





**THE EFFECT OF SPRINT TRAINING ON HUMAN SKELETAL MUSCLE  
PURINE NUCLEOTIDE METABOLISM**



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## ABSTRACT

The major focus of this thesis was to examine the effect of sprint training on skeletal muscle purine nucleotide (PnN) metabolism. Specifically, this thesis studied the effects of sprint training on skeletal muscle PnN and metabolites of purine catabolism at rest, during a 30 s "all out" cycle bout and after 3 min of recovery. Furthermore, the accumulation of purine degradation products in the plasma (ammonia, hypoxanthine) during exercise, and in recovery from a 30 s "all out" sprint was also examined. Finally, this thesis studied the influence of sprint training on performance variables pertinent to sprint exercise.

Eight untrained subjects (six male and two female), performed a 30 s maximal sprint bout on a bicycle ergometer before and after a seven week intense sprint training regime. Venous blood was sampled at rest, after exercise and during recovery from the maximal sprint bout in all subjects. Muscle biopsies were obtained from six of the subjects at rest, immediately after and 3 min into recovery from exercise.

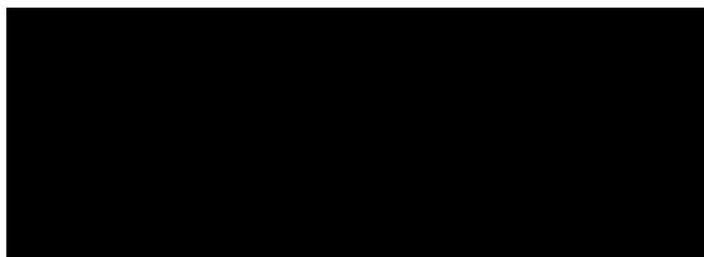
After sprint training there was; 1) a reduction in the resting muscle ATP content, whilst the concentration of the other metabolites measured did not change; 2) a decrease in the magnitude of skeletal muscle ATP degradation and the accumulation of IMP and ammonia during a 30 s "all out" sprint bout; 3) a fall in the level of IMP and the accumulation of inosine 3 min after a 30 s sprint; 4) an increased plasma ammonia concentration 2 min post exercise; 5) an attenuation of the plasma hypoxanthine concentration 45 and 60 min into recovery; 6) an increase in peak power, mean power and total work for a 30 s sprint.

The decreased ATP and adenine nucleotide content in skeletal muscle was probably a result of the acute effects of the exercise performed in the sprint training regime, however a training induced downregulation of the ATP stores cannot be

excluded. The training-induced reduction in the magnitude of ATP depletion during a 30 s sprint bout reflects an improved balance between ATP hydrolysis and resynthesis, resulting in a decreased accumulation of IMP and ammonia. The mechanism leading to this improved balance is unknown. Ultimately, these metabolic adaptations are likely to result in smaller purine loss from sprint trained muscle.

## DECLARATION

This dissertation summarises original, previously unpublished work conducted at the Victoria University of Technology in the Departments of Chemistry and Biology and Physical Education and Recreation. With the exception of data collection which required collaboration and invasive procedures which were conducted by qualified medical personnel, this dissertation is the result of work performed solely by the author.



Christos G. Stathis

## PREFACE

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# CHAPTER 1

## INTRODUCTION

Skeletal musculature is a component of the largest organ system in the body and is specialised for contraction, hence there are a number of biochemical processes which supply energy for this function. The variability in the metabolic responses of the muscle over a large range of exercise intensities, and its adaptability to training and other stresses demonstrates the plasticity of muscle.

The total adenine nucleotide (AdN) pool is defined as the sum of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) and adenosine 5'-monophosphate (AMP) concentrations (Katz et al. 1986a), and the metabolism of these compounds is essential to the role of the muscle as a transducer of chemical energy to mechanical work. Precise regulation of muscle AdN metabolism is imperative to enable the performance of high intensity exercise. Stored ATP represents the immediate source of muscular energy, and its hydrolysis liberates energy which is used by the myofilaments to power muscle contraction. During high intensity exercise, the ATP hydrolysis rate exceeds the capacity for its resynthesis producing an increase in free ADP (fADP) (Fig. 2.1; Tullson and Terjung, 1991a). Subsequently, a series of reactions catalysed by myokinase and AMP deaminase (AMPd) rapidly degrade ADP and AMP, respectively, resulting in the formation of inosine 5'-monophosphate (IMP) and ammonia<sup>1</sup> (Fig. 2.1, Graham et al. 1990; Jansson et al. 1987; Katz et al. 1986a&b; Lowenstein, 1972). With intense exercise, muscle IMP and ammonia accumulation are closely matched to the magnitude of the ATP degradation (Graham et al. 1990; Jansson et al. 1987, Katz et al. 1986a). This demonstrates that AMPd is the predominant pathway for AdN degradation. IMP production, enables the muscle fibre to maintain low ADP and AMP concentrations with respect to ATP. This helps to maintain the energy charge ( $EC = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$ ) within the muscle (Atkinson, 1968; Chapman and Atkinson, 1973; Coffee and Solano, 1977; Sahlin et al. 1978; Solano

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<sup>1</sup> Throughout this thesis the term ammonia is used to refer to the sum of ammonia (NH<sub>3</sub>) and ammonium (NH<sub>4</sub><sup>+</sup>). The symbols NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> will refer to the non-protonated and protonated forms, respectively.

and Coffee, 1978) to sustain muscle contraction.

A small proportion of the IMP produced may be degraded to inosine, which can be further catabolised to hypoxanthine (Fig. 2.1; Tullson and Terjung, 1991a). Hypoxanthine can diffuse across the sarcolemma into the plasma and represents a loss of purine base from the muscle (Bangsbo et al. 1992). Purine bases lost from the muscle cell are replenished by the AdN *de novo* biosynthesis pathway which is a slow, energy consuming process in comparison with the purine salvage pathway (Tullson and Terjung, 1991a). The purine salvage pathway provides a mechanism for the retention of AdN within the muscle via the conversion of hypoxanthine to IMP. Further reamination of IMP to AMP in the purine nucleotide cycle (PNC) (Fig. 2.1; Lowenstein, 1972), and subsequent phosphorylation substantially restores the resting levels of ATP. Hence the purine salvage pathway and the PNC reduce the potential loss of AdN from skeletal muscles.

Sprint exercise places a large metabolic stress on skeletal muscle. The magnitude of AdN decrease, particularly ATP, after 30 s of sprint exercise is around 30-40% of resting levels (Boobis et al. 1983a,b; Nevill et al. 1989; Withers et al. 1991). Although the products of AdN degradation have not been measured during "all out" exercise, such a decrease in ATP should lead to an accumulation of muscle IMP and ammonia. Subsequent degradation should lead to increased inosine and hypoxanthine in the muscle during recovery. This hypothesis is supported by increases in plasma ammonia (Itoh et al. 1990; Snow et al. 1992) and hypoxanthine concentration (Hellsten-Westring et al. 1991, Stathis et al. 1992) during recovery from maximal sprinting.

Sprint training involves repeated bouts of intense exercise which induces a large stress on muscle AdN metabolism (Balsom et al. 1992; McCartney et al. 1986). A number of studies have examined the effects of sprint training on various components of purine nucleotide (PnN) metabolism in man (Boobis et al. 1983b; Hellsten-Westring et al. 1993; Nevill et al. 1989; Sjödín and Hellsten-Westring, 1991; Snow et al. 1992; Stathis et al. 1992; Thorstensson et al. 1975). As yet, however, no study has simultaneously examined the metabolites produced with AdN degradation in human skeletal muscle during, and in recovery from, a maximal

exercise bout, before and after an intense sprint training regime. Information from such a study would provide greater insight into the possible adaptation of PnN metabolism to an intermittent metabolic stress.

This thesis examined the hypothesis that sprint training influences PnN metabolism during a maximal sprint bout. Specifically, this thesis examined the effect of sprint training on:

1. resting concentrations of AdN and their degradation products in skeletal muscle.
2. the change in concentrations of AdN and their degradation products within skeletal muscle during a 30 s sprint and after 3 min of recovery.
3. the accumulation of ammonia and purine bases in the plasma during recovery from a 30 s sprint.

Additionally, the performance variables during an "all out" 30 s cycle sprint were investigated to ascertain the effect of the sprint training regime employed.

## CHAPTER 2

### REVIEW OF LITERATURE

#### 2.1 **Introduction**

This review presents literature relevant to the biochemical pathways of AdN degradation and the recovery/synthesis of the AdN pool in skeletal muscle, focusing on maximal sprint exercise and recovery. For the purpose of this review, sprint or "all out" exercise refers to a maximal voluntary exertion over a specified duration. Where evidence is unavailable on "all out" exercise, other studies are reviewed which utilise high intensity exercise close to the nature of maximal sprinting. In addition, the review focuses on sprint training, with respect to muscle PnN metabolism and the degradation products of AdN which accumulate in the blood during, and in recovery from intense exercise.

#### 2.2. **Adenine Nucleotide and Purine Degradation Pathways.**

During intense muscular contraction the rate of ATP hydrolysis exceeds the rate of its resynthesis. Under such circumstances, there is an increase in the production of fADP and a cascade of reactions which subsequently result in a reduction of the AdN pool (Tullson and Terjung, 1991a). The decrease in ATP concentration observed during high intensity exercise results in transient increases in fADP and fAMP which are subsequently removed by the action of myokinase and AMP catabolic enzymes (AMPd and 5'-nucleotidase), respectively (Fig 2.1). The myokinase reaction involves the transfer of a phosphate moiety from one ADP molecule to another. The end result is the production of a molecule of both ATP and AMP. Subsequent degradation of AMP can be effected by AMPd, which rapidly deaminates AMP to produce IMP and ammonia (Fig 2.1; Lowenstein 1972). Studies demonstrating an equimolar increase in IMP and ammonia relative to the decrease in ATP during intense exercise (Graham et al. 1990; Jansson et al. 1987; Katz et al. 1986a; Sahlin et al. 1978), clearly indicate that AMP deamination is the major pathway for AMP catabolism. Dephosphorylation of AMP by 5'-nucleotidase to produce adenosine and orthophosphate can occur (Fig 2.1; Meghji et al. 1988;

Rubio et al. 1973; Truong et al. 1988), however this pathway is relatively inactive in skeletal muscle (Sabina et al. 1984; Schopf et al. 1986, Tullson and Terjung, 1990; Zoref-Shani et al. 1987).

The high level of activity of the myokinase and AMP deaminase enzymes ensure that changes in fADP and fAMP concentrations are small and transient, which effectively maintains a high ATP/ADP ratio in the muscle under stress (Lowenstein, 1990; Tullson and Terjung, 1990). This maintenance of low ADP and AMP concentrations is important for muscle function due to their effect on the energy charge (Atkinson et al. 1968; Cooke and Pate, 1985; Dawson et al. 1980; Lowenstein, 1972; Sahlin and Katz, 1988).

The IMP produced within skeletal muscle has two potential fates, viz: reamination to AMP via reamination in the purine nucleotide cycle (PNC) (Lowenstein 1972) and dephosphorylation to inosine by 5'-nucleotidase (Fig. 2.1; Meghji et al. 1988; Schütz et al. 1981). During high intensity exercise there is little, if any, reamination of IMP (Meyer and Terjung, 1980) and only a small amount of the IMP produced is dephosphorylated to inosine during exercise and subsequent recovery (Sabina et al. 1984; Tullson and Terjung, 1990). Inosine can be further oxidized to hypoxanthine by purine nucleoside phosphorylase (PNP) (Schopf et al. 1986; Sinkeler et al. 1986; Sjödin and Hellsten-Westing, 1991; Tullson and Terjung, 1991a). Both inosine (Harkness et al. 1983, Sabina et al. 1984) and hypoxanthine (Bangsbo et al. 1992; Harkness et al. 1983; Hellsten-Westing et al. 1989; Sutton et al. 1980) can diffuse out of the muscle into the blood. From muscle hypoxanthine efflux data, Bangsbo et al. (1992) estimate that at least 5% of the fall in the AdN pool can be lost from the muscle after an intense exercise bout. Hypoxanthine can be further oxidised to uric acid in the capillary endothelial cells (Hellsten-Westing, 1991) or the liver (Newsholme and Leech, 1983). Uric acid is removed from the blood by the kidney (Newsholme and Leech, 1983) and subsequently excreted (Nasrallah and Al-Khalidi, 1964), thus representing a loss of purine base from the muscle.

## 2.2.1 Regulation of Adenine Nucleotide and Purine Degradation Pathways

### 2.2.1.1 AMP deaminase

An important step in the regulation of AdN metabolism in skeletal muscle is the AMPd pathway (Fig. 2.1). The activity of AMPd in skeletal muscle is higher than that of any other tissue (Lowenstein, 1972). The regulation of AMP deaminase is complex and involves isozyme variation, substrate availability, allosteric control and myosin binding.

Two distinctively different isozymes have been isolated in skeletal muscle of rabbits (Ogasawara et al. 1983; Raggi et al. 1975), rats (Raggi et al. 1975) and humans (Kaletha et al. 1987; Kaletha and Nowak, 1988; Ogasawara et al. 1982; Raggi et al. 1975). Raggi et al. (1975) have adopted a nomenclature wherein these isozymes are identified as isoform A and B. Fast twitch (FT) fibres contain only the B isoform, whilst slow twitch (ST) fibres contain both isoforms (Raggi et al. 1975).

Allosteric regulation is another factor likely to play a role in the activity of AMPd. Many *in vitro* studies have investigated potential modulators of AMPd and have provided some insight as to which metabolites may regulate the *in vivo* activity in skeletal muscle (Ashby and Frieden, 1978; Barshop and Frieden, 1984; Raggi and Ranieri-Raggi, 1987; Ronca-Testoni et al. 1970; Smiley and Suelter, 1967; Wheeler and Lowenstein, 1979). AMPd is inhibited by physiological levels of GTP (Ashby and Frieden, 1978; Barshop and Frieden, 1984; Raggi and Ranieri-Raggi, 1987; Ronca-Testoni et al. 1970; Wheeler and Lowenstein, 1979) and inorganic phosphate (Pi) (Raggi and Ranieri-Raggi, 1987; Ronca-Testoni et al. 1970; Wheeler and Lowenstein, 1979). Additionally, ATP inhibits AMPd at low concentrations but at higher concentrations, in the physiological range, this inhibition is diminished substantially (Ashby and Frieden, 1978; Raggi and Ranieri-Raggi, 1987; Ronca-Testoni et al. 1970; Wheeler and Lowenstein, 1979). This apparent conflict can be explained by the presence of two binding sites on the enzyme with different affinities for nucleotides. Binding of ATP to the high affinity site reduces activity whilst binding to the low affinity site alleviates this inhibition (Ashby and Frieden 1978). Furthermore, it has been highlighted that the two isoforms exhibit different regulatory properties at physiological concentrations of ATP (Barsacchi et al. 1979).

These authors suggest that the A isoform is constantly in the activated state as a result of its activation by ATP, whereas the B isoform is inhibited by ATP and will become more active during intense exercise when ATP levels decrease within the cell.

Positive modulation of AMPd by fAMP, fADP and  $H^+$  has been reported (Ronca-Testoni et al. 1970; Wheeler and Lowenstein, 1979). These findings are further supported by several reports demonstrating that a decreased energy charge results in the activation of this enzyme (Coffee and Solano, 1977). The most effective metabolite activating AMPd is likely to be ADP, which acts by reducing the inhibition of ATP, GTP and Pi (Ronca-Testoni et al. 1970; Rundell et al. 1992b; Wheeler and Lowenstein, 1979). An increase in fADP, however, is not the only change which occurs with decreasing energy charge during metabolic stress. A concurrent decrease in muscle pH usually occurs and may also enhance AMPd activity (Raggi and Ranieri-Raggi, 1987; Wheeler and Lowenstein, 1979). The optimum pH for the enzyme is around 6.3 (Raggi and Ranieri-Raggi 1987), thus during intense contraction, a simultaneous decrease in the energy charge and pH act to increase AMPd activity. A decrease in pH is not necessary for the activation of AMPd (Sahlin et al. 1990a; Dudley et al. 1985b) but is often coincident with IMP formation.

Substrate availability is an important factor in the AMPd reaction. The  $K_m$  for AMPd is around 1.0 mM AMP (Raggi and Ranieri-Raggi, 1987; Wheeler and Lowenstein, 1979) which is well above resting fAMP concentrations, calculated from the myokinase equilibrium at less than 1  $\mu$ M (Tullson and Terjung, 1990). Hence any increase in the fAMP concentration will increase the rate of deamination. A major factor in the availability of substrate for the AMPd enzyme may be the rate of ATP turnover. The production of AMP transients has been suggested as a major factor in AMP deamination. Sahlin et al. (1990b) followed the production of IMP in rat FT muscle as a result of electrical stimulation and 5 min of anoxic recovery. In spite of the continued high levels of allosteric activators in the muscle during recovery there was no further production of IMP once stimulation had ceased. Thus, a high rate of ATP hydrolysis/resynthesis cycle is also required for the production of

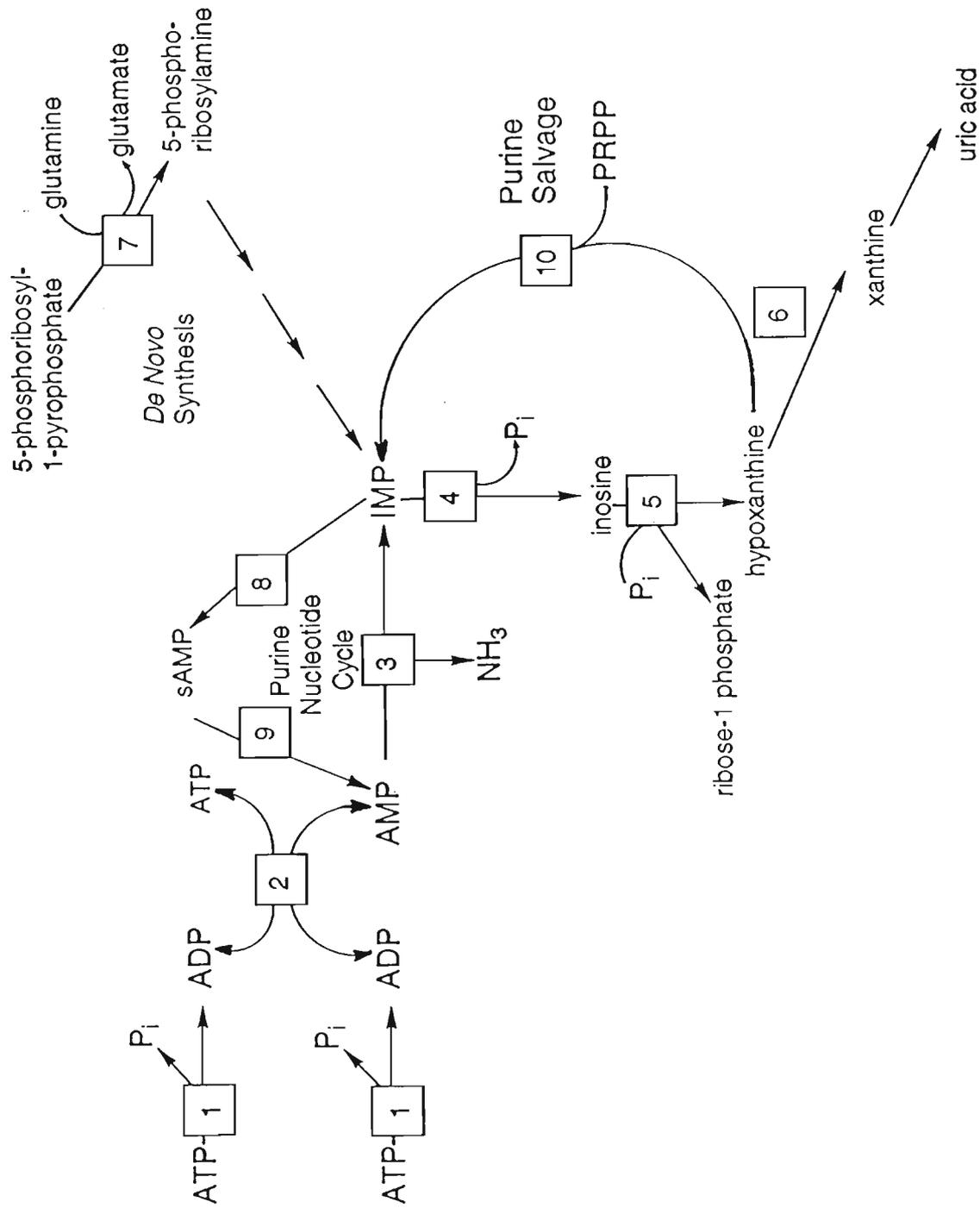


Figure 2.1: Major pathways of purine nucleotide synthesis and degradation in human skeletal muscle. 1. ATPase, 2. adenylate kinase, 3. AMP deaminase, 4. cytoplasmic 5'-nucleotidase, 5. purine nucleoside phosphorylase, 6. xanthine oxidase, 7. 5-phosphoribosyl-1-pyrophosphate (PRPP) aminotransferase, 8. adenylosuccinate synthetase, 9. adenylosuccinate lyase, 10. hypoxanthine/guanine 5-phosphoribosyl-1-pyrophosphate transferase.

IMP, not just the conditions of the internal milieu of the cell.

The binding of AMPd to myosin provides another potential regulatory mechanism of AMPd activity. The binding of AMPd to native myosin and myosin fragments (Ashby and Frieden, 1977; Barshop and Frieden, 1984; Cooper and Trinick, 1984), has been confirmed *in vivo* in the resting hindlimb muscles of rats (Rundell et al. 1992a) and chicken myofibrils (Ashby et al. 1979) where approximately 10% of the AMPd is bound. Intense exercise and electrical stimulation of muscle increase the proportion of the enzyme bound to myosin (Rundell et al. 1992a; Rundell et al. 1993; Shiraki et al. 1981). It has also been demonstrated both *in vitro* (Shiraki et al. 1979a&b) and *in vivo* (Rundell et al. 1992a; Rundell et al. 1993; Shiraki et al. 1981) that the binding is readily reversible. Binding increases the activity of the enzyme by decreasing the GTP induced inhibition (Ashby and Frieden, 1978) and increasing allosteric activation by ADP (Barshop and Frieden, 1984). The binding of AMPd also alters the kinetics of the enzyme (Rundell et al. 1992b). Additionally, at concentrations of AMP less than 0.15 mM the bound enzyme exhibits 20% of the maximal activity ( $V_{max}$ ) of the cytosolic component, whilst the affinity for the substrate increases 10-20 fold; the substrate concentration at half maximal activity ( $K_m$ ) is 0.05-0.10 mM compared to that of about 1 mM for the soluble component (Rundell et al. 1992b). Furthermore, at concentrations of AMP above 0.15 mM the kinetic characteristics are much the same as the cytosolic component. The 10-20 fold lower  $K_m$  in the bound enzyme effectively allows a higher rate of AMP deamination at physiological concentrations of the substrate.

AMPd binding to myosin appears to be coincident with conditions which also produce IMP. Rundell et al. (1992a) demonstrate that binding precedes any increase in the activity, which indicates the binding kinetics may play a major role in the control of AMPd activity. As yet the mechanism triggering the binding process is unclear, however investigations have demonstrated that  $P_i$  (Ashby and Frieden, 1977) and  $H^+$  (Barshop and Frieden, 1984) also influence binding to the myosin fragments thus suggesting that the accumulation of these metabolites with exercise may also influence the affinity of the enzyme for this myofibrillar protein.

### 2.2.1.2 AMP Deamination in Various Muscle Fibre Types

Less extensive IMP accumulation has been demonstrated in ST relative to FT fibres, in rat (Meyer and Terjung, 1979; Meyer et al. 1980) and human skeletal muscle (Jansson et al. 1987). There also seems to be a species difference in the capacity of IMP production in skeletal muscle fibres. Early investigations reported that intense exercise induced little IMP accumulation in slow oxidative soleus muscle of rats during intense exercise (Meyer et al. 1980). This is in contrast with human studies, which have demonstrated substantial increases in IMP in ST fibres after an intense exercise bout (Jansson et al. 1987).

Different isoforms of AMPd may account for much of the variation in AMPd activities of skeletal muscle fibres (Raggi et al. 1975). As discussed earlier, whilst FT fibres contain exclusively the isozyme found only in skeletal muscle, the ST muscle contains a portion of the isozyme which is also located in the heart and this may provide an explanation for the intrinsic difference in AMPd activity. Raggi et al. (1975), isolated the different isoforms of AMPd in rat, rabbit and humans using a column chromatography technique. Although not specifically mentioned by the authors, it appears that the type A isozyme of the rat and rabbit eluted earlier when compared to the human type A isozyme. These results suggest that there may be a species difference in the type A isozyme. This may explain, at least in part, why rat ST muscle exhibits no significant AMP deamination with intense exercise (Meyer et al. 1980) in contrast to the relatively large AMP deamination reported in human ST muscle (Jansson et al. 1987).

The maximal activity of AMPd of FT fibres is higher compared with ST fibres in both rat (Raggi et al. 1969; Winder et al. 1974) and human muscle (Norman and Jansson, 1991), although the difference is less pronounced in humans. The reported *in vitro* maximal AMPd activity in homogenised FT white quadriceps muscle of rats ( $83 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  wet weight (ww)) (Winder et al. 1974), is much higher than the *in vivo* rate of IMP accumulation during intense exercise ( $0.65 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  ww.) (Meyer et al. 1980). Furthermore a difference is also reported in the ST soleus muscle in rats where the maximal *in vitro* activity is  $33 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  ww (Winder et al. 1974) and the *in vivo* production rate measured after intense

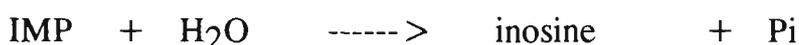
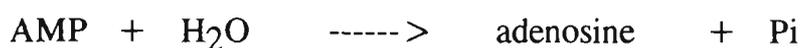
exercise is much lower at  $0.2 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}\text{ww}$  (Meyer et al. 1980). This discrepancy suggests that the *in vitro* maximal activity of the isozymes probably does not fully account for the different AMP deamination rates observed in FT and ST fibres. Nor does it explain the difference in IMP production in ST fibres observed between rats and humans with intense exercise. It is more likely that these differences are best explained by the factors influencing enzymatic control of the isoforms. Both of the isoforms are activated by a fall in pH, with a pH optimum of 6.5 (Raggi and Ranieri-Raggi, 1987). Furthermore, the B isoform (FT fibre form), shows a greater degree of activation as a result of increases in ADP or decreases in ATP concentrations compared to the A isoform (Raggi and Ranieri-Raggi, 1987). Due to their metabolic potential FT fibres experience a greater decrease in pH (Park et al. 1987) and ATP (Greenhaff et al. 1992; Jansson et al. 1987) concentration and a potentially greater ATP turnover (Sahlin and Katz, 1988). Thus for these reasons activation of AMPd is likely to be greater in FT fibres than ST fibres during intense exercise.

Another alternative which needs to be considered in relation to the control of AMPd activity is the availability of substrate. Since AMP production results from an imbalance in ATP degradation vs. resynthesis, fibre differences in the capacity of ATP hydrolysis and/or ATP resynthesis mechanisms will influence AMP deamination. These include differences in myosin-ATPase activity (Bárány, 1967; Essén, 1975; Baldwin et al. 1975), oxidative capacity (Baldwin et al. 1972, Dudley et al. 1985a; Saltin and Gollnick, 1983), and glycolytic capacities (Baldwin et al. 1973; Barnard et al. 1971; Saltin and Gollnick, 1983; Peter et al. 1972) of the different fibre types which may result in different fAMP production rates. The importance of the imbalance between ATP hydrolysis and its resynthesis in the regulation of AMP deamination is highlighted in rodent ST muscle where intense exercise does not induce significant IMP formation, since the capacity to resynthesize ATP in ST muscle, via glycolytic and oxidative mechanisms, may be sufficient to match its hydrolysis. Conversely, extreme conditions of intense electrical stimulation ( $120 \text{ tetani}\cdot\text{min}^{-1}$ ) or mild stimulation ( $12 \text{ tetani}\cdot\text{min}^{-1}$ ) combined with ischaemia, result in an imbalance in ATP hydrolysis vs. resynthesis

and hence an accumulation of IMP in rodent ST muscle (Meyer and Terjung, 1979; Tullson et al. 1990; Whitlock and Terjung, 1987). It is also possible that differences in the binding kinetics of the two different isoforms of AMPd to the contractile filaments in muscle fibres may account, at least in part, for differences in fibre type response. As yet, no studies have examined this possibility.

### 2.2.1.3 5'-Nucleotidase

The total maximal activity of human skeletal muscle 5'-nucleotidase is reported to be  $4.0 \pm 0.1 \mu\text{moles.g protein}^{-1}.\text{min}^{-1}$  (Schopf et al. 1986). The following reactions are catalysed by 5'-nucleotidase.



The regulation of this enzyme is important in limiting unnecessary degradation and subsequent loss of the muscle nucleotide pool, since adenosine and inosine can diffuse from muscle (Van Belle et al. 1987). Factors influencing 5'-nucleotidase regulation include isozyme variation, cellular location, allosteric modulation and substrate supply.

At least two isozymes, a membrane bound ecto-enzyme and a soluble cytosolic enzyme, have been found in cardiac (Meghji et al. 1988; Schütz et al. 1981) and skeletal muscle (Camici et al. 1985; Frick and Lowenstein, 1976; Tullson and Terjung, 1992). The relative contribution of the isozymes to adenosine/inosine production in skeletal muscle is not clear. The different cellular locations of the enzyme may influence adenosine and/or inosine production in muscle. Considering that the site of muscle AMP/IMP production is intracellular, the cytosolic enzyme is potentially more influential in dephosphorylating these nucleotides as a result of its locational advantage. Very little information exists relating to the *in vivo* activity of this isozyme in skeletal muscle. In ischaemic rat heart, however, an increase in

adenosine content parallels a decrease in adenine nucleotides (Rubio et al. 1973). Additionally, inhibition of the ectozyme does not influence the release of adenosine and its degradative products from the heart during hypoxic conditions (Schütz et al. 1981). These results indicate that the soluble cytosolic form of 5'-nucleotidase is probably responsible for most, if not all, of the *in vivo* adenosine production in the heart. A significant involvement of the ectozyme in intracellular nucleoside production in skeletal muscle cannot be discounted, however, as there is some evidence to suggest that the membrane bound ectozyme may gain access to the sarcoplasm (Stanley et al. 1980).

*In vitro* studies, which enable the purification of the 5'-nucleotidase isozymes, have demonstrated some differences in their regulation. Nucleoside di (ADP, uridine 5'-diphosphate; UDP, cytidine 5'-diphosphate; CDP, Guanosine 5'-triphosphate; GDP) and tri (ATP, uridine 5'-triphosphate; UTP, cytidine 5'-triphosphate; CTP, GTP) phosphates were powerful inhibitors of the 5'-nucleotidase ectozyme in guinea-pig skeletal muscle (Camici et al. 1985.). Itoh et al. (1986) have also observed an inhibition of the ectozyme by ATP in rat cardiac muscle. This inhibition by ATP and ADP is partially reversed by  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Ca^{2+}$  (Camici et al. 1985). In circumstances where the ectozyme does gain access to the sarcoplasm inhibition via the intracellular ATP levels may be offset by the presence of divalent cations in the cytosol (Camici et al. 1985). In contrast, the cytosolic 5'-nucleotidase found in cardiac muscle is activated by ATP, ADP and decreasing energy charge and is inhibited by Pi (Itoh et al. 1986). Studies examining the allosteric regulation of cytosolic 5'-nucleotidase in skeletal muscle have not been conducted.

Finally, the finding of two soluble cytosolic 5'-nucleotidases with differing affinities for AMP and IMP (Truong et al. 1988), demonstrates that the availability of substrate is another factor regulating 5'-nucleotidase activity. The production of AMP and IMP, which occurs when a high metabolic challenge is imposed upon skeletal muscle, is important for an increase in activity of both cytosolic isozymes. However, the AMP favoring 5'-nucleotidase activity is likely to be curtailed by the availability of AMP as a result of competition with AMPd

(Tullson et al, 1990). Patients deficient in AMPd exhibit elevated adenosine production with exercise relative to control subjects (Sabina et al. 1984). This finding supports the argument of limited substrate availability, for adenosine production, during exercise in healthy subjects. This is not the case with the IMP favoring 5'-nucleotidase, which can be exposed to its substrate over a longer time period.

In the rat, soluble 5'-nucleotidase activity is significantly higher in the soleus muscle when compared to other muscle groups (Tullson and Terjung, 1992). Tullson and Terjung (1992) argue that the pattern of soluble 5'-nucleotidase activities among muscle fibre sections suggests that red ST muscle is more likely to form nucleosides and bases in contrast to FT muscle which retains purine metabolites as nucleotides. This is supported by other studies on cats and rats (Bockman and McKenzie, 1983; Rubio et al. 1973). As yet, there are no reports of human skeletal muscle soluble cytosolic 5'-nucleotidase activity in different fibre types.

#### 2.2.1.4 AMP Deaminase vs 5'-nucleotidase in AMP Degradation

The pathway of AMP catabolism, and ultimately AdN degradation, is influenced by the relative activities, kinetic characteristics and cellular distribution of the enzymes AMPd and 5'-nucleotidase (Tullson and Terjung, 1990). The location of the enzyme within the cell, relative to the site of AMP production, is likely to play an important role in AMP degradation. The binding of AMPd enzyme to myosin (Ashby and Freiden, 1977; Cooper and Trinick, 1984; Rundell et al. 1992a; 1993) brings it into close proximity to myokinase (Savabi et al. 1986) thus increasing the likelihood that AMP will be deaminated rather than dephosphorylated by the cytosolic 5'-nucleotidase enzyme.

During severe metabolic stress, competition arises between AMPd and 5'-nucleotidase for the fAMP produced within the muscle (Tullson and Terjung, 1990). The extent of IMP accumulation, with intense exercise, appears to be in amounts equimolar with AdN degradation in human skeletal muscle (Jansson et al. 1987; Meyer and Terjung, 1980; Sahlin and Ren, 1989). This suggests that the AMPd

pathway is more prominent than AMP dephosphorylation. Sabina et al. (1984) demonstrated that the extent of dephosphorylation during exercise is small and can be accentuated in AMPd deficient patients. Reports from *in vitro* studies demonstrate relatively greater activities and higher affinities of skeletal muscle AMPd for fAMP, compared with that of 5'-nucleotidase (Meghji et al. 1988; Newsholme et al. 1985; Tullson and Terjung, 1990; Winder et al. 1974). Furthermore, in cultured muscle cells the ratio of the flux through AMPd and 5'-nucleotidase was 19:1 (Zoref-Shani et al. 1987). Although this is not necessarily representative of the magnitude of AMP degradation to IMP in skeletal muscle, it further supports the contention that the AMPd pathway is the primary route of AMP degradation.

#### 2.2.1.5 Physiological Role of AMP Deaminase and 5'-nucleotidase

The relative activities of the AMP degradation pathways in the different muscles may be an adaptation specific to their physiological role. Adenosine is a potent vasodilator (Rubio et al. 1973) and the preferential production of adenosine in the heart (Tullson and Terjung, 1990) is an advantage to the local cardiac circulation during stressful conditions (Rubio et al. 1973). This hyperaemic effect may also occur to a degree in skeletal muscle however adenosine production in human skeletal muscle during exercise is normally very low (Sabina et al. 1984) and therefore its a role in the control of blood flow in skeletal muscle is probably small (Bockman and McKenzie, 1983; Schwarz and McKenzie, 1990; Phair and Sparks, 1979).

Human skeletal muscle readily produces IMP when metabolically stressed. The combined function of myokinase and AMPd is to maintain a high energy charge (Lowenstein, 1972; Sahlin and Katz, 1988; Tullson and Terjung, 1991a). The maintenance of the energy charge is important for contractile function because it influences the amount of free energy released as a result of ATP hydrolysis (Sahlin et al. 1978) and an increase in ADP relative to ATP decreases tension development of the muscle fibre (Cooke and Pate. 1985). Individuals who are AMPd deficient highlight the importance of the enzyme as they exhibit markedly impaired tolerance to intense exercise, characterised by cramps and rapid fatigue (Fishbein et al. 1978). Additionally, the dominance of AMPd in skeletal muscle and the production of IMP

may enable the conservation of AdN (Sabina et al. 1980). The inability of IMP to diffuse from the muscle permits the transient storage of IMP during exercise and subsequent rapid resynthesis in recovery minimises the loss of purine base (Tullson and Terjung, 1991a).

#### 2.2.1.6 Purine Nucleoside Phosphorylase

The degradation of IMP to hypoxanthine is the result of the action of 5'-nucleotidase and purine nucleoside phosphorylase (PNP) (Fig. 2.1; Tullson and Terjung, 1990). Firstly IMP is dephosphorylated to form inosine by 5'-nucleotidase (Truong et al. 1988) and then inosine is subsequently degraded to form hypoxanthine by PNP in skeletal muscle (Schopf et al. 1986), endothelial cells (Bowditch et al. 1985) or in the plasma (Harkness et al. 1983). Relatively little information exists on the regulation of PNP. The maximal *in vitro* activity of PNP in human skeletal muscle has been reported to be  $2.6 \pm 0.3 \mu\text{mol.g protein}^{-1}.\text{min}^{-1}$  (Schopf et al. 1986). Both the substrate (Bockman and McKenzie, 1983; Harkness et al. 1983) and the product (Bangsbo et al. 1992; Harkness et al. 1983; Hellsten-Westling et al. 1989; Patterson et al. 1983; Sutton et al. 1980), of this reaction have been found in the plasma. The loss of hypoxanthine from active skeletal muscle (Bangsbo et al. 1992) provides evidence of the ability of this base to cross the sarcolemma. The efflux of hypoxanthine from the muscle ultimately results in a loss of AdN from the muscle. The examination of inosine efflux from skeletal muscle has not been conducted.

### 2.3 Adenine Nucleotide Resynthesis/Synthesis Pathways

The pathways involved in the recovery of AdN are purine salvage, reamination pathways of the PNC and the relatively slow AdN *de novo* synthesis pathway (Fig. 2.1; Tullson and Terjung, 1991a). The purine salvage pathway is catalysed by hypoxanthine/guanosine 5-phosphoribosyl 1-pyrophosphate transferase (HGPRT). This enzyme is responsible for the conversion of hypoxanthine to IMP and plays an important role in minimising the muscular loss of purine base (Edwards et al. 1979). Circulating hypoxanthine can be taken up by inactive muscle (Bangsbo

et al. 1992) and may be converted to IMP and ultimately to ATP (Fig 2.1; Lowenstein, 1972).

The reamination of IMP to AMP is a two step process (Fig 2.1; Lowenstein, 1972). The first reaction, catalysed by adenylosuccinate synthetase (AdSS), involves the consumption of one aspartate and one molecule of guanosine 5'-triphosphate (GTP) for every IMP molecule converted to adenylosuccinate. The second reaction, catalysed by adenylosuccinate lyase (AdSL), results in the production of AMP and fumarate (Lowenstein 1972).

Little, if any IMP reamination occurs during intense exercise (Katz et al. 1986a&b; Meyer and Terjung, 1980). Investigations into the operation of the PNC suggest, however, that there is some concurrent reamination during exercise of moderate intensity (Aragón and Lowenstein, 1980). This is further supported by an increase in adenylosuccinate concentration during exercise (Goodman and Lowenstein, 1977; Lowenstein and Goodman, 1978). Reamination in contracting fibres during high intensity exercise, if any, is minimal (Meyer and Terjung, 1980). The substrate inhibition of high IMP concentrations on AdSS activity (Goodman and Lowenstein, 1977; Stayton et al. 1983) may, in part, reduce the reamination rates. Meyer and Terjung (1980) show that most of the IMP is converted to ATP during recovery from intense exercise. The reamination rates of IMP after exercise in humans have been estimated indirectly from the ATP resynthesis rates (Katz et al. 1986a) and the difference in IMP concentration at various timepoints during recovery (Fig. 2.2; Graham et al. 1990; Sahlin and Ren, 1989). From these measurements it appears that the maximal *in vivo* rate of IMP reamination is between 0.4 and 0.6 mmol.kg<sup>-1</sup>dry weight (dw).min<sup>-1</sup>, which is not as rapid as the maximal rate of IMP production, estimated from AdN degradation to be between 14.4-31.0 mmol.kg<sup>-1</sup>dw.min<sup>-1</sup> (Boobis et al. 1983b; Gaitanos et al. 1993; Nevill et al. 1989).

The *de novo* purine biosynthesis pathway is critical to skeletal muscle AdN metabolism in the longer term, continually supplying and replenishing the AdN pool. The initial substrate for this process is phosphoribosylpyrophosphate (PRPP). In an elaborate series of reactions carbon and nitrogen atoms, provided by glutamine, aspartate, glycine, formate and bicarbonate ions, are added to PRPP to

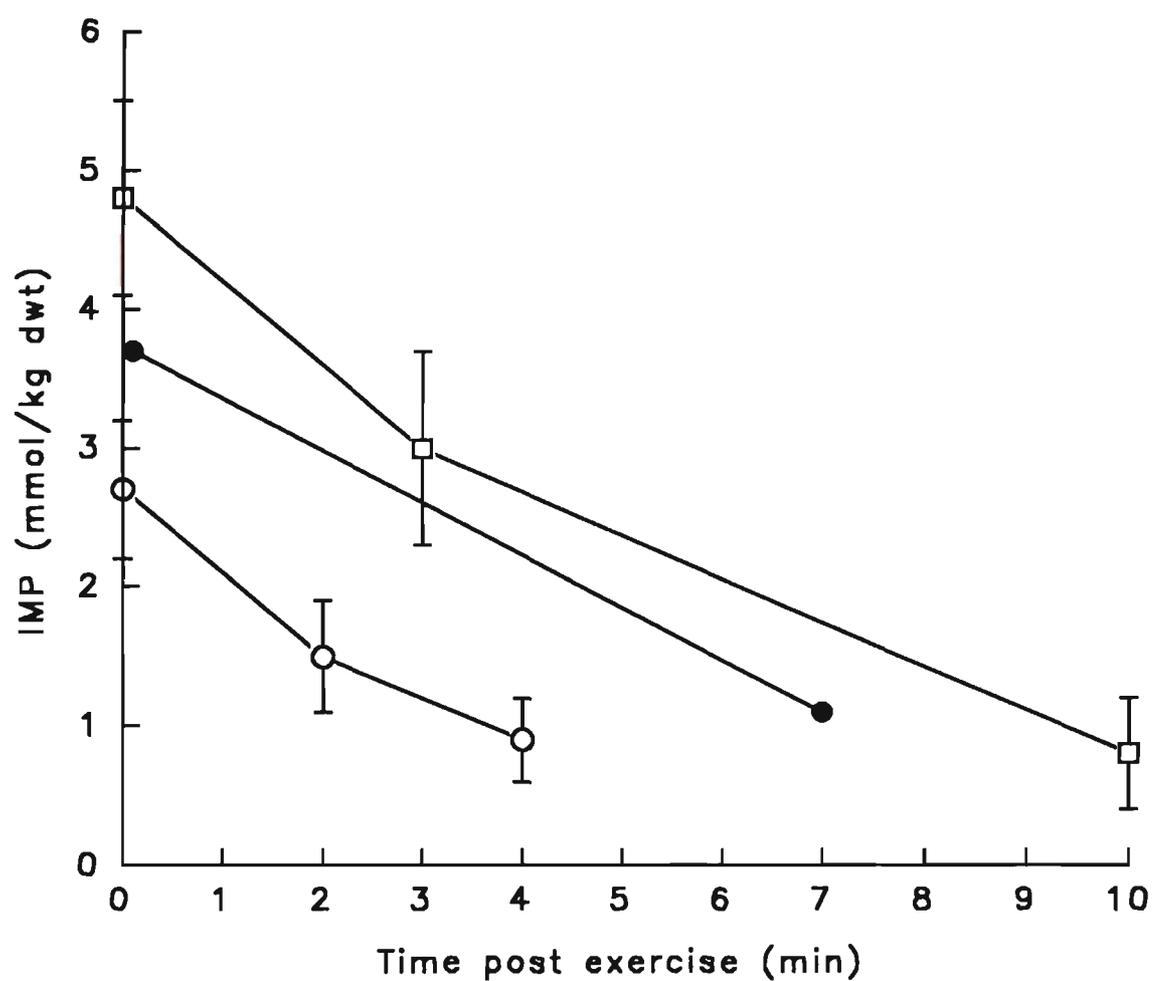


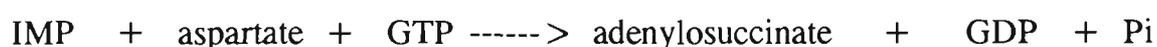
Figure 2.2. The recovery of IMP in skeletal muscle during recovery from intense exercise; ● – Sahlin et al. 1978 (Cycling >80%  $\dot{V}O_2\text{max}$ ), ○ – Sahlin and Ren, 1989 (leg extension, 66%MVC), □ – Graham et al. 1990 (leg extension, ~140%  $\dot{V}O_2\text{max}$  of the leg).

produce IMP, which is then incorporated into the AdN pool via the PNC (Newsholme and Leech, 1983). The production of an IMP molecule via this mechanism involves the consumption of six high energy phosphate bonds (Newsholme and Leech, 1983) and the estimated rate of synthesis for mixed muscle in rats has been measured at between 32-37 nmol.kg<sup>-1</sup>.ww.hr<sup>-1</sup> (Tullson et al. 1988). There are no equivalent human data.

### 2.3.1 Regulation of Recovery/Synthesis Pathways

#### 2.3.1.1 Adenylosuccinate Synthetase

AdSS catalyses the conversion of IMP and aspartate to adenylosuccinate as described in the following equation,



The maximal activity of this enzyme in rodent skeletal muscle is 0.74 μmol.g<sup>-1</sup> ww.min<sup>-1</sup> (Goodman and Lowenstein, 1977) and it is the rate limiting reaction of the PNC (Fig 2.1; Lowenstein, 1990). The regulation of AdSS is influenced by substrate availability. The enzyme requires three different substrates (IMP, GTP, aspartate) to catalyse the reaction and the substrate binding sites for each are quite specific (Stayton et al. 1983). Several authors (Goodman and Lowenstein, 1977; Muirhead & Bishop 1974; Ogawa et al 1977; Stayton et al. 1983) report that skeletal muscle AdSS has a Km for IMP in the range 0.2-0.7 mM. The IMP concentration in resting skeletal muscle is around 0.07 mM (Goodman and Lowenstein, 1977) and therefore small increases in IMP would be expected to elevate enzyme activity. The Km of AdSS for aspartate is 0.3 mM and for GTP between 0.01-0.4 mM (Goodman and Lowenstein, 1977; Muirhead & Bishop 1974; Ogawa et al 1977; Stayton et al. 1983). Of these three substrates only IMP is considered to be the one which exerts any significant regulatory influence during muscular activity (Stayton et al. 1983).

Inhibition of AdSS by GDP, adenylosuccinate and AMP has been demonstrated using purified AdSS (Muirhead and Bishop, 1974; Ogawa et al. 1977). Nucleoside di- and monophosphates are also inhibitors of AdSS (Ogawa et al. 1977),

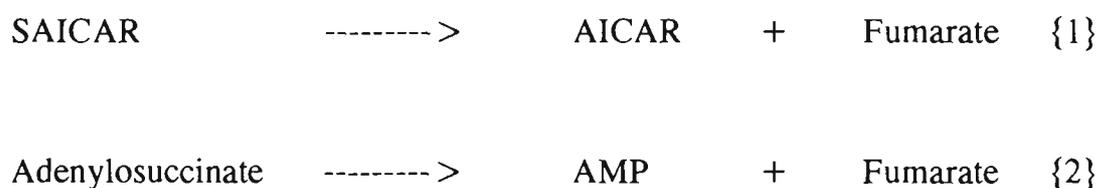
of which GDP is the most potent (Stayton et al. 1983). Although small increases in IMP activate AdSS, elevated concentrations of IMP inhibit the rate of the reaction *in vitro* (Goodman and Lowenstein, 1977; Stayton et al. 1983). Thus with intense exercise where IMP concentrations are substantially elevated one would expect marked inhibition of AdSS. The extent of this inhibition *in vitro* does not appear to be confirmed by *in vivo* studies since the rate of IMP disappearance post-exercise is not apparently dependent upon IMP concentration (Fig. 2.2). It is acknowledged that this argument presupposes that IMP reamination rates account for most of its disappearance, as supported by the close relationship between ATP recovery and IMP removal (Katz et al. 1986a). Additionally, creatine phosphate (CP) and Pi inhibit AdSS at physiological levels *in vitro* (Stayton et al. 1983). Hence the inhibition by high resting CP stores are probably relieved during intense exercise. Conversely, Pi accumulates during the exercise tending to inhibit the enzyme (Stayton et al. 1983). Clearly the regulation of this enzyme is complex and activation of the enzyme is determined by the balance of activators and inhibitors. During intense exercise it has been suggested that AdSS is inhibited such that the PNC is not operational and that reamination occurs only during recovery (Meyer and Terjung, 1979&1980). There is evidence, however, which suggests that AdSS may be active and the PNC operative during high intensity exercise (Flanagan et al. 1986; Manfredi and Holmes, 1984). Flanagan et al. (1986) inhibited AdSS using 5-amino-4-imidazolecarboxamide riboside (AICARiboside) in rats and found a decreased performance in intensely stimulated skeletal muscle. Additionally, Manfredi and Holmes (1984), investigated the individual PNC enzyme activities and their response to changes in energy charge in protein extracts of rat skeletal muscle. In this model they were able to manipulate the composition of the nucleotide pool and demonstrate concurrent increases in the activities of all the PNC enzymes with a reduced ratio of ATP/ADP. An increase in AMPd was expected due to the increased AMP and ADP concentrations (Wheeler and Lowenstein, 1979). An increase in the activity of AdSS, however, with decreasing energy charge was unexpected considering the purified enzyme is inhibited by ADP (Muirhead and Bishop, 1974; Ogawa et al. 1977). Regardless of the AdSS activity during high intensity exercise,

it is clear that the marked accumulation of IMP (Graham et al. 1990; Sahlin et al. 1978) results from a far greater rate of AMP deamination than IMP reamination.

It has been reported that AdSS binds to purified F-actin, actin tropomyosin complexes and reconstructed thin filaments (Manfredi et al. 1989; Ogawa et al. 1978) but does not bind to myosin (Manfredi et al. 1989). Manfredi et al. (1989), further postulate that at least 99% of the enzyme is estimated to be bound to the myofibrils at rest. Factors which may influence the binding of AdSS to actin have not been reported but it has been proposed by Manfredi et al. (1989) that tropomyosin could be a competitor with AdSS for actin binding sites, since an apparent decrease in affinity of AdSS towards purified actin, in the presence of tropomyosin, was demonstrated. Although speculative, binding of AdSS may alter the kinetic properties of the enzyme and fluxes through AMP deaminase and AdSS may be coupled as a result of their close proximity to one another once bound.

### 2.3.1.2 Adenylosuccinate Lyase

AdSL is a bifunctional enzyme and is involved in catalysing the conversion of 4-(N-succino)-5-aminoimidazole-4-carboxamide ribonucleoside 5'-phosphate (SAICAR) to 5-aminoimidazole-4-carboxamide ribonucleoside 5'-phosphate (AICAR) and fumarate {1}, one of the reactions in *de novo* AdN synthesis. The enzyme is also involved in the conversion of AdSS to AMP and fumarate {2};



The enzyme has been purified from rat skeletal muscle and the  $K_m$  for adenylosuccinate and SAICAR was found to be 1.5  $\mu\text{M}$  and 1  $\mu\text{M}$ , respectively (Casey and Lowenstein, 1987). Its activity in converting AdSS to AMP at 25°C has been reported to be 11  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$  of protein (Casey and Lowenstein, 1987). Adenylosuccinate concentrations range from undetectable levels at rest to

approximately 20 nmol.g<sup>-1</sup>dw after *in situ* stimulation of rat skeletal muscle (approximately 8 μM) (Lowenstein and Goodman, 1978). The K<sub>m</sub> values, therefore, suggest that AdSL is sensitive to small changes in adenylosuccinate concentrations but during high intensity exercise may be operating at its maximal rate. Studies examining the regulation of AdSL have not been conducted. However the importance of AdSL has been demonstrated as inhibition of the enzyme using substrate analogue results in a decrease in muscle performance (Swain et al. 1984).

### 2.3.1.3 Purine Salvage Pathway

The pathway catalysed by hypoxanthine-guanosine phosphoribosyltransferase (HGPRT) combines hypoxanthine with 5-phosphoribosyl-1-pyrophosphate (PRPP) to form IMP (Fig. 2.1; Liu and Feinberg, 1971; Namm, 1973; Wiedmeier et al. 1972). Hypoxanthine and inosine can readily efflux from muscle and result in a loss of AdN precursors from skeletal muscle after intense exercise (Harkness et al. 1983). The purine salvage pathway acts to reduce the loss of hypoxanthine from skeletal muscle and plays a significant role in the recovery of IMP from hypoxanthine (Fig 2.1; Edwards et al. 1979). The purine salvage pathway effectively utilises preformed purine bases for nucleotide synthesis (Manfredi and Holmes, 1984) avoiding the metabolically expensive *de novo* synthesis pathway.

At rest HGPRT is estimated to be responsible for approximately 75% of hypoxanthine recovery (Edwards et al. 1979). The extent of purine base recovery following exercise is unknown. Control of the activity of this enzyme has not been thoroughly investigated but patients deficient in HGPRT have provided some insight into the valuable role it plays in resting AdN metabolism (Edwards et al. 1979; Lesch and Nyhan, 1964). The maximal activity of HGPRT in human myocardium and quadriceps femoris muscles is 134±40 and 280±56 nmol.g protein<sup>-1</sup>.min<sup>-1</sup>, respectively (Schopf et al. 1986). The factors which determine the activity of HGPRT have not been extensively studied. To date, most studies of the salvage pathway have used rodent heart (Harmsen et al. 1984) and liver (Kim et al. 1992). These studies have demonstrated that the availability of PRPP limits the rate of IMP formation. Kim et al. (1992) suggest that the provision of ribose-5-phosphate may

ultimately determine the availability of PRPP as they report that changes in PRPP are correlated with changes in ribose-5-phosphate. This is further supported by enhanced PnN synthesis from hypoxanthine when ribose was added to the fluid perfusing the myocardium (Harmsen et al. 1984). Studies investigating the factors regulating HGPRT in human skeletal muscles have not been reported.

#### 2.3.1.4 *De novo* Adenine Nucleotide Synthesis

The *de novo* biosynthesis pathway involves 11 sequential reactions and utilises 6 high energy phosphate bonds in the formation of an ATP molecule (Newsholme and Leech, 1983). The resting *de novo* AdN synthesis rates are reportedly about 35 nmol.g ww<sup>-1</sup>.h<sup>-1</sup> for mixed muscle in rats (Tullson et al. 1988) and as yet no measurement has been made in human skeletal muscle.

The rate limiting factor of *de novo* synthesis appears to be substrate availability, particularly that of PRPP (Fox and Kelly, 1971; Harmsen et al. 1984; Zimmer and Gerlach, 1978; Zoref-Shani et al. 1982). PRPP synthesis is catalysed by PRPP synthetase and this enzyme is inhibited by various nucleotides, particularly AMP, ADP and GDP (Mathews and van Holde, 1990). An increase in the concentration of ribose, a precursor of PRPP, may also activate PRPP synthetase as the addition of ribose to perfusate of rat skeletal muscle increased the *de novo* AdN synthesis 3-4 fold (Tullson and Terjung, 1991b). It is also postulated that the rate of *de novo* synthesis may be limited by the flow through the hexose monophosphate shunt (Zimmer and Gerlach, 1978) which produces the ribose for PRPP production. None of the substrates which are sequentially added in the purine *de novo* biosynthesis pathway, including glycine, glutamine, formate and bicarbonate (Newsholme and Leech, 1983), are likely to be limiting due to their relative abundance in skeletal muscle.

Tullson et al. (1988) and Tullson and Terjung, (1991b) have proposed that *de novo* AdN synthesis occurs in favorable energetic conditions, particularly where a high energy charge exists. These authors have demonstrated that, during intense contraction in rat muscle, *de novo* synthesis rates are reduced possibly as a result of a decrease in the energy charge. The decrease in energy charge may reduce *de novo*

AdN synthesis by inhibiting PRPP-aminotransferase (Fig. 2.1). This enzyme catalyses the conversion of PRPP to 5-phosphoribosylamine and is inhibited allosterically by AMP and GMP (Mathews and van Holde, 1990).

Skeletal muscle exhibits comparatively higher *de novo* synthesis when compared to that of the liver (Brosh et al. 1982). Higher *de novo* synthesis rates in red muscle compared to white muscle have also been reported (Tullson et al. 1988). The capacity for *de novo* synthesis is higher in ST fibres and this may be related to the greater potential of these fibres to produce purines which can cross the sarcolemma (Bockman and McKenzie, 1983; Tullson and Terjung, 1990). Thus to maintain constant AdN levels in the face of a greater loss of purines from the muscle, the ST fibres would require a higher *de novo* synthesis rate (Tullson et al. 1988).

## 2.4 Adenine Nucleotide Management

### 2.4.1 Rest

Clearly, the ATP concentration found at rest must result from the balance of degradation and resynthesis pathways. The factors which control and maintain skeletal muscle ATP concentrations at rest, are not fully understood. Evidence of the incorporation of radiolabelled glycine into AdN in resting rat skeletal muscle (Tullson et al. 1988; Tullson and Terjung, 1991b) demonstrates that a basal turnover rate of ATP occurs.

Resting ATP concentrations in human skeletal muscle are usually reported between 21-28 mmol.kg<sup>-1</sup>dw (Boobis et al. 1983a,b; Cheetham et al 1986; Katz et al 1986a&b; Nevill et al. 1989). Studies which have examined the resting ATP concentrations in human single muscle fibres of untrained subjects have observed no difference between FT and ST fibres (Ball-Burnett et al. 1991; Essen, 1978; Greenhaff et al. 1992; Jansson et al. 1987; Söderlund and Hultman, 1990).

Training has produced variable outcomes in relation to resting ATP concentrations in human muscle homogenates. A decrease (Hellsten-Westling et al. 1993), or no change (Boobis et al. 1983b; Nevill et al. 1989), in the resting levels of ATP content in human muscle has been reported after sprint training. The effect of

sprint training on resting ATP levels will be discussed subsequently (Section 2.5.1.1). In contrast, increases in ATP content has been observed after intermittent strength (Houston and Thompson, 1977) and endurance (Karlsson et al. 1972) training. These changes demonstrate that there are underlying mechanism(s) controlling the resting ATP levels which may be manipulated. Increased AdN *de novo* synthesis is unlikely to explain elevations in resting ATP content with endurance training since the capacity for *de novo* synthesis in rats is not altered with endurance training (Tullson and Terjung, 1991b). In the endurance trained state, an elevated aerobic capacity may increase the rate of ATP resynthesis at rest, during exercise, and/or assist in ATP recovery after exercise. As a consequence, there may be lesser reliance on the *de novo* synthesis mechanisms to maintain ATP concentrations at rest. The effect of sprint training, however, requires examination.

A reduction in ATP in resting samples may also be the direct effect of prior intense exercise regimes. Sprint exercise can produce substantial amounts of purine base in the blood (Hellsten-Westling et al. 1989; Stathis et al. 1992) and AdN *de novo* synthesis may not be rapid enough to completely restore the loss of purine base. Hence exercise prior to muscle sampling may increase the efflux and net loss of purine base from the muscle, resulting in lower ATP levels at the time of sampling.

The question of diet manipulation has not been addressed in relation to resting ATP concentrations. Studies of ribose feeding have demonstrated an increase in the flux through the *de novo* synthesis (Tullson and Terjung, 1991b) and purine salvage pathways (Kim et al. 1992), thus enhancing ATP production. Whether this results in an increase in the resting AdN content, or an increase in hypoxanthine in the muscle and therefore uric acid in the blood, is unknown and is worthy of future investigation.

#### 2.4.2 During High Intensity Exercise

The management of AdN is central to the function of the muscle in transforming chemical energy into mechanical energy. The hydrolysis of ATP by myosin ATPase provides the energy for muscle contraction. Anaerobic ATP turnover

rates during 6 s of intense voluntary exercise, have been estimated at about 15 mmol.kg<sup>-1</sup>dw.sec<sup>-1</sup> (Gaitanos et al. 1993). At this rate, total hydrolysis of the muscle ATP stores alone would only be able to meet the energy requirements of the first 1-2 s of maximal exercise. Thus, the supply of energy in this form of exercise must be derived from ATP stores in conjunction with other energy sources. These other sources include a combination of CP degradation, glycolysis and oxidative metabolism, all of which contribute to supply the muscle with ATP during exhaustive exercise of short duration (Boobis et al. 1983a; Cheetham et al. 1986; Medbø and Tabata 1989; Withers et al. 1991).

Strenuous maximal exercise imposes extreme metabolic demands upon the muscle, and the high rates of ATP degradation can be matched by the ADP rephosphorylation mechanisms for only a limited period of time. Maximal sprint exercise of 6 s duration induces a 13% reduction in the resting ATP concentration (Gaitanos et al. 1993) demonstrating that even after 6 s there is an imbalance between ATP hydrolysis and resynthesis. In contrast, Boobis et al. (1983a) did not demonstrate such an effect after 6 s of maximal cycling, probably due to an insufficient subject number (n=4). Studies using extended sprint durations have demonstrated a much larger decrement in ATP ranging between 36-40% of the resting concentrations (Boobis et al. 1983a&b; Cheetham et al. 1986; McCartney et al. 1986; Nevill et al. 1989; Withers et al. 1991). Maximal sprinting extended beyond 30 s does not further reduce the muscle ATP content below the levels reached at 30 s (Withers et al. 1991)

With high intensity exercise, the magnitude of ATP degradation appears to be matched by the increase in IMP (Graham et al. 1990; Jansson et al. 1987). This is due to a severe imbalance in ATP utilisation relative to its resynthesis rate. A greater magnitude of ATP degradation is reported in FT compared with ST fibres after 30 s of maximal sprinting (Greenhaff et al. 1992). Additionally, Jansson et al. (1987) demonstrated a two fold higher degradation of ATP and an increase in IMP in human FT fibres compared with ST fibres with maximal leg kicking exercise. As yet, no studies have measured IMP content of the muscle after "all out" maximal sprint exercise but it is likely that such substantial decreases in ATP would be

reflected by large equimolar increases in IMP content.

#### 2.4.3 During Recovery From High Intensity Exercise

No studies have investigated the recovery of the AdN pool after "all out" sprint exercise. Studies of metabolite concentrations several minutes into recovery following intense exercise have demonstrated substantial recovery of the AdN pool from IMP within 4-10 min (Fig. 2.2; Graham et al. 1990; Sahlin and Ren, 1989). Sahlin and Ren (1989), using a knee extension protocol, reported that with 66% of maximal voluntary contraction to fatigue of the quadriceps, muscle IMP concentration ( $2.7 \text{ mmol}\cdot\text{kg}^{-1}\text{dw}$ ) recovered significantly after 2 min ( $1.5 \text{ mmol}\cdot\text{kg}^{-1}\text{dw}$ ) and was back to resting values after 4 min ( $0.9 \text{ mmol}\cdot\text{kg}^{-1}\text{dw}$ ). Graham et al. (1990) used a similar protocol, working at a capacity of about 140%  $\text{VO}_2\text{max}$  of the leg extensors to fatigue. In this study the IMP content at fatigue ( $4.8 \text{ mmol}\cdot\text{kg}^{-1}\text{dw}$ ) was well above resting value, which was undetectable, and the muscle contents at 3 and 10 min were  $3.0$  and  $0.8 \text{ mmol}\cdot\text{kg}^{-1}\text{dw}$ , respectively. In addition, Sahlin et al. (1978) demonstrated that the ATP was back to resting levels by 7 min recovery from exercise greater than 80%  $\text{VO}_2\text{max}$ . From the above data it appears that the time taken for the recovery of ATP is proportional to the extent of IMP accumulation post-exercise (Fig 2.2).

The magnitude of IMP accumulation is 2-3 fold higher after a 30 s maximal sprint bout ( $7.5$ - $10.7 \text{ mmol}\cdot\text{kg}^{-1}\text{dw}$ ), estimated from ATP depletion, (Boobis et al. 1983a&b; Cheatham et al. 1986; McCartney et al. 1986; Nevill et al. 1989) than for the knee extension protocols reported above (Fig. 2.2). During recovery a majority of IMP is reaminated to AMP and further phosphorylated to ATP (Graham et al. 1990). It has been estimated that approximately 5% of the fall in ATP during an intense supramaximal exercise bout is lost from the muscle as purine bases (Bangsbo et al. 1992). On this basis about 2 % of the total ATP stores would be lost after a 30 s maximal sprint bout. As yet no study has examined the recovery of AdN or purine base efflux from the muscle with a maximal sprint bout, hence the magnitude of the loss is unknown. The loss of AdN from the muscle, represented by the net efflux of purine base, is replaced by the relatively slow AdN

*de novo* biosynthesis pathway (Tullson and Terjung, 1991a).

As previously mentioned, the recovery of ATP involves two relatively rapid pathways, namely, the reamination of IMP and purine salvage, and *de novo* synthesis which is slow by comparison. Quantitatively reamination is responsible for the greatest proportion of AdN recovery as indicated by the close relationship between the ATP recovery and IMP disappearance in recovery from exercise (Sahlin and Ren, 1989). Differences in proportions of fibre types within a muscle could play a large part in the rate of recovery of ATP from IMP for the following reasons. The accumulation of IMP after intense exercise is greater in FT relative to ST fibres in humans (Jansson et al. 1987). As mentioned previously (section 2.3.1.1) IMP partially exerts a substrate inhibition on AdSS at high IMP concentrations (Goodman and Lowenstein, 1977; Stayton et al. 1983). Thus potentially the greater IMP accumulation in FT fibres may prolong the recovery of ATP. Additionally, Winder et al. (1974) report the highest *in vitro* AdSS activity in the FT red muscle and lowest in FT white muscle of the rat skeletal muscle which could influence the differences in ATP recovery rates. In mixed skeletal muscle, a higher production of IMP in skeletal muscle results in a recovery time which is simply proportional to the initial concentration of IMP (Fig 2.2), thus suggesting no difference in the rate of ATP resynthesis over time. Measurements in rodent skeletal muscle suggest there may be a slight difference in the IMP disappearance rate in ST relative to FT muscle (Arabadjis et al. 1992). A difference in ATP resynthesis rates in humans skeletal muscle fibres needs to be examined to clarify this point.

The complete recovery of resting ATP levels is reliant upon the *de novo* synthesis mechanism to replace purine bases lost after intense exercise. As mentioned above the time to recover the ATP via AdSS is potentially longer in FT muscle due to the larger extent of accumulation. This may then allow an extended duration for 5'-nucleotidase to degrade IMP to purines which can efflux from the muscle leading to a loss of ATP precursors. In addition, the potential for purine loss (via adenosine and inosine production) may be greater in ST compared with FT muscle fibres (Tullson and Terjung, 1990). The extent of purine base loss from each fibre type by these mechanisms is unknown and probably related to the 5'-

nucleotidase/AMPd activity ratio of the different fibres (Tullson and Terjung, 1990).

A fibre type difference is also reported in the capacity of AdN *de novo* synthesis in rat (Tullson et al. 1988) with the highest rate exhibited in FT red, then the ST red muscle and the lowest rate in FT white muscle. This trend appears to be related to the relative oxidative capacity of these fibres as evidenced by the strong correlation between *de novo* synthesis rates and citrate synthase activity (Tullson et al. 1988). In rat muscle, endurance training does not appear to increase AdN *de novo* synthesis (Tullson and Terjung, 1991b). However, these results may be influenced by limitations in the substrate supply (Section 2.3.1.4). There is, as yet, no information on the *de novo* synthesis capacity of human skeletal muscle.

## 2.5 Sprint Training

### 2.5.1 Effect on Human Adenine Nucleotide Metabolism

#### 2.5.1.1 Resting Muscle

The resting concentration of AdN, particularly that of ATP is reduced after six weeks of interval sprint training (Hellsten-Westring et al. 1993). This is in contrast to other reports which found no difference (Boobis et al. 1983b; Nevill et al. 1989), or an increase (Thorstensson et al. 1975) in resting ATP levels in the post-trained relative to the pre-trained status. These conflicting findings may be explained by the variation in the sprint training protocols employed by the different studies. The ATP decrease observed by Hellsten-Westring et al. (1993) must be due to a greater loss of purines relative to AdN resynthesis over the duration of the training regime. The efflux of purine bases from the muscle is affected by the different intensities, number of exercise bouts, and/or the duration of the exercise bouts. Hence, the more intense and demanding the exercise performed in training the greater the magnitude of purine base loss which may occur with training (Hellsten-Westring et al. 1993). This is supported by the fact that the rise in plasma concentration of purines is directly related to exercise intensity (Sjödin and Hellsten-Westring, 1990). Furthermore, a smaller work to rest ratio employed in a sprint training regime may enhance the net decrease in AdN since shorter recovery durations result in greater plasma hypoxanthine concentrations (Balsom et al. 1992).

Therefore, the more intense the exercise performed in the training session, and the lower the work to recovery time ratio, the greater the likelihood of purine loss from the muscle. The duration of the exercise also influences the extent of the accumulation of hypoxanthine in the blood (Fig. 2.3).

Interestingly, the decrease in AdN content of the muscle in the initial 6 wk of sprint training was not reduced further by the additional week of more intense training (Hellsten-Westring et al. 1993). This may indicate a training adaptation. Furthermore, there does not appear to be any significant recovery of the AdN level after 72 hours of recovery compared with 3 hours recovery after 6 weeks of training (Hellsten-Westring et al. 1993). Such a result suggests that there may be a down regulation of the resting levels of AdN. These authors also show that one week of intense sprint training may be enough to stimulate a down regulation. Further research is needed to establish the occurrence of down regulation and if it occurs and what mechanism(s) are responsible. The levels of degradation products of AdN at rest are not likely to be influenced by sprint training since ATP hydrolysis rates are low and matched by aerobic ATP synthesis.

#### 2.5.1.2 During Intense Exercise

During a 30 s sprint on a non-motorised treadmill the vastus lateralis muscle is apparently maximally activated since substantial CP degradation occurs in both FT and ST muscle fibres (Greenhaff et al. 1992). Training does not alter the extent of CP degradation in mixed muscle over a 30 s sprint bout (Boobis et al. 1983b; Hellsten-Westring et al. 1993), indicating that both fibre types are utilised throughout the training regime. Greenhaff et al. (1992) also reported a substantial drop in ATP in both fibre types during a 30 s sprint. The magnitude of ATP degradation in mixed muscle after a maximal sprint bout is not different with training (Boobis et al. 1983b; Nevill et al. 1989). On this basis it would be expected therefore, that the IMP content immediately after a sprint bout is also likely to be of similar magnitude pre and post-training, although no such measurements have been made in humans. Westra et al. (1985), however, report that the sprint training

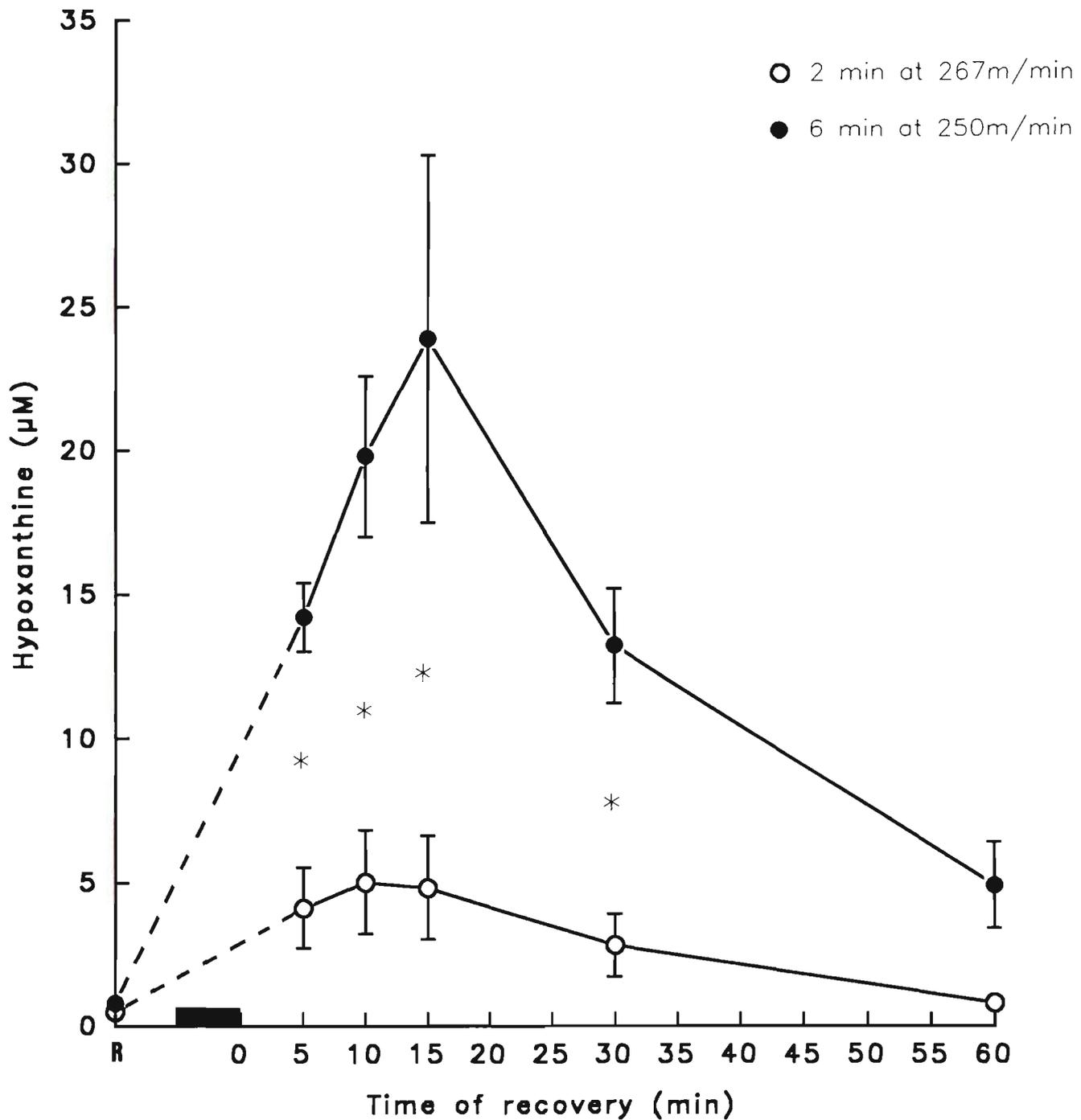


Figure 2.3; Forearm venous plasma hypoxanthine concentration at rest (R) and during recovery from 2 rowing bouts of different duration but similar intensity, unpublished data taken from Baldwin, 1993; (n=6, ■ denotes exercise, \* significantly different from 2 min trial P<0.05). Reproduced with permission Baldwin, 1993.

induced accumulation of IMP is not different in anaerobically trained rats compared with the control animals. Furthermore, no information exists on the influence of sprint training on the degradation products of IMP during sprint exercise in humans. Sjödin and Hellsten-Westling (1991) report that PNP activity is unchanged with sprint training indicating that the capacity of hypoxanthine production is probably unaltered by training.

#### 2.5.1.3 During Recovery From Intense Exercise

There are no data examining the influence of sprint training on muscle AdN metabolite concentrations during recovery from a maximal exercise bout. However, the *in vitro* hypoxanthine guanine phosphoribosyltransferase (HGPRT) activity is increased following high intensity training (Sjödin and Hellsten-Westling, 1991) implying that there may be an enhanced salvage of hypoxanthine during recovery. This is indirectly supported by the observation that training reduces the post-exercise plasma levels of hypoxanthine (Stathis et al. 1992) and uric acid (Sjödin and Hellsten-Westling, 1991). It must be noted however that Hellsten-Westling et al. (1993), reported no difference in the accumulation of hypoxanthine 15 min into recovery from a sprint training session in the trained compared to the untrained state.

## 2.6 Markers of Muscle Purine Metabolism in Plasma

### 2.6.1 Ammonia

The basal venous concentration of ammonia in normal healthy individuals ranges between 5-45  $\mu\text{M}$  in plasma (Mineo et al. 1985; Snow et al. 1992; Svensson and Anfalt, 1982) and 40-85  $\mu\text{M}$  in venous whole blood (Babij et al. 1983; Dudley et al. 1983; Wilkerson et al. 1977). The predominant source of resting blood ammonia is a continual protein catabolism in the gut (Onstad and Zieve, 1979). The maintenance of low basal concentrations is due predominantly to a constant removal by the liver (Eriksson et al. 1985, Lockwood et al. 1979) and to a lesser extent the brain (Lockwood et al. 1979), skeletal muscle (Eriksson et al. 1985; Lockwood 1979) and the lung (Larson et al. 1979).

Exercise induced hyperammonaemia was first documented by Parnas (1929) and it has since been established that in the transition from rest to exercise the muscle changes from a major sink to a major source of ammonia (Eriksson et al. 1985; Graham et al. 1990; Katz et al. 1986a&b; Lockwood et al. 1979). The removal of ammonia from the blood by the splanchnic region is not altered during exercise compared with rest (Eriksson et al. 1985). Additionally significant amounts of ammonia have been reported in the sweat (Czarnowski and Gorski, 1991). Although still to be determined, the lung may play a greater role in the removal of ammonia during exercise and recovery than during rest since the concentration within the blood are elevated in the former conditions and thus could increase the concentration within expired air (Larson et al. 1979). Taken as a whole, these data indicate that the rise in plasma ammonia concentration with exercise is primarily due to an increase in the ammonia efflux from contracting muscle as the ammonia removal rates from the plasma may increase or remain unaltered.

Ammonia production in contracting skeletal muscle is the result of AdN degradation (Graham et al. 1990; Katz et al. 1986a&b; Lowenstein 1972) and/or amino acid catabolism (Goodman and Ruderman, 1982; Goldberg and Chang, 1978; Graham et al. 1987). The relative contribution of each pathway is influenced by the intensity of the exercise (Wagenmakers et al. 1990). As mentioned earlier in this review, the degradation of AdN is the major metabolic pathway of ammonia production during high intensity exercise (Graham et al. 1990; Katz et al. 1986a&b).

Ammonia is a relatively small molecule which exists predominantly as  $\text{NH}_4^+$  at physiological pH and this form effluxes only slowly from the muscle. As  $\text{NH}_3$  the molecule can readily traverse the sarcolemma (Graham et al. 1987) and it appears in the blood soon after accumulation within the muscle. Estimates of ammonia release from the muscle range from 10 % (Katz et al. 1986a) to 25 % (Graham et al. 1990) of that which is produced during exercise. The discrepancies reported may be due to differences in exercise intensities and/or the mode of exercise. Intracellular acidosis results in a greater proportion of the ammonia in the protonated form and this may act to retain ammonia within the muscle (Harris and Dudley, 1989; Katz et al. 1986a). However, a dissociation between the muscle to

venous ratios of  $H^+$  and ammonia (Graham et al. 1990) suggests that there are other factors which may be more important in the exchange of ammonia across the sarcolemma.  $NH_4^+$  translocation may be effected by alterations in the membrane potential (Kleiner, 1981) and the competition of  $NH_4^+$  with  $K^+$  for the  $K^+$  channels in the sarcolemma (Knepper et al. 1989). It has also been suggested that a portion of ammonia may exit the muscle incorporated into amino acids (Graham et al. 1990).

The level to which the concentration of ammonia rises after an intense exercise bout is dependent on the duration (Graham et al. 1987) and intensity (Babij et al. 1983; Eriksson et al. 1985; Sahlin et al. 1991) of the exercise, muscle fibre type composition (Dudley et al. 1983) and training status of the individual (Lo and Dudley, 1987; Snow et al. 1992). Venous plasma concentrations of ammonia reach a peak 8-10 fold higher than resting levels in recovery from 30 s of "all out" cycle exercise, with values reaching 80  $\mu M$  (Snow et al. 1992). In a fatiguing exercise involving the knee extensors, and lasting an average 3.2 min, plasma levels of ammonia in excess of 150  $\mu M$  have been reported (Graham et al. 1990). Plasma ammonia concentrations are reported to be elevated above resting levels immediately after a 30 s sprint bout (Snow et al. 1992), and peak at about 2 min post-exercise (Graham et al. 1990; Itoh et al. 1990; Snow et al. 1992). Subsequent to the peak ammonia concentrations, which occurs early in recovery, there is a decrease towards resting levels after approximately 30 min (Graham et al. 1990; Katz et al. 1986a).

There is to date, only one study which has examined the influence of sprint training on plasma ammonia accumulation in humans (Snow et al. 1992). This study found that sprint training attenuated plasma ammonia concentration during recovery from a maximal 30 s cycle sprint bout on a cycle ergometer (Snow et al. 1992). These authors suggest that this is due to either a reduced ammonia efflux from the muscle after exercise, and/or an increased capacity of blood ammonia removal mechanisms. The precise mechanisms need to be confirmed by examining the effects of sprint training on muscle ammonia concentration.

### 2.6.2 Adenosine

Resting plasma adenosine concentrations are around  $1\mu\text{M}$  (Harkness et al. 1983; Sinkeler et al. 1986). As mentioned in an earlier section of this review, the production of adenosine in skeletal muscle is not considered to be the major fate of AMP degradation. Although elevated levels of skeletal muscle adenosine are reported after intense exercise in individuals deficient in AMPd (Sabina et al. 1984), the levels measured at the point of fatigue are not as great as the magnitude of IMP production in healthy subjects after high-intensity exercise. Sinkeler et al. (1986), show a greater change in plasma adenosine in AMPd deficient following ischaemic forearm exercise highlighting the potential for this pathway in skeletal muscle which is not realised in healthy subjects. Additionally, the absence of an increase in plasma adenosine whilst plasma inosine shows a small rise after exercise (Sinkeler et al. 1986) supports the point that skeletal muscle favours AMP deamination. Alternatively, the half-life of adenosine in human blood, reported to be about 10 s (Klabunde, 1983), is likely to maintain low levels of adenosine in the blood. Also the adenosine within the plasma could be rapidly converted to inosine (Harkness et al. 1983).

### 2.6.3 Inosine

Plasma inosine levels at rest have been measured at around  $1\mu\text{M}$  (Harkness et al. 1983; Mineo et al. 1985). Cycle exercise up to 40 W (Kono et al. 1986) does not increase plasma inosine concentrations in healthy individuals, but 2 min of intense cycle exercise (Harkness et al. 1983) and 2 min of ischaemic forearm exercise (Mineo et al. 1985) produces a small but significant rise in this metabolite. Differences in exercise mode and intensity may explain the varied response. Inosine is produced from either adenosine or IMP and the marked accumulation of IMP after intense exercise suggests that it is probably the major substrate for inosine formation in skeletal muscle in these circumstances. Additionally, the intensity of the exercise is likely to have some influence on the production of inosine. Inosine is subsequently converted to hypoxanthine by PNP and the activity of this enzyme may explain the relatively low inosine levels in the plasma. This is supported by the magnitude of

hypoxanthine relative to inosine in the plasma following exercise (Harkness et al. 1983; Mineo et al. 1985). The extensive accumulation of IMP in skeletal muscle supports the notion that the production of inosine is the rate limiting reaction in the degradation of AdN. The rapid return of plasma inosine to resting values during recovery from exercise is probably due to the presence of PNP in the plasma (Harkness et al. 1983) and/or endothelial cells (Bowditch et al. 1985).

#### 2.6.4 Hypoxanthine

The resting concentration of hypoxanthine is around 1-2  $\mu\text{M}$  (Harkness et al. 1983; Ketai et al. 1987; Mineo et al. 1985; Sahlin et al. 1991; Stathis et al. 1992). The basal ATP turnover in tissues is probably responsible for the small but constant production of hypoxanthine. The removal of hypoxanthine in the blood at rest is due to direct excretion (Nasrallah and Al-Khalidi, 1964), conversion of hypoxanthine to uric acid (Hellsten-Westling et al. 1989) and/or hypoxanthine uptake by tissues (Harmsen et al. 1984).

It has been demonstrated that skeletal muscle production of hypoxanthine and its efflux into the plasma (Bangsbo et al. 1992) is primarily responsible for this elevation in plasma hypoxanthine during recovery from intense exercise (Harkness et al. 1983; Hellsten-Westling et al. 1991; Ketai et al. 1987; Sjödin and Hellsten-Westling, 1990; Stathis et al. 1992). The intensity of the exercise is a major determinant in plasma hypoxanthine levels (Hellsten-Westling et al. 1991; Ketai et al. 1987; Sahlin et al. 1991). Sjödin and Hellsten-Westling, (1990), report that there is an inflexion point around 110%  $\text{VO}_2\text{max}$  for the rise in plasma hypoxanthine. Data by Ketai et al. (1987) shows that hypoxanthine increases in the plasma at intensities above the ventilatory threshold ( $V_t$ ). Maximal sprint exercise for 30 s does not increase plasma levels of hypoxanthine at the end of exercise but there is an increase to a peak level around 15-20 min into recovery (Stathis et al. 1992). This reflects the time course of hypoxanthine production in, and efflux from, the muscle and is supported by other studies investigating the recovery levels of hypoxanthine which report peak concentrations 15-30 fold higher than resting levels 15-20 min after intense exercise (Hellsten-Westling et al. 1989; Ketai et al. 1987; Sjödin and

Hellsten-Westling, 1990). Plasma concentrations remain elevated above resting levels for at least 30 min after 30 s of "all out" cycling (Stathis et al. 1992) and for 120 min after approximately 2 min of intense maximal running (Hellsten-Westling et al. 1989). Hypoxanthine could be removed from the plasma by inactive skeletal muscle when circulating hypoxanthine concentrations are high (Bangsbo et al. 1992), or other tissues such as the heart (Harmsen et al. 1984) and liver (Kim et al. 1992).

Hellsten-Westling et al. (1993) report that there is no difference in plasma hypoxanthine 15 min into recovery after an intense intermittent sprint training session in the sprint trained relative to the control subjects. In contrast, Stathis et al. (1992) reported that sprint training attenuates the plasma hypoxanthine concentrations 20 and 30 min into recovery from a 30 s sprint bout. Differences may be explained by the sampling times, of which only one is reported by Hellsten-Westling et al. (1993), compared to several serial samples measured by Stathis et al. (1992). The difference may also be due to the total duration of the sprint exercise performed before the sampling period. The sprint induced attenuation of plasma hypoxanthine reported by Stathis et al. (1992) may arise from a decreased efflux into the blood or an increased capacity of the plasma hypoxanthine removal mechanisms with sprint training. Sjödin and Hellsten-Westling (1991), demonstrated an increased maximal activity of HGPRT after training which may support an increased reuptake of the hypoxanthine from the blood. There are no studies available which have directly examined the effects of training on the removal mechanisms of hypoxanthine from the blood.

#### 2.6.5 Uric Acid

The concentration of uric acid in the blood in healthy individuals is around 250-300  $\mu\text{M}$  at rest (Hellsten-Westling et al. 1989; Sjödin and Hellsten-Westling, 1990). The production of uric acid at rest arises from the conversion of hypoxanthine to uric acid catalysed by xanthine oxidase (Fig. 2.1) which is found in endothelial cells of blood vessels (Jarasch et al. 1981). The major mechanism of removal of uric acid from the blood is excretion by the kidney (Edwards et al. 1979; Sahlin and Katz, 1988).

Increases in plasma uric acid levels are observed 20-30 min after intense exercise (Hellsten-Westling et al. 1989; Hellsten-Westling et al. 1991; Sjödín and Hellsten-Westling 1990). The intensity (Sjödín and Hellsten-Westling, 1990) and duration (Hellsten-Westling et al. 1991) of the exercise influences the rise in concentration of uric acid in the blood. Like hypoxanthine, plasma uric acid levels post-exercise are unchanged unless the exercise intensity is high (Sjödín and Hellsten-Westling, 1990).

The levels of plasma uric acid begin to rise 15 min into recovery from maximal, short-distance running efforts and peak after 45 min (Hellsten-Westling et al. 1989). This delay is probably due to the fact that the formation of uric acid is the final step in a cascade of reactions (Fig. 2.1), in which many of the enzymes have low maximal reaction rates. The time taken to rise above resting concentrations and the time to reach peak levels of plasma uric acid is delayed relative to the same points for hypoxanthine (Hellsten-Westling et al. 1989). The fact that it takes somewhat longer for uric acid to peak suggests that much of the time taken for the accumulation of uric acid is probably due to the activity of xanthine oxidase. Xanthine oxidase, the enzyme responsible for the production of uric acid, is located in skeletal muscle (Wajner and Harkness, 1988) and endothelial cells of the blood vessels (Jarasch et al. 1981) and other tissues (Wajner and Harkness, 1988). Xanthine oxidase activity is relatively low in skeletal muscle compared to other tissues, particularly the liver (Wajner and Harkness, 1988) hence formation of uric acid occurs predominantly when hypoxanthine diffuses out of the muscle. This explains in part, the delay in uric acid accumulation. The absolute change in uric acid is three times greater than hypoxanthine after intense exercise (Hellsten-Westling et al. 1989), which is partly due to the fact that uric acid is the end point of AdN degradation. Sprint training attenuates uric acid concentration (Sjödín and Hellsten-Westling, 1991). It is unclear whether this attenuation is due to a reduction in uric acid production and/or an increased capacity for removal by the kidneys following training. No human study has investigated the effects of sprint training on uric acid concentration in recovery from intense exercise.

## CHAPTER 3

### METHODS

#### 3.1 Subjects

Six male and two females, who were active but non-specifically trained, were recruited for this study. All subjects were fully informed of the experimental procedures and signed informed consent (see Appendix A). This experiment was approved by the Human Ethics Committee of Victoria University of Technology.

#### 3.2 Maximal Oxygen Consumption ( $\text{VO}_2\text{max}$ )

Incremental cycle ergometer tests were conducted at least one week before the first, and one week after the last sprint test in order to determine the effect of the sprint training program on the subject's  $\text{VO}_2\text{max}$ . The  $\text{VO}_2\text{max}$  test initially required the subjects to cycle (Monark 814E ergometer) for 2 min at each of three progressively increasing submaximal workloads. Immediately following this, the workload was further increased every min until volitional exhaustion. Expired air was directed, by a Hans-Rudolf valve, through a ventilometer (Pneumoscan S30) into a mixing chamber and analysed for oxygen and carbon dioxide by gas analysers (Applied Electrochemistry S-3A  $\text{O}_2$  and CD-3A  $\text{CO}_2$ ) which were calibrated prior to each test using commercially prepared gas mixtures. The criterion used to determine the attainment of  $\text{VO}_2\text{max}$  was the achievement of a plateau in oxygen consumption. A plateau was defined as an increase of less than  $100 \text{ ml}\cdot\text{min}^{-1}$  with increasing workrate.

#### 3.3 Sprint Testing Protocol

The subjects were required to perform a 30 s maximal "all out" sprint bout before and after a training regime. Blood sampling and muscle biopsies were obtained before and following these exercise bouts (invasive trials), as summarised in Fig 3.1. The final sprint test was performed 2 to 3 days after the last training session. Each subject was instructed to refrain from exercise and alcohol consumption 24 hours prior to each test and to report to the laboratory in the

Figure 3.1. Summary of blood and muscle sampling during maximal sprint bout; R, rest; ■, denotes 30 s sprint; ↑, denotes blood sampling; ↓, denotes muscle sampling.

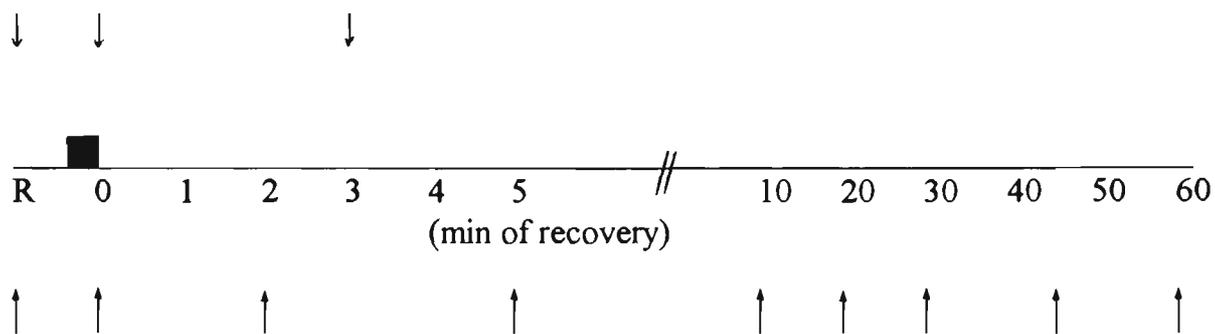


Table 3.1. Summary of the sprint training regime

week number	number of sessions per week	number of 30 s sprint bouts per session	rest interval between bouts (min)
1	3	3	4
2	3	4	4
3	3	6	4
4	3	8	4
5	3	10	4
6	3	10	3
7	3	10	3

morning after an overnight fast. The 30 s maximal sprint bout was performed on an air-braked cycle ergometer (Series A, Repco, Melbourne, Australia), modified to enable computerised determination of peak power, mean power, total work, fatigue index and the power output from the ergometer at 5 s intervals. The power output of the air-braked cycle ergometer is related to the cube of the wheel velocity which was measured using a tachometer (Hall effect device and a cog at the wheel hub). The fatigue index was calculated as  $[(\text{peak power} - \text{the power output at 30 s}) / \text{peak power}] \times 100$ . The subjects were instructed to remain seated and pedal as fast as possible for the complete 30 s. Once the sprint was completed and the immediate post-exercise biopsy (exercise sample) was obtained, the subjects rested in the supine position for the 3 min biopsy and until blood sampling procedures were completed. A 30 s sprint familiarisation session, during which performance parameters were measured, was conducted at least three days prior to the first invasive trial. Due to technical difficulties only six of the subjects were measured non-invasively pre-training. Performance parameters were also measured during the first sprint bout of the final training session and were compared to the final sprint test. The conditions in these sessions (non-invasive trials), except for muscle biopsies and blood collection, were maintained as close as possible to those of the invasive trials. The results of these non-invasive trials were used to ascertain the effect of invasive procedures on performance and to provide an indication that performances in the invasive trials were maximal.

### 3.4 Training Protocol

Training involved 3 sessions per week and each training session was separated by at least one day but, where possible, no more than three days. Over a period of seven weeks, the subjects completed a series of 30 s maximal "all out" sprint bouts on an air-braked cycle ergometer (Repco). In the first five weeks the number of sprint bouts per session increased weekly from three to ten with four min rest between bouts. During the final two weeks, ten maximal sprint bouts of 30 s duration were completed each session with three min recovery between sprints. Details of the training protocol are summarized in Table 3.1.

### 3.5 Blood Sampling, Treatment and Analysis

Venous blood samples (5 ml) were taken from an antecubital vein, via an indwelling catheter, at rest, immediately post-exercise (0 min) and at 2, 5, 10, 20, 30, 45 and 60 min of recovery. Blood was placed in tubes containing lithium heparin and then spun in a centrifuge. Subsequently, 500  $\mu$ l of blood or plasma were added to ice cold 3M PCA, spun again and the supernatant stored at  $-80^{\circ}\text{C}$  prior to analysis for lactate. The remainder of the plasma was stored in liquid nitrogen for later ammonia and hypoxanthine analysis. The plasma stored for the measurement of hypoxanthine required deproteinisation with 1.5 M PCA and subsequent neutralisation with 2.1 M  $\text{KHCO}_3$  immediately prior to analysis. Blood and plasma lactate were determined in duplicate, using an enzymatic spectrophotometric technique (Lowry and Passonneau, 1972). Plasma ammonia analysis was performed in duplicate within 72 hours of collection, using flow injection analysis (Svensson and Anfält, 1982). Plasma hypoxanthine was measured on neutralised PCA extracts, using a modification of the reverse-phase high performance liquid chromatography (HPLC) method described by Wynants & Van Belle (1985). Separation was achieved by a Merck Hibar Lichrosphere 100 CH - 18/2 250 mm x 4 mm column using a Bio-Rad Model 700 Chromatography Workstation. Separate venous blood samples (2mls) were drawn into syringes lined with heparin ( $5000 \text{ IU}\cdot\text{ml}^{-1}$ ) and kept on ice, under anaerobic conditions, until analysed for pH within 90 min of collection. Plasma pH was determined from whole blood, in duplicate, using a blood gas analyser (Radiometer, Copenhagen, ABL-30 acid base analyser). For further details of analytical procedures for blood and plasma metabolites see Appendix B.

### 3.6 Muscle Sampling, Treatment and Analysis

Muscle biopsies were obtained from the vastus lateralis under local anaesthesia (1% lignocaine) by the percutaneous needle biopsy technique (Bergström, 1962) modified for suction. Muscle samples were taken at rest, immediately following, and 3 min after exercise. These were obtained from 3 separate incisions (3 cm apart) in one leg with the resting sample taken from the distal incision and with each subsequent biopsy from the next proximal incision. Leg

selection was random and in the second trial the contralateral leg was biopsied. Each muscle biopsy sample was obtained approximately 10 s after the cessation of exercise and frozen in liquid nitrogen about 5 s subsequent to this. Each sample was divided into two portions which were weighed at  $-30^{\circ}\text{C}$ . One portion (8-15 mg) was extracted at  $-20^{\circ}\text{C}$  using 0.6M PCA-10% methanol, neutralised with KOH and analysed for ammonia by a flow injection analysis technique as described by Katz et al. (1986a). Muscle ammonia is reported per unit dry weight, using the wet weight to dry weight ratio determined on the second portion of the sample. The second muscle portion was freeze dried, weighed, dissected free of any connective tissue, powdered and extracted according to Harris et al. (1974). The neutralized extract was assayed enzymatically for creatine phosphate (CP), creatine and lactate using fluorometric detection (Lowry and Passonneau, 1972). Reverse-phase HPLC was used to quantify ATP, ADP, AMP, IMP, inosine, adenosine, adenine and hypoxanthine concentrations using the method of Wynants & Van Belle (1985). Separation was achieved using the same HPLC apparatus described above. For details of all muscle analysis see Appendix C. Muscle metabolites, except for lactate and ammonia (due to their extracellular presence), were adjusted to the peak total creatine for each subject in each trial. This was done to correct for variability in blood, connective tissue and other non-muscle constituents between biopsies.

### 3.7 Statistical Analyses

Peak power, mean power, total work, subject weight,  $\text{VO}_2\text{max}$ , fatigue index and the changes in muscle metabolite concentrations during exercise and recovery were analysed using Student's t-test for paired data. The 5 s interval power data and the muscle and plasma metabolite concentrations were analysed using two factor (time and training) ANOVA with repeated measures using the Bio-Medical Data Processing (BMDP) statistical software. Simple main effects analyses and Newman-Keuls post-hoc tests were used to locate differences when ANOVA revealed a significant interaction. The level of probability to reject the null hypothesis was set at  $P < 0.05$ . All values are reported as means  $\pm$  standard error of the mean (SE).

## CHAPTER 4

### RESULTS

#### 4.1 Subject Characteristics

The average age of the subjects was  $22.1 \pm 1.0$  years and their weight was  $70.1 \pm 3.4$  kg. There were no differences in the weight of the subjects in the pre compared with the post-trained state ( $P > 0.05$ ).

#### 4.2 Performance Variables and Maximal Oxygen Consumption

There were no differences in peak power, mean power and total work between the familiarisation session and the first invasive trial, respectively (Fig 4.1;  $n=6$ ). Similarly, no differences in these variables were found between the first bout of the the final training session and the post-training invasive trial (Fig 4.1). Sprint training resulted in significant increases in peak power (16.8 %), mean power (11.8 %) and total work (11.9 %) (Fig. 4.2) over the 30 s sprint. Furthermore, training resulted in a significantly greater power output, at every 5 s interval throughout the trial except at 30 s (Fig. 4.3). The seven week sprint training program did not influence  $VO_2\max$  ( $49.6 \pm 3.6$  vs  $51.7 \pm 1.8$  ml.kg<sup>-1</sup>.min<sup>-1</sup>,  $n=8$ ,  $P > 0.05$ , untrained vs trained) nor did it affect the fatigue index ( $42.0 \pm 5.9$  vs  $45.1 \pm 7.8$  %,  $n=8$ ,  $P > 0.05$ , untrained vs trained).

#### 4.3 Muscle Metabolite Concentrations

Due to technical difficulties, muscle data are presented for six of the eight subjects who completed the training regime (four males and two females). The muscle metabolite concentrations at rest, end-exercise and 3 min post-exercise before and after sprint training are summarised in Table 4.1. The mean total creatine concentration was similar pre and post-training ( $117.8 \pm 3.7$  vs  $123.7 \pm 4.4$  mmol.kg<sup>-1</sup> dw, untrained vs trained,  $P > 0.05$ ). There were no systematic changes in total creatine content comparing rest with exercise, before or after training, when examining non-adjusted values. All measured resting metabolites were similar pre and post-training except for ATP and the total adenine nucleotide (TAN =

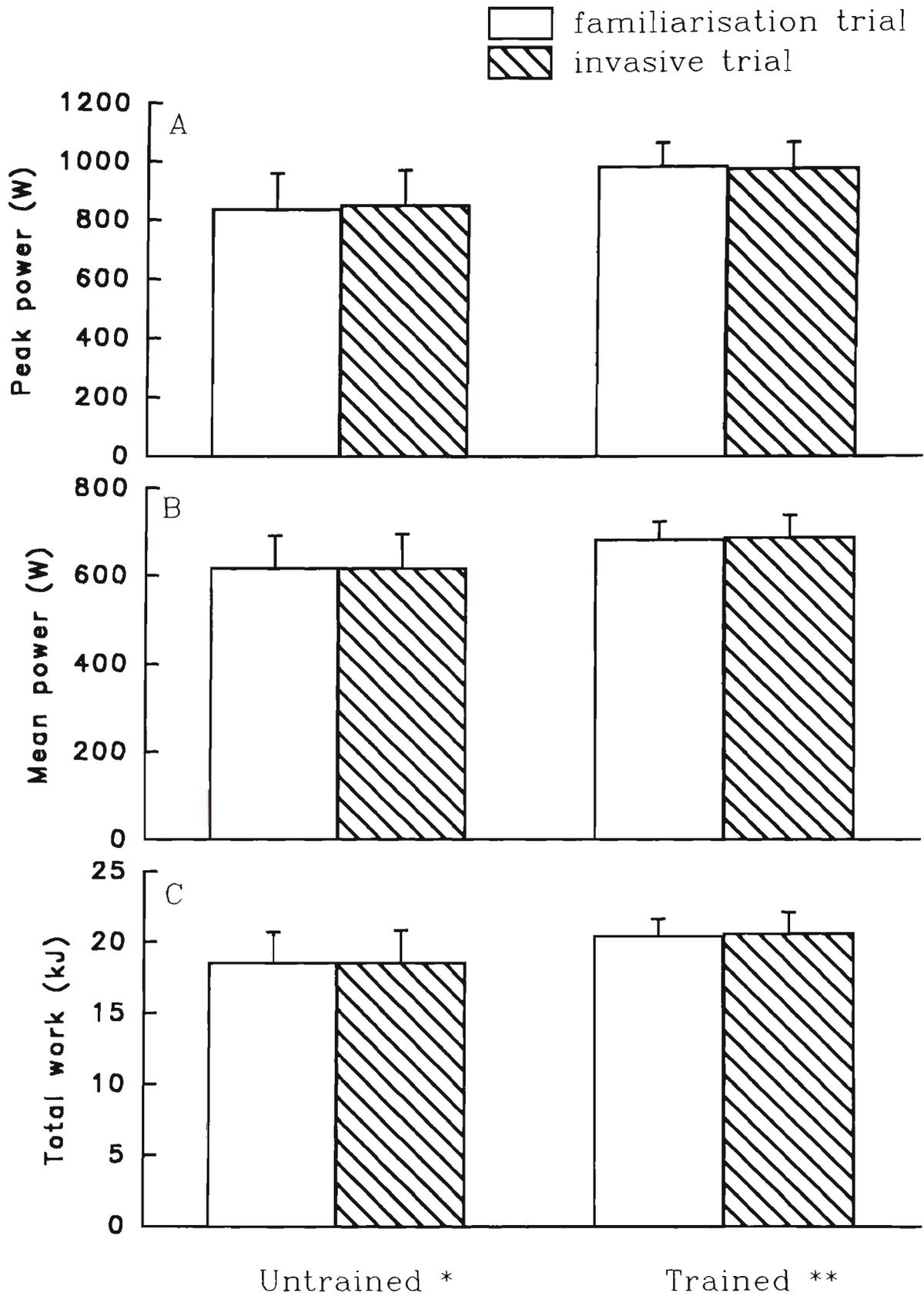


Figure 4.1. The peak power (A), mean power (B) and total work (C) obtained in a 30 s maximal sprint bout on a cycle ergometer after non-invasive (familiarisation) and invasive trials, before and after sprint training; (\*\* n=8, \* n=6).

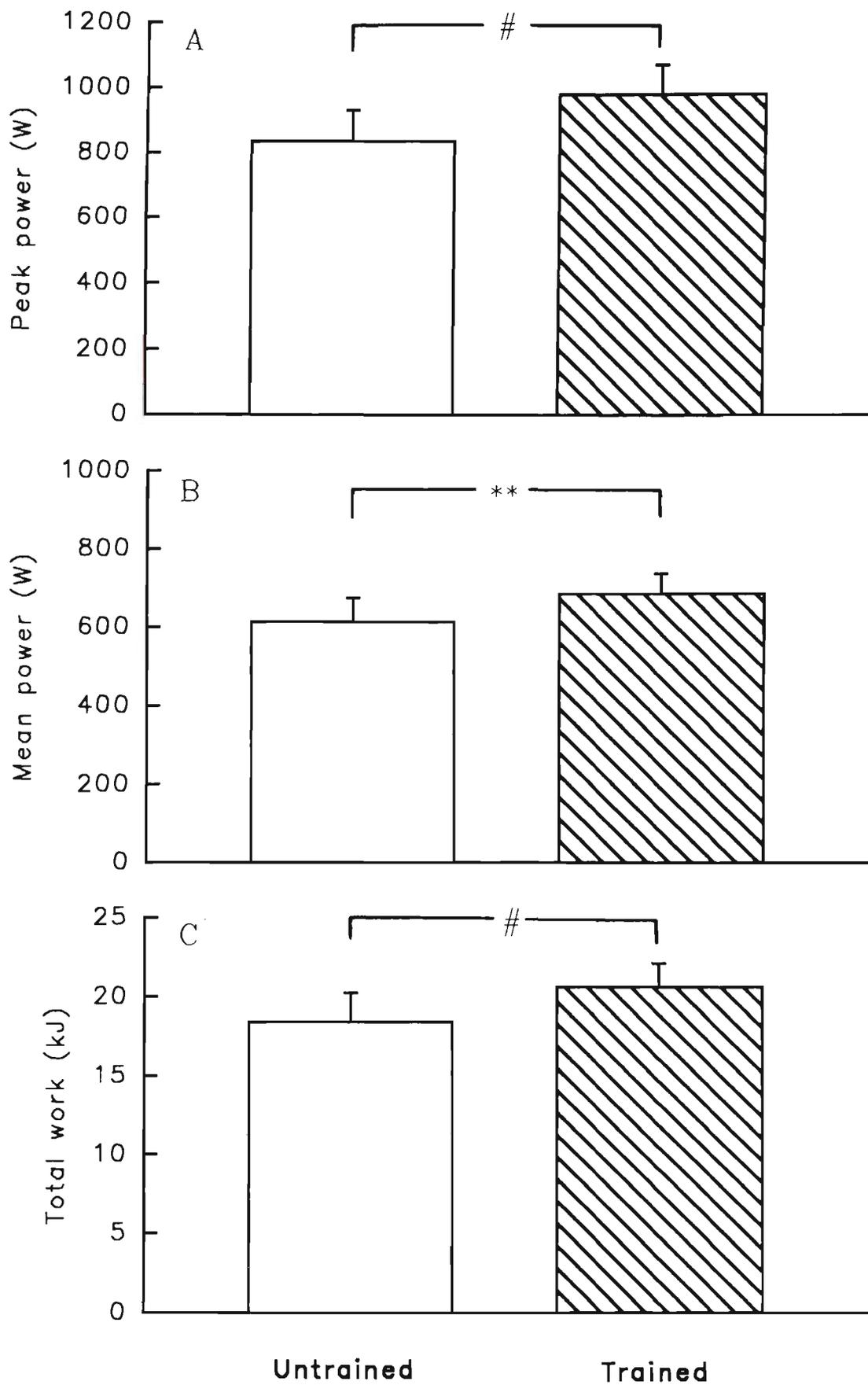


Figure 4.2. The peak power (A), mean power (B) and total work (C) obtained in a maximal sprint bout on a cycle ergometer before and after sprint training; (n=8, significantly different from untrained #  $P < 0.001$ , \*\*  $P < 0.01$ )

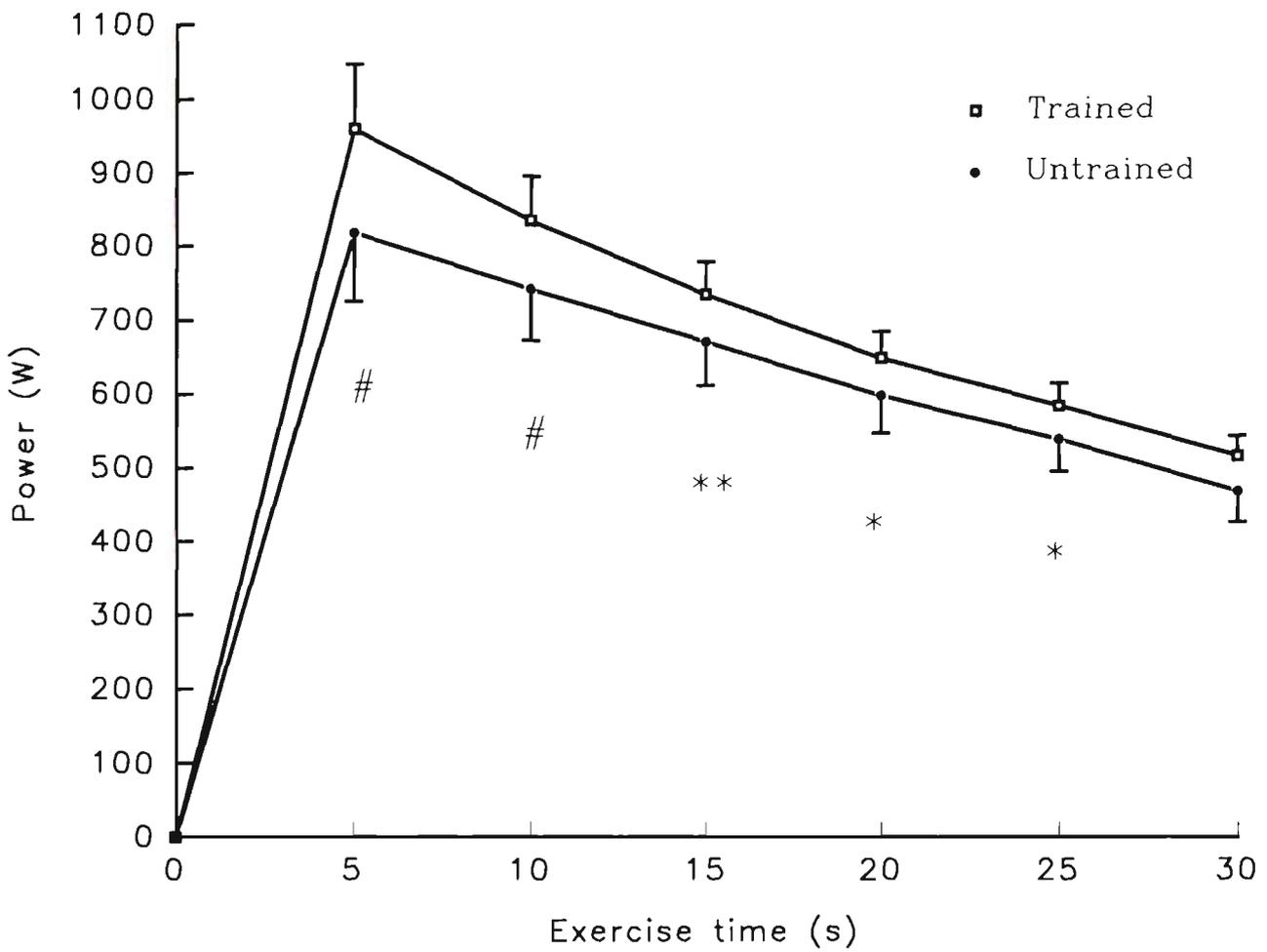


Figure 4.3. Power output at 5 s intervals during a 30 s maximal sprint on a cycle ergometer before and after sprint training; (n=8, significantly different from untrained #  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ ).

Table 4.1. Muscle metabolite concentrations in vastus lateralis at rest, at the completion of 30 s of maximal sprint cycling and after 3 min of recovery before (Untrained) and after (Trained) 7 weeks of sprint training.

	UNTRAINED			TRAINED		
	Rest	Exercise	3 min recovery	Rest	Exercise	3 min recovery
ATP	22.8±0.8	13.6±1.0 a	16.3±1.4 a	18.5±0.6 *	14.1±1.5 a	16.0±1.7 a
ADP	1.97±0.06	1.79±0.11	1.88±0.11	1.61±0.11	1.74±0.17	1.83±0.017
AMP	0.07±0.01	0.06±0.01	0.04±0.01 a	0.06±0.01	0.05±0.01	0.07±0.03
IMP	0.08±0.01	7.40±0.90 a	6.25±0.98 a	0.08±0.02	4.42±0.76 *a	3.95±0.87 *a
Ammonia	0.6±0.2	8.7±1.7 a	5.7±1.3 a	0.6±0.1	4.3±0.8 *a	3.4±0.5 a
Inosine	0.02±0.01	0.10±0.01 a	0.44±0.03 a,b	0.02±0.01	0.06±0.01	0.21±0.04 **a,b
Hypoxanthine @	<0.01	0.03±0.01	0.14±0.07	<0.01	0.03±0.01	0.05±0.01
CP	85.4±3.6	27.6±2.8 a	65.5±5.0 a,b	92.0±5.3	29.6±4.9 a	71.2±6.8 a,b
Creatine	42.9±2.2	100.7±2.6 a	62.8±4.4 a,b	40.9±1.5	103.3±3.0 a	60.3±2.3 a,b
Lactate	6.8±0.9	92.4±7.3 a	69.6±7.0 a,b	6.1±0.4	99.2±4.8 a	69.0±3.4 a,b
TAN	24.8±1.1	15.5±1.1 a	18.2±1.4 a	20.3±0.7 *	15.8±1.6 a	17.9±1.9 a
TAN + IMP #	24.9±1.1	22.9±0.8	24.5±0.9	20.4±0.7	20.3±1.5	21.8±1.8

Values are means ± SE, (n=6); units are mmol.kg<sup>-1</sup>.dw; CP, creatine phosphate; IMP, inosine 5'-monophosphate; TAN, total adenine nucleotide pool (ATP+ADP+AMP); \* P<0.05, \*\* P<0.01, significantly different from Untrained; a, significantly different from rest, P<0.05; b, significantly different from exercise, P<0.05, # significant main effect trained less than untrained, P<0.05, @ significant main effect for time, 3 min significantly greater than exercise and rest, P<0.05.

ATP+ADP+AMP) pool which were reduced by 19% and 18%, respectively ( $P < 0.05$ ) after training.

Muscle ADP and AMP levels did not change with exercise either before or after training. In contrast, ATP and CP decreased ( $P < 0.05$ ) to a similar end-exercise level, irrespective of training status. Muscle creatine, lactate, ammonia and IMP increased markedly ( $P < 0.05$ ) in the two sprint trials. Training decreased ( $P < 0.05$ ) the content of ammonia and IMP after exercise but had no influence on lactate and creatine levels. Prior to training there was an exercise-induced increase ( $P < 0.05$ ) in muscle inosine concentration, however, no increase occurred after training.

Muscle ATP, ADP and AMP content after 3 min of recovery were not different from exercise values and were unaffected by training. AMP concentration was lower ( $P < 0.05$ ) in recovery compared with rest in the untrained state but this was not the case after training. After 3 min of recovery muscle IMP and ammonia contents were similar to exercise values and still elevated ( $P < 0.05$ ) above resting levels. IMP at 3 min of recovery was lower ( $P < 0.05$ ) after training, however, training did not influence muscle ammonia content at this time point. During recovery, muscle inosine concentration increased ( $P < 0.05$ ) above resting and exercise levels both before and after training. Furthermore training reduced ( $P < 0.01$ ) the inosine levels during recovery. The recovery values for lactate and creatine were lower ( $P < 0.05$ ) than exercise values but greater ( $P < 0.05$ ) than resting concentrations. The concentrations of creatine and lactate after 3 min of recovery were not influenced by training. The 3 min recovery values for CP were higher ( $P < 0.05$ ) than exercise values but lower ( $P < 0.05$ ) than resting concentrations and training had no effect on the recovery of this metabolite.

Adenosine (detection limit  $< 0.04 \text{ mmol.kg}^{-1} \text{ dw}$ ) and adenine (detection limit  $< 0.15 \text{ mmol.kg}^{-1} \text{ dw}$ ) were undetectable in all biopsy samples. Hypoxanthine levels at rest were not detectable in all but one sample (detection limit  $< 0.01 \text{ mmol.kg}^{-1} \text{ dw}$ ). There was no significant interaction evident when analysing the muscle hypoxanthine data. There was a main effect, however, ( $P < 0.05$ ) for time which showed that the 3 min recovery values were greater than rest and exercise.

The latter hypoxanthine values were not different.

The effect of sprint training on the change in muscle metabolite concentration during exercise and recovery is summarised in Table 4.2. The magnitude of the exercise-induced fall in ATP was 52% lower ( $P < 0.05$ ) after training. In addition, training reduced ( $P < 0.05$ ) the accumulation of IMP and ammonia after 30 s of sprinting by 41% and 52%, respectively. The changes in concentration of the remaining metabolites during exercise were not influenced by training. Training had no effect on the change in any of the muscle metabolite concentrations during recovery except for inosine, which increased ( $P < 0.05$ ) during the recovery period to a greater extent before training.

#### 4.4 Plasma and Blood Metabolite Concentrations

The resting concentrations of all measured plasma and blood metabolites were similar pre and post-training. After exercise plasma ammonia increased ( $P < 0.05$ ) approximately 8 fold from rest, reaching a peak between 5 and 10 min into recovery (Fig. 4.4A). Prior to training, ammonia increased ( $P < 0.05$ ) above resting values after 2 min of recovery and remained elevated until at least 30 min. In contrast, after training the ammonia levels were greater ( $P < 0.05$ ) than rest at the end of exercise and remained so up until 20 min. Training resulted in an increase ( $P < 0.05$ ) in ammonia at 2 min recovery, however, these levels tended to be lower ( $P = 0.06$ ) after training at 20 min. Plasma hypoxanthine concentration was higher ( $P < 0.05$ ) than resting concentrations after 5 min recovery, before training, compared with 2 min, after training (Fig. 4.4B). Hypoxanthine concentrations had not returned to resting levels ( $P < 0.05$ ) within 60 min of recovery in either trial. Training resulted in a decreased ( $P < 0.05$ ) plasma hypoxanthine concentration at 45 and 60 min of recovery. Plasma pH decreased immediately post-exercise in both trials and remained significantly lower until 20 and 30 min of recovery in the untrained and trained states, respectively (Fig 4.4C). The post-exercise plasma pH was significantly lower in the trained state at the end of exercise and at 2, 5 and 10 min of recovery when compared to the untrained state. Plasma and blood lactate levels exceeded ( $P < 0.05$ ) resting concentrations at the end of exercise and

Table 4.2. The change in muscle metabolite concentrations at the completion of 30 s of maximal sprint cycling and after 3 min of recovery before (Untrained) and after (Trained) 7 weeks of sprint training.

	Exercise a		Recovery b	
	Untrained	Trained	Untrained	Trained
ATP	-9.2±1.6	-4.4±1.1 *	2.7±0.8	2.0±0.9
ADP	-0.18±0.10	0.13±0.07	0.09±0.08	0.09±0.12
AMP	-0.02±0.01	-0.01±0.01	-0.03±0.01	-0.01±0.04
IMP	7.30±0.87	4.33±0.78 *	-1.13±0.37	-0.45±0.31
Ammonia	8.1±1.0	3.7±0.4 *	-3.0±0.8	-0.9±0.4
Inosine	0.08±0.02	0.04±0.01	0.34±0.03	0.15±0.03 *
CP	-57.9±2.9	-62.4±3.1	37.9±2.8	43.0±4.1
Creatine	57.9±2.9	62.4±3.1	-37.9±2.8	-43.0±4.1
Lactate	85.6±7.9	93.1±4.9	-22.8±3.25	-30.2±6.0

Values are means ± SE, (n=6); units are mmol.kg<sup>-1</sup>.dw; CP, creatine phosphate; IMP, inosine 5'-monophosphate; \* P<0.05, significantly different from Untrained; a, end 30 s sprint exercise concentration - resting concentration; b, 3 min recovery concentration - end 30 s exercise concentration.

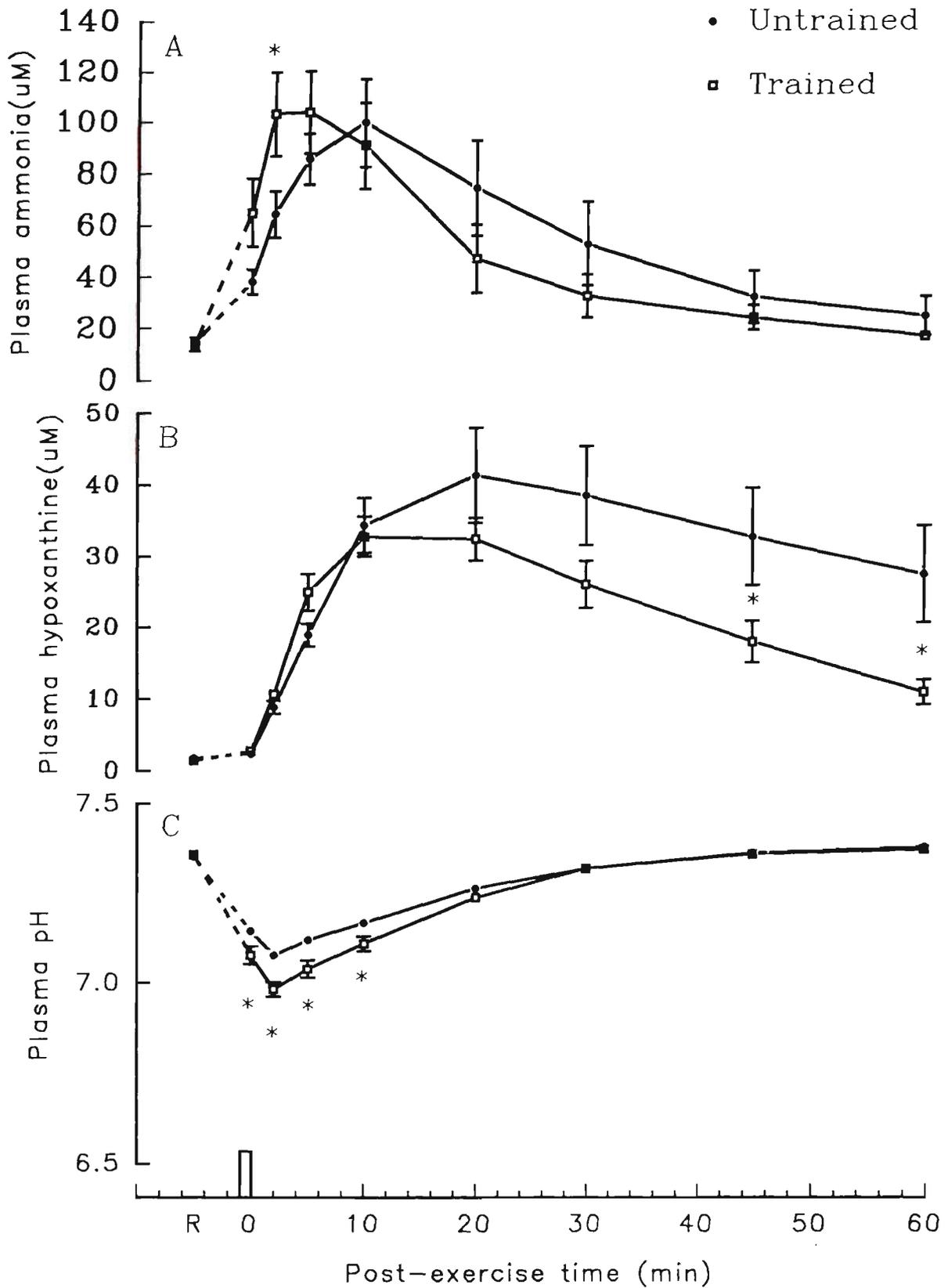


Figure 4.4. Forearm venous plasma ammonia (A), hypoxanthine (B), and pH (C) at rest (R) and during recovery from a 30 s maximal sprint bout before and after sprint training; (n=8,  $\square$  denotes exercise significantly different from untrained \* P<0.05)

throughout the 60 min recovery period in both the trained and untrained condition (Fig. 4.5 A,B). There was a greater increase ( $P < 0.05$ ) in plasma lactate after training with the concentrations being higher immediately after exercise and at 2, 5, 10 and 20 min of recovery. Blood lactates were elevated ( $p < 0.05$ ) after training, immediately after exercise and at 2, 5 and 10 min of recovery.

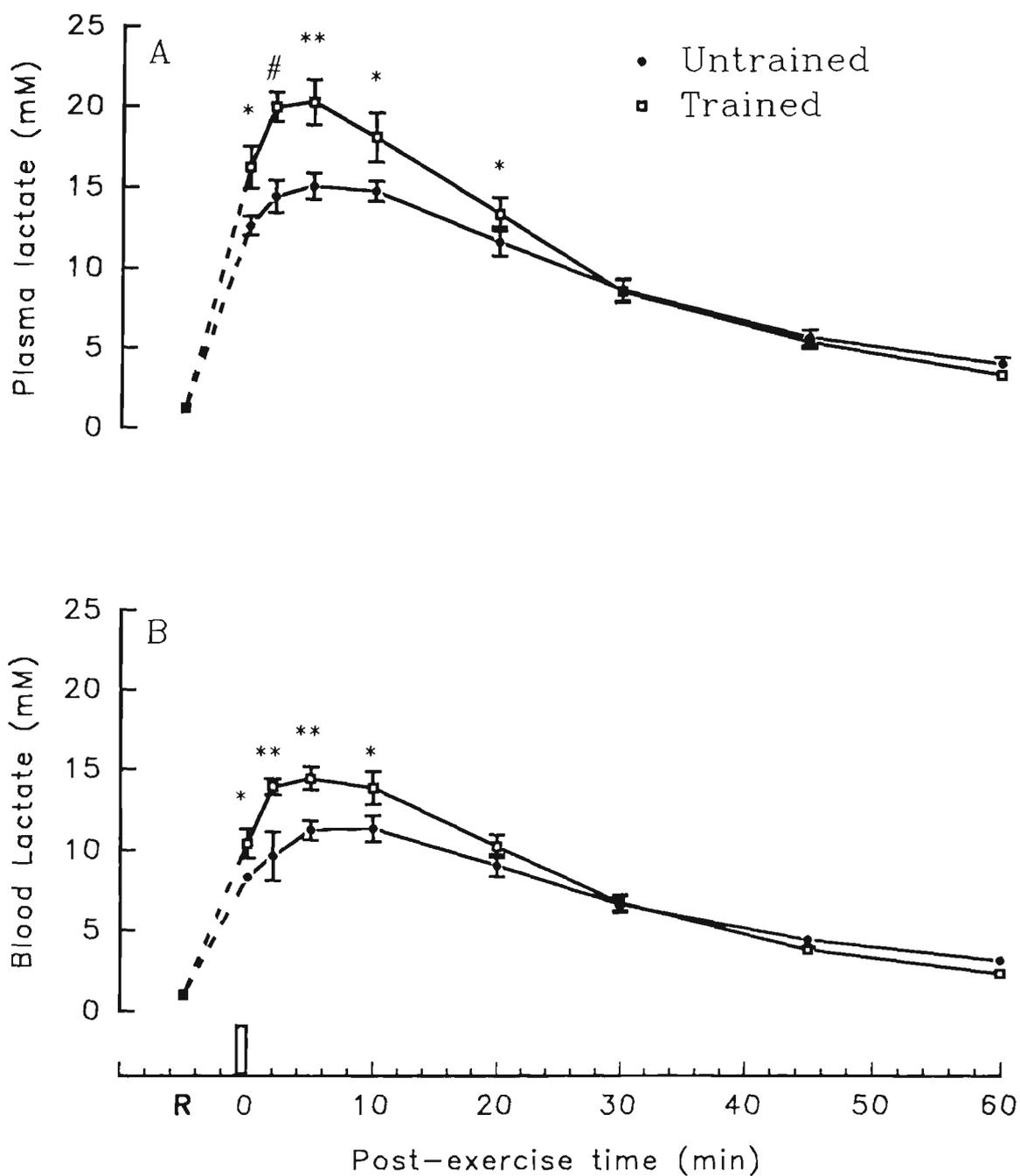


Figure 4.5. Forearm venous plasma lactate (A), and blood lactate (B) concentration at rest (R) and during recovery from a 30 s maximal sprint bout before and after sprint training; (n=8,  $\square$  denotes exercise, significantly different from untrained #  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ ).

## CHAPTER 5

### DISCUSSION

#### 5.1 The Effects of Sprint Training on Performance Variables with a Maximal Sprint Cycling Bout

Relatively few investigations into the effectiveness of sprint training regimes on performance variables in humans have been reported. The criteria for successful sprint training protocols have not been defined. Previous sprint training studies have reported an improvement in peak power (Nevill et al. 1989), mean power (Boobis et al. 1983b) or total work output (Snow et al. 1992) over 30 s of maximal exercise. Sharp et al. (1986) reported a training increase in peak torque and total work over a 45 s maximal exercise bout, while Thorstensson et al. (1975) found that the time to fatigue, performing isometric contractions of the leg at 50% MVC, was prolonged after training. Other investigators have reported no change in any measured performance variables as a result of sprint training (Jacobs et al. 1987). One of the purposes of this study was to investigate the effect of intense sprint training on total work, peak and mean power over a 30s maximal sprint cycle bout.

Similar sprint performances in the invasive and non-invasive trials suggest that exercise performance was not adversely affected by biopsy and cannulation procedures (Fig 4.1). This is consistent with the findings of Withers et al. (1991) and furthermore indicates that the performances in the invasive trials were probably maximal. The training-induced increase in exercise performance observed in the present study was similar to, or slightly higher, than other studies which have used a 30 s maximal exercise test (Boobis et al. 1983b; Nevill et al. 1989; Snow et al. 1992). It is interesting to note that the power profiles reported by Nevill et al. (1989) over the 30 s sprint bout before and after training, are similar to those recorded in this study (Fig 4.2). Training enhanced the peak power output but no difference in power output at the 30 s mark was observed. The major difference was a reduced time taken to obtain peak power in the study by Nevill et al. (1989), and this may be explained by differences in the mode of exercise employed in the testing protocol.

The power generated by skeletal muscle is a function of the force it generates and the velocity of shortening (Edgerton et al. 1986). The power output measured during exercise is representative of a whole body system of muscle and levers which coordinate to perform the task (Jones and Round, 1990). Hence, power output during maximal exercise is influenced by the interaction between neuromuscular recruitment (e.g., type of motor unit, size of motor unit pool, frequency and pattern of discharge), contractile properties and metabolic processes.

Both before and after training, there is probably maximum recruitment of muscle fibres in the prime movers involved in an "all out" sprint bout, such as has been employed in this study. This conclusion is supported by muscle metabolite data which demonstrates that substantial CP degradation occurs during 30 s of maximal running in FT and ST fibres in the vastus lateralis (Greenhaff et al. 1992). Furthermore, the magnitude of CP degradation in mixed muscle is not altered by sprint training (Table 4.1; Boobis et al. 1983b; Nevill et al. 1989; Sharp et al. 1986).

The maximal recruitment of prime mover muscles does not exclude the possibility that neural adaptations occur with training, which may lead to an increased sprint performance. There is the potential for a "learned" component, manifesting itself in a better coordination of synergistic muscles and/or reducing the inhibitory effect of the antagonists specific to that activity (Rutherford et al. 1986; Sale, 1986). A more appropriate recruitment could also be due to an improved pattern of discharge from the CNS with training optimising the force production of the muscle (Green, 1986).

As mentioned previously, power is determined by muscle force generation and contraction velocity. In turn, the latter is influenced by myosin ATPase activity and the functional characteristics of the sarcoplasmic reticulum (Bárány, 1967; Briggs et al. 1977). Increases in myosin ATPase activity after sprint and high velocity resistance training (Bell et al. 1992; Thorstensson et al. 1975) may explain, at least in part, the observed increase in peak power in this study. An increase in the proportion of FT fibres with sprint training (Esbjörnsson et al. 1992; Jansson et al. 1990) may account for this increase in myosin ATPase activity. Furthermore,

compared to ST fibres, FT fibres also have a greater amount (Eisenberg et al. 1974), and increased functional capacity of the sarcoplasmic reticulum (Briggs et al. 1977; Fiehn and Peter, 1971; Salviati et al. 1984). Hence any increases in peak power after training may also be related to changes in fibre type composition. Studies examining the adaptations of the sarcoplasmic reticulum to sprint training have not been conducted. Additionally, a greater number of FT fibres could potentially enhance the power generation as they are also adapted for greater absolute force development than ST fibres (Green et al. 1986) since they usually have a greater cross sectional area (Saltin and Gollnick, 1983). Bell et al. (1992) demonstrate a significant increase in hypertrophy, as measured by the cross sectional area of the knee extensor, following high velocity resistance training. This is further supported by a report of an increased FT muscle fibre area and % fibre area occupied by FT fibres after high velocity resistance training (Coyle et al. 1981) and explosive resistance training at high velocity (Hakkinen et al. 1985) and may explain the improvement in sprint performance in the present study.

Measured values of mean power and total work are reliant to a considerable extent upon the maintenance of power over the whole duration of the exercise, subsequent to the peak power being attained. The energy provision for power generation during a maximal exercise bout is reliant on contributions from both aerobic and anaerobic mechanisms (Medbø and Tabata, 1989; Withers et al. 1991). The glycolytic rate is probably a major factor determining mean power and total work over a 30 s maximal sprint bout. In support of this, the levels of lactate at 6 s maximal exercise account for around half of the ATP produced up to this time point (Hultman and Sjöholm, 1986). Furthermore, the aerobic contribution to a 30 s sprint bout has been reported to account for 40% of the total energy production (Medbø and Tabata, 1989), hence the anaerobic energy system is the major contributor during a 30 s sprint. The increases in mean power and total work found with training in this study may be explained, at least in part, by a training-induced increase in glycolytic capacity. This is supported by sprint training studies which have reported increases in PFK activity in post-trained muscle (Sharp et al. 1986; Jacobs et al. 1987). Additionally, an increased exercise induced muscle lactate accumulation

following training (Nevill et al. 1989; Sharp et al. 1986) demonstrates an increased glycolytic capacity of the fibres. Muscle lactate concentration at the end of exercise were similar pre and post-training in the present study. There was an increase in plasma and blood lactate concentration as a result of sprint training immediately following exercise (Fig 4.5 A,B). These blood data suggest that training may increase lactate efflux from the muscle. Therefore, an enhanced muscle glycolytic capacity cannot be excluded. Pilegaard et al. (1993) have reported an increased lactate transport from rat sarcolemmal giant vesicles after high intensity training. Alternatively, it is possible that the increased post-exercise blood lactate levels after training are due to a greater contribution to the exercise from synergistic muscles (Nevill et al. 1989).

The cause of the decline in power output during the latter phase of a maximal sprint exercise bout is unclear and was not specifically examined in this study. The decline in power, however, may be associated with an accumulation of metabolic end-products ( $H^+$ , ADP, Pi). These metabolites have been associated with disruption of the muscle contractile apparatus and energy supply pathways. (Sahlin et al. 1983; Schädler et al. 1967). The mechanisms involved may include an inhibition of myosin ATPase activity by  $H^+$  and Pi (Fabiato and Fabiato, 1978), a blocking of the troponin  $Ca^{2+}$  binding site by  $H^+$  (Fabiato and Fabiato, 1978) and inhibition of the sarcoplasmic reticulum ATPase (Nakamura, 1972). Another effect of an increase in  $H^+$  concentration is an inhibition of PFK (Ui, 1966), disrupting metabolic energy supply.

The decline in power, as measured by the fatigue index, was not different before and after training in spite of a potentially greater lactate production in the trained state (Refer section 5.4). Sharp et al. (1986) however, demonstrated an increased buffering capacity of the muscle after sprint training and an increased exercise performance. It is possible that the increased mean power and total work reported in the present study may also be attributed to an enhanced muscle buffering capacity attenuating the deleterious effects of increased  $H^+$  on muscle function.

Unlike previous sprint training studies this training protocol demonstrated increases in peak power, mean power and total work. This is probably indicative of

the training protocol employed, which gradually increased the workload by progressively increasing the number of bouts per session, presenting a continual overload stimulus. Other training protocols also gradually increased the number of bouts over the duration of the training but did not report an increase in performance (Jacobs et al. 1987) or did not show increases in more than one of the performance variables measured (Snow et al. 1992). There are two possible explanations for this. In the study by Jacobs et al. (1987), although there was a progressive increase in the number of bouts, the resistance was set at  $75 \text{ gm.kg}^{-1}$  which may not have been sufficient to produce the desired stimulus for sprint training. In the study by Snow et al. (1992), a mechanically braked cycle ergometer was utilised for training purposes and an air-braked cycle ergometer was used in the performance trials. The specific nature of the training adaptation (i.e. neural factors) on a mechanically-braked cycle ergometer may not lead to the optimal performance increase on an air-braked cycle ergometer. An alternative method using a gradual progressive increase in resistance during the training bouts, was employed by Sharp et al. (1986). The number of bouts was maintained at 8 per session for the duration of the training and the resistance was progressively increased by selecting a pedal resistance which the subject could just maintain for 30 sec at 90 pedal revolutions per minute. These authors reported an improvement in peak torque and total work over the 45 sec "all out" test indicating that a progressive overload stimulus was required for improved sprint performance.

## 5.2 Sprint Training and Maximal Oxygen Uptake ( $\text{VO}_2\text{max}$ )

As mentioned above the aerobic metabolism during a maximal 30 sec sprint bout is about 40% of the total energy required (Medbø and Tabata, 1989). Sprint training protocols, which involve multiple sprint bouts, may therefore enhance aerobic capacity (Nevill et al. 1989; Sharp et al. 1986) which may contribute to an improvement in sprint performance. The similar  $\text{VO}_2\text{max}$  scores observed in the present study when comparing the pre and post-training data confirms some previous reports (Henriksson and Rietman, 1976; Sjödín et al. 1976; Thornsténsson et al. 1975) but conflicts with others (Nevill et al. 1989; Sharp et al. 1986). The training

protocols employed across studies vary substantially from one another possibly accounting for such diverse results. For example, Thorstensson et al. (1975), utilised 5 s sprints in the training protocol which is unlikely to tax the aerobic system. In addition, differences in the initial training level of the subjects in the studies may explain these conflicting findings. McKenna (1991) suggests that the lower the pre-training  $\text{VO}_2\text{max}$  score of the subject, the greater the induced aerobic stimulus of a training protocol. With relatively high pre-training levels, it is feasible that the training stress employed in this study was not high enough to induce an increase in the aerobic capacity of the muscle.

### **5.3 The Effects of Sprint Training on Resting Adenine Nucleotide Concentrations**

Earlier studies demonstrated that sprint training had no effect on the resting skeletal muscle ATP concentrations (Boobis et al. 1983b; Nevill et al. 1989; Thorstensson et al. 1975). Evidence has since been reported showing a reduction in resting skeletal muscle ATP concentrations after 1 week and 6 weeks of intense sprint training (Hellsten-Westling et al. 1993). This indicates that training may influence the resting concentration of the AdN pool, particularly ATP. One aim of this study was to reinvestigate the effects of sprint training on resting ATP levels in human skeletal muscle.

One of the major findings of this study was the observation that resting ATP levels were reduced by 19 % after training (Table 4.1). The training-induced fall in ATP resulted in a similar decrease in muscle TAN content at rest. This decrease in ATP could not be explained by a change in total creatine content as this value was similar pre and post-training. Hence the observation of a reduced resting ATP concentration 48-72 hours after the last training session must therefore be due to ATP degradation being greater than synthesis during the 7 week training period. It should be noted that this study not only compares resting muscle in the trained and untrained state, but also the muscle in the trained state is potentially affected by the prior exercise protocol. Hence, the imbalance between ATP degradation and synthesis could be attributed to two possible factors; training and/or the acute effects

of prior exercise.

It is feasible that the decreased resting AdN concentration is a potential training adaptation of the muscle in an attempt to minimise the metabolic expense of constant AdN replenishment by the AdN *de novo* biosynthesis pathways. Evidence which best supports this possibility is found in the manipulation of two training protocols by Hellsten-Westing et al. (1993). The design of their experiment utilised an extremely stressful one week training protocol with, and without, 6 weeks of prior sprint training. These authors found that muscle AdN decreased after 6 weeks of sprint training, however, this group subsequently maintained this lower AdN concentration up to 3 days after the one week intense training protocol. This was in contrast to the untrained control group which demonstrated marked reductions in resting AdN levels at least 72 hours after the last exercise session. These results indicate that sprint training can reduce resting muscle ATP concentrations. In addition, the fact that the trained group had similar ATP levels 3 hours after the last session compared with the start of the week, and that these levels did not increase with a further 69 hours of recovery, suggests that training down regulates resting ATP levels.

An alternative explanation is that the reduced AdN levels at rest in the post-trained state are an unavoidable consequence of the performed exertion in the prior training protocol. Enhanced ATP degradation probably occurs primarily as a result of an acute imbalance in ATP hydrolysis and synthesis during the sprint training sessions. High intensity exercise results in significant ATP degradation, large increases in muscle IMP accumulation (Jansson et al. 1987; Sahlin et al. 1978) and elevated inosine and hypoxanthine in the blood (Balsom et al. 1992; Bangsbo et al. 1992; Sinkeler et al. 1986). Furthermore, depleted ATP levels observed after one maximal 30 s bout are also found when exercise bouts are subsequently repeated (McCartney et al. 1986). A close association between the exercise-induced reduction in ATP stores and elevated IMP concentrations (Sahlin and Ren, 1989), coupled with the fact that IMP recovery is reasonably slow (Table 2, Arabadjis et al. 1992; Graham et al. 1990; Sahlin and Ren, 1989), suggests that throughout the duration of each sprint training session, there is probably a sustained elevated IMP

concentration. This provides favorable conditions for significant IMP catabolism leading to enhanced inosine and hypoxanthine production. Both inosine and hypoxanthine diffuse from the muscle and can be converted to uric acid in the endothelial cells (Hellsten-Westling, 1991) and result in the loss of purine bases. This argument is supported by Bangsbo et al. (1992) who observed a greater hypoxanthine efflux from contracting muscle when intense exercise was repeated. They have also estimated that hypoxanthine efflux after a single short duration, exhaustive exercise bout is equivalent to at least 5% of the fall in the TAN pool. If this figure is applied to the post-training data of the present study the total purine loss from the active muscle after ten 30 s sprints is at least  $2.2 \text{ mmol.kg}^{-1} \text{ dw}$ . Assuming a rate of *de novo* synthesis of  $35 \text{ nmol.g}^{-1} \text{ ww.hr}^{-1}$ , as found in rodent mixed skeletal muscle (Tullson et al. 1988), the estimated loss would take just over half a day to restore. This being the case, one would assume that the levels of AdN would be well recovered after at least 48 hours. It is possible that the rates of AdN synthesis observed for rat muscle are markedly different to human skeletal muscle thus prolonging the restoration time. Presently, rates of *de novo* AdN synthesis in human skeletal muscle and the levels of precursors for their synthesis after high intensity exercise are unknown. The availability of PRPP may restrict *de novo* synthesis, at least during the early phase of recovery (Hellsten-Westling et al. 1993), since PRPP synthetase is the rate limiting enzyme in the AdN biosynthesis pathway (Harmsen et al. 1984; Zoref-Shani et al. 1982; Fox and Kelly, 1971) and the purine salvage pathway utilises PRPP to resynthesise ATP (Fig. 4.1). A further consideration is that the calculated muscle hypoxanthine loss occurring with a single exercise bout (based on Bangsbo et al. (1992) data), may be an underestimate. If this is the case, the calculated time required for *de novo* AdN synthesis to restore the net loss in the present study would be underestimated.

A decrease in ATP resynthesis may be partially responsible for the lower resting ATP levels after training. Initial recovery of ATP content is predominantly due to increased flux through the IMP reamination (Meyer et al. 1980) and purine salvage pathways (Edwards et al. 1979). A sprint training-induced increase in enzyme activity in the purine salvage pathway provides evidence of a

greater potential for purine salvage (Sjödín and Hellsten-Westling, 1991). The present data indicate that net ATP resynthesis during 3 min of recovery was not significant either before or after training, making it impossible to ascertain any training influence (Table 4.2). *De novo* synthesis of ATP plays a minor role in ATP restoration during the early recovery phase, but is critical in the long term replenishment of purine base lost from skeletal muscle (Tullson and Terjung, 1991a). Little is known about skeletal muscle *de novo* synthesis rates of AdN during recovery from high intensity exercise or the possible effects of sprint training on this process. Future research needs to examine whether the continued loss of ATP from the muscle during training provides a stimulus for an adaptation in the rate of *de novo* synthesis.

In contrast with the present study and the work of Hellsten-Westling et al. (1993), other sprint training studies have not observed a decrease in resting ATP levels 48-72 hours after the last training session (Boobis et al. 1983b; Nevill et al. 1989; Thorstensson et al. 1975). This discrepancy may be due to differences in training protocols (i.e., intensity, duration of exercise and recovery, etc). Based on the above arguments, sprint training programs which are more extreme, such as that employed in the present study, probably result in greater levels of ATP degradation (via elevated ATP turnover) and subsequent purine loss than less strenuous programs utilized by others (Boobis et al. 1983b; Nevill et al. 1989; Thorstensson et al. 1975). The demanding training sessions used in the present investigation probably require a longer recovery period to restore the large purine loss from the muscle providing that no down regulation of resting ATP content occurs with training.

Although resting ATP levels were reduced by 19 % after the sprint training regimen this did not prevent significant improvements in sprint performance being achieved (Fig 4.2). This result confirms the finding of previous work (Hellsten-Westling et al. 1993). It is not clear, however, if sprint performance would be further improved if the post-training bout was begun with higher resting ATP levels. Improvements in sprint performance with higher resting ATP concentration, if they occurred, are likely to be small because the fall in ATP content during a 30 s sprint bout is estimated to contribute only 2-4 % of the estimated total anaerobic ATP

turnover (Boobis, 1987). Furthermore, studies on skinned muscle fibres show that maximal isometric tension is maintained even at low levels of ATP (Ferenczi et al. 1984).

In agreement with the results of other sprint training studies no differences were observed in resting muscle ADP, AMP, IMP, lactate, creatine and CP as a result of training (Table 4.1, Boobis et al. 1983b; Hellsten-Westling et al. 1993; Nevill et al. 1989; Sharp et al. 1986, Thorstensson et al 1975). It was also found that training status appeared to have no effect on the resting muscle concentrations of ammonia, inosine and hypoxanthine (Table 4.1). The effect of sprint training on these metabolite concentrations at rest has not been previously reported.

#### **5.4 The Effect of Sprint Training on the Management of Adenine Nucleotides and their Degradation Products During a Maximal Sprint Bout**

Sprint training attenuated the magnitude of muscle ATP degradation by 52% during the sprint bout (Table 4.2). In contrast to previous reports (Boobis et al. 1983b; Nevill et al. 1989), the data in the present study show that sprint training decreases the amount of ATP degradation occurring during high intensity exercise. A reduction in the magnitude of ATP degradation must be the result of a closer match between ATP resynthesis and hydrolysis rates during the post-training, compared with the pre-training, sprint bout. This is reflected in the reduced accumulation of IMP within the muscle. Unfortunately, the design of the present study does not allow a determination of the effect of sprint training on ATP hydrolysis and resynthesis rates. One can speculate that ATP hydrolysis rates may need to be increased in the vastus lateralis in order to fuel the elevated power outputs observed after training. Alternatively, Nevill et al. (1989) suggest that ATP hydrolysis in the vastus lateralis may not change and the increased power output may be due to a more active involvement of synergist muscle groups. There is also the possibility that training may lead to increases in contractile efficiency allowing more power to be produced at similar or reduced ATP cost (Westra et al. 1985). Furthermore, resting levels of ATP alone may influence the energetic cost of contraction, as it has been reported that in rat FT muscle the ATP cost of twitch and tetanic isometric

contractions were markedly reduced in muscles partially depleted of ATP prior to exercise (30% of normal resting values) (Foley et al. 1991). The possibility that lower ATP levels at rest may reduce the rates of ATP hydrolysis for the same power output, at least in part, provides an attractive explanation of these findings.

Very few investigations have examined the effect of sprint training on ATP resynthesis rates during a 30 s sprint bout. Training probably does not affect ATP resynthesis rates from CP, as the magnitude of CP degradation during a sprint bout was similar pre and post-training (Table 4.2; Boobis et al. 1983b; Nevill et al. 1989). The present study demonstrates a training-induced decrease in IMP accumulation (Table 4.2) and no change in AMP concentration (Table 4.2) after exercise which tends to suggest that ATP resynthesis by the myokinase reaction was lowered by training. In addition, although the aerobic capacity of muscle fibres may increase with sprint training (Jacobs et al. 1987) there is no conclusive evidence that training enhances muscle oxygen consumption during a maximal sprint bout. No differences in the pulmonary oxygen uptake (McKenna, 1991, Nevill et al. 1989) and arterial-femoral venous oxygen content (McKenna, 1991) have been observed during maximal sprinting as a consequence of sprint training. Hence the most likely source of an improved ATP resynthesis rate is an increased glycolytic rate (Jacobs et al. 1987; Nevill et al. 1989; Sharp et al. 1986). The finding that training had no effect on muscle lactate concentration (Table 4.1) and increased blood/plasma lactate early in recovery from exercise (Fig. 4.5) is best explained by an increased muscle lactate production in conjunction with an enhanced efflux into the circulation. Alternatively, an increased muscle lactate production and efflux from synergist muscles after training may explain the elevated plasma lactate data without any change in muscle lactate production in the vastus lateralis.

A gross comparison of the glycolytic activity can be made using an estimate of the lactate accumulation in the muscle and the blood over the exercise period before and after training. To calculate this, the difference in the lactate content of the muscle at the end of exercise and rest is multiplied by the estimated mean weight of muscle in both legs (3.55 kg dry muscle - from calculations reported by Katz et al. 1986a). This is added to an estimate of the net flux across the sarcolemma,

calculated from the difference in venous plasma lactate concentration before and immediately after exercise multiplied by the extracellular water (ECW) volume which is approximately 20% of the body weight. The resultant calculated values for the total lactate accumulation during exercise are 458 and 536 mmol, pre and post training, respectively. These calculations support the suggestion that the glycolytic capacity is increased with training. It must be acknowledged, that these calculations involve certain assumptions, since venous blood lactate is reflective of the instantaneous content of the blood and does not account for the addition and/or the removal of lactate from the circulation, or the oxidation of lactate and the effects of sprint training on these processes. Additionally it is assumed in these calculations that there are no changes in the distribution of lactate in the ECW, exercising muscle mass or ECW volume with training.

The exercise-induced accumulation of IMP and ammonia in the muscle was closely matched by the magnitude of ATP degradation, irrespective of training status (Table 4.2). This observation suggests that the training-induced reductions in IMP and ammonia accumulation are caused by a reduced reliance on AMP deamination, as a result of the improved balance between ATP hydrolysis and resynthesis. This data, in conjunction with the observation that any change in muscle adenosine or adenine concentrations must be relatively small, confirm that AMP deamination is the major adenine nucleotide degradation pathway utilised by human skeletal muscle during high intensity exercise (Jansson et al. 1987; Katz et al. 1986a&b; Sabina et al. 1984; Sahlin et al. 1978). This also strongly indicates that the predominance of this reaction is not altered by sprint training. An interesting point to note is that the IMP content in the muscle is markedly lower at the 30 s mark in the pre and post-training sprint and yet the power output is not significantly different. This suggests that IMP may not be a factor responsible for fatigue during "all out" exercise.

The accumulation of muscle inosine after maximal exercise observed prior to, but not after, training (Table 4.1) may be explained by an earlier accumulation of substrate (IMP), during sprinting prior to training. This would allow an increased time for hydrolysis by 5'-nucleotidase. In addition, *in vivo* 5'-nucleotidase activity maybe reduced with training due to a smaller allosteric activation ( $Mg^{2+}$ ,  $Ca^{2+}$ ,

GDP) of the enzyme and/or a reduction in its concentration (Cutmore et al. 1986). Muscle hypoxanthine did not accumulate during 30 s of sprinting, irrespective of sprint training status (Table 4.1). The lack of an increase was probably due to the rate of inosine formation due to the low activity of 5'-nucleotidase (Schopf et al. 1986).

### **5.5 The Effect of Sprint Training on Adenine Nucleotide Recovery up to 3 min After a Maximal Sprint Bout**

The effect of sprint training on the recovery of AdN after a maximal sprint bout have not previously been reported. The changes in muscle metabolite concentrations during 3 min of recovery before and after training are summarised in Table 4.2. In agreement with the data reported in this study, Sharp et al. (1986) found that sprint training had no effect on CP recovery after exhaustive incremental exercise. Sharp et al. (1986) also indicated that the return of muscle lactate concentration to resting values was enhanced by sprint training, which is in contrast to our results. The reason for the conflict is not readily apparent; however it may be related to differences in training and/or exercise testing protocols.

Since there was no significant recovery of ATP, IMP and ammonia 3 min after the sprint bout, pre or post-training (Table 4.1), it is not possible to ascertain any effect training may have on the recovery of these metabolites. In hindsight, it would appear that the muscle biopsy taken 3 min post-exercise was too early to evaluate any training effects on the AdN, IMP and ammonia recovery rates. In contrast, muscle inosine accumulated to a lesser extent during recovery after training (Table 4.2). This finding is best explained by an attenuated inosine production rate during recovery in the trained state, rather than an increased rate of removal. A decreased inosine production rate probably results from a reduced activity of cytoplasmic 5'-nucleotidase possibly caused by a smaller substrate supply (IMP) (Itoh et al. 1986), an attenuated allosteric activation, and/or decreased enzyme concentration (Cutmore et al. 1986). Importantly, the decreased muscle inosine concentrations found during recovery after training may lead to a smaller muscle purine loss.

It is also likely that the failure to observe a training effect for hypoxanthine was due to the fact that muscle samples were obtained too early in recovery. Muscle hypoxanthine production is a reasonably slow process due to the low activities of cytoplasmic 5'-nucleotidase (Tullson and Terjung, 1992) and purine nucleoside phosphorylase (Schopf et al. 1986). Sahlin et al. (1991) have observed that the release of hypoxanthine from muscle was three times higher 10 min after termination of exercise than at fatigue. Additionally, plasma hypoxanthine levels peak around 15-20 min into recovery, suggesting that up until this time the muscle was still releasing hypoxanthine and thus the level in the muscle was higher than plasma concentrations creating a gradient favoring the efflux of hypoxanthine. The present data indicate that during recovery there was an emerging trend towards a higher muscle hypoxanthine content before training. Although speculative, it would be reasonable to expect this difference to be accentuated as more time is allowed for hypoxanthine to accumulate. This expectation is made more reasonable since inosine, the immediate precursor of hypoxanthine, is elevated to a greater extent in untrained muscle early in the recovery period. In addition, a decreased muscle hypoxanthine accumulation after training may also occur as a result of an increased activity in the purine salvage pathway (Sjödín et al. 1991). The decrease in plasma hypoxanthine concentration (Fig. 4.4B; Stathis et al. 1992) provides indirect evidence that training reduces hypoxanthine production and its efflux from the muscle during recovery. Importantly, a sprint training-induced reduction in hypoxanthine loss from the muscle may also be an adaptation to conserve the purine nucleotide pool in the face of an intermittent metabolic stress.

Interestingly, the training-induced attenuation of ammonia within the muscle after 30 s of maximal exercise (Table 4.2) does not appear to be reflected in a lower plasma ammonia concentration during the recovery period (Fig. 4.4A). Furthermore, an earlier peak in plasma ammonia concentration is found after training which suggests an increased muscle ammonia efflux during exercise or early into recovery. This interpretation is not supported by the fact that training had little influence on the change in muscle ammonia concentration during this period (Table 4.2). Although speculative, these apparent contradictions may be explained by a

training-induced alteration in the mechanism of nitrogen efflux from muscle. Ammonia can exit from muscle fibres as free ammonia or be incorporated into amino acids which may subsequently exit the cell (Graham et al. 1990). Sprint training may markedly reduce the rate of incorporation of ammonia into amino acids allowing a greater proportion of the ammonia produced to efflux from the muscle in the free form. Future research needs to examine this possibility.

In contrast to the present study, it has previously been reported (Snow et al. 1992) that sprint training attenuates plasma ammonia concentration after a maximal sprint bout. Unfortunately, there are no obvious reasons which explain these conflicting results. There are, however, training response differences between these results and those of the previous study, which may provide a clue. There was a greater improvement in sprint performance after training in this investigation compared with that of Snow et al. (1992). This may result in a greater depletion of the TAN pool and an increased muscle ammonia production during the post-training sprint bout in the present, compared with the earlier study. Furthermore, greater training specificity was probably achieved in the present investigation as training was performed on an air-braked, as opposed to a mechanically braked, cycle ergometer.

## CHAPTER 6

### CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

#### 6.1 Summary

In conclusion, the high intensity sprint training regime employed influenced PnN metabolism in skeletal muscle. This study observed a reduction in resting ATP and AdN after training which may be attributed to an inability of the muscle to completely restore the purine bases lost as a result of high ATP turnover rates during the intense training sessions. It is also possible that training down regulates resting ATP levels, therefore, a recovery to pre-training levels may not occur.

The present study has also demonstrated that there was an attenuation of the magnitude of ATP depletion during exercise after training, resulting in a reduced accumulation of its degradation products (i.e., IMP and ammonia). This observation indicates that there is an improved balance between ATP hydrolysis and resynthesis during sprint exercise after training. The mechanism for this improvement is unknown and requires further examination. Additionally, the decrease in ATP was equimolar to the increase in IMP and ammonia before and after sprint training, suggesting that the mechanism of AdN degradation is unchanged with training.

Additionally, no change was evident in ATP, IMP, hypoxanthine and ammonia in the muscle after 3 min of recovery compared to immediately after a 30 s sprint both before and after training. Muscle sampling was probably performed too soon after exercise to observe any training adaptations which may influence the recovery of these metabolites. The level of inosine accumulation at 3 min of recovery, however, was reduced after training, a likely consequence of a decreased IMP accumulation during exercise in the trained state. A training induced increase in plasma ammonia is evident at 2 min recovery from exercise however no difference was observed subsequent to the 2 min mark. This did not reflect the reduced post-trained muscle ammonia accumulation, which was almost half the pre-trained values. Plasma hypoxanthine levels were attenuated in the trained state at 45 and 60 min post-exercise, which is indicative of a reduced muscle production and efflux and/or an increased removal.

With such adaptations occurring at the metabolic level, the sprint training protocol employed in this study was successful in enhancing peak power, mean power and total work during a 30 s "all out" exercise bout. The likely reason for these increases in performance was the specific nature and progressive loading of the training. Mechanisms which produce the increases in the measured performance variables could arise from altered recruitment of the muscle with training and/or an increase in the metabolic and contractile capacity of the fibres involved in the sprint.

## 6.2 Recommendation for Future Research

The information from this study reveals the effects of sprint training on the management of AdN metabolism at rest, during and in recovery from a 30 s maximal exercise bout. This is the first time the major metabolites in the cascade of reactions involved in AdN degradation to hypoxanthine have been studied simultaneously and the results obtained have raised some interesting questions.

The mechanisms responsible for the decrease in resting ATP concentrations after sprint training need further controlled investigation to ascertain whether an excessive purine base loss over the duration of sprint training or a down regulation of the ATP levels is (are) the mechanism(s) responsible. Skeletal muscle AdN degradation to hypoxanthine during "all out" sprint exercise, and the efflux of the metabolites of purine degradation which can traverse the sarcolemma during, and in recovery from, a single maximal exercise bout and repeated sprint bouts require investigation. The effect of sprint training on the rate of efflux of these metabolites also needs further research. This will enable quantification of the total loss of AdN precursors after a maximal sprint exercise bout before and after sprint training.

Furthermore, the sprint-training induced reduction in the magnitude of AdN degradation after sprint training indicates that there is an improved balance between ATP hydrolysis and ATP resynthesis. The mechanism for this improvement is unknown and requires further examination.

The recovery of ATP from IMP was investigated in this study. However, the sampling time 3 min into recovery was too early to observe any recovery, thus no training effect on the recovery of ATP could be established. Therefore, an

investigation into the recovery of ATP from IMP, before and after training, is needed to further understand AdN metabolism after a maximal exercise bout and the adaptations which occur in recovery after sprint training.

Another question which arises from this study is the influence of sprint training on *de novo* AdN synthesis in skeletal muscle. Information from such a study may enhance the understanding of the mechanism(s) for the decrease in resting ATP levels observed after sprint training in the present study.

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**APPENDIX A**

**SUBJECT INFORMATION AND  
INFORMED CONSENT STATEMENTS**

## INFORMATION FOR SUBJECTS ON EXPERIMENTAL PROCEDURE

The experiment in full involves the following;

Procedure:(in chronological order)

-Pre training maximal oxygen uptake ( $\dot{V}O_{2max.}$ ) test to be performed as soon as possible before the sprint test.

-Sprint test familiarisation trial

-Pre training sprint test which involve muscle and blood sampling

### TRAINING PROGRAMME:

-7 weeks of training requiring attendance 3 times/week. Training will begin with 3 sprint bouts / session and build up to 10 sprint bouts / session.

-Post training sprint test performed within 2-3 days of finishing the last training session. If training starts at the above time this should be around the middle to the end of May.

-Post training  $\dot{V}O_{2max.}$  test performed as soon as possible after the sprint test.

I \_\_\_\_\_, certify that I have read the above details on the experiment and that the information on the muscle biopsy and catheterisation for blood collection has been supplied in writing to me.

Signed:.....

Date:.....

## 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.

## CATHETERISATION

At specific intervals throughout the trials a small blood sample will be taken via a catheter placed into an arm vein. The catheter consists of a needle and teflon tubing. The tubing is fed over the top of the needle (which has punctured the vein) and into the vein. A tap (stopcock) is placed into 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 the vein). Each time a blood sample is taken a small volume of sterile heparinised saline will be injected to clear the catheter and keep it patent. Catheterisation of subjects is slightly discomfoting and can lead to the possibility of bruising and infection. The use of sterile, disposable catheters, syringes, swabs etc. will reduce markedly the possibility of infection caused by the catheterisation procedure. The use of experienced and qualified staff will reduce the likelihood of bruising as this is primarily caused by poor venapuncture techniques. Although the possibility of infection and significant bruising is quite small, if by chance it does eventuate, we suggest you consult your doctor immediately.

STANDARD CONSENT FORM FOR SUBJECTS INVOLVED IN EXPERIMENTS

I. 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 SPRINT TRAINING ON METABOLISM  
.....  
....."

being conducted at Footscray Institute of Technology by: Assoc. Prof. Michael Carey,  
Mr. Rod Snow and Mr. C. Stathis  
.....

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:

Mr. C. Stathis  
.....  
.....

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

Procedures

Muscle Biopsy  
.....

Catheterisation  
.....

Maximal Oxygen Consumption Test  
.....

Maximal Sprint Bout ("All Out" 30 sec. test)  
.....  
.....

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: .....)

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

Date: .....

.....)

2. CERTIFICATION

I, .....CHRISTOS STATHIS.....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.

In order to be eligible to participate in the experiment investigating  
The Effects of Sprint Training on Metabolism

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

Name: \_\_\_\_\_ Date: \_\_\_\_\_

Age: \_\_\_\_\_ years                      Height: \_\_\_\_\_ cms

Weight: \_\_\_\_\_ kgs

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

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

- |    |  |     |     |                    |
|----|--|-----|-----|--------------------|
| 1. | Are you overweight?  | Yes | No  | Don't know*        |
| 2. | Do you smoke?  | Yes | No  |                    |
| 3. | Has your family a history of premature cardiovascular problems (e.g., heart attack, stroke)? | Yes | No  | Don't know         |
| 4. | Are you a diabetic?  | Yes | No  | Don't know         |
| 5. | Do you have a high blood cholesterol level?  |     | Yes | No      Don't know |
| 6. | Do you have high blood pressure?   |     | Yes | No      Don't know |
| 7. | Are you on any medication?   | Yes | No  |                    |

If so what is the medication \_\_\_\_\_

8. Do you have any medical complaint or any other reason which you know of which you think may prevent you from participating in strenuous exercise?

Yes                      No

If so please elaborate \_\_\_\_\_

\* Circle appropriate response

I \_\_\_\_\_, believe that the answers to these  
 (Name)

questions are true and correct.

Signed: \_\_\_\_\_

Date: \_\_\_\_\_

NAME: \_\_\_\_\_

ADDRESS: \_\_\_\_\_

Post Code: \_\_\_\_\_

'PHONE No.: \_\_\_\_\_

DATE: \_\_\_\_\_

AGE: \_\_\_\_\_ Years

1. Have you or your family suffered from any tendency to excessively bleed? (e.g., Haemophilia)

Yes

No

Don't know

If yes please elaborate \_\_\_\_\_

2. Are you allergic to local anaesthetic?

Yes

No

Don't know

If yes please elaborate \_\_\_\_\_

3. Do you have any skin allergies?

Yes

No

Don't know

If yes please elaborate \_\_\_\_\_

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

Yes

No

Don't know

If yes please elaborate \_\_\_\_\_

5. Are you currently on any medication?

Yes

No

Don't know

If yes what is the medication? \_\_\_\_\_

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

Yes

No

If yes please elaborate \_\_\_\_\_

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

Signature: \_\_\_\_\_

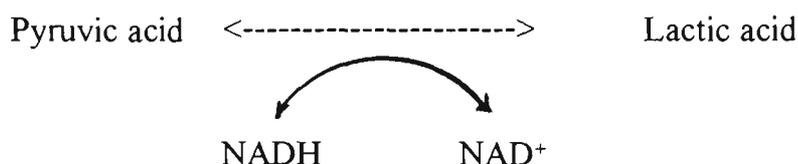
Date: \_\_\_\_\_

**APPENDIX B**

**DETAILS OF ANALYTICAL CHEMICAL METHODS**  
**FOR BLOOD AND PLASMA METABOLITES**

### Plasma and Blood Lactate

Principle: The enzyme lactate dehydrogenase (LDH) catalyses the following reaction:



In the presence of an excess of  $\text{NAD}^+$  and LDH, and at a highly alkaline pH, nearly all of the lactate is converted to pyruvate. To ensure maximal conversion of lactate to pyruvate, hydrazine is utilised converting pyruvate to pyruvatehydrazone. The concurrent equimolar appearance of NADH converted from  $\text{NAD}^+$ , is measured spectrophotometrically (Shimadzu UV-120-02) and becomes a measurement of the lactate originally present. A set of lactate standards, ranging from 1 mM to 35 mM was run with each analysis (see Fig. B.1).

### Plasma Hypoxanthine

Plasma hypoxanthine was determined using high performance liquid chromatography (HPLC). Mobile phase A was a 0.15 M ammonium dihydrogen phosphate solution, adjusted to a pH of 6.0, and mobile phase B was a solution of acetonitrile/methanol (50/50, vol/vol). The flow rate of the pump was set at  $1.0 \text{ ml} \cdot \text{min}^{-1}$ , the UV detector wavelength 254 nm and the gradient profile for the separation achieved was set as follows;

Run time (min)	% mobile phase A	% mobile phase B
0.0	100	0
5.5	100	0
25.5	85	15
30.0	100	0

Three standard solutions (1, 10 and  $60 \mu\text{M}$ ) were run with each sample set (Fig. B.2) Chromatographs of standards, typical resting and recovering samples are shown (Fig. B.3, B.4, B.5, B.6) to demonstrate the separation achieved with this method.

### Plasma Ammonia

Plasma ammonia was quantified using flow injection analysis (FIA) and gas diffusion. This involves the injection of a  $200 \mu\text{l}$  aqueous sample containing ammonium ions into a carrier steam (0.9% NaCl) which further combines with an alkaline stream of sodium hydroxide (0.1M). All of the ammonium ions are converted to ammonia in the alkaline stream and it can diffuse through a gas permeable membrane into an acid-base indicator stream. The basic form of the indicator is measured spectrophotometrically. A standard curve is constructed prior to each sample set (Fig. B.7) and a copy of the printout of a typical set of ammonia standards (Fig. B.8) and samples (Fig. B.9) is shown.

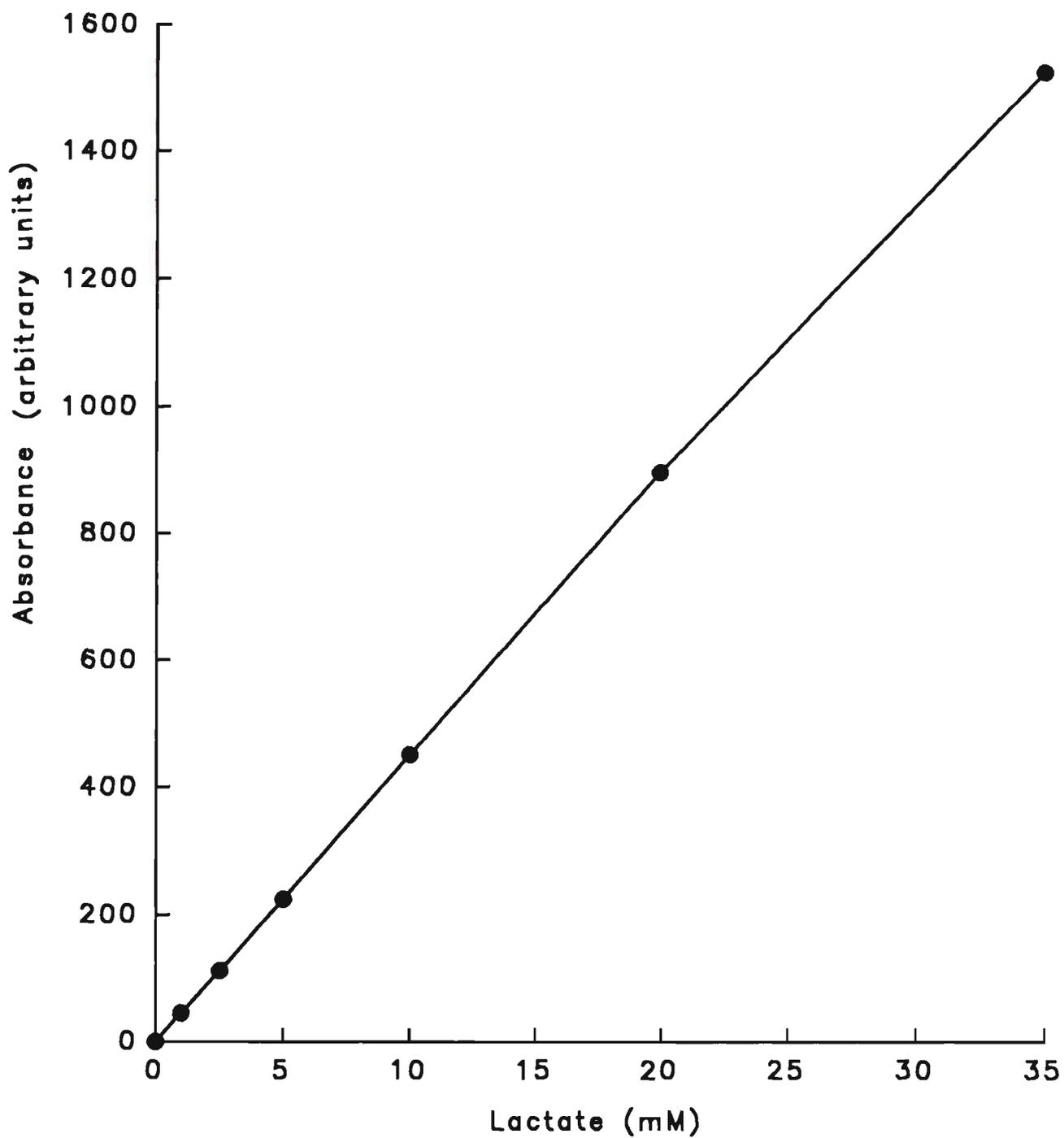


Figure B.1; A typical standard curve for blood and plasma analyses.

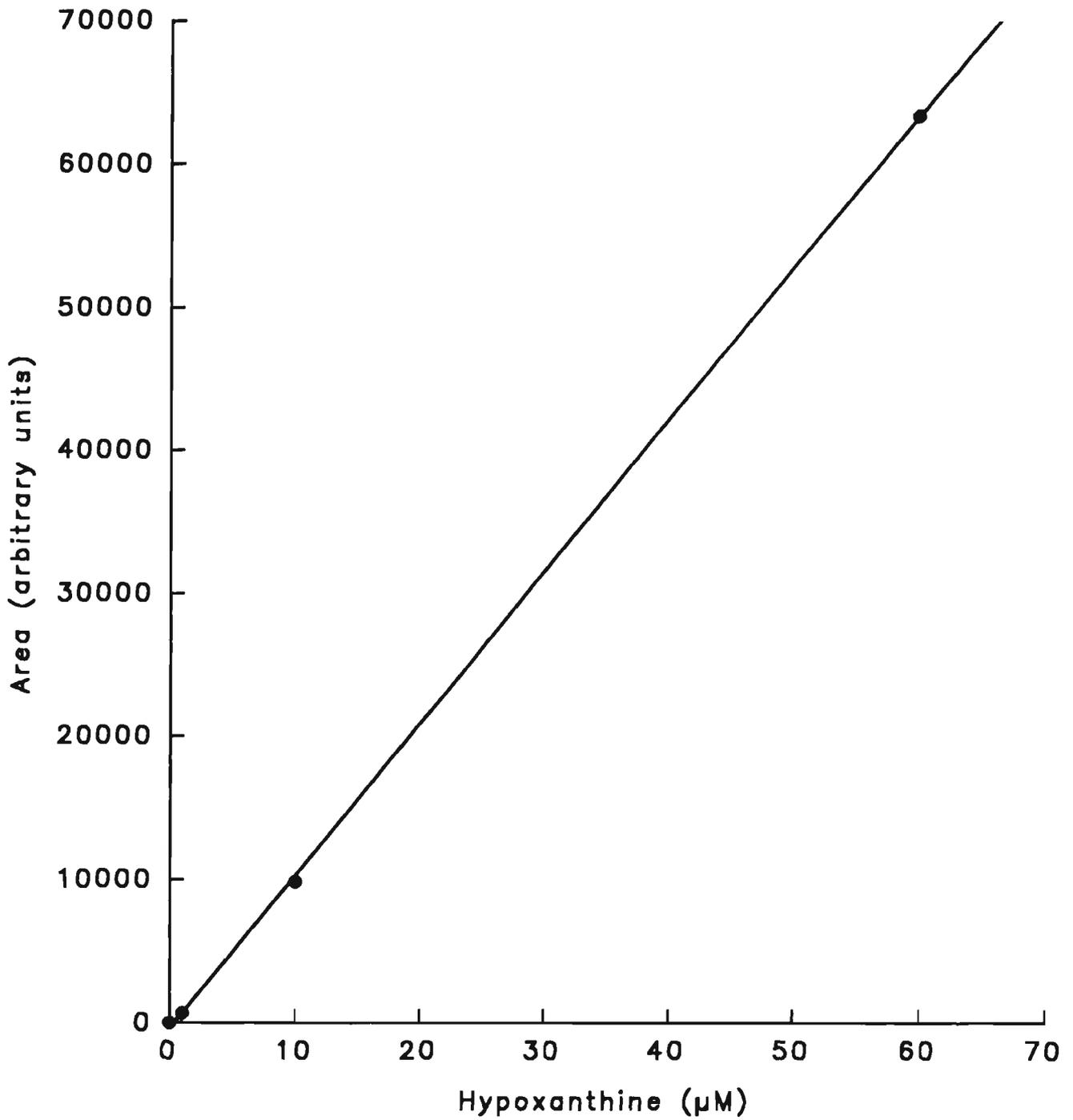
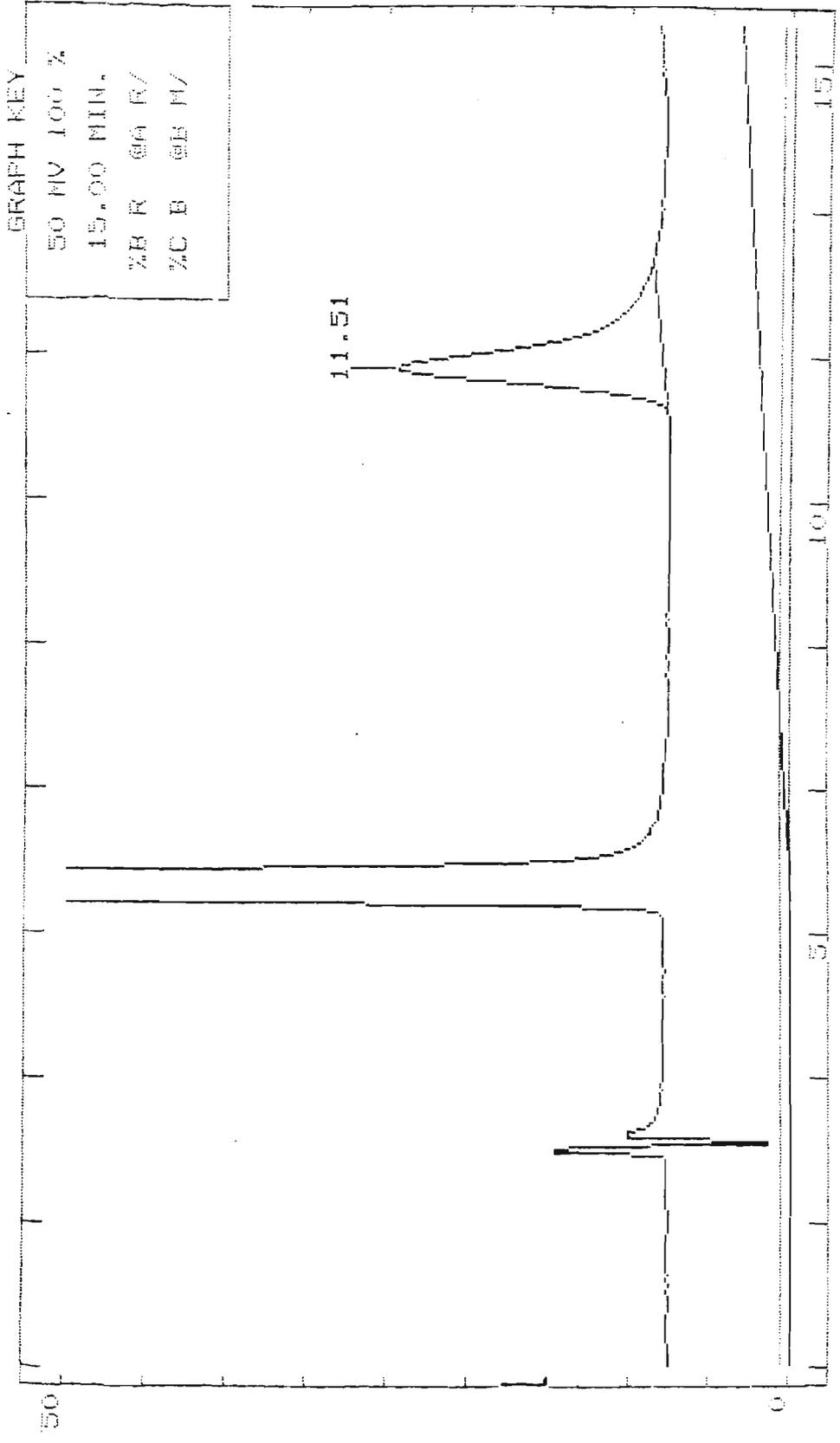


Figure B.2; A typical standard curve for plasma hypoxanthine analysis



**Standard 2**

SEQ INJ 1 / 1 SYSTEM1 / 1  
DATA FILE: HX252809.  
01:101

Figure B.3; A typical HPLC chromatograph of a hypoxanthine standard.

## Standard 2

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

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

METHOD: [ 13] [staph-hypoxanthine] [ ]  
 INTEGRATION: [ ] [ ] [ ]  
 SEQUENCE: [ ] [ ] [ ]

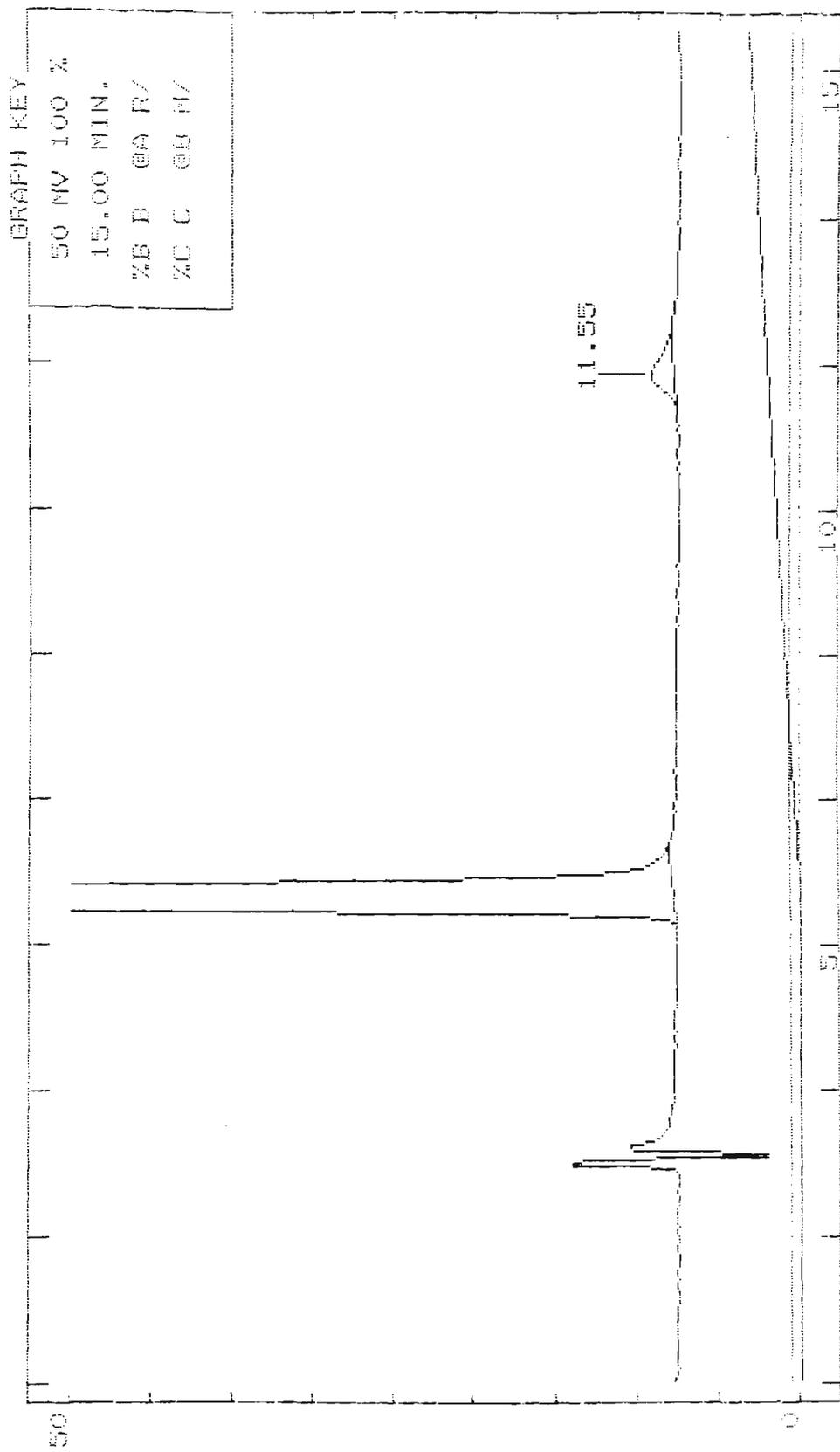
SIGNAL ACQUISITION FROM [ ] MIN] TO [ 25.00 MIN]

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

RETEN.	COMPOUND	AREA	AREAX	HEGT
11.51	<b>hypoxanthine</b>	9495	100.00%	18411

TOTALS: 9495 100.00%

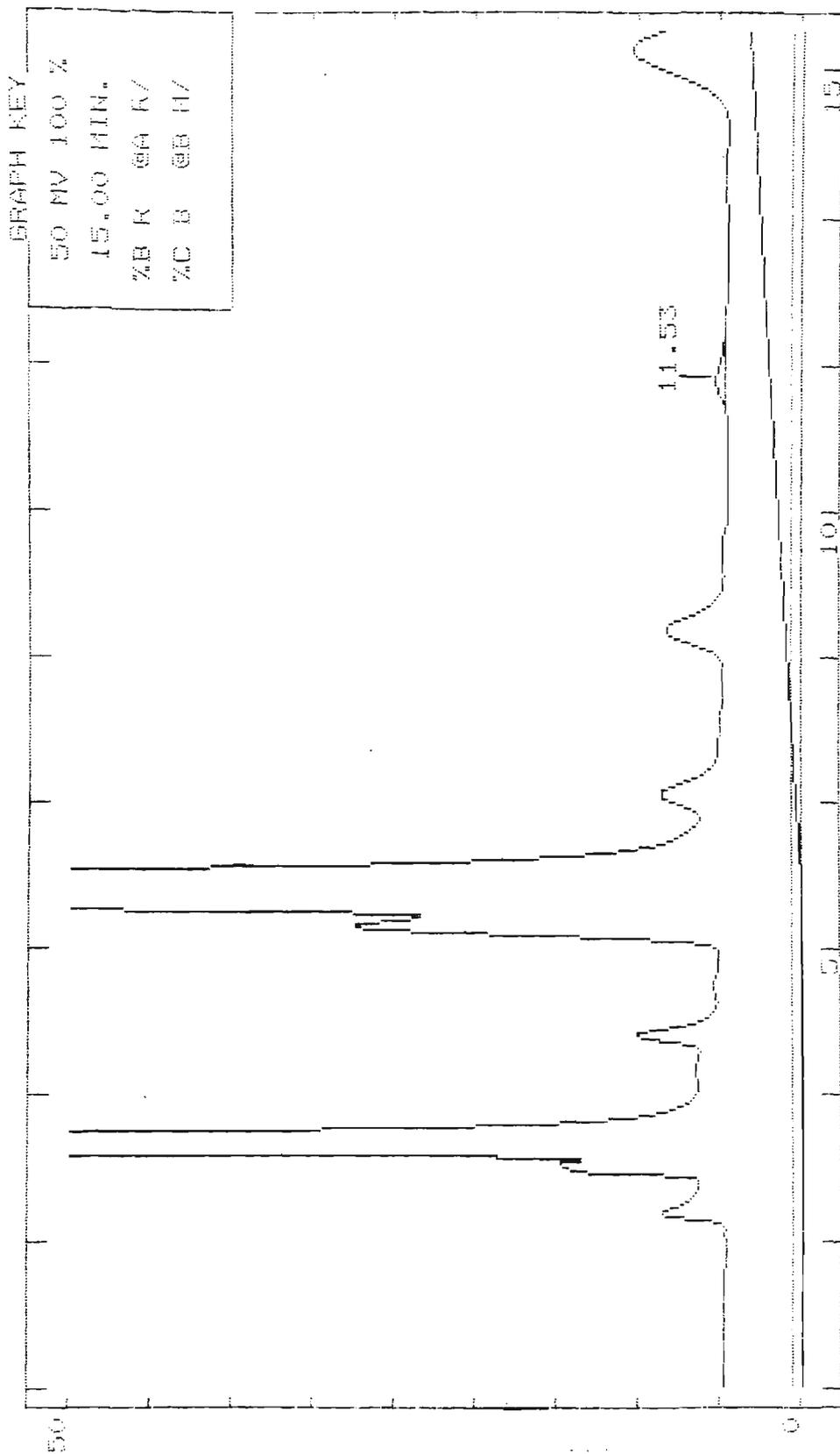
SEQ INQ 1/ 1 SYSTEM11  
 DATA FILE: RX282809.  
 00:58

**Standard 1**

SEQ 114 / / SYSTEM11  
DATA FILE: HX152807,  
05:02

Figure B.4; A typical HPLC chromatograph of a hypoxanthine standard.





Resting sample

Figure B.5; A typical HPLC chromatograph of a resting plasma sample.

## Resting sample

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

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

METHOD: [ 13] [staph-hypoxanthine] ]  
 INTEGRATION: [ ] [ ] ]  
 SEQUENCE: [ ] [ ] ]

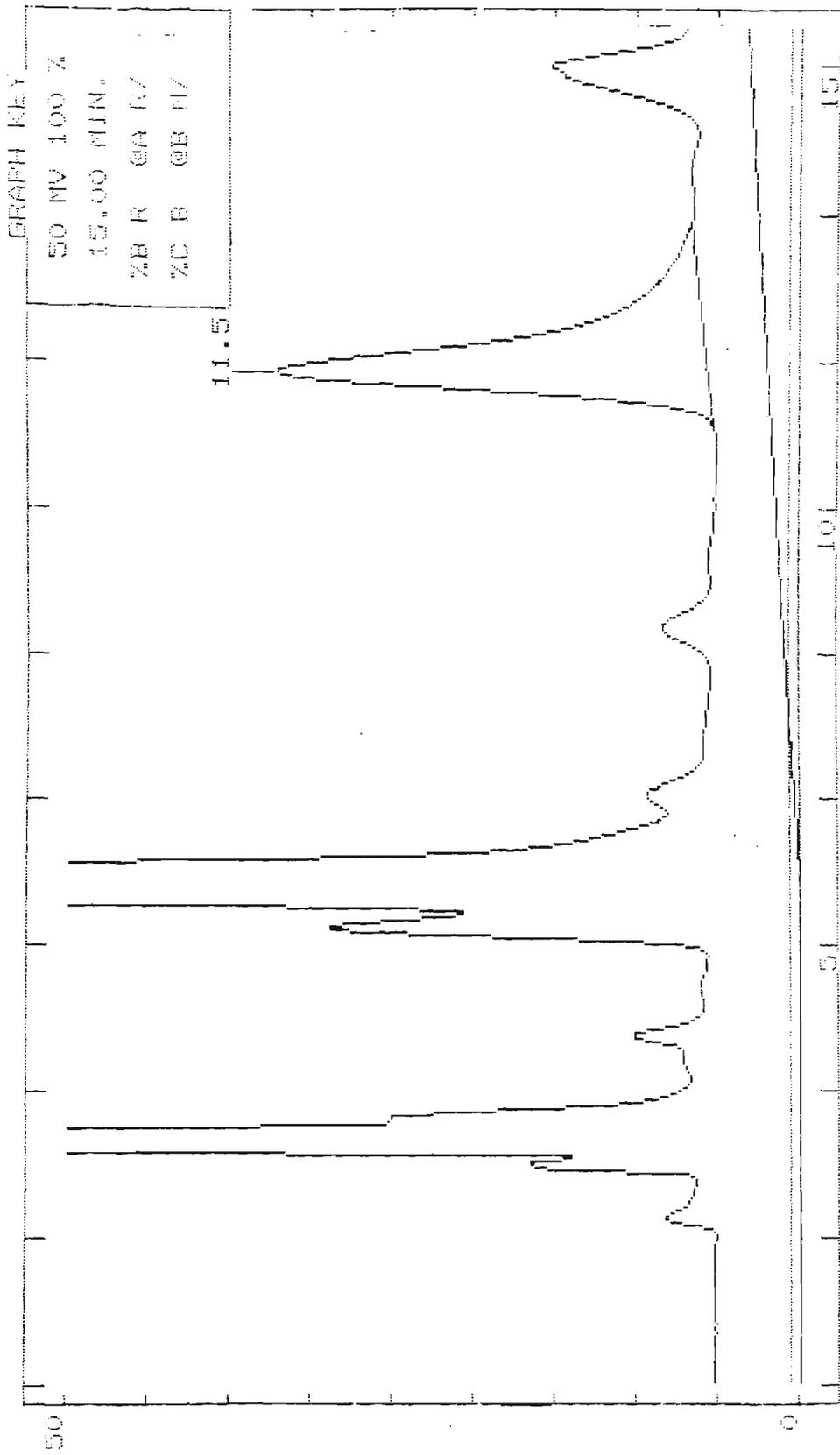
SIGNAL ACQUISITION FROM [ ] MIN TO [ 25.00 ] MIN

IS AMT: [ ] WGT: [ ] DIL FAD: [ ]

RETEN.	COMPOUND	AREA	AREAX	HGHT
11.53	<b>hypoxanthine</b>	280	100.00%	661

TOTALS: 280 100.00%

SEQ INQ 1/11 SYSTEM11  
 DATA FILE: HX0272rest.  
 01:02



**20 min recovery sample**

SEP 11 11 51 SYSTEM111  
DATA FILE: HXGDI2.COM  
02:12

Figure B.6; A typical HPLC chromatograph of a recovery plasma sample.

## 20 min recovery sample

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

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

METHOD: [ 13] [staph-hypoxanthine] [ ]  
 INTEGRATION: [ ] [ ] [ ]  
 SEQUENCE: [ ] [ ] [ ]

SIGNAL ACQUISITION FROM [ ] MIN] TO [ 25.00 MIN]

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

RETEN.	COMPOUND	AREA	AREA%	HGMT
11.55	<b>hypoxanthine</b>	19774	100.00%	29472

TOTALS: 19774 100.00%

SEQ INO 11 SYSTEM13  
 DATA FILE: HXGDT20min.  
 02110

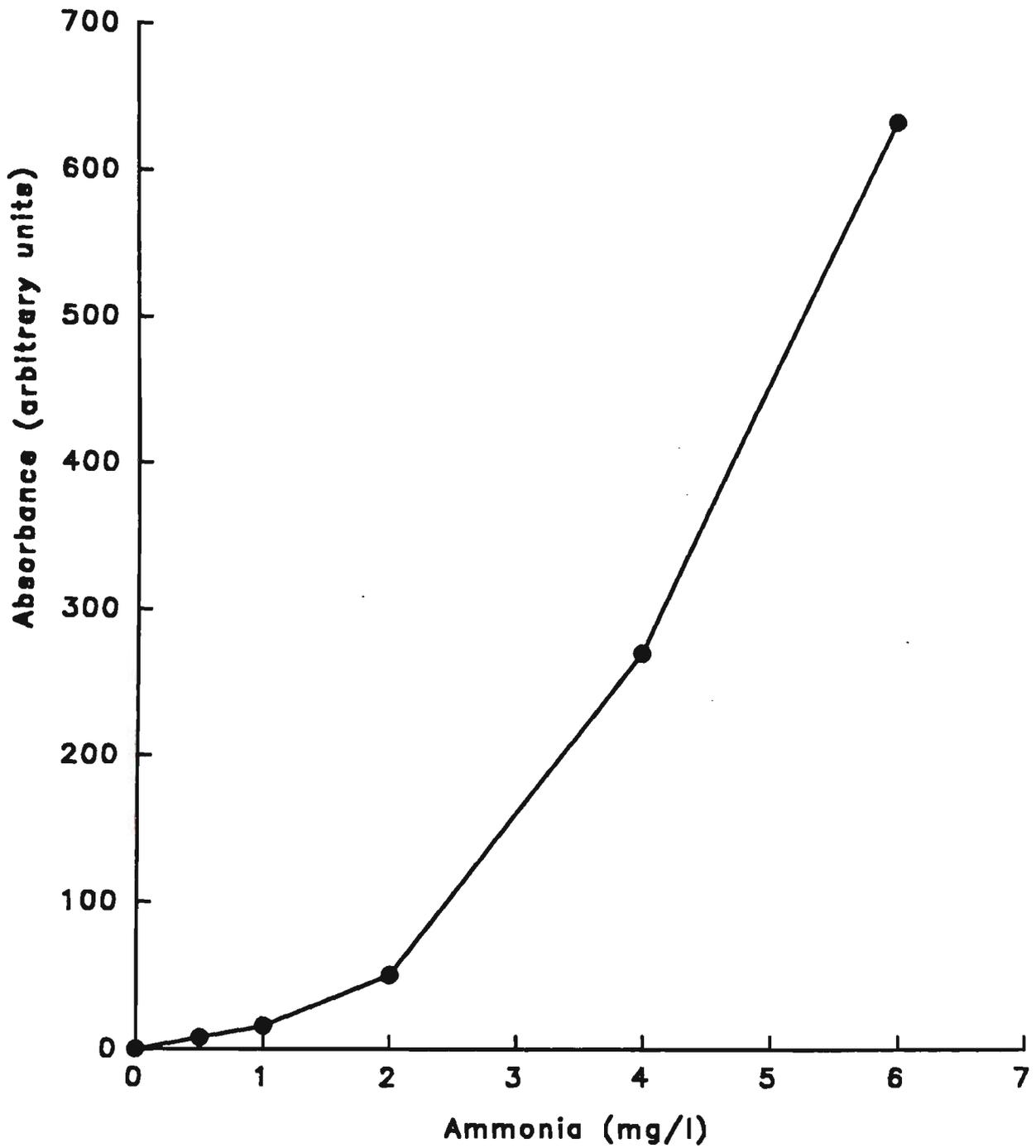


Figure B.7; A typical standard curve for plasma ammonia analysis

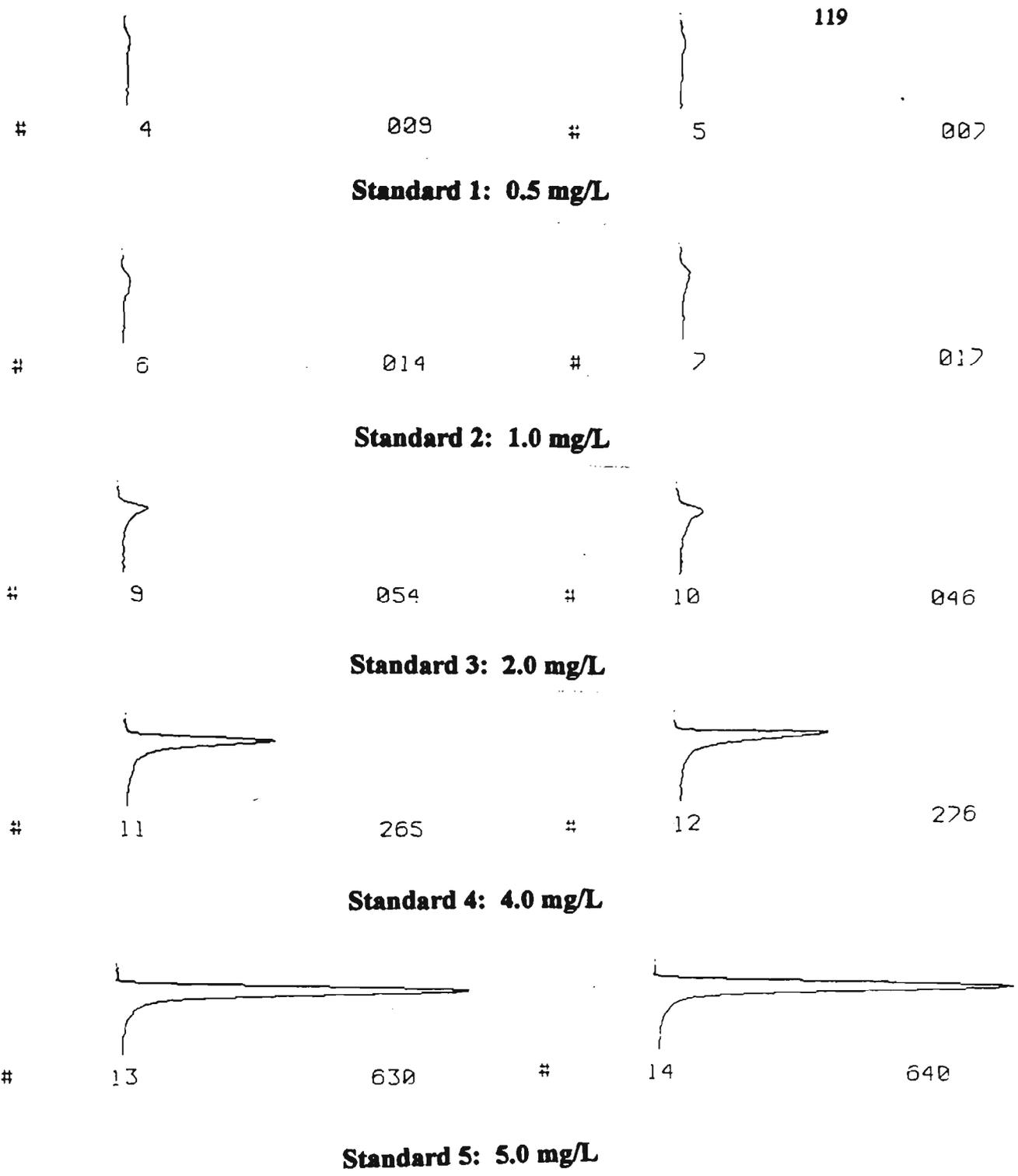
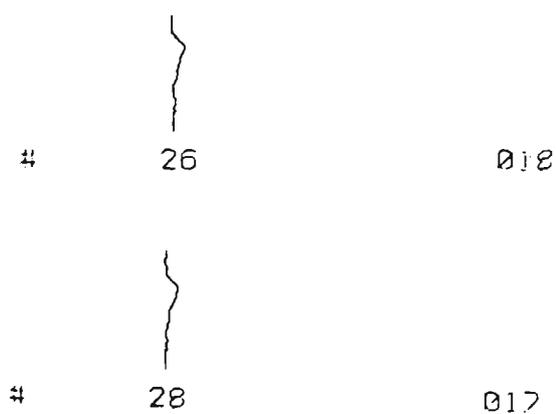
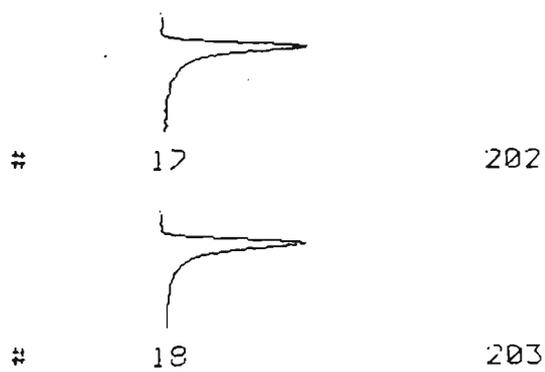


Figure B.8; Plasma ammonia FIA peaks for standard solutions.



**Plasma - resting**



**Plasma - post-exercise**

Figure B.9; Plasma ammonia FIA peaks for a resting and post-exercise sample.

**APPENDIX C**

**DETAILS OF ANALYTICAL CHEMICAL METHODS**  
**FOR MUSCLE METABOLITES**

## Muscle extraction

Approximately 2mg of freeze dried muscle was extracted in 0.5 M PCA and neutralized in 2.1 M KHCO<sub>3</sub> and subsequently analysed enzymatically for lactate, ATP, CP, Cr and using high performance liquid chromatography (HPLC) for ATP, ADP, AMP, IMP, inosine, hypoxanthine, adenosine and adenine.

## Muscle ATP, ADP, AMP, IMP, inosine and hypoxanthine

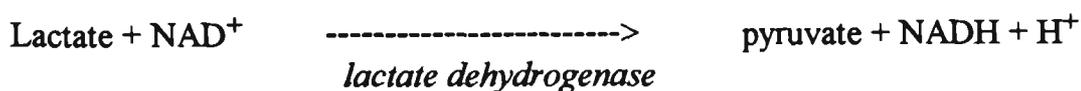
These metabolites were determined using high performance liquid chromatography (HPLC). Mobile phase A was ammonium dihydrogen phosphate solution, adjusted to a pH of 6.0, and mobile phase B was a solution of acetonitrile/methanol (50/50, vol/vol). The flow rate of the pump was set at 1.0 ml.min<sup>-1</sup>, the UV detector wavelength at 254 nm and the gradient profile for the separation achieved was set as follows.

Run time (min)	% mobile phase A	% mobile phase B
0.0	100	0
5.5	100	0
25.5	85	15
30.0	100	0

Standard solutions containing all of these and other metabolites (uric acid, xanthine, NADP<sup>+</sup>, xanthosine, NAD<sup>+</sup>, adenosine, adenine) were run during the analysis of the samples and the subsequent chromatographs demonstrate the separation achieved with the standards and muscle extracts of approximately 2mg dw of resting, post-exercise and 3 min recovery samples (Fig. C1, C2, C3, C.4, C.5). Typical standard curves for all the measured metabolites are also presented (Fig. C.6, C.7, C.8, C.8, C.9, C.10, C.11) to demonstrate the level of separation achieved using the methods as described in this thesis.

## Muscle Lactate

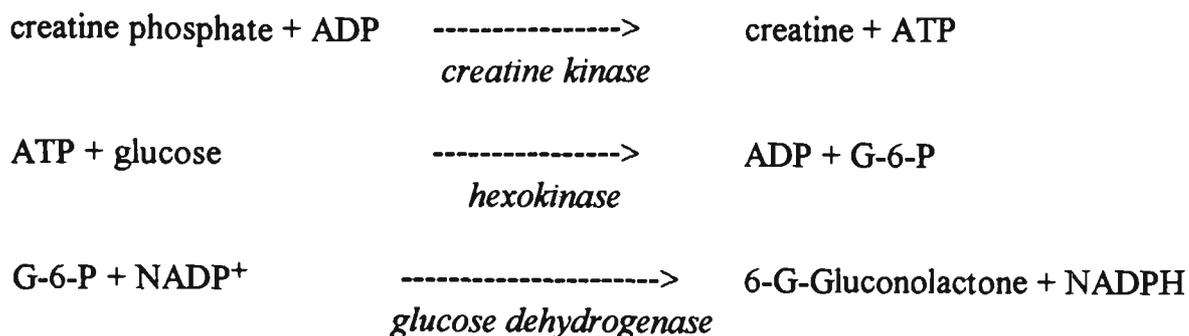
### Principle



Lactate standards of 50 and 500 μM were used in the assay along with a set of NADH standards ranging from approximately 50-400 μM. The NADH standards were quantified spectrophotometrically at 340 nm ( $E^0 = 6220$ ) and the actual concentration was used to construct a standard curve for the assay (NADH concentration vs fluorescence). This was used to confirm the metabolite standard fluorescence. The samples were analysed in triplicate and the assay was considered successful if the metabolite standard fluorescences were within 10% of the predicted value (using NADH standards; Fig. C.12)).

## Muscle ATP and CP

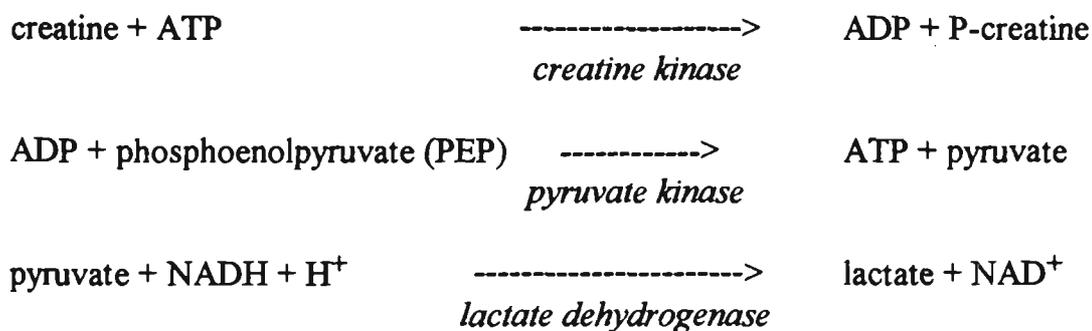
### Principle



This assay was run with ATP and CP standards of 250 and 500  $\mu\text{M}$ , and a set of NADH standards ranging from approximately 50-400  $\mu\text{M}$ . The NADH standards were measured spectrophotometrically at 340 nm (as in the muscle lactate assay above) and were used to estimate the expected fluorescence of the ATP and CP standards. Standards and samples were measured in triplicate and if the actual metabolite standards (ATP and CP) were within 10% of the predicted value, the assay was considered successful (Fig. C.13, C.14). The ATP concentrations measured were used to confirm the ATP measured by HPLC.

## Muscle creatine

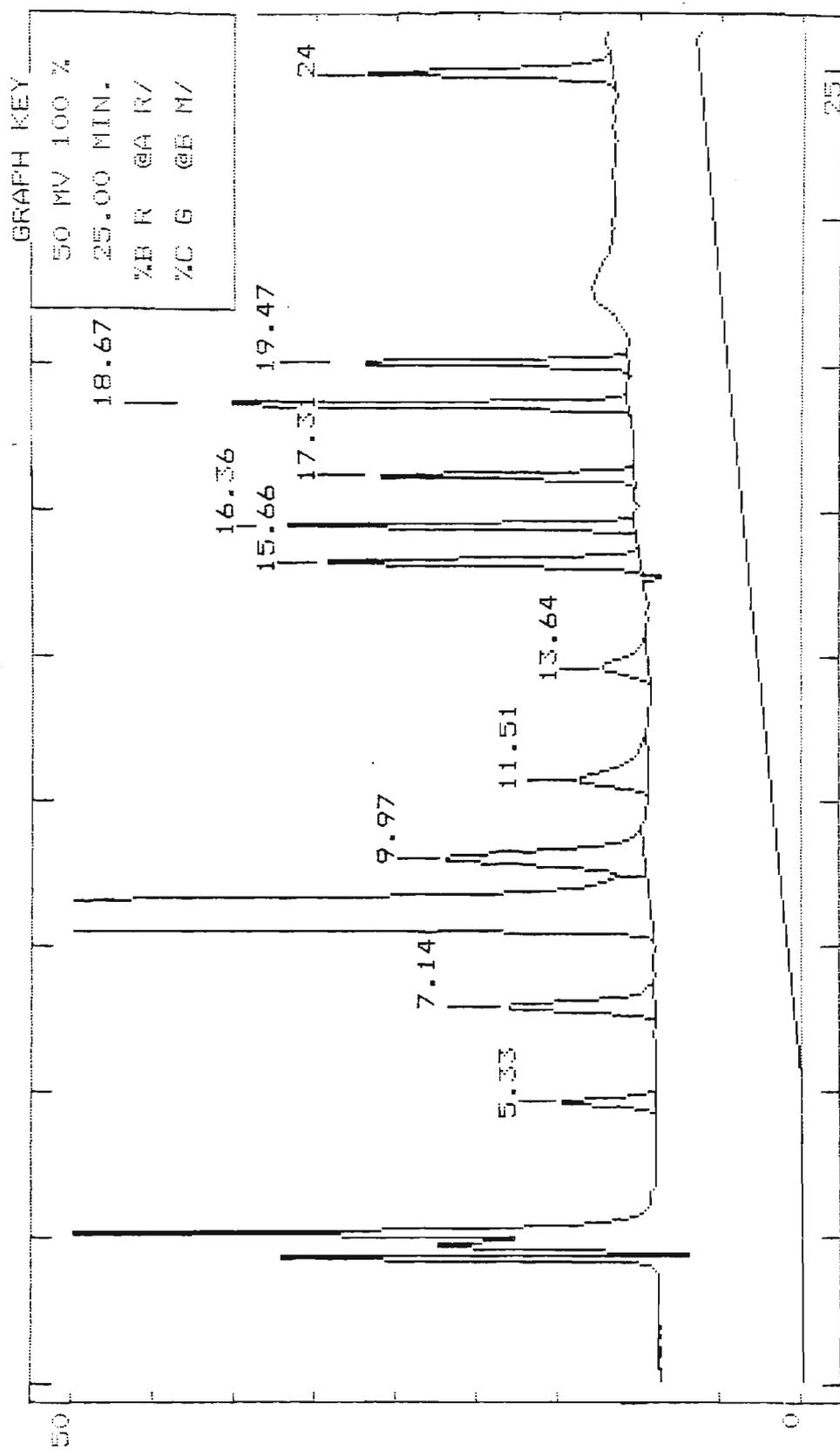
### principle



This assay requires the addition of NADH to the assay solution and the calculation of the disappearance of NADH is used to determine the content of creatine in the sample. The final concentration of NADH in the analysis "cocktail" was determined spectrophotometrically allowing the assays performance to be assessed.

## **Muscle Ammonia**

Muscle ammonia was quantified using flow injection analysis (FIA) and gas diffusion. The principle is the same as that of plasma ammonia analysis except that the carrier stream was deionised distilled water and the injected sample was deproteinized muscle extract. A copy of the printout of a typical set of ammonia standards (Fig. C.15), treatment standards and samples (Fig. C.16) and a standard curve was constructed prior to each sample set (Fig. C.17) is also presented. The treatment standard controls for the influence of the extraction procedure on the ammonia content in the muscle. The weight of the samples extracted varied between samples thus the wet weight is included with each printout.

**Standard 2**

SEQ INJ 1/1 SYSTEM111  
DATA FILE: 77250510.  
00:25

Figure C.1; A typical HPLC chromatograph of a standard.

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

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

METHOD: [ 12 ] [staphTANmethod] ]  
 INTEGRATION: [ ] [ ] ]  
 SEQUENCE: [ ] [ ] ]

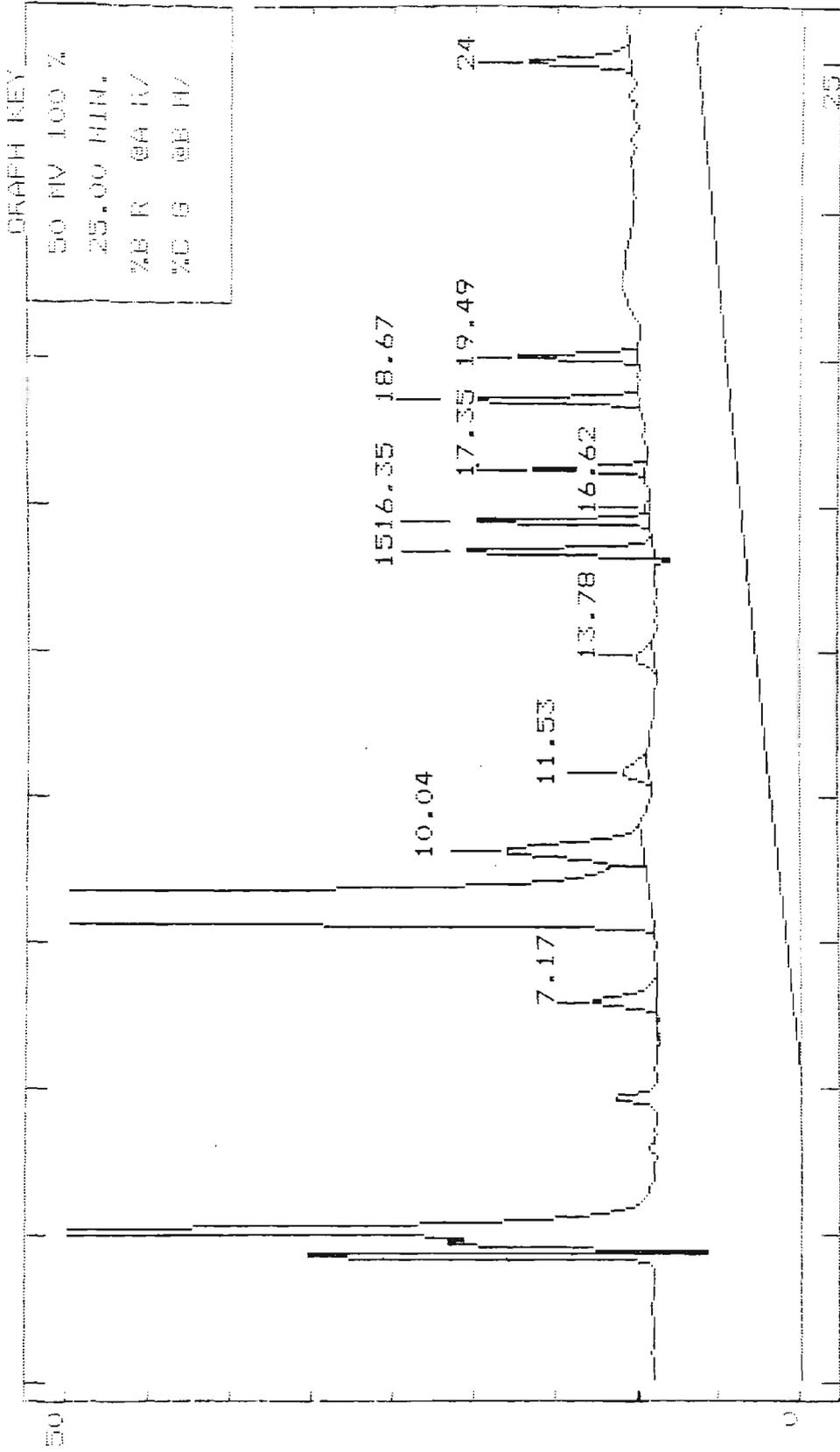
SIGNAL ACQUISITION FROM [ ] MIN] TO [ 35.00 MIN]

IS AMT: [ ] [ ] WGT: [ ] [ ] DIL FAD: [ ] [ ]

RETEN.	COMPOUND	AREA	AREA%	HGHT
5.33	<b>uric acid</b>	1135	0.49%	6519
7.14	<b>IMP</b>	2549	1.10%	10511
8.63	<b>ATP</b>	200137	86.76%	676502
9.97	<b>ADP</b>	4667	2.02%	13846
11.51	<b>hypoxanthine</b>	2007	0.87%	4766
13.64	<b>xanthine</b>	1165	0.50%	3157
15.66	<b>AMP</b>	3674	1.59%	21976
16.36	<b>NADP<sup>+</sup></b>	3071	1.33%	24974
17.31	<b>xanthosine</b>	2383	1.03%	16301
18.67	<b>NAD<sup>+</sup></b>	5943	1.70%	30902
19.47	<b>inosine</b>	2712	1.17%	20309
24.66	<b>adenosine</b>	3218	1.39%	16647
TOTALS:		230664	100.00%	

**Standard 2**

SEQ = 100 17 2 SYSTEMS  
 DATA FILE: 70230510.  
 00125



SEQ INJ 1/1 SYSTEM  
DATA FILE: 7030510.  
02:04

Standard 3

Figure C.2; A typical HPLC chromatograph of a standard.

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

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

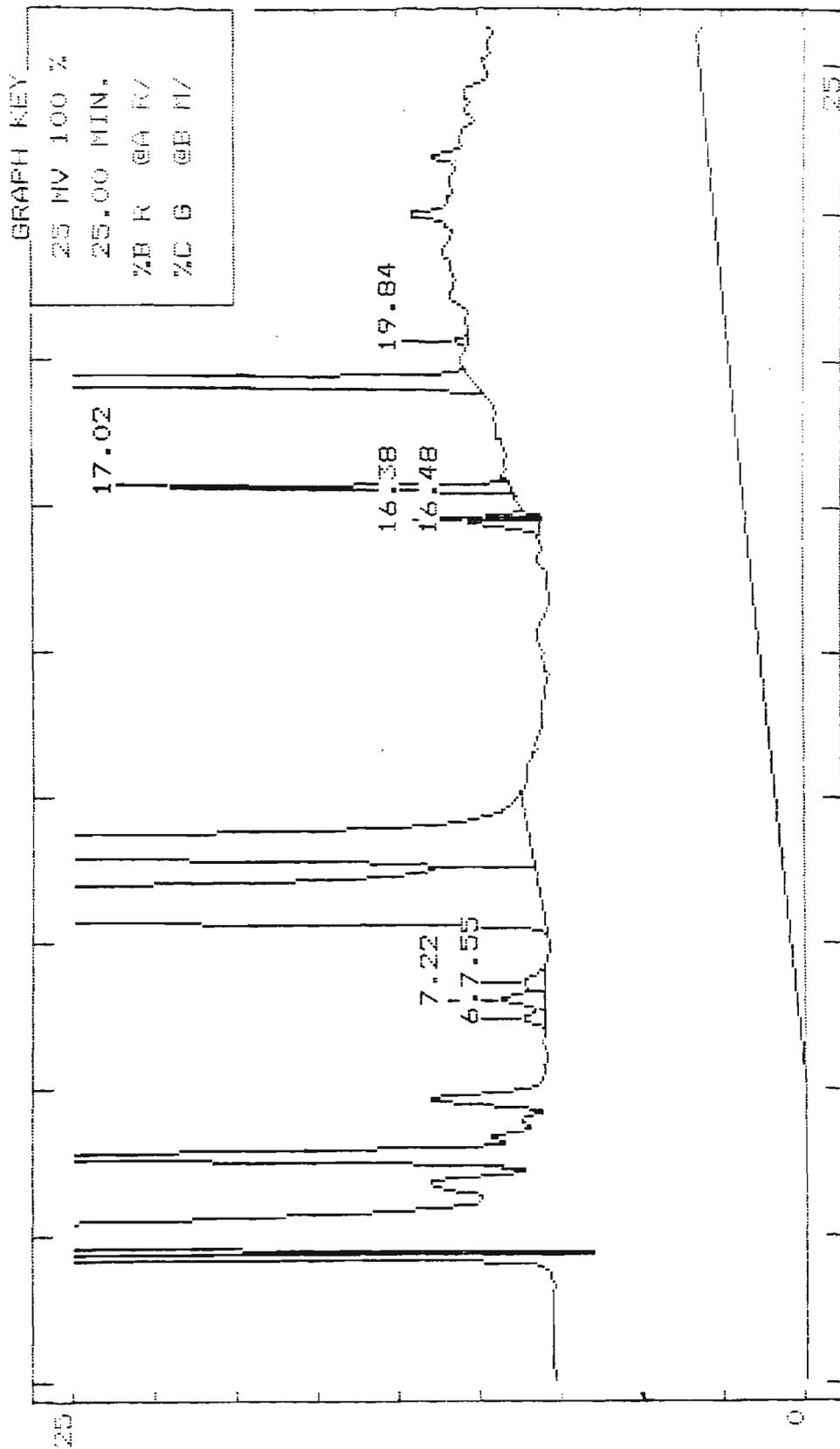
METHOD: [ 123 ] [staphTANmethod ]  
 INTEGRATION: [ ] [ ]  
 SEQUENCE: [ ] [ ] [ ]

SIGNAL ACQUISITION FROM [ ] MIN] TO [ 35.00 MIN]

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

RETEN.	COMPOUND	AREA	AREA%	HGHT
7.17	<b>IMP</b>	1163	0.44%	4403
8.88	<b>ATP</b>	247321	94.54%	798468
10.04	<b>ADP</b>	3343	1.27%	9562
11.53	<b>hypoxanthine</b>	567	0.21%	1842
13.78	<b>xanthine</b>	394	0.15%	1205
15.77	<b>AMP</b>	2089	0.79%	13693
16.35	<b>NADP<sup>+</sup></b>	1471	0.56%	13375
16.82		60	0.02%	477
17.35	<b>xanthosine</b>	976	0.37%	7849
18.67	<b>NAD<sup>+</sup></b>	1714	0.65%	13344
19.49	<b>inosine</b>	1134	0.43%	8401
24.99	<b>adenosine</b>	1366	0.52%	7020
TOTALS:		261603	100.00%	

Standard 3



**Resting sample**

SEQ INJ 1/1 SYSTEM111  
DATA FILE: APT1-rest.  
02/20

Figure C.3: A typical HPLC chromatograph of a resting muscle sample.

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

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

METHOD: [ 12] [staphTANmethod] ]  
 INTEGRATION: [ ] [ ] ]  
 SEQUENCE: [ ] [ ] ]

SIGNAL ACQUISITION FROM [ ] MIN] TO [ 35.00 MIN]

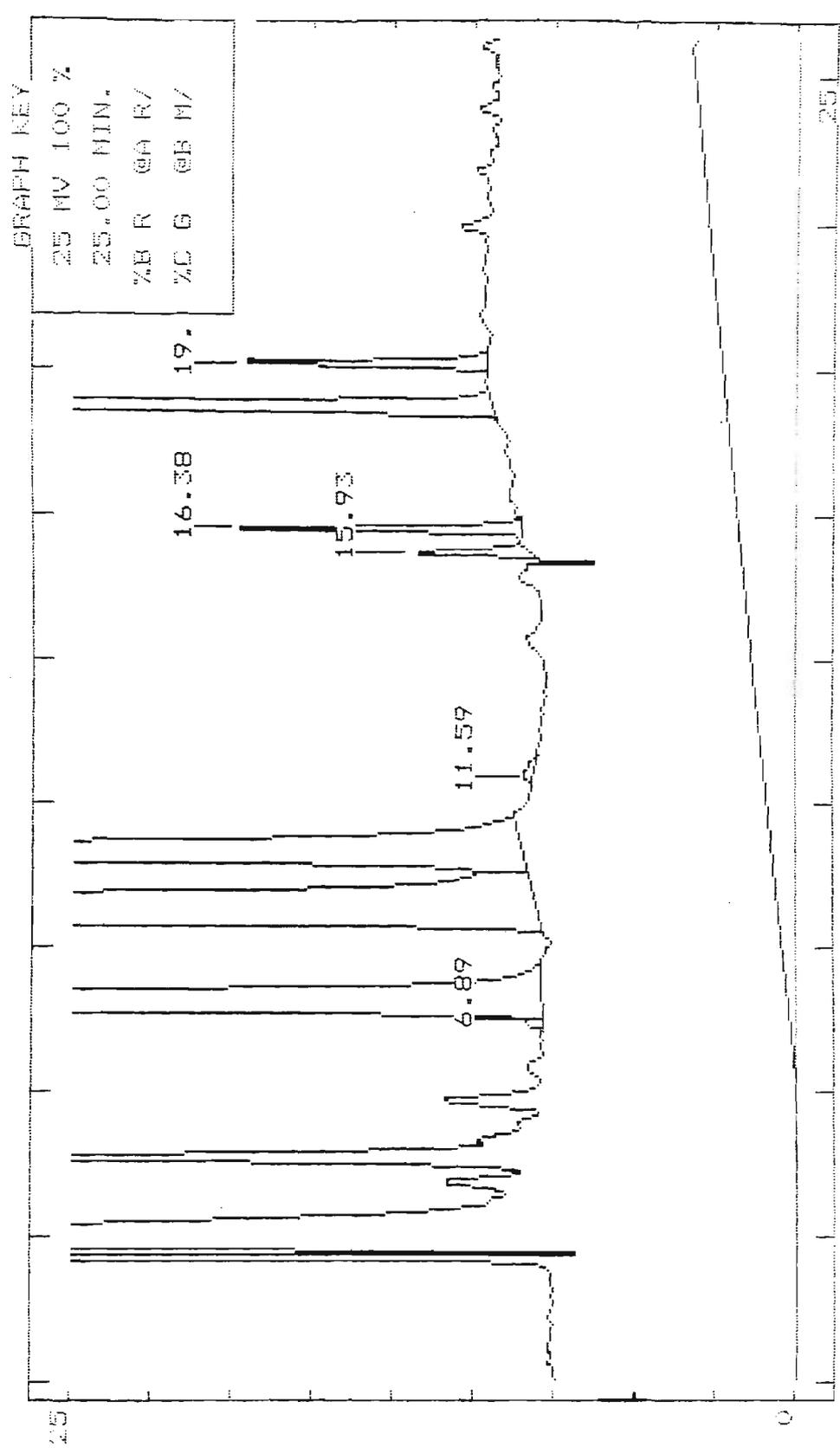
IS AMT: [ ] 1] WGT: [ ] 1] DIL FAC: [ ] 1]

RETEN.	COMPOUND	AREA	AREA%	HGHT
6.86		143	0.05%	694
7.22	<b>IMP</b>	323	0.12%	1458
7.55		175	0.06%	718
8.97	<b>ATP</b>	218216	83.76%	707897
10.12	<b>ADP</b>	22330	8.57%	64224
16.36	<b>AMP</b>	371	0.14%	2605
16.48		114	0.04%	1541
17.02	<b><u>NADP<sup>+</sup></u></b>	1180	0.45%	11658
18.99	<b><u>NAD<sup>+</sup></u></b>	17592	6.75%	143895
19.84	<b>inosine</b>	49	0.01%	440

TOTALS: 260501 100.00%

**Resting sample**

SEP 11 11:18 INJ 1/ 1 SYSTEM113  
 DATA FILE: AP11-rest.  
 02:18



**Immediate post-exercise sample**

SEP INJ 11 1 SYSTEM111  
DATA FILE: APT1-0min.  
02:30

Figure C.4; A typical HPLC chromatograph of an immediate post-exercise muscle sample.

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

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

METHOD: [ 12] [staphTAmethod] ]  
 INTEGRATION: [ ] [ ] ]  
 SEQUENCE: [ ] [ ] ]

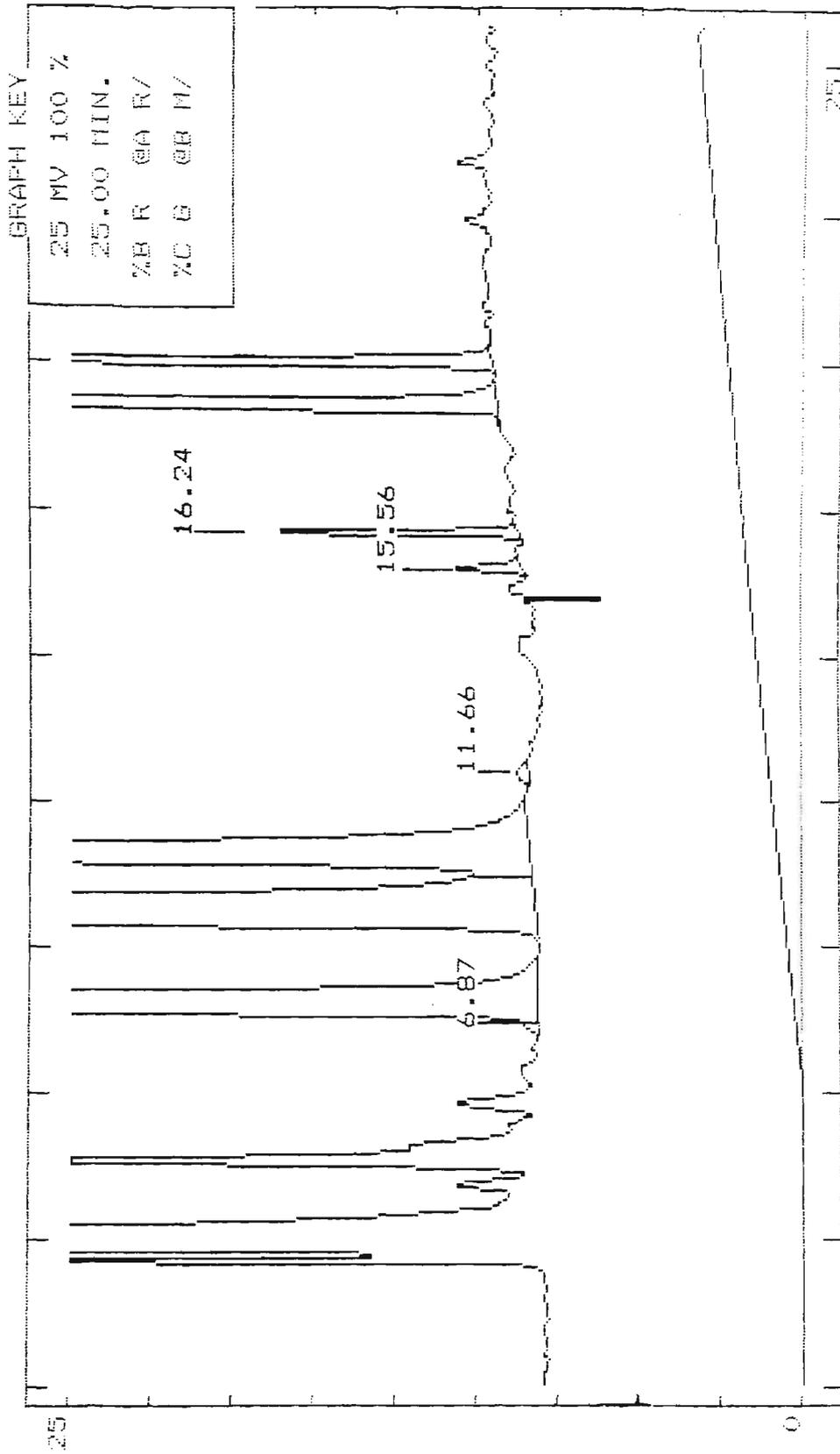
SIGNAL ACQUISITION FROM [ ] MINS TO [ 35.00 ] MINS

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

RETEN.	COMPOUND	AREA	AREA%	HGHT
6.89		70	0.03%	615
7.24	<b>IMP</b>	64035	30.00%	270719
8.99	<b>ATP</b>	113417	53.15%	372927
10.13	<b>ADP</b>	16907	7.92%	50352
11.59	<b>hypoxanthine</b>	99	0.04%	295
15.93	<b>AMP</b>	557	0.26%	4254
16.36	<b>NADP<sup>+</sup></b>	1170	0.54%	9866
18.71	<b>NAD<sup>+</sup></b>	15953	7.47%	116595
19.55	<b>inosine</b>	1156	0.54%	5484
TOTALS:		213351	100.00%	

**Immediate post-exercise sample**

SEG INJ 17 SYSTEMIC  
 DATA FILE: APT1-0m17.  
 02:28



### 3 min recovery sample

SEQ INJ 1/1 SYSTEMC/1  
DATA FILE: AP11-3min.  
02:33

Figure C.5; A typical HPLC chromatograph of a recovery muscle sample.

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

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

METHOD: [ 12] [staphTANmethod ]  
 INTEGRATION: [ ] [ ]  
 SEQUENCE: [ ] [ ]

SIGNAL ACQUISITION FROM [ ] MIN] TO [ 35.00 MIN]

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

RETEN.	COMPOUND	AREA	AREA%	HGHT
6.87		79	0.03%	520
7.22	<b>IMP</b>	59399	28.66%	251819
8.95	<b>ATP</b>	108590	52.39%	361667
10.09	<b>ADP</b>	18185	8.77%	52314
11.66	<b>hypoxanthine</b>	114	0.05%	443
15.56	<b>AMP</b>	335	0.16%	2300
16.24	<b>NADP<sup>+</sup></b>	995	0.48%	9272
18.63	<b>NAD<sup>+</sup></b>	15749	7.59%	122463
19.44	<b>inosine</b>	3796	1.83%	28334

TOTALS: 207245 100.00%

3 min recovery sample

SEQ INJ 1/ 1 SYSTEM[1]  
 DATA FILE: APT1-3min.  
 02:37

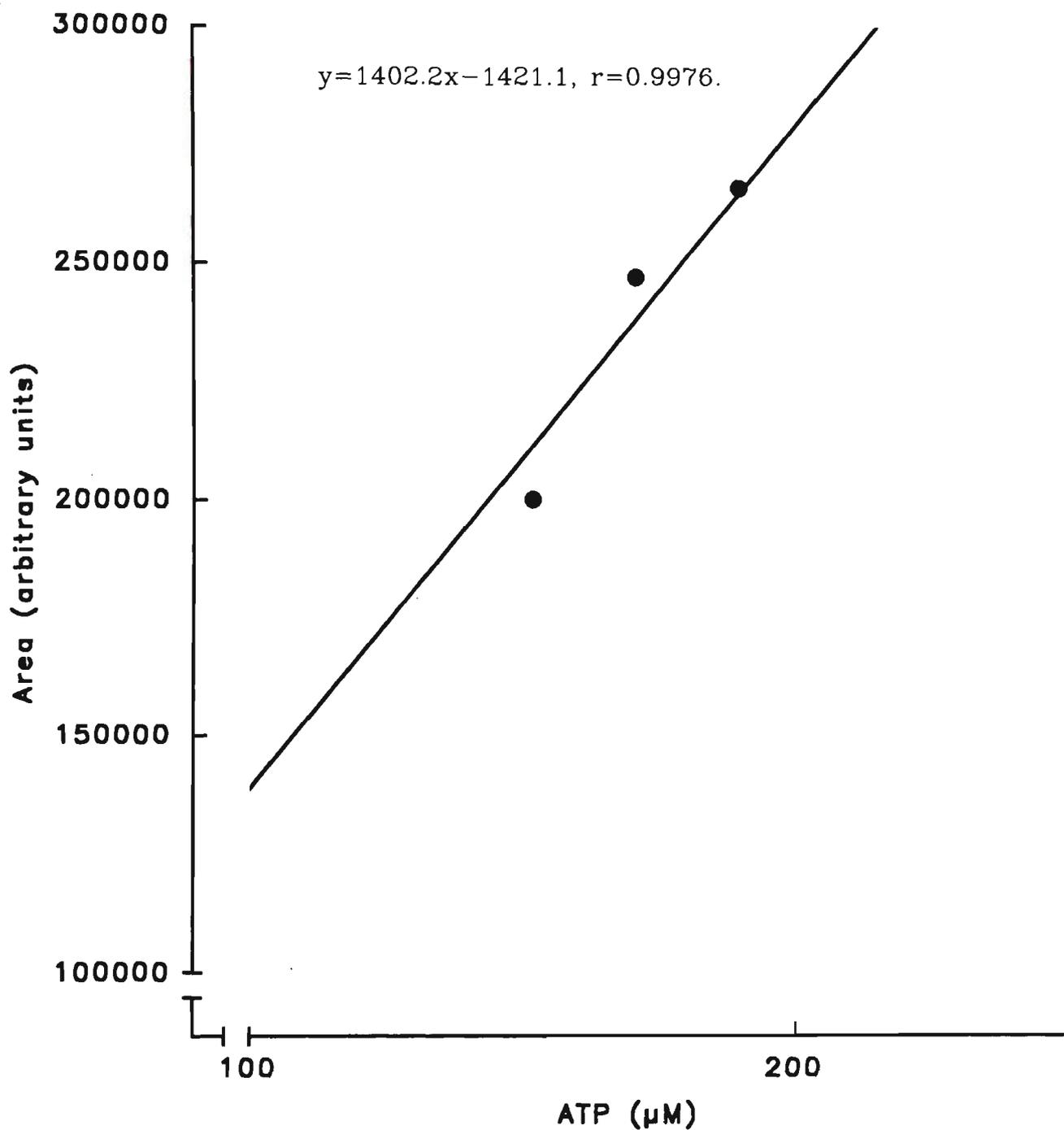


Figure C.6; A typical standard curve for muscle ATP analysis performed using a HPLC technique.

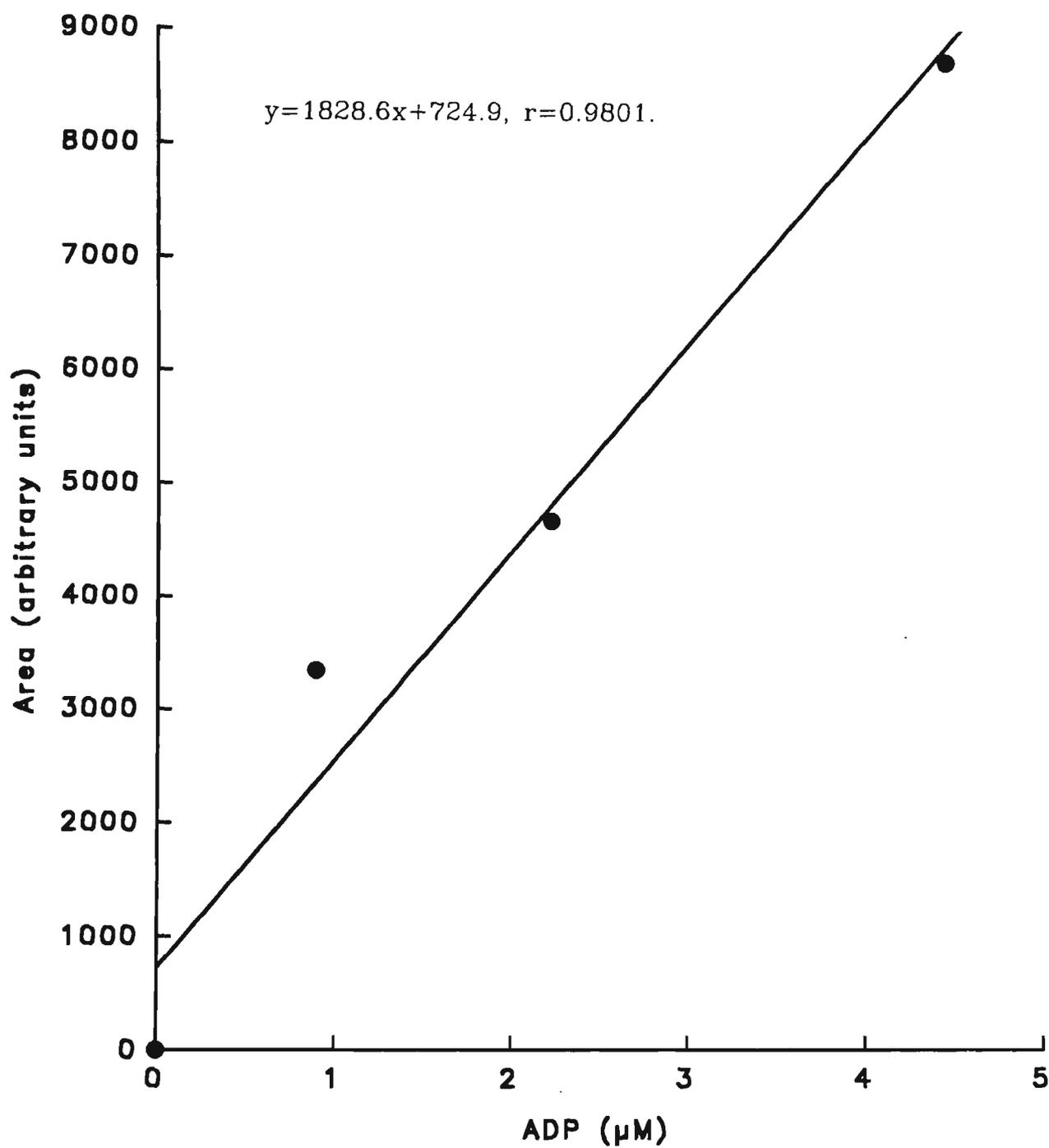


Figure C.7; A typical standard curve for muscle ADP analysis performed using a HPLC technique.

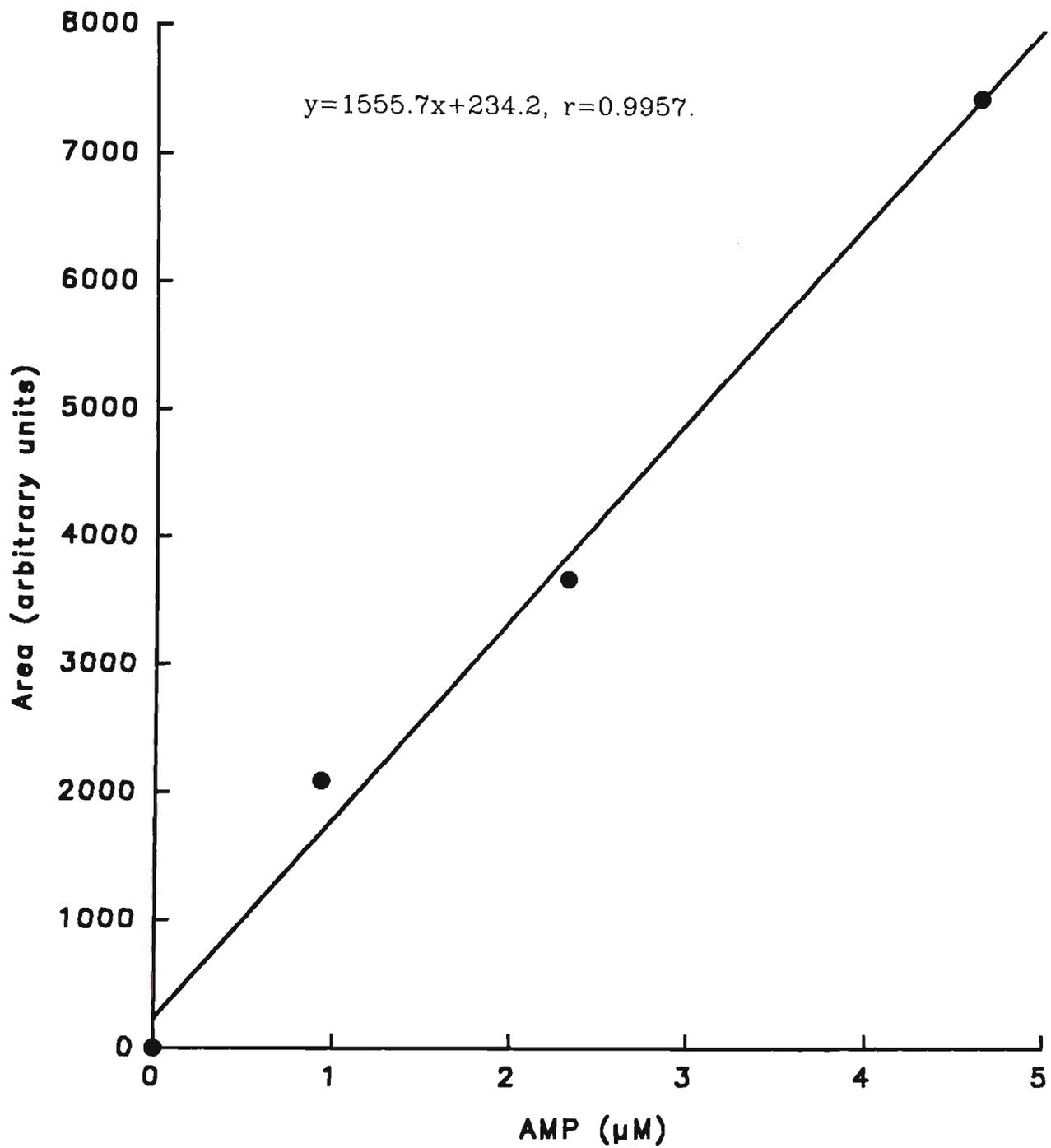


Figure C.8; A typical standard curve for muscle AMP analysis performed using a HPLC technique.

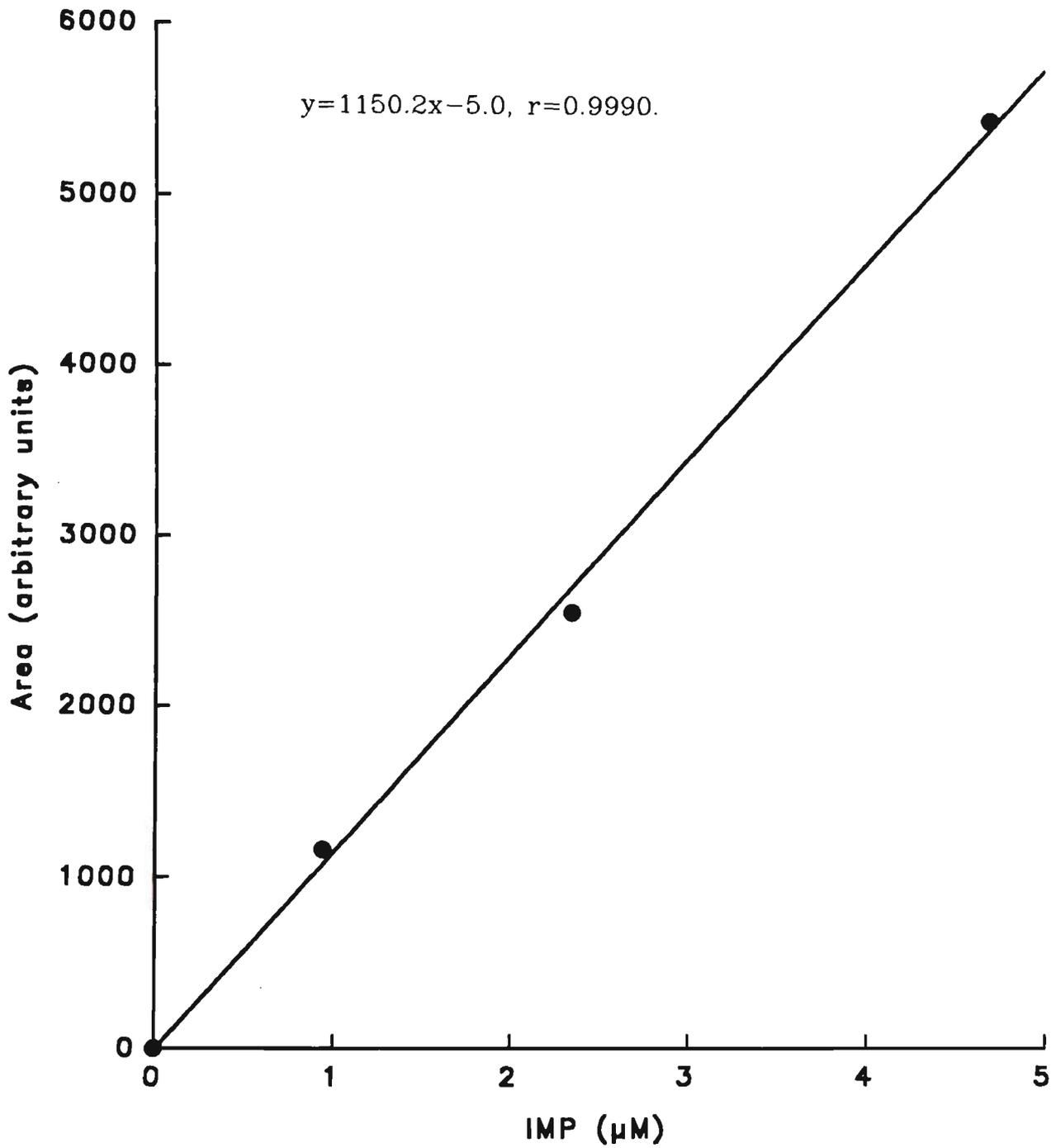


Figure C.9; A typical standard curve for muscle IMP analysis performed using HPLC technique.

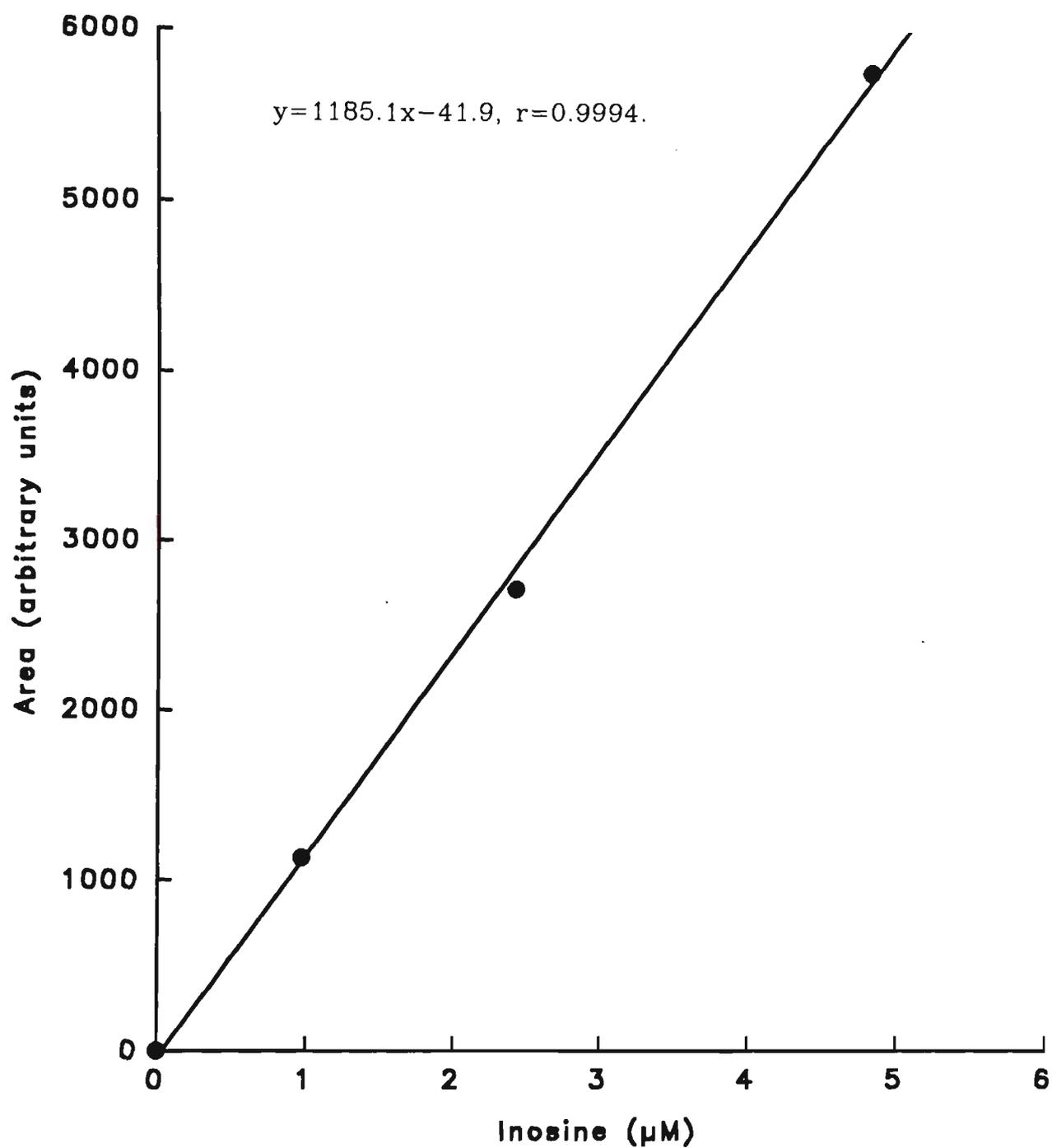


Figure C.10; A typical standard curve for muscle inosine analysis performed using a HPLC technique.

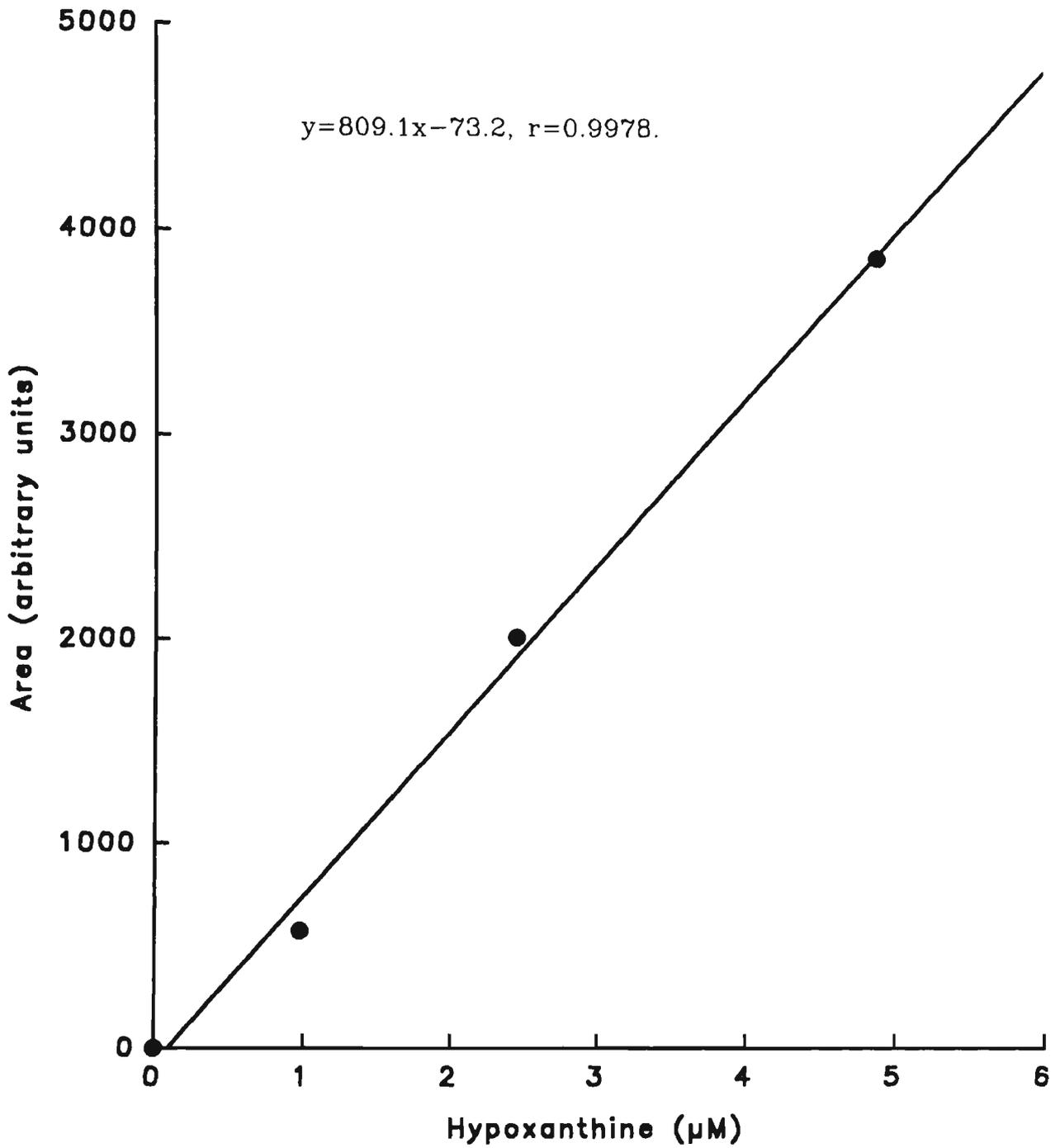


Figure C.11; A typical standard curve for muscle hypoxanthine analysis performed using a HPLC technique.

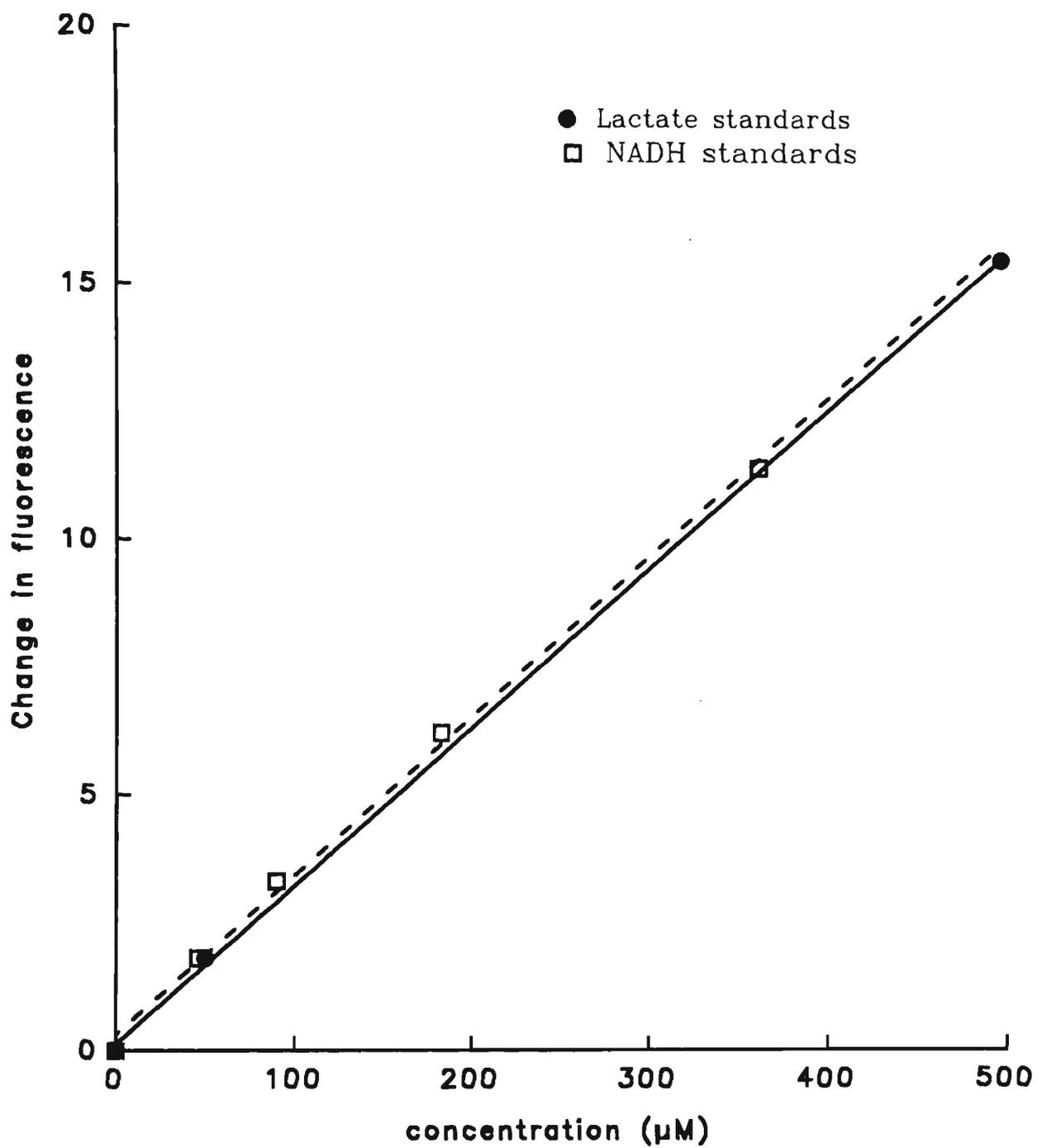


Figure C.12; A typical standard curve for muscle lactate analysis performed using an enzymatic technique with fluorometric detection; both lactate and NADH standards are presented.

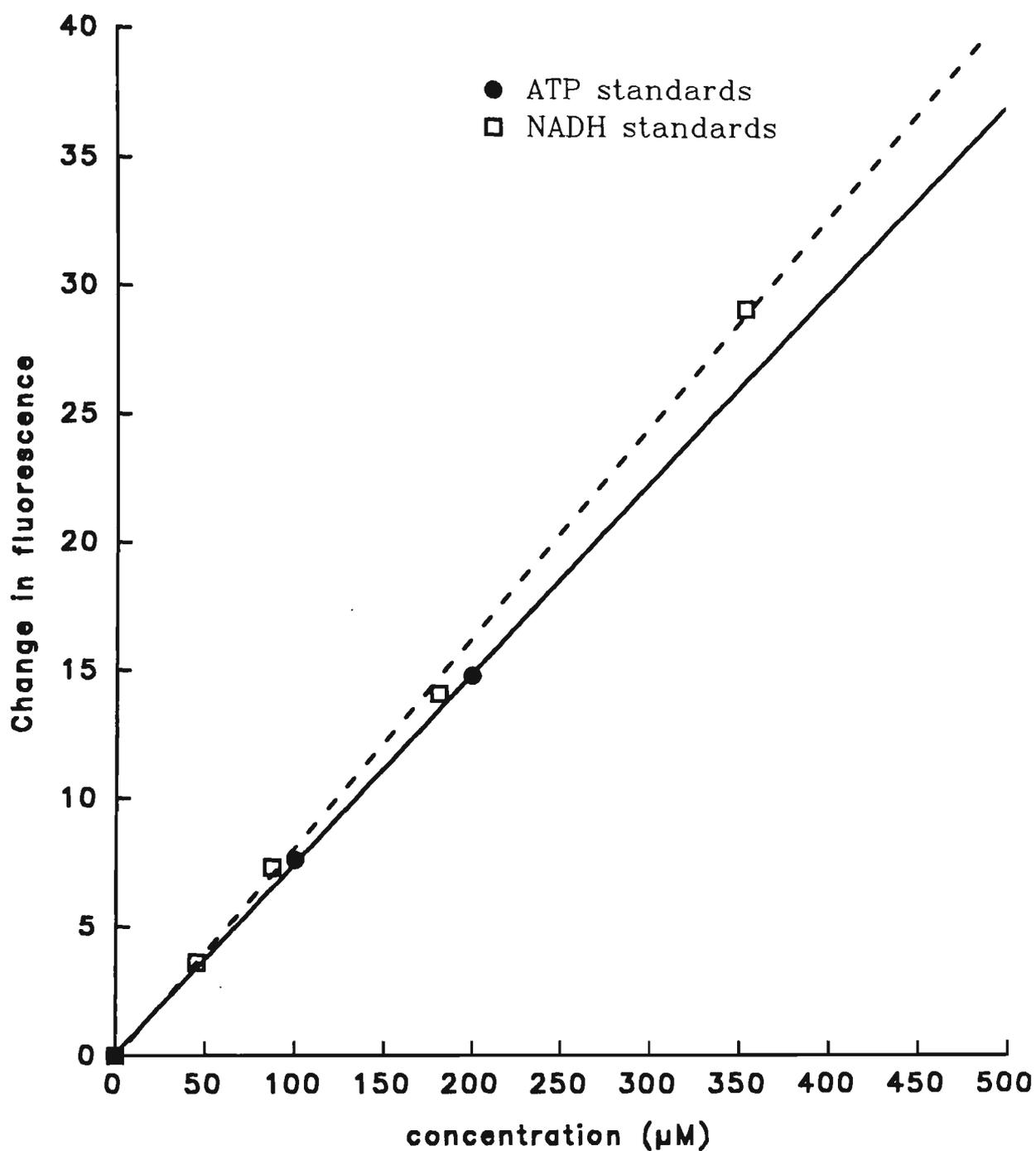


Figure C.13; A typical standard curve for muscle ATP analysis performed using an enzymatic technique with fluorometric detection; both ATP and NADH curves are presented.

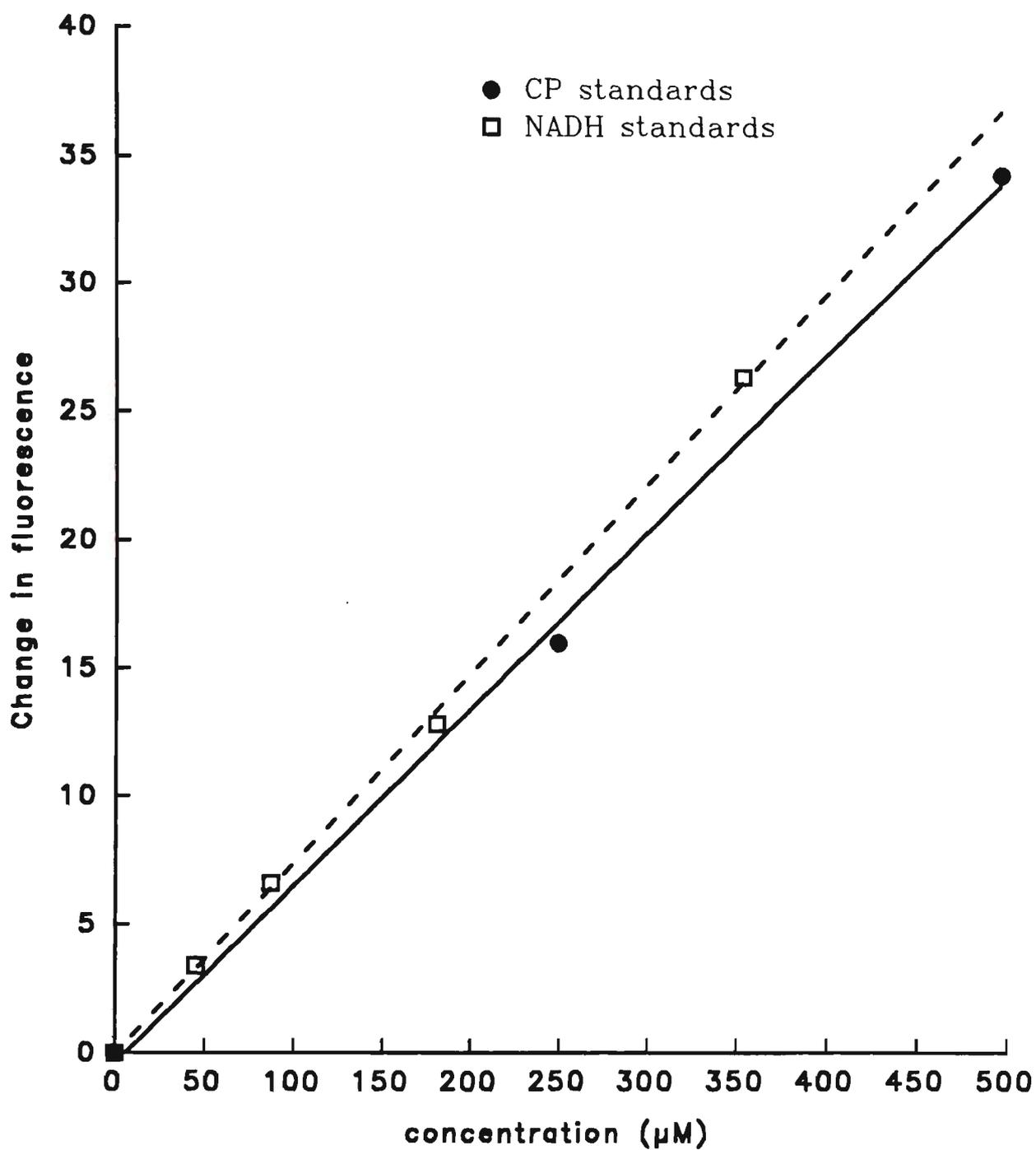


Figure C.14; A typical standard curve for muscle CP analysis performed using an enzymatic technique with fluorometric detection; both CP and NADH curves are presented.

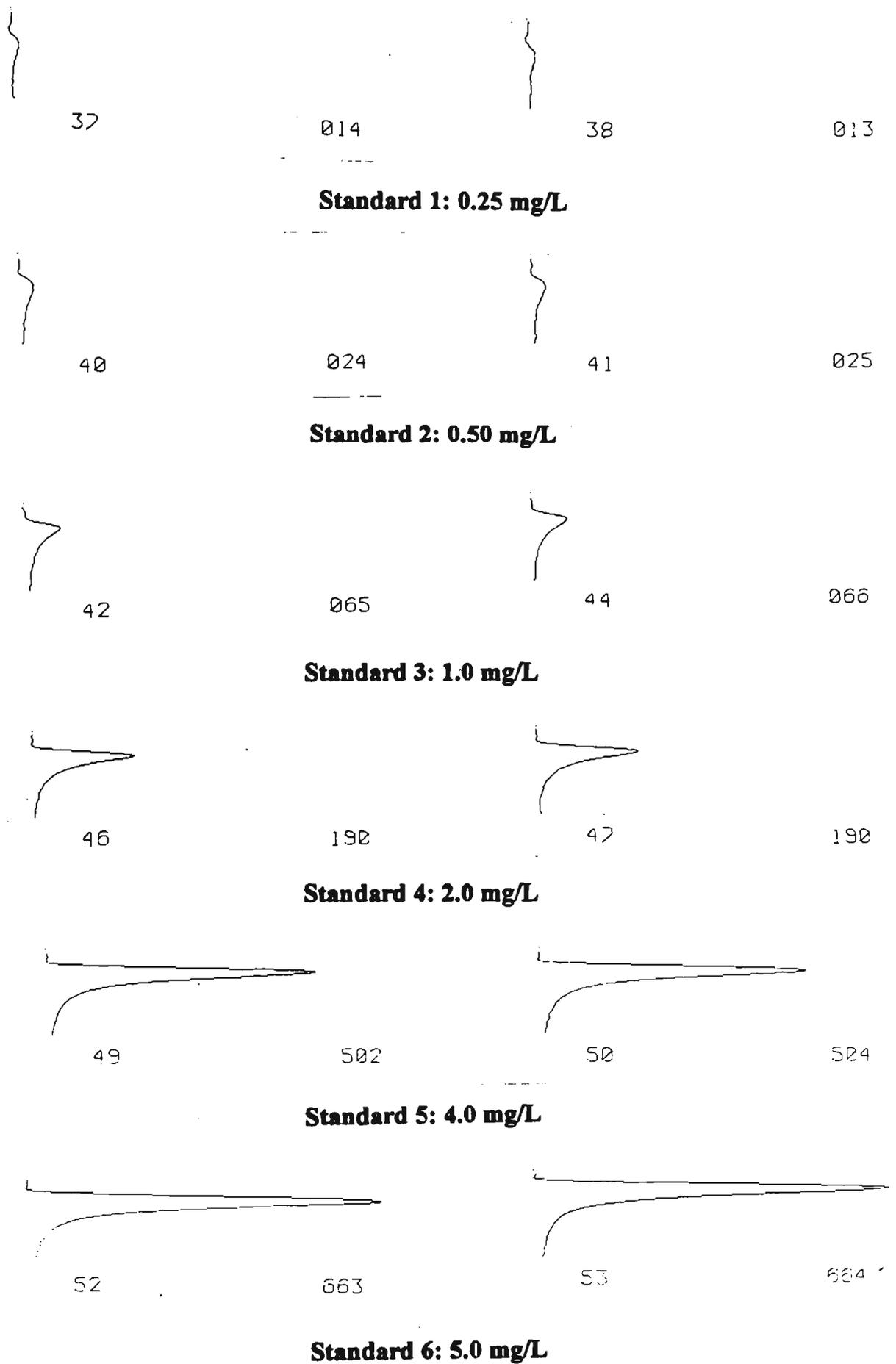


Figure C.15; Muscle ammonia FIA peaks for standard solutions.

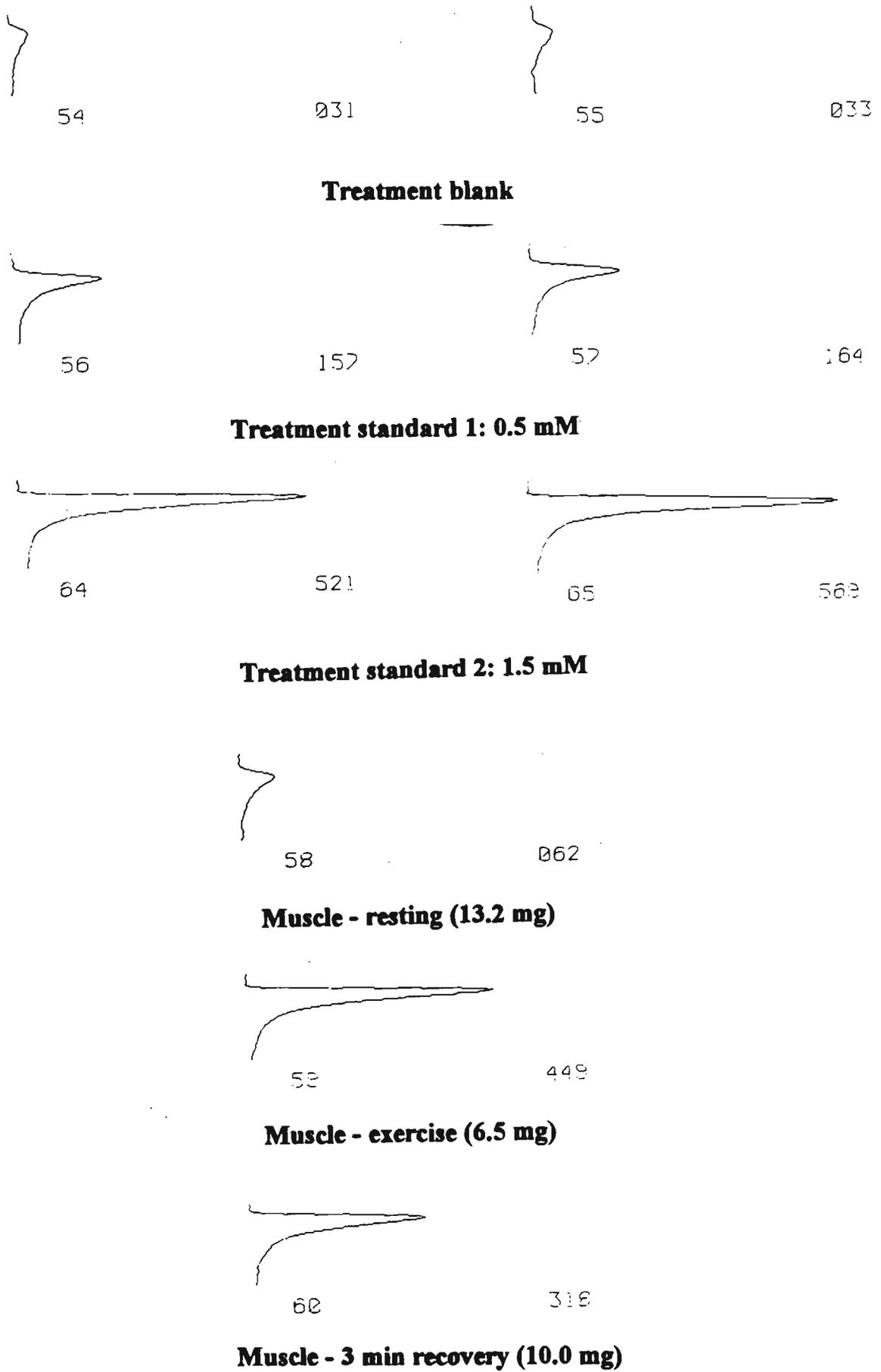


Figure C.16; Muscle ammonia FIA peaks for the treatment blank, standards and muscle samples at rest, after a 30 s sprint and after 3 min of recovery.

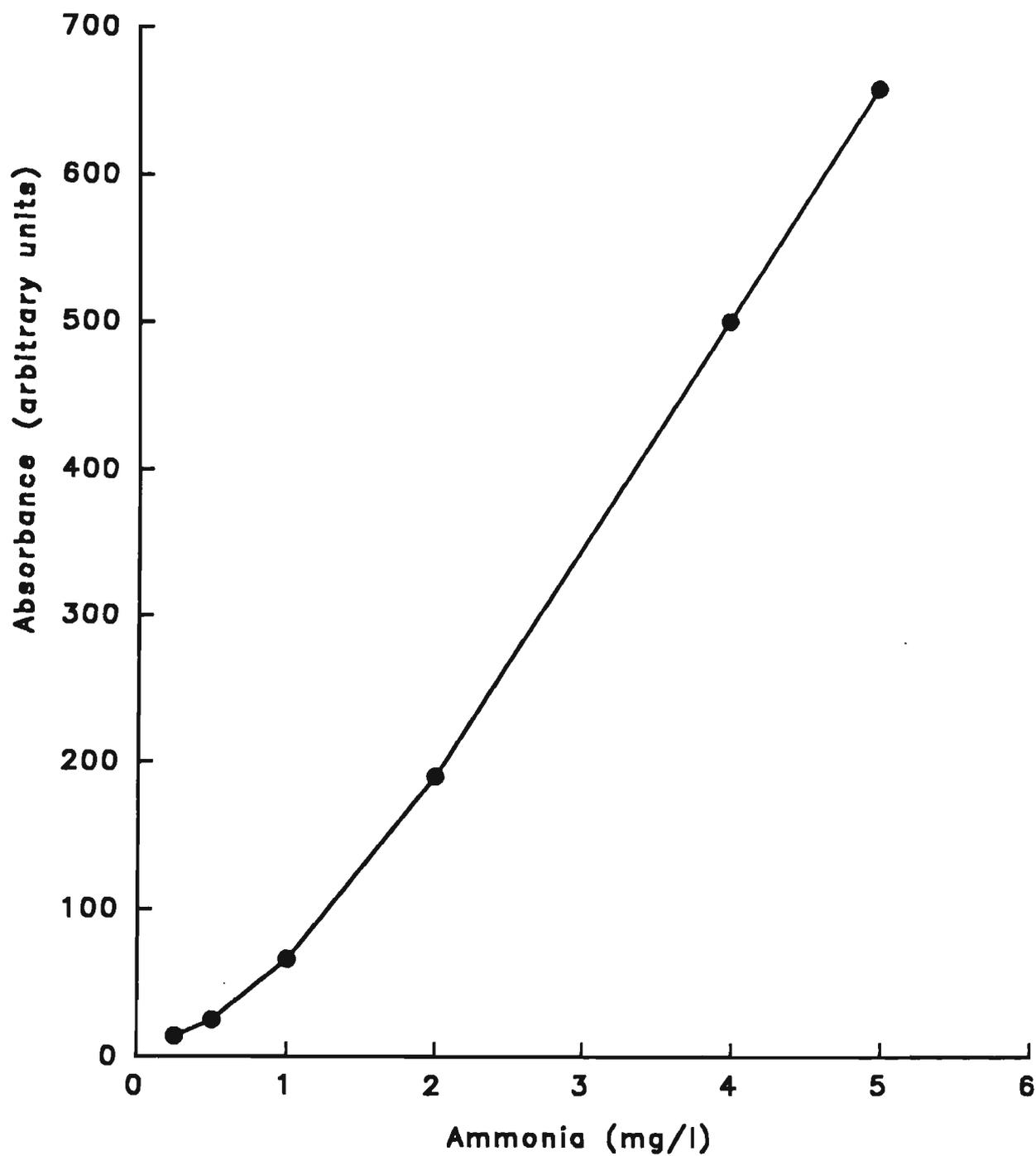
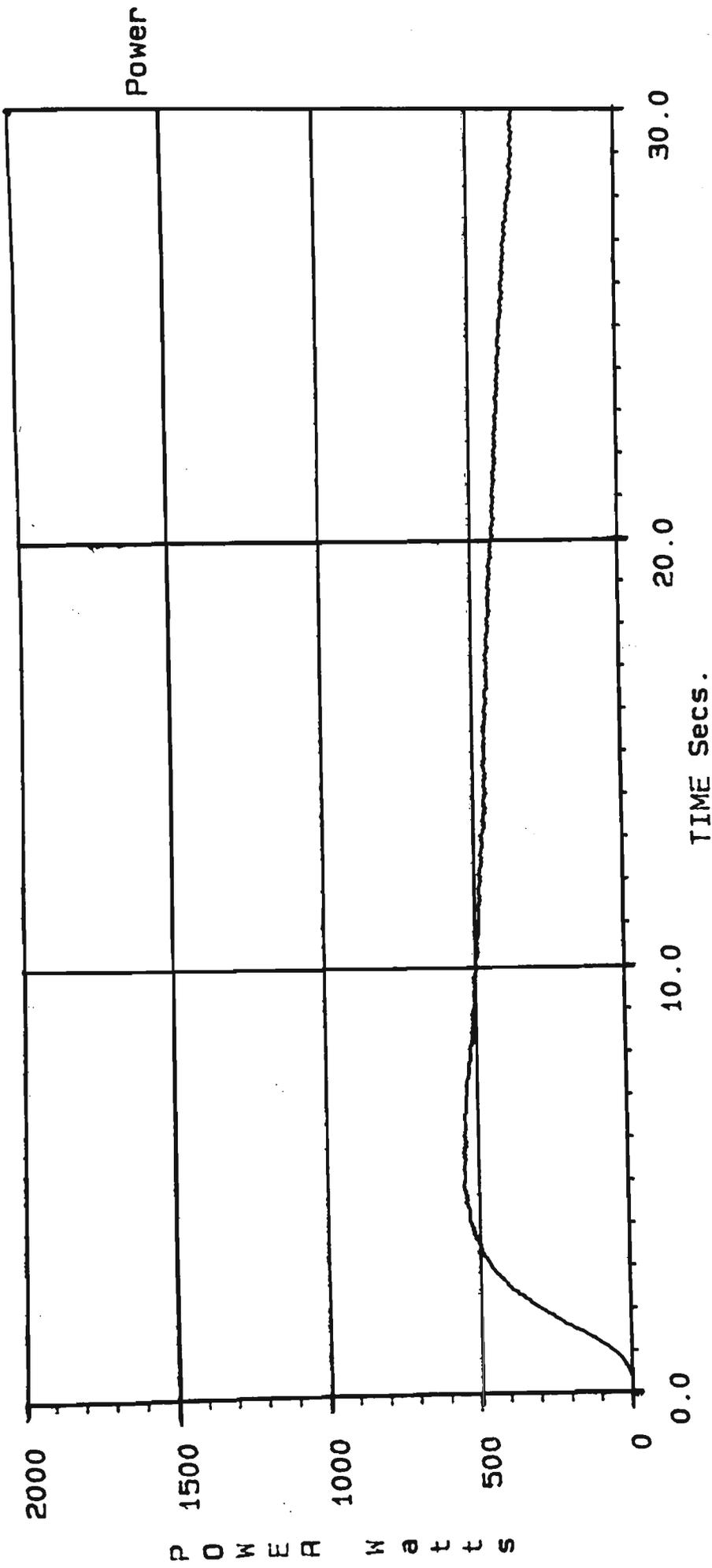


Figure C.17; A typical standard curve for muscle ammonia analysis.

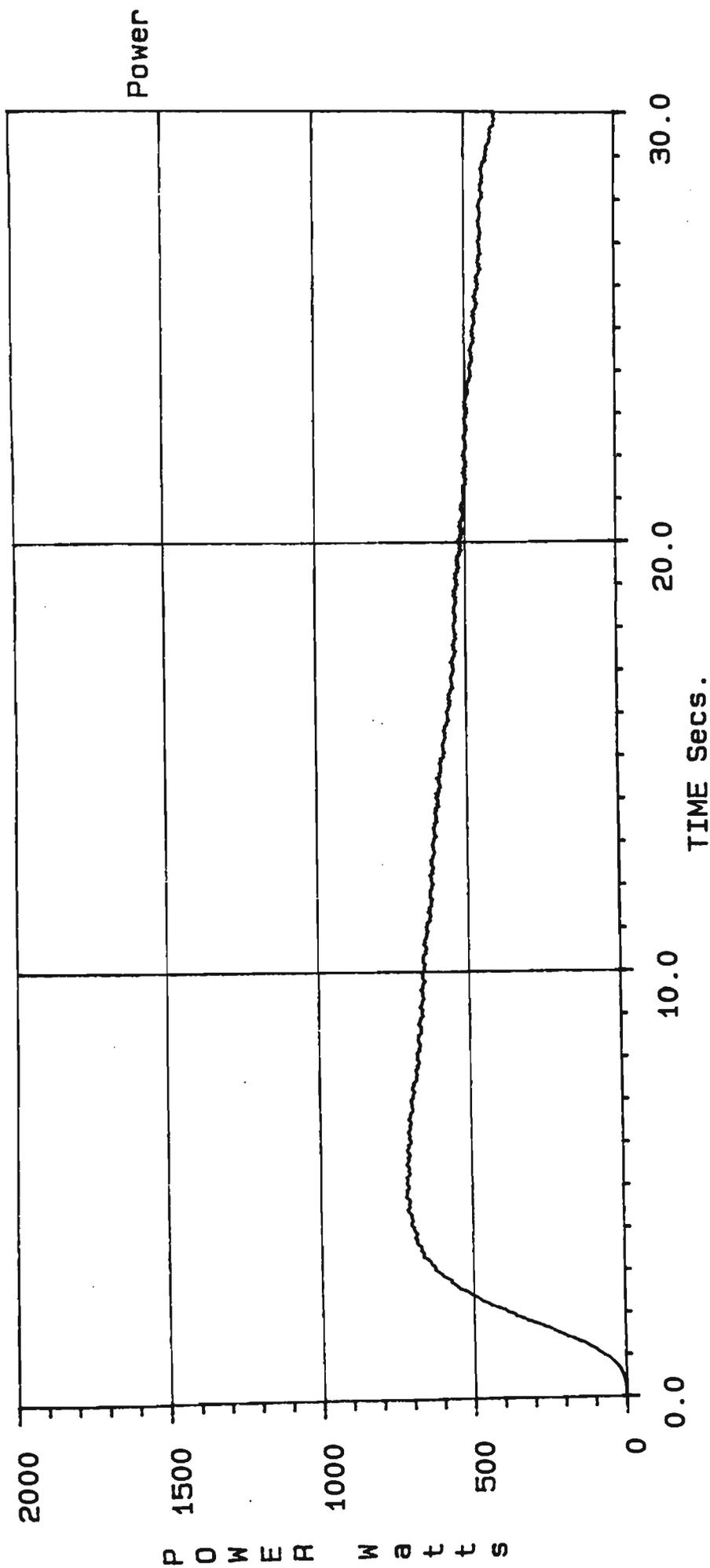
**APPENDIX D**  
**SPRINT PROFILES**



Time (Secs.)	INST. POWER (Watts)	TIME OF PEAK (Secs.)	WORK (Kj)
5.0	541.5	5.7	1.6
10.0	489.8		4.2
15.0	455.6		6.5
20.0	412.6		8.7
25.0	384.8		10.7
30.0	340.8		12.5

PEAK POWER (Watts) = 544.9      TIME SECS. = 5.7      % DECR. = 37.5  
 MEAN POWER = 417.3      TOT WORK = 13

Figure D.1; Profile of the power output of subject Nat; Untrained.



Time (Secs.)	INST. POWER (Watts)	WORK (Kj)
5.0	708.5	2.1
10.0	647.5	5.5
15.0	580.6	8.6
20.0	523.4	11.3
25.0	467.8	13.8
30.0	402.8	16.0

PEAK POWER (Watts) = 720.2      TIME OF PEAK (Secs.) = 4.8  
 MEAN POWER = 533.4      TOT WORK = 16      % DECR. = 44.1

Figure D.2; Profile of the power output of subject Nat; Trained.





