]m}{[NH_3]m} = 2.2 \quad (1)$$

$$\text{Log } \frac{[NH_4^+]b}{[NH_3]b} = 1.9 \quad (2)$$

where m and b denote muscle and blood respectively.

Since NH_3 in the non-protonated form is freely diffusible, (Hindfelt, 1975) the concentration of this form will rapidly reach equilibrium across the muscle membrane hence $[NH_3]m = [NH_3]b$. Therefore the ratio of (1) over (2) gives:

$$\text{Log}\frac{[NH_4^+]m}{[NH_4^+]b} = 0.3$$

$$\frac{[NH_4^+]m}{[NH_4^+]b} = 1.995$$

The above calculations show that at rest, the total protonated and non-protonated NH_3 concentration is approximately twofold greater in muscle than blood. A similar analysis using data taken after short term exhaustive exercise yields a ratio of 3.76 and 2.51, for untrained and trained individuals, respectively. These ratios are based on the following data. Trained individuals (i.e., endurance trained cyclists, soccer players, ice-hockey players and basketball players) at exhaustion have a muscle pH = 6.8-6.9 (Sahlin and Henriksson, 1984; Sharp et al. 1986) and blood pH = 7.25 (Rassmussen et al. 1975). At exhaustion untrained individuals have a muscle pH = 6.6-6.65 (Sahlin et al. 1976; Sahlin and Henriksson, 1984; Sharp et al. 1986) and a blood pH = 7.2 (Hermansen and Osnes, 1972). In the present study the plasma NH_3 concentration just prior to exhaustion was 73.9 ± 13.1 and 70.7 ± 10.7 μM, for the trained and untrained, respectively. If the appropriate NH_3 ratios are applied this would yield an estimated muscle NH_3 concentration of 185 ± 32.9 and 266 ± 40.2 μmoles per litre of intracellular water for the trained and untrained

individuals, respectively. On this basis untrained human muscle is calculated to have an NH_3 concentration which is larger than trained muscle at exhaustion.

Admittedly, the above argument is based on comparisons of trained and untrained individuals and therefore some doubt exists whether changes in the muscle-blood pH ratio occur as a result of training or other factors. Results published by Sahlin and Henriksson (1984) indicate that the difference in muscle pH between trained and untrained groups cannot be attributed solely to muscle fibre type. Sahlin and Henriksson (1984) found no difference in fibre type composition between their untrained and trained subjects and still found a significantly higher muscle pH in the latter group after exhaustive exercise.

The above argument is also based upon plasma NH_3 concentrations determined from blood sampled from an inactive limb. This will lead to an underestimate of the actual plasma NH_3 concentration draining the exercising muscle and therefore underestimate the actual muscle NH_3 concentration (Eriksson et al. 1985). Training may increase the ability of inactive muscle to remove NH_3 (see below) thereby exacerbating the underestimation problem.

5.3.2.3 Plasma Ammonia Removal

The rate of plasma NH_3 removal is an important factor which influences plasma NH_3 accumulation. Plasma removal is performed by various tissues (i.e., resting

skeletal muscle, liver and brain) and is probably influenced by the amount of NH_3 delivered to these tissues (i.e., blood flow and plasma NH_3 concentration), and by various processes within the tissue (i.e., relevant enzyme activities) (Ericksson et al. 1985; Lockwood et al. 1979; Meyer et al. 1980). Endurance training enhances blood flow to liver and inactive muscle during a given absolute work intensity (Clausen, 1977). Increases in the perfusion of the liver and inactive skeletal muscle suggests that training may increase plasma NH_3 removal rates. Increased NH_3 removal rates may occur provided there are no major changes in the activity of enzymes involved with plasma NH_3 removal. If training increases the body's ability to remove NH_3 from the circulation, there is the possibility that the reduced plasma NH_3 accumulation observed during exercise is caused by this factor. Research investigating the effects of endurance training on plasma NH_3 removal rate at a given workload is not apparent in the published literature.

5.3.3 Training and Plasma Ammonia Accumulation During Recovery

The untrained subjects exhibited a larger increase in plasma NH_3 accumulation after 5 minutes of recovery when compared to the trained group (Figure 5.1). There was a strong tendency for this difference to remain significant even when muscle fibre type was statistically controlled

(i.e., ANCOVA, n=11, min 5 rec., p=0.07). This indicates that training, rather than muscle fibre type, is responsible for the lower plasma NH_3 accumulation found in the trained individuals after 5 minutes of recovery. Training may produce these results via changes in rates of muscle NH_3 efflux and/or plasma NH_3 removal. It is interesting to note that NH_3 accumulation 5 minutes post exercise was significantly related to the %ST fibres in the untrained but not the trained group (Chapter 6). This relationship suggests that the 5 minute recovery value observed for the untrained group was influenced by NH_3 effluxing from untrained ST fibres. If this is true, perhaps NH_3 leaving these fibres causes the untrained subjects to accumulate more NH_3 in the circulation than their trained counterparts. Training may, therefore, reduce the absolute NH_3 efflux rate from ST fibres during recovery from exhaustive exercise.

In the trained group, plasma NH_3 concentration did not rise after the cessation of exercise but remained constant for at least 10 minutes before decreasing. In contrast, plasma NH_3 concentration of the untrained group continued to increase significantly during the first few minutes of recovery with peak values occurring 5 minutes after exercise. The observation that plasma NH_3 concentration either rises or remains stable after intense exercise is consistent with the findings of Buono et al. 1984; Katz et al. 1986a and Wilkerson et al. 1977 but not those of Babij et al. 1983 or Banister et al. 1983. Buono

et al. (1984) have argued that conflicting patterns of plasma NH_3 recovery may result from differing types of recovery (i.e. active or passive). Unfortunately, in most studies the type of recovery has not been reported. Training status does not appear to explain the existing conflict as an immediate fall in plasma NH_3 was not observed in either group.

5.3.4 Exercise at the Same Relative Intensity

5.3.4.1 Plasma Ammonia Accumulation

Plasma NH_3 accumulation tended to be lower in the trained individuals at minute 3 ($p=0.07$). After 3 minutes of exercise the amount of plasma NH_3 accumulated in the trained group tended to decrease while the NH_3 values of the untrained tended to increase (Figure 5.2). In a similar experiment, Dudley et al. (1983) found that NH_3 accumulation, measured immediately after 3 minutes of exercise at 85% $\dot{V}O_2$max, was related to muscle fibre type. Individuals with a high %ST muscle fibres displayed a nonsignificant decrease, while individuals with a low %ST fibres produced a significant increase in blood NH_3 accumulation (Dudley et al. 1983). The results of the present study are consistent with the concept that it is muscle fibre type that is responsible for the near significant differences in NH_3 accumulation during the exercise conditions examined. When muscle fibre type was used as a covariate the tendency for group NH_3 values to be different at minute 3 disappeared ($p=0.31$).

Endurance training appears to have little influence on the magnitude of plasma NH_3 accumulation during exercise at the same relative intensity because no difference in NH_3 accumulation was found between the groups when muscle fibre type was statistically controlled. This finding is consistent with previous research (Hurley et al. 1984; Lo and Dudley, 1987). Very little is known about why training fails to alter plasma NH_3 accumulation during this type of exercise. The remainder of this discussion will address this issue.

5.3.4.2 Muscle Ammonia Efflux and Plasma Ammonia Removal

Blood flow to the important plasma NH_3 removing organs (liver, inactive muscle and brain) is unlikely to be altered by training when exercising at similar relative intensities (Clausen, 1977). If the activities of NH_3 consuming enyzmes are also unaffected by training, then plasma NH_3 removal rates probably remain the same during exercise of the same relative intensity. If the rate of NH_3 removal is unaffected by training, then muscle NH_3 efflux rates must also remain unchanged in order to permit the similar plasma NH_3 accumulation values for the trained and untrained groups observed in the present study. Muscle NH_3 efflux rates can be influenced by ALA synthesis and alterations in the muscle-blood pH ratio (see pages 54-56). In the present study, plasma ALA accumulation was not significantly different between the trained and untrained groups, even when muscle fibre type was

statistically controlled. This finding indicates that plasma ALA accumulation is unaltered by training when exercising at the same relative intensity. This finding also indicates, albeit indirectly, that muscle ALA synthesis has little effect on trained or untrained muscle NH_3 concentration or muscle NH_3 efflux during exercise at 85% $\dot{V}O_2$max. Very little, if anything, is known about the effects of training on the muscle-blood pH ratio during submaximal exercise. If the rate of plasma NH_3 removal and its rate of accumulation in blood are unaltered by training the rate of NH_3 efflux must also remain unchanged. Therefore the muscle-blood pH ratios during submaximal exercise must also remain unaffected. Further research is required.

5.3.4.3 Muscle Ammonia Production

If it is assumed that both muscle NH_3 efflux and plasma NH_3 removal rate are unaltered with training at the same relative workload, then it appears that muscle NH_3 production must also be similar. It has been suggested that muscle NH_3 production remains unchanged because the depletion of ATP in mixed human muscle is unaffected by training during exercise at the same relative intensity (Lo and Dudley, 1987).

Lo and Dudley's hypothesis faces at least one major problem as it implies that NH_3 production is closely linked to ATP depletion. During very intense, short

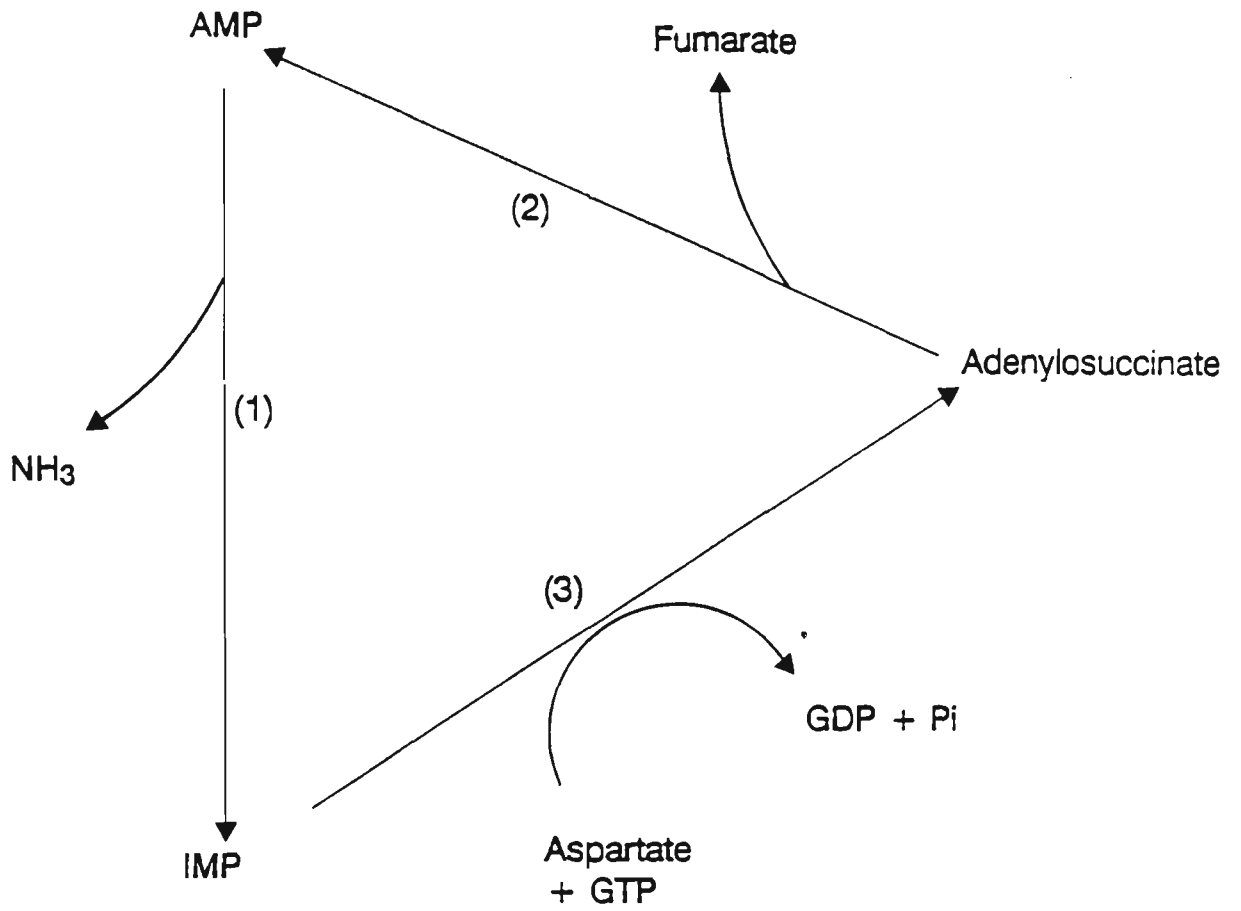
duration exercise, the magnitude of ATP depletion is matched by an equal increase in muscle NH_3 accumulation (Katz et al. 1986a,b). Therefore the hypothesis is supported. However, during more moderate, prolonged exercise, muscle NH_3 accumulation is no longer associated with a stoichiometric decrease in ATP (Graham et al. 1987). Graham et al. (1987) observed a continual increase in both muscle and blood NH_3 concentration during 30 minutes of exercise at 75% $\dot{V}O_2$max. The evidence suggests that at this exercise intensity there is little, if any, change in ATP concentration (Katz, 1986). In the present study, plasma NH_3 values continued to rise throughout the exercise period in both groups, even though most of the physiological parameters (e.g., $\dot{V}O_2$, LA, HR) indicated that the trained group was in steady state and therefore presumably maintaining a stable concentration of ATP.

During prolonged, moderately intense exercise NH_3 may be produced via operation of the purine nucleotide cycle (PNC), an active 5'-nucleotidase / adenosine deaminase pathway or via amino acid catabolism. Both the PNC and amino acid catabolism can produce NH_3 without stoichiometric decrements in ATP (Aragòn and Lowenstein, 1980; Flanagan et al. 1986; Graham et al. 1987; Swain et al. 1984). The operation of the PNC during exercise and the production of NH_3 via amino acid catabolism are processes which are controversial.

The PNC (see Figure 5.7) initially involves the deamination of AMP and ultimately the reconstitution of AMP. One turn of the cycle results in the net production of NH_3, fumarate, guanosine diphosphate (GDP) and inorganic phosphate (Pi). Water, aspartate and guanosine triphosphate (GTP) are consumed (Lowenstein, 1972).

It has been demonstrated that the PNC is apparently not active to any appreciable extent during either light (i.e, phosphocreatine depletion less than 25%) (Katz et al. 1986a; Meyer and Terjung, 1980) or strenuous exercise (phosphocreatine depletion greater than 75%) in either rat or human skeletal muscle (Katz et al. 1986a,b; Meyer and Terjung, 1980). However, during muscle contraction of sufficient intensity to produce a 30 to 65% decrease in phosphocreatine (i.e., moderate exercise) the PNC was shown to be operative in the gastrocnemius muscle of mice and rats (Aragòn and Lowenstein, 1980; Flanagan et al. 1986; Swain et al. 1984). Meyer and Terjung (1980) have also presented data that IMP formation and reamination occur in rat gastrocnemius muscle during 30 minutes of moderate exercise (approximately 65% decrease in phosphocreatine). However, these authors contend that the processes of deamination and reamination do not occur concurrently. According to Meyer and Terjung (1980) reamination only occurs in muscle fibres that are no longer contracting.

In 1986, Flanagan et al. investigated PNC function using similar techniques to those reported by Meyer and Terjung (1980). Both the studies of Flanagan et al. (1986)



(1) AMP deaminase

(2) Adenylosuccinate lyase

(3) Adenylosuccinate synthetase

Figure 5.7 The purine nucleotide cycle

and Meyer and Terjung (1980) aimed to investigate PNC operation by inhibiting the enzymes involved with reaminating IMP. These studies assumed that if the PNC was operating during exercise, inhibition of IMP reamination would result in a greater than normal accumulation of IMP in stimulated muscle. Flanagan et al. (1986) inhibited adenylosuccinate lyase with 5-amino-imidazolecarboxamide riboside, while Meyer and Terjung (1980) inhibited adenylosuccinate synthetase with Hadacin. Flanagan et al. (1986) found that after 10 minutes of _in situ_ stimulation (single pulses, 8 volts, 5 ms duration, 0.75 pulses per second) IMP accumulation increased significantly and rat gastrocnemius twitch tension was significantly reduced when the PNC was inhibited. Meyer and Terjung (1980) also observed an increased IMP accumulation when rat gastrocnemius muscles were stimulated for 30 minutes _in situ_ (6 volts, 0.1 ms duration, 5 Hz). In contrast to Flanagan et al. (1986), Meyer and Terjung (1980) reported no drug effect on gastrocnemius twitch tension during stimulation. Reasons for the conflict are unclear. Resolution of the conflict is important because an increased IMP accumulation observed after PNC inhibition indicates only that the PNC operates, but not whether it operates in contracting muscle fibres. Attenuation of twitch tension with inhibition of the PNC provides evidence that the PNC operates within active fibres.

Amino acid catabolism may also contribute to NH_3 production and may contribute to the continual rise in NH_3 concentration found in both human muscle and blood during steady state exercise (Graham et al. 1987). Graham et al. (1987) suggest that it is the catabolism of branched-chain amino acids which is most likely to contribute to NH_3 production during this type of exercise. Evidence obtained from isotope studies indicates that most of the amino groups from branched-chain amino acids are not released as free NH_3, but are transferred by a series of reactions to form alanine which then leaves the muscle cell (Wolfe et al. 1982; Wolfe et al. 1984). Evidence from these same studies indicates that a small proportion of the amino groups obtained from branched-chain amino acids is not transferred to alanine and therefore may end up as free NH_3. Oxidative deamination of glutamate, catalysed via glutamate dehydrogenase, has also been regarded as a major NH_3 producing reaction in skeletal muscle (Newsholme and Leech, 1983). The direction in which this enzyme works during muscle contraction is not known. Skeletal muscle has biochemical pathways involving the catabolism of other amino acids (e.g. glycine, threonine and asparagine) which can produce NH_3 (Goodman and Ruderman, 1982; Haralambie and Mössinger, 1980). Very little is known about the activation of these pathways during exercise and as such the contribution of these amino acids to muscle NH_3 production is also unknown.

CHAPTER 6

THE RELATIONSHIP BETWEEN PLASMA AMMONIA, BLOOD LACTATE AND MUSCLE FIBRE TYPE

6.1 Introduction

The absence of a positive relationship normally found between blood NH_3 and blood lactate (LA) after intense, short term exercise has been used as an aid in identifying AMP deaminase deficiency (Fishbein et al. 1978; Sinkeler et al. 1986), McArdle's disease (Haller et al. 1985; Sinkeler et al. 1986) and idiopathic hypoparathyroidism (Hara et al. 1987). The ability of such tests to aid in the diagnosis of these disorders is dependent upon a thorough understanding of non-clinical factors which can effect the NH_3-LA relationship. Existing evidence suggests that the NH_3-LA relationship is influenced by muscle fibre type (Dudley et al. 1983), exercise duration (Graham et al. 1987) and hyperoxia (Graham et al. 1987). An aim of the present chapter is to examine the relationship between plasma NH_3 and LA, during and after exercise (maximal and submaximal), in trained and untrained individuals.

Dudley et al. (1983) reported that blood NH_3 levels, measured after short term exhaustive exercise, were inversely related to the %ST muscle fibres. Based on this data Banister et al. (1985) suggested that peak changes in blood NH_3 concentration after such exercise tests may provide a relatively non-invasive estimate of human muscle fibre type composition. Recent evidence suggests that the relationship between muscle fibre type and blood NH_3 does not hold during less intense exercise performed for a prolonged period (Graham et al. 1987). This data indicates that various factors can effect the NH_3-fibre type relationship and suggests that this relationship may be an unreliable predictor of muscle fibre type. Training may effect the relationship between plasma NH_3 and muscle fibre type, however, very little information is available on this issue. This chapter aims to investigate the muscle fibre type - peak change in plasma NH_3 accumulation relationship in trained and untrained individuals.

6.2 Results

Nonsignificant correlation coefficients between peak LA and peak NH_3 accumulation were found for both untrained ($r=0.66$, $n=6$) and trained subjects after maximal exercise ($r=0.84$, $n=5$). When the data for both groups were pooled, a significant correlation and linear relationship was found ($r=0.84$, $n=11$) (Figure 6.1). Positive relationships were found between LA and NH_3 in both groups (trained: $r=0.73$, $n=30$; untrained: $r=0.82$, $n=28$)

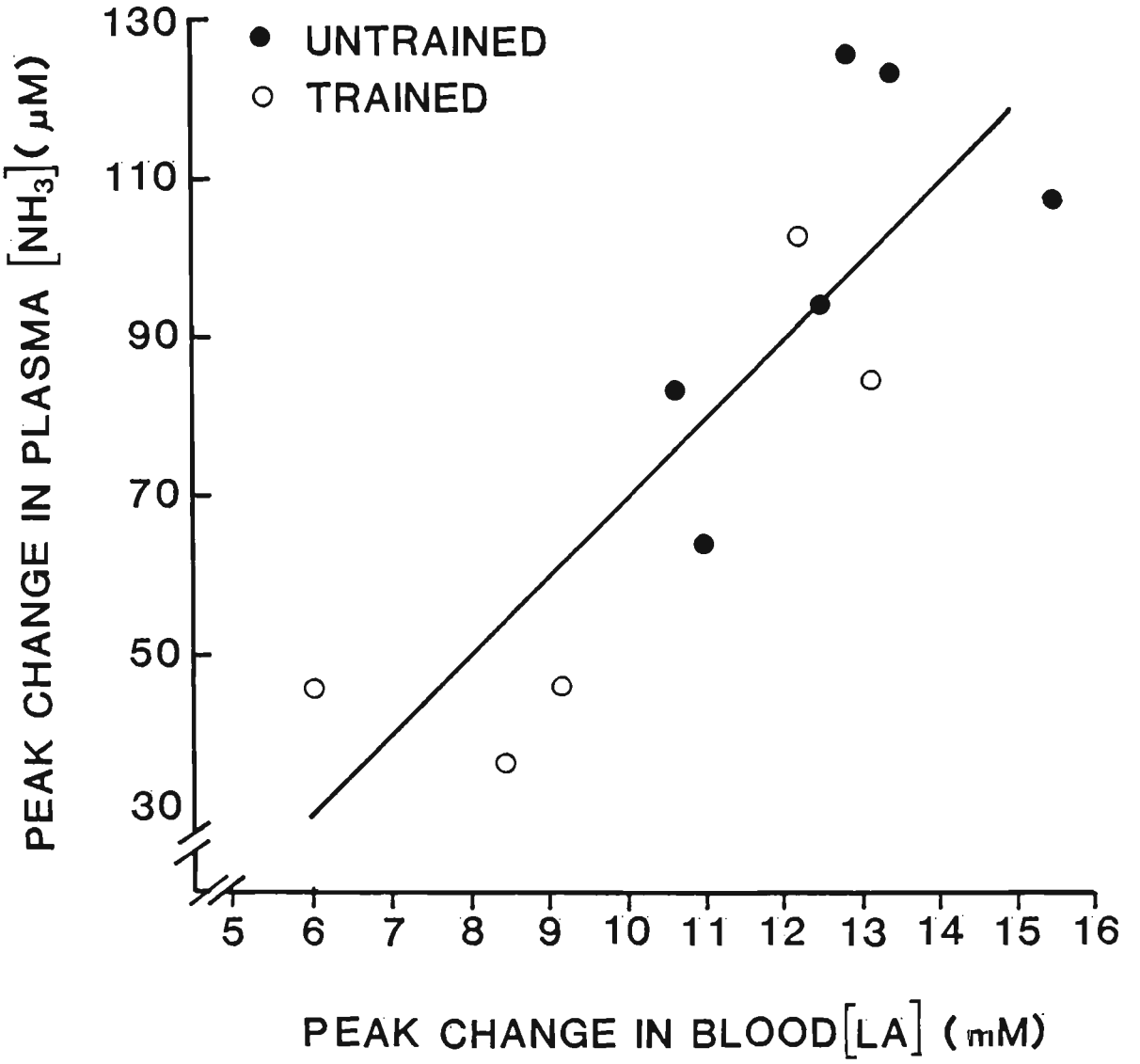


Figure 6.1 - The relationship between peak plasma ammonia accumulation $[NH_3]$ and peak blood lactate accumulation [LA] when the results for both trained and untrained individuals are pooled.

(Pooled results: $[NH_3] = 10.0\,[LA] - 30.3$, SEE = 18.1, $r = 0.84$, $n = 11$, $p < 0.05$).

during the submaximal exercise bout (Figure 6.2). The slopes of the linear regression equations relating plasma NH_3 accumulation and LA during the submaximal exercise bout were significantly different between the groups. For every unit increase in LA the trained group displayed twice the NH_3 accumulation of the untrained.

The relationship between peak plasma NH_3 accumulation and muscle fibre type (Figure 6.3) was linear in both groups. However, an inverse relationship between plasma NH_3 accumulation and muscle fibre type was found for the trained group ($r=-0.92$, $n=5$), while a positive relationship was found for the untrained individuals ($r=0.84$, $n=6$). These relationships should be interpreted with caution as the number of data points used to calculate both correlations was small. The slopes of the linear regression equations relating peak plasma NH_3 accumulation and %ST fibres for both groups were significantly different.

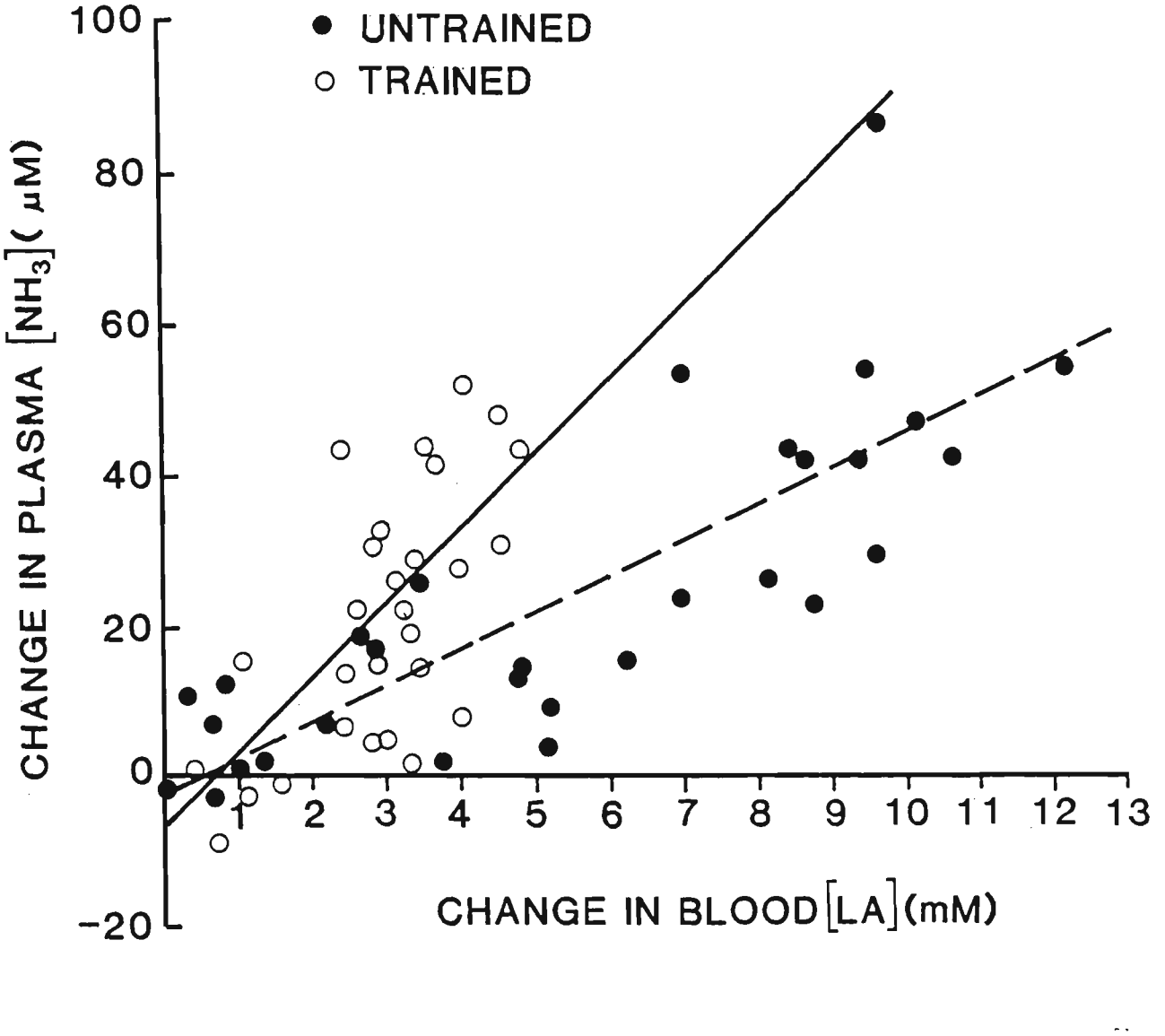


Figure 6.2 - The relationship between plasma accumulation [NH_3] and blood lactate accumulation [LA], for both trained and untrained individuals during submaximal exercise (≈ 85% $\dot{V}O_2max$).

Untrained: $[NH_3] = 4.84\,[LA] - 2.44$, SEE = 13, $r = 0.82$, $n = 28$, $p < 0.05$.

Trained: $[NH_3] = 9.89\,[LA] - 7.25$, SEE = 11.5, $r = 0.73$, $n = 30$, $p < 0.05$.

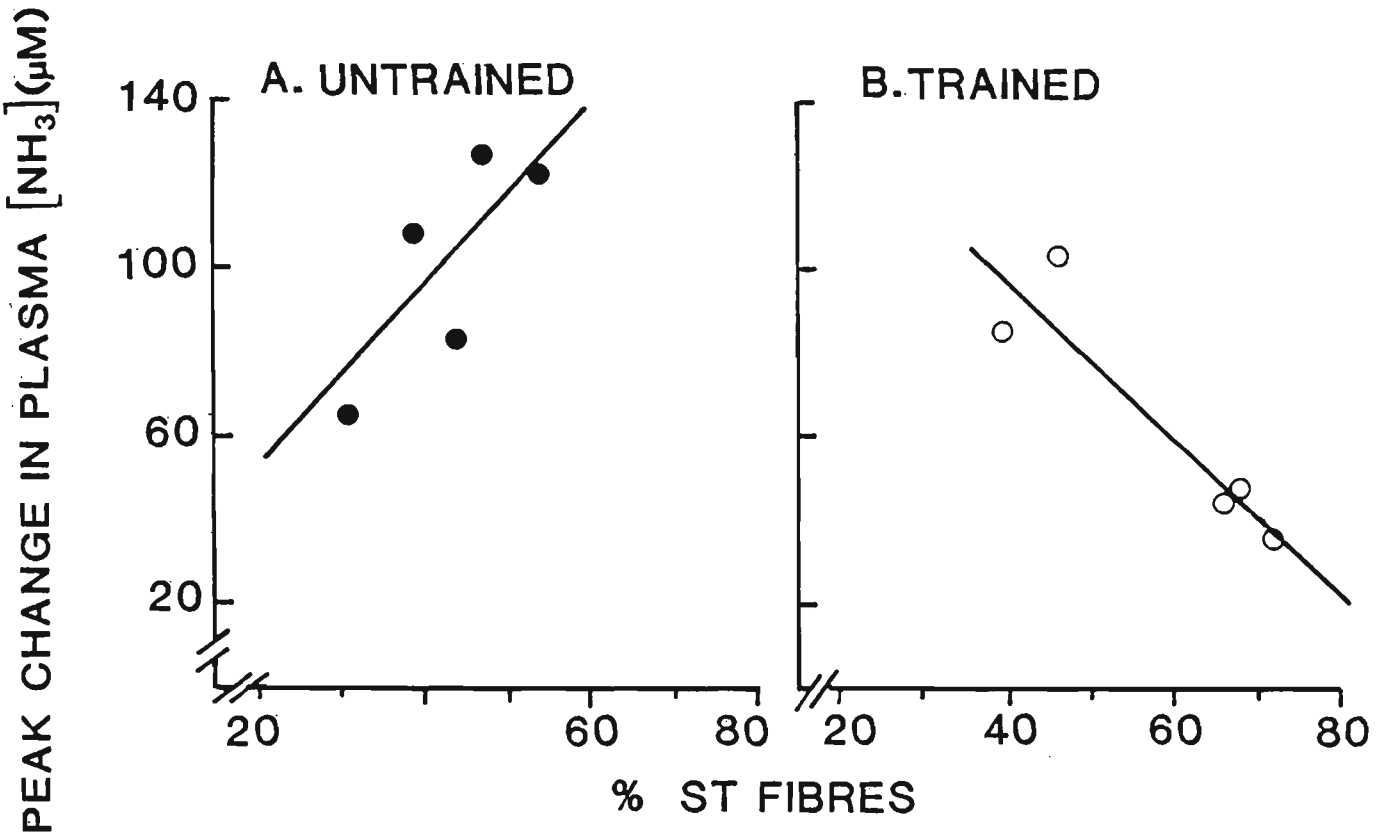


Figure 6.3 - The relationship between peak plasma ammonia accumulation [NH_3] and percentage slow twitch fibres (% ST) for both trained and untrained individuals.

Untrained: $[NH_3] = 12.24 + 2.11$ (% ST), SEE = 14.7, n = 6, r = 0.84, $p < 0.05$.

Trained: $[NH_3] = 170.4 - 1.84$ (% ST), SEE = 12.9, n = 5, r = 0.92, $p < 0.05$.

6.3 Discussion

6.3.1 The Relationship Between Plasma Ammonia and Blood Lactate

Previous work has demonstrated a positive linear relationship between NH_3 and LA concentration in blood during and after short duration, high intensity exercise (Babij et al. 1983; Buono et al. 1984; Dudley et al. 1983; Ericksson et al. 1985; Fishbein et al. 1978; Lo and Dudley, 1987). The present study found a positive relationship between peak NH_3 accumulation and peak LA accumulation after maximal exercise. Unfortunately, a significant relationship was only found when the data for both groups were pooled. This finding probably reflects a problem of sample size. As a result of this limited data it is impossible to ascertain the effects training may or may not have on the plasma NH_3-blood LA relationship.

A positive relationship between blood LA and plasma NH_3 was found for both groups during the 15 minute submaximal exercise bout. This is consistent with previously published results (Graham et al. 1987). It is known that training decreases the rate of LA efflux from muscle (Henriksson, 1977; Saltin et al. 1976) and increases the rate of LA removal from blood (Donovan and Brooks, 1983) during exercise. These factors alone may be sufficient to explain the differences in the blood LA-plasma NH_3 relationship observed between the groups in

the present study. As previously mentioned, training may also increase the rate of muscle NH_3 efflux and plasma NH_3 removal, however little, if any, research has investigated this possibility. Future research needs to establish whether the difference in the slope of the blood LA-plasma NH_3 relationship observed between the groups is the result of training per se, and not other factors such as muscle fibre type.

A number of researchers have attempted to explain the connection between NH_3 and LA. Some authors believe that muscle NH_3 production may be the trigger that causes increased muscle lactate production (Banister et al. 1983; Buono et al. 1984; Mutch and Banister, 1983), while others believe that the production of lactate leads to changes in cellular conditions which promote NH_3 production (Dudley and Terjung, 1985b; Terjung et al. 1986). It is unlikely that lactate causes NH_3 production, or vice versa, because either metabolite can be produced in the absence of the other. For example Dudley and Terjung (1985b) inhibited lactic acid production with iodoacetic acid before electrically stimulating rodent muscle and found an accelerated production of IMP when compared to normal muscle. IMP production is linked stoichiometrically to NH_3 production via the AMP deaminase reaction (Lowenstein, 1972). Therefore, the accelerated production of IMP found by Dudley and Terjung (1985b) implies that the production rate of NH_3 was also increased. Similarly, studies investigating McArdle's disease (i.e.,

phosphorylase deficient muscle) have found an abnormally high blood NH_3 concentration during exercise (presumably caused by an increased efflux from exercising skeletal muscle) in the absence of muscle lactic acid production (Haller et al. 1985; Lewis and Haller, 1986). It is also unlikely that NH_3 causes the production of lactic acid. Dudley and Terjung (1985a) reported that lactic acid accumulation occurred in stimulated muscle before NH_3 levels increased. Similarly, AMP deaminase deficient individuals, who are incapable of producing large quantities of NH_3 during short duration exercise, display normal post-exercise blood LA levels (Sinkeler et al. 1986).

Recent evidence suggests the production of the two metabolites during intense exercise are linked via intramuscular AMP concentration (Katz et al. 1986b). An increase in AMP is known to be a potent stimulator of both phosphofructokinase (Hultman and Sahlin, 1980), the rate limiting enzyme in glycolysis, and AMP deaminase (Dudley and Terjung, 1985b). For this reason Katz et al. (1986b) argue that lactate and NH_3 tend to be produced, in normal muscle, at similar times.

As previously mentioned there is considerable evidence that blood NH_3 and LA accumulation are positively related, however it appears that this relationship is only found in certain circumstances. Factors that may alter, or totally uncouple, the positive relationship between plasma NH_3 and blood LA include

muscle fibre type, exercise duration and hyperoxia (Dudley et al. 1983; Graham et al. 1987). In the present study blood LA and plasma NH_3 were related during the 15 minute exercise bout, however, there was a tendency for this relationship to disappear toward the end of the bout, especially in the trained group (Figure 6.4). Extending the exercise time of the trained group beyond 15 minutes would have probably seen the disappearance of the positive blood LA-plasma NH_3 relationship. That an uncoupling of the positive relationship between blood LA and plasma NH_3 can occur is not surprising, as concentrations of these metabolites are not only dependent upon muscle production rates, but are also dependent upon rates of muscle efflux and rates of uptake by other tissues. Any imbalance in the way NH_3 and LA are produced, released, and removed will lead to a reduction in the correlation between the two metabolites. Recent evidence suggests that imbalances in NH_3 and LA metabolism can exist. For example lactate concentration has been observed to increase before NH_3 in electrically stimulated rodent skeletal muscle (Dudley and Terjung, 1985a). Ericksson et al. (1985) found that human splanchnic uptake of NH_3 was unaltered during exercise, while LA uptake was significantly increased. In preliminary trials, Graham and colleagues (personal communication) have found that muscle lactate efflux decreases to basal levels during prolonged exercise while muscle NH_3 efflux is maintained.

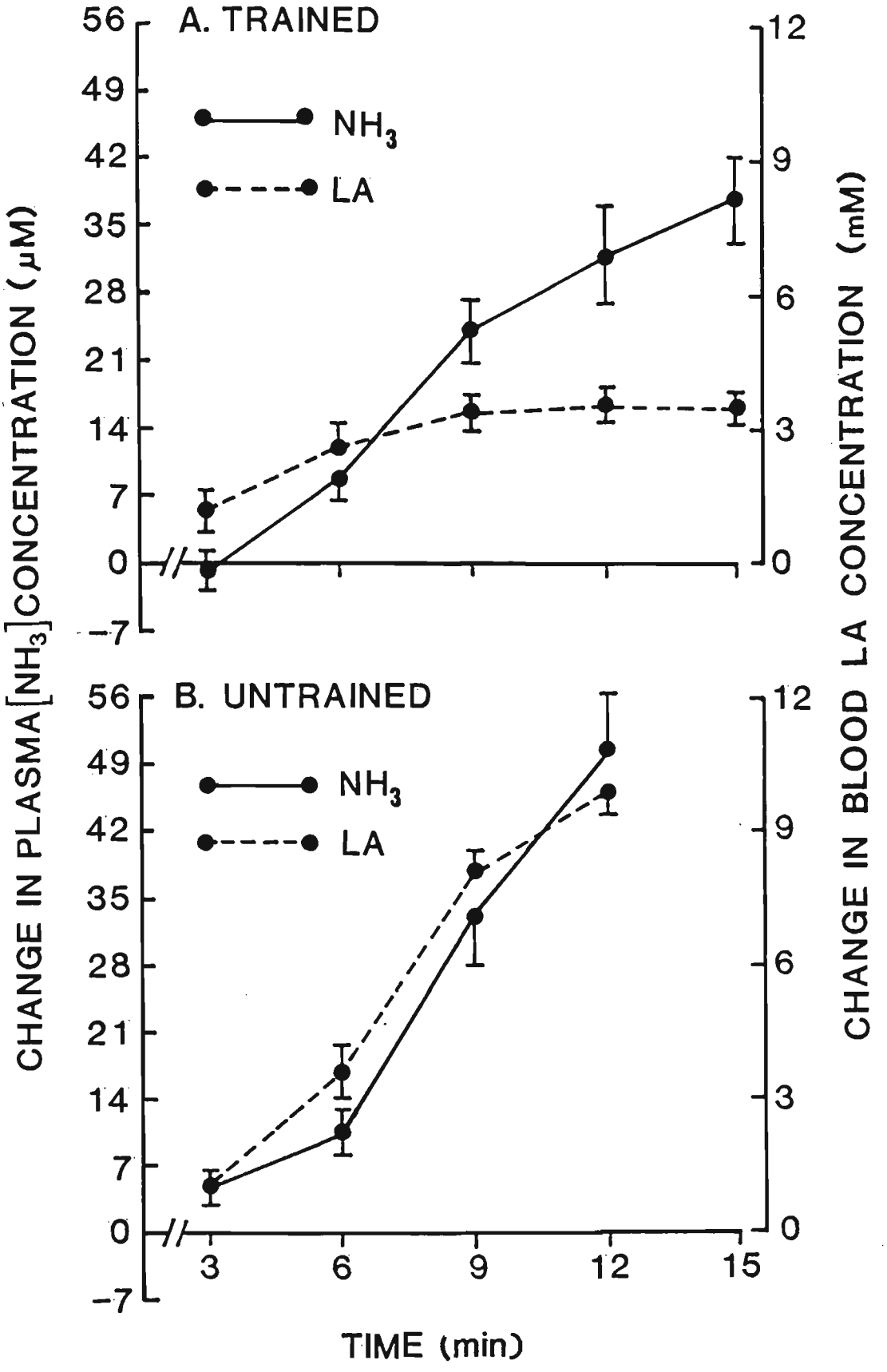


Figure 6.4 - The change in plasma [NH_3] and blood [LA] concentration for trained and untrained individuals during intense cycling.

(≈85% $\dot{V}O_2$ max).

6.3.2 The Relationship Between Plasma Ammonia and Muscle Fibre Type

Methodological differences may explain the discrepancy between the results of this study and those of Dudley et al. (1983). Dudley et al. (1983) determined the change in blood NH_3 from blood samples taken immediately post-exercise. In the present study, the relationship between fibre type and NH_3 was calculated using peak NH_3 values, which for the untrained subjects, occurred 5 minutes post-exercise.

The relationship between %ST fibres and NH_3 in the present study was re-calculated using NH_3 values obtained from plasma samples drawn immediately prior to exhaustion. This analysis revealed no relationship between the two variables for the untrained group ($r=0.46$, $n=6$) and a significant inverse relationship for the trained ($r=-0.91$, $n=5$). The change in NH_3 sample time significantly affected the relationship between muscle fibre type and NH_3 for the untrained group. This indicates that the timing of blood sampling is important when establishing relationships.

Data from the present study (using NH_3 values obtained immediately prior to exhaustion) and that of Dudley and his colleagues (1983) were used to determine the relationship between the two variables (NH_3 and %ST muscle fibres) when the fibre type range of the two subject populations were similar (i.e., 25 to 55 %ST fibres). Data from Dudley et al. (1983) were obtained by interpolation

from Figure 1 in their publication. This analysis revealed no significant relationship between %ST fibres and NH_3 in either the present study ($r=0.46$, $n=6$), or in the work conducted by Dudley et al. ($r=0.39$, $n=6$). This indicates that when groups with similar training status and fibre types are analysed similar results are found.

Evidence that the relationship between muscle fibre type and peak NH_3 accumulation differed according to training status is worthy of further examination. The data indicate that ST fibres are a significant contributor to peak plasma NH_3 accumulation in untrained individuals. However, in trained individuals ST fibres may make only a small contribution to plasma NH_3 accumulation. This finding is in conflict with the claim, based upon research using rodent skeletal muscle (Meyer and Terjung, 1979), that ST fibres do not produce significant quantities of NH_3. However, several studies (Graham et al. 1987; Jansson et al. 1987) have reported evidence which indicates that human ST muscle can produce NH_3. The different relationship between peak NH_3 accumulation and %ST fibres displayed by the two groups may be explained by differences in ST muscle NH_3 production and muscle NH_3 efflux. During intense exercise, trained human ST muscle probably produces NH_3 in small quantities (presumably via amino acid catabolism or 5'-nucleotidase / adenosine deaminase pathway) and therefore function more like rodent ST muscle (Meyer and Terjung, 1979). As a result trained FT fibres are the only fibres producing large quantities of NH_3

(via AMP deaminase pathway), hence the inverse relationship found for the trained group. In contrast, untrained human ST fibres produce significant quantities of NH_3 (mainly via AMP deaminase activity) during intense exercise (Janssen et al. 1987). The NH_3 produced in ST fibres may enter the blood more readily during and after exercise because the pH ratio between these fibres and blood is relatively high due to a comparatively low rate of lactic acid accumulation in these fibres (Essén and Häggmark, 1975; Ivy et al. 1987; Tesch, 1980). NH_3 produced in ST fibres may also efflux readily because ST blood flow is relatively large (Frisk-Holmberg et al. 1981) and the diffusion distance between ST fibres and capillaries is relatively small (Andersen, 1975). Undoubtedly, untrained FT fibres will also produce NH_3 during intense exercise. FT fibre NH_3 production rates will probably exceed ST muscle (Jansson et al. 1987), however, rates of NH_3 efflux from FT fibres may not exceed ST fibre rates during recovery because of an unfavourable pH gradient, a relatively small blood flow and relatively large diffusion distances (Andersen, 1975; Essén and Häggmark, 1975; Frisk-Holmberg et al. 1981; Tesch, 1980). As a result peak NH_3 accumulation is positively related to ST fibre type in untrained individuals after maximal exercise.

CHAPTER 7

AMMONIA AND LACTATE BREAK POINT AND TRAINING STATUS

7.1 Introduction

NH_3 has been observed to accumulate in plasma (or blood) at various work intensities (i.e., 35-70 $\%\dot{V}O_2$ max) (Babij et al. 1983; Buono et al. 1984; Ericksson et al. 1985; Katz et al. 1986a; Wilkerson et al. 1977). The wide variation reported for the relative workload at which NH_3 begins to accumulate (NH_3 break point - NH_3 BP) has been attributed, in part, to testing procedures and, in part, to individual human variability (Banister et al. 1985). Training status is an important factor in determining individual variability and may also be important in determining the intensity of work at which the NH_3 BP occurs. It is the intention of this chapter to examine the effects of training status on the NH_3 BP.

This chapter also intends to examine the relationship between the NH_3 BP and the blood lactate breakpoint (LABP) for trained and untrained subjects, respectively. Buono et al. (1984) reported that the NH_3 BP and LABP of untrained individuals was positively related. The NH_3 BP-LABP relationship has not been determined for trained individuals.

7.2 Results

The oxygen consumption ($l.min^{-1}$) corresponding to the NH_3 BP did not differ between the groups (Table 7.1). There was a tendency for the trained subjects to have a lower NH_3 BP expressed as $\%\dot{V}O_2$ max when compared to the untrained, however this difference was not significant ($t=-1.25$, $n=12$, $p>0.2$). The $\dot{V}O_2$ ($l.min^{-1}$) at which

Table 7.1: NH_3 BP and LABP of trained and untrained individuals (All values means ± SE of mean)

	NH_3 BP ($\dot{V}O_2$ $l.min^{-1}$)	NH_3 BP ($\%\dot{V}O_2$ max)	LABP ($\dot{V}O_2$ $l.min^{-1}$)	LABP ($\%\dot{V}O_2$ max)
Trained (n=5)	2.18 ±0.23	43.3 ±7.91	2.84 ±0.23 *	55.3 ±3.52
Untrained (n=7)	2.15 ±0.21	55.5 ±5.93	1.79 ±0.11	46.3 ±3.95

* Significant difference between groups ($p<0.05$)

the LABP occurred was significantly higher in the trained group ($t=4.56$, $n=12$), however when the LABP was expressed as $\%\dot{V}O_2$ max no difference between the groups was found ($t=1.61$, $n=12$, $0.2>p>0.1$). The $\dot{V}O_2$ ($l.min^{-1}$) at which the NH_3 BP and LABP occurred was not statistically different in either group (trained: paired $t=1.18$, $p>0.2$; untrained: paired $t=1.5$, $0.2>p>0.1$).

In the trained group, the NH_3 BP ($l.min^{-1}$) was inversely related to the LABP ($r=-0.90$, $n=5$, $p<0.05$) while no relationship between the two variables was found for the untrained ($r=0.32$, $n=7$) (Figure 7.1). Admittedly, a small number of data points were used to construct the LABP-NH_3 BP relationships for both groups, therefore interpretation of these relationships must be viewed with caution. In an attempt to overcome this problem the data for both groups were pooled. This analysis found no significant relationship between the two break points ($r=-0.30$, $n=12$) (Figure 7.2). No relationship between the NH_3 BP ($l.min^{-1}$) and %ST fibres was found even when the data was pooled (pooled data; $r=-0.07$, $n=11$) (Figure 7.3).

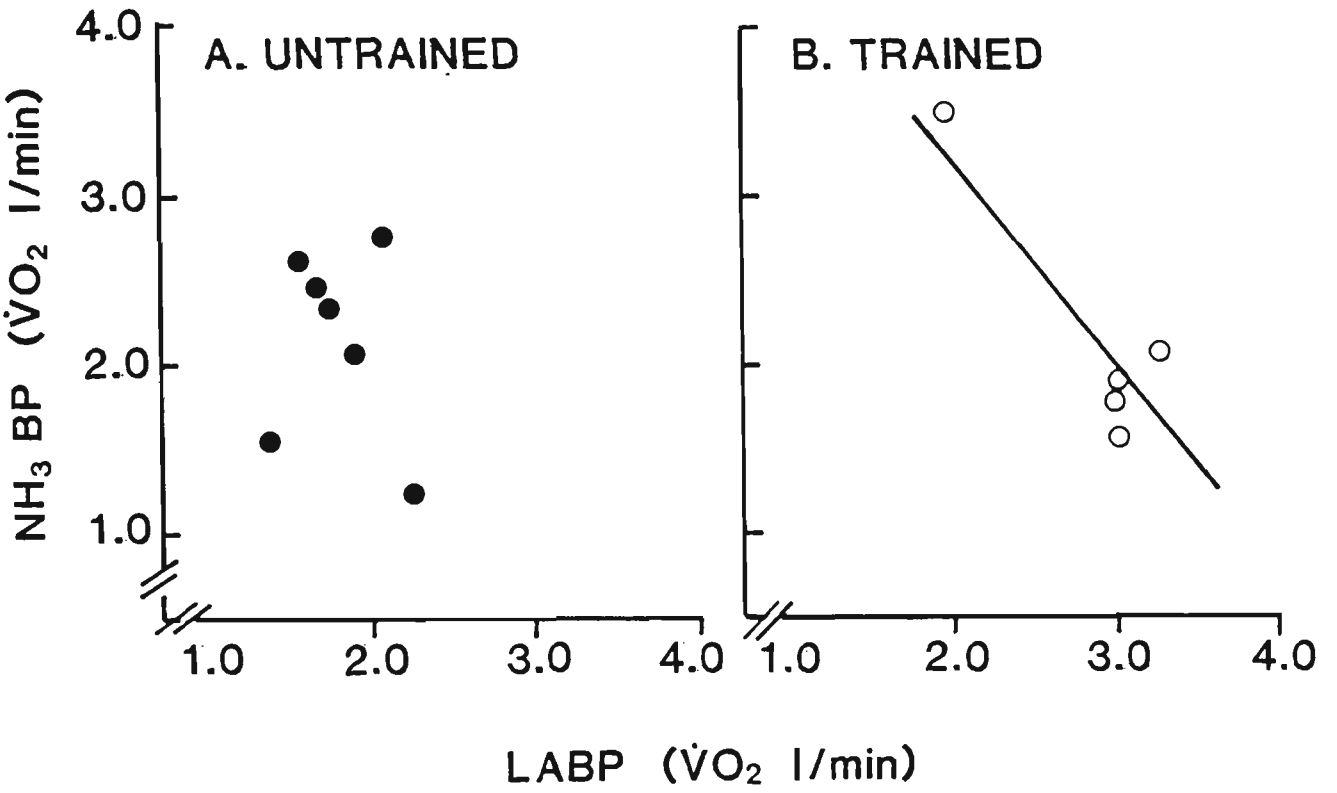


Figure 7.1 - The relationship between the ammonia breakpoint (NH_3BP) and lactate breakpoint (LABP) in trained and untrained individuals.

(Untrained: $NH_3BP = 1.83 - 0.021$ (LABP), $r = 0.04$, SEE = 0.32, $n = 7$, $p > 0.05$

Trained: $NH_3BP = 6.01 - 1.35$ (LABP), $r = -0.90$, SEE = 0.39, $n = 5$, $p < 0.05$).

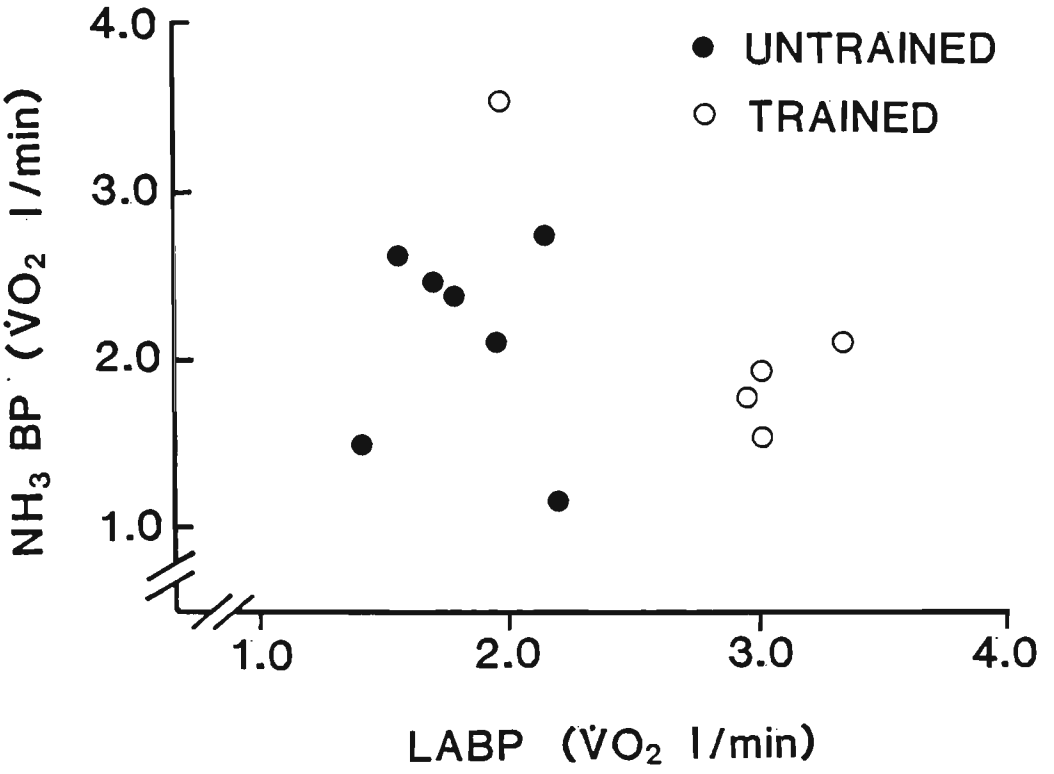


Figure 7.2 - The relationship between the ammonia breakpoint (NH_3BP) and lactate breakpoint (LABP) when the results for both trained and untrained individuals are pooled.

(Pooled results: $NH_3BP = 2.91 - 0.32$ (LABP), $r = -0.30$, SEE = 0.66, $n = 12$, $p > 0.05$).

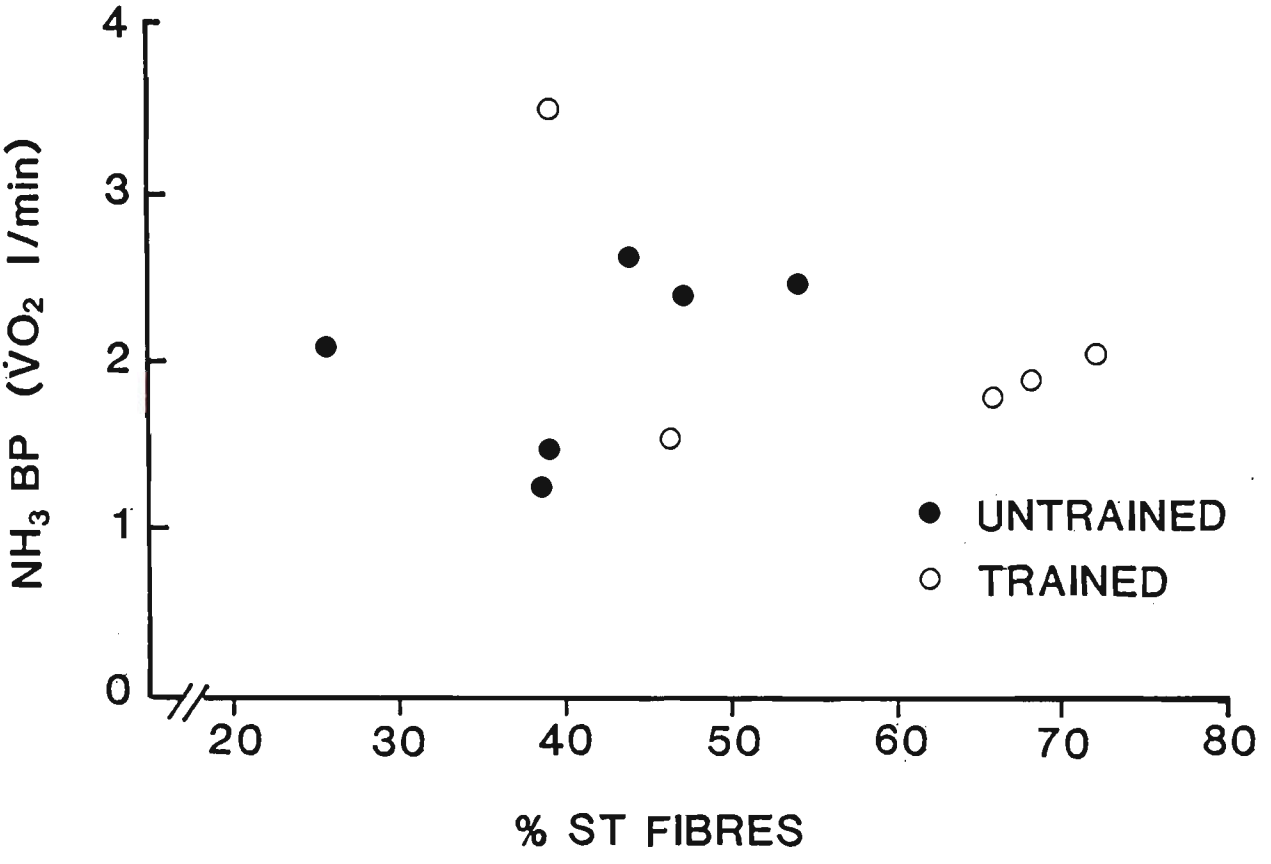


Figure 7.3 - The relationship between the ammonia breakpoint (NH_3BP) and muscle fibre type (% ST fibres) when the results for both trained and untrained individuals are pooled.

(Pooled results: $NH_3BP = 2.27 - 0.003$ (% ST), $r = -0.07$, SEE = 0.66, $n = 11$, $p > 0.05$).

7.3 Discussion

7.3.1 Ammonia Breakpoint and Training

The oxygen consumption (absolute and relative) at which the NH_3 BP occurred was not different between the two groups (Table 7.1). The mean NH_3 BP (absolute and relative) reported for the untrained subjects in this study are in close agreement to those reported by Buono et al. (1984) (i.e., 1.96 $\dot{V}O_2$ $l.min^{-1}$ or 58.6% $\dot{V}O_2$max). The NH_3 BP of trained athletes has not been determined previously.

As previously defined (page 71), the NH_3 BP is the exercise intensity at which the rate of NH_3 entering the blood begins to rapidly exceed the rate at which NH_3 leaves the blood. In the present study the NH_3 BP's of both groups were similar indicating that training may not alter the NH_3 BP. Definitive conclusions in this regard must await longitudinal training studies. Failure to observe a training effect on the NH_3 BP may indicate that muscle NH_3 production is similar at moderate work intensities. Alternatively, NH_3 production at moderate work intensities may be reduced by training and yet have very little effect on NH_3 BP values. An increased NH_3 efflux from trained muscle, or a training-induced decrease in plasma NH_3 removal rate may prevent NH_3 BP changes despite reductions in muscle NH_3 production. It is unlikely that plasma NH_3 removal rate will be decreased by training for reasons that have been detailed previously

(see pages 103 and 106). Efflux of NH_3 from trained muscle may increase, despite a decrease in NH_3 production, via two possible mechanisms. First, NH_3 efflux may be increased by a decreased intramuscular ALA synthesis from GLU and pyruvate (Palaiologos and Felig, 1976). Very little, if any, data are available on the effects of endurance training on muscle ALA production, therefore, this avenue of increasing NH_3 efflux is purely speculative. The second method by which training could increase NH_3 efflux rates in the face of reduced NH_3 production is via alterations in the muscle-blood pH ratio. If appropriate changes in the muscle-blood pH ratio occur at moderate work intensities, less of the total NH_3 produced will be trapped in muscle. As previously stated, untrained muscle is likely to have a greater proportion of the total NH_3 concentration trapped within muscle at exhaustion (i.e., approximately 33% more) because untrained individuals display a lower muscle-blood pH ratio than trained athletes (see page 101). If training alters the muscle-blood pH ratio during moderate exercise intensities, then there would be evidence to support the proposition that NH_3 efflux may increase with training (i.e. at a given muscle NH_3 concentration a greater proportion can leave the cell). The results of the present study indirectly suggest that training may alter the muscle-blood pH ratio allowing NH_3 to leave conditioned muscle fibres more easily. The trained individuals accumulated LA in the blood at significantly higher absolute workloads than the

untrained subjects. This finding suggests that significant muscle LA accumulation also occurred in untrained individuals at lower absolute workloads when compared to the trained group. As a result, intramuscular acidosis may be greater in the untrained at lower absolute workloads thereby retarding NH_3 efflux.

Although indirect evidence suggests that training may alter the muscle-blood pH ratio at moderate exercise intensities direct pH measurements indicate that this may not be the case. Sjogaard et al. (1985) determined the pH ratio between mixed muscle and femoral venous blood during moderately intense exercise (i.e., one-legged dynamic knee extensions at a workload demanding 55% of the knee extensor $\dot{V}O_2$ max) in six male subjects. Training status of the subjects was not reported. At an exercise intensity of 55% $\dot{V}O_2$ max no change in the muscle-blood pH ratio was seen when compared to resting values (i.e. rest = 0.965, exercise = 0.969). Their failure to observe a change in the muscle-blood pH ratio during exercise when compared to rest gives strong, but not definitive evidence, that training will not increase NH_3 efflux by altering the pH ratio at moderate work intensities. More definitive evidence requires the determination of training effects on the pH gradients across the membranes of single muscle fibres after moderate exercise. Research of this type has not been published.

Catabolism of AMP is unlikely to be the source of NH_3 associated with the NH_3 BP as previous studies have reported that both ATP concentrations (Katz, 1986; Katz et

al. 1986a, Rusko et al. 1986; Taylor et al. 1986), and the total adenine nucleotide pool (Katz et al. 1986a; Sahlin et al. 1978) do not decrease significantly at low exercise intensities (i.e., 50% $\dot{V}O_2$max). Additionally, the purine nucleotide cycle is unlikely to be functional at work intensities less than 50% $\dot{V}O_2$max (Katz et al. 1986a) thereby limiting the amount of NH_3 that could be produced without stoichiometric decreases in muscle ATP concentration (see page 109).

If AMP catabolism is not the source of NH_3 at the NH_3BP then amino acid catabolism is the only reasonable alternative. Amino acid catabolism can occur at low exercise intensities (i.e., 30% $\dot{V}O_2$max) and may increase with increasing work rates (Haralambie and Mössinger, 1980; Henderson et al. 1985; White and Brooks, 1981; Wolfe et al. 1982). It must be noted that endurance training increases skeletal muscle amino acid catabolism and the activity of various enzymes associated with this process (Askew et al. 1979; Dohm et al. 1977; Henderson et al. 1985; Holloszy et al. 1970; Yarasheski and Lemon, 1983). A training-induced increase in amino acid catabolism may lead to an increased NH_3 production. Logically, any increase in NH_3 production would tend to reduce the work rate at which the NH_3BP occurs. Although the present study did not observe a training-induced reduction in the NH_3BP, as would be expected if amino acids were the source of NH_3 at the NH_3BP, there was a tendency for the trained individuals to display a lower NH_3BP when expressed as %$\dot{V}O_2$max.

Although there is indirect evidence which suggests that amino acid catabolism can contribute to NH_3 production (Haralambie and Mössinger, 1980; Holloszy et al. 1970; Lowenstein and Goodman, 1978), definitive evidence is still lacking. Future research needs to address this issue.

7.3.2 Lactate and Ammonia Break Points

Buono et al. (1984) reported that the NH_3 BP and LABP occurred at similar oxygen consumptions (absolute) for untrained individuals. They also reported that their was a positive relationship between the two breakpoints ($r=0.96$, $n=6$, $p<0.05$). The present study also found the VO_2 at which the NH_3 BP and the LABP occurred were nonsignificantly different for both the trained and untrained groups, respectively. However, in contrast to Buono et al. (1984), the present study found no relationship between the LABP and the NH_3 BP in the untrained subjects and a significant inverse relationship between the two break-points for the trained group. Admittedly, the LABP-NH_3 BP relationships reported in the present study could be criticised on the basis of insufficient data. Nevertheless, when the data for both groups was pooled the poor correlation found ($r=-0.3$, $p>0.05$) in the present study is still inconsistent with the significant positive relationship published by Buono et al.

(1984). It would appear from the data presented that the two break points are either not related, or in the case of the trained subjects negatively related. The negative relationship found between the LABP and the NH_3 BP for the trained subjects needs to be confirmed by further research.

Methodological differences may explain why the relationship for the untrained group differed from that previously reported. In the present study the protocol for the maximal exercise test involved 50 watt increments every four minutes, with blood sampling occurring toward the end of each work stage (steady-state protocol). Buono et al. (1984) used an exercise protocol in which workloads were increased 25 watt every minute with blood being sampled at the end of each minute (ramp protocol). Previous research has shown that the LABP, determined using venous blood, is dependent upon the exercise protocol used to determine it (Yoshida, 1984). Investigations into the effects of differing protocols on the NH_3 BP have yet to be conducted. The NH_3 BP, like the LABP, may be influenced by different testing procedures. The factors that determine the NH_3 BP (i.e., rates of amino acid catabolism, intramuscular alanine synthesis, NH_3 uptake from plasma by various organs and tissues) probably have more time to exert their influence on plasma NH_3 accumulation during "steady-state" rather than "ramp" exercise tests. As a consequence the "steady-state" test may highlight the different physiological treatments of LA and NH_3, and

this may explain why the two break-points were not correlated.

CHAPTER 8

CONCLUSIONS

Skeletal muscle produces ammonia (NH_3) during exercise. Potentially, NH_3 may be produced via three biochemical pathways. These include the AMP deaminase pathway, the 5'-nucleotidase / adenosine deaminase pathway, and various pathways involved with amino acid catabolism. Results from previous investigations suggest that the AMP deaminase pathway is utilized more readily in fast twitch muscle fibres and is the major pathway of ammonia production during intense exercise. On the other hand, the other potential NH_3 producing pathways occur more readily in slow twitch fibres and may be responsible for NH_3 production during low intensity exercise. With increasing exercise intensity both the NH_3 concentration of muscle and plasma rise significantly.

Endurance training has resulted in a reduction of both muscle and blood NH_3 concentration in animals during exercise at the same absolute intensity. In humans, endurance training has produced equivocal results during exercise at the same absolute intensity, while no change in plasma NH_3 accumulation has been observed during exercise at the same relative intensity. As the effects of endurance training on human plasma NH_3 accumulation are unclear further investigation appears to be warranted. The effects of endurance training on other important areas of NH_3

metabolism have not been examined. Therefore the purpose of this study was to investigate the effects of endurance training on: 1) plasma NH_3 accumulation during exercise at similar absolute and relative work intensities; 2) plasma NH_3 accumulation during recovery from maximal work; 3) the absolute and relative work intensities at which blood lactate (LA) and plasma NH_3 concentrations begin to rapidly accumulate (i.e., the LA breakpoint - LABP, and NH_3 breakpoint - NH_3 BP); 4) the relationship between the LABP and NH_3 BP; and 5) the relationship between plasma NH_3, blood LA, and muscle fibre type.

Six enduranced trained male cyclists and seven active untrained males performed two exercise tests, each on a separate occasion. On the first occasion, each subject underwent an incremental, exhaustive exercise test. This test involved 4 minutes of upright, rested sitting, 4 minutes of unloaded pedalling, and there after, workload increments of 50 watt every 4 minutes to exhaustion. During the final minute of each 4 minute period and during the final minute of exercise, venous blood was sampled and oxygen uptake ($\dot{V}O_2$) and heart rate (HR) were determined. Metabolic data collection and blood sampling continued during the second, fifth, tenth and twentieth minute of recovery. On the second occasion each subject was required to ride for up to 15 minutes at a workload that would elicit 85% of each individuals $\dot{V}O_2$max. Collection of $\dot{V}O_2$, HR, and venous blood occurred after 3 minutes of rest and during every third minute of exercise. All venous

blood samples were analysed for blood lactate and plasma NH_3 concentration. Only blood samples drawn during the submaximal exercise test were used to determine plasma alanine (ALA) concentration. Muscle biopsies were taken from 11 of the original 13 subjects (5 trained and 6 untrained) approximately 3 months after the last exercise test in order to determine the %ST fibre type composition. Where appropriate, data was analysed using ANCOVA where the %ST fibres was used as a covariate.

Differences in activity patterns and physiological responses indicated that the groups differed in training status. For example, the $\dot{V}O_2$ max, OBLA (%$\dot{V}O_2$ max), and LABP ($\dot{V}O_2$ $l.min^{-1}$) were significantly greater in the trained group. During the incremental maximal exercise test, trained individuals were found to accumulate significantly less plasma NH_3 than untrained individuals at 300 watt. When group differences in %ST fibres were statistically controlled, trained individuals accumulated significantly less NH_3 at 250 and 300 watt. During the 15 minute exercise bout performed at 85% $\dot{V}O_2$ max, the trained subjects tended to have a lower accumulation of NH_3 at 3 minutes of exercise ($p=0.07$). However, when muscle fibre type was used as a covariate the tendency for group NH_3 values to be different at minute 3 disappeared ($p=0.31$). No differences in plasma ALA concentration were observed between the groups throughout the submaximal exercise test. Plasma NH_3 accumulation during recovery from maximal exercise was different between the groups. Five minutes

post maximal exercise plasma NH_3 accumulation was significantly greater in the untrained group. There was a strong tendency for this difference to remain significant even when muscle fibre type was statistically controlled ($p = 0.07$).

The NH_3 BP expressed either in absolute ($\dot{V}O_2$ $l.min^{-1}$) or relative terms (%$\dot{V}O_2$ max) was not significantly different across the groups. The NH_3 BP was inversely related to the LABP in the trained group ($r=-0.90$), while no significant relationship was found between the two variables in the untrained group ($r=0.32$). No relationship between the LABP and NH_3 BP was found when the data for both groups were pooled ($r=-0.30$). Nonsignificant correlation coefficients between peak blood LA and peak plasma NH_3 accumulation were found for both untrained ($r=0.66$) and trained subjects ($r=0.84$) after maximal exercise. However, significant positive relationships between blood LA and plasma NH_3 accumulation were found for both groups during the 15 minute submaximal exercise bout. The slopes of the linear regression equations relating plasma NH_3 and blood LA accumulation during the submaximal exercise bout were significantly different between the groups. For every unit increase in LA the trained group displayed twice the NH_3 accumulation of the untrained. Peak plasma NH_3 accumulation was inversely related to the %ST fibres in the trained group ($r=-0.92$). In contrast, a significant positive relationship between %ST fibres and peak plasma

NH_3 accumulation was found for the untrained subjects ($r=\emptyset.84$).

In conclusion, the results of this study indicate that NH_3 metabolism is different in trained athletes when compared to untrained individuals. Furthermore, these results suggest that endurance training alters NH_3 metabolism in exercising humans.

REFERENCES

Abrahams, S.L., and E.S. Younathan. Modulation of the kinetic properties of phosphofructokinase by ammonium ions. J. Biol. Chem. 246: 2464-2467, 1971.

Akedo, H., H. Nishihara, K. Shinkai, K. Komatsi, and S. Ishikawa. Multiple forms of human adenosine deaminase: I. Purification and characterization of two molecular species. Biochim. Biophys. Acta 276: 257-271, 1972.

Andersen, P. Capillary density in skeletal muscle of man. Acta Physiol. Scand. 95: 203-205, 1975.

Andersen, P., and J. Henriksson. Training induced changes in the subgroups of human type II skeletal muscle fibres. Acta Physiol. Scand. 99: 123-125, 1977.

Aragòn, J.J., and J.M. Lowenstein. The purine nucleotide cycle: Comparison of the levels of citric acid cycle intermediates with the operation of the purine nucleotide cycle in rat skeletal muscle during exercise and recovery from exercise. Eur. J. Biochem. 110: 371-377, 1980.

Aragòn, J.J., K. Tornheim, M.N. Goodman, and J.M. Lowenstein. Replenishment of citric acid cycle intermediates by the purine nucleotide cycle in rat skeletal muscle. Curr. Top. Cell. Reg.:18, 131-149, 1981.

Askew, E.W., G.J. Klain, J.F. Lowder, W.R. Wise. Influence of exercise on amino acid mobilization and oxidation. Med. Sci. Sports 11: 106, 1979 (abstract).

Astrand, P.O., and K. Rodahl. Textbook of Work Physiology New York, McGraw-Hill, 1977.

Babij, P., S.M. Matthews, and M.J. Rennie. Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. Eur. J. Appl. Physiol. 50: 405-411, 1983.

Baldwin, K.M., P.J. Campbell, and D.A. Cooke. Glycogen, lactate, and alanine changes in muscle fiber types during graded exercise. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 43: 288-291, 1977.

Banister, E.W., M.E. Allen, I.B. Mekjovic, A.K. Singh, B. Legge, and B.J.C. Mutch. The time course of ammonia and lactate accumulation in blood during bicycle exercise. Eur. J. Appl. Physiol. 51: 192-202, 1983.

Banister, E.W., W. Rajendra, and B.J.C. Mutch. Ammonia as an indicator of exercise stress: Implications of recent findings to sports medicine. Sports Med. 2: 34-46, 1985.

Barnes, R.H., B.A. Labadan, B. Siyamoglu, and R.B. Bradfield. Effects of exercise and administration of aspartic acid on blood ammonia in the rat. Am. J. Physiol. 207: 1242-1246, 1964.

Baumann, H., M. Jäggi, F. Soland, H. Howald, and M.C. Schaub. Exercise training induces transitions of myosin isoform subunits within histochemically typed human muscle fibres. Pflugers Arch. 409: 349-360, 1987.

Beaver, W.L., K. Wasserman, and B.J. Whipp. Improved detection of lactate threshold during exercise using a log-log transformation. J. Appl. Physiol.:Resp. Environ. Exercise Physiol. 59: 1936-1940, 1985.

Berg, A., E. Jakob, G. Huber, C. Anhert, and J. Keul. Influence of maximum aerobic stress and physical performance capacity on the behaviour of blood ammonia (NH_3). Int. J. Sports Med. 8: 140, 1987 (Abstract).

Bergström, J., P. Fürst, and E. Hultman. Free amino acids in muscle tissue and plasma during exercise in man. Clin. Physiol. 5: 155-160, 1985.

Bessman, S.P., and J.E. Bradley. Uptake of ammonia by muscle: its implications in ammoniagenic coma. N. Engl. J. Med. 253: 1143-1147, 1955.

Blomqvist, C.G., and B. Saltin. Cardiovascular adaptations to physical training. Ann. Rev. Physiol. 45: 169-189, 1983.

Blomstrand, E., and B. Ekblom. The needle biopsy technique for fibre type determination in human skeletal muscle -a methodological study. Acta Physiol. Scand. 116: 437-442, 1982.

Bloom, S.R., R.H. Johnson, D.M. Park, M.J. Rennie, and W.R. Sulaiman. Differences in the metabolic and hormonal response to exercise between racing cyclists and untrained individuals. J. Physiol. (Lond.) 258: 1-18, 1976.

Bockman, E.L., and J.E. McKenzie. Tissue adenosine content in active soleus and gracilis muscles of cats. Am. J. Physiol. 244 (Heart Circ. Physiol. 13) H552-H559, 1983.

Brooke, M.H., and K.K. Kaiser. Three "myosin adenosin-triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. J. Histochem. Cytochem. 18: 670-672, 1970.

Brooks, G.A. Anaerobic threshold: review of the concept and directions for future research. Med. Sci. Sports and Exercise 17: 22-31, 1985.

Bryla, J., and A. Niedzwiecka. Relationship between pyruvate carboxylation and citrulline synthesis in rat liver mitochondria: the effect of ammonia and energy. Int. J. Biochem. 10: 235-239, 1979.

Buono, M.J., T.R. Clancy, and J.R. Cook. Blood lactate and ammonium ion accumulation during graded exercise in humans. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 57: 135-139, 1984.

Burke, E.R., F. Cerny, D. Costill, and W. Fink. Characteristics of skeletal muscle in competitive cyclists. Med. Sci. Sports 9: 109-112, 1977.

Camici, M., G. Donadel, and P.L. Ipata. Properties and possible physiological role of 5'-nucleotidase from skeletal muscle. In Benzi, G., L. Packer, and N. Siliprandi (eds). Biochemical Aspects of Physical Exercise Elsevier Science Publishers, 1986, pp 497-489.

Cardelli-Cangiano, P., C. Cangiano, J.H. James, F. Ceci, J.E. Fischer, and R. Strom. Effect of ammonia on amino acid uptake by brain microvessels. J. Biol. Chem. 259: 5295-5300, 1984.

Casaburi, R., T.W. Storer, I. Ben-Dov, and K. Wasserman. Effect of endurance training on possible determinants of $\dot{V}O_2$ during heavy exercise. J. Appl. Physiol. 62: 199-207, 1987.

Chang, T.W. and A.L. Goldberg. The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. J. Biol. Chem. 253: 3685-3695, 1978.

Clausen, J.P. Effect of physical training on cardiovascular adjustments to exercise in man. Physiol. Rev. 57: 779-815, 1977.

Coffee, C.J., and C. Solando. Rat muscle 5'-adenylic acid aminohydrolase. J. Biol. Chem. 252: 1606-1612, 1977.

Consolazio, C.G., R.E. Johnson, and L.J. Pecora. Physiological Measurements of Metabolic Functions in Man. New York, McGraw-Hill, 1963.

Constable, S.H., R.J. Favier, J.A. Mclane, R.D. Fell, M. Chen, and J.O. Holloszy. Energy metabolism in contracting muscle: adaptation to exercise training. Am. J. Physiol. 253 (Cell Physiol. 22): C316-C322, 1987.

Deyl, Z., J. Hyanek, and M. Horakova. Profiling of amino acids in body fluids and tissues by means of liquid chromotography. J. Chromotogr. Biomed. Appl. 379: 177-250, 1986.

Dohm, G.L., A.L. Hecker, W.E. Brown, G.J. Klain, F.R. Puente, E.W. Askew, and G.R. Beecher. Adaptation of protein metabolism to endurance training. Increased amino acid oxidation in response to training. Biochem. J. 164: 705-708, 1977.

Donovan, C.M., and G.A. Brooks. Endurance training affects lactate clearance, not lactate production. Am. J. Physiol. 244 (Endocrinol. Metab. 7): E83-E92, 1983.

Dudley, G.A., and R.L. Terjung. Influence of aerobic metabolism on IMP accumulation in fast-twitch muscle. Am. J. Physiol. 248 (Cell Physiol. 17): C37-C42, 1985a.

Dudley, G.A., and R.L. Terjung. Influence of acidosis on AMP deaminase activity in contracting fast-twitch muscle. Am. J. Physiol. 248 (Cell Physiol. 17): C43-C50, 1985b.

Dudley, G.A., R.S. Staron, T.F. Murray, F.C. Hagerman, and A. Luginbuhl. Muscle fibre composition and blood ammonia levels after intense exercise. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 54: 582-586, 1983.

Durschlag, R.P., and R.J. Smith. Regulation of glutamine production by skeletal muscle in culture. Am. J. Physiol. 248 (Cell Physiol. 17): C442-C448, 1985.

Einspar, K.J., and G.D. Tharp. Plasma alanine concentrations in trained and non-trained humans at rest and after intense exercise. Med. Sci. Sports and Exercise 17: 232, 1985 (abstract).

Eriksson, L.S., S. Broberg, O. Björkman, and J. Wahren. Ammonia metabolism during exercise in man. Clin. Physiol. 5: 325-336, 1985.

Essén, B., and T. Häggmark. Lactate concentration in Type I and II muscle fibres during muscular contraction in man. Acta Physiol. Scand. 95: 344-347, 1975.

Felig, P. Amino acid metabolism in man. Ann. Rev. Biochem. 44: 933-955, 1975.

Felig, P. Amino acid metabolism in exercise. Ann. N. Y. Acad. Sci. 301: 56-63, 1977.

Felig, P., and J. Wahren. Amino acid metabolism in exercising man. J. Clin. Invest. 50: 2703-2714, 1971.

Fishbein, W.N., V.W. Armbrustmacher, and J.L. Griffin. Myoadenylate deaminase deficiency: A new disease of muscle. Sci. 200: 545-548, 1978.

Flanagan, W.F., E.W. Holmes, R.L. Sabina, and J.L. Swain. Importance of purine nucleotide cycle to energy production in skeletal muscle. Am. J. Physiol. 251 (Cell Physiol 20): C795-C802, 1986.

Frick, M., R. Elovainio, and T. Somer. The mechanism of bradycardia evoked by physical training. Cardiologia 51: 46-54, 1967.

Frisk-Holmberg, M., L. Jorfeldt, A. Juhlin-Dannfelt, and J. Karlsson. Leg blood flow during exercise in man in relation to muscle fibre composition. Acta Physiol. Scand. 112: 339-342, 1981.

Goldberg, A.L., and T.W. Chang. Regulation and significance of amino acid metabolism in skeletal muscle. Fed. Proc. 37: 2301-2307, 1978.

Goldstein, A. Biostatistics, an Introductory Text. New York: MacMillan, 1964.

Gollnick, P.D., R.B. Armstrong, B. Saltin, C.W. Saubert IV, W.L. Sembrowich, and R.E. Shepherd. Effect of training on enzyme activity and fibre composition of human skeletal muscle. J. Appl. Physiol. 34: 107-111, 1973.

Gollnick, P.D., R.B. Armstrong, C.W. Saubert IV, K. Piehl, and B. Saltin. Enzyme activity and fibre type composition in skeletal muscle of trained and untrained men. J. Appl. Physiol. 33: 312-319, 1972.

Goodman, M.N., and J.M. Lowenstein. The purine nucleotide cycle: Studies of ammonia production by skeletal muscle in situ and in perfused preparations. J. Biol. Chem. 252: 5054-5060, 1977.

Goodman, M.N., and N.B. Ruderman. Influence of muscle use on amino acid metabolism. Exercise Sport Sci. Rev. 10: 1-26, 1982.

Graham, T.E., P.K. Pederson, and B. Saltin. Muscle and blood ammonia and lactate responses to prolonged exercise with hyperoxia. J. Appl. Physiol. 63: 1457-1462, 1987.

Graham, T.E., and B. Saltin. Estimation of the mitochondrial redox state in human muscle during exercise. Can. J. Sport Sci. 13: 13P, 1988.

Green, H.J., H. Reidmann, and D. Pette. Fibre type transformations in the enzyme activity pattern of rat vastus lateralis muscle by prolonged endurance training. Pflugers Arch. 399: 216-222, 1983.

Haller, R.G., S.F. Lewis, M. Gunder, and M. Dennis. Ammonia production during exercise in McArdle's syndrome: An index of muscle energy supply and demand. Neurology 35: 207, 1985 (abstract).

Hara, N., I. Mineo, N. Kono, T. Shimizu, Y. Yamada, M. Kawachi, K. Suzuki, Y. Fukumuto, and S. Tarui. Enhanced release of ammonia and hypoxanthine from exercising muscles in patients with idiopathic hypoparathroidism. Muscle and Nerve 10: 599-602, 1987.

Haralambie, G., and M. Mössinger. Metabolites of the aminoacetone pathway in blood after exercise. Metabolism 29: 1258-1261, 1980.

Henderson, S.A., A.L. Black, and G.A. Brooks. Leucine turnover and oxidation in trained rats during exercise. Am. J. Physiol. 251 (Endocrinol. Metab. 12): E137-E144, 1985.

Henriksson, J. Training induced adaptations of skeletal muscle and metabolism during submaximal exercise. J. Physiol. (Lond.) 270: 661-675, 1977.

Henritze, J., A. Weltman, R.L. Schurrer, and K. Barlow. Effects of training at and above the lactate threshold on the lactate threshold and maximal oxygen uptake. Eur. J. Appl. Physiol. 54: 84-88, 1985.

Hermansen, L., and J.B. Osnes. Blood and muscle pH after maximal exercise in man. J. Appl. Physiol. 32: 304-308, 1972.

Hermansen, L., and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am. J. Physiol. 233 (Endocrinol. Metab. Gastrointest. Physiol. 2): E422-E429, 1977,

Hindfelt, B. The distribution of ammonia between extracellular compartments of the rat brain. Clin. Sci. Mol. Med. 48: 33-37, 1975.

Hindfelt, B., and B.K. Siesjo. The effect of ammonia on the energy metabolism of the rat brain. Life Sci. 9: 1021-1028, 1970.

Hintz, C.S., M.M.Y. Chi, R.D. Fell, J.L. Ivy, K.K. Kaiser, C.V. Lowry, and O.H. Lowry. Metabolite changes in individual rat muscle fibres during stimulation. Am. J. Physiol. 242 (Cell Physiol. 11) C218-C228, 1982.

Holloszy, J.O., and E.F. Coyle. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 56: 831-838, 1984.

Holloszy. J.O., L.B. Oscai, I.J. Don, and P.A. Molé. Mitochondrial citric acid cycle and related enzymes. Biochem. Biophys. Res. Comm. 40: 1368-1373, 1970.

Howanitz, J.H., P.J. Howanitz, C.A. Skrodzki, and J.A. Iwanski. Influences of specimen processing and storage conditions on results for plasma ammonia. Clin. Chem. 30: 906-908, 1984.

Hurley, B.F., J.M. Hagberg, W.K. Allen, D.R. Seals, J.C. Young, R.W. Cuddihee, and J.O. Holloszy. Effect of training on blood lactate levels during submaximal exercise. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 56: 1260-1264, 1984.

Hultman, E., and K. Sahlin. Acid-Base balance during exercise. Exercise Sport Sci. Rev. 41-128, 1980.

Ingjer, F. Effects of endurance training on muscle fibre ATPase activity, capillary supply and mitochondrial content in man. J. Physiol. (Lond.) 294: 419-432, 1979.

Ivy, J.L., M.M.Y. Chi, C.S. Hintz, W.M. Sherman, R.P. Hellendall, and O.H. Lowry. Progressive metabolite changes in individual human muscle fibers with increasing work rates. Am. J. Physiol. 252 (Cell Physiol. 21): C630-C639, 1987.

Jansson, E., G.A. Dudley, B. Norman, and P.A. Tesch. ATP and IMP in single human muscle fibres after high intensity exercise. Clin. Physiol. 7: 337-345, 1987.

Karlsson, J., and I. Jacobs. Onset of blood lactate accumulation during maximal exercise as a threshold concept I. Theoretical considerations. Int. J. Sports Med. 3: 190-201, 1982.

Katanuma, N., M. Okada, and Y. Nishii. Regulation of the urea cycle and TCA cycle by ammonia. Advances in Enzyme Regulation 4: G. Wever (Ed.), Pergamon Press, New York, 317-335, 1966.

Katz, A. Regulation of lactic acid production during muscle contraction. Doctoral Thesis, Karolinska Institute, Stockholm, 1986.

Katz, A., S. Broberg, K. Sahlin, and J. Wahren. Muscle ammonia and amino acid metabolism during dynamic exercise in man. Clin. Physiol. 6: 365-379, 1986a.

Katz, A., K. Sahlin, and J. Henriksson. Muscle ammonia metabolism during isometric contraction in man. Am. J. Physiol. 250 (Cell Physiol. 19): C834-C840, 1986b.

Keul, J., E. Doll and D. Keppler. Energy metabolism of human muscle. In Jokl, E. (Ed) Medicine and Sport 7: 1-313, 1972.

Knochel, J.P., J.D. Blachey, J.H. Johnston, and N.W. Carter. Muscle cell electrical hyperpolarization and reduced exercise hyperkalemia in physically conditioned dogs. J. Clin. Invest. 75: 740-745, 1985.

Lawrence, W., A.E. Schwartz, and H.T. Randall. Alterations in blood ammonia in dogs following total hepatectomy and abdominal evisceration. Surg. Gynecol. Obstet. 107: 69-78, 1958.

Lewis, S.F., and R.G. Haller. The pathophysiology of McArdle's disease: clues to regulation in exercise and fatigue. J. Appl. Physiol. 61: 391-401, 1986.

Lo, P.Y., and G.A. Dudley. Endurance training reduces the magnitude of exercise-induced hyperammonemia in humans. J. Appl. Physiol. 62: 1227-1230, 1987.

Lockwood, A.H., J.M. McDonald, R.E. Reiman, A.S. Gelbard, J.S. Laughlin, T.E. Duffy, and F.Plum. The dynamics of ammonia metabolism in man, effects of liver disease and hyperammonemia. J. Clin. Invest. 63: 449-460, 1979.

Lowenstein, J.M. Ammonia production in muscle and other tissues: the purine nucloeotide cycle. Physiol. Rev. 52: 382-414, 1972.

Lowenstein, J.M. and M.N. Goodman. The purine nucleotide in skeletal muscle. Fed. Proc. 37: 2308-2312, 1978.

Lowry, O.H., and J.V. Passonneau. Kinetic evidence for multiple binding sites on phosphofructokinase. J. Biol. Chem. 241: 2268-2279, 1966.

Ma, P.F., and T.A. Magers. Comparative studies of human adenosine deaminases. Int. J. Biochem. 6: 281-286, 1975.

MacDougall, J.D. The anaerobic threshold: its significance for the endurance athlete. Can. J. Appl. Sports Sci. 2: 137-140, 1977.

Mackovà, E., J. Melichna, L. Havlickovà, Z. Placheta, D. Blahovà, and B. Semiginovsky. Skeletal muscle characterics of sprint cyclists and nonathletes. Int. J. Sports Med. 7: 295-297, 1986.

Mathews, D.K., and E.L. Fox. The Physiological Basis of Physical Education and Athletics. (Ed 2), Philadelphia: Saunders College, 1976, p 506.

Maughan, R.J., and D.J.M. Sadler. The effects of oral administration of salts of aspartic acid on the metabolic response to prolonged exhausting exercise in man. Int. J. Sports Med. 4: 119-123, 1983.

McDaniel, H. Regulation of glutamate dehydrogenase in heart mitochondria. Fed. Proc. 46: 1402, 1987 (abstract).

Mckann, G.M., and D.B. Tower. Ammonia toxicity and cerebral oxidative metabolism. Am. J. Physiol. 200: 420-424, 1961.

McLellan, T.M. Ventilatory and plasma lactate response with different exercise protocols: A comparison of methods. Int. J. Sports Med. 6: 30-35, 1985.

Meyer, R.A., G.A. Dudley, and R.L. Terjung. Ammonia and IMP in different skeletal muscle fibres after exercise in rats. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 49: 1037-1041, 1980.

Meyer, R.A., and R.L. Terjung. Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. Am. J. Physiol. 237 (Cell Physiol. 6): C111-C118, 1979.

Meyer, R.A., and R.L. Terjung. AMP deamination and IMP reamination in working skeletal muscle. Am. J. Physiol. 239 (Cell Physiol. 8): C32-C38, 1980.

Miller, P., and L. Lawrence. The effect of conditioning on blood lactate and ammonia in horses. Fed. Proc. 45: 647, 1986 (abstract).

Molé, P.A., K.M. Baldwin, R.L. Terjung, and J.O. Holloszy. Enzymatic pathways of pyruvate metabolism in skeletal muscle: adaptations to exercise. Am. J. Physiol. 224: 50-54, 1973.

Molé, P.A., S. Tangsakul, and D. Vaughn. Adaptation to exercise: blood alanine, lactate and pyruvate as related to workload in man. In Yakelov (Ed.) Metabolism and Biochemical Evaluation of Fitness of Sportsmen. Lenigrad, 1974, pp 232-235.

Mutch, B.J.C. Chronic cannulation allows serial blood sampling and reinfusion during exercise in rats. Can. J. Appl. Sports Sci. 8: 27-28, 1983.

Mutch, B.J.C., and E.W.Banister. Ammonia metabolism in exercise and fatigue. Med. Sci. Sports and Exercise 15: 41-50, 1983.

Newsholme, E.A., and R. Leech. Biochemistry for the Medical Sciences New York: J.Wiley and Sons, 1983.

Onstad, G.R., and L. Zieve. What determines blood ammonia? Gastroenterology 77: 803-805, 1979.

Owen, E.E., M.P. Tyor, J.F. Flanagen, et al. The kidney as a source of blood ammonia in patients with liver disease:the effect of acetazoleamide. J. Clin. Invest. 39: 288-294, 1960.

Palaiologos, G., and P. Felig. Kinetic studies of glutamate dehydrogenase (aminating) from rat diaphragm mitochondria. Fed. Proc. 35: 299, 1976 (abstract).

Parnas, J.K. Ammonia formation in muscle and its source. Am. J. Physiol. 90: 467-472, 1929.

Poole, D.C., and G.A. Gaesser. Response of ventilatory and lactate thresholds to continuous and interval training. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 58: 1115-1121, 1985.

Randle, P.J., H.R. Fatania, and K.S. Lau. Regulation of the mitochondrial branched-chain 2-oxoacid dehydrogenase complex of animal tissues by reversible phosphorylation. In Cohen, P. (ed), Enzyme Regulation by Reversible Phosphorylation - Further Advances, Elsevier Science, New York, 1984, pp 1-26.

Rasmussen, B., K. Klausen, J.P. Clausen, and J. Trap-Jenson. Pulmonary ventilation, blood gases, and blood pH after training of the arms or the legs. J. Appl. Physiol. 38: 250-256, 1975.

Rennie, M.J., R.H.T. Edwards, S. Krywawych, C.T. Davies, D. Halliday, J.C. Waterlow, and D.J. Millward. Effects of exercise on protein turnover in man. Clin. Sci. Lond. 61: 627-639, 1981.

Rennie, M.J., and P.W. Watt. Kinetics of glutamate and glutamine transport in perfused rat skeletal muscle J. Physiol. (Lond.) 361: 37P, 1985 (abstract).

Robinson, S., and P.M. Harmon. The lactic acid mechanism and certain properties of the blood in relation to training. Am. J. Physiol. 132: 757-769, 1941.

Ronca-Testoni, S., A. Raggi, and G. Ronca. Muscle AMP aminohydrolase. III. A comparitive study on the regulatory properties of skeletal muscle enzyme from various species. Biochim. Biophys. Acta 198: 101-112, 1970.

Rosado, A., G. Flores, J. Mora, and G. Soberòn. Distribution of an ammonia load in the normal rat. Am. J. Physiol. 203: 37-42, 1962.

Rowe, W.B. Glutamine synthetase from muscle. Methods Enzymol. 113: 199-212, 1985.

Rubio, R., R.M. Berne, and J.G. Dobson. Sites of adenosine production in cardiac and skeletal muscle. Am. J. Physiol. 225: 938-953, 1973.

Ruderman, N.B., and M. Berger. The formation of glutamine and alanine in skeletal muscle. J. Biol. Chem. 249: 5500-5506, 1974.

Ruderman, N.B., and P. Lund. Amino acid metabolism in skeletal muscle. Israel J. Med. Sci. 8: 295-301, 1972.

Rusko, H., P. Luhtanen, P. Rahkila, J. Viitasalo, S. Rehunen, and M. Härkönen. Muscle metabolism, blood lactate and oxygen uptake in steady state exercise at aerobic and anaerobic thresholds. Eur. J. Appl. Physiol. 55: 181-186, 1986.

Sabina, R.L., J.L. Swain, B.M. Patten, T. Ashizawa, W.E. O'Brien, and E.W. Holmes. Disruption of the purine nucleotide cycle: A potential explanation for muscle dysfunction in myoadenylate deaminase deficiency. J. Clin. Invest. 66: 1419-1423, 1980.

Sabina, R.L., J.L. Swain, C.W. Olanow, W.G. Bradley, W.N. Fishbein, S. DiMauro, and E.W. Holmes. Myoadenylate deaminase deficiency: functional and metabolic abnormalities associated with disruption of the purine nucleotide cycle. J. Clin. Invest. 73: 720-730, 1984.

Sahlin, K., R.C. Harris, B. Nylind, and E. Hultman. Lactate content and pH in muscle samples obtained after dynamic exercise. Pflugers Arch. 367: 143-149, 1976.

Sahlin, K., and J. Henriksson. Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. Acta Physiol. Scand. 122: 331-339, 1984.

Sahlin, K., G. Palmskog, and E. Hultman. Adenine nucleotide and IMP contents of the quadriceps muscle in man after exercise. Pflugers Arch. 374: 193-198, 1978.

Sale, D.G. Influence of exercise and training on motor unit activation. Exercise Sport Sci. Rev. 15: 95-151, 1987.

Saltin, B., and P.O. Astrand. Maximal oxygen uptake in athletes. J. Appl. Physiol. 23: 353-358, 1967.

Saltin, B., and P.D. Gollnick. Skeletal muscle adaptability significance for metabolism and performance. In: Peachy, L.D. (ed), Handbook of Physiology, Section 10, Skeletal muscle. Amer. Physiol. Soc., Maryland, USA, pp 555-631, 1983.

Saltin, B., B. Blomqvist, J. Mitchell, R.L. Johnson, Jr., K. Wildenthal, and C.B. Chapman. Response to submaximal and maximal exercise after bed rest and training. Circulation 38: Suppl. 7, VII-1 - VII-78, 1968.

Saltin, B., K. Nazar, D.L. Costill, E. Stein, E. Jansson, B. Essen, and P.D. Gollnick. The nature of the training response: peripheral and central adaptations in one-legged exercise. Acta Physiol. Scand. 96: 289-305, 1976.

Schantz, P.G. Plasticity of human skeletal muscle with special reference to effects of physical training on enzyme levels of the NADH shuttles and phenotypic expression of slow and fast myofibrillar proteins. Acta Physiol. Scand. Suppl 558: 1-62, 1986.

Schantz, P., R. Billeter, J. Henriksson, and E. Jansson. Training-induced increase in myofibrillar ATPase intermediate fibers in human skeletal muscle. Muscle and Nerve 5: 628-636, 1982.

Schoolwerth, A., W. Hoover, C. Daniel and K. LaNoue. Effect of aminooxyacetate and 2-oxoglutarate on glutamate deamination by rat kidney mitochondria. Int. J. Biochem. 12: 145-149, 1980.

Schwartz, A.E., W. Lawrence, and K.E. Roberts. Elevation of peripheral blood ammonia following muscular exercise Proc. Soc. Exp. Biol. 98: 548-550, 1958.

Senay, L.C., and R. Kok. Effects of training and heat acclimatization on blood plasma contents of exercising men. J. Appl. Physiol. 43: 591-599, 1977.

Setlow, B., and J.M. Lowenstein. Adenylate deaminase. II. Purification and some regulation properties of the enzyme from calf brain. J. Biol. Chem. 242: 607-615, 1967.

Sharp, R.L., D.L. Costill, W.J. Fink, and D.S. King. Effects of eight weeks of bicycle ergometer sprint training on human muscle buffer capacity. Int. J. Sports Med. 7: 13-17, 1986.

Simon, J. J.L. Young, D.K. Blood, K.R. Segal, R.B. Case, and B. Gutin. Plasma lactate and ventilation thresholds in trained and untrained cyclists. J. Appl. Physiol. 60: 777-781, 1986.

Sinkeler, S.P., H.A. Daanen, R.A. Wevers, T.L. Oei, E.M. Joosten, and R.A. Binkhorst. The relation between blood lactate and ammonia in ischemic handgrip exercise. Muscle and Nerve 8: 523-527, 1985.

Sinkeler, S.P., E.M. Joosten, R.A. Wevers, R.A. Binkhorst, F.T. Oerlemans, C.A. van Bennekom, M.M. Coerwinkel, and T.L. Oei. Ischaemic exercise test in myoadenylate deaminase deficiency and McArdle's disease: measurement of plasma adenosine, inosine and hypoxanthine. Clin. Sci. 70: 399-401, 1986.

Sinniah, D., T.T. Fulton, and H. McCullough. The effect of exercise on the venous blood ammonium concentration in man. J. Clin. Path. 23: 715-719, 1970.

Sjogaard, G., R.P. Adams, and B. Saltin. Water and ion shifts in skeletal muscle of humans with intense dynamic knee extension. Am. J. Physiol. 248(Regulatory Intergrative Comp. Physiol. 17): R190-R196, 1985.

Snell. K. Muscle alanine synthesis and hepatic gluconeogenesis. Biochem. Soc. Trans. 8: 205-213, 1980.

Snell, K., and D.A. Duff. Alanine and glutamine formation in muscle. Biochem. Soc. Trans. 8: 501-504, 1980.

Snow, D.H., R.C. Harris, and S.P. Gash. Metabolic response of equine muscle to intermittent maximal exercise. J. Appl. Physiol. 58: 1689-1697, 1985.

Sugden, P.H., and E.A. Newsholme. The effects of ammonium, inorganic phosphate and pottasium ions on the activity of phosphofructokinases from muscle and nervous tissues of vertebrates and invertebrates. Biochem. J. 150: 113-122, 1975.

Svensson, G., and T. Anfält. Rapid determination of ammonia in whole blood and plasma using flow injection analysis. Clin. Chim. Acta. 119: 7-14, 1982.

Swain, J.L., J.J. Hines, R.L. Sabina, O.L. Harbury, and E.W. Holmes. Disruption of the purine nucleotide cycle by inhibition of adenylosuccinase lyase produces skeletal muscle dysfunction. J. Clin. Invest. 74: 1422-1427, 1984.

Taylor, D.J., P. Styles, P.M. Mathews, D.A. Arnold, D.G. Gadian, P. Bore, and G.K. Radda. Energetics of human muscle: exercise-induced ATP depletion. Magn. Reson. Med. 3: 44-54, 1986.

Tejwani, G.A., A. Ramaiah, and M. Ananthanarayanan. Regulation of glycolysis in muscle. Archiv. Biochem. Biophys. 158: 195-199, 1973.

Terjung, R.L., G.A. Dudley, R.A. Meyer, D.A. Hood, and J. Gorski. Purine nucleotide cycle function in contracting muscle. In Saltin, B., E. Richter, and H. Galbo (Eds). Biochemistry of Exercise 6TH International Symposium, Champaign Illinois, Human Kinetics Publishers, 131-147, 1986.

Tesch, P. Muscle fatigue in man with special reference to lactate accumulation during short term intense exercise. Acta Physiol. Scand. Suppl 480: 1-40, 1980.

Troup, J.P., J.M. Metzger, and R.H. Fitts. Effect of high intensity exercise training on functional capacity of limb skeletal muscle. J. Appl. Physiol. 60: 1743-1751, 1986.

Uyeda, K., and E. Racker. Regulatory mechanisms of carbohydrate metabolism. J. Biol. Chem. 240: 4682-4688, 1965

Viru, A. Mobilisation of structural proteins during exercise. Sports Med. 4: 95-128, 1987.

Visek, W.J. Some aspects of ammonia toxicity in animal cells. J. Dairy Sci. 51: 286-295, 1968.

Weast, R.C. (Ed). Handbook of Chemistry and Physics Ohio, CRC Press, 1975-1976, p D151.

Wheeler, T.J., and J.M. Lowenstein. Adenylate deaminase from rat muscle. Regulation by purine nucleotides and orthophosphate in the presence of 150 mM KCl. J. Biol. Chem. 254: 8994-8999, 1979.

White, T.P. and G.A. Brooks. [U-^{14}C]glucose, -alanine, -leucine oxidation in rats at rest and during two intensities of running. Am. J. Physiol. 240 (Endocrinol. Metab. 3): E155-E165, 1981.

Wilkerson, J.E., D.L. Batterton, and S.M. Horvath. Ammonia production following maximal exercise: treadmill vs bicycle testing. Eur. J. Appl. Physiol. 34: 164-172, 1975.

Wilkerson, J.E., D.L. Batterton, and S.M. Horvath. Exercise induced changes in blood ammonia levels in humans. Eur. J. Appl. Physiol. 37: 255-263, 1977.

Winer, B.J. Statistical Principles in Experimental Design. Ed 2, New York, McGraw-Hill, 1971.

Winder, W.W., R.L. Terjung, K.M. Baldwin, and J.O. Holloszy. Effect of exercise on AMP deaminase and adenylosuccinase in rat skeletal muscle. Am. J. Physiol. 227: 1411-1414, 1974.

Whitlock, D.M., and R.L. Terjung. ATP depletion in the slow twitch red muscle of rat. Am. J. Physiol. 253 (Cell Physiol. 22) C426-C432, 1987.

Wolfe, R.R. Does exercise stimulate protein breakdown in humans?: Isotopic approaches to the problem. Med. Sci. Sports and Exercise 19 (Suppl): S172-S178, 1987.

Wolfe, R.R., R.D. Goodenough, M.H. Wolfe, F.T. Royle, E.R. Nadel. Isotopic analysis of leucine and urea metabolism in exercising humans. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 52: 458-466, 1982.

Wolfe, B.R., T.E. Graham, and J.K. Barclay. Hyperoxia, mitochondrial redox state, and lactate metabolism of in situ canine muscle. Am. J. Physiol. 253 (Cell Physiol. 22): C263-C268, 1987.

Wolfe, R.R., M.H. Wolfe, E.R. Nadel, and J.H.F. Shaw. Isotopic determination of amino-acid urea interactions in exercise in humans. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 56: 221-229, 1984.

Worcel, A., and M. Erecinska. Mechanism of inhibitory action of ammonia on the respiration of rat-liver mitochondria. Biochim. Biophys. Acta 65: 27-33, 1962.

Yarasheski, K.E., and P.W.R. Lemon. Effect of endurance training on protein catabolism during prolonged exercise in males. Can. J. Appl. Sport Sci. 8: 195, 1983 (abstract).

Yoshida, T. Effect of exercise duration during incremental exercise on the determination of anaerobic threshold and the onset of blood lactate accumulation. Eur. J. Appl. Physiol. 53: 196-199, 1984.

APPENDIX A

INFORMED CONSENT STATEMENTS

APPENDIX

FOOTSCRAY INSTITUTE OF TECHNOLOGY

STANDARD CONSENT FORM FOR SUBJECTS INVOLVED IN EXPERIMENTS

1. 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:

"..Effects of Endurance training on human ammonia metabolism.....

.....

.....

being conducted at Footscray Institute of Technology by:.....

...Rod Snow.....

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

...See attached sheet.....

.....

.....

.....

.....

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.

Signed:.....)

)Date:.....

Witness other than the experimenter.....)

.../2

Procedure

For the untrained subjects the experiment will involve exercise on two occassions, for the trained it will involve exercise on three separate occassions. For all subjects the first occassion will involve riding a stationary bicycle at a work output which is very low and easy to achieve. Every 4 minutes the resistance to pedalling will be increased until the subject can no longer maintain the required pedalling speed. During the second and third test (in the case of the trained subjects) you will be required to ride the bicycle for 15 minutes at a workload which is quite physically demanding, but not exhausting.

During any vigorous exercise bout there is always a risk that something physically can go wrong. However this risk is very small given that you are young (< 30 years of age), active, and lack any symptoms which indicate that exhausting exercise could be hazardous. Although the risk is quite remote, young active, asymptomatic people could experience dizzy spells, alterations in heart rate and rythm, even heart attack. In an attempt to further reduce the risk of injury to the subjects, you are asked to immediately cease exercise if you feel dizzy, pain in the chest or left arm, nausea, severe fatigue, or marked breathlesness.

At specific intervals throughout each exercise test a small blood sample (3 mls) will be taken via a catheter placed into an arm vein. The catheter consists of a needle and teflon tubing. The tubing is feed over the top of the needle (which has punctured the vein) and into the vein. A tap (stopcock) is placed onto the tubing so the flow of blood along the tubing can be altered at will. This procedure allows the taking of multiple blood samples without the need for multiple venapunctures (puncturing of vein). Catheterization of subjects is slightly discomforting and can lead to the possibility of bruising and infection. The use of sterilized disposable catheters, syringes, swabs, etc. will reduce markedly the possibility of infection caused by the catheterization procedure. The use of experienced and qualified staff will reduce the likelihood of bruising as bruising is primarily caused by poor venapuncture technique. Although the probability of infection and significant bruising is quite small, if by chance it does eventuate, we suggest you consult with your doctor immediately.

2. CERTIFICATION

I, ... have fully explained the objectives, risks and procedures of the abovenamed experiment to the subject named herein.

Signed:..... ..... Date:..... .....

NOTES:

1. Those signing this form are reminded that while research workers have a duty to advance knowledge by research, the rights of the individual subject take precedence over expected benefits to knowledge or to the community.

2. The experimenter is reminded of the need to observe confidentiality, when appropriate, to protect the interests of subjects.

3. Subjects who are employees of the Institute should be advised that participation in the experiment does not affect in any way their entitlement or right to receive workers' compensation.

APPENDIX

FOOTSCRAY INSTITUTE OF TECHNOLOGY

STANDARD CONSENT FORM FOR SUBJECTS INVOLVED IN EXPERIMENTS

1. 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:

"....The effects of muscle fibre type on the accumulation .....
....of plasma ammonia during exercise."

.....

being conducted at Footscray Institute of Technology by:.....
Dr. Warren Payne and Dr. Michael Carey.

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

See attached sheet

.....

.....

.....

.....

.....

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.

Signed:.....)

)Date:.....

Witness other than the experimenter.....)

.../2

INFORMATION FOR SUBJECTS

MUSCLE BIOPSY PROCEDURE

The muscle biopsy is a relatively painless procedure that is used to obtain small samples of skeletal muscle tissue for metabolic analysis. A small incision is made in the skin overlying the muscle, under local anaesthetic. The biopsy needle is then inserted into the muscle and a small piece of tissue removed from the muscle. During this part of the procedure you may feel some pressure and a tendency for the muscle to cramp, however, this only persists for a few seconds. Following the biopsy the incision will be closed and a pressure bandage applied for 24 hours. It is common for subjects to experience some mild soreness in the muscle over the next 2-3 days, however this does pass and does not restrict movement. In some rare cases mild haematomas have been reported, but these symptoms disappear within a week. The whole procedure will be performed under sterile conditions by a qualified medical practitioner.

2. CERTIFICATION

I, ... have fully explained the objectives, risks and procedures of the abovenamed experiment to the subject named herein.

Signed:..... Date:.....

NOTES:

1. Those signing this form are reminded that while research workers have a duty to advance knowledge by research, the rights of the individual subject take precedence over expected benefits to knowledge or to the community.

2. The experimenter is reminded of the need to observe confidentiality, when appropriate, to protect the interests of subjects.

3. Subjects who are employees of the Institute should be advised that participation in the experiment does not affect in any way their entitlement or right to receive workers' compensation.

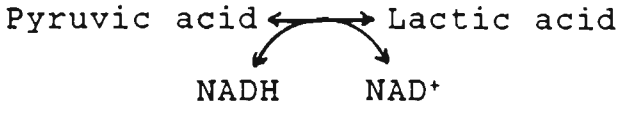
APPENDIX B

DETAILS OF ANALYTICAL CHEMICAL METHODS

FOR BLOOD LACTATE AND PLASMA ALANINE

Blood Lactate

Principle:- The enzyme lactate dehydrogenase catalyzes the following reaction:



In the prescence of an excess of NAD^+ and LDH, and at a highly alkaline pH, nearly all the lactate is converted to pyruvate. The amount of NAD+ which is converted to NADH is measured spectrophotometrically and becomes a measurement of the lactate originally present.

A set of standards ranging in concentration from 1 mM to 20 mM was run with each analysis. A typical standard curve is shown in Figure B.1. The relative standard deviation (RSD) for multiple measurements of each aqueous standard ranged between 3.8% (1 mM, n=6) and 1.2% (20 mM, n=9).

Plasma Alanine

Plasma alanine (ALA) concentration was determined using high performance liquid chromatography (HPLC). A standard ALA solution (240 µM) was run before each set of samples. The RSD for multiple measurements (n=6) of the ALA standard was 3.15 %. A chromatogram of a plasma sample is shown in Figure B.2 to demonstrate the degree of amino acid separation achieved using the methods described in this thesis.

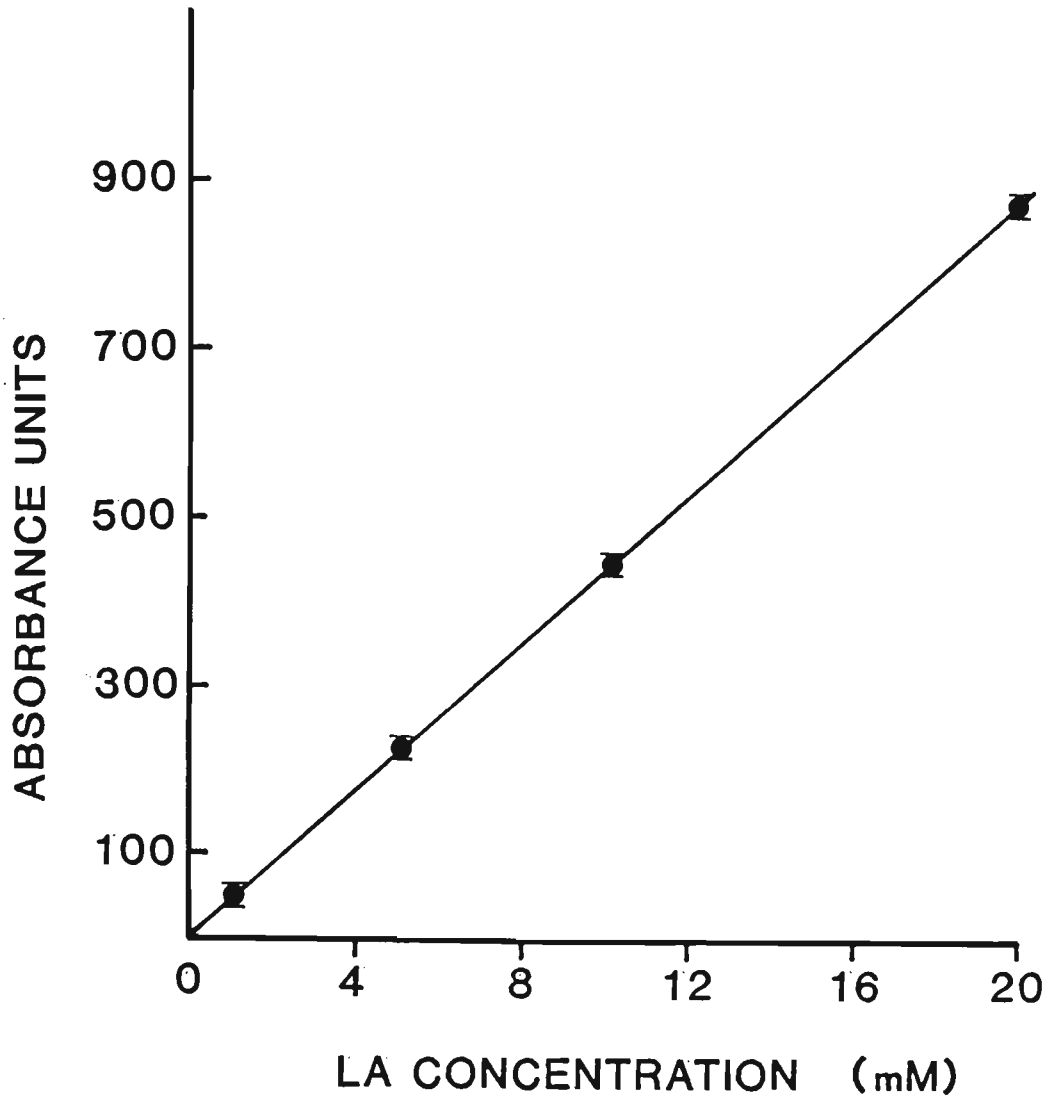


Figure B.1 - Lactate standard curve (error bars represent relative standard deviations).

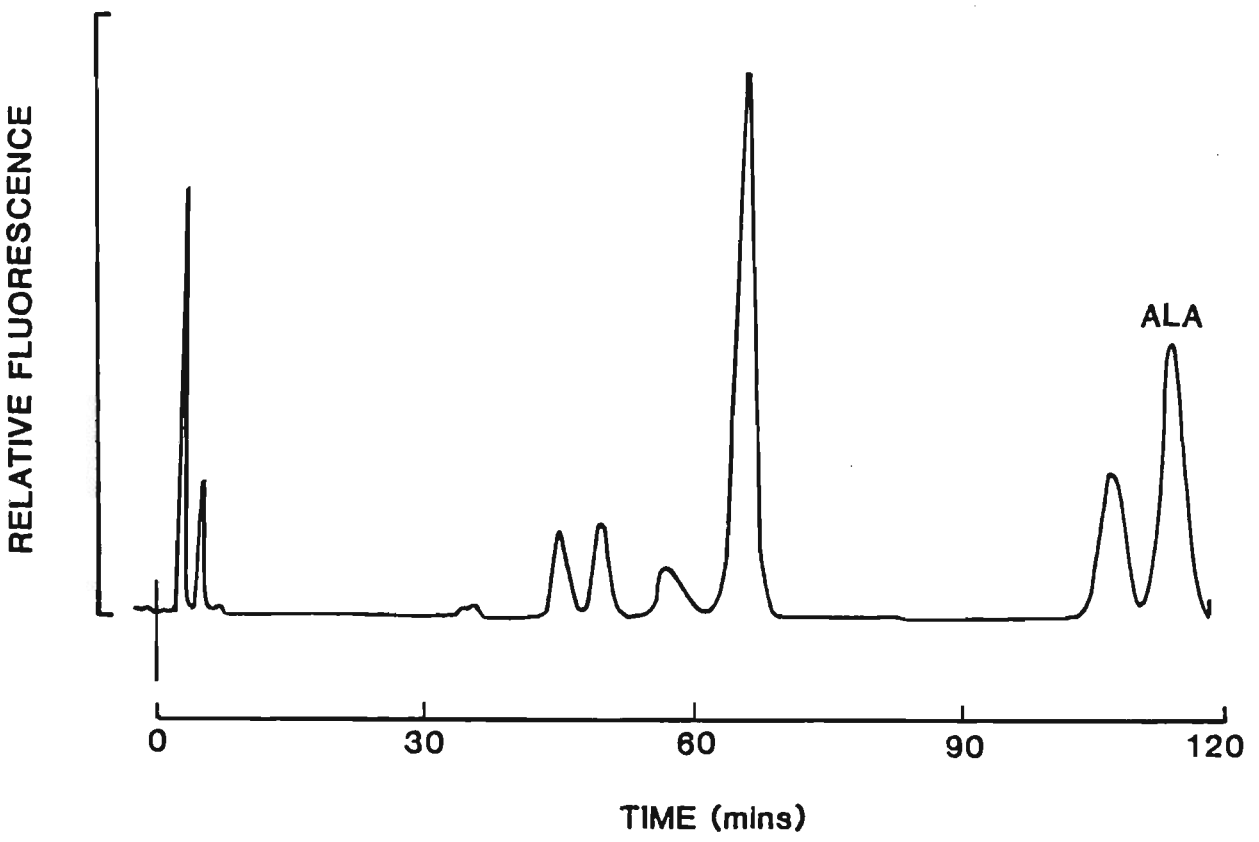


Figure B.2 - Example of chromatogram for analysis of alanine (ALA) in plasma.

APPENDIX C

DETAILS OF ANALYTICAL CHEMICAL METHODS

FOR PLASMA AMMONIA

THE DETERMINATION OF TOTAL AMMONIA/AMMONIUM IN HUMAN PLASMA BY FLOW INJECTION ANALYSIS

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ABSTRACT

This paper describes the determination of total plasma ammonia / ammonium (NH_3) using flow injection analysis (FIA) and presents data which compare the concentration of plasma NH_3 in endurance trained and untrained subjects during exercise and recovery. The detection limit obtained with this method was 0.05 mg/L, the precision was 8% RSD, and the rate of sampling was in the order of 20 - 30 samples per hour. The mean resting plasma NH_3 concentration for 13 subjects was 0.24 (SD±0.09) mg/L. During incremental exercise plasma NH_3 concentration rose significantly above resting values. A significant difference was also found between the plasma NH_3 concentration of trained and untrained subjects during certain periods of exercise and recovery.

INTRODUCTION

The measurement of plasma ammonium has important medical and scientific significance. Changes in plasma ammonium concentration across various tissue beds (muscle, kidney, brain, and liver) can give valuable information about a number of metabolic processes (e.g., adenine nucleotide metabolism, urea cycle etc.) and certain disease states (e.g., malfunctioning liver, enzyme deficiencies)[1,2]. Until recently, conventional analytical techniques, such as the ammonia probe and enzymatic analysis, have been used to determine the ammonium concentration in plasma samples. Such techniques are slow and imprecise at the ammonium concentrations found in human plasma [3]. In this paper, we describe the determination of total plasma ammonia / ammonium (NH_3) using Flow Injection Analysis (FIA) with a gas diffusion cell. Data are also presented from a study comparing the concentration of plasma NH_3 in endurance trained and untrained subjects during intense bicycle exercise.

Ammonia determination by FIA and gas diffusion involves the injection of an aqueous sample containing ammonium ions into a carrier stream which subsequently merges with a sodium hydroxide stream. In the resulting alkaline stream ammonia is formed which diffuses through a gas permeable membrane into an acid-base indicator stream. The basic form of the indicator is measured spectrophotometrically.

EXPERIMENTAL

The analysis was carried out using a Tecator (5020) FIA analyser which contains two peristaltic pumps, an injection valve (L 100-1) and a thermostatically controlled compartment (30°C) which houses the gas diffusion cell (Chemifold type V). The alkaline and indicator streams were separated in the gas diffusion cell by a 0.076mm teflon membrane. Spectrophotometric detection (5023) was carried out at 590nm and peaks were recorded on a Tecator printer (5021).

The injection loop (150µL) was filled manually by attaching a syringe to the waste side of the injection loop and applying suction. The loop was flushed manually between sample injections using 5ml of 0.9% NaCl.

Reagents and solutions

All reagents were of analytical grade and solutions were prepared with water purified by a Milli Q water purification system (Millipore, USA). The indicator stock solution was prepared by dissolving 0.1g cresol red (Hopkins & Williams) and 0.3g of thymol blue (May & Baker) in 10ml of 0.1M NaOH. This solution was then diluted to 200ml with 0.9% NaCl. The indicator stream was prepared daily by adding 500ml of 0.9% NaCl to 10ml of indicator stock solution. This solution was filtered, degassed and then titrated with NaOH or HCl until the absorbance at 590nm was between 0.25 and 0.35. The alkaline stream and carrier stream were 0.1M NaOH and 0.9% NaCl, respectively. The solutions in both streams were filtered and degassed. All reagent streams were protected from CO_2 absorption using soda lime. A set of standards was repared by diluting a stock solution of 1000mg/L NH_4Cl (May & Baker) using 0.9% NaCl.

Sample Preparation

Venous blood from six endurance trained and seven untrained males was drawn from the subjects at rest and during various stages of exercise and recovery. Blood was centrifuged at 15000 G for 3 minutes. The plasma was separated from the red blood cells, snap frozen using liquid nitrogen and stored at -80° C until analysis. Analysis of plasma NH_3 was completed within 72 Hrs of sampling to avoid increases in ammonia due to deamination of plasma amino acids [4].

RESULTS AND DISCUSSION

Previous work [3,5] suggests the concentration range for NH_3 in plasma samples lies between 0.15 and 2.25 mg/L. A calibration graph of FIA peak heights verses NH_3 concentration over this range is shown in Fig. 1. The average RSD over this range was 8%. This compares well with the value of 14% quoted for an enzymatic method for NH_3 determination [6]. Svensson and Anfalt [3] reported an RSD of 1% for their FIA method. The precision of our determination was probably limited by the need, imposed by the conditions of this study, to work with small volume plasma samples. Consequently the automatic injector could not be used and the 150 µL sample loop had to be flushed manually with only 250 µL of plasma sample. The rate of analysis

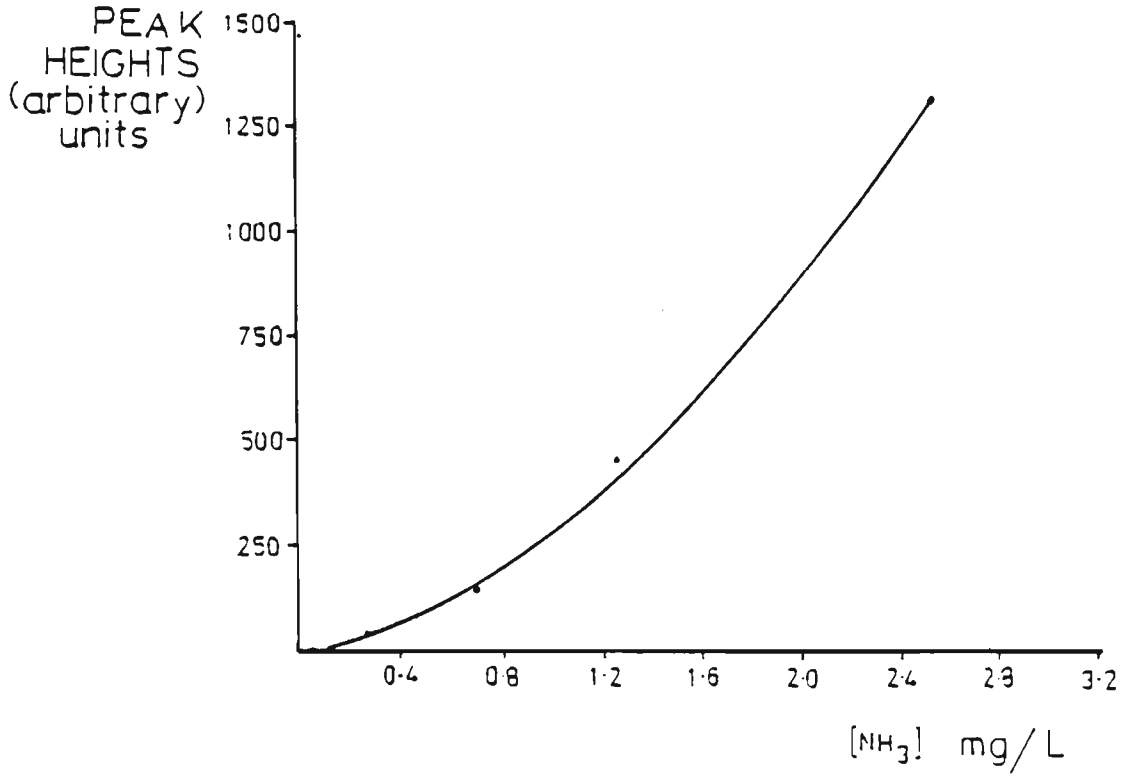

Fig 1. Calibration graph using aqueous NH_4Cl solutions.

was approximately 20 - 30 samples per hour. This rate compares favourably with other analytical techniques [6,7]. The detection limit with our system was found to be 0.05mg/L.

The production of exogenous ammonia by alkaline hydrolysis of compounds containing amine groups in plasma was insignificant in the time scale of this FIA technique. This was demonstrated by the injection of a urea solution three times the normal plasma urea concentration. No signal for NH_3 was detected.

The plasma NH_3 results for trained and untrained subjects during rest, incremental exercise and recovery are presented in Fig 2. The mean resting plasma NH_3 concentration for 13 subjects was found to be 0.24 (SD $\pm$ 0.09) mg/L. This is lower than the value obtained by Svensson and Anfalt [3], 0.42 (SD $\pm$ 0.19) mg/L. Despite the large standard deviations in these populations these results are significantly different at the $p < 0.05$ level based on a t-test of means. The higher values recorded by Svensson and Anfalt may be due to the use of heparin as an anticoagulant. Conn [8] reported that commercially available heparins contain significant amounts of NH_3. Also, differences could arise due to the methods of population sampling. Our study used highly trained endurance athletes and active untrained subjects. This may have skewed the sample compared with that of Svensson and Anfalt who reported only that their plasma came from healthy subjects.

During exercise the NH_3 concentration increased significantly above the rest value at workloads greater than 250 and 300 watts for the untrained and trained subjects, respectively. Significance was determined by a one-way ANOVA and Newman-Keuls post hoc analysis. For all subjects the mean peak NH_3 concentration during maximal exercise was 1.22 (SD $\pm$ 0.50)

mg/L. This value agrees with that reported by Buono et al. [5], using a phenol-hypochlorite method, during a similar maximal exercise test, 1.7 (SD ± 0.67) mg/L. Maximum NH_3 concentration occurred after 5 and 10 minutes of recovery for the trained and untrained subjects, respectively . Maximum values were in close agreement to those found in previous research [5,9]. A significant difference in NH_3 concentration was found between trained and untrained subjects at a workload of 300 watts and after 5 minutes of recovery. Significance was determined by a two-way ANOVA with repeated measures on one factor. Differences between the means were uncovered by simple main effects analysis. Due to the cross sectional design of the study further research is required to ascertain whether this difference is a result of training or other factors (e.g. muscle fibre type).

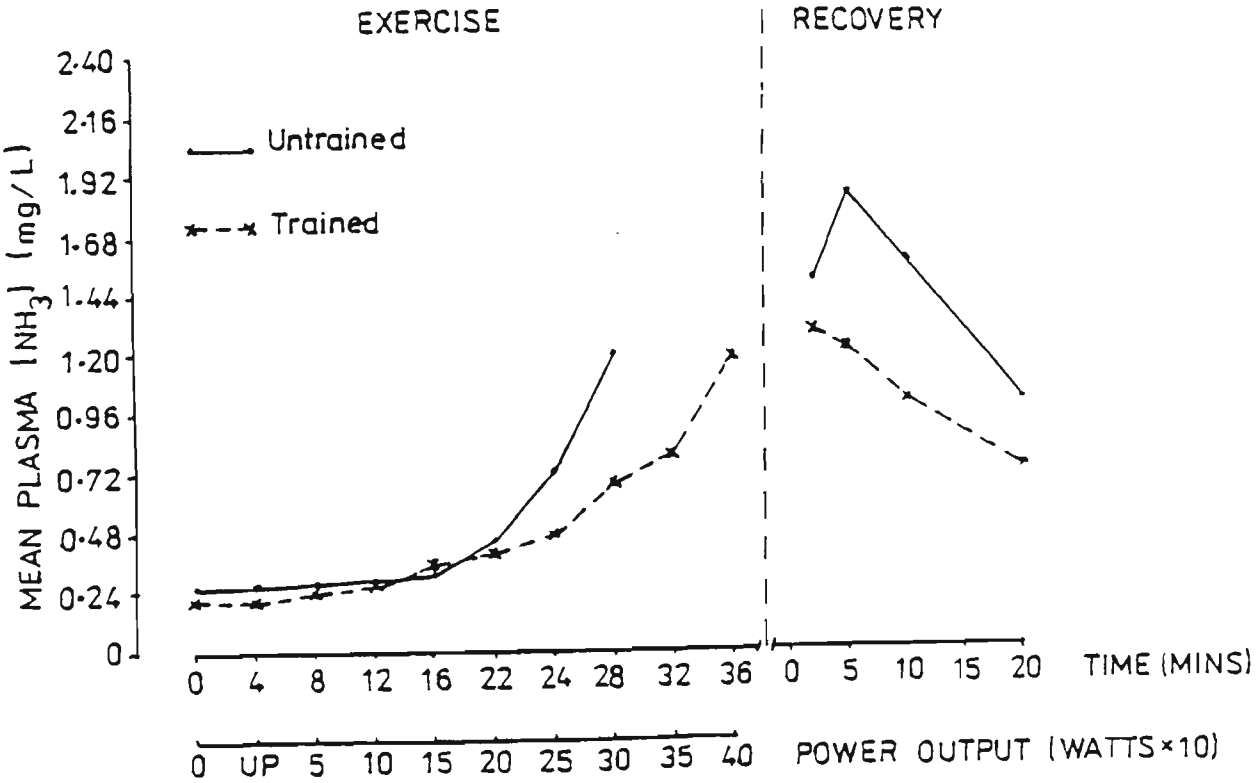

Fig 2. Plasma NH_3 during rest, exercise and recovery in trained and untrained males (UP denotes unloaded pedalling)

REFERENCES

1. A.H. Lockwood, et al., J. Clin. Invest., 63 (1979) 449.
2. B. Hindfelt, Clin. Sci. Mol. Med., 48 (1975) 33.
3. G. Svensson and T. Anfalt, Clin. Chim. Acta., 119 (1982) 11.
4. J. H. Howanitz, et al., Clin. Chem., 30 (1984) 906.
5. M.J. Buono, et al., J. Appl. Physiol.: Respirat.Environ. Exercise Physiol., 57 (1984) 136.
6. Sigma Chemical Company, Technical procedure # 170-UV.
7. E. Kun and E.B Kearney in Bergmeyer (Ed.), Methods Of Enzymatic Analysis, Academic Press, New York, (1974) p.1802
8. H.O. Conn, N. Engl. J. Med., 262 (1960) 1103.
9. J.E. Wilkerson, et al., Euro. J. Appl. Physiol., 34 (1975) 171.

APPENDIX D

PLOTS USED TO DETERMINE LABP AND NH_3 BP

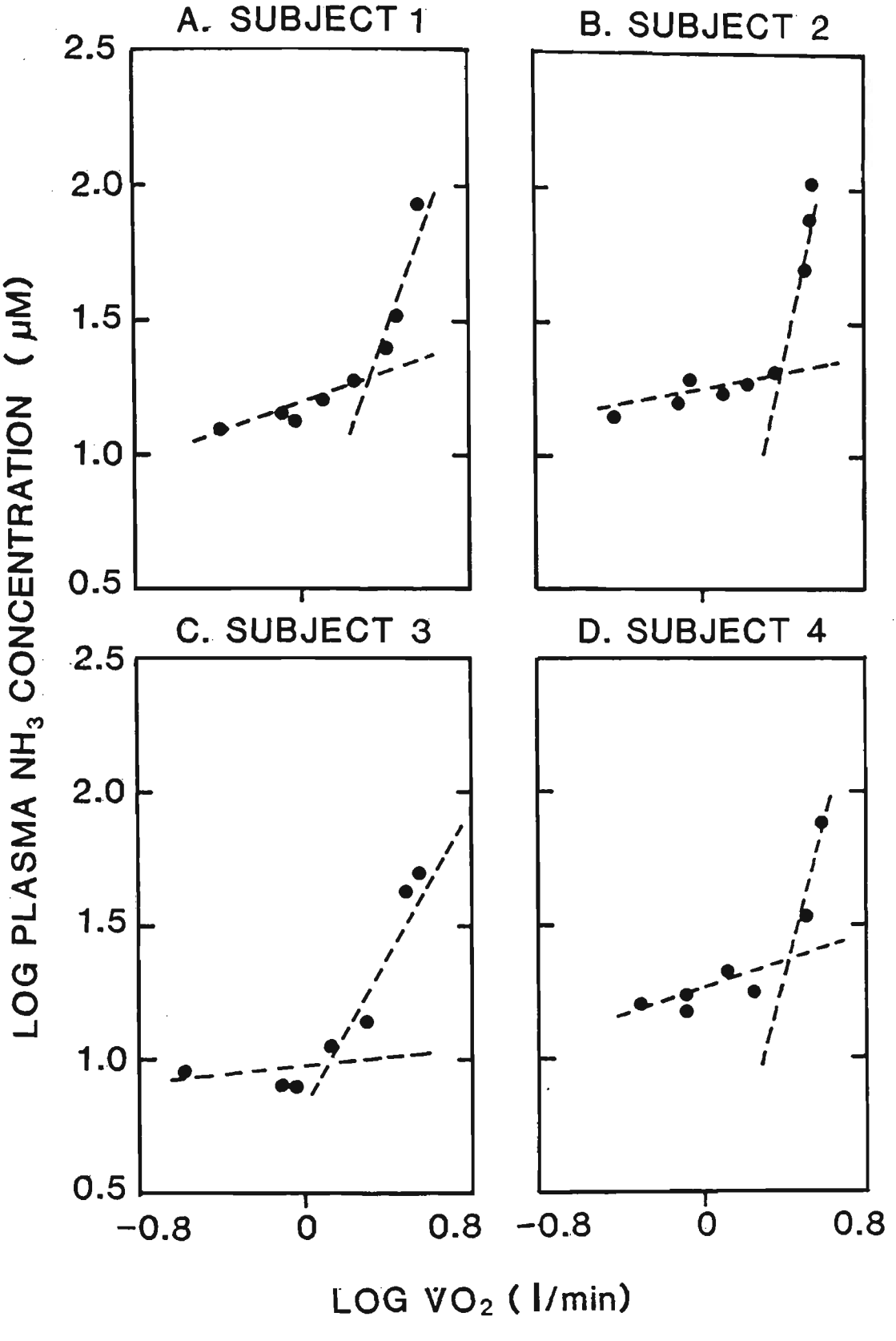

Figure D.1 - Log plasma NH_3 concentration versus Log O_2 uptake ($\dot{V}O_2$) for each untrained subject. NH_3BP occurs at intersection of dashed line.

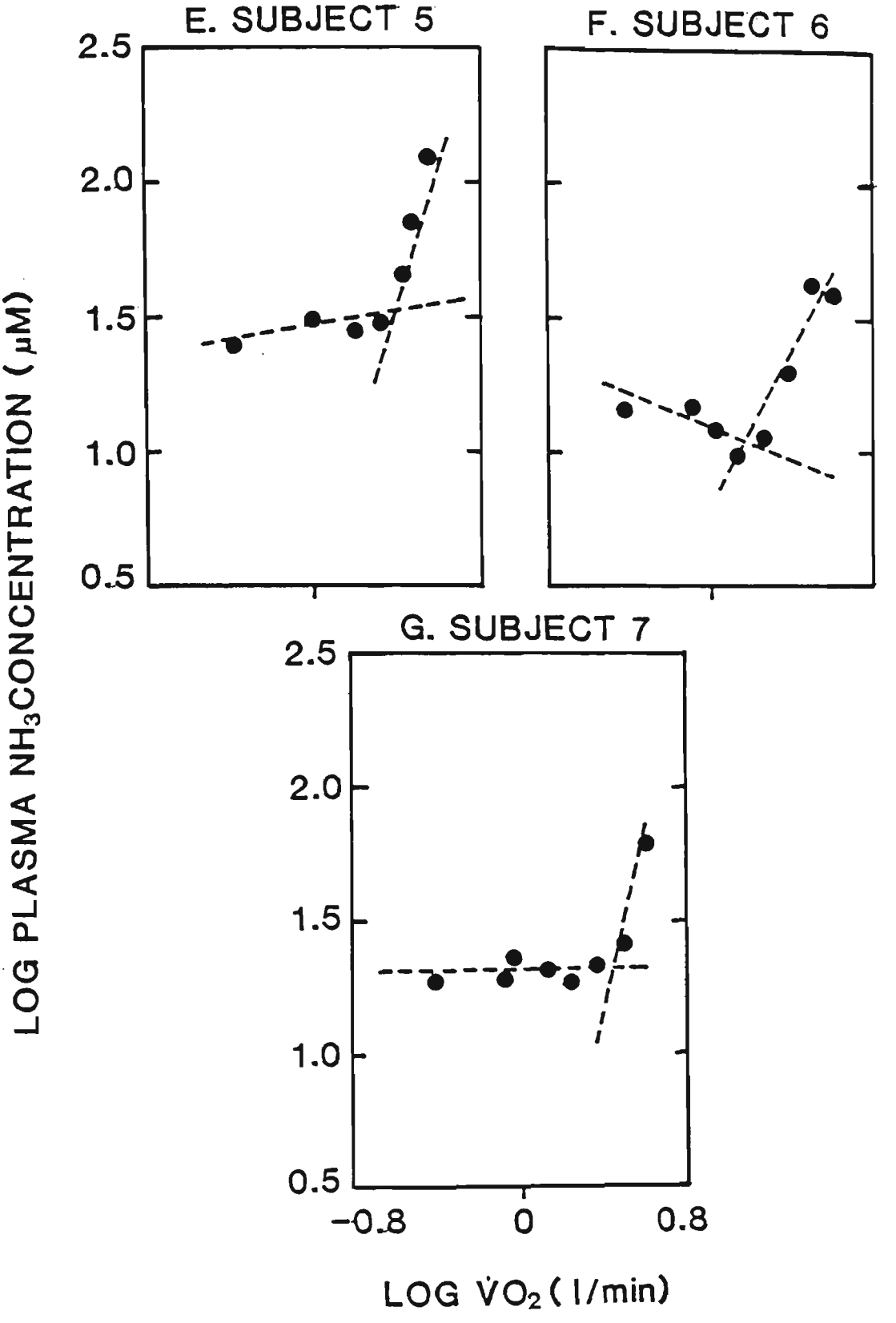

Figure D.1 (Continued)

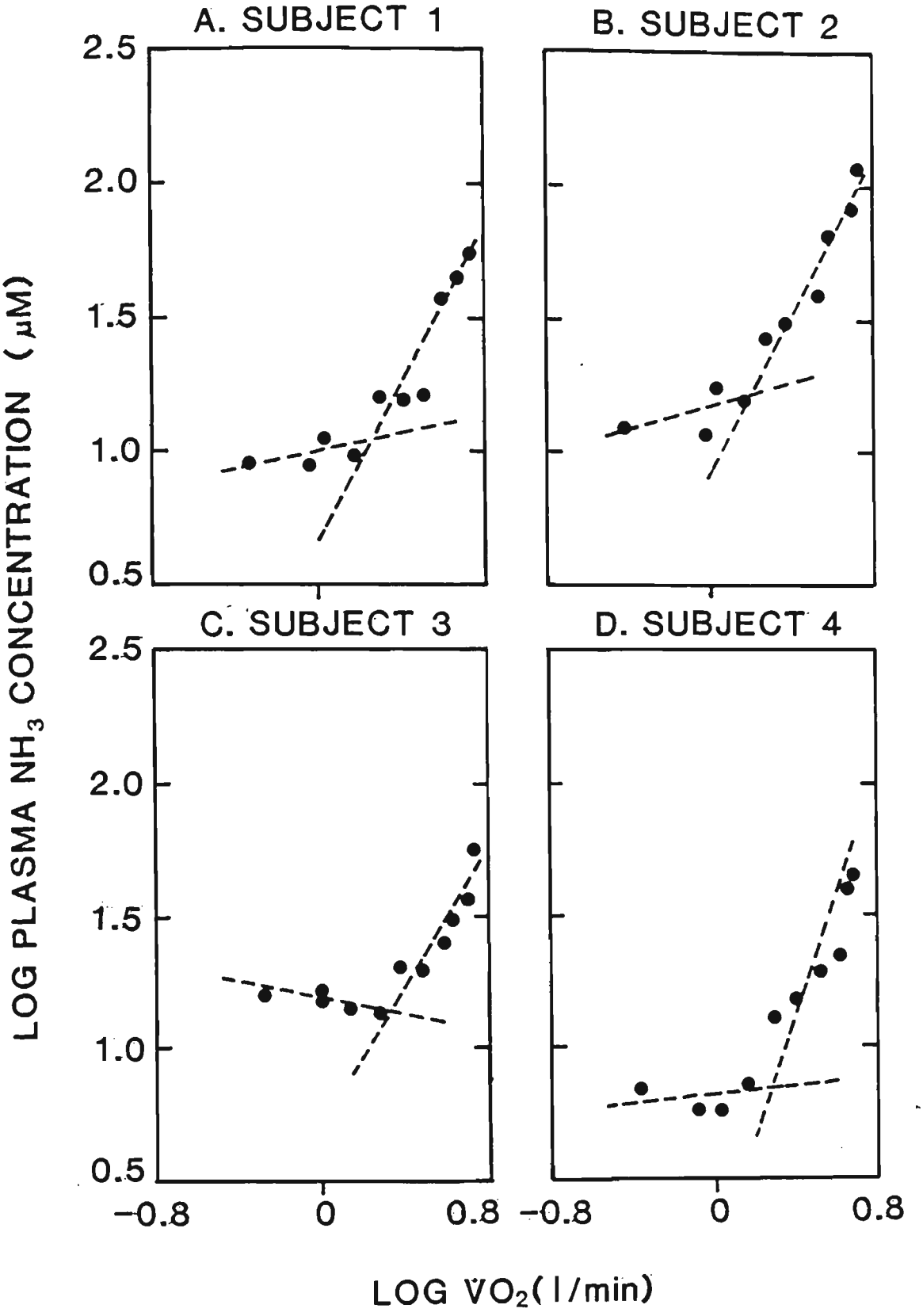

Figure D.2 - Log plasma NH_3 concentration versus Log O_2 uptake ($\dot{V}O_2$) for each trained subject. NH_3BP occurs at intersection of dashed lines.

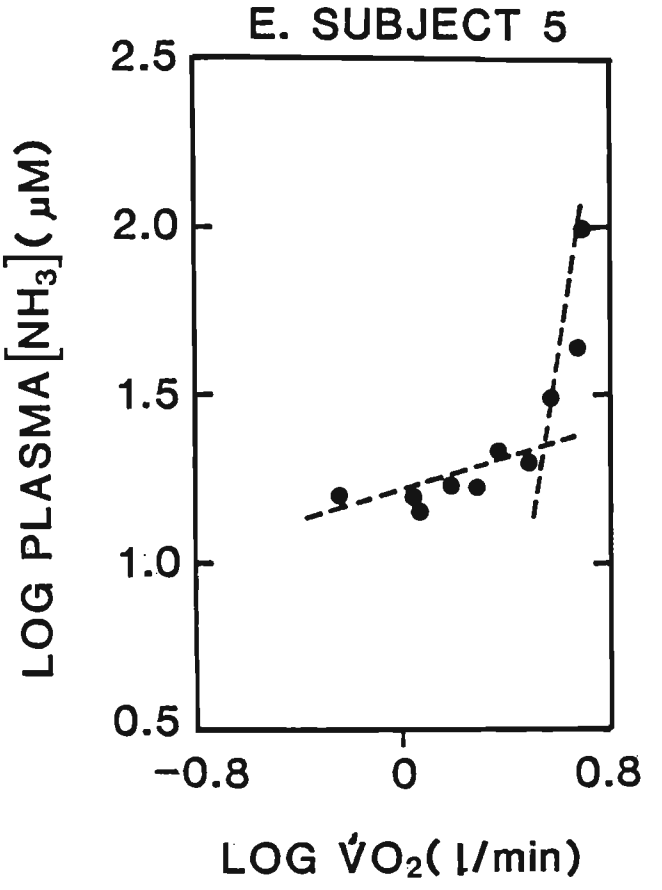

Figure D.2 (Continued)

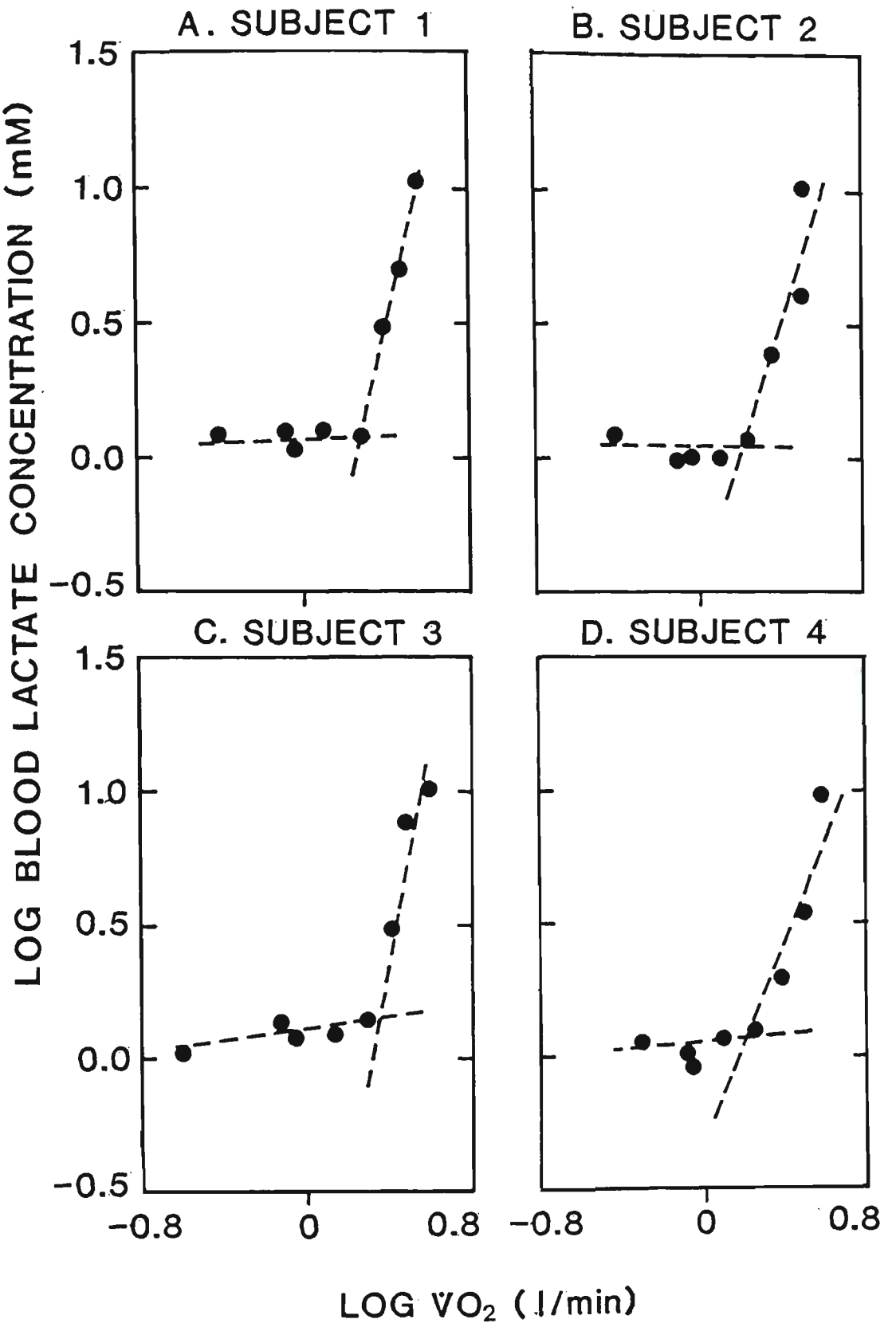

Figure D.3 - Log blood lactate concentration versus Log O_2 uptake ($\dot{V}O_2$) for each untrained subject. LABP occurs at intersection of dashed line.

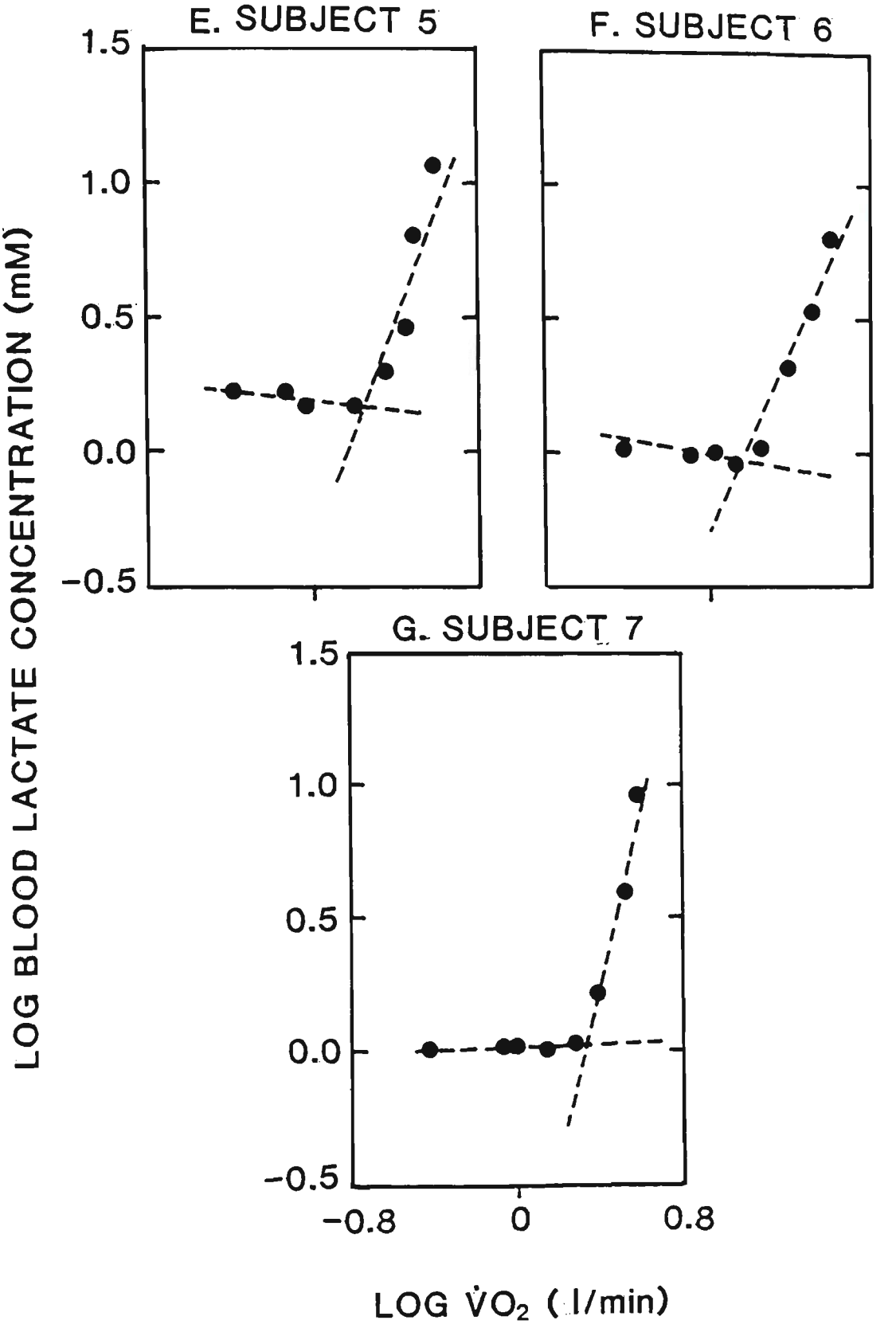

Figure D.3 (Continued)

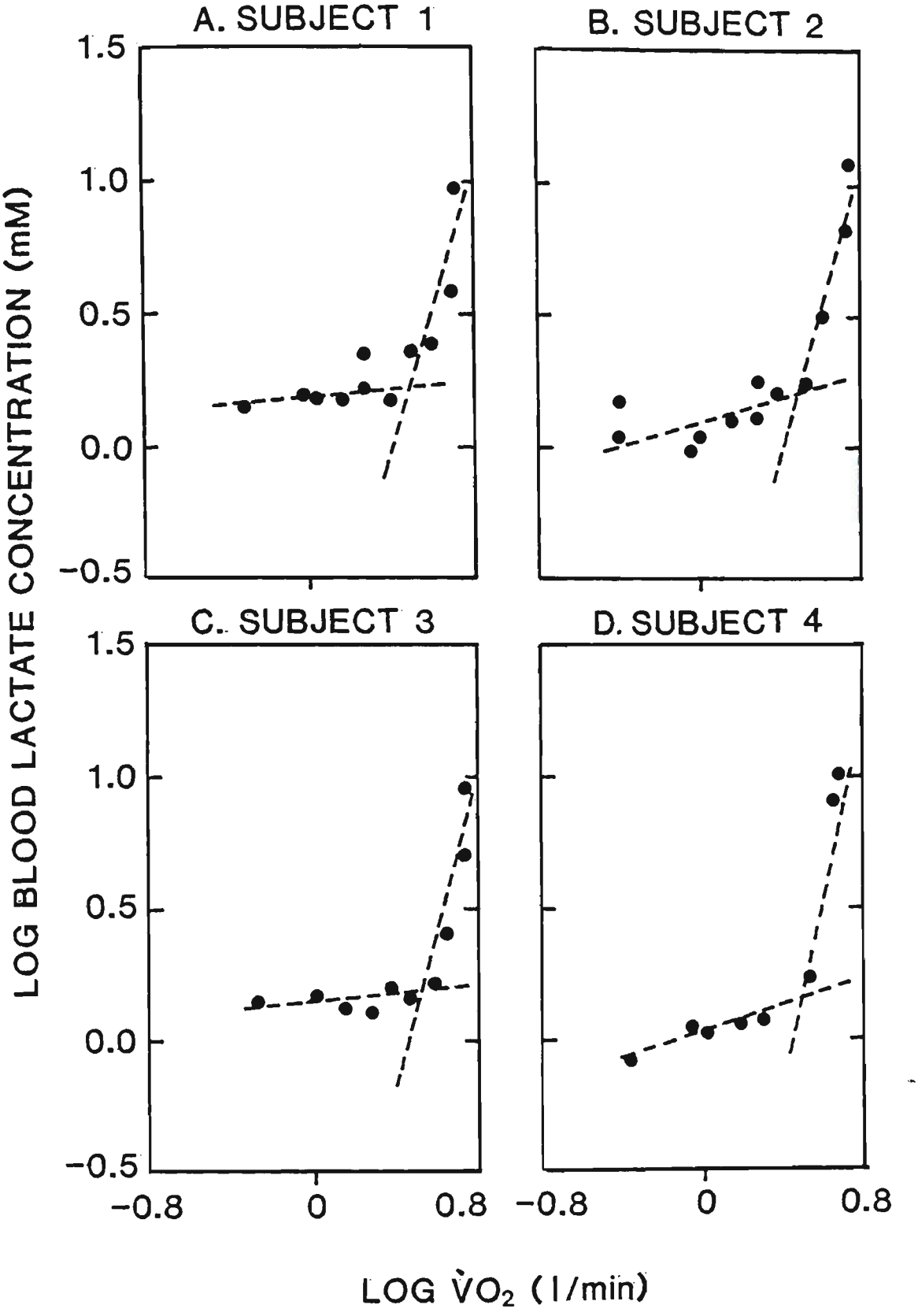

Figure D.4 - Log blood lactate versus log O_2 uptake ($\dot{V}O_2$) for each trained subject. LABP occurs at intersection of dashed line.

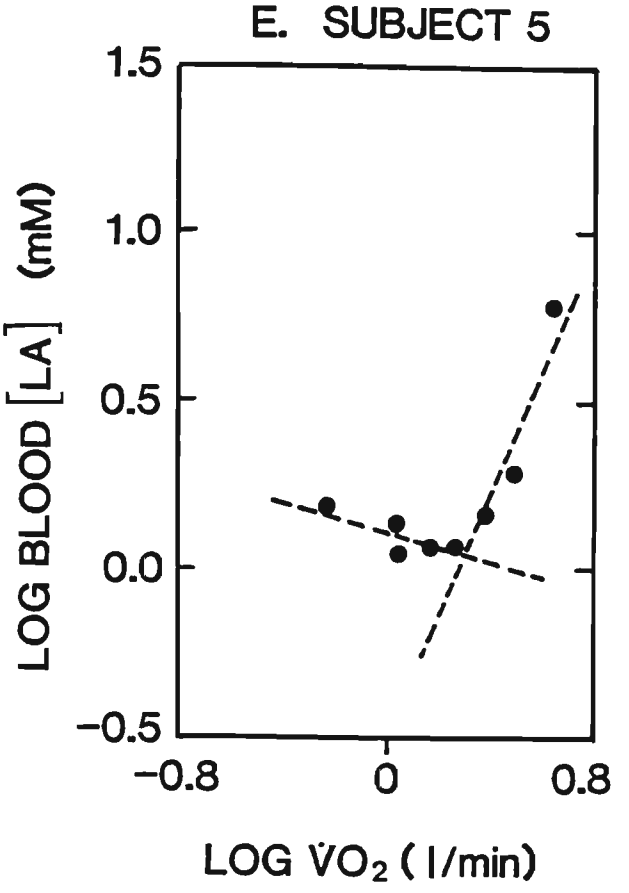

Figure D.4 (Continued)

APPENDIX E

STATISTICAL TABLES

TABLE E.1

STATISTICAL ANALYSIS OF MAIN EFFECTS AND INTERACTIONS USING ANOVA

(Note: probability levels which are reported as 0.00 were actually $\leq$ 0.005).

A. HR DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
Training (A)	1	9.66	19.30	0.00
Error	11	0.498		
Time (B)	11	13572	182.6	0.00
A x B	11	225.5	3.03	0.00
Error	121	74.4		

B. CHANGE IN BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
Training (A)	1	124.8	21.14	0.00
Error	11	5.91		
Time (B)	10	246.5	154.4	0.00
A x B	10	14.8	9.25	0.00
Error	110	1.6		

C. % $\dot{V}O_2$max DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	120.7	2.18	0.17
Error	11	55.4		
Time (B)	4	13988.4	1856.7	0.00
A x B	4	105.0	13.9	0.00
Error	44	7.53		

D. HR DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	3323.1	11.28	0.01
Error	11	294.6		
Time (B)	4	24184.5	643.7	0.01
A x B	4	14.08	0.37	0.83
Error	44	37.57		

E. CHANGE IN PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
Training (A)	1	3817	2.41	0.15
Error	11	1587		
Time (B)	10	10814	70.7	0.00
A x B	10	641.6	4.19	0.00
Error	110	153.0		

F. CHANGE IN PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	1008	3.3	0.10
Error	11	305.3		
Time (B)	3	4120.5	85.44	0.00
A x B	3	179.2	3.71	0.02
Error	33	48.2		

G. CHANGE IN PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	219.3	0.03	0.87
Error	10	7428.6		
Time (B)	3	59142	33.0	0.00
A x B	3	1964	1.09	0.37
Error	30	1795		

H. BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
Training (A)	1	107.1	16.9	0.00
Error	11	6.33		
Time (B)	11	239.4	163.4	0.00
A x B	11	13.4	9.12	0.00
Error	121	1.47		

I. PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
Training (A)	1	7156.5	3.36	0.09
Error	11	2133		
Time (B)	11	10797	71.5	0.00
A x B	11	612.2	4.05	0.00
Error	121	151.1		

J. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	348.8	0.67	0.43
Error	11	521.2		
Time (B)	4	4126.1	80.0	0.00
A x B	4	178.5	3.46	0.02
Error	44	51.6		

K. PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	229.7	0.01	0.91
Error	10	16551		
Time (B)	4	77109	44.6	0.00
A x B	4	1429.9	0.83	0.52
Error	40	1728.7		

L. CHANGE IN BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST

	df	MS	F	P
Training (A)	1	115.4	64.5	0.00
Error	11	1.79		
Time (B)	3	82.7	87.4	0.00
A x B	3	29.6	31.3	0.00
Error	33	0.95		

M. BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
Training (A)	1	120.6	84.6	0.00
Error	11	1.43		
Time (B)	4	105.7	132.3	0.00
A x B	4	27.9	35.02	0.00
Error	44	0.79		

TABLE E.2

STATISTICAL ANALYSIS OF MAIN EFFECTS

AND INTERACTIONS USING ANCOVA

(Note: probability levels which are

reported as 0.00 were actually $\leq$ 0.005)

A. CHANGE IN BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	13.83	2.75	0.14
Training (A)	1	46.9	9.34	0.02
Error	8	5.03		
Time (B)	10	210.9	118.7	0.00
A x B	10	14.5	8.18	0.00
Error	90	1.77		

B. CHANGE IN PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	180.1	0.66	0.44
Training (A)	1	334.4	1.22	0.30
Error	8	274.2		
Time (B)	3	3792.4	84.9	0.00
A x B	3	197.9	4.43	0.01
Error	27	44.7		

C. CHANGE IN PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	3363.3	0.41	0.54
Training (A)	1	44.0	0.01	0.94
Error	8	8211.9		
Time (B)	3	57353.3	29.9	0.00
A x B	3	2046.2	1.07	0.38
Error	27	1912.9		

D. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	1.83	0.00	0.95
Training (A)	1	639.1	1.24	0.30
Error	8	516.9		
Time (B)	4	3864.4	81.98	0.00
A x B	4	197.0	4.18	0.01
Error	36	47.1		

E. PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	22292	1.56	0.25
Training (A)	1	3765.1	0.26	0.62
Error	8	14295		
Time (B)	4	74495	40.69	0.00
A x B	4	1542.8	0.84	0.51
Error	36	1830.8		

F. BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	13.9	2.68	0.14
Training (A)	1	40.2	7.77	0.02
Error	8	5.17		
Time (B)	11	204.7	125.1	0.00
A x B	11	12.9	7.93	0.00
Error	99	1.64		

G. PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	386.2	0.14	0.71
Training (A)	1	5040	1.88	0.21
Error	8	2675		
Time (B)	11	9986	60.9	0.00
A x B	11	557.3	3.40	0.00
Error	99	163.8		

H. CHANGE IN BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	0.17	0.08	0.78
Training (A)	1	69.8	33.6	0.00
Error	8	2.08		
Time (B)	3	75.0	77.6	0.00
A x B	3	28.1	29.1	0.00
Error	27	0.97		

I. CHANGE IN PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	4.16	0.00	0.96
Training (A)	1	4839	3.72	0.09
Error	8	1299		
Time (B)	10	9001	56.2	0.00
A x B	10	805.2	5.03	0.00
Error	90	160.2		

J. BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST.

	df	MS	F	P
%ST fibres (Covariate)	1	1.19	0.86	0.38
Training (A)	1	57.6	41.4	0.00
Error	8	1.39		
Time (B)	4	94.6	115.5	0.00
A x B	4	26.3	32.1	0.00
Error	36	0.82		

TABLE E.3

ONE-WAY ANOVA SUMMARY TABLES

(Note: probability levels which are reported as 0.00 were actually $\leq$ 0.005)

A. % $\dot{V}O_2$ max DURING SUBMAXIMAL EXERCISE TEST: TRAINED

	df	MS	F	P
Time	5	5293.4	1323	0.00
Error	25	3.99		

B. % $\dot{V}O_2$ max DURING SUBMAXIMAL EXERCISE TEST: UNTRAINED

	df	MS	F	P
Time	4	7852.9	790	0.00
Error	24	9.94		

C. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST: TRAINED

	df	MS	F	P
Time	5	1664.9	58.01	0.00
Error	25	28.7		

D. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST: UNTRAINED

	df	MS	F	P
Time	4	3026.3	55.39	0.00
Error	24	54.6		

E. PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST: TRAINED

	df	MS	F	P
Time	5	52656	20.95	0.00
Error	25	2512.3		

F. PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST: UNTRAINED

	df	MS	F	P
Time	4	34314	19.15	0.00
Error	20	1791.8		

G. BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST: TRAINED

	df	MS	F	P
Time	5	12.6	21.49	0.00
Error	25	0.59		

H. BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST: UNTRAINED

	df	MS	F	P
Time	4	129.1	126.8	0.00
Error	24	1.02		

I. PLASMA [NH_3] DURING INCREMENTAL EXHAUSTIVE EXERCISE TEST: TRAINED

	df	MS	F	P
Time	12	3589	24.3	0.00
Error	60	147.9		

J. PLASMA [NH_3] DURING INCREMENTAL EXHAUSTIVE EXERCISE TEST: UNTRAINED

	df	MS	F	P
Time	11	8536	45.6	0.00
Error	66	187.2		

TABLE E.4

SIMPLE MAIN EFFECTS ANALYSIS USING ANOVA

(Note: probability levels which are reported as 0.00 were actually $\leq$ 0.005)

A. HR DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS tr	MS error	F	P
Rest	1	532.1	216.6	2.46	0.15
UP	1	111.7	109.8	1.02	0.33
50 W	1	546.0	83.3	6.56	0.03
100 W	1	508.7	66.8	7.62	0.02
150 W	1	2073	44.1	47.0	0.00
200 W	1	2537	72.1	35.2	0.00
250 W	1	2629	125.0	21.1	0.00
300 W	1	1839	163.0	11.3	0.01
Rec 2	1	534.1	222.2	2.40	0.15
Rec 5	1	270.1	81.5	3.31	0.10
Rec 10	1	456.0	50.0	9.12	0.01
Rec 20	1	97.7	81.4	1.20	0.30
Error	11				

B. CHANGE IN BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS tr	MS error	F	P
UP	1	0.009	0.037	0.25	0.63
50 W	1	0.017	0.038	0.46	0.51
100 W	1	0.0004	0.064	0.01	0.94
150 W	1	0.12	0.074	1.66	0.22
200 W	1	2.62	0.15	17.3	0.00
250 W	1	28.9	1.19	24.2	0.00
300 W	1	144.5	2.05	70.5	0.00
Rec 2	1	1.14	4.86	0.23	0.64
Rec 5	1	23.4	4.63	5.06	0.05
Rec 10	1	37.1	5.37	6.89	0.02
Rec 20	1	34.8	3.4	10.2	0.01
Error	11				

C. CHANGE IN PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS tr	MS error	F	P
UP	1	3.66	1.10	3.32	0.10
50 W	1	1.06	9.33	0.11	0.74
100 W	1	7.74	31.3	0.25	0.63
150 W	1	107.4	43.7	2.45	0.15
200 W	1	15.4	64.2	0.24	0.63
250 W	1	333.8	181.1	1.84	0.20
300 W	1	2412	448.9	5.37	0.04
Rec 2	1	255.8	634.1	0.40	0.54
Rec 5	1	3598	689.0	5.22	0.04
Rec 10	1	2947	716.0	4.12	0.07
Rec 20	1	549.8	297.5	1.85	0.20
Error	11				

D. CHANGE IN PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS tr	MS error	F	P
Min 3	1	104.9	25.6	4.1	0.07
Min 6	1	8.1	38.1	0.21	0.65
Min 9	1	262.9	128	2.05	0.18
Min 12	1	1169.5	258.3	4.53	0.06
Error	11				

E. % $\dot{V}O_2$max DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS tr	MS error	F	P
Rest	1	14.8	3.39	4.36	0.06
Min 3	1	75.5	32.2	2.34	0.15
Min 6	1	0.32	14.7	0.02	0.89
Min 9	1	203.9	18.3	11.1	0.01
Min 12	1	246.3	16.8	14.7	0.00
Error	11				

F. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS tr	MS error	F	P
Rest	1	17.9	54.7	0.33	0.58
Min 3	1	36.2	63.8	0.57	0.48
Min 6	1	1.93	73.2	0.03	0.87
Min 9	1	143.7	202.9	0.71	0.42
Min 12	1	863.2	332.9	2.59	0.14
Error	11				

G. BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS tr	MS error	F	P
Rest	1	0.38	0.14	2.70	0.13
Min 3	1	0.21	0.59	0.36	0.56
Min 6	1	6.09	1.70	3.57	0.09
Min 9	1	79.5	1.04	76.6	0.00
Min 12	1	146.4	1.15	127.6	0.00
Error	11				

H. BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS tr	MS error	F	P
Rest	1	0.003	0.059	0.04	0.84
UP	1	0.002	0.06	0.03	0.86
50 W	1	0.007	0.04	0.16	0.69
100 W	1	0.005	0.06	0.08	0.78
150 W	1	0.16	0.08	1.92	0.19
200 W	1	2.80	0.19	14.6	0.00
250 W	1	29.5	1.35	21.0	0.00
300 W	1	145.7	2.27	64.3	0.00
Rec 2	1	2.09	7.08	0.29	0.60
Rec 5	1	19.7	4.31	4.58	0.06
Rec 10	1	29.0	4.64	6.3	0.03
Rec 20	1	25.1	2.34	10.7	0.01
Error	11				

I. PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS tr	MS error	F	P
Rest	1	53.8	23.0	2.33	0.15
UP	1	85.8	30.1	2.85	0.12
50 W	1	39.9	38.9	1.02	0.33
100 W	1	20.9	47.9	0.43	0.52
150 W	1	9.10	50.9	0.18	0.68
200 W	1	11.7	83.7	0.14	0.72
250 W	1	656.3	206.1	3.18	0.10
300 W	1	3188	561.2	5.68	0.04
Rec 2	1	543.4	789.8	0.69	0.42
Rec 5	1	4533	784.5	5.78	0.04
Rec 10	1	3799	828.9	4.58	0.06
Rec 20	1	948.4	349.8	2.71	0.13
Error	11				

J. CHANGE IN BLOOD [LA] DURING SUBMAXIMAL TEST.

Time	df	MS tr	MS error	F	P
Min 3	1	0.024	0.69	0.03	0.86
Min 6	1	3.44	1.77	1.94	0.19
Min 9	1	68.9	1.07	64.2	0.00
Min 12	1	131.9	1.09	120.9	0.00
Error	11				

TABLE E.5

SIMPLE MAIN EFFECTS ANALYSIS USING ANCOVA

(Note: probabilities reported as 0.00

were actually $\leq$ 0.005)

A. CHANGE IN BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE, EXERCISE TEST.

Workload	df	MS cv	MS tr	MS error	F tr	P
UP	1	0.005	0.0004	0.048	0.01	0.93
50 W	1	0.05	0.00009	0.043	0.00	0.96
100 W	1	0.004	0.002	0.068	0.03	0.86
150 W	1	0.008	0.08	0.09	0.85	0.38
200 W	1	0.07	1.37	0.16	8.66	0.02
250 W	1	0.26	14.2	1.55	9.12	0.02
300 W	1	0.20	79.4	2.57	30.9	0.00
Rec 2	1	19.5	2.29	3.66	0.63	0.45
Rec 5	1	15.4	3.73	3.61	1.03	0.34
Rec 10	1	6.35	11.7	6.2	1.88	0.21
Rec 20	1	0.49	23.3	3.46	6.75	0.03
Error	8					

B. CHANGE IN PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS cv	MS tr	MS error	F tr	P
Min 3	1	2.28	28.4	24.2	1.17	0.31
Min 6	1	131.4	17.3	22.5	0.77	0.41
Min 9	1	56.3	133.5	122.1	1.09	0.33
Min 12	1	40.5	568.4	249.9	2.27	0.17
Error	8					

C. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS cv	MS tr	MS error	F tr	P
Rest	1	23.83	17.3	36.7	0.47	0.51
Min 3	1	11.4	90.2	74.2	1.21	0.30
Min 6	1	43.2	0.00	63.3	0.00	0.99
Min 9	1	6.88	247	183.3	1.35	0.28
Min 12	1	4.32	736.7	360.5	2.04	0.19
Error	8					

D. BLOOD [LA] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS cv	MS tr	MS error	F	P
Rest	1	0.001	0.006	0.07	0.08	0.79
UP	1	0.002	0.003	0.07	0.04	0.85
50 W	1	0.03	0.004	0.05	0.08	0.78
100 W	1	0.0009	0.0008	0.06	0.01	0.91
150 W	1	0.003	0.13	0.089	1.41	0.27
200 W	1	0.09	1.56	0.16	9.59	0.01
250 W	1	0.29	14.7	1.71	8.62	0.02
300 W	1	0.22	80.7	2.82	28.7	0.00
Rec 2	1	24.9	2.23	5.63	0.40	0.55
Rec 5	1	16.2	2.48	3.04	0.81	0.39
Rec 10	1	5.56	8.81	5.31	1.66	0.23
Rec 20	1	0.24	18.1	2.18	8.31	0.02
Error	8					

E. PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS cv	MS tr	MS error	F	P
Rest	1	0.53	19.7	29.2	0.67	0.44
UP	1	2.98	41.4	38.4	1.08	0.33
50 W	1	10.1	22.4	47.9	0.47	0.51
100 W	1	8.46	8.05	58.1	0.14	0.72
150 W	1	0.07	10.9	66.7	0.16	0.70
200 W	1	0.78	4.15	100.4	0.04	0.84
250 W	1	119.8	658.2	216.4	3.04	0.12
300 W	1	236.5	2760	726.8	3.80	0.09
Rec 2	1	18.6	140.2	990.9	0.14	0.72
Rec 5	1	6.07	2469	876.2	2.82	0.13
Rec 10	1	378.2	3114	1043	2.99	0.12
Rec 20	1	208.9	1104	432.5	2.55	0.15
Error	8					

F. CHANGE IN PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST.

Workload	df	MS cv	MS tr	MS error	F	P
UP	1	0.90	4.15	1.30	3.18	0.11
50 W	1	0.45	1.78	4.65	0.38	0.55
100 W	1	3.85	0.62	7.13	0.09	0.78
150 W	1	17.7	31.3	23.7	1.32	0.28
200 W	1	24.4	0.29	29.9	0.01	0.92
250 W	1	6.9	654.5	103.1	6.35	0.04
300 W	1	75.8	2655	461.1	5.76	0.04
Rec 2	1	229.4	174.5	693.8	0.25	0.63
Rec 5	1	125.4	2774	620.2	4.47	0.07
Rec 10	1	89.5	3213	801.4	4.01	0.08
Rec 20	1	68.4	1020	275.8	3.70	0.09
Error	8					

G. CHANGE IN BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST.

Time	df	MS cv	MS tr	MS error	F	P
Min 3	1	0.62	0.03	0.84	0.04	0.85
Min 6	1	0.06	2.34	2.41	0.97	0.35
Min 9	1	0.13	43.8	1.02	43.0	0.00
Min 12	1	0.31	70.2	0.96	73.4	0.00
Error	8					

H. BLOOD [LA] DURING SUBMAXIMAL EXERCISE

Time	df	MS cv	MS tr	MS error	F	P
Rest	1	0.43	0.003	0.14	0.02	0.89
Min 3	1	0.02	0.06	0.76	0.07	0.79
Min 6	1	0.17	2.50	2.32	1.08	0.33
Min 9	1	0.08	44.5	0.91	48.7	0.00
Min 12	1	1.48	71.1	0.82	86.6	0.00
Error	8					

TABLE E.6

NEWMAN-KEULS POST-HOC ANALYSIS

A. % $\dot{V}O_2max$ DURING SUBMAXIMAL EXERCISE TEST: TRAINED

Time		0	3	12	15	9	6	r	q	cv
	$\bar{x}$	8.82	78.93	81.38	81.42	82.28	83.40			
0	8.82		70.11*	72.56*	72.60*	73.46*	74.58	6	4.37	3.58
3	78.93			2.45*	2.49	3.35	4.47*	5	4.17	3.42
12	81.38				0.04	0.90	2.02	4	3.90	3.20
15	81.42					0.86	1.98	3	3.53	2.89
9	82.28						1.12	2	2.92	2.39
6	83.40									

B. %$\dot{V}O_2$max DURING SUBMAXIMAL EXERCISE: UNTRAINED

Time		0	3	6	12	9	r	q	cv
	$\bar{x}$	10.96	74.10	83.10	90.10	90.20			
0	10.96		63.14*	72.14*	79.14*	79.24*	5	4.17	4.96
3	74.10			9.00*	16.00*	16.10*	4	3.90	4.64
6	83.10				7.00*	7.10*	3	3.53	4.20
12	90.10					0.10	2	2.92	3.47
9	90.2								

C. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE TEST: TRAINED

Time		3	0	6	9	12	15	r	q	cv
	$\bar{x}$	14.48	15.47	24.32	39.32	47.18	53.57			
3	14.48		0.99	9.84*	24.84*	32.7*	39.09*	6	4.37	9.56
0	15.47			8.85*	23.85*	31.71*	38.10*	5	4.17	9.12
6	24.32				15.00*	22.86*	29.30*	4	3.90	8.53
9	39.32					7.86*	14.25*	3	3.53	7.72
12	47.18						6.39*	2	2.92	6.39
15	53.57									

D. PLASMA [NH_3] DURING SUBMAXIMAL EXERCISE: UNTRAINED

Time		0	3	6	9	12	r	q	cv
	$\bar{x}$	13.11	17.83	23.54	45.99	62.10			
0	13.11		4.72	10.43*	32.88*	48.99*	5	4.17	11.63
3	17.83			5.71	28.16*	44.27*	4	3.90	10.88
6	23.54				22.45*	38.56*	3	3.53	9.85
9	45.99					16.11*	2	2.92	8.15
12	62.10								

E. PLASMA [ALA] DURING SUBMAXIMAL EXERCISE TEST: TRAINED

Time		0	3	6	9	12	15	r	q	cv
	$\bar{x}$	360.5	387.8	448.1	512.0	567.8	586.7			
0	360.5		27.30	87.60*	151.5*	207.3*	226.2*	6	4.37	89.6
3	387.8			60.30*	124.2*	180.0*	198.9*	5	4.17	85.5
6	448.1				63.90*	119.7*	138.6*	4	3.90	79.95
9	521.0					55.80	74.70*	3	3.53	72.40
12	567.8						18.90	2	2.92	59.9
15	586.7									

F. PLASMA [ALA] DURING SUBMAXIMAL EXERCISE: UNTRAINED

Time		0	3	6	9	12	r	q	cv
	$\bar{x}$	367.6	407.0	449.8	535.2	535.9			
0	367.6		30.40	73.20*	158.6*	159.3*	5	4.23	73.2
3	407.0			42.80	128.2*	128.9*	4	3.96	68.5
6	449.8				85.40*	86.10*	3	3.58	61.9
9	535.2					0.70	2	2.95	51.0
12	535.9								

G. BLOOD [LA] DURING SUBMAXIMAL EXERCISE: TRAINED

Time		0	3	6	9	15	12	r	q	cv
	$\bar{x}$	0.87	2.00	3.40	4.16	4.34	4.38			
0	0.87		1.13*	2.53*	3.29*	3.47*	3.51*	6	4.37	1.37
3	2.00			1.40*	2.16*	2.34*	2.38*	5	4.17	1.31
6	3.40				0.76	0.94	0.98	4	3.90	1.22
9	4.16					0.94	0.98	3	3.53	1.11
15	4.34						0.04	2	2.92	0.92

H. BLOOD [LA] DURING SUBMAXIMAL EXERCISE TEST: UNTRAINED

Time		0	3	6	9	12	r	q	cv
	$\bar{x}$	1.21	2.25	4.77	9.12	11.11			
0	1.21		1.04*	3.56*	7.91*	9.90*	5	14.7	1.58
3	2.25			2.52*	6.87*	8.86*	4	3.90	1.48
6	4.77				4.35*	6.34*	3	3.53	1.34
9	9.12					1.99*	2	2.92	1.10
12	11.11								

I. PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST: TRAINED

Workload		Up	Rest	50W	100W	150W	200W	250W	300W	Rec20	350W	Rec10	Rec5	400W	Rec2	r	q(r,65)	c.v.
	x	11.3	11.7	14.1	15.3	20.0	23.8	27.8	39.3	42.7	51.8	58.7	72.1	73.9	75.8			
Up	11.3		0.4	2.8	4.0	8.7	12.5	16.5	28.0*	31.4*	40.5*	47.4*	60.8*	62.6*	64.5*	14	4.94	23.9
Rest	11.7			2.4	3.6	8.3	12.1	16.1	27.6*	31.0*	40.1*	47.0*	60.4*	62.2*	64.2*	13	4.88	23.62
50W	14.1				1.2	5.9	9.7	13.7	25.2*	28.6*	37.7*	44.6*	58.0*	59.8*	61.7*	12	4.81	23.30
100W	15.3					4.7	8.5	12.5	24.0*	27.4*	36.5*	43.4*	56.8*	58.6*	60.5*	11	4.73	22.89
150W	20.0						3.8	7.9	19.4*	22.7*	31.9*	38.8*	52.1*	53.9*	55.9*	10	4.65	22.51
200W	23.8							4.0	15.5	18.9*	28.0*	34.9*	48.3*	50.1*	52.0*	9	4.55	22.02
250W	27.8								11.5	14.9	24.0*	30.9*	44.3*	46.0*	50.0*	8	4.44	21.49
300W	39.3									3.4	12.5	19.4*	32.8*	34.6*	36.5*	7	4.31	20.86
Rec20	42.7										9.1	16.1	29.4*	31.2*	33.2*	6	4.16	20.13
350W	51.8											6.9	20.3*	22.1*	24.0*	5	3.98	19.26
Rec10	58.7												13.4	15.1	17.1	4	3.74	18.10
Rec5	72.1													1.8	3.7	3	3.40	16.46
400W	73.9														2.0	2	2.83	13.70
Rec2	75.8																	

J. PLASMA [NH_3] DURING INCREMENTAL, EXHAUSTIVE EXERCISE TEST: UNTRAINED

Workload		Rest	Up	50W	100W	150W	200W	250W	Rec20	300W	Rec2	Rec10	Rec5	r	q(r,66)	c.v.
	$\bar{x}$	15.8	16.5	17.6	17.9	18.3	25.7	42.1	59.8	70.7	88.8	93.0	109.5			
Rest	15.8		0.7	1.8	2.1	2.5	9.9	26.3*	44.0*	54.9*	73.0*	77.2*	93.8*	12	4.81	24.87
Up	16.5			1.1	1.4	1.8	9.2	25.6*	43.3*	54.2*	72.3*	76.5*	93.0*	11	4.73	24.46
50W	17.6				0.3	0.7	8.1	24.5*	42.2*	53.1*	71.2*	75.4*	91.9*	10	4.65	24.05
100W	17.9					0.4	7.8	24.2*	41.9*	52.9*	70.9*	75.2*	91.7*	9	4.55	23.53
150W	18.3						7.4	23.8*	41.5*	52.4*	70.5*	74.8*	91.3*	8	4.44	22.96
200W	25.7							16.4*	34.1*	45.0*	63.1*	67.3*	83.8*	7	4.31	22.29
250W	42.1								17.7*	28.6*	46.7*	50.9*	67.5*	6	4.16	21.51
Rec20	59.8									10.9	29.0*	33.2*	49.7*	5	3.98	20.58
300W	70.7										18.1*	22.3*	38.8*	4	3.74	19.34
Rec2	88.8											4.2	20.8*	3	3.40	17.58
Rec10	93.0												16.5*	2	2.83	14.63
Rec5	109.5															