EFFECTS OF ENDURANCE TRAINING ON CYTOPLASMIC ALANINE AMINOTRANSFERASE IN THE RAT SKELETAL MUSCLE FIBRE TYPES

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ABSTRACT

In order to determine the adaptability of alanine metabolism in the cell, cytosolic alanine aminotransferase (AAT) isoenzyme activity was determined in the cardiac, soleus and the white and red portions of the vastus lateralis muscles of; 1) young rats, 2) rats subjected to an 8 week endurance training program, 3) rats subjected to an equal period of inactivity. It was found that the activity of cytosolic AAT in the skeletal muscle fibre types did not change significantly with age or training whether enzyme activity was expressed relative to wet weight of muscle or to mass of protein. However, cytosolic AAT activity in cardiac muscle increased significantly with age. Protein concentration in any of the muscles types was not affected by age or training. Significantly greater cytosolic AAT activity existed in SO and FOG skeletal muscle fibre types than in heart and the FG fibre in all groups of rats. It was concluded that aerobic training does not significantly increase the capacity of any of the muscle types to synthesize alanine but that the mechanism for alanine synthesis may be linked to the oxidative energy metabolism of the skeletal muscle fibre cell. The cytosolic AAT activity does not follow a similar relationship with the capacity for oxidative metabolism in the heart.

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INTRODUCTION

In the attempt to understand the complexity of physiological adjustment to exercise, considerable research in recent years has been concerned with the effects of chronic exercise at the molecular level. Prolonged running has been shown to induce major biochemical adaptations in the intermediary metabolism of skeletal muscle. The adaptive responses at the level of the enzymes affect the rate capacity of numerous metabolic events which underlie the physiological processes.

An increase in the number of muscle cell mitochondria with an increased capacity to oxidize pyruvate and fatty acids (Holloszy, 1967) induced by prolonged running may effectively contribute to the individual's maximal capacity to utilize oxygen. As well, several enzymes in the muscle cell located in the cytoplasm have been shown to be responsive to endurance training. The cytosolic malate dehydrogenase and aspartate aminotransferase enzymes which are directly involved with the malate-aspartate shuttle system, a mechanism for the transport of reducing equivalents from the cytoplasm to the mitochondrion for reoxidation, have been shown to increase in activity (Holloszy, 1975). Similarly, the cytosolic alanine aminotransferase isoenzyme

(AAT) which converts pyruvate to alanine via a transamination reaction, has been implicated with the functioning of the malate-aspartate shuttle (Safer, 1975) and has also been shown to increase in activity with endurance training (Molé et al., 1973).

These cytoplasmic adaptations indicate an improved capacity for entry of NADH generated by glycolysis into the malate-aspartate shuttle, thus lessening the need for pyruvate conversion to lactate to regenerate NAD. Muscles of trained individuals appear to produce less lactate than those of nontrained individuals working at similar relative workloads (Saltin and Karlsson, 1971b). Since the activity of AAT is increased the capacity for a larger fraction of pyruvate formed in the cytoplasm to be converted to alanine may be one mechanism by which lower lactate levels are maintained.

The adaptive response of muscular tissue occurs in those muscles that directly participate in the exercise (Pattengale and Holloszy, 1967). Muscular tissue is not homogenous in its metabolic structure. Differentiation in the metabolic structure at the level of enzymatic organization has distinguished three functionally distinct skeletal muscle fibre types in rats (Pette and Dolken, 1975). Adaptation occurs specifically in those fibres that are recruited (Terjung, 1976), the recruitment of the muscle

fibre types varying according to the exercise workload intensity (Burke and Edgerton, 1975). Therefore, a muscle or muscle section having a predominance of one particular fibre type will demonstrate a relatively homogeneous enzymatic adaptation characteristic of its promiment fibre type.

Studies that have characterized AAT activity in whole skeletal muscle (DeRosa and Swick, 1975; Krebs, 1975) and those that have shown increases of the enzyme in muscle with endurance training (Molé et al., 1973) were performed on muscles composed of mixed fibre type populations. Therefore documentation characterizing the muscle fibre types and their relative adaptability with respect to the cytosolic AAT isoenzyme has not been established. It was deemed important therefore, to characterize and to investigate the effect of an endurance training program on the activity of the cytosolic AAT isoenzyme in the three skeletal muscle fibre types and in cardiac muscle.

Purpose

The aim of the present study is to characterize cytosolic alanine aminotransferase isoenzyme activity within the rat heart and skeletal muscle fibre types. It is also the objective to define the relative adaptability of the cytoplasmic isoenzyme within these tissues to growth and endurance training.

Hypothesis

It is hypothesized that the oxidative muscle types (heart, SO, and FOG) will possess greater cytosolic AAT activity than the FG fibre type. The chronic stress of an aerobic training program will result in skeletal muscle adapting biochemically. It is hyposized that if the cytosolic AAT isoenzyme is closely linked to oxidative energy metabolism in each of the muscle types, the aerobic stress of the training regime will lead to an increase in this isoenzyme's activity. It is further hypothesized that the increases in cytosolic AAT activity will be greatest in the more oxidative skeletal muscle fibre types. No cytosolic AAT activity changes are expected in the heart and FG skeletal muscle fibre.

Limitations

Since absolute homogeneity of a particular muscle fibre type is lacking in any muscle of the rat, tissue analyses were limited to the soleus and the white and red portions of the vastus lateralis muscles.. Although these tissues are relatively homogeneous for one particular fibre type their use as representative samples does pose limitations.

A major limitation in enzyme activity studies, as reviewed by Youdim and Woods (1975), is the fact tthat the optimum conditions of the medium in which the biochemical analysis

takes place does not necessarily simulate cellular conditions *in vivo*. Therefore, caution should be exercised when interpretations are derived from results of such studies.

Scope of Study

The study was delimited to the spectrophotometric determination of cytosolic AAT activity in the white and red portions of the vastus lateralis, the soleus and heart muscles of male Wistar rats: 1) 6 to 7 weeks old (n=10), 2) 14 to 15 weeks old (n=10), and 3) subjected to an eight week endurance training program (n=8).

Abbreviations

AAT:	alanine aminotransferase
AMP:	adenosine monophosphate
ASP-T:	aspartate aminotransferase
ATP:	adenosine triphosphate
EDTA:	ethylenediamine tetracyclic acid
FG:	fast-twitch, high glycolytic fibre
FOG:	fast-twitch, high oxidative, glycolytic fibre
GS:	glutamine synthetase
IMP:	inosine monophosphate
Km:	Michaelis constant
LDH:	lactate dehydrogenase
MDH:	malate dehydrogenase (NADH linked)
MVO_2:	maximal oxygen uptake capacity
NAD^+:	nicotinamide adenine dinucleotide (oxidized form)
NADH:	nicotinamide adenine dinucleotide (reduced form)

PDH:	pyruvate dehydrogenase
PEPCK:	phosphoenolpyruvate carboxykinase
PFK:	phosphofructokinase
SO:	slow-twitch, oxidative fibre

CHAPTER II

REVIEW OF THE LITERATURE

The Skeletal Muscle Fibre Types

An attempt to understand the mechanisms which underlie skeletal muscle metabolism necessitates an appreciation of the biochemical nature of the tissue. Muscle is composed of fibres possessing distinct metabolic characteristics which presuppose different patterns of function. The study of whole muscle should therefore be treated as an integration of functionally related subunits.

The indications of heterogeneity found within and between mammalian skeletal muscles have led to a classification of muscle fibre types (Close, 1972). Based on myofibrillar ATPase activity these two categories have been designated as slow-twitch (low levels of myofibrillar ATPase activity) and fast-twitch (high levels of myofibrillar ATPase activity) fibres (Barnard et al., 1971). Within the fast-twitch fibre population two fibre types have been distinguished on the basis of differences in their capacities for oxidative metabolism (Peter et al., 1972). The characteristics of the three fibre groups designated as, fast-twitch glycolytic (FG), fast-twitch oxidative (FOG), and slow-twitch oxidative (SO) fibres are described in Table 1.

TABLE I

Summary of the major characteristic metabolic differences among the three skeletal muscle fibre types in rat.

Metabolic capacities	Fibre Type		
	FG	FOG	SO
glycolysis	High	High	Low
glycogenolysis	High	High	Low
carbohydrate oxidation	Low	High	Intermediate
oxidative capacity	Low	High	High
lactate: Production	High	Intermediate-High	Low
Uptake	Low	High	High

The above data is derived from Burke and Edgerton, 1975; Edgerton and Simpson, 1969; Peter et al., 1972.

The excitability of a motor neuron is related to its dimensions. Consequently, progressively larger motor neurons discharge under increasing amounts of excitatory input (Henneman, 1974). The order of motor unit recruitment depends therefore on the size of the motor neurons. Each motor unit contains fibres of the same type (Burke and Edgerton, 1975) as is indicated by a homogeneity in the enzyme profiles of fibres associated with a common motor unit (Kugelberg and

Edstrom, 1968). The differential degree of glycogen depletion within the fibres of heterogeneous muscle following exercise of varying intensities demonstrates a specificity of motor unit recruitment for such activity. At lower work intensities corresponding to lower levels of excitatory input, only the smaller motor units associated with oxidative muscle fibres are recruited (Saltin and Karlsson, 1971a). In the rat oxidative or 'red' fibres (histochemically divided into FOG and SO fibres), glycogen depletion studies have demonstrated that both these fibres become activated at low submaximal work intensities (Armstrong et al., 1974; Terjung, 1976). At greater work intensities, the white (FG) fibres of the larger motor units become activated. Muscle fibre glycogen depletion studies have indicated that the speed of treadmill running (Armstrong et al., 1974; Baldwin et al., 1973a) and the degree of treadmill incline (Terjung, 1976) are important criteria in the mediation of fibre type activation. The metabolic enzyme profile of the fibre types within skeletal muscle tends to support specialized functions of muscle in relation to the fibre type composition (Pette and Dölken, 1975). The soleus, a highly oxidative muscle possessing a relatively homogeneous composite of SO fibres (84-96% SO) (Ariano et al., 1973; Baldwin et al., 1972), is mainly involved in low intensity work such as required in postural function. In contrast, the superficial,

white portion of the quadriceps which consists prodominantly of FG fibres (Baldwin et al., 1972) functions primarily during activity requiring an enhancement to the speed and/or tension-force component developed by the earlier recruited oxidative fibres (Armstrong et al., 1974). Since glycogen utilization in FG fibres occurs essentially under anaerobic conditions, much of the stored potential energy is not converted to ATP, but is released instead from the cell as lactate. The observed rates of blood lactate accumulation during exercise of varying intensity (Depocas et al., 1969; Hubbard, 1973; Jorfeldt, 1970; Minaire et al., 1971) support the proposed greater use of FG fibres in higher intensity exercise. As a result of a less efficient use and greater capacity to breakdown glycogen, activated FG fibres deplete their stored glycogen supply more rapidly than do the SO fibres despite initially higher concentrations of glycogen in the FG fibre type (Armstrong et al., 1974; Gollnick et al., 1974).

The underlying, red portion of the quadriceps muscle, being comprised predominantly of FOG fibres (Baldwin et al., 1972) is essentially fast contracting and fatigue-resistant. The high oxidative FOG fibres are considered to be the main muscle fibre type involved in the mild to moderate spectrum of exercise intensities (Armstrong et al., 1974).

Pyruvate Metabolism

For many years muscular fatigue has been associated with lactate accumulation (Asmussen et al., 1948; Hill et al., 1924; Karlsson and Saltin, 1970). Katz (1970) has pointed out that since hydrogen ions compete with Ca^{++} for the binding site that controls the acto-myosin interaction, acidosis resulting from lactic acid accumulation would be expected to interfere with muscular contraction. Since lactate concentration is closely related to pyruvate levels (Krebs, 1975; Simonson, 1971) and since pyruvate is an intermediate for several metabolic pathways which are affected by acute and chronic exercise, pyruvate is an important point of focus at the molecular level of energy metabolism.

The concentration of intracellular pyruvate is affected by both its rate of production and utilization. Since it is readily diffusible across the muscle membrane, the production rate of pyruvate is dependent on the quantitative and qualitative involvement of the various muscle fibre types within the muscle. It has been suggested that in addition to the glycolytic contribution to the intracellular pyruvate pool, amino acids, which are metabolized within muscle, may serve as precursors of pyruvate (Garber et al., 1976b; Goldstein and Newsholme, 1976). The removal of intracellular pyruvate may occur by simple diffusion into the blood circulation or

by its metabolic dissipation within the fibre. Conversion to lactate by the lactate dehydrogenase enzyme (L-lactate: NAD oxidoreductase, EC 1.1.1.27) (LDH) is an important fate of pyruvate and in effect, reflects the cellular cytosolic redox state, a critical regulatory factor in glycolysis (Edington, 1970). The formed lactate may diffuse into the blood from which it may be taken up by the liver for reconversion into glucose, this being a portion of the Cori cycle (Cori, 1931). A second metabolic sink for pyruvate is its oxidation to acetyl CoA via the pyruvate dehydrogenase enzyme system (EC 1.2.4.1) (PDH) in the mitochondria. However, during anaerobic glycolysis an imbalance develops between the rate of glycolysis and pyruvate oxidation. Due to the lack of oxygen availability for the oxidation processes and/or the change in the pyridine nucleotide redox state (Edington, 1970) pyruvate conversion to lactate becomes the favored metabolic route. During aerobic conditions, the formation of acetyl CoA resulting from beta-oxidation of fatty acids inhibits the PDH system by favoring the inactive form of the enzyme (Hiltunen and Hassinen, 1976). Hence, under steady state conditions in exercise fat becomes the predominant energy supplying substrate (Holloszy, 1975) while pyruvate oxidation may be secondary. An alternative for removal of pyruvate in muscle may be its conversion to

alanine via the reaction catalysed by alanine aminotransferase (L-alanine: α-oxoglutarate aminotransferase, EC.2.6.1.2) (AAT) (Molé et al., 1973).

Alanine Metabolism

The peripheral release of amino acids has long been associated with states of protein catabolism as occurs in the fasted condition (Lehninger, 1975, pp. 840-845). It has been more recently shown however that the mixture of amino acids released does not reflect their proportional content in skeletal muscle protein (Felig et al., 1970). Measurements of arterial-femoral venous free amino acid differences have revealed a net release of alanine from skeletal muscle both in the rat (Ruderman and Lund, 1972) and in man (Felig and Wahren, 1971b; Marliss et al., 1971; Pozefsky et al., 1969) in quantities far beyond those which could be attributed to protein degradation alone. The comparatively large release of alanine has been attributed to a peripheral synthesis resulting from a transamination reaction involving the AAT enzyme (Felig et al., 1970). The skeletal muscle's ability to directly aminate pyruvate is insignificant (Krebs, 1975; Ruderman and Lund, 1972).

Cytosolic and mitochondrial isoenzymes of AAT exist within several mammalian tissues and vary in concentration according to the function of the tissue (DeRosa and Swick, 1975). Alanine production is believed to occur in the

cytosol (Hochachka and Dressendorfer, 1976). Since cellular pyruvate concentrations vary about the isoenzyme's Michaelis constant while the Km value for alanine lies at least 30 times higher than its concentration in muscle (DeRosa and Swick, 1975), it is suggested that the cytosolic AAT reaction proceeds in the direction of alanine formation. In cardiac muscle, where mitochondrial AAT activity is virtually absent (DeRosa and Swick, 1975), a notable increase in intracellular alanine, and a large release of alanine occurs during metabolic alterations. The predominant form of the AAT enzyme in skeletal and cardiac muscle is the cytosolic form accounting for more than 90% of the total cellular enzyme activity (DeRosa and Swick, 1975).

The mitochondrial isoenzyme may function in the alternate direction in view of a considerably lower (4 times) Km value for alanine and its greater specific activity for alanine conversion to pyruvate in comparison to the cytosolic form (Swick et al., 1965). Since the mitochondrial isoenzyme's activity is significantly higher in hepatic tissue (DeRosa and Swick, 1975) this form may be a cellular adaptation for gluconeogenic purposes.

The contributory significance of the AAT reaction to the net release of alanine is made evident by marked decreases in the efflux of alanine from skeletal muscle (Garber et al., 1976a) and changes to a net uptake of alanine in cardiac

muscle (Taegtmeyer et al., 1977) when incubated in an aerobic medium containing aminooxyacetic acid, an AAT inhibitor. Similarly, a reduced peripheral release of alanine has been demonstrated subsequent to perfusion of L-cycloserine, another inhibitor of AAT, into the intact organism (Blackshear et al., 1975; Rudernan and Berger, 1974). Approximately 80% of the alanine released by the extrasplanchnic tissues has been estimated to be derived from the AAT pathway rather than directly from proteolysis (Blackshear et al., 1975)

It has been generally held that the intracellular availability of pyruvate influences the rate of alanine formation in skeletal muscle (Blackshear et al., 1975; Felig and Wahren, 1971a, 1974; Odessey et al., 1974; Snell, 1976) as well as in cardiac muscle (Taegtmeyer et al., 1977). Ruderman and Berger (1974) have demonstrated an increased release of alanine from isolated rat hindquarter when perfused with lactate or lactate and pyruvate. Pozefsky and Tancredi (1972) had previously observed similar results upon infusion of pyruvate into the brachial artery of man. The rate of alanine release from muscle has been shown to increase in relation to exercise intensity (Felig and Wahren, 1971b) higher intensities being concomitant with greater muscular glycolytic activity (Saltin and Karlsson, 1971a). The rises in the circulating levels of alanine which parallel increases in blood pyruvate levels with exercise (Felig and Wahren,

1971b; Wahren et al., 1973b) suggest that the cellular level of pyruvate is important in the regulation of alanine synthesis in the physiologic situation. The evidenced maximal two-fold elevation in arterial alanine concentration with exercise (Felig and Wahren, 1971b) could not be ascribed to decreased hepatic uptake. The reduced hepatic blood flow associated with physical activity is offset by a greater fractional extraction of the amino acid (Felig and Wahren, 1974). It was pointed out by Marliss and co-workers (1972), however, that an impairment in the hepatic mechanism for alanine uptake occurs when the lactate concentration is elevated to high levels.

The relative importance of glycolysis in supplying the carbon skeleton of alanine is unclear. Muscle lactate formation or CO_2 production cannot account for a significant proportion of glycogen and glucose utilization (Hultman, 1967). Consequently it was proposed (Felig and Wahren, 1971a, 1971b) that the carbon skeleton of alanine actually represents an important end-product of glycolysis with the glycolytic rate being a limiting factor in alanine synthesis.

Although pyruvate utilized in the synthesis of alanine may to a large extent be glucose derived, the regulatory influence of glycolysis on the amino acid's formation has been challenged (Garber et al., 1976a, 1976b; Ozand et al., 1973).

The rate of glycolysis can be augmented by electrical stimulation of muscle via the innervating nerve. This has been demonstrated by the utilization of glycogen stores within the fast twitch fibres in particular (Baldwin and Tipton, 1972). The increased flux of carbohydrate carbon through glycolysis, by electrically stimulated rat hindhimb (Ruderman and Berger, 1974) however, revealed negligible increases in the release of alanine.

A number of pathological conditions seemingly support an important role of carbohydrate as the carbon skeleton source of alanine. Such is the case of a disease involving an enzymatic disorder in the glycogenolytic pathway, known as McArdle's syndrome. The resulting failure to metabolize glycogen to pyruvate is reflected by below normal levels of circulating alanine during exercise (Wahren et al., 1973a). Measurements of leg alanine exchange under these conditions have revealed a net uptake rather than the normal release of the amino acid, suggesting that alanine may serve as an auxiliary fuel for muscle (Wahren et al., 1973a). Diabetes, another pathological instance associated with muscle's inability to metabolize carbohydrate is also characterized by markedly lower circulating alanine levels (Wahren et al., 1972). The cause for decreased blood alanine levels, however, appears to be primarily due to a hepatic rather than a peripheral effect of insulin (Felig, 1973).

The normal relative contribution of exogenous glucose to the total alanine release by muscle has been estimated to be within 60-80% as determined by muscle incubation experiments (Odessey et al., 1974) and A-V difference studies in human forearm (Felig, 1975). Based on arterio-deep venous differences, Felig (1973) has demonstrated that glucose-derived alanine may account for 18% of the basal glucose uptake by human forearm muscle.

These observations lend support to an earlier proposed 'glucose-alanine cycle' (Felig et al., 1970; Malette et al., 1969). The formulation of the glucose-alanine cycle is based on the entry of glucose into the skeletal muscle cell, its metabolism to pyruvate and its subsequent transamination to alanine. The newly formed alanine is then released from muscle and eventually extracted from the circulation by the liver where it is reconverted to glucose (Felig, 1973). Essentially, the concept implies that alanine is merely a recirculating product of glucose metabolism.

During the postabsorptive condition muscle is in a state of negative nitrogen balance which is associated with decreases of the peripheral protein stores. The increased alanine efflux from muscle that occurs in such a state (Pozefsky et al., 1969) indicates a possible link between muscle amino acid metabolism and alanine formation. Since the glucose-alanine cycle concept did not

provide for the net flux of carbon from protein to carbohydrate, the concept of the glucose-alanine cycle was therefore extended (Davis and Bremer, 1973) to include other amino acids.

Amino Acid: Carbon Source for Alanine Synthesis

The early stages (first week) of starvation invariably result in a hypoglycemic state which leads to increased muscle protein degradation and an augmented release of alanine in amounts much above those of other amino acids (Felig, 1975). A shift in the rate of amino acid catabolism therefore appears to influence alanine synthesis (Garber et al., 1976b; MacDonald et al., 1976).

The complete mechanism by which the glucogenic amino acids, other than glycine, cysteine, serine, and aspartate lead to net alanine synthesis is not well defined (Garber et al., 1976b). The initial pathway by which the ketoacids of the deaminated amino acids are metabolized to pyruvate is suggested to be via the TCA cycle (Davis and Bremer, 1973; Goldstein and Newsholme, 1975; Spydevold, 1976). The ketocacids enter the TCA cycle as illustrated in Figure 1, and exit the cycle at the level of oxaloacetate. The mitochondrial efflux of TCA cycle intermediates derived from amino acid catabolism probably would not occur during a state of elevated energy metabolism within muscle cells dependent essentially on the malate-aspartate shuttle for

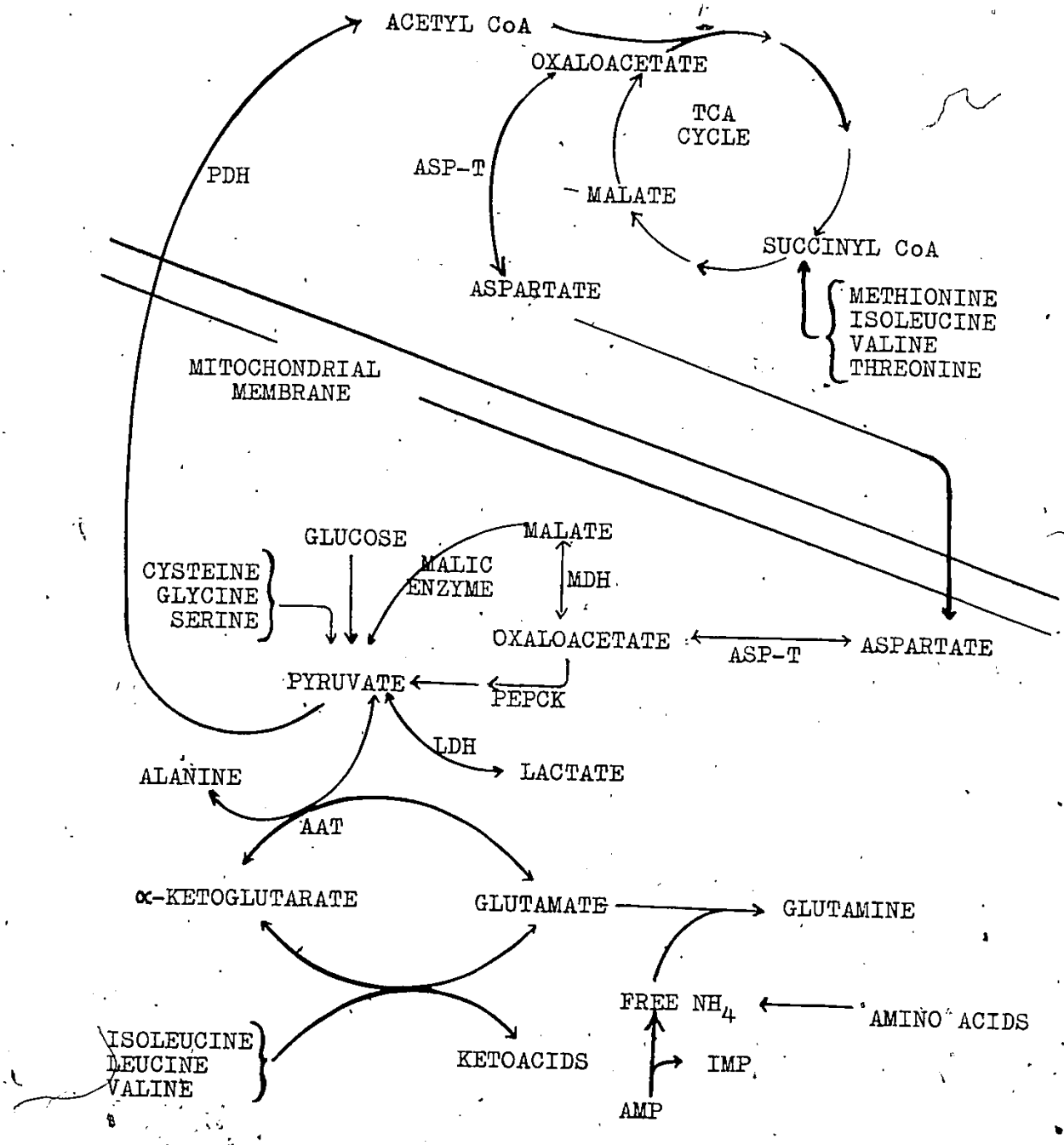


FIGURE 1. Scheme for interaction between amino acid metabolism and alanine synthesis.

hydrogen transport across the mitochondrial membrane. In order to preserve the cytosolic redox potential in a more oxidized state, oxaloacetate can be converted to aspartate via the mitochondrial aspartate aminotransferase isoenzyme (EC 2.6.1.1) (ASP-T). Upon entering the cytoplasmic compartment via the malate-aspartate shuttle system, aspartate is reconverted to oxaloacetate. Subsequent conversion of oxaloacetate to pyruvate can follow either the pathway involving malate dehydrogenase (EC 1.1.1.37) (MDH) and malic malic enzyme (EC 1.1.1.40) or the pathway involving the phosphoenolpyruvate carboxykinase enzyme (EC 4.1.1.32) (PEPCK).

The activity of the malic enzyme responsible for malate decarboxylation is however, quite low in mixed skeletal muscle (Molé et al., 1973). The concentration of PEPCK, the decarboxylating enzyme of oxaloacetate, is also extremely low in both red and white skeletal muscle, with white fibres possessing the higher concentration of the fibre types (Opie and Newsholme, 1967). The low activity of these enzymes therefore may restrict the amino acid carbon flux towards pyruvate formation. The malic enzyme pathway is nevertheless considered to be a significant source for pyruvate formation (Karl et al., 1976). Furthermore, Snell (1976) has demonstrated a significant reduction in alanine formation when PEPCK is inhibited. It has been estimated from perfusion experiments that

extrahepatic proteolysis can account for a maximum of 18% of alanine synthesized (Blackshear et al., 1975).

Branched Chain Amino Acid Metabolism.

The liver is a primary organ for amino acid catabolism in the mammaliam organism. Arterial-hepatic venous differences for the branched chain amino acids, leucine, valine, and isoleucine, however, demonstrate net negative balances during prolonged mild exercise (Ahlborg et al., 1974) and brief periods of heavy exercise (Wahren et al., 1972). Although several extrahepatic tissues can oxidize the branched chain amino acids, skeletal muscle, due to its mass, is considered to be the principle site of branched chain amino acid catabolism (Goldberg and Odessey, 1972). It is generally accepted that muscle relies essentially on carbohydrate and fatty acid metabolism for its energy requirements (Felig and Wahren, 1975). The contribution of amino acid oxidation in supplying energy to the muscle cell has been overshadowed by the extensive research on the latter two fuels. In terms of ATP production complete oxidation of valine, isoleucine and leucine yield 32, 43 and 42 ATP per mol respectively (Odessey et al., 1974). Under conditions imposed by prolonged exercise (40 minutes) the arteriofemoral venous differences for the branched chain amino acids become positive (Felig and Wahren, 1971b; Wahren et al., 1973b) indicating a net uptake by the exercising muscles. The

existence of an intimate relationship between branched chain amino acid catabolism and alanine synthesis in muscle such as to complement the glucose-alanine cycle has been suggested (Odessey et al., 1974). Diaphragm muscle which contains approximately 60% FOG, 20% SO and 20% FG (Gauthier, 1970), incubated in a medium containing leucine effectively results in an increased release of alanine from the preparation (Odessey et al., 1974; Ruderman and Berger, 1974). These findings are in agreement with the results of earlier investigations which demonstrated an increased release of alanine by isolated perfused rat hindquarters (Ruderman and Lund, 1972). Since leucine is strictly a ketogenic amino acid, the sole product of its catabolism is acetyl CoA. Acetyl CoA is metabolized via the TCA cycle to CO_2 and therefore does not allow leucine to provide the essential carbon intermediate, pyruvate, for alanine synthesis. Leucine (as well as valine and isoleucine) preferentially transaminates with α-ketoglutarate instead of pyruvate (Odessey et al., 1974). These transamination reactions permit a cycling of α-ketoglutarate carbon and thereby contribute to the maintenance of cellular glutamate levels which would otherwise decrease as a result of the reactions involving AAT and glutamine synthetase (EC 6.3.1.2) (GS) (see Figure 1). The formation of acetyl CoA from leucine, however, may inhibit PDH, thus blocking a metabolic route for pyruvate removal and permitting increased cellular levels

of pyruvate and thus of alanine (Blackshear et al., 1975). The carbon chains of valine and isoleucine are metabolized to the TCA cycle intermediate, succinyl CoA (Lehninger, 1975, p. 562). The resulting formation of the TCA cycle intermediates, malate and oxaloacetate can then be converted to pyruvate (Goldstein and Newsholme, 1975.

The nature of the regulatory influence by branched chain amino acids on alanine formation has been suggested to involve the amino group (Odessey et al., 1974). The amino group of newly formed alanine is essentially derived from the branched chain amino acids (Odessey et al., 1974) with the requirement of glutamate as an intermediary amino carrier in the transfer. The metabolism of the branched chain amino acids is therefore important for the maintenance of sufficiently high levels of glutamate for the AAT reaction.

Glutamine Metabolism.

Like alanine, glutamine is released from muscle in amounts far in excess of what could be expected from protein degradation alone (Marliss et al., 1971; Ruderman, 1975). Together, these two amino acids account for 20-25% of the amino acid content in muscle protein (Odessey et al., 1974) while accounting for 50% (Ruderman and Lund, 1972) to greater than 60% (Garber et al., 1976a) of the amino acid efflux from muscle. Glutamine's immediate carbon source, glutamate, (Ruderman, 1975) is directly

aminated via the glutamine synthetase enzyme. Since the concentrations of glutamate and ATP effectively saturate the glutamine synthetase enzyme it is suggested (Iqbal and Ottaway, 1970) that the rate of glutamine formation is dependent mainly on the level of ammonia in the cell. The amino group for this reaction differs therefore from the amino source for alanine synthesis. The addition of NH_4Cl (free ammonia) to a muscle incubating medium resulted in an increased rate of glutamine release from the preparation with a concurring decrease (Garber et al., 1976b) or an absence of change (Ruderman and Lund, 1972) in cellular alanine levels. The importance of glutamine synthesis lies in the removal of free NH_3 accumulating in the cell (Blackshear et al., 1975; MacDonald et al., 1976) in contrast to the primary role of alanine in carbohydrate metabolism (Felig, 1973). The cellular concentration of ammonia appears to be an important rate determinant for the synthesis of glutamine (Garber et al., 1976a; Ruderman and Berger, 1974; Ruderman and Lund, 1972).

Whereas the fate of alanine is in liver gluconeogenesis, glutamine is extracted from circulation by the kidney for purposes of gluconeogenesis and for the maintenance of cation levels while excreting an acid urine (Ruderman, 1975). The gut as well accounts, in part, for the splanchnic uptake of glutamine (Felig et al., 1973) which is concomitant with this organ's release of alanine.

Alanine-Glutamine Interrelationship.

The existence of an interdependent mechanism for regulation of the formation of alanine and glutamine has been suggested (Blackshear et al., 1975; Garber et al., 1976a). An actual cellular control mechanism, however, for the synthesis of alanine and glutamine remains obscure. It has been shown that an increased activity of PDH, a removal system of pyruvate, results in decreased glutamine levels in functionally hepatectomized rats (Blackshear et al., 1975). The rates of glutamine or alanine formation are affected by a decreased synthesis of the alternate amino acid. Infusion of an AAT inhibitor into an intact physiologic system stimulates the rate of glutamine formation (Blackshear et al., 1975) as determined by changes in the arterial-venous differences across muscle. These observations are in agreement with muscle incubation experiments more recently reported by Garber and co-workers (1976a). Increased alanine release as a result of glutamine synthetase inhibition indicates that alanine nitrogen may offset an efflux of free NH_3 from the cell by increasing the cell's rate of alanine release (Blackshear et al., 1975; Krebs, 1975). Since free NH_3 is not used for direct amination of pyruvate (Ruderman and Lund, 1972) the reason for increased formation of alanine is unclear. Kaletha and co-workers (1976) have demonstrated that alanine exerts a non-competitive form of

inhibition at pH 7.0 on skeletal muscle AMP deaminase, thus affecting NH_3 production. Since NH_3 is important in the glutamine synthetase reaction, this inhibition may partially explain the reciprocity phenomenon observed in alanine and glutamine synthesis (Garber et al., 1976a).

Amino Source, Effect of Exercise.

The major source of free ammonia for the amidation of glutamate to glutamine is not clear. Glutamate dehydrogenase (EC 1.4.1.3) may contribute to cellular NH_3 formation though the activity of this enzyme is extremely low (Lowenstein, 1972). Since protein degradation must occur with the normal metabolic turnover of protein, ammonia may arise from the subsequent deamination of certain amino acids released from protein (Ruderman and Berger, 1974; Ruderman and Lund, 1972).

Protein turnover has been associated with exercise (Felig, 1975). Since the rate of protein synthesis within the muscle cell decreases proportionally with the exhaustion of its glycogen stores (Gerber, 1976) a shift toward net protein catabolism occurs with exercise induced glycogen depletion. Disturbances in skeletal muscle protein metabolism are made evident by alterations in the flux of circulating amino acids across muscle (Felig and Wahren, 1971b).

Gerez and Kirsten (1965) (as reviewed by Poortmans, 1973) have reported a greater production of ammonia in

white than in mitochondrial rich, red muscle during exercise. The major source of NH_3 during exercise is probably the deamination of AMP reaction of the purine nucleotide cycle (Ruderman and Berger, 1974). The activity of the AMP deaminating enzyme, adenylate deaminase is highest in FG (white) muscle fibres and lowest in SO (red) fibres (Winder et al., 1974).

During exercise of various intensities there is no significant change in glutamine release from muscle (Felig, 1973) in contrast to the increase in the rate of alanine output. The activity of the glutamine synthetase enzyme in skeletal muscle is low and is virtually absent in heart muscle (Iqbal and Ottaway, 1970). The absence of an increased glutamine outflow from muscle during exercise then may be explained by the fact that the enzyme is functioning close to its maximal capacity in resting conditions (Ruderman and Lund, 1972). Thus, it has been suggested (Felig,1973) that while glutamine and alanine share an important role in the transfer of NH_3 from muscle to the splanchnic organs, during exercise alanine appears to be of greater importance.

Metabolism of Other Amino Acids.

The molecular involvement of the remaining amino acids in peripheral alanine synthesis is not clear. At the cellular level however, the negative net exchange for most amino acids (in addition to alanine, glutamine and

the branched chain amino acids) across muscle tissue contrast with a consistent uptake of serine, cysteine and glutamate (Felig, 1973). Both serine and cysteine can be converted directly to pyruvate (Lehninger, 1975, pp. 698-699). Glutamate occupies a central role in cellular metabolism. Apart from being a possible source of carbon for alanine synthesis, glutamate is involved in most transamination reactions, serving as a specific amino group donor (Lehninger, 1975, pg. 563). It has been suggested that cellular glutamate levels may be regulatory in the synthesis of alanine (Ruderman and Lund, 1972). The effects of cellular fluxes of glutamate affecting the rate of alanine synthesis, however, is not conclusive since equivocal evidence has been reported (Blackshear et al., 1975; Goldstein and Newsholme, 1975). Increased rates of alanine release from incubated muscle tissue are also demonstrable when either aspartate, methionine, threonine or glycine is present in the incubation medium (Garber et al., 1976. Their relationship to alanine synthesis is illustrated in Figure 1. The characteristic pattern of amino acid release from muscle into an incubating medium (Garber et al., 1976a; Jefferson et al., 1974) and from human forearm arterio-deep venous differences (Aoki et al., 1974; Pozefsky and Tancredi, 1972) is marked by a tissue retention or uptake of aspartate.

Aspartate has been described as an important

precursor for alanine synthesis by Garber and co-workers (1976b; 1976c). This research group has shown that muscle incubated in an aspartate medium results in an increased alanine release (Garber et al., 1976b) while during conditions of decreased intracellular aspartate availability alanine synthesis also decreased (Garber et al., 1976c). In a perfusion study involving hemicorpus preparations (Jefferson et al., 1977), large releases of alanine and glutamine were observed while concomitant decreases in cellular aspartate and glutamate concentrations occured. Two mechanisms by which aspartate may influence these intracellular fluxes of alanine have been described. Aspartate conversion to oxaloacetate followed by conversion to pyruvate via aspartate aminotransferase and malic enzyme (Garber et al., 1976b) will allow a greater substrate availability for the alanine synthetic reaction to occur. It has also been shown that aspartate metabolism is indirectly responsible for cellular alanine concentration changes (Safer, 1975) by supplying cytosolic oxaloacetate for the malate-aspartate shuttle system which in turn influences alanine synthesis through an interaction with the TCA cycle (discussed under the section of malate-aspartate shuttle).

Hormonal Influence.

Hormonal factors have been shown to effect quantitative as well as qualitative changes in the releases of

alanine and glutamine from skeletal muscle (Karl et al., 1976). In addition to its function in blood glucose homeostasis, insulin serves a role in the regulation of the metabolic protein turnover in muscle (Newsholme and Start, 1973). Insulin stimulates the uptake of certain amino acids by muscle as well as their incorporation into muscle protein. An inhibition of skeletal muscle protein synthesis occurs when the levels of plasma insulin are low (Jefferson et al., 1974). Decreased insulin levels as a result of prolonged, mild or moderate exercise (Galbo et al., 1975) may precipitate a shift from glucose to amino acid derived carbon for the synthesis of alanine. The greater supply of amino acids for oxidation may further provide for an increased production of glutamine and/or alanine. Consistent with this suggestion is the finding of proportionally larger rates of release of glutamine and particularly of alanine from perfused mixed skeletal muscle in comparison to the remaining amino acids when insulin was omitted from the perfusate. (Jefferson et al., 1974). Although the flux of alanine into and out of the muscle cell appears to be an integral component of the regulation of amino acid metabolism in skeletal muscle, Ruderman and co-workers (1977) have recently suggested that the transport of alanine may be directly modulated by insulin.

In contrast to the decreased blood insulin

concentration associated with exhaustive, prolonged exercise are the augmented levels of another gluco-regulatory hormone, glucagon (Galbo et al., 1975). The general systemic response to glucagon is generally regarded as antagonistic to insulin. The stimulatory effect of glucagon on hepatic gluconeogenesis and glycogenolysis effectively contribute to a release of glucose into the blood circulation (Felig and Wahren, 1974). It has been shown that alanine stimulates the release of this hormone (Müller et al., 1971). Such an interrelationship may underscore a general systemic importance of alanine with respect to the metabolism of carbohydrate under metabolically stressful situations such as exercise. However, Galbo et al., (1976) have shown that blood alanine levels declined in man to preexercise levels following an initial increase during the course of repeated 30 minute runs at 60% MVO_2. In view of this decline attributed to increased splanchnic fractional extraction, the significance of alanine as psysiologic stimulus for glucagon release is contested (Galbo et al., 1976).

The effect of glucocorticoid hormones on amino acid metabolism is not well understood. Augmented blood glucocorticoids levels lead to an increase in plasma amino acid levels and in particular that of alanine (Felig, 1973) as a result of a catabolic effect on muscle protein.

Incubation of muscle in a cortisone medium resulted in an increased release of alanine with a reciprocal change in glutamine release (Karl et al., 1976). In man, cortisol levels have been shown to increase significantly above resting levels with heavy exercise (Hartley et al., 1972).

Fibre Types and Alanine Metabolism.

The metabolism of alanine may not be uniform across whole muscle in view of the obvious metabolic differences which exist among the fibres which comprise skeletal muscle. Therefore consideration of the fibre types is necessary in the analysis of the production and flux of the energy metabolites, alanine and lactate within whole muscle. Muscle lactate formation originates predominantly in the FG, and possibly in the FOG fibres as suggested by the large increases in plasma lactate levels (Felig and Wahren, 1971b) during recruitment of these fibres in higher intensity exercise (Terjung, 1976).

The survey of the energy metabolism enzyme profile (Pette and Dölken, 1975) and in particular of the LDH isoenzyme activity profile across the various fibre types (Baldwin et al., 1973b) indicate a favorable enzymatic adaptation for the FG fibre to produce lactate when utilized. While the FG fibres produce lactate it is conceivable that other fibre types within the same muscle, as well as cardiac and other skeletal muscles take up

lactate (Hermansen and Stensvold, 1972; Jorfeldt, 1971; Pette, 1971). The lactate dehydrogenase isoenzyme profile in SO fibres favors the adaptation for oxidation of lactate to pyruvate. Since the AAT reaction is not energy driven and depends solely on the accumulation of substrate (Krebs, 1975), the possibility exists for a metabolic interaction between fibre types based on metabolite diffusion.

Lactate formed via anaerobic glycolysis in white muscle or sections of muscle possessing predominantly FG fibres may serve as a substrate for oxidative metabolism in muscle and sections of muscle composed of red oxidative fibres (Essén et al., 1975) whose oxidative capacities have not been exceeded by the rate of glycolysis. However, an increasing fraction of the lactate formed may be deviated to other metabolic pathways such as that of alanine (Freminet et al., 1974; Mudge et al., 1976) as a result of a mass action effect of elevated tissue lactate levels retarding the conversion of pyruvate to lactate. The pathway of alanine formation may be further enhanced by the decreased Km of AAT for pyruvate with acidosis (Owen and Hochachka, 1974).

The existence of a predominant fibre type source in the peripheral synthesis of alanine remains unknown. When incubated in a lactate medium the release of alanine from the extensor digitorum longus, a muscle of

predominantly FOG and FG fibre (Ariano et al., 1973) was considerably less than the efflux of alanine observed from the soleus, a slow oxidative muscle, under identical conditions (Garber et al., 1976a). Also suggestive of the alanine formation within oxidative muscle tissue is the release of the amino acids from myocardial tissue in a pattern similar to that observed for skeletal muscle (Jefferson et al., 1974). Felig (1975) has calculated the release of alanine from heart to account for more than 80% of the total amino acid release in comparison to 30-40% release reported for skeletal muscle (Felig and Wahren, 1974). The difference in the proportional release of amino acid may relate to the differences in the myocardium's enzymatic arrangement, in particular, with respect to the LDH enzyme profile and absence of glutamine synthetase (Iqbal and Ottaway, 1970). The heart lactate dehydrogenase isoenzyme (H_4), also found in oxidative skeletal muscle, is strongly substrate-inhibited, so whenever pyruvate concentrations rise (Edington et al., 1973), lactate dehydrogenase is inhibited hence favoring alternative channels for pyruvate metabolism. When the heart is anoxic, an accelerated rate of glycolysis occurs, culminating in a maximal four fold increase in the intracellular level of alanine (Kao et al., 1976).

Malate - Aspartate Shuttle.

Since the mitochondrial membrane is impermeable to

NADH, electron displacement from cytoplasmic NADH to a mitochondrial equivalent must occur in complete oxidation of glucose by any fibre. The malate-aspartate electron shuttle system possesses a functional significance in heart as well as in red skeletal muscle for the transportation of such reducing equivalents while the α-glycerophosphate shuttle is probably more important in the FG fibres (Holloszy, 1975; Holloszy, 1976; McGilvery, 1973). The reason for a greater activity of the malate-aspartate shuttle with concomitant lower α-glycerophosphate shuttle activity in the more oxidative muscle tissue is not clear. Since the arrangement of the composition of mitochondrial protein varies according to the different rates of protein turnover (Swick et al., 1965), the mitochondria may be essentially different.

The collective participation of cytosolic and mitochondrial forms of malate dehydrogenase (NADH linked) and ASP-T as well as two membrane carriers structure the electron shuttle system (Krebs, 1975) as illustrated in Figure 2. An increased rate of generation of glycolytic NADH at the glyceraldehyde 3-P dehydrogenase step results in an increased reduction of oxaloacetate in the cytoplasm to malate catalysed by the cytoplasmic malate dehydrogenase. Malate immediately enters the mitochondrion via a stoichiometric exchange with α-ketoglurarate and is

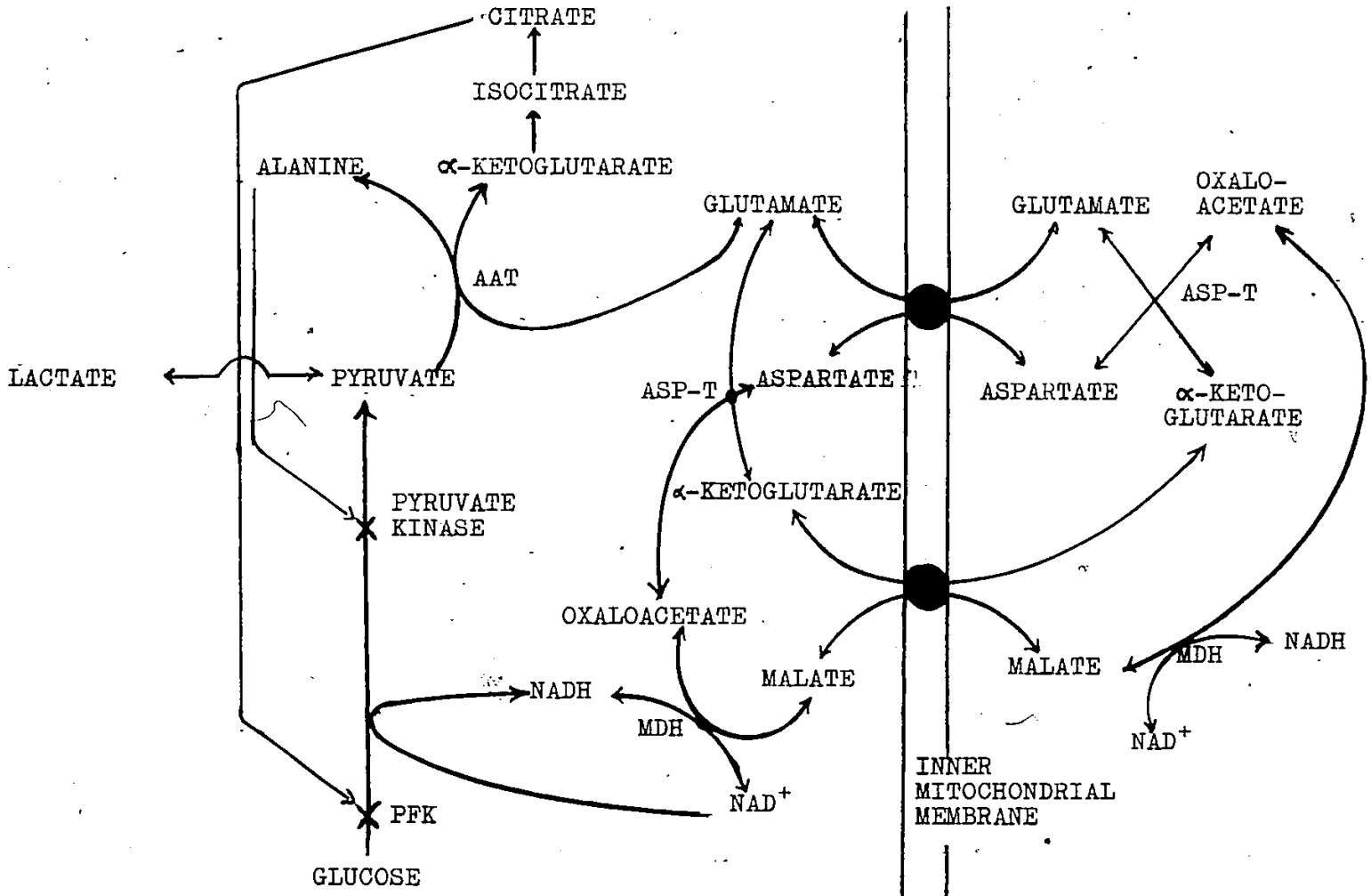


FIGURE 2. Metabolic link between the malate-aspartate electron shuttle and glycolysis.

reoxidized to oxaloacetate by the mitochondrial malate dehydrogenase. Since the inner mitochondrial membrane is poorly permeable to oxaloacetate the involvement of a transamination reaction between oxaloacetate and glutamate by means of the cytosolic and mitochondrial ASP-T isoenzymes permits the oxaloacetate carbon to return to the cytosol as aspartate before reconversion to complete the cycle. Safer and Williamson (1973) have shown that there exists a delay in the onset of increased glutamate-aspartate exchange and flux through mitochondrial aspartate aminotransferase. Due to the nonequal flux across the mitochondrial membrane through the α-ketoglutarate-malate and glutamate-aspartate exchange reactions a nonuniform flux in the TCA cycle results between the two spans from oxaloacetate to α-ketoglutarate and from α-ketoglutarate to malate. The key enzymes of the TCA cycle which are located at the crucial points of interaction with the malate-aspartate shuttle are α-ketoglutarate dehydrogenase and citrate synthase. A decreased flux occurs in the former enzyme while an increased flux occurs in the latter during the delay period until the onset in the increase of the glutamate-aspartate exchange, at which time a reciprocal return to common steady state value occurs (Safer and Williamson, 1973).

In this way the metabolic interaction of the malate-aspartate shuttle system, cytosolic AAT, isocitrate dehydrogenase and aconitase together with the TCA cycle within the mitochondria provides a mechanism for rapid changes in the TCA cycle intermediate levels in the cytosolic compartment (Safer, 1975). Furthermore, Safer and Williamson (1973) have shown that during this transition phase the rate of the sum accumulation of the TCA cycle intermediates is similar to that of alanine accumulation. Although evidence for the cellular flux of TCA cycle intermediates is drawn from studies performed with heart muscle, these authors (Davis and Bremer, 1973) assume that cardiac and skeletal muscles are qualitively similar with respect to these metabolic parameters.

Subsequent return of the TCA cycle intermediates to normal cytosolic levels can be accomplished in at least two manners. A mitochondrial membrane exchange between cytosolic α-ketoglutarate and mitochondrial succinate (Owen and Hochachka, 1974) and subsequent diffusion of succinate into the blood resulting in elevated blood succinate levels has been reported (Hochachka and Dressendorfer, 1976). Secondly, cytosolic α-ketoglutarate can transaminate with the branched chain amino acids to form glutamate and following

amination via glutamine synthetase to form glutamine, is subsequently released from muscle.

The glycolytic flux can be largely mediated by the redox state of pyridine mucleotides or energy state of the muscle cell (Safer, 1975). During states of increased oxidation the TCA cycle intermediate, citrate, rises to levels in the cytosol which affect the rate of glycolysis in skeletal muscle (Essén and Kaijser, 1976) by inhibiting the activity of the glycolytic enzyme phosphofructokinase (PFK) (Hochachka and Storey, 1975). Probably more important in the regulation of the glycolytic flux however, is the cellular accumulation of alanine. The levels of the TCA cycle intermediates in the muscle cell are regulated to a large extent by shifts in the concentrations of free alanine and aspartate via their respective transaminases (Davies and Bremer, 1973). Moreover, alanine has an inhibitory influence on pyruvate kinase (Hochachka and Storey, 1975).

The consequent metabolic events which occurs following diffusion of exogenous lactate into the oxidative muscle cell for oxidation would be expected to be essentially similar to an increased glycolytic flux in the cell itself since lactate conversion to pyruvate results in the production of NADH as occurs

in glycolysis. The cellular redox state would be maintained through the malate-aspartate shuttle system.

Koziol and Edington (1975) have suggested a relationship between the malate-aspartate shuttle system and the disposal of lactate in skeletal muscle. Their experiment involved the electrical stimulation of the rat gastrocnemius-plantaris muscle group (composed of mixed fibre population) followed by a monitoring of the changes in cellular metabolite concentration during the recovery period. While lactate levels decreased and pyruvate levels remained relatively unchanged 15 minutes into recovery, cellular alanine concentration increased two-fold (Koziol and Edington, 1975; McCafferty and Edington, 1975), suggesting a carbon flux through the LDH and AAT enzyme system. This was accompanied by a decreased cellular aspartate concentration to provide oxaloacetate for the acceptance of the reducing equivalents (Koziol and Edington, 1975).

Garber and co-workers (1976a) have provided evidence to support the postulate for a malate-aspartate shuttle, alanine synthesis relationship. With the addition of low concentrations of artificial electron acceptors to an incubating medium the muscle preparation exhibited a shift to a more oxidized state in the cytosolic redox potential thus lowering the activity of the

malate-aspartate shuttle system. Despite elevated pyruvate levels resulting from increased glucose uptake and subsequent glycolysis, alanine synthesis in the muscle tissue was markedly decreased (Garber et al., 1976a).

Training - Metabolic Adaptation.

Regularly performed exercise has been shown to induce major biochemical adaptations in skeletal muscle with the specificity of the adaptive response being fundamental to the nature of the stress (Kowalski et al., 1969). The effects of endurance training are manifested notably by the modification of several enzymic capacities associated with the oxidative processes. Specifically, the alteration of the capacities for β-oxidation of fatty acids, flux of the citric acid cycle and respiratory chain result in skeletal muscle becoming increasingly similar to cardiac muscle in its capacities for energy metabolism (Holloszy, 1975).

The improved oxidative capacity is associated with an adaptive response in the mechanism for transport of reducing equivalents into the mitochondrion. An alteration in the transport mechanism appears to be related to an increased capacity of the malate-aspartate shuttle (Holloszy et al., 1975) while α-glycerophosphate

dehydrogenase activity decreased significantly when expressed per mg of mitochondrial protein (Holloszy et al., 1969).

The general responses of the three fibre types with respect to relative increases in the oxidative function are similar (Baldwin et al., 1972). The quantitative response of each type, however, appears to vary according to the degree of training stimulus the fibre type is subjected to during the exercise (Holloszy, 1975; Terjung, 1976). Prolonged exercise of a low intensity nature will involve the predominant recruitment of the FOG and SO fibres, hence the greater stimulus for adaptation in these fibres. The response of the cytosolic energy-metabolism enzymes are of increases or decreases in activity which are specific to the fibre type. Hexokinase activity, however, increase in parallel to the increases in the respiratory capacity in the three fibre types (Baldwin et al., 1973b). Of the glycolytic enzymes the decreased activities of phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase in FOG fibre contrast with the increased activity for these enzymes in SO fibre. No significant changes were observed in FG fibre (Baldwin et al., 1973b). The small but consistent decrease in the activity of LDH observed in skeletal muscle (Gollnick et al., 1967)

appears to be specifically located in the FG and FOG fibres (Baldwin et al., 1973b; Holloszy, 1975).

The lower levels of blood lactate (Saltin and Karlsson, 1971a; Short et al., 1969) and of muscle lactate (Saltin and Karlsson, 1971b) observed in the aerobically trained compared to non trained men following a submaximal exercise of an equal relative intensity are considered to be primarily a result of the increased function of the oxidative processes in the muscle. The apparent reduction in the net production of lactate suggests it acts as an indicator of the dynamic balance which exists between the production rate and the efficiency of the removal processes (Eldridge, 1975). Cytosolic AAT which competes for the same 3 carbon fragment (pyruvate) as LDH has been reported to increase with endurance training (Molé et al., 1973) in skeletal muscle. However, the relative activities, as well as the influence endurance training has on AAT activities within the three fibre types, has not been clearly documented.

An alteration in the LDH:AAT enzyme activity ratio as a result of endurance training may signify an adjustment in muscle intermediary metabolism (Orlicky et al., 1977). Reduced pyridine nucleotides arising from cellular metabolism may become reoxidized to a greater extent by the malate-aspartate shuttle system, shifting from reoxidation of NADH via reduction of pyruvate to

lactate. A reduced rate of lactate formation in the trained individuals working at similar relative submaximal workloads as non trained individuals (Holloszy, 1975).

Although the activity of the AAT enzyme is rather high in skeletal muscle in general (Goldstein and Newsholme, 1975), the loss of alanine by cellular diffusion is sufficiently large to maintain a non-equilibrium state (Krebs, 1975) in the AAT reaction. The efflux of alanine from skeletal muscle has been reported to closely approximate the rate of its net formation (Garber et al., 1976a) therefore an increase in the activity of AAT may alter the relationship that exists between alanine, pyruvate and lactate demonstrated by Felig and Wahren (1971b).

CHAPTER III

RESEARCH METHODOLOGY

This chapter presents the methods and procedures employed in the investigation of the effects of a chronic aerobic stress upon the cytosolic alanine aminotransferase isoenzyme in heart muscle and in the SO, FOG and FG fibre types in rat skeletal muscle.

Animal Selection.

A total of 30 male Wistar strain rats purchased from Bio Breeding Laboratories of Canada in Ottawa were used in the experiment described in this thesis. The animals were between six to seven weeks of age and their weights ranged from 145 to 166 grams. On delivery to the laboratory, the rats were weighed and placed in separate cages under a 12 hour light-dark cycle (6 am-6 pm dark). They were provided with a diet of Purina laboratory chow and water ad libitum for the duration of the experiment. All the animals were handled daily and were weighed every second day throughout the study.

Ten rats were randomly selected for an experimental (EXP) group which underwent an eight week endurance training program as described below. Another group of ten rats were assigned to a pre-training control group (CON) to be sacrificed after acclimation, at 7 weeks of

age. The remaining 10 animals were designated as a sedentary control group (SED) to be sacrificed at the same time as the experimental group. This group served as a post-training control for the age affect.

Adaptation.

All animals were introduced to the treadmill (Quinton model 42-15) and adapted to treadmill running for a period of four days prior to beginning the EXP group on the running program. Once daily during the acclimation period the animals were submitted to running at a speed of 10 meters/min for a duration of 10 minutes while the treadmill was inclined to a 10% grade.

Experimental Design.

The experimental group followed a modified low-intensity treadmill running program to the one described previously by Pattengale and Holloszy (1967). An exercise program of this intensity has been found to result in a large increase in capacity for prolonged running and does not result in muscle hypertrophy (Holloszy, 1967; Pattengale and Holloszy, 1967). The rats were exercised five days per week for the first 3 weeks and six days per week for the subsequent weeks. The animals were exercised twice daily with four hours separating the exercise sessions. The program was made progressively more vigorous for the first 7 weeks, at which time the

exercise workload was maintained constant for the remainder of the program until sacrifice (Table 2).

TABLE 2

Protocol for the exercise program

Week	Exercise Duration (min.)	Exercise frequency		Speed (m/min.)	Grade (%)
		times/day	days/week		
1	15	2	5	22	10
2	30	2	5	22	10
3	40	2	5	28	10
4	40	2	6	28	10
5	50	2	6	28	10
6	50	2	6	31	10
7	60	2	6	31	10
8	60	2	6	31	10

Removal and Preparation of Muscles.

The animals of the EXP and SED groups were sacrificed after the eighth week of the training program, when both groups were 14 to 15 weeks of age. The EXP group was not exercised for 48 hours prior to sacrifice in order to avoid any acute effects of exercise. Following an overnight fast, the animals were weighed then killed by

decapitation and exsanguinated. The complete right hind limb musculature was exposed by cutting the skin at the ankle and slitting the skin with a scalpel from the ankle to the anus; the skin was then peeled back towards the body. The white vastus lateralis was first removed with scissors, followed by extraction of the red vastus lateralis, then soleus. All muscle samples were immediately placed in a beaker containing Ringer solution (Horowicz et al., 1970) maintained at 0°C. The muscles were then washed and trimmed free of connective tissue on a plastic plate placed on ice. The tissues were quickly weighed then minced in a small beaker containing an ice-cold medium composed of 0.225 M mannitol, 0.075 M sucrose, 0.01 M phosphate buffer, pH 7.6 and 0.05 mM EDTA (Tyler and Gonze, 1967). The homogenates contained 1.0 gram of muscle per 20 ml. Usually, samples weighed between 120 and 200 mg. The homogenates were prepared in an all-glass Duall tissue grinder. These were then centrifuged at 15,000 g for 45 minutes at 2° in a Sorvall Superspeed RC 2-B centrifuge to obtain a mitochondria-free fraction (DeRosa and Swick, 1975). Although no AAT activity was detected in the mitochondria in an earlier pilot study, DeRosa and Swick (1975) did detect a minute amount in this compartment but no activity was found in the microsomal fraction.

Assay Procedures for Enzymatic Activity.

The technique used for analysis of the AAT activity is essentially that which is described by Karmen (1955)

for aspartate aminotransferase and modified for AAT by Wroblewski and Ladue (1955). The reaction medium contained the following (in concentrations similar to those used by Molé et al. (1973): phosphate buffer 0.1 M, pH 7.6; substrate (L-alanine) 193mM; NADH 0.125 mM; α-ketoglutarate 5mM; lactate dehydrogenase 8 units; enzyme preparation 3.75-5.0 mg muscle. The mixture was prepared in triplicate in spectrophotometer cells, omitting α-ketoglutarate and was then placed in the thermospaced spectrophotometer for 2 minutes to allow exhaustion of any endogeous substrates. Following the period of incubation the rection was started by the addtion of the α-ketoglutarate. The reaction scheme is as follows:

(1) alanine + α-ketoglutarate ⟶ pyruvate + glutamate

(2) pyruvate + NADH ⟶ lactate + NAD^+

The transamination reaction is coupled with lactate dehydrogenase to determine the AAT activity. The reaction product, pyruvate, is reductively trapped by lactate dehydrogenase and the decrease in absorption at 340 nm due to the conversion of NADH to NAD^+ was followed on a Unicam spectrophotometer model SP.800. The reaction was carried out at 28° C, pH 7.6. The blank used contained all the components except alanine. Each homogenate was measured in triplicate using samples

of 5.0, 5.0, and 3.75 mg of muscle.

The international unit of transaminase is the amount catalyzing the transformation of μmole of substrate per minute. Since NADH has a molecular extinction coefficient (ϵ_{340}) of 6.2×10^3, an absorbance change of 1.0 for a 10 mm light path and a volume of 3 ml in which the assays are carried out corresponds to oxidation of .484 μmoles NADH (.484 μmoles pyruvate). The enzyme activity was expressed in terms of μmoles of pyruvate reduced per minute per gram of wet weight of muscle and mg of protein.

Protein Estimation.

The concentration of protein in the 5% muscle homogenate was estimated by a Lowry et al. (1951) method modified by Schacterle and Pollack (1973). To avoid possible interference of Tris buffer from the 'mannitol' medium with the method, the following preliminary steps were carried out. Triplicates of 0.04 ml of each sample were added to test tubes, 5 ml of 12.5% TCA was added to each aliquot, and allowed to stand on ice for 30 minutes, after which they were centrifuged at 7,500 x g for 30 minutes. The supernatants were discarded, and the pellets (protein) each disolved in 1.0 ml of 0.5 N NaOH. The preceeding steps were those of Schacterle and Pollack (1973). Measurements for protein estimation procedure and measurements were performed in triplicate using 2 mg

samples of muscle.

Analysis of the Data.

The statistical analysis of the cytosolic AAT isoenzyme activity was done by a two-way analysis of variance with repeated measures using the Full Rank Multivariate Linear Model (FRMLM) computer program. The independent variables were muscle with three subcategories; white vastus lateralis, red vastus lateralis, soleus and heart and treatment also with three subcategories; EXP, SED, and CON. The dependent variables were enzyme activity, specific enzyme activity and protein estimation. A multivariate post hoc analysis was used to identify the specific contrasts between groups which caused significance ($p < .01$) in the analysis of the main effects.

CHAPTER IV

RESULTS

The purpose of this study was to determine the distribution of cytosolic AAT activity in the following muscle types: FOG, FG, and SO skeletal muscle fibres and heart. It was also the aim of this study to investigate the effect of an 8 week endurance running program on the activity of the AAT isoenzyme within these tissues. The results will be presented as follows: a) the training aspect of the experiment, which includes training performance and body weight data, b) the activity of cytosolic AAT in all sample tissues, expressed in terms of muscle wet weight, c) the specific activity of cytosolic AAT in the sample tissues, expressed in terms of mg of protein and, d) protein estimation. All of the raw data appears in the Appendices while the means and standard errors are summarized in Table 3.

a) Training aspect

The training program was of 8 to 9 weeks duration, depending on the day of sacrifice of the EXP rat. By the beginning of the eighth week of the program the EXP rats were able to run 2 x 60 minute sessions at 31 m/min up a 10% incline.

TABLE 3

MEAN ENZYME ACTIVITIES AND PROTEIN ESTIMATIONS

	CONTROL Mean ± S.E. (n = 10)	SEDENTARY Mean ± S.E. (n = 10)	EXPERIMENTAL Mean ± S.E. (n = 8)
SOLEUS			
Enzyme Activity (µm/min/g muscle)	3.19 ± .40	3.93 ± .55	4.55 ± .58
Enzyme Activity (µm/min/mg protein)	.102 ± .015	.122 ± .020	.149 ± .020
Protein Estimation (µg/mg muscle)	31.7 ± 4.0	32.6 ± 4.8	30.8 ± 3.7
RED VASTUS			
Enzyme Activity (µm/min/g muscle)	4.32 ± .54	4.70 ± .51	5.07 ± .47
Enzyme Activity (µm/min/mg protein)	.120 ± .017	.144 ± .027	.155 ± .023
Protein Estimation (µg/mg muscle)	36.6 ± 6.8	33.7 ± 7.1	33.3 ± 5.4
WHITE VASTUS			
Enzyme Activity (µm/min/g muscle)	1.93 ± .17	2.57 ± .40	2.17 ± .16
Enzyme Activity (µm/min/mg protein)	.056 ± .011	.092 ± .021	.068 ± .016
Protein Estimation (µg/mg muscle)	35.3 ± 6.1	29.5 ± 4.7	32.6 ± 4.2
HEART			
Enzyme Activity (µm/min/g muscle)	1.47 ± .13	2.15 ± .23	2.02 ± .17
Enzyme Activity (µm/min/mg protein)	.042 ± .004	.067 ± .013	.058 ± .008
Protein Estimation (µg/mg muscle)	34.9 ± 3.3	33.1 ± 7.0	35.5 ± 6.9

The average weight gain for the EXP group (158 g) was less than that of the SED group (230 g). A student t test indicated that the final gross weights of the EXP group animals were significantly less ($p < .001$) than the gross weights of the SED group rats. The growth of the rats in relation to body weight of EXP and SED groups is graphically represented in Figure 3. The mean weight values and standard errors are tabled in Appendix A.

b) Activity of cytosolic AAT in the sample tissues

The data on cytosolic AAT activity in relation to the wet weight of the individual muscle samples are in Appendix B.

Although the Full Rank Multivariate Linear Model program for statistical analysis employed in this study provided a univariate analysis of variance with repeated measures, the F statistic could not be used in the analysis of enzyme activity. Since a significant cross-interaction occurred ($p < .01$) between repeated measures and treatment groups, due primarily to a decrease in AAT activity in White vastus concomitant to an increase in Soleus with training (illustrated in Figure 4), this violates one of the assumptions (symmetry) of a repeated measures analysis (Timm, 1975).

The analysis of the main effects (Table 4) revealed statistically significant differences in enzyme activity

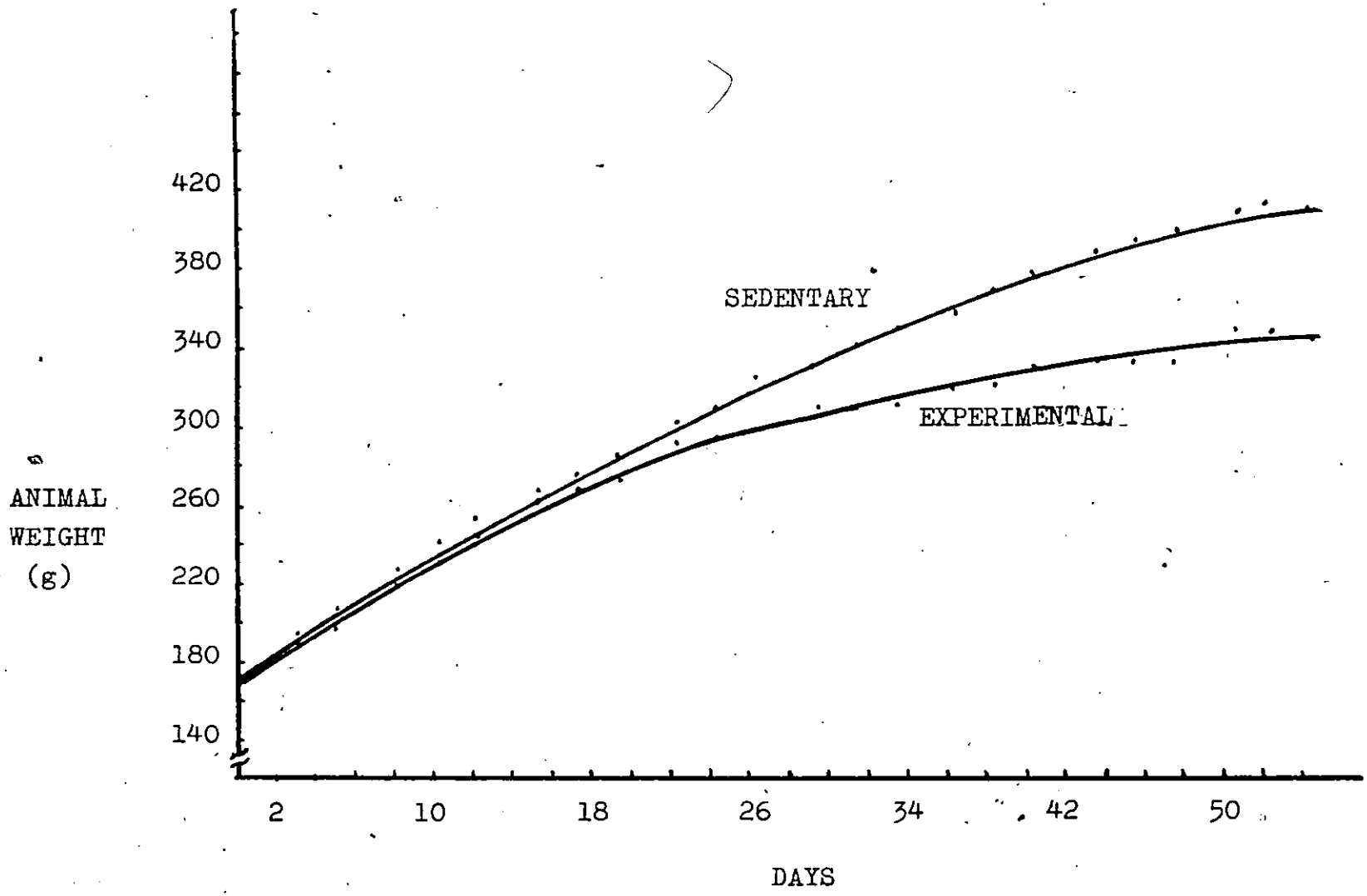


FIGURE 3. The change in the mean body weights of rats (EXPERIMENTAL) subjected to an 8 week endurance training program (n=8) and a sedentary (SEDENTARY) control group (n=10).

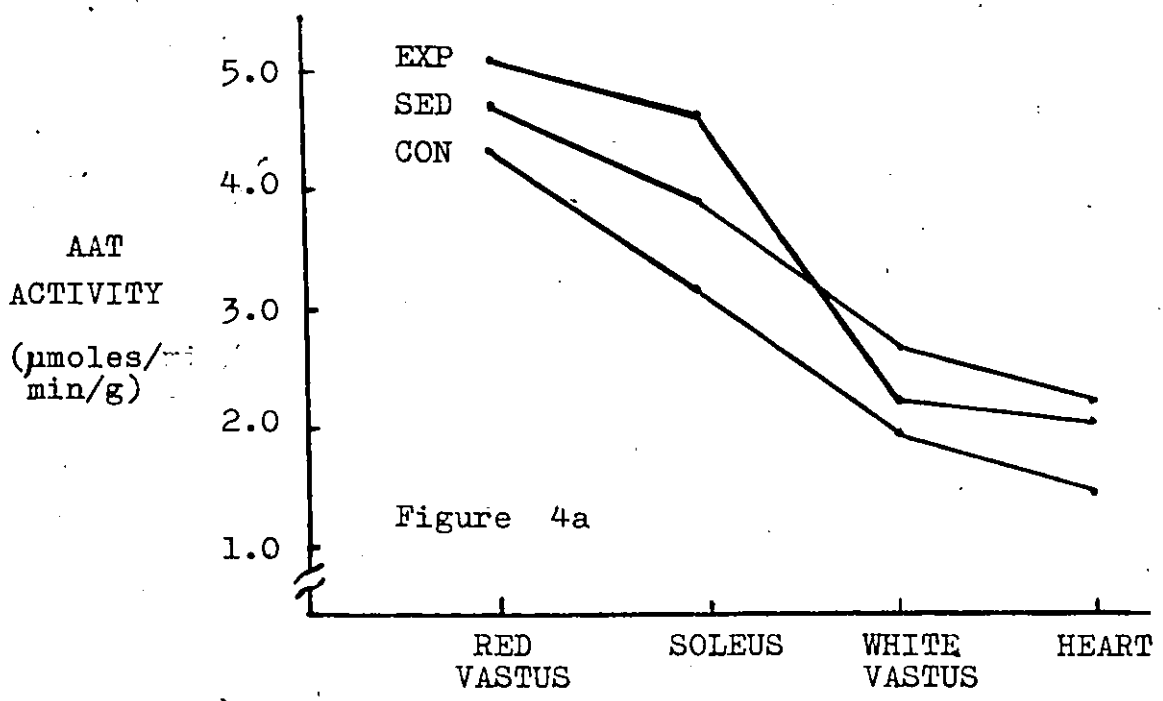


Figure 4a

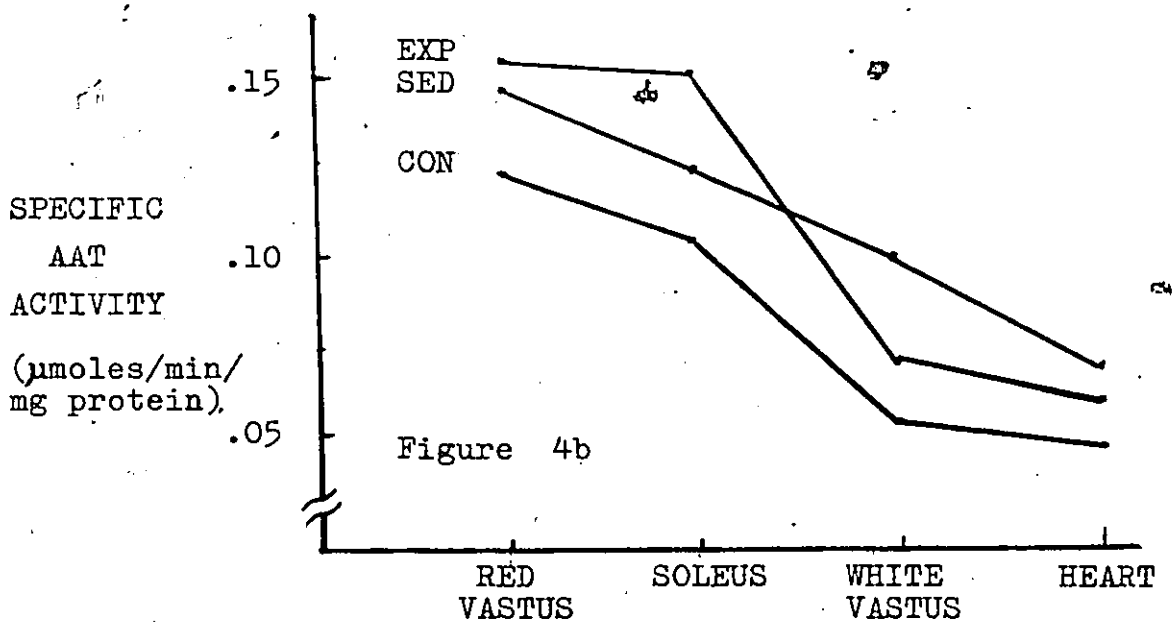


Figure 4b

FIGURE 4. Graphic representation of means for enzyme activity (figure 4a) and for specific enzyme activity (figure 4b) in the muscle types of the treatment groups (EXP, SED, and CON).

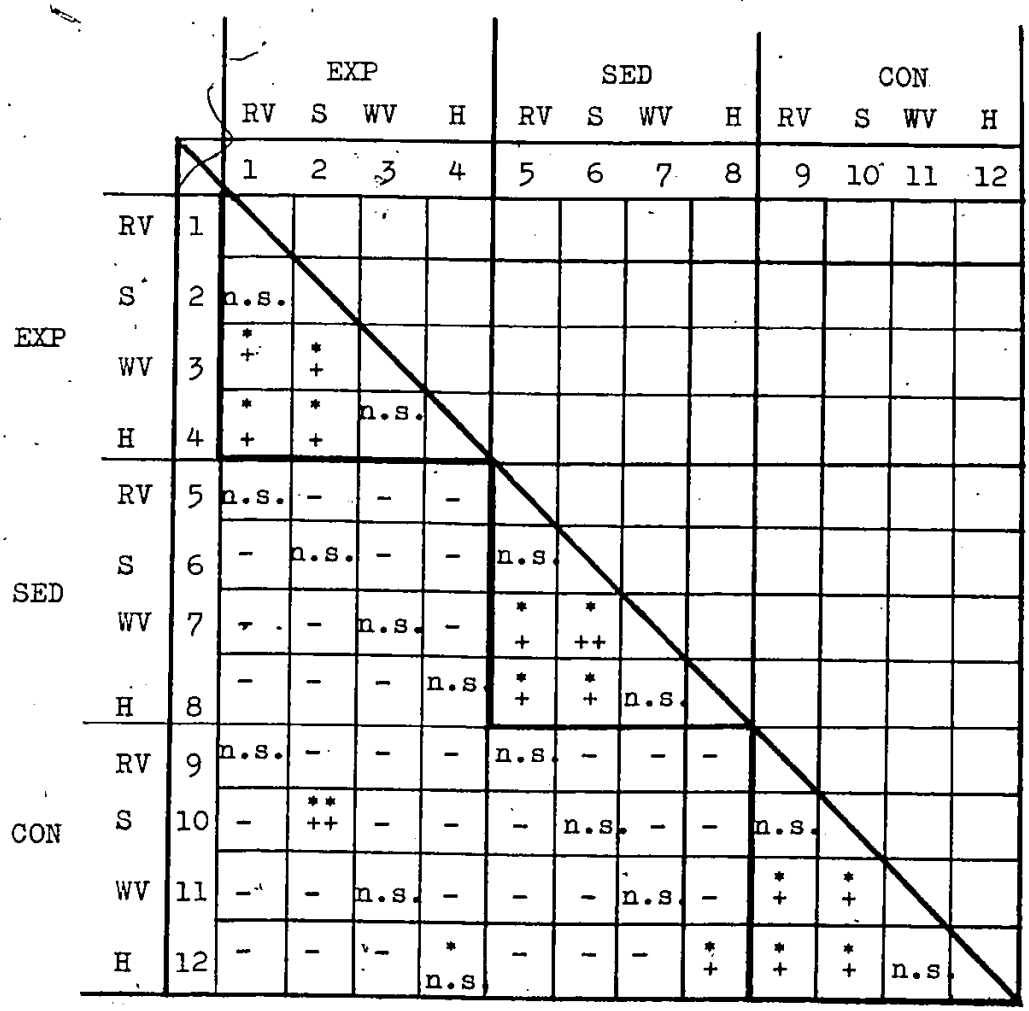
TABLE 4

ANALYSIS OF MAIN EFFECTS

Dependent Variable	Analysis	Differences Between Muscles		Differences Between Treatments		Interaction	
		Calculated values	Critical values	Calculated values	Critical values	Calculated values	Critical values
Enzyme Activity	Multivariate						
	1. Wilks	.074*	.424	.014*	.412	.402*	.412
	2. Roy	.884*	.507	.974*	.505	.572*	.465
	3. Pillai	1.366*	.653	1.438*	.700	.633*	.600
Specific Enzyme Activity	Multivariate						
	1. Wilks	.094*	.424	.027*	.412	.337*	.412
	2. Roy	.744*	.507	.957*	.505	.652*	.465
	3. Pillai	1.378*	.653	1.344*	.700	.682*	.600
Protein	Univariate						
	1. Anova (F statistic)	1.702	2.43	2.022	2.43	.92	2.43

* $p < .01$

between the treatment groups (EXP, SED, and CON). A multivariate post hoc analysis was carried out to determine which contrasts were significant. Since the multivariate post hoc analysis incorporated three post hoc tests; Wilks, Roy, and Pillai, a contrast was determined significant if two of the three tests showed significance for the specific contrast. The specific contrasts are tabled in Appendix E along with the critical ranges for the most conservative test for the specific contrast. The analysis indicated that a significant difference existed between the CON and SED heart muscles ($p < .01$). A significant difference in AAT activity also existed between CON and EXP soleus muscles ($p < .05$). The treatments had no significant effect (see Figure 5) on enzyme activity in any of the other muscle types. A statistically significant difference also existed in enzyme activity between the muscle types. The post hoc analysis indicated that when Red vastus was contrasted with Soleus of the same treatment group, no significant differences existed in AAT activity. Similarly, when White vastus was contrasted with Heart AAT activity of the same treatment group, no significant differences existed. However, both Red vastus and Soleus AAT activities were significantly higher than in White vastus and Heart in all of the treatment groups.



* Enzyme activity, significant, $p < .01$
** Enzyme activity, significant, $p < .05$
\+ Specific enzyme activity, significant, $p < .01$
++ Specific enzyme activity, significant, $p < .05$
n.s. non significant

FIGURE 5. Results of the multivariate post hoc analysis on enzyme activity and specific enzyme activity. RV (red vastus), S (soleus), WV (white vastus), H (heart).

c) Specific activity of cytosolic AAT in the muscle samples

The data representing individual samples for specific enzyme activity, expressed in μmoles of alanine transaminated to pyruvate/min/mg of protein are in Appendix C. The statistical treatment for these data is presented in Tables 3 and 4 and in Figure 5. Since the statistical analysis of the main effects indicated a significant cross-interaction (Table 4), a multivariate analysis was used as in the statistical treatment for enzyme activity. The analysis also indicated a significant difference in specific enzyme activity ($p < .01$) between treatment groups. The post hoc analysis (Figure 5) indicated that significant differences existed between CON and SED heart muscles ($p < .01$) and between CON and EXP soleus muscles ($p < .05$). As well, a statistically significant differences ($p < .01$) existed in specific activity when muscle types of one treatment group were contrasted. The post hoc analysis indicated that specific AAT activities in RED vastus and Soleus were not significantly different in any treatment group. Similarly, no significant differences existed between enzyme activity in White vastus and Heart of the same treatment group. However, both Red vastus and Soleus were significantly higher ($p < .01$) than White vastus and Heart within each treatment group, with the exception of differences between

Soleus and White vastus in the SED group which were significant beyond the 5% level.

d). Protein estimation

The data tabled in Appendix D represents the mean value of protein concentration (µg/mg muscle wet weight) for each muscle sample. Tables 3 and 4 show the statistical analysis of the data.

No statistically significant differences existed between treatment groups or between muscle types for protein concentration in the muscle samples.

CHAPTER V

DISCUSSION

Training aspect

The training program was progressive in daily workload and enabled the rats to reach a peak endurance performance of 31 m/min on a 10% incline for 1 hour, twice daily. Although most rats showed no sign of fatigue (refusal to run) during the final (eighth) training week, some rats exhibited some difficulty in maintaining the 31 m/min running pace toward the end of several training sessions and began falling back on to the electric shock grid. Towards the final stages of the training program the endurance running ability of the rats may have been adversely affected by their weight. Gollnick and co-workers (1970) have noted a consistent decrease in endurance of rats as they become heavier. In spite of the low intensity nature of the training program, two EXP rats died in their cages, sometime between exercise sessions.

A student t test indicated that the EXP group weighed significantly less than the age control SED group ($p < .001$) at the end of the 8 week endurance running program. As graphically illustrated in Figure 3 (in chapter IV), training had a pronounced effect on both the

pattern and amount of weight gain acheived by the EXP group. When compared with the SED group, there was a trend towards a progressive decrease in the rate of weight gain as the training program progressed. The observed decrease in the rate of weight gain of the EXP in relation to the SED group may have been due to reductions in both lean body mass and fat accumulation as reviewed by Reed et al. (1973).

Cytosolic AAT activity in the muscle types

Molé and co-workers (1973) have reported AAT activity for gastrocnemius muscle homogenates to be 22.7 µmoles/min/g wet weight. This value for AAT activity in skeletal muscle deviates considerably from several other studies reporting the following values for AAT activity in skeletal muscle (in µmoles/min/g): 4.25 (DeRosa and Swick, 1975), 5.48 (Krebs, 1975), and 4.44 (Snell and Walker, 1973). The data from the present study for AAT activity of homogenates of the skeletal muscle fibre types of sedentary rat (Red vastus, FOG, 4.7; Soleus, SO, 3.93; White vastus, FG, 2.57) are in accord with the enzyme activities reported in previous studies on rat skeletal muscle. Cytosolic AAT activity is greatest in the oxidative skeletal muscle fibre types (FOG, SO) in the EXP, SED and CON groups. The FG fibre, whose enzyme profile is oriented for anaerobic metabolism, had between 43% and 65% of the AAT activity found in the other two fibre types. In contrast to the oxidative fibre types, the α-glycerophosphate shuttle appears to be the important mechanism for the transport of cytosolic electrons to the

mitochondrion in the FG fibre (Holloszy, 1975). Since the function of the cytosolic AAT enzyme has been linked to an alternative electron transport system, the malate-aspartate shuttle (Safer and Williamson, 1973), its activity may therefore be expected to be less in the FG fibre.

The AAT activity found in heart muscle in this study (2.15 μmoles/min/g) was consistent with activities of 1.94 and 2.87 μmoles/min/g muscle for rat heart homogenates obtained by DeRosa and Swick (1975) and Krebs (1975) respectively. Cytosolic AAT activity in Heart was lower than the activity found in the skeletal muscle fibre types whether activity was expressed either as μmoles/min/g wet weight or as μmoles/min/mg protein. These data are in agreement with previous studies (DeRosa and Swick, 1975; Krebs, 1975) which have demonstrated a lower AAT activity in rat heart homogenates than in rat skeletal muscle. Since heart muscle is a highly oxidative tissue, AAT activity would be expected to be high if the trend found in the skeletal muscle fibre types were to continue. A lower AAT activity suggests a possible difference in the role of this enzyme in cardiac as compared to skeletal muscle metabolism. Also consistent with the results of this study, Ozand and co-workers (1973) observed that incubated heart tissue released less alanine per unit

time than did skeletal muscle under similar conditions. However, since the synthesis of alanine in heart muscle has been quantitatively related to myocardial oxygen deprivation (Taegtmeyer et al., 1977), the pyruvate to alanine pathway may not be important in an in vivo condition. The cytosolic AAT activity may therefore be expected to be low.

Cytosolic AAT activity: effect of age and training

Comparable age related increases in AAT activity, albeit insignificant, have been shown to occur in all muscle types between the ages of 6.5 and 14.5 weeks. One study by Snell and Walker (1973), has graphically illustrated the increase in skeletal muscle AAT concentration during the development period of the rat. These authors showed that AAT activity reached approximately 80% of the adult value of 4.44 μmoles/min/g wet weight by four weeks of age. In the present study, AAT activity levels in the skeletal muscle fibre types of 6.5 week old rats (CON group) were; 92% (FOG), 81% (SO), and 75% FG of the sedentary adult rat levels. Although the search of the literature revealed no studies on AAT activity changes in heart muscle with age, the present study showed that 6 to 7 week old rats had 68% of the cytosolic AAT activity found in sedentary adults rat hearts. The percentage of adult levels is similar whether

enzyme activity is expressed relative to muscle wet weight or mass of protein.

When compared to the SED group, the endurance trained rats (EXP) showed no significant differences in AAT activity in the three skeletal muscle fibre types and heart muscle when enzyme activity is expressed relative to muscle wet weight (Figure 6). This is also true when enzyme activity is expressed relative to mass of protein. The lack of a significant increase in cytosolic AAT activity in any of the skeletal muscle fibre types is not in accord with the results of an earlier study (Molé et al., 1973) which showed a significant 46% increase in cytosolic AAT activity in gastrocnemius muscle.
A significant difference existed in AAT activity in soleus muscle (SO fibres) due to a combined effect of age and training (CON vs EXP group). The separate effects of age and training each seem to have contributed to raise the AAT activity level to significance ($p < .05$). The significant increase observed in soleus muscle may be related to the ultrastructural changes, indicative of protein changes that occur in SO fibres with age and training. Maxwell and co-workers (1973) reported a reduction in the number of fibres per square millimeter of cross-sectional area in soleus muscle of guinea pigs (100% SO fibre population) due to hypertrophy of individual skeletal muscle fibres with age and training.

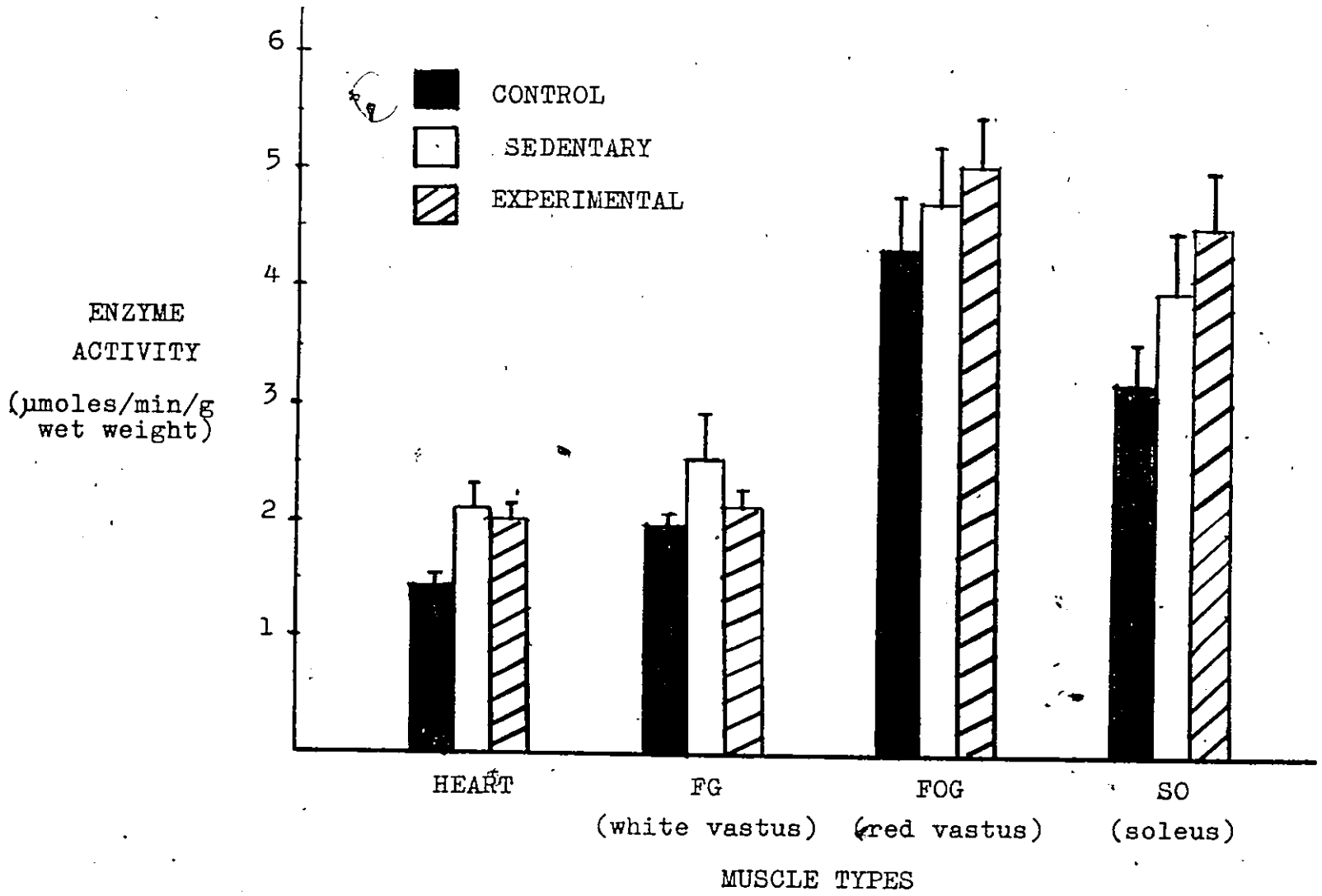


FIGURE 6. The effect of an 8 week endurance running program or an 8 week period of inactivity on cytosolic AAT activities in heart and skeletal muscle fibre types of rats initially 6 to 7 weeks old.

The significant increase in soleus AAT activity may also be releated to the recruitment pattern of this muscle's SO fibres. Armstrong and co-workers (1974) have studied the relative extent of glycogen depletion (an indicator of muscle fibre recruitment) within the various fibre types of the plantaris muscle of rats running on a treadmill at a similar exercise intensity to the workload used in this study. These authors noted 75% of the FG fibre population stained dark (saturating glycogen levels for the staining method) and 24% stained moderate, indicating very little recruitment of the fibre type. Total glycogen depletion occured in approximately 40% of the FOG and SO fibres. The remaining 60% of the SO and 50% of the FOG fibres stained lightly for glycogen (glycogen stores mostly depleted). Only ten per cent of the FOG fibre population continued to stain moderate, indicating little recruitment. On the basis of these results, the present training program would have been expected to induce a recruitment of virtually all motor units of the soleus muscle and most motor units of the FOG fibre population, while recruitment of the FG population would have been less frequent. As well, since soleus muscle serves a postural function, its fibres are therefore being recruited constantly for the support of body weight.

In contrast to the skeletal muscle fibre types, enzyme activity in the heart muscle has not been shown to

change with endurance training (Oscai et al., 1971). The data of the present study are in accord with the findings of these authors.

Significance of the results

Pette (1971) has well established that within various tissues there are groups of enzymes whose activities exist in constant proportions. Despite variations in absolute enzyme activities, due to training for instance, constant molar ratios can be maintained between reference enzymes of different constant-proportion groups (or metabolic systems) suggesting that the represented systems are metabolically related (Pette and Dölken, 1975). This concept is of particular importance when considering the shift that occurs in the enzyme pattern of soleus muscle towards that of FG fibres when soleus is cross-innervated by FG neurons (Golisch et al., 1970). In this latter case, glycogenolytic and glycolytic enzyme activities increased while a decrease occurred in enzymes of the TCA cycle and β-oxidation of fatty acids. Concomitant with these changes, the same authors noted a decrease in AAT activity in a similar proportion to enzymes of the TCA cycle. These results suggest that not only is AAT activity higher in soleus (SO fibres) than in FG fibres, which is in agreement with the results of the present study, but also that the AAT enzyme may be closely related to the

oxidative metabolism of the cell.

Since the nature of the endurance training stimulus is distinct from that of cross-innervation, the pattern of the enzyme activity shift may also be different. Some question exists as to whether parallel increases should occur with endurance training, in the activities of AAT and other enzymes of oxidative metabolism. The rationale of Pette and Dölken (1975), of constant-proportion systems, supports an increase in AAT activity in view of the increases in activity of aspartate aminotransferase and NADH-linked malate dehydrogenase enzymes (Holloszy et al., 1975), which are also part of the malate-aspartate shuttle. Holloszy (1975), reviewing the biochemical adaptations of muscle to endurance training, has discussed at length the reasoning for the increase in AAT activity in skeletal muscle earlier proposed by Molé and co-workers (1973). These researchers theorized that elevated AAT activity levels would allow for a greater capacity to shunt pyruvate towards alanine formation, hence reducing lactate formation during anaerobic conditions. This could help to explain the lower concentration of blood lactate in the trained than in the untrained state during submaximal exercise of similar relative intensity. The present study however, does not support this hypothesis.

Baldwin and co-workers (1972) have observed that

enzymes linked with the oxidative metabolism of the skeletal muscle fibres increase in activity, towards levels of the more oxidative heart muscle, with endurance training. Since heart muscle has a very low level of AAT activity, while possessing a highly oxidative metabolic system, an increase in the oxidative enzyme capacity of the skeletal muscle fibre types may not require a comparable increase in AAT activity. This lack of an increase in AAT activity, comparable to that of enzymes involved in the oxidative metabolism of the muscle cell, may possibly indicate that the cytosolic AAT isoenzyme is not closely related to the oxidative energy metabolism of the cell. The lack of an increase in cytosolic AAT activity in the muscle types with endurance training may, however, indicate sufficient cytosolic AAT activity for the turnover of the components during elevated metabolic states and/or that cytosolic AAT activity can be acutely regulated by the intracellular biochemical environment. Evidence of a mechanism for the regulation of the pyruvate to alanine flux stems from the observation that the K_m of AAT for pyruvate decreases with acidosis (Owen and Hochachka, 1974). Further support of this concept stems from the report of an increase in AAT activity with acute muscle stimulation (Hajek and Perry, 1967).

CHAPTER VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of this study was to characterize cytosolic alanine aminotransferase isoenzyme (AAT) activity within the rat heart and skeletal muscle fibre types. It was also the purpose to define the relative adaptability of this isoenzyme within these tissues to endurance training. It was reasoned that the nature and the magnitude of the adaptation in the skeletal muscle fibre types would relate to the degree of their recruitment during the exercise sessions.

Following an eight week endurance training program, the exercised rats (EXP) were sacrificed along with their sedentary controls (SED). Cytosolic AAT was determined in sections of muscle predominant in one fibre type as well as in heart muscle. It was found that FOG and SO fibres possessed a significantly higher cytosolic AAT activity than in the FG fibre and heart muscle within the sedentary and exercised rats as well as in young rats (CON). Cytosolic AAT activity within all skeletal muscle fibre types showed small, but insignificant, increases with age, while heart muscle showed a significant increase. The endurance trained rats did not show any significant

changes in cytosolic AAT activity when compared to the sedentary control group. However, small but insignificant increases occurred in the soleus and red vastus muscles which are representative of the SO and FOG fibre types respectively.

Conclusions.

On the basis of the findings of this study, the following conclusions are warranted:

1. Prolonged low intensity exercise, performed regularly, results in a significant depression in body weight gain in growing rats.

2. Within skeletal muscle, FOG and SO fibre types possess a significantly greater capacity for alanine synthesis than does the FG fibre type. This association of high activity with the oxidative capacity in the skeletal muscle fibre types supports the contention that the cytosolic AAT isoenzyme is linked to the oxidative metabolism in these tissues. The tendency, albeit not significant, for AAT activity to increase in response to aerobic training reinforces this suggestion, though some of this observed effect could be attributed to growth changes.

3. The role of AAT in heart muscle seems to be at a variance to that in skeletal muscle. Although heart muscle is highly oxidative, the AAT activity level resembles that found in FG fibres and shows no tendency to increase with endurance training.

4. Beyond seven weeks of age a developmental pattern for the increase in AAT activity in the rat muscle types was not significant, except for heart muscle. This could reflect a slower developmental pattern for the alanine pathway in heart than in skeletal muscle.

Recommendations.

In view of the outcome of this study, the following recommendations are made for further study:

1. Rats used for exercise studies should first be selected on the basis of their willingness to run and should then be randomly assigned to either control or experimental groups.

2. A similar study, if conducted, should include a) the analysis of the activity changes of an oxidative enzyme such as cytochrome oxidase as a marker for oxidative adaptation, b) the analysis of LDH so that cytosolic AAT: LDH isoenzyme activity ratios could be measured; c) the analysis of aspartate aminotransferase so that other changes in the malate-aspartate shuttle system could be followed.

3. Other procedures which could add to the interpretations of similar studies in the future would be: the application of histochemistry to characterize the exact fibre population present in the samples and a determination of changes in body components (fat vs lean body mass effects).

4. This study should be succeeded with a study involving the measurement of the changes in the arteriovenous alanine differences in trained and nontrained individuals undergoing maximal and submaximal exercise.

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APPENDIX A

ANIMAL WEIGHTS

TABLE 5

Gross Animal Weights: Mean Weight (g) of Experimental and Sedentary Rats

DAY	GROUP	
	Experimental	Sedentary
1	177	180
3	187	196
5	197	207
8	219	226
10	229	241
12	241	250
15	260	265
17	266	277
19	270	284
22	288	301
24	290	310
26	289	325
29	304	333
31	303	340
33	305	348
36	315	359
38	314	367
40	325	378
43	333	389
45	328	395
47	327	400
50	343	411
52	340	414
54	335	410

APPENDIX B

ALANINE AMINOTRANSFERASE ACTIVITY

TABLE 6

ALANINE AMINOTRANSFERASE ACTIVITY
(μmoles NADH oxidized/min/g)*

MUSCLE		TREATMENT		
		Control	Sedentary	Experimental
RED VASTUS		4.59	4.73	4.99
		3.39	5.21	5.01
		3.86	4.01	4.37
		3.92	4.70	5.00
		4.72	5.65	5.63
		4.24	4.70	5.82
		4.35	4.00	4.69
		5.13	4.37	5.04
		4.99	4.84	
		4.03	4.94	
	Mean ± S.E.	4.32 ± .54	4.70 ± .51	5.07 ± .47
SOLEUS		3.01	4.04	4.12
		3.59	3.50	4.23
		3.09	3.44	3.87
		3.46	2.89	4.60
		3.17	3.80	4.20
		3.23	4.63	5.30
		2.54	4.24	5.28
		3.96	4.68	4.78
		2.92	3.94	
		2.88	4.09	
	Mean ± S.E.	3.19 ± .40	3.93 ± .55	4.55 ± .58

* Individual AAT activity values represent a mean of three experiments.

TABLE 6 (con't)

ALANINE AMINOTRANSFERASE ACTIVITY
(μmoles NADH oxidized/min/g)*

MUSCLE		TREATMENT Control	Sedentary	Experimental
WHITE VASTUS		1.98	2.88	2.06
		1.80	2.47	2.25
		2.04	2.04	2.13
		2.10	2.42	1.83
		1.74	2.29	2.35
		1.72	3.07	2.22
		1.96	2.50	2.30
		1.89	3.22	2.18
		1.80	2.74	
		2.26	2.08	
	Mean ± S.E.	1.93 ± .17	2.57 ± .40	2.17 ± .16
HEART		1.78	2.02	2.01
		1.39	1.71	2.25
		1.45	2.22	2.00
		1.40	2.26	2.12
		1.47	2.40	1.83
		1.56	2.39	1.81
		1.48	2.15	2.19
		1.29	2.04	1.84
		1.45		
		1.41		
	Mean ± S.E.	1.47 ± .13	2.15 ± .23	2.02 ± .17

* Individual AAT activity values represent a mean of three experiments.

APPENDIX C

ALANINE AMINOTRANSFERASE SPECIFIC ACTIVITY

TABLE 7

ALANINE AMINOTRANSFERASE ACTIVITY
(μmoles NADH oxidized/min/mg protein)*

MUSCLE	TREATMENT		
	Control	Sedentary	Experimental
RED VASTUS	.143	.118	.143
	.154	.121	.179
	.114	.108	.132
	.109	.181	.132
	.124	.149	.148
	.099	.118	.146
	.118	.167	.168
	.119	.182	.194
	.108	.156	
	.115	.145	
Mean ± S.E.	.120 ± .017	.144 ± .027	.155 ± .023
SOLEUS	.131	.104	.121
	.100	.117	.146
	.094	.127	.138
	.108	.107	.124
	.099	.112	.162
	.111	.136	.166
	.091	.128	.165
	.113	.167	.171
	.083	.119	
	.085	.098	
Mean ± S.E.	.102 ± .015	.122 ± .020	.149 ± .020

* Individual specific AAT activity values represent a mean of three experiments.

TABLE 7 (con't)

ALANINE AMINOTRANSFERASE ACTIVITY
(μmoles NADH oxidized/min/mg protein)*

MUSCLE		TREATMENT Control	Sedentary	Experimental
WHITE VASTUS		.083	.103	.054
		.060	.073	.075
		.057	.073	.071
		.057	.061	.050
		.046	.076	.078
		.052	.110	.058
		.050	.104	.079
		.063	.107	.075
		.042	.129	
		.053	.087	
	Mean ± S.E.	.056 ± .011	.092 ± .021	.068 ± .016
HEART		.043	.075	.057
		.048	.066	.063
		.040	.093	.053
		.038	.065	.046
		.046	.073	.061
		.049	.057	.070
		.042	.051	.050
		.037	.057	.066
		.040		
		.039		
	Mean ± S.E.	.042 ± .004	.067 ± .013	.058 ± .008

* Individual specific AAT activity values represent a mean of three experiments.

APPENDIX D

PROTEIN ESTIMATION

TABLE 8

PROTEIN ESTIMATION
(µg/mg protein) *

MUSCLE		TREATMENT Control	Sedentary	Experimental
RED VASTUS		33	40	35
		22	43	28
		34	37	33
		36	26	38
		38	38	38
		43	40	40
		37	24	28
		43	24	26
		46	31	
		35	34	
	Mean ± S.E.	36.6 ± 6.8	33.7 ± 7.1	33.3 ± 5.4
SOLEUS		23	39	34
		36	30	29
		33	27	28
		32	27	37
		32	34	26
		29	34	32
		28	33	32
		35	28	28
		35	33	
		34	41	
	Mean ± S.E.	31.7 ± 4.0	32.6 ± 4.8	30.8 ± 3.7

* Individual protein values represent a mean of three experiments.

TABLE 8 (con't)

PROTEIN ESTIMATION
(µg/mg protein) *

MUSCLE		TREATMENT Control	Sedentary	Experimental
WHITE VASTUS		24	28	38
		30	34	30
		36	28	30
		37	40	37
		38	30	30
		33	28	38
		39	24	29
		30	30	29
		43	29	
		43	24	
	Mean ± S.E.	35.3 ± 6.1	29.5 ± 4.7	32.6 ± 4.2
HEART		41	27	37
		29	26	36
		36	24	38
		37	35	46
		32	33	30
		32	42	26
		35	42	38
		35	36	31
		36		
		36		
	Mean ± S.E.	34.9 ± 3.3	33.1 ± 7.0	35.5 ± 6.9

* Individual protein values represent a mean of three experiments.

APPENDIX E

POST HOC. TREATMENT ON ENZYME ACTIVITY

TABLE 9

Post Hoc Treatment on Enzyme Activity Expressed as μmoles/min/g (EA) and as μmoles/min/mg protein (SEA): Contrasts Between Similar Muscle Types of Each Treatment Group.

		ESTIMATED CONTRAST		RANGE (±)	TEST
1) EXP vs CON					
	a) EA	R vastus	.747	1.41	Wilks
		Soleus	1.363	1.376	"
		W vastus	.236	.75	"
		Heart	.549*	.50	"
	b) SEA	R vastus	.035	.075	Pillai
		Soleus	.048	.062	"
		W vastus	.011	.048	"
		Heart	.016	.027	"
2) EXP vs SED					
	a) EA	R vastus	.356	1.66	Pillai
		Soleus	.622	1.62	"
		W vastus	-.460	.89	"
		Heart	-.13	.59	"
	b) SEA	R vastus	.011	.075	Pillai
		Soleus	.028	.062	"
		W vastus	-.025	.048	"
		Heart	-.009	.027	"
3) SED vs CON					
	a) EA	R vastus	.319	1.73	Pillai
		Soleus	.740	1.69	"
		W vastus	.642	.93	"
		Heart	.68*	.62	"
	b) SEA	R vastus	.024	.058	Wilks
		Soleus	.020	.047	"
		W vastus	.036	.041	"
		Heart	.025*	.023	"

* Significant, $p < .01$

TABLE 10

Post Hoc Treatment on Enzyme Activity Expressed as μmoles/min/g (EA) and as μmoles/min/mg protein (SEA): Contrasts Between Muscle Types of Same Treatment Group. *

GROUP		ESTIMATED CONTRAST		RANGE (±)	TEST
Experimental					
	1) EA	RV vs S	.521	1.89	Pillai
		RV vs WV	2.904*	1.62	"
		RV vs H	3.051*	1.52	"
		S vs WV	2.382*	1.28	"
		WV vs H	.148	.89	"
		S vs H	2.53*	1.49	"
	2) SEA	RV vs S	.006	.061	"
		RV vs WV	-	-	
		RV vs H	.097*	.069	"
		S vs WV	.082*	.046	"
		WV vs H	.009	.053	"
		S vs H	.091*	.053	"
Sedentary					
	1) EA	RV vs S	.788	1.70	"
		RV vs WV	2.142*	1.45	"
		RV vs H	2.565*	1.36	"
		S vs WV	1.354*	1.15	"
		WV vs H	.423	.79	"
		S vs H	1.77*	1.33	"
	2) SEA	RV vs S	.023	.053	"
		RV vs WV	-	-	
		RV vs H	.077*	.061	"
		S vs WV	.029	.038	"
		WV vs H	.025	.046	"
		S vs H	.054*	.046	"
Control					
	1) EA	RV vs S	1.137	1.52	"
		RV vs WV	2.393*	1.36	"
		RV vs H	2.854*	1.36	"
		S vs WV	1.256*	1.15	"
		WV vs H	.46	.79	"
		S vs H	1.717*	1.33	"
	2) SEA	RV vs S	.019	.053	"
		RV vs WV	-	-	
		RV vs H	.078*	.061	"
		S vs WV	.045*	.038	"
		WV vs H	.014	.046	"
		S vs H	.059*	.046	"

* RV (red vastus), S (soleus), WV (white vastus), H (heart)