SKELETAL MUSCLE METABOLISM AND PERFORMANCE DURING HEAVY MUSCULAR CONTRACTION IN THE ISOLATED PERFUSED RAT HINDQUARTER

by

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ABSTRACT

Direct assessments of the relative contributions of the major energy releasing pathways in human skeletal muscle during heavy exercise are difficult to obtain due to the invasive measurements required. With an isolated muscle preparation the muscles' environment is carefully controlled and all metabolic measurements are directly obtained. For this reason the isolated perfused rat hindquarter model, previously used to study resting muscle metabolism, was developed to examine the metabolism and performance of heavily contracting skeletal muscle.

Energy calculations based upon measurements of O_2 uptake (aerobic metabolism), lactate production (anaerobic glycolysis) and CP hydrolysis (alactic anaerobiosis) were made during 20 minutes of repetitive tetanic stimulation. During the initial 5 minutes of stimulation isometric tension production was high but fatigued rapidly and anaerobic involvement in energy production was large (30%), especially in the fast-twitch glycolytic muscle fibers. Muscle glycogenolysis provided the majority of substrate for both anaerobic glycolysis and aerobic metabolism. During the final 15 minutes of stimulation aerobic metabolism dominated (90%) while 60% of peak tension was held, mainly by the fast-twitch oxidative, glycolytic muscle fibers. Glycogen utilization was minimal and intramuscular triacylglycerol became the dominant fuel for oxidative metabolism,

contributing 62% of the energy produced.

Perfusions with acidotic mediums (metabolic and respiratory) reduced muscle glycogenolysis and lactate accumulation by 35% during the initial 5 minutes of stimulation. The decreased glycolytic flux reduced the availability of carbohydrate substrate for aerobic metabolism and O_2 uptake decreased. The associated reduction in energy release produced an increased rate of tension decay. Total energy release and tension production were also reduced during acidosis in the final 15 minutes of stimulation. The decreased glycolytic flux appeared to be due to an earlier fall in muscle pH during acidosis and subsequent inhibition of key regulatory enzymes such as phosphorylase and phosphofructokinase. However an alternate hypothesis is that acidosis exerted a direct negative effect on the excitation-contraction coupling mechanism, thereby reducing the need for energy production.

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1. HISTORICAL INTRODUCTION

1.1 Introduction

Man is capable of performing large amounts of external work through the conversion of chemical to mechanical energy in the skeletal muscles of the body. The required chemical energy is ultimately provided through the ingestion of foodstuffs and their subsequent uptake and metabolism by the muscles. During periods of physical exertion the metabolism of the entire body is dominated by the metabolism of the working muscles.

The study of muscle metabolism originated soon after the discovery and isolation of oxygen (O_2) in the late eighteenth century and the subsequent development of methods to examine the amount of substances taken up and released by living organisms (Asmussen, 1971). Over the course of the next century work centered on attempts to determine the nature of the foodstuffs responsible for the energy utilized at rest and during physical work. Studies by Pettenkofer and Voit (1862) examining the urinary nitrogen (N_2) output suggested that protein was not a major source of fuel during work (Asmussen, 1971). Zuntz and colleagues (1894) believed that work was performed through the combustion of both fat and carbohydrate fuels as at rest, while Chauveau and

coworkers (1896) maintained that carbohydrate was the sole energy source for muscular contraction (Asmussen, 1971). Both groups arrived at their conclusions through measurements of the respiratory exchange ratio (R), defined as the ratio of carbon dioxide (CO_2) produced over O_2 utilized by the body as measured indirectly from the inspired and expired air. Direct measurements of the CO_2 produced and O_2 utilized during complete combustion of carbohydrate and fat produced ratios of 1.0 and 0.7, respectively. When direct measurements are made the ratio is referred to as the respiratory quotient (RQ). With the beginning of the twentieth century this uncertainty persisted and would not be resolved for another 40 years.

At the same time a new and more direct line of research in muscle metabolism began with the classic study of Fletcher and Hopkins (1907). Using improved techniques for extracting lactic acid from amphibian muscle they demonstrated the existence of lactic acid in surviving resting muscle and the production of lactic acid by working muscle. An insufficient O_2 supply to resting or working muscle increased lactic acid production while the administration of O_2 caused the lactic acid to disappear. Muscle fatigue was always greatest in the presence of lactic acid and least in its absence. Fletcher and Hopkins (1907) also maintained that carbohydrate, in the form of glycogen was the precurser of lactic acid formation.

In the following 20 years work in muscle physiology and metabolism was dominated by two individuals; Otto Meyerhof working in Germany and A.V. Hill in England, who shared the 1922 Nobel prize for physiology for their work with frog muscles. Meyerhof integrated his chemical findings with the thermodynamic and mechanical findings of the time to formulate an hypothesis explaining the physiology of muscular contraction (Sacks and Sacks, 1933). Hill combined his myothermic findings with those of the biochemists and published his explanation of muscular exertion (Hill and Lupton, 1923; Hill et al, 1924). The Hill-Meyerhof theory seemed to explain most of the mechanical and metabolic changes produced by muscular contraction.

1.2 The Hill-Meyerhof Theory of Muscle Metabolism

The primary event in a contracting muscle was held to be the anaerobic breakdown of glycogen to lactic acid which evoked the muscle's mechanical response. During exercise CO_2 was driven off, the heat release was proportional to the lactic acid production and the hydrogen ion (H^+) content of the muscle increased. This acidification was mentioned as the possible mechanism for the activation of the contractile proteins. During recovery in the presence of O_2, approximately one-fifth of the produced lactic acid was oxidized, to provide the energy for the reconversion of the remaining lactic acid to glycogen. During recovery from

exercise the O_2 uptake did not immediately return to resting levels. This additional O_2 uptake was referred to as the O_2 debt since it was used to remove the lactic acid which had accumulated early in the exercise before the circulation responded to the work load by increasing O_2 delivery. In prolonged exercise a steady state was attained when available lactic acid production matched the aerobic removal, resulting in a constant blood lactic acid concentration.

Although Hill and Lupton (1923) were aware that O_2 was ultimately used in the combustion of foodstuffs to supply the energy required for body functions, they felt it was not used in the primary breakdown processes, only in the recovery processes. Additionally, at that time most researchers believed carbohydrate was the sole precursor for energy production and lactic acid was a central intermediary in metabolism with points of contact with carbohydrate, fat and protein metabolism (Jervell, 1923).

1.3 Phosphate Compounds in Muscle

While the Hill-Meyerhof theory dominated the thinking concerning skeletal muscle physiology for a number of years, evidence was accumulating to suggest the theory was incorrect or too simplistic. Two groups of investigators working independently published results demonstrating the existence of a phosphate compound as a substance in muscle. Eggleton and Eggleton (1927) in London improved the existing methods

for extracting phosphates from muscle enabling the measurement of both inorganic and organic phosphate compounds. Previous extraction methods degraded the unstable organic phosphates to inorganic phosphate (Pi). Work producing rapid fatigue in frog muscles decreased organic phosphate and increased Pi levels. Comparisons of heart, skeletal and smooth muscles suggested that phosphagen levels correlated with the muscle's ability to respond to sudden demands for violent activity. Fiske and Subbarow (1927) in Boston also noted the importance of quickly neutralizing acid muscle extracts to ensure accurate measurements of organic phosphates. They reported decreasing organic phosphates with fatiguing contractions in frog muscle and a total depletion during stimulation with muscle blood flow occluded. The compound in question was identified as a derivative of creatine and labelled creatine phosphate (CP). Following contractions CP stores were resynthesized quickly in the presence of O_2. These discoveries suggested that CP degradation and not lactic acid formation from glycogen might be the immediate precursor of the mechanical events of muscular contraction.

The largest setback to the lactic acid theory of energy provision came from the findings of Lundsgaard (working in Meyerhof's laboratory in 1930), who stimulated frog muscles bathed in iodoacetic acid, a compound which prevents the formation of lactic acid (Margaria, 1933).

Under anaerobic conditions the muscles were able to produce a series of contractions. This work, as summarized by Sacks and Sacks (1933), clearly showed that lactic acid production was not the event directly linked to muscular contraction, but more likely served to resynthesize CP stores which subsequently provided both the energy and stimulus for muscular contraction.

However, Lohmann (Bessman and Geiger, 1981) and Fiske and Subbarow (1929) both discovered the presence of an additional high energy phosphate compound in muscle, adenosine triphosphate (ATP). In 1934, Lohmann demonstrated, using dialyzed muscle extracts, that CP breakdown occurred only when adenosine diphosphate (ADP) was present to accept the phosphate from CP, producing ATP and creatine (Bessman and Geiger, 1981). Consequently, it appeared that CP stores were used to replenish ATP levels which transferred energy directly to the contractile mechanism, although an earlier study reported no decrease in ATP concentration with muscular stimulation producing tetanus (Sacks and Sacks, 1933). Englehardt and Ljubimowa (1939) supported this premise by reporting that myosin, a contractile constituent of muscle fibrils, possessed the enzyme ATPase which hydrolyzes ATP to ADP and Pi (Asmussen, 1971). Cain and Davies (1962) conclusively established that ATP was the immediate source of energy for muscular contraction by chemically inhibiting CP hydrolysis and demonstrating a decreased ATP concentration

following a single contraction.

1.4 Additional Findings Contradicting the Hill-Meyerhof Theory

In the thirties, other lines of research produced results suggesting that additional aspects of the Hill-Meyerhof theory of energy provision for muscular contraction were untenable. Studies by Cori and Cori (1929), Eggleton and Evans (1930b), and Himwich, Koshoff and Nahum (1930) examined the fate of muscle lactate that diffused into the circulation. If the Hill-Meyerhof concept was correct and all lactic acid was oxidized or reconverted to glycogen only in muscle, the escaped lactic acid should be taken back up by the same muscles or by other muscles not involved in its production. However, the majority of circulating lactate was taken up by the liver and converted to glycogen. Since it was known that circulating glucose originated from the liver some of the lactic acid converted to glycogen was released as glucose. This glucose could be taken up by the muscle and ultimately produce lactic acid, completing a cycle between skeletal muscle and liver which became known as the Cori cycle. Resting muscles also took up smaller amounts of lactic acid, presumably most being oxidized since little or no increase in muscle glycogen occurred. Infusions of lactic acid also produced no increase in muscle glycogen suggesting that muscle oxidized all it took up and other organs

contributed significantly to blood lactic acid removal (Himwich, Koshoff and Nahum, 1930; Eggleton and Evans, 1930a; Sacks and Sacks, 1935).

Owles (1930), Margaria (1933), and Bang (1936) all reported little or no lactic acid increase in blood during light to moderate exercise in humans. Lactic acid began to accumulate in the blood at a threshold coinciding with an O_2 uptake representing two-thirds of the maximal O_2 uptake. By assuming low blood lactic acid levels reflected low muscle concentrations during light exercise, these authors postulated that aerobic processes provided the energy for mechanical work during this type of exercise. Sacks and Sacks (1933) examined both muscle and blood lactic acid levels during various forms of contractions using an isolated rabbit gastrocnemius preparation which enabled muscle to be frozen "in situ". During moderate prolonged stimulation no lactic acid appeared in the working muscles or blood while muscle glycogen decreased. The authors interpreted these findings as evidence that glycogen derivatives were directly oxidized to provide the energy for muscular work. These findings replaced the earlier concept that O_2 was used only in metabolic processes of recovery, with the belief that the major energy releasing reactions in muscle were aerobic and not anaerobic. Anaerobic energy production occurred only when the O_2 supply was inadequate, serving as a stopgap during periods of circulatory adjustment or insufficiency.

Owles (1930) even had the foresight to suggest that lactic acid could appear in well-oxygenated muscle if some fibers had large O_2 diffusion distances.

Hill and Lupton (1923) described the O_2 debt as an expression of an oxidative process in which the energy liberated was repaying the amount of energy set free anaerobically during muscular contractions. According to this theory the extra O_2 utilized above rest values during recovery was the O_2 debt since it served to reconvert lactic acid to glycogen through the combustion of a small fraction of the acid. Margaria (1933) revised our understanding of the O_2 debt with three main findings. Firstly, during prolonged moderate exercise a 3 l. O_2 debt was incurred yet neglible changes in blood lactic acid and pH were seen. Secondly, the regeneration of CP stores was known to occur following exercise in the presence of O_2 and thirdly, distinct fast and slow phases in the return of O_2 uptake to rest levels were seen. The initial phase was present after every bout of exercise, unrelated to the lactic acid concentration and during this phase O_2 uptake decreased rapidly. This phase was labelled the "alactacid O_2 debt" and appeared to account for the oxidative regeneration of CP stores. The second phase was slower and prolonged, responsible for the oxidative removal of lactic acid and referred to as the "lactacid O_2 debt". Additionally, Hill and Meyerhof had reported that for every 5-6 g of lactic acid

reconverted to glycogen, 1 g was oxidized but Margaria (1933) found this combustion coefficient to be nearer 10 to 1.

1.5 Summary

By the late thirties the Hill-Meyerhof theory no longer adequately explained how muscles provided the chemical energy required to produce external work. The major drawback to the theory was not that all its postulations were wrong but that it was far too simplistic and incomplete. For example, the theory stated that all energy was derived from the anaerobic breakdown of glycogen to lactic acid. Although, anaerobic glycolysis was confirmed as an important energy pathway in muscle in certain situations, aerobic metabolism was identified as the dominant pathway of energy provision in most activities common to man. Additionally, anaerobic metabolism was shown to include an alactacid component due to the hydrolysis of CP which regenerated muscle stores of ATP. The integration of aerobic and anaerobic metabolic reactions were designed to provide a continual supply of ATP, the immediate source of energy in the muscle. Aerobic metabolism dominated when exercise was of low to moderate intensity in the presence of O_2 while CP hydrolysis occurred at the onset of physical exertion at all intensities while the circulaton adjusted to the increased demands for O_2. Anaerobic glycolysis also contributed to the energy production at the onset of exercise, increasing in

importance with the severity of exercise. Lactic acid production from anaerobic glycolysis was also of major importance during exercise at and above two-thirds of the maximal O_2 uptake, presumably due to an inadequate O_2 supply to the muscles. Keul et al (1972) provided a schematic summary of the relative contributions of the energy releasing pathways to the total supply during exercise (Fig. 1). The initial work by Hill and Meyerhof had stimulated researchers to uncover the major reactions or pathways which provided the energy for muscular contraction. With these discoveries, research efforts shifted to examine the nature of the foodstuffs or fuels metabolized by the resting and working muscles.

1.6 Fuel Sources for Muscle Metabolism

1.6.1 Early Research

At the outset of the twentieth century the controversy over the nature of the fuel(s) responsible for providing the energy for muscular work was unresolved; was carbohydrate the sole energy source or was fat also utilized directly or only following initial conversion to carbohydrate? Benedict and Cathcart (1913) found a lower R during cycle exercise when body carbohydrate stores were reduced following a low carbohydrate diet and higher R values following a carbohydrate rich diet (Asmussen 1971). Also, R

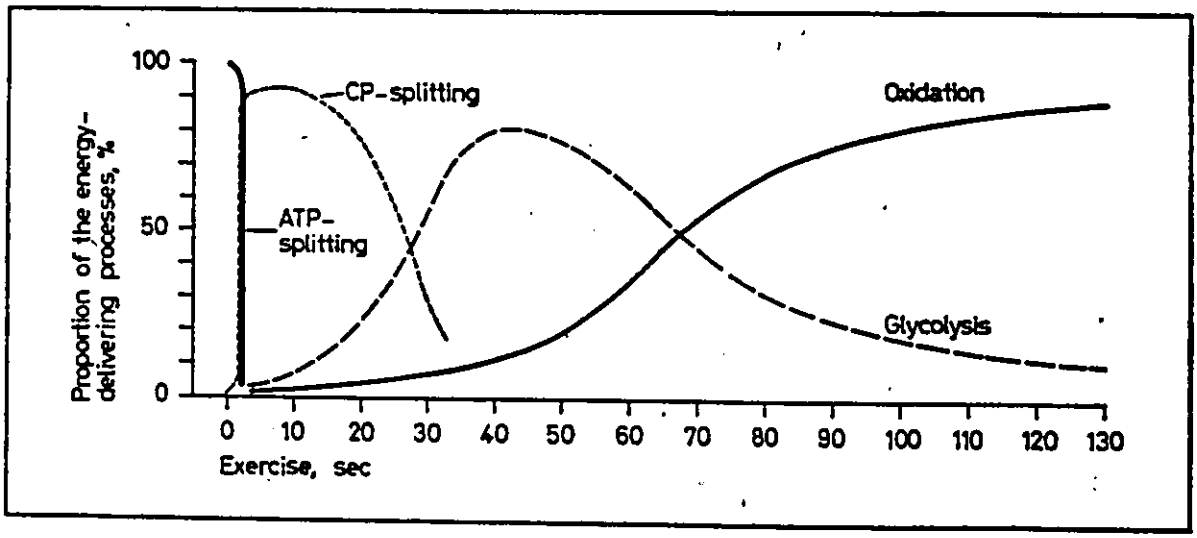


Fig. 1. Schematic representation of the proportion of the major energy supplying pathways to the total energy supply with strenuous exercise (Keul et al, 1972).

continued to increase over resting levels with increasing severity of work. Krogh and Lindhard (1920) using improved methods for gas analysis concluded that both fats and carbohydrates were used as fuel at rest and during exercise (Asmussen, 1971). Again R varied as a function of the substrate availability. Measured in terms of O_2 utilized/energy released, fat was 11% less efficient than carbohydrate presumably due to the conversion of fat to carbohydrate prior to catabolism. Support for the fat to carbohydrate conversion cnncept came from the earlier findings of Fletcher and Hopkins (1907) and the later theory of Hill and Lupton (1923) suggesting that the breakdown of carbohydrate to lactic acid was the immediate energy source for muscular contraction. In 1928, Jervell summarized the feelings of most investigators of his day by writing, "The metabolism (conversion of energy) in the muscle is bound up with the breakdown of carbohydrate. For, according to the modern conception, fat and proteins cannot be utilized as energy-producing material for the muscles without first being converted into carbohydrate."

It remained for Christensen and Hansen (1939), in a series of classic papers, to reaffirm the thought that both fat and carbohydrate were used as immediate fuels for muscular exercise (Gollnick, 1971). Although their results were also based upon the indirect method of measuring R, their great regard for standard conditions and attainment of

steady state conditions free from anaerobic metabolism produced many important findings; First, in work of light to moderate intensity of short duration the relative amounts of fat and carbohydrate utilization depended mainly on the diet. Second, as work of light to moderate intensity was increasingly prolonged, the relative energy contribution from fat was increased due to a decreased carbohydrate supply. A high fat diet (lower body carbohydrate stores) decreased the ability to perform this type of work and fatigue coincided with hypoglycemia, apparently due to liver glycogen depletion. This finding led to the belief that liver glycogen was the main source for aerobic muscular exercise of this intensity. Muscle glycogen was important mainly during anaerobic work. Third, as the intensity of exercise increased above a certain workload, the contribution from carbohydrate began to increase, reaching nearly 100% at maximal work intensities. This workload above which carbohydrate utilization became more important was dependent on the working capacity of both the subject and the specific muscle group doing the work (this workload was reached at a lower O_2 uptake in arm vs leg work). The workload also coincided with the intensity at which blood lactate began to accumulate.

However, during heavy exercise the CO_2 released by the body is the sum of the aerobically produced CO_2, the CO_2 released from body CO_2 stores and the CO_2 produced through

the bicarbonate buffering of H^+ derived from lactic acid production. This makes it difficult to interpret the relative amounts of fat and carbohydrate used in the aerobic portion of the exercise, from the measurements of R.

1.6.2 Circulating Free Fatty Acids

In the years following Christensen and Hansen's findings, efforts centered on confirming their results with direct and quantitative measurements of fat utilization by resting and exercising muscle. In the 1950's two key findings implicated free fatty acids (FFA) as the major circulating fat utilized as fuel in muscles; starvation caused an increase in plasma FFA and FFA turnover was very rapid even though its concentration was low (Havel et al, 1980). Dole (1956) employed an improved method for the measurement of FFA and reported fluctuations in plasma FFA which followed changes in the nutritional state. Fasting increased FFA while a subsequent meal and infusions of glucose or insulin decreased the fasted levels. The infusion of norepinephrine (NE), a known stimulator of adipose tissue triacylglycerol (TG) lipolysis to FFA and glycerol, increased plasma FFA. Since alterations in the FFA concentration occurred rapidly and were reciprocal to the changes in glucose concentration, Dole (1956) suggested FFA entered a metabolic process common to fat and carbohydrate. Andres et al (1956) examined the metabolism of resting human forearms

and found glucose uptake could account for only 10-20% of the total oxidative metabolism. Since the forearm RQ was 0.80 and proteins and ketone bodies were not metabolized, they reasoned that fats provided the bulk of the fuel for aerobic metabolism. However, forearm FFA extraction was difficult to detect, probably due to the error of existing FFA measurement techniques, the high energy value of lipids and the low resting oxidative metabolism.

Infusions of ^{14}C labelled fatty acids (palmitate, linoleate and oleate) and the rapid appearance of $^{14}CO_2$ in the expired air of humans demonstrated the ability of the body's tissues to metabolize FFA even though plasma concentrations changed little (Fredrickson and Gordon, 1958a). The authors (1958b) reviewed the current knowledge regarding fatty acids and drew a number of conclusions. First, FFA were carried in plasma bound to the protein albumin with a maximum transport capacity of approximately 2 mM presumably due to saturation of the albumin molecules with FFA. Second, the release of FFA from adipose tissue was the quantitatively important source of plasma FFA. Third, all major tissues except the brain appeared capable of extracting and metabolizing circulating FFA. Finally, esterified FFA in the form of circulating TG did not contribute significantly to the plasma FFA level or the metabolism of body tissues.

Havel and Goldfien (1959) demonstrated that activation of the sympathetic nerve endings in adipose tissue

and the release of NE rapidly increased FFA mobilization. Blocking the sympathetic nerve endings decreased plasma FFA but increasing circulating levels of NE could increase plasma FFA in spite of the blockage. Other investigators reported the FFA mobilizing effect of NE and also the inhibitory effect of glucose and insulin which decreased adipose tissue lipolysis directly and/or increased the reesterification of FFA (Carlson et al, 1963a; Carlson, 1965). The net mobilization of FFA from adipose tissue was dependent on the hydrolysis of TG to FFA and glycerol and the reesterification rate of FFA and alpha-glycerophosphate to TG. (Each mole TG releases 3 moles FFA and 1 mole glycerol). Using infusions of radiolabelled palmitate and glycerol Havel and Carlson (1963) demonstrated that the ratio of FFA/glycerol released from adipose tissue to plasma was 3 and therefore stoichiometrically equivalent. This ratio remained constant following a NE infusion but was significantly reduced following the addition of insulin and glucose. The primary inhibitory effect of insulin and glucose on FFA mobilization was an enhanced FFA reesterification secondary to an increased adipose tissue glucose uptake and provision of the glycolytic intermediate alpha-glycerophosphate, a precursor of TG synthesis. These results collectively confirmed the earlier suppositions by Wertheimer and Shapiro (1948) that fat metabolism and deposition were active ongoing processes governed by nervous and endocrine factors.

The advent of radiolabelled FFA tracer techniques permitted direct measurements of plasma FFA turnover rates and fractional uptakes by the entire body or isolated muscle groups at rest and during exercise. Exercise increased the turnover rate and fractional uptake of FFA in fasted and fed athletes (Havel et al, 1963). At the onset of prolonged low intensity exercise FFA efflux from plasma to the tissues (dominated by skeletal muscle) increased quickly while FFA influx from adipose tissue to plasma increased more slowly, resulting in an increasing turnover rate in the first hr. In a subsequent study these results were confirmed for untrained subjects and changes in the tracer palmitate were shown to accurately represent the metabolic changes in the total FFA pool (Havel et al, 1964). Using similar techniques, Issekutz et al (1964) also reported FFA uptake increased in exercising dogs, as a function of the increasing plasma FFA concentration. Havel et al, (1967) measured the uptake and release of FFA across the legs of fasted men cycling for 90-120 min at an O_2 uptake of 1.0 l. The fractional extraction of FFA was lower during exercise than at rest but the total delivery was greater due mainly to the increased blood flow, resulting in an increased turnover rate. Leg FFA fractional extraction was greater than that of the whole body and the output of radiolabelled CO_2 nearly equalled the input from plasma FFA within 1 hr. Therefore in working legs, FFA uptake was roughly equivalent to utilization. It

also appeared that a maximal capillary transport for FFA existed in muscle during exercise, similar to that for other readily diffusable substances. Later studies reaffirmed these findings of direct FFA utilization by working skeletal muscle in dogs (Paul, 1970) and humans (Hagenfeldt and Wahren, 1968; Ahlborg et al, 1974). Hagenfeldt and Wahren (1971) reported a linear relationship between muscle FFA delivery and FFA uptake (arterial concentration x muscle plasma flow) at rest and exercise. They found no evidence for saturation of the plasma to muscle FFA diffusion process over a wide range of FFA inflows. Therefore in exercise where muscle blood flow is constant, the factors regulating plasma FFA concentration determine muscle uptake and utilization. Since adipose tissue is the major source of FFA, these factors include local NE release from sympathetic nerves, circulating levels of NE and epinephrine, plasma glucose and insulin concentrations and adipose tissue blood flow (Mayerle and Havel, 1969; Larsen et al, 1981).

1.6.3 Intramuscular Triacylglycerol

The radiolabelled FFA turnover studies quantified the metabolic importance of FFA during prolonged low to moderate intensity exercise (Havel et al, 1963, 1967; Hagenfeldt and Wahren, 1968). These studies also noted that the oxidation of plasma FFA by the muscle accounted for only 50% of the total fat metabolism. This suggested that intramuscular

stores of triacylglycerol (TG) may be hydrolyzed to provide FFA for aerobic metabolism during this type of work.

Froberg (1971) subjected rats to 3 hr of low intensity treadmill running and directly measured decreases of 44 and 31% in red and white gastrocnemius TG, respectively. Baldwin et al (1973) also found 2 hr of running produced a 40% decrease in the TG of the red vastus lateralis, which possesses both high oxidative and glycolytic capacities. No changes were found in the glycolytic white muscle or highly oxidative red soleus. Prolonged low to moderate intensity swimming (2-5 hr) also produced utilization of skeletal muscle TG in rats (Reitman et al, 1973; Stankiewicz-Choroszucha and Gorski, 1977; Oscai et al, 1982). Red vastus lateralis TG decreased by 44-68% and soleus levels dropped 20-25%.

Several direct measurements of intramuscular TG were made on men exercising for prolonged periods (60-150 min) at 55-80% of their maximal O_2 uptakes (VO_2max). Ski racing for 7 hr at an average heart rate of 156 beats.min^{-1} depleted vastus lateralis TG by 50% (Froberg and Massfeldt, 1971) while 100 min of cycling at 67% VO_2max produced a 25% drop in TG levels of the same muscle (Froberg et al, 1971). Costill et al (1973) found vastus lateralis TG decreased 31% following a 30 km foot race which required participants to run at 65-80% VO_2max for 147 min. Even cycling for 60 min at 55% VO_2max decreased muscle TG by 34% (Essen, 1978).

Animal and human studies suggest that intramuscular fat may also be utilized during heavy exercise. Froberg et al (1971) reported decreases of 25% in both white and red hindlimb muscles of rats electrically stimulated at 5 Hz for 2 hr. Barclay and Stainsby (1972) demonstrated that dog hindlimb TG was metabolized only when the muscle's metabolic rate was increased 15-20 fold above rest. This corresponded to a stimulation rate of 5 Hz and produced a 20% decrease in 1 hr. Therriault et al (1973) ran dogs at various workloads for 60-75 min and reported decreases in biceps femoris TG only after heavy work. Direct evidence of intramuscular TG metabolism during heavy exercise in humans was presented by Essen (1978). Continuous intense exercise producing maximal O_2 uptakes and intermittent exercise at the same workload (15 sec work, 15 sec rest) produced respective decreases in vastus lateralis TG of 27 and 20% in just 5 min. Continuous exercise of moderate intensity (55% VO_2max) for 5 min also produced a 20% decrement. Jones et al (1980) provided evidence suggesting that intramuscular TG was utilized during heavy exercise (70% VO_2max) lasting 40 min. This exercise intensity in untrained men required a heavy anaerobic energy component as judged by the high plasma lactates (10 mM). The elevated lactate concentration coincided with a lower plasma FFA concentration and decreased FFA turnover rate. Low FFA levels were reported earlier in dogs during heavy exercise (Issekutz et al, 1963) and humans exercising at 85-90% VO_2max

(Pruett, 1970). The decreased plasma FFA concentraton was due to a fall in the FFA influx to the plasma pool, lending support to an earlier hypothesis that high lactate levels inhibit the mobilization of adipose tissue FFA (Issekutz and Miller, 1962; Fredholm, 1971). However, the plasma glycerol concentration continued to increase throughout the exercise, prompting Jones et al (1980) to suggest the glycerol originated in the working muscle as a result of endogenous TG metabolism while the released FFA were metabolized locally.

Therefore, evidence does exist suggesting that intramuscular fat stores contribute significantly to the energy metabolism of working muscle during all intensities of exercise. However, the number of investigations examining the metabolic role of this fuel are surprisingly few. This may be partly due to the variability of measuring TG due to its inhomogeneity in skeletal muscle. Hence our knowledge of the involvement of intramuscular TG in many forms of activities and of the regulation of its metabolism is extremely limited.

1.6.4 Circulating Glucose

The work of Christensen and Hansen (1939) implied that circulatory glucose originating from liver glycogen was the major carbohydrate source for prolonged aerobic work. Although it was known that muscle took up glucose from the blood (Reichard et al, 1961; Sanders et al, 1964), the

quantitative importance of blood glucose was not examined until the work of Wahren and colleagues (1971a, b). Arterial and venous cannulations of the appropriate vessels and blood flow measurements permitted the measurement of the uptake or release of several metabolites by the liver and working muscles of exercising men. In arm work of moderate intensity lasting 60 min exogenous glucose accounted for nearly all of the carbohydrate oxidation and 50% of the total oxidative metabolism. Arterial glucose concentration remained constant while forearm blood flow and extraction increased. During leg exercise for 60 min at low to moderate intensities glucose uptake accounted for 60-80% of the carbohydrate oxidation and 25-30% of the total oxidative metabolism. In heavy exercise, with no net lactate accumulation in the plasma, exogenous glucose provided 60% of the carbohydrates burned aerobically and up to 40% of the total aerobic metabolism. Ahlborg et al, (1974) also examined the glucose uptake of leg muscles exercised for 4 hr at 30% VO_2max. Blood glucose oxidation by the muscles accounted for 27, 41, 36 and 30% of the O_2 uptake after 40, 90, 180 and 240 min of exercise, respectively. Arterial glucose concentration fell by 30% after 4 hr suggesting liver glucose output was less than muscle utilization. These findings demonstrated the quantitative importance of exogenous glucose to total muscular energy production during low to moderate intensity work.

Liver glycogen was very low or depleted following prolonged bouts of exercise (Hultman and Nilsson, 1971; Baldwin et al, 1973). The liver attemps to cope with glycogen depletion by enhancing the production of de-novo glucose (gluconeogenesis). In the study by Ahlborg et al (1974), the portion of liver glucose output derived from gluconeogenesis increased from 20-25% at rest and following 40 min of exercise to 45% after 4 hr. At the onset of heavy exercise gluconeogenesis contributed 20% of the total liver glucose output but only 6% following 40 min (Wahren et al, 1971a).

In these studies the major gluconeogenic precursors were lactate, pyruvate, glycerol and selected amino acids. Felig and Wahren (1971) reported that alanine was the major amino acid released by muscle (40% of total) and taken up by the liver (50% of total) at rest. Mild and heavy exercise produced a 2 fold increase in alanine release from muscle while liver uptake increased 60% in mild exercise and 10% in heavy exercise. The increase in hepatic uptake occurred via an enhanced fractional extraction as hepatic blood flow was greatly reduced. The authors postulated the existence of a glucose-alanine cycle between muscle and liver; glucose from blood or muscle glycogen is metabolized to pyruvate in muscle, transamination of pyruvate produces alanine which diffuses into the circulation, alanine is taken up by the liver and reconverted to glucose and released into the

circulation to complete the cycle.

Felig and Wahren (1971) estimated that alanine release may account for 12-18% of the glucose taken up by resting muscle but recent work has questioned the quantitative significance of this cycle (Newsholme and Leech, 1983). Grubb (1976), using radiolabelled glucose and alanine, reported that 33% of the alanine released from muscle was derived from glucose but this represented only 2.7% of the total glucose taken up at rest. Recent work by White and Brooks (1981) showed that alanine and leucine oxidation also increased with the metabolic rate during exercise and contributed to energy production without prior conversion to glucose but again the quantitative significance of these pathways was small. Additionally, glutamine appears to be released from skeletal muscles and taken up by the liver in significant amounts (Newsholme and Leech, 1983).

1.6.5 Intramuscular Glycogen

Early attempts to identify the major energy sources in exercising men with O_2, CO_2 and R measurements were only capable of estimating the relative amounts of total fat and carbohydrate used (Christensen and Hansen, 1939; Hedman, 1957). Animal studies had shown that muscle glycogen was utilized during exercise (Sacks and Sacks, 1933). A study with radiolabelled glucose demonstrated that circulating

glucose and an additional carbohydrate, presumably muscle glycogen were important during exercise (Reichard et al, 1961). The reintroduction by Bergstrom (1962) of the needle biopsy technique for sampling of skeletal muscle permitted the direct examination of the importance of muscle glycogen during exercise.

Studies employing the biopsy technique produced several important findings (Bergstrom and Hultman, 1967; Bergstrom et al, 1967; Hultman, 1967; Hultman et al, 1971). Starvation for 10 days produced only a 30-40% decrease in muscle glycogen stores and following exercise that depleted muscle glycogen, resynthesis to pre-exercise levels occurred in 24 hr when a carbohydrate rich diet was given. Further increases up to 2-3 times normal values were found over the course of the next 8-10 days and a carbohydrate diet without prior exercise did not enhance normal glycogen levels. During exercise, non-working muscles did not release glucose into the circulation or reveal any glycogen depletion. Glycogen utilization was positively correlated to the work intensity and related to a subject's relative workload not absolute workloads. Infusions of glucose decreased the glycogen utilization by 25% during 60 min of moderate to heavy exercise and the maximal rate of glycogen resynthesis was only 1% of the maximal glycogenolytic rate found in heavy exercise.

Additional studies closely examined the rates of

glycogen utilization at various intensities maintained for as long as possible (Hermansen et al, 1967; Saltin and Karlsson, 1971). The corresponding rates of glycogen utilization during exercise at 25, 50, 75, 100, 125 and 150% VO_2max were 0.3, 0.7, 1.4, 3.4, 6.0 and 10.0 mmoles glucosyl units.kg^{-1} wet muscle.min^{-1}. Since normal resting glycogen levels were 80-100 mmoles.kg^{-1} in the quadriceps femoris muscle of the leg, more than one-tenth of the muscle's glycogen store was used in 1 min of supramaximal exercise. Glycogen depletion correlated with exhaustion only at workloads between 65 and 89% of VO_2max. Bergstrom et al, (1967) reported a good correlation between work performance times at 75% VO_2max and the initial muscle glycogen levels, varied through diet manipulation. These studies directly demonstrated the major importance of carbohydrates stored in the muscle for energy provision during heavy exercise, supporting the indirect findings of Christensen and Hansen (1939).

1.6.6 The Interaction of Fat and Carbohydrate Fuels in Muscle

During most forms of exercise the majority of the required muscular energy is derived through the oxidation of carbohydrate and fat. Carbohydrate fuel is obtained from glycogen stored in the muscle or from circulating glucose released from liver glycogen stores. Similarly, fat is provided from intramuscular stores and from the blood as FFA,

following its release from the body's adipose tissue. There are many factors which determine the relative proportion of each fuel utilized during aerobic metabolism, such as the diet, the intensity, type and duration of exercise, hormonal status, O_2 availability, blood flow, state of aerobic training and intramuscular acidity.

Numerous studies have noted the effects of dietary manipulation on the relative utilization of fat and carbohydrate during exercise through R measurements or direct measurements of exogenous and endogenous fuels (Christensen and Hansen, 1939; Bergstrom et al, 1967; Bergstrom, 1969; Gollnick, 1972; Jansson and Kaijser, 1982). Generally the ingestion of carbohydrate rich diets will increase R and result in greater utilization of glucose and/or muscle glycogen. Fat rich diets decrease R and increase the proportion of fuel derived from circulating fats. In situations of reduced carbohydrate availability (prolonged exercise), FFA levels and utilization were elevated and/or intramuscular fat was metabolized (Baldwin et al, 1973). When FFA levels were elevated through artificial means (caffeine, heparin or FFA infusions) glucose uptake and muscle glycogen depletion rates were decreased during exercise. This demonstrated a carbohydrate sparing effect of plasma FFA (Carlson et al, 1963b; Rennie et al, 1976; Rennie and Holloszy, 1977; Costill et al, 1977). Randle et al (1963, 1964) demonstrated this effect in heart and diaphragm

muscle. When FFA are available they are oxidized producing elevated citrate levels which directly inhibit the glycolytic enzyme phosphofructokinase (PFK). Kemp and Krebs (1967) demonstrated a direct inhibitory effect of citrate on PFK in vitro and Rennie and Holloszy (1977) reported that in exercising red muscle perfused with 1.8 mM FFA, citrate levels were elevated, PFK was inhibited and glucose uptake was decreased. These findings confirmed the existence of the glucose-fatty acid cycle in red skeletal muscle as in the heart as originally proposed by Randle et al (1963).

Studies by Bergstrom et al (1967) and Hultman (1967) demonstrated that the amount of glycogen stored in the working muscles was a limiting factor in prolonged cycling exercise at 75% VO_2max. Enhancing muscle glycogen stores increased endurance time while reducing muscle glycogen through ingestion of a low-carbohydrate, high-fat diet decreased endurance time. Performance time could also be increased by elevating plasma FFA concentration in the face of normal glycogen stores by slowing the rate of carbohydrate utilization (Hickson et al, 1977; Ivy et al, 1979). However, recent work has challenged the relationship between muscle glycogen and endurance time during prolonged exercise. Dohm et al (1983) reported that fasting rats for 24 hr depleted liver glycogen and reduced muscle glycogen, yet run time to exhaustion was longer compared to rats fed ad libitum. Plasma FFA and ketone bodies were elevated in the fasted rats

prompting the authors to suggest that enhanced fat utilization and the resultant glycogen sparing accounted for the increased endurance. Miller et al (1984) also found that rats fed a high-fat, low-carbohydrate diet for up to 5 weeks (decreased liver and muscle glycogen) ran longer than rats on a mixed diet. Increases in the activities of key oxidative enzymes suggested that the muscles adapted to the diet by enhancing their ability to oxidize fat and therefore spare glycogen.

1.7 Anaerobic Metabolism

Early work showed that anaerobic metabolism in skeletal muscle consisted of two components; hydrolysis of the endogenous phosphagens CP and ATP and the production of lactic acid from carbohydrates. It was held that anaerobic energy pathways were utilized only in situations of O_2 lack such as at the onset of exercise or during heavy activity. Also, it was uncertain whether working skeletal muscle could remove lactic acid directly since the majority was removed by liver, heart, non-working skeletal muscle and kidney.

Andres et al (1956) questioned this first concept after finding a constant lactate release in resting forearm muscle in men. Using a dog muscle preparation, Stainsby and Welch (1966) reported a net lactate release at rest and in 50% of the exercising preparations. In dogs running at 30% VO_2max lactate production increased over rest, resulting in

elevated steady state plasma lactate levels in some animals. (Depocas et al, 1969). Wahren et al (1971a) also measured lactate release across the leg muscles of men at rest and during 40 min of low to moderate intensity exercise. Studies by Jorfeldt (1970, 1971) with radiolabelled lactate conclusively demonstrated lactate production and release by human forearm muscle during low intensity work. Jorfeldt (1970) stressed the fact that mammalian skeletal muscle contains fibers differing in their metabolic capabilities as a potential explanation of simultaneous O_2 utilization and lactate production during rest or light work. Fast-twitch white fibers contain low oxidative capacities and when recruited will contribute to force production with a heavy anaerobic component while slow-twitch red muscles are constantly recruited and are predominantly aerobic in nature. Fast-twitch red fibers have high aerobic and anaerobic capabilities and while the energy they produce is predominantly aerobic, a small anaerobic involvement is a possibility. In addition to this explanation work with stimulated dog muscles and fluorometric measurements of the intracellular redox state of nicotinamide adenine dinucleotide indicated that O_2 was not limiting at the onset of exercise (Jobsis and Stainsby, 1968). The lactate formation which occurs was not caused by hypoxic stimulation of anaerobic glycolysis but was the result of a relative imbalance between the rates of pyruvate production by aerobic

glycolysis and pyruvate utilization in the tricarboxylic cycle. Recent work with fully aerobic, working, dog gracilis muscle has shown a linear relationship between muscle lactate concentration and twitch rate while subcellular volume PO_2 values were not limiting (Connett et al, 1984). It was concluded that lactate accumulation resulted from causes other than a simple O_2 limit on mitochondrial ATP production.

At rest and during all forms of exercise some lactate is produced and released into the blood but an equilibrium between production and removal exists during rest and low to moderate intensity exercise such that circulating blood concentrations are kept low. This accounts for the existence of low amounts of lactate in muscle even at rest. Therefore, it appears that lactate formation occurs when glycolysis proceeds at a rate which exceeds the rate pyruvate is accepted into the tricarboxylic cycle. This may occur during situations of O_2 lack, in heavy exercise or in a situation where the activity of the rate limiting enzyme pyruvate dehydrogenase is low, as found in untrained subjects (Ward et al, 1982).

The tracer work of Jorfeldt (1970, 1971) also demonstrated that lactate was taken up by working muscle as a function of its delivery. Fifty percent was acutely oxidized to CO_2, 20% was released as alternate metabolites, and the remainder presumably entered metabolite pools with slow turnover rates. Previous tracer work by Depocas et al (1969)

reported that 74% of the lactate produced during submaximal running in dogs was metabolized directly by the working muscles. Ryan et al (1979) infused lactate into resting and exercising humans and demonstrated an increased capacity to metabolize lactate aerobically during exercise. The major site of lactate removal appeared to be the aerobic muscle fibers. Recent work by Donovan and Brooks (1983) also reported lactate removal from the blood as a function of the arterial concentration during low and high intensity running in rats. Lactate was directly oxidized during exercise, partly by the working muscles. These results explain the often cited phenomenon of enhanced lactate removal in recovery when the subject exercises up to 50% of VO_2max (Jervell, 1928; Hermansen and Stensvold, 1972).

There has been considerable controversy regarding the ability of skeletal muscle to synthesize glycogen from lactate. Originally it was believed that lactic acid was reconverted to glycogen (Hill and Lupton, 1923) but Krebs (1964) reported that skeletal muscle did not contain adequate concentrations of key regulatory enzymes required in lactate to glycogen conversion (fructose-1,6-diphosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase). Only the liver and kidney were equipped for gluconeogenesis. Recently, McLane and Holloszy (1979) were able to show the conversion of ^{14}C-lactate into glycogen in resting fast-twitch red and fast-twitch white types of muscle. This

work was performed in an isolated rat hindquarter preparation where lactate was delivered as the only exogenous fuel in high but physiologic concentrations. No glycogen accumulation occurred in the slow-twitch red muscle due to the low concentration of the regulatory enzyme fructose-1,6-diphosphatase. Although this pathway does exist it is unlikely to contribute significantly to glycogen resynthesis in the muscles of intact man.

Margaria et al (1964, 1969) reported that only the alactacid component of anaerobic metabolism was involved during repeated bouts of heavy exercise lasting 10-15 sec. No lactate buildup in the blood occurred provided the recovery periods were long enough to permit the replenishment of phosphagen stores. The authors postulated that anaerobic glycolysis was used only when CP was completely depleted. Later studies employed the biopsy technique to demonstrate the simultaneous production of lactic acid and CP hydrolysis (Karlsson, 1971a, b; Saltin, 1973). A series of recent studies examined muscle following 6-10 sec of supramaximal cycling in men and confirmed the simultaneous activation of both the alactacid and lactacid components of anaerobic metabolism (Jacobs et al, 1983; Boobsis et al, 1983; Jones et al, 1984). Karlsson (1971b) summarized his findings on the involvement of anaerobic processes during dynamic exercise; (1) at workloads less than 50% VO_2max there is a moderate anaerobic yield which is all alactic, (2) at workloads

between 50 and 90% VO_2max the energy required from anaerobic metabolism is derived 50-50 from CP hydrolysis and anaerobic glycolysis with lactate production, (3) at workloads above 90% VO_2max CP hydrolysis is maximal and anaerobic glycolysis is maximally stimulated producing high levels of lactate.

During exercise at 90, 100 and 130% VO_2max sustained for 16, 7 and 2 min, respectively, ATP and CP levels consistently dropped to 70 and 20% of normal values within 2 min. Glycogen was not depleted and muscle lactate at exhaustion was lower during the lowest workload (Karlsson, 1971a, b). The author postulated that some additional factor besides ATP, CP, glycogen depletion or lactate buildup per se was responsible for fatigue. The inability of the muscle to buffer H^+ released from the dissociation of lactic acid and the resultant lower pH was suggested as an alternate mechanism.

1.8 The Effects of Acidosis on Muscle Metabolism and Performance

Hill and Lupton (1923) noted that fatigue in a stimulated frog muscle was related to the buildup of the metabolic byproduct lactic acid. They also knew that lactic acid in muscle and blood existed mainly in its dissociated form (H^+ and lactate ion bound to Na^+ or K^+) due to its low dissociation constant. The phenomonen of fatigue appeared to occur locally since Meyerhof reported that immersion of a

muscle into a solution of alkaline buffers increased the amount of lactic acid that could accumulate prior to fatigue. Meyerhof believed this increased amount of stimulation before fatigue was due to a decreased H^+ concentration or a blocking of the hindering effect of H^+ by the alkali (Hill and Lupton, 1923). These results led Hill and Lupton (1923) to suggest that the efficiency of buffering in the muscles determined the fatigue maximum of lactic acid and therefore the duration of a short-lived violent effort. The neutralization of alkali-protein was hypothesized as important in negating the acidotic effect of lactic acid production. Long maintained exercise was more dependent on the provision of O_2 and the efficiency of oxidations, since the H^+ concentration did not change appreciably. Eggleton and Evans (1930a) also demonstrated that the rate of lactic acid formation in surviving muscle was accelerated in alkaline fluids and retarded in acidotic solutions.

Work by Fiske and Subbarow (1927) suggested that CP hydrolysis during stimulation released base and aided in the buffering of lactic acid although the quantitative significance of this reaction was not known. The importance of both protein buffers and CP hydrolysis in maintaining muscle pH during asphyxia at the onset of exercise was noted from work with an isolated muscle preparation (Sacks and Sacks, 1933).

Several investigators reported decreases in the CO_2 combining capacity, pH and PCO_2 and increases in the lactic acid concentration of blood taken from humans subjected to intense exercise (Barr et al, 1923; Barr and Himwich, 1923; Jervell, 1928; Dennig et al, 1931; Laug, 1934). The production of lactic acid was partly buffered through the binding of H^+ to bicarbonate ions (HCO_3^-) to form carbonic acid, which dissociated to H_2O and CO_2. The CO_2 was blown off at the lungs through the increased ventilation during exercise. However during intense exercise this system was unable to prevent a significant acidosis from occurring. Dennig et al (1931) were the first to discuss the importance of all three known buffering mechanisms (carbonic acid, protein and CP hydrolysis) in combating the development of acidosis in heavily exercising humans.

In an attempt to enhance the buffering capacity of the body, Jervell (1928) had 2 subjects ingest sodium bicarbonate ($NaHCO_3$) prior to 12-15 min of heavy work. Total work done was unaffected but lactate accumulation was lower in the alkalotic state. Dennig et al, (1931) took the opposite approach by administering ammonium chloride (NH_4Cl) and showed a reduced endurance time in 1 subject. Later studies reported that artificial alkalosis increased the time to exhaustion and produced higher peak blood lactate values at exhaustion (Dill et al, 1932; Margaria et al, 1933). Asmussen et al (1948) gave NH_4Cl to 2 subjects and saw no

change in work capacity, VO_2max or maximum blood lactate during heavy exercise.

More recently, numerous studies have reported that acidosis decreases muscle performance, lowers the rate of muscle glycolysis and decreases muscle lactate release in isolated animal muscles during stimulation (Hirche et al, 1975; Mainwood and Worsley-Brown, 1975; Steinhagen et al, 1976; Donaldson et al, 1978; Fretthold and Garg, 1978; Gimenez and Florentz, 1979). In heavily exercising man, Jones et al (1977) demonstrated that NH_4Cl ingestion decreased peak blood lactate concentration and time to exhaustion as compared to control conditions. Induced alkalosis ($NaHCO_3$) produced prolonged endurance times and higher peak blood lactates. A later study of similar design examining working muscle demonstrated an inhibition of glycolysis at PFK during acidosis and also a possible lactate efflux impairment (Sutton et al, 1981).

A decrease in pH inhibits the regulatory enzyme PFK (Gevers and Dowdle, 1963; Trivedi and Danforth, 1966) and phosphorylase b (inactive) to phosphorylase a (active) conversion (Danforth, 1965; Chasiotis et al, 1983). The result of these inhibitions is a decreased glycolytic flux and a slower rate of lactate and/or acetyl CoA production. Therefore, both anaerobic and aerobic energy production may be decreased, although activation of pyruvate dehydrogenase by acidosis may contribute to lower lactate production and

maintained aerobic metabolism (Newsholme and Leech, 1983). The reduced anaerobic energy production will lead to enhanced fatiguability, accounting for the shortened endurance times during heavy exercise. A reduced ability to remove lactate and/or H^+ from the interior of the muscle cell would serve to enhance the acidosis.

Given this speculation, the relationship between the metabolic effects and impaired performance of acidosis remains unclear. Respiratory and metabolic acidosis may have different metabolic effects (Mainwood and Worsley-Brown, 1975; Ehrsam et al, 1982). Muscle O_2 uptake may be depressed by acidosis during stimulation through a decrease in muscle blood flow and O_2 delivery (Hirche et al, 1975; Steinhagen et al, 1976). Sahlin et al (1983) have recently reported that acidosis may produce fatigue indirectly by depletion of the high energy phosphate stores and the effect of acidosis on Ca^{2+} and excitation-contraction coupling has not been clarified.

1.9 Purpose of the Thesis

The intent of this thesis was to directly quantify the relative contributions of the major energy releasing pathways in skeletal muscle during heavy stimulation. In order to accomplish this, direct measurements of endogenous and exogenous fuel utilization, O_2 uptake, lactate production and phosphagen hydrolysis were required.

Studies of contracting muscle in vivo are complicated by unknown or varying amounts of working muscle, changes in blood substrate and metabolite concentrations, uncertain extracellular volume size and composition and poor precision of circulatory and respiratory measurements. Additionally, arterial and venous cannulations and muscle biopsies are necessary to adequately characterize the working muscle. In an isolated muscle preparation these factors can be controlled and sampling requirements are more easily met. For these reasons the isolated perfused rat hindquarter preparation was chosen to study muscle metabolism and performance during heavy stimulation.

Several groups of investigators have successfully used the isolated perfused rat hindquarter preparation for the study of resting skeletal muscle metabolism (Lewis et al, 1977; Van Hardeveld and Kassenaar, 1978; Dietz et al, 1980; Ivy and Holloszy, 1981; Richter et al, 1982b; Walker et al, 1982a) following the pioneering work of Houghton (1971) and the evaluation and characterization of this model by Ruderman

et al (1971). A number of factors make this preparation particularly suitable for this work. The hindquarter preparation consists mainly of skeletal muscle with very little adipose tissue. The muscle receives exogenous substrates through normal vascular channels and the normal bone, muscle and nerve relationships are maintained. All components of the arterial perfusate may be tightly controlled and manipulated: blood gas, acid-base and electrolyte status, hemoglobin concentration and hematocrit and the concentrations of energy substrates, metabolites, and hormones. Simultaneous sampling of arterial and venous perfusates permits the measurement of uptake and release of energy substrates and metabolites. Finally, pre and post-perfusion muscle biopsies are readily obtained for the measurement of intramuscular energy stores, metabolites, enzyme activities and pH.

For the purposes of this thesis the resting hindquarter model was extended for use during heavy muscular contraction. Following the development of the exercising preparation this model was used to examine some of the unknowns, gaps and controversies concerning the present understanding of metabolism and performance of heavily contracting skeletal muscle. What are the relative contributions of the aerobic and anaerobic energy producing pathways during sustained maximal stimulation? How do the major fiber types in muscle differ in their contribution to

total energy production and muscular performance and which energy releasing pathways are used? What are the relative contributions and order of preference of the available fuel sources throughout the stimulation? Are heavily working muscles able to shift to alternate fuels such as intramuscular fats when preferred fuels (carbohydrates) are depleted or unavailable? Can the fatigue which occurs in heavily contracting muscle be linked to biochemical changes in fuel stores, metabolite concentrations or intracellular acidity? What is the effect of perfusing with an acidotic medium on the metabolism and performance of heavily contracting muscle? Are the changes in performance during acidosis biochemically mediated?

The following chapter describes the development of the model to the point where prolonged high tension production by the muscle of the perfused hindquarter was obtained.

2. DEVELOPMENT OF THE ISOLATED PERFUSED RAT HINDQUARTER MODEL

Houghton (1971) and Ruderman et al (1971) established the viability of the isolated perfused rat hindquarter preparation for the study of resting muscle metabolism. Subsequently, several groups of investigators used this model to examine insulin-glucose uptake dynamics (Reimer et al, 1975; Lewis et al, 1977; Ivy and Holloszy, 1981; Richter et al, 1982b; Walker et al, 1982a) and glycogen metabolism (Dietz et al, 1980) in skeletal muscle and the effects of thyroid status on muscle metabolism (Van Hardeveld and Kassenaar, 1978).

At the outset of this project few attempts at stimulating the perfused hindquarter had been reported and most assessed the quality of the stimulation simply by palpation of the working muscles (Ruderman et al, 1971; Berger et al, 1975, 1976; Ruderman et al, 1980). Rennie and Holloszy (1977) were the first to quantify the force produced by the gastrocnemius-plantaris-soleus (GPS) muscle group of the hindquarter. This thesis was intended to extend their work by characterizing the model during heavy muscular contraction with measurements of force production, substrate and metabolite uptake and release and muscle fuel and metabolite concentrations.

2.1 Early Perfusions

2.1.1 Perfusion Protocol

Perfusions were performed on anesthetized male rats prepared as outlined by Ruderman et al, (1971). The left hindquarter was isolated and perfused with an artificial medium via the abdominal aorta. Venous perfusate left the animal via the inferior vena cava. Control muscle biopsies were taken prior to the surgery from the right hindquarter. The perfusate medium contained Krebs-Henseleit (KH) bicarbonate buffer solution and aged human red blood cells (RBCs) at a hemoglobin (Hb) concentration of 8 $g.dl^{-1}$. The perfusate was continually gassed with 95% O_2 and 5% CO_2 as the medium was recirculated through the hindquarter. The tendon of the GPS muscle group was attached to a force transducer and the muscles were stimulated via the sciatic nerve with a train of impulses every 2 sec. Trains lasted 50 msec and contained 5 separate impulses (100 Hz), each lasting 0.2 msec (Rennie and Holloszy, 1977). The stimulation intensity of each impulse was supramaximal (5 volts). During stimulation the perfusion flow rate was 10 $ml.min^{-1}$ and muscle biopsies immediately followed.

2.1.2 Hindquarter Performance

Early attempts at stimulation produced peak isometric tensions of approximately 2000 g with tension falling rapidly to 0-100 g in 10 min (Fig. 2). Rennie and Holloszy, (1977) had reported peak tensions of only 1000 g but fatigue was less pronounced since 400-500 g of tension were held after 10 min (Fig. 2). McLane et al (1981), working in the same laboratory, reported peak isometric tensions of 1500 g which fell to 800 g following 10 min of stimulation (Fig. 2). Intact anesthetized rats produced peak tensions of approximately 2000 g. During the first 5 min tension decreased by only 25% and following 60 min, 1300-1400 g of tension were maintained (Fig. 2).

It did not seem likely that inadequate conduction of the electrical impulses from the nerve to the working muscles was responsible for the extreme fatigue in the present preparation. Visible contractions were seen every 2 sec and it was unlikely that only certain muscles or motorunits of the GPS muscle group were not stimulated. Additionally, action potentials recorded at various times from the contracting semitendonosis muscles of intact and perfused hindquarters of the same rat were identical.

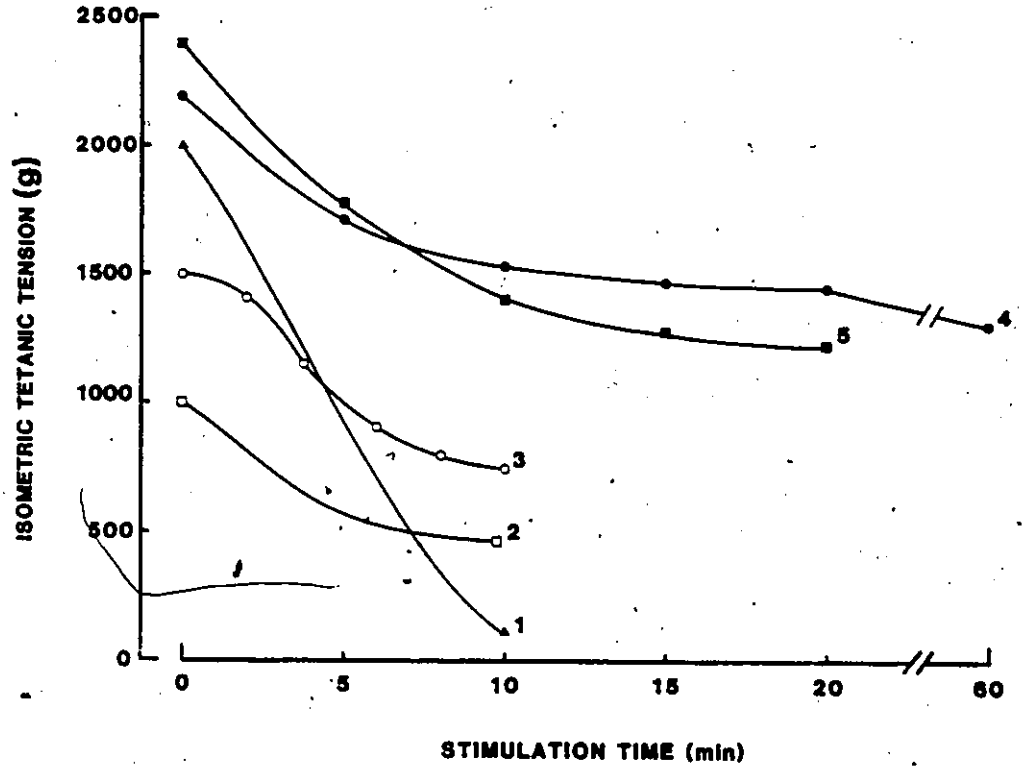


Fig. 2. Isometric tetanic tension production by the rat gastocnemius-plantaris-soleus muscle group under varying conditions and in previous investigations. 1. Early perfusions. 2. Rennie and Holloszy (1977). 3. McLane et al (1981); stimulation rate was 100 msec trains (50Hz) at a 0.5 Hz frequency and a 5 volt intensity. 4. Intact anesthetized rats. 5. Perfusions with fresh and rejuvenated red blood cells. Stimulation rate for conditions 1, 2, 4 and 5 was 100 msec trains (100Hz) at a 0.5 Hz frequency and a 5 volt intensity.

2.1.3 Oxygen Delivery

Long-term exercise is heavily dependent on aerobic metabolism and it therefore seemed reasonable to assess the adequacy of O_2 delivery to the hindquarter as the factor responsible for the rapid fatigue. Time-expired RBCs were known to possess low ATP and 2,3-diphosphoglycerate (DPG) concentrations following storage at $4^{o}C$ for 21 days. The low 2,3-DPG produces a left-shifted Hb-O_2 dissociation curve which translates into a greater Hb-O_2 affinity and results in decreased O_2 offloading at the tissues. Enhanced RBC fragility and hemolysis were also problems with aged RBCs as evidenced by the rise in perfusate K^+ concentration from 5.9 to 8 mM or over following 1 hr of perfusion. To negate these problems fresh human RBCs were obtained and used in the perfusions. Also, the perfusing Hb concentrations and flow rates were increased from 8 to 12 $g.dl^{-1}$ and 10 to 16 $ml.min^{-1}$, respectively since these factors may have also contributed to a low O_2 delivery.

With these modifications, peak isometric tensions were not significantly altered but the rate of tension fatigue was much slower and more closely resembled the performance of the intact anesthetized rat (Fig. 2). Unfortunately, it was not practical to routinely secure adequate volumes of fresh human RBCs so time-expired cells were subjected to a rejuvenation procedure (Deuticke et al,

1971). The cells were incubated in a solution containing inosine, glucose, pyruvate and sodium phosphate. This procedure increased the P_{50} (PO_2 at which 50% of Hb is saturated with O_2) of the RBCs from 19 mmHg or lower to 25 mmHg, returning the offloading characteristics to near normal levels (fresh RBCs P_{50} = 26-27 mmHg). Perfusions with rejuvenated RBCs produced tension curves similar to those with fresh cells (Fig. 2) and reduced the amount of RBC hemolysis.

2.1.4 Non-Recirculating Perfusion System

These early perfusions employed a recirculating system where the initial 50 ml of perfusate were discarded after one pass through the hindquarter and the remaining 200-250 ml were recirculated. Therefore, the metabolite concentrations of the arterial perfusate were continually changing and it was difficult to sample this blood frequently to assess the changes since the blood was constantly in the oxygenators being gassed. However, the major drawback of this system was the inability to separate the metabolism of the RBCs from that of the resting or working hindquarter. Both the RBCs and hindquarter took up glucose and released lactate. In order to accurately assess the metabolism of the perfused muscle only, a one pass or non-recirculating system was used. One reservoir of arterial perfusate was prepared

and continual gassing was unnecessary. Samples of the reservoir perfusate were taken every 5 or 10 min to assess the RBC metabolism while simultaneous arterial and venous sampling identified substrate and metabolite uptake or release by the hindquarter. Acid-base manipulations of the arterial perfusate were also easily accomplished with this system. The drawback was the need for larger volumes of RBCs.

2.1.5 Collection of Fresh Red Blood Cells

One experiment required 800 ml of perfusate (400 ml of packed RBCs) for 30 min of rest perfusion and 20 min of stimulation. In one day 3-4 experiments were completed requiring approximately 1200-1600 ml of packed RBCs. Sources included time-expired cells from the local blood banks and RBCs obtained from polycythemic patients bled at local hospitals. These sources could not meet the volume demands and delivery was not on a regular basis. It was not practical to obtain the required volumes from human donors so a local abbatoir was contacted. Initially, RBCs from pigs were collected directly into anticoagulant but perfusions with these cells resulted in severe edema and behaved as if the rat microvasculature was plugged. Since the animals were killed in a traumatic manner it appeared that their clotting mechanisms were activated prior to collection. The clots

were not picked up by the 40 um filter in the perfusion system or may have formed when in contact with the microcirculation. This problem was overcome by collecting large quantities of bovine blood. The cattle were killed in a considerably less traumatic manner. Identical collection and washing procedures resulted in successful perfusions free from clot formation.

The quality of the transported blood was maintained by direct collection into siliconized plastic reservoirs and bottles containing chilled acid-citrate-dextrose (ACD) anticoagulant. The blood was transported on ice and each volume of RBCs was washed with 25-30 volumes of KH bicarbonate buffer. With the establishment of an adequate and reliable source of RBCs, a number of additional aspects of the exercising hindquarter model were assessed and altered where necessary.

2.2. Perfusion Related Alterations

Several adjustments to the perfusate and perfusion apparatus were incorporated during the course of the model's development. Perfusions with albumin concentrations of 4,5 and 6 $g.dl^{-1}$ demonstrated that 4 $g.dl^{-1}$ was the optimal protein concentration, producing appropriate osmotic pressures which combated the forces tending to drive the perfusate out of the circulation. During rest and stimulation fluid gain by the tissues was maintained at

acceptable levels.

The water used to wash the RBCs and needed to form the plasma portion of the perfusate required a high degree of purity. It was not practical to sterilize sufficient quantities of water but cleanliness was necessary. All glass or plasticware was carefully washed and kept free from contamination. Initially, distilled water obtained from the hospital's central store was passed through deionizing columns and a carbon filter before use. The life of these columns was 6 months with normal use, but due to the large quantities of water needed for blood washing they expired after 2-3 months. Perfusions with unclean water led to constant contamination of the hindquarter with subsequent tissue reactivity, high perfusion pressures and edema. Since the replacement cost of columns was high it became necessary to install a distilling apparatus in the laboratory and use only double distilled water. This improvement maintained the quality of the water and alleviated the related perfusion problems.

As mentioned, a one-pass perfusion system was used. The perfusate was gassed with a tank of 95% O_2, 5% CO_2 gas, transferred to a one litre reservoir and covered with heavy mineral oil to maintain the blood gases. Although this system was adequate it did not permit the fine tuning necessary to manipulate the acid-base state of the perfusate. For this reason separate CO_2 and O_2 tanks were used to

achieve the desired blood gas and acid-base status. The mineral oil covering of the reservoir was also replaced with a reservoir capped with a rubber stopper. A constant flow of the required gases was bled into the air space between the perfusate and the rubber stopper. This maintained gases exactly as desired for as long as required and alleviated possible contamination of the blood by the mineral oil and the slow equilibration which occurred between the perfusate and the atmosphere in spite of the mineral oil.

Perfusions of either fresh bovine or rejuvenated human RBCs decreased cell fragility, maintained normal O_2 onloading-offloading characteristics and enhanced the O_2 delivery by the perfusate. An advantage of using bovine cells over rejuvenated human cells was related to the size of the RBCs. The volume of a bovine erythrocyte (50 um^3) is nearer the rat erythrocyte volume (60 um^3) than human erythrocytes (90 um^3). In the normal microcirculation, the diameters of many capillaries are smaller than a red blood cell, requiring the cells to elongate and pass through in single file. This may cause potential problems for tissue oxygenation especially at the high flow rates of exercise when perfusing the large and potentially more fragile rejuvenated human cells.

Since RBC volume was near the normal for rat and the Hb concentration in the perfusate was adjusted to the normal rat value of 14 $g.dl^{-1}$, perfusions were attempted with more

normal O_2 levels and not hyperoxic partial pressures. Experiments with PaO_2 values of approximately 150 mmHg produced lower perfusion pressures during rest (40 mmHg) but subsequent inability to produce tension during stimulation. These preparations behaved as though the tissues were anoxic during rest although similar flow rates were used (6 $ml.min^{-1}$). Oxygen uptake measurements were also lower during normoxic perfusions suggesting that the flow was bypassing many of tissue capillary beds. The vasoconstrictor response to hyperoxia (>300 mmHg) resulted in higher perfusion pressures at rest (80 mmHg) which appeared to open all capillary vascular channels and maintain tissue oxygenation. Although it was essential to perfuse with hyperoxic mixtures, pharmacological agents were not needed to obtain adequate vasodilation and tissue oxygenation. Several groups (Ruderman et al, 1971; Walker et al, 1982a) reported the use of phentolamine, an alpha-adrenergic blocker, to produce adequate vasodilation. As the group's expertise with this model improved, these agents were no longer needed (Walker et al, 1982b).

2.3 Surgical Alterations

The surgical isolation of the hindquarter as described by Ruderman et al (1971) results in the perfusion of approximately 15 g of muscle, of which 30-50% contracts when stimulated via the sciatic nerve (Rennie and Holloszy,

1977). . In the present investigation 55% of the perfused muscle contracted during stimulation (Chapter 3). In order to enhance the working to non-working ratio of the preparation, cannulations of the femoral artery and vein were attempted. Walker et al (1982a, 1982b) used this technique to perfuse all the muscles of the lower leg and the distal sections of the upper leg muscles and reported that approximately 75% of the perfused muscle could be stimulated. In the present work femoral perfusions were not successful, resulting in high perfusion pressures and subsequent tissue edema.

Only minor modifications were made to the surgical techniques of Ruderman et al (1971). Weighing of the rat pre and post-perfusions during early experiments revealed a significant increase in animal weight. This was due to an engorgement of the animal's abdominal cavity with perfusate. Investigation revealed that perfusate was slipping by the venous catheter and an additional suture around the catheter was required to alleviate this problem.

2.4 Hindquarter Stimulation and Performance

With the production of high isometric tensions by the GPS muscle group it became necessary to develop a method of adequately and reliably stabilizing the rat hindquarter during stimulation. Secondly a reliable method of securing the GPS tendon to the force transducer was needed. Initial

attempts at stimulation often resulted in the hindquarter pulling free of its stabilization at the knee joint and the GPS tendon tearing from its attachment to the transducer. The stabilization system was improved by securing the hindquarter with support brackets through which metal screws held the bones at the knee and ankle joints. The lower leg was held parallel to the platform while the hip angle was approximately 135° (Fig. 3). This stabilization procedure did not interfere with blood flow at the knee and ensured uniform fixation of the hindquarter during isometric contractions. Suture material was used to reinforce the GPS tendon and attach it to a flexible wire which could be secured to the force transducer. The resting length of the muscle was moved to the position producing optimal tetanic tension by moving the transducer. These improvements were instrumental in the achievement of reliable tension recordings during stimulation. They also contributed to the higher tension measurements in the present model as compared to previous reports using similar stimulation frequencies (Rennie and Holloszy, 1977; McLane et al, 1981).

Stimulation was produced by direct attachment of an electrode to the distal section of the transected sciatic nerve. At times improper grounding of the animal to the metal platform led to the escape of the stimulation signal. To alleviate this problem the platform and metal support brackets were coated with a nonconductive material.

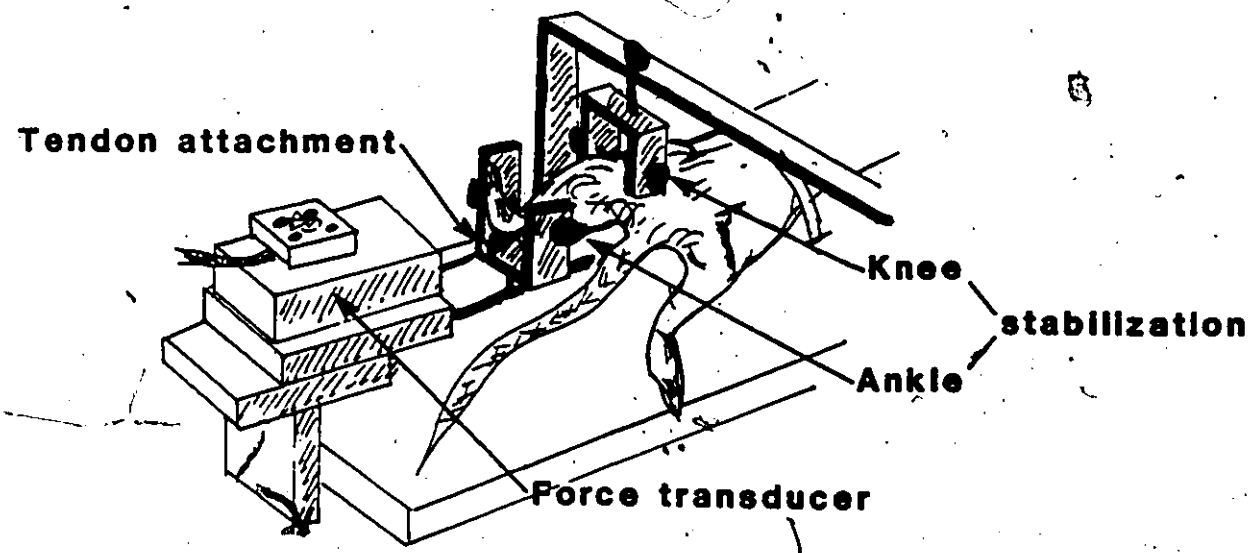


Fig. 3. Rat hindquarter stabilization and attachment of the gastrocnemius-plantaris-soleus tendon to the force transducer.

The hindquarter was stimulated every 2 sec with a train of supramaximal stimuli, producing tetanic isometric contractions. Trains of stimuli were used to simulate physiological stimulation during heavy exercise. Supramaximal stimuli ensured that each train fully activated all motorunits and prevented motorunit recruitment from changing over time as it does during stimulation with submaximal stimuli. Stimulation with double or single pulses produced lower workloads, peak tensions and O_2 uptake values (Table 1). The present work was limited to stimulation rates producing high workloads.

The isometric tension generated by the hindquarter was dependent on the fed state of the rat. A 15 hr overnight fast depleted glycogen levels by 30-35% in the GPS muscles (Table 2). Tension decayed at a faster rate during perfusions with fasted rats. The findings in Chapter 3 demonstrate the rapid breakdown of muscle glycogen during the first 5 min of stimulation and therefore explain the importance of pre-exercise glycogen levels on muscle performance during heavy stimulation.

Table 1. Performance variables at rest and selected stimulation rates (V, volts; T, train; SP, single pulse).

Workload	Stimulation Rate	O_2 Uptake (umol/min/ hindquarter)	Peak Tension (g)
Rest		8-10	
Low (a)	0.5 Hz, 5 V, (100 Hz)	12-17	800
(b)	0.5 Hz, 5 V, 0.2 msec SP	12-17	600
High	0.5 Hz, 5 V, 100 msec T, (100 Hz)	20-25	2100-3000

Table 2. Effects of a 15 hour fast on muscle glycogen content (mean ± SE, n = 7).

	Glycogen (umol glucosyl units/ g wet weight)		
	Fed Rats	Overnight Fast (15 hr)	Percent of Fed Glycogen
SOLEUS	21.6 ± 2.9	14.7 ± 1.2	68.1
PLANTARIS	31.6 ± 3.4	20.3 ± 3.0	64.2
RED GAST.	34.2 ± 3.1	23.3 ± 2.6	68.1
WHITE GAST.	34.8 ± 2.5	22.9 ± 3.8	65.8

2.5 Measurement Related Improvements

Simultaneous sampling of arterial and venous perfusate samples permitted the measurement of arterio-venous differences across the hindquarter for metabolites such as glucose, lactate and FFA. Blood flow was measured from timed venous collections. Perfusate samples were dispensed into the appropriate anticoagulants or deproteinizing agents and measured later. A more immediate system was needed for the measurement of O_2 uptake. Anaerobic perfusate samples were analyzed within 2 min for PO_2, PCO_2 and pH with a blood gas analyzer and for Hb concentration and percent O_2 saturation with an automatic hemoximeter. This permitted the immediate calculation of arterial and venous O_2 contents and together with the flow rate gave the O_2 uptake.

A pressure transducer in the arterial line continuously monitored the arterial perfusion pressure and a force transducer quantitated the force production during stimulation.

Resting perfusions of varying lengths determined that 30 min of rest perfusion at 6 $ml.min^{-1}$ was required for glucose uptake and lactate production by the hindquarter to reach a steady state following the surgery.

Muscle biopsies were immediately freeze clamped and stored in liquid N_2 until analyzed. Control biopsies were taken from the contralateral hindquarter prior to the surgery. At the end of each perfusion, biopsies were taken

from the exercising muscles without disturbing the stimulation or blood flow of the hindquarter.

2.6 Summary

The improvements and alterations made to the isolated perfused rat hindquarter model discussed in this chapter were designed to produce a preparation capable of sustaining heavy muscular contraction for prolonged periods of time. Only after solving these problems could the model be considered viable for use in the study of muscle metabolism. Chapter 3 describes the working model in detail and examines the metabolic response of the hindquarter to prolonged heavy stimulation.

3. MUSCLE METABOLISM AND PERFORMANCE DURING HEAVY EXERCISE IN THE PERFUSED RAT HINDQUARTER

3.1 Introduction

Early attempts at studying the metabolism and performance of skeletal muscle during exercise in the perfused rat hindquarter were poorly characterized. Submaximal stimulation rates were used and the quality of stimulation was assessed only by palpation of the muscles (Ruderman et al, 1971, 1980; Berger et al, 1975, 1976). Only Rennie and Holloszy (1977) reported an attempt at force quantification during rhythmic tetanic stimulation. During the course of the present work additional investigators have reported the use of this model during submaximal stimulation (Everts et al, 1981, 1983; Walker et al, 1982a, b; Richter et al, 1982a, c). Additional attempts at tetanic stimulation have also appeared (Everts et al, 1981; McLane et al, 1981; Hood et al, 1983).

The present work fully characterizes this preparation and permits the examination of the muscle's metabolism and performance during heavy stimulation. To accomplish this simultaneous measurements of isometric force production, substrate and metabolite uptake and release and intramuscular fuel utilization and metabolite accumulation were made.

3.2 Methods

3.2.1 Animals

Male Sprague-Dawley rats weighing 283 ± 7 g (x ± SD, n=23) were used in the perfusions. The animals were fed Purina laboratory chow ad libitum and housed in a temperature, humidity and light controlled environment (12 hr on, 12 hr off).

3.2.2 Perfusion Medium

The perfusion medium consisted of KH buffer (Krebs and Henseleit, 1932) and fresh bovine erythrocytes to give a final Hb concentration of 14 $g.dl^{-1}$. The perfusate also contained 24 mM sodium bicarbonate, 4 $g.dl^{-1}$ dialyzed bovine albumin, 5.6 mM glucose, 0.15 mM FFA (bound to the albumin), 2.5 mM calcium chloride and 22.0 uM choline chloride. The resulting plasma ionic concentrations (mM) were Na^+, 143; Cl^-, 125; K^+, 5.9; Mg^{2+} 1.21; Ca^{2+}, 2.5; and HCO_3^-, 25. The initial lactate concentration in the perfusion medium was 1.02 ± 0.07 mM (x ± SE, n=23) resulting from erythrocyte metabolism. Following a 50 min perfusion the lactate concentration rose to 1.23 ± 0.08 mM. Sodium pyruvate was added to give an initial lactate/pyruvate ratio of 10-15.

The bovine erythrocytes were obtained from a local abbatoir 1-2 days prior to perfusion days. The erythrocytes

were collected directly into ACD anticoagulant (1 l ACD per 3.5 l whole blood), transported to the laboratory on ice and washed with 25-30 volumes of KH buffer containing 30 mM bicarbonate and 11.1 mM glucose. The RBCs were passed through a column of glass beads to remove fibrin and fibrinogen and stored as packed cells at 4°C. The morning of perfusions they received a final wash with 2-3 volumes of KH buffer. (HCO_3^- = 24 mM) and were passed through an intravenous blood line filter (Abbott Laboratories). The non-erythrocyte portion of the perfusate containing the albumin was passed through a 22 um filter (Millipore) and added to the erythrocytes. Calcium chloride was added to the perfusate only after the gassing with CO_2 and O_2.

3.2.3 Hindquarter Surgical Preparation

The surgical isolation of the rat hindquarter was performed as described by Ruderman et al (1971) with modifications. The rats were anesthetized with an intraperitoneal injection (6 mg.100 g^{-1} body wt) of sodium pentobarbital. Following the midline incision through the abdomen and ligation of the vessels supplying the abdominal wall and skin, two sutures were placed around the right common iliac artery and vein near their origins. At this point, following as little surgical intervention as possible, pre-perfusion muscle biopsies were taken from the right hindquarter. The right common iliac artery and vein were

quickly ligated, the remaining major vessels were exposed and the isolation of blood flow to the left hindquarter was completed. The tendon of the superficial posterior crural muscle group (gastrocnemius, plantaris and soleus muscles) was exposed, sutured to a wire ring and cut distal to the ring. The sciatic nerve was exposed in its gluteal course, ligated and cut proximal to the ligation. A rubber collar was used to attach a stimulating electrode to the nerve. Ringer's solution was used to keep the nerve moist and manipulation was minimized.

The rat was placed on the perfusion platform on its dorsal surface and the left leg was fixed by support brackets at the knee and ankle joints such that the lower leg was parallel to the platform and the hip angle was approximately 135^{o} (Fig. 3). Care was taken to ensure proper stabilization at the knee and prevent dislodging during stimulation. The force transducer was attached to the platform and secured to the metal ring on the exposed tendon.

The abdominal aorta was cannulated (22 gauge catheter, Deseret Co.) and flushed with a 0.4 ml of heparinized saline (100 U heparin. ml^{-1}). The vena cava was cannulated with a 14 gauge catheter (Deseret Co.) and the hindquarter was flushed with 3-4 ml of 0.9% saline. The animal was killed with an intracardiac injection of 20 mg sodium pentobarbital and moved to the perfusion box where the perfusion was started. The hindquarter was ischemic for 2-3

min during the cannulations. In all experiments the skin of the perfused leg was left intact except for sections around the ankle and knee and the leg was covered with plastic sheeting to prevent surface evaporation. The entire operative procedure required 30-35 min.

3.2.4 Perfusion Apparatus

During the surgical preparation of the hindquarter the perfusate was circulated through disc oxygenators for 5-10 min inside the perfusion box (37 $\pm$ 1°C). The perfusate was equilibrated with a combination of humidified gases from pure CO_2 and O_2 tanks transferred to a sealed arterial reservoir and continually mixed. The gassing resulted in approximate PaO_2, $PaCO_2$, pH and plasma HCO_3^- concentrations of >350 mmHg, 40 mmHg, 7.41 and 24 mM, respectively. The humidified gases were slowly bled into the reservoir to maintain the blood gas and acid-base status throughout the duration of the perfusion. Side ports on the arterial reservoir permitted anaerobic sampling of the perfusate.

Following preparation of the perfusate, a sample was tonometered with a 95% N_2, 5% CO_2 gas. Samples of tonometered gas were taken at selected times to give percent O_2 saturations of 70, 40 and 20%. Exact percent O_2 saturations and PO_2 values were measured at 37°C, corrected to a pH of 7.4 and plotted to give a Hb-O_2 dissociation curve. The P_{50} of the perfusion medium was read directly

from the plot.

A roller pump (Universal Electric Co.) was used to maintain a constant flow of perfusate through a 40 um filter (Millipore), gas debubbler, physiological pressure transducer (Gould-Statham No. 48703) and into the rat hindquarter (Fig. 4). The pressure transducer was attached to a carrier preamplifier (Hewlett-Packard No. 8805B) and the continuous mean arterial pressure was displayed on a chart recorder (Hewlett-Packard No. 7754A). The perfusate left the hindquarter through a short piece of tubing with a rubber port for repeated sampling of venous blood. The venous perfusate was collected for measurement of flow rate, substrates and metabolites. All experiments employed a one-pass or non-recirculating perfusion system.

3.2.5 Stimulation of Perfused Skeletal Muscle

A shielded bipolar platinum stimulating electrode was fixed to the sciatic nerve during the surgical preparation and connected to an electrical stimulator (Grass Instruments No.S48). The muscles innervated by the sciatic nerve were stimulated to contract with 100 msec trains (100 Hz) at a supramaximal 5 volt intensity and a 0.5 Hz frequency. Every train consisted of 10 separate impulses in 100 msec, each 0.2 msec in duration. At the onset of perfusion, the resting length of the GPS muscle group was adjusted to produce maximal tetanic tension by altering the position of the force

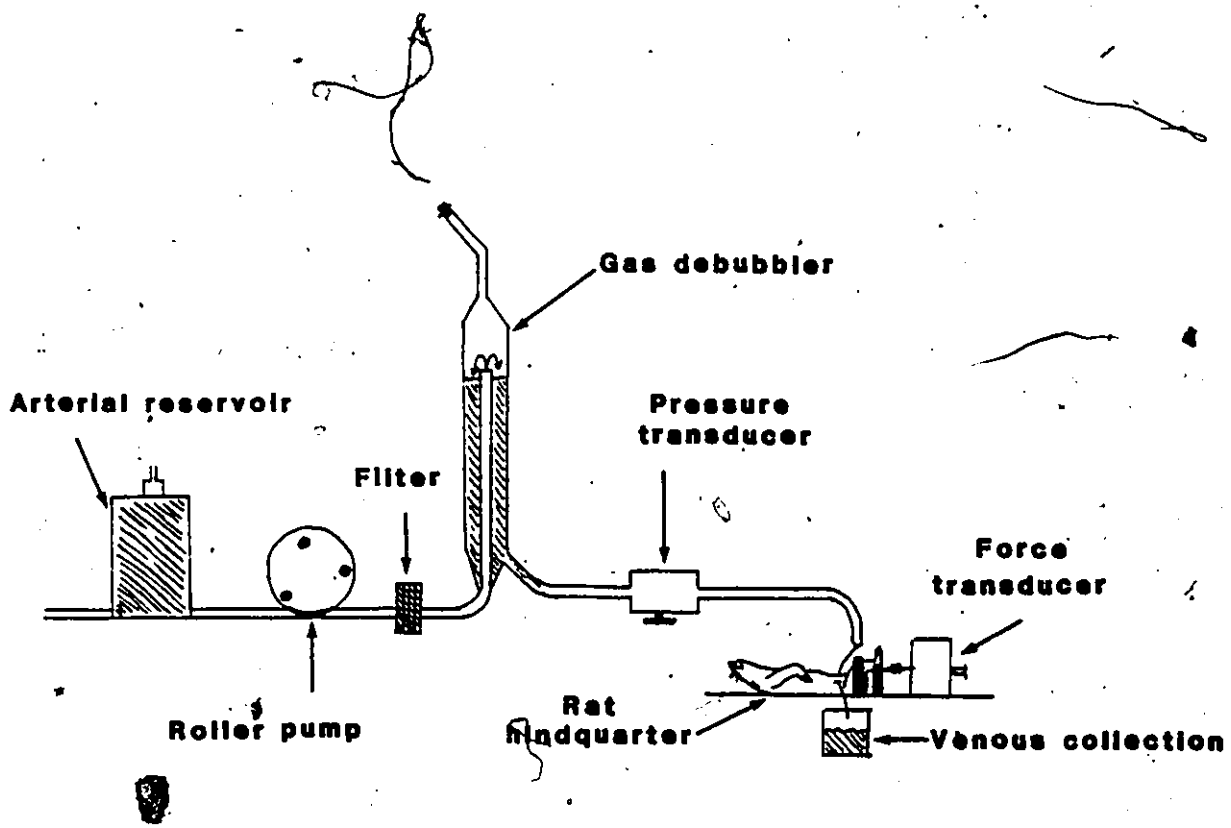


Fig. 4. Schematic representation of the rat hindquarter perfusion apparatus.

transducer. The generated isometric tension was measured with a force transducer (Statham No. 10713) and recorded on a Hewlett-Packard chart recorder. Previous investigations have reported that the contracting muscle mass represented 30-50% of the total perfused hindquarter muscle mass (Ruderman et al, 1971; Rennie and Holloszy, 1977). In the present investigation the perfused hindquarter tissue was determined by dissection of stained muscle tissue following perfusion with a dye. The total perfused hindquarter muscle mass weighed 13.74 ± 0.89 g (n=6, Appendix F). The stimulated or working muscle groups (anterior, lateral, deep posterior and superficial posterior crural groups and the bulk of the posterior femoral group) weighed 7.72 g and represented 56.2% of the total perfused muscle mass. The non-working muscle groups (anterior and medial femoral groups and a small portion of the posterior femoral group) weighed 6.02 g, respresenting 43.8% of the total perfused muscle mass. Perfusion of gluteal and lower back muscles was minimal.

3.2.6 Sampling of Perfused Skeletal Muscle

Muscle biopsies were taken from the GPS muscle group. The wet weights from six resting rats were 1,785 ± 68 mg, 294 ± 13 mg and 117 ± 9 mg for the gastrocnemius, plantaris and soleus muscles, respectively (Appendix F). The total weight of the group (2,196 mg) represented 16.0% of the total perfused muscle mass and 28.5% of the total working muscle

mass. A hemostat was attached to the distal end of the GPS tendon and used to pull the muscle group free. The entire soleus and plantaris muscles were sequentially removed and immediately frozen in large metal tongs precooled in liquid N_2. The soleus consists of 84% slow oxidative (SO) fibers and 16% fast oxidative glycolytic fibers (FOG) while the plantaris contains 6% SO, 41% fast glycolytic (FG) and 53% FOG fibers (Adriano et al, 1973). Additionally, samples of superficial white gastrocnemius muscle, containing predominantly FG fibers and the deep portion of the gastrocnemius medial head, containing predominantly FOG fibers, were removed and frozen (Hickson et al, 1976). The plantaris muscle best represents the working muscles of the rat hindquarter being comprised of roughly equal numbers of FOG and FG fibers (Ivy and Holloszy, 1981). The total time taken for the preparation, sampling and freezing of all four biopsies was 90-120 sec. During this time, the flowrate was maintained and the stimulation remained constant. The tissue samples were wrapped in aluminum foil while frozen and stored in liquid N_2 for subsequent analysis.

3.2.7. Perfusion Protocol

The experimental design presented in Figure 5 was employed to examine the metabolism and performance of skeletal muscle during heavy stimulation. During surgery, the perfusion medium was gassed and its P_{50} measured. Upon

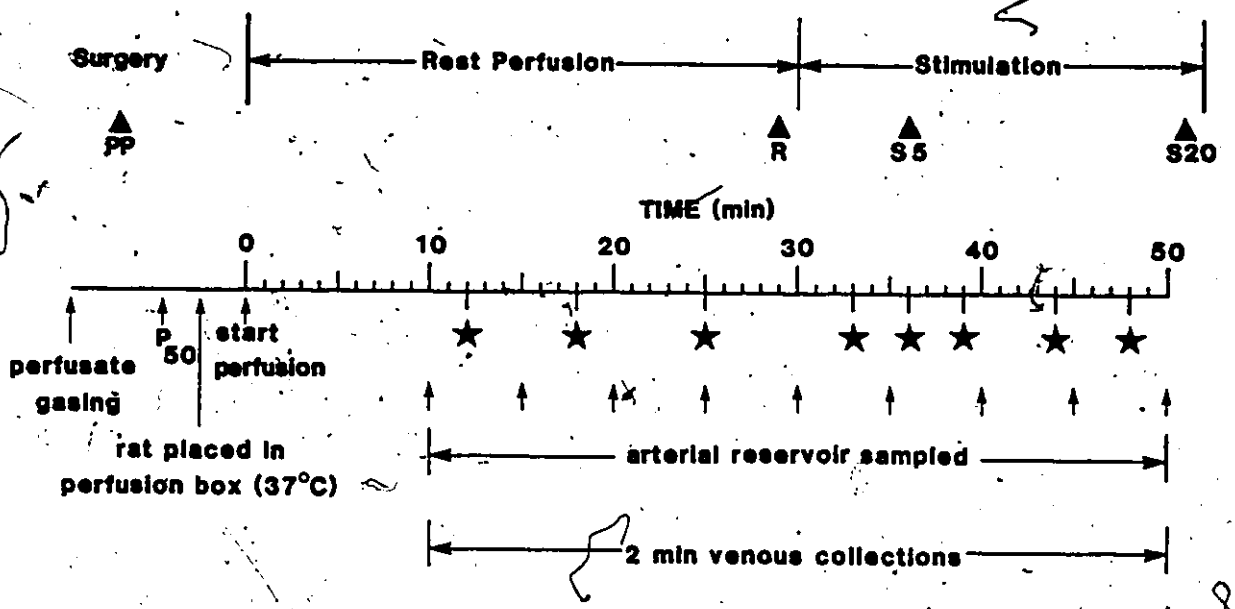


Fig. 5. Perfusion protocol. ▲, muscle biopsies; ★, O_2 uptake measurements. PP, pre-perfusion; R, 30 min rest perfusion; S5, 30 min rest and 5 min stimulation; S20, 30 min rest and 20 min stimulation.

completion of the surgery the rat was placed in the perfusion box and perfused at a flowrate of 5.9 ± 0.1 $ml.min^{-1}$ (resting O_2 uptake did not increase above 4 $ml.min^{-1}$). Following 30 min of rest perfusion the flowrate was increased to 18.0 ± 0.6 $ml.min^{-1}$ and the hindquarter stimulated for 20 min. Throughout the 50 min perfusion the arterial perfusate was sampled every 5 min and continuous 2 min venous collections were made. Anaerobic arterial and venous perfusate samples were drawn at the times indicated for the measurement of blood gas and acid-base status and O_2 uptake. Muscle biopsies were taken at the end of the stimulation period. Perfusions of two additional durations were used and followed the same protocol as the 50 min perfusions (S20) but ended after 5 min of stimulation (S5) or following 30 min of rest (R). A second set of 50 min perfusions were performed to obtain muscle samples (S20) exclusively for the measurement of intramuscular triacylglycerol stores.

3.2.8 Analytical Methods

Electrodes were used to measure PO_2, PCO_2 and pH (Radiometer BMS 3) and the percent O_2 saturation of Hb and Hb concentration were measured with a hemoximeter (Radiometer OSM2). Total O_2 content was calculated from the PO_2 values, Hb concentration and percent saturation of Hb (Appendix A). The hindquarter O_2 uptake was calculated as the product of

the arterial-venous O_2 content difference and the flowrate. Plasma bicarbonate was calculated from pH and PCO_2 (Siggaard-Anderson, 1963). Arterial hematocrit (Hct) was measured by the centrifuge method.

Four ml of each perfusate sample were immediately dispensed into glass tubes containing powdered sodium fluoride, mixed and stored on ice. An aliquot was removed, deproteinized in cold 6% (w/v) $HClO_4$ and stored on ice. Both samples were centrifuged within 1 hr. The acid extract supernatant was frozen for subsequent analysis of glucose and lactate concentrations by enzymatic fluorometric techniques (Bergmeyer, 1965). The plasma supernatant was frozen for subsequent analysis of glycerol, FFA, protein and electrolyte concentrations. Glycerol was analyzed by the enzymatic technique of Garland and Randle (1962). The FFA concentration was measured as described by Nixon and Chan (1979) and protein using the method of Lowry et al (1951). Routine laboratory methods using Technicon auto-analyzers were used to measure plasma electrolyte concentrations of Na^+, K^+, Cl^- and Ca^{2+}. The release and uptake of metabolites and substrates across the hindquarter were calculated as the product of the flowrate and the arterial-venous concentration difference.

Frozen muscle biopsies were pulverized under liquid N_2 and all visible connective tissue was removed. A portion of the sample (50 100 mg) was weighed wet added to

preweighed plastic tubes containing cold 6% $HClO_4$ and homogenized (Polytron). The sample was stored on ice and centrifuged at 4°C within 1 hr. The supernatant was removed and neutralized with KOH. The acid precipitate was washed with H_2O, freeze dried and weighed to give the tissue dry weight. The precipitated $KClO_4$ of the neutralized extracts was removed by centrifugation and the supernatant used to measure lactate, ATP, CP, ADP and AMP concentrations using standard enzymatic fluorometric techniques as outlined by Bergmeyer (1965). The second portion of muscle was weighed wet, added to a plastic tube containing chilled 0.2 M acetate buffer and homogenized. The sample was stored on ice and centrifuged within 1 hr. The supernatant was used for the measurement of glycogen content (Keppler and Decker, 1974).

Total adenine concentration (TA), the ATP/ADP and CP/ATP ratios and the energy charge potential (ECP = ATP + 1/2 ADP/ATP + ADP + AMP) were calculated from the measured values. All reported values were expressed per g dry wt since the muscle wet/dry wt ratios increased following stimulation by approximately 17% in the soleus (SOL) and plantaris (PL) muscles and 25% in the red (RG) and white gastrocnemius (WG) muscles.

Muscle samples obtained from the second set of 50 min perfusions were homogenized under liquid N_2 and all visible connective tissue was removed. A small aliquot of tissue (20-40 mg) was removed and freeze dried for determination of

the muscle wet/dry weight ratio. The remaining muscle was divided into 2 equal aliquots (40 100 mg) for the quantitative determination of muscle TG content as originally outlined by Denton and Randle (1967) and later modified by Frayn and Maycock (1980).

Each aliquot was weighed wet and added to a glass tube containing 5 ml of a chloroform-methanol (2/1, V/V) extraction solution. Three ml of 4 mM $MgCl_2$ were added and the tube centrifuged at 1000 g for 1 hr at 4°C for separation of the phases. The upper aqueous phase was aspirated and discarded without disturbing the muscle powder "cake" at the interface of the two phases Two ml of the lower organic phase was aspirated into a clean glass tube, evaporated and the residue redissolved with 5 ml of chloroform. Phospholipids were removed by adding 500 mg of silicic acid to each tube. Following mixing and centrifugation the supernatant was evaporated and saponified with ethanolic KOH (0.5 ml of 4% KOH in 95% ethanol/tube; 5 parts of aqueous 80% KOH in 95 parts ethanol) at 60°C for 1 hr. One ml of 0.15 M $MgSO_4$ was added and following centrifugation, portions of the supernatant were analyzed for glycerol by the enzymatic technique of Garland and Randle (1962). A triolein standard processed through the extraction procedure gave a glycerol recovery of 94-100%.

All chemicals were obtained from Sigma Chemical Co., St. Louis, U.S.A. and enzymes from Boehringer Mannheim,

Montreal, Canada.

3.2.9 Statistics

All reported values are means ± SE unless otherwise noted. A paired t-test was used to assess pre and post-perfusion muscle measurements. All other dependent variables were assessed by a series of oneway analyses of variance (dependent variable x time points) for the evaluation of time effects on the dependent variable. The Student Newman-Keuls test was used to compare means when a significant F ratio was obtained. Statistical significance was accepted at $P<0.05$.

3.3 Results

Blood gas and acid-base status remained constant throughout the perfusions (Table 3) and there were no significant differences between the arterial and venous electrolyte values at rest and during stimulation (Table 4).

The peak isometric tension generated by the GPS muscle group was 2648 ± 55 g (Fig. 6). Tension decreased to 85.8%, 69.3% and 59.7% of peak tension after 2, 5 and 10 min of stimulation, respectively. During the final 10 min of stimulation, the rate of tension decay was much slower; the generated tension after 20 min of stimulation representing 51.6% of peak tension. Tension values after 15 min and 20 min of stimulation were not significantly different.

Table 3. Characteristics of the perfusion mediums (mean ± SE, n = 23).

PaO_2, mmHg	$PaCO_2$, mmHg	pH	HCO_3^-, mmol/l	P_{50}, mmHg	[Hb], g/dl	Hct, %	Protein, g/dl
360	39.7	7.412	25.2	27.0	14.3	40.9	3.8
±12	±0.9	±0.008	±0.7	±0.5	±0.3	±0.7	±0.1

Table 4. Electrolyte data at rest and during stimulation (mean ± SE, n = 19 at rest; n = 6-9 during stimulation).

	Na^+	K^+	Cl^-	Ca^{2+}
	mmol/l			
REST				
20 min arterial	148	5.8	121	2.4
	± 1	± 0.1	± 1	± 0.1
STIMULATION				
40 min arterial	149	5.7	122	2.4
	± 1	± 0.1	± 1	± 0.1
40 min venous	149	6.0	121	2.4
	± 1	± 0.1	± 1	± 0.1

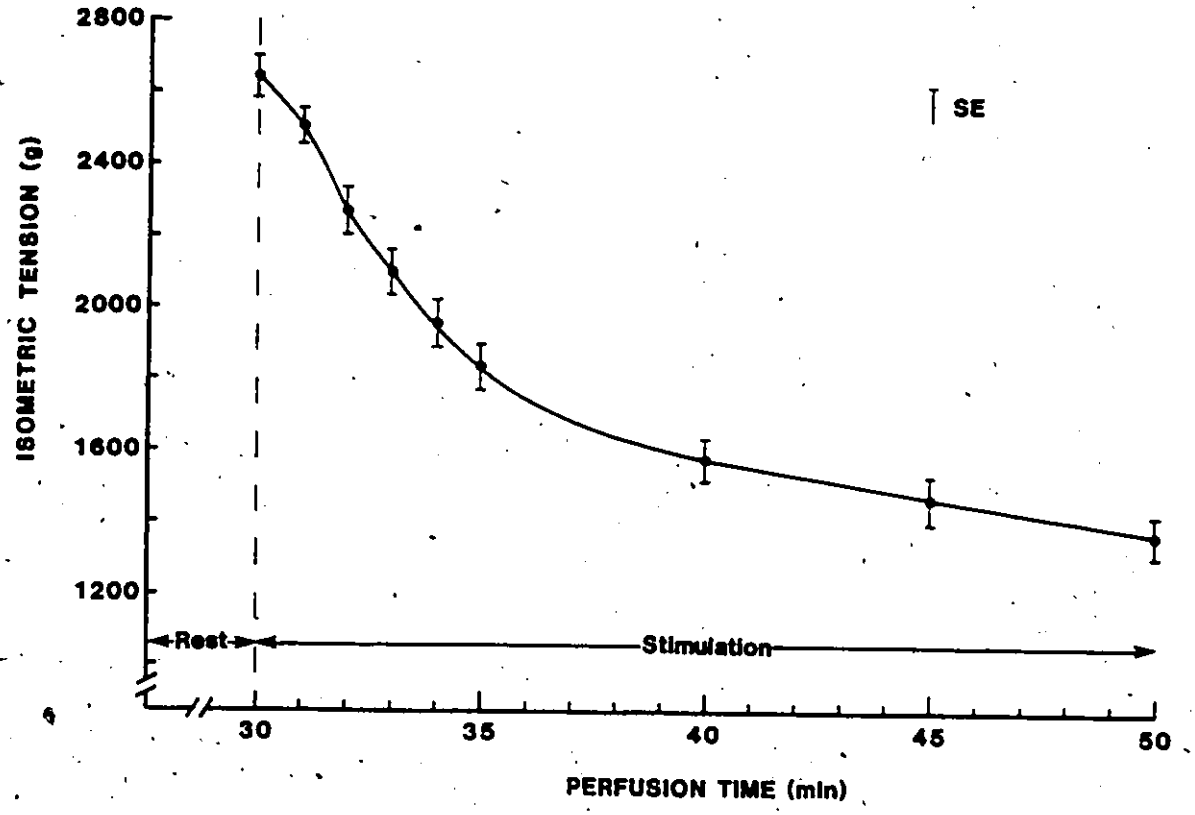


Fig. 6. Isometric tension generated by the gastrocnemius-plantaris-soleus muscle group during stimulation (100 msec trains (100Hz), 5 volts, 0.5 Hz).

Hindquarter O_2 uptake was 7.7 ± 0.3 umol.min^{-1} at 12 min and remained unchanged for the duration of the rest perfusion (Fig. 7). Three min after the onset of stimulation at 33 min, O_2 uptake increased approximately 3 fold to 22.5 ± 0.7 umol.min^{-1}, peaked at 36 min (23.4 ± 1.3 umol.min^{-1}) and decreased thereafter such that the 48 min O_2 uptake of 18.8 ± 1.1 umol.min^{-1} was significantly lower than the 33 and 36 min values. The resting O_2 uptake expressed per g wet wt of perfused muscle was 0.56 umol.min^{-1}. The corresponding peak O_2 uptake value during stimulation was 2.60 umol.min^{-1}.g^{-1} wet wt working muscle, assuming that 56.2% of the 13.74 g hindquarter was working (see above) and the non-working muscle O_2 uptake remained unchanged. The arterial pressure at 10 min was 82 ± 2 mmHg and remained constant at rest (Fig. 7). With the increased flow rate during stimulation, the pressure increased to 197 ± 6 mmHg and continued to rise gradually but non-significantly throughout the stimulation period. The pressures required to overcome the resistance of only the perfusion system at the flow rates used at rest and during stimulation were 24 and 104 mmHg, respectively.

The lactate release by the hindquarter at 21 min was 1.39 ± 0.24 umol.min^{-1} and remained constant over the final 10 min of rest perfusion (Fig. 8). Stimulation caused an immediate increase in lactate release and a peak at 33 min (23.40 ± 1.40 umol.min^{-1}). No significant differences existed between any of the data points at 43 min (10.82 ±

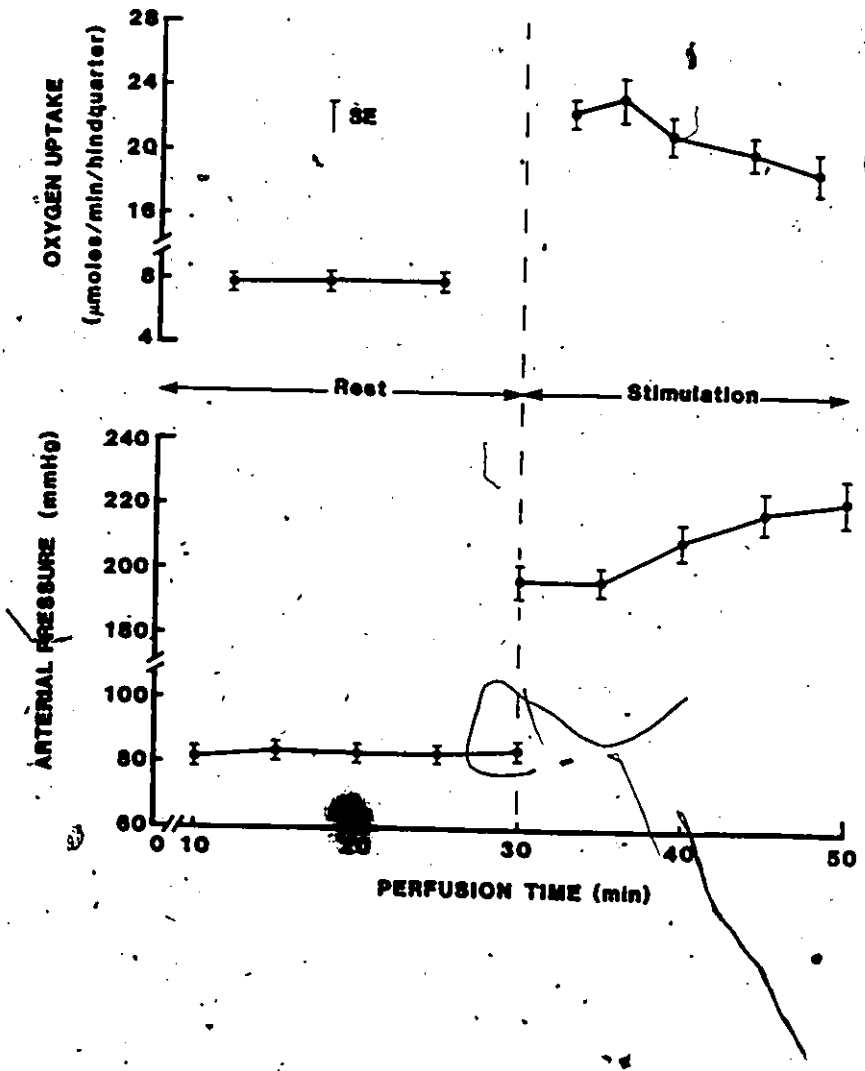


Fig. 7. Hindquarter O_2 uptake and perfusion pressure at rest and during stimulation.

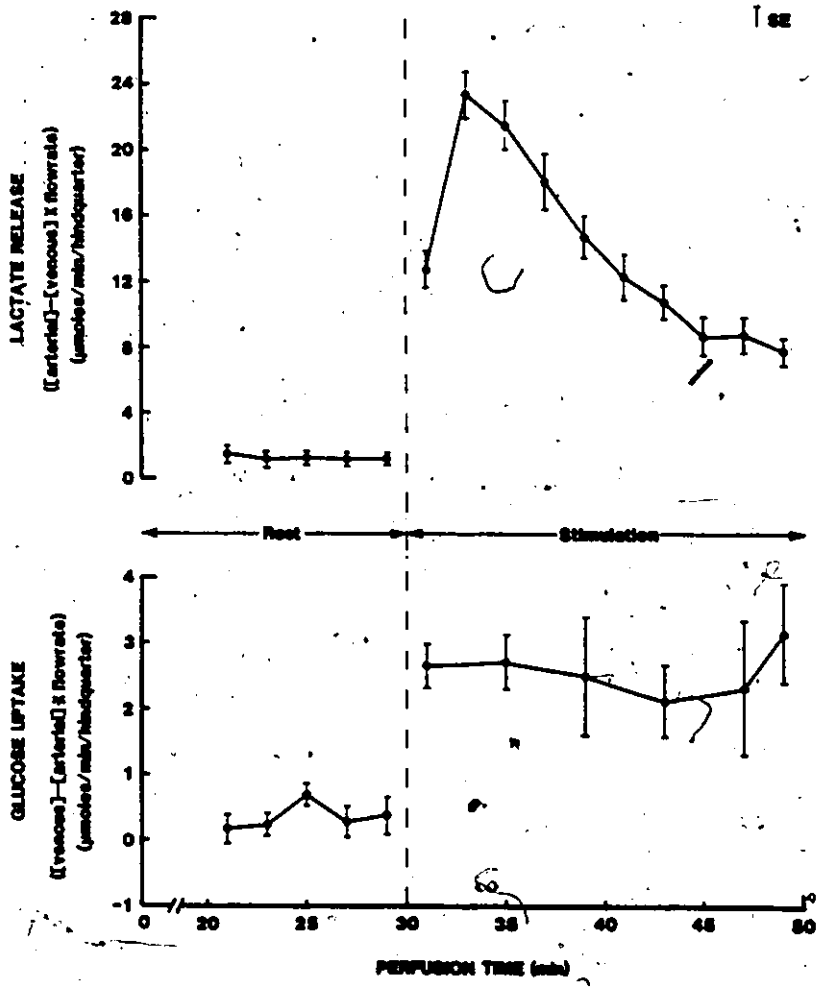


Fig. 8. Hindquarter lactate release and glucose uptake at rest and during stimulation.

1.22 umol.min^{-1}) and beyond, indicating that a steady state of lactate release was achieved. The lactate released at rest expressed per g of perfused muscle was 0.10 umol.min^{-1} and increased to a peak of 2.95 umol.min^{-1}.g^{-1} working muscle, representing a 30 fold increase in the lactate released from working muscle, during heavy stimulation.

The hindquarter glucose uptake was low both at rest and during stimulation since no insulin was added to the perfusate (Fig. 8). Stimulation produced a seven-fold increase in uptake over rest with mean values of 2.63 $\pm$ 0.23 and 0.35 $\pm$ 0.10 umol.min^{-1}, respectively. The glucose uptake remained constant over time at rest and during stimulation.

No free fatty acids were added to the perfusate but the measured arterial FFA concentration was 0.15 $\pm$ 0.01 mM. The FFA entered the perfusate bound to the bovine albumin. The venous FFA concentration was higher than arterial at rest and during stimulation leading to a FFA release from the hindquarter of 87-89 nmol.min^{-1} at rest and 168-220 nmol.min^{-1} during stimulation (Fig. 9). Glycerol release by the hindquarter also increased from rest to stimulation (Fig. 9).

Pre-perfusion biopsy measurements were not significantly different in the R, S5 and S20 conditions (Appendix B, Tables 13-16), therefore the control values in Fig. 10-11 represent mean values. Rest perfusion for 30 min (R) had no effect on the measured variables (Fig. 10-11,

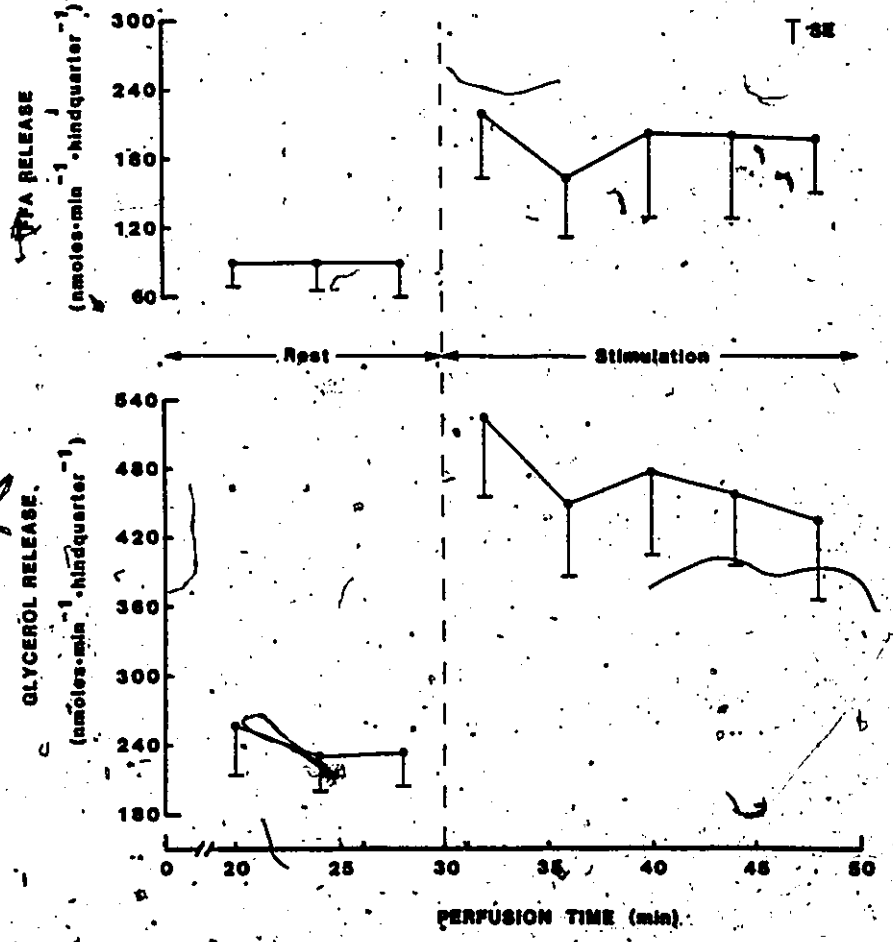
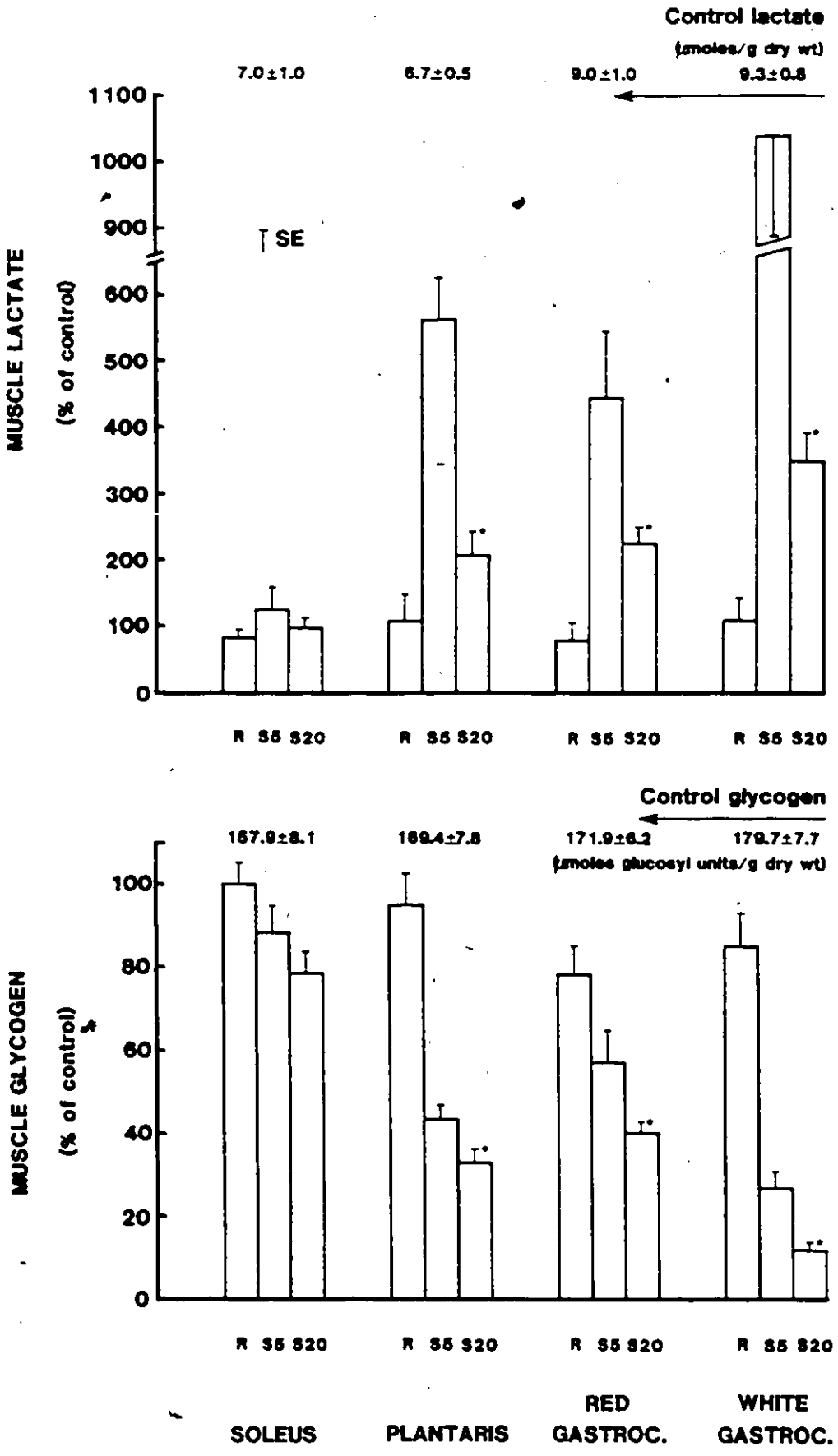
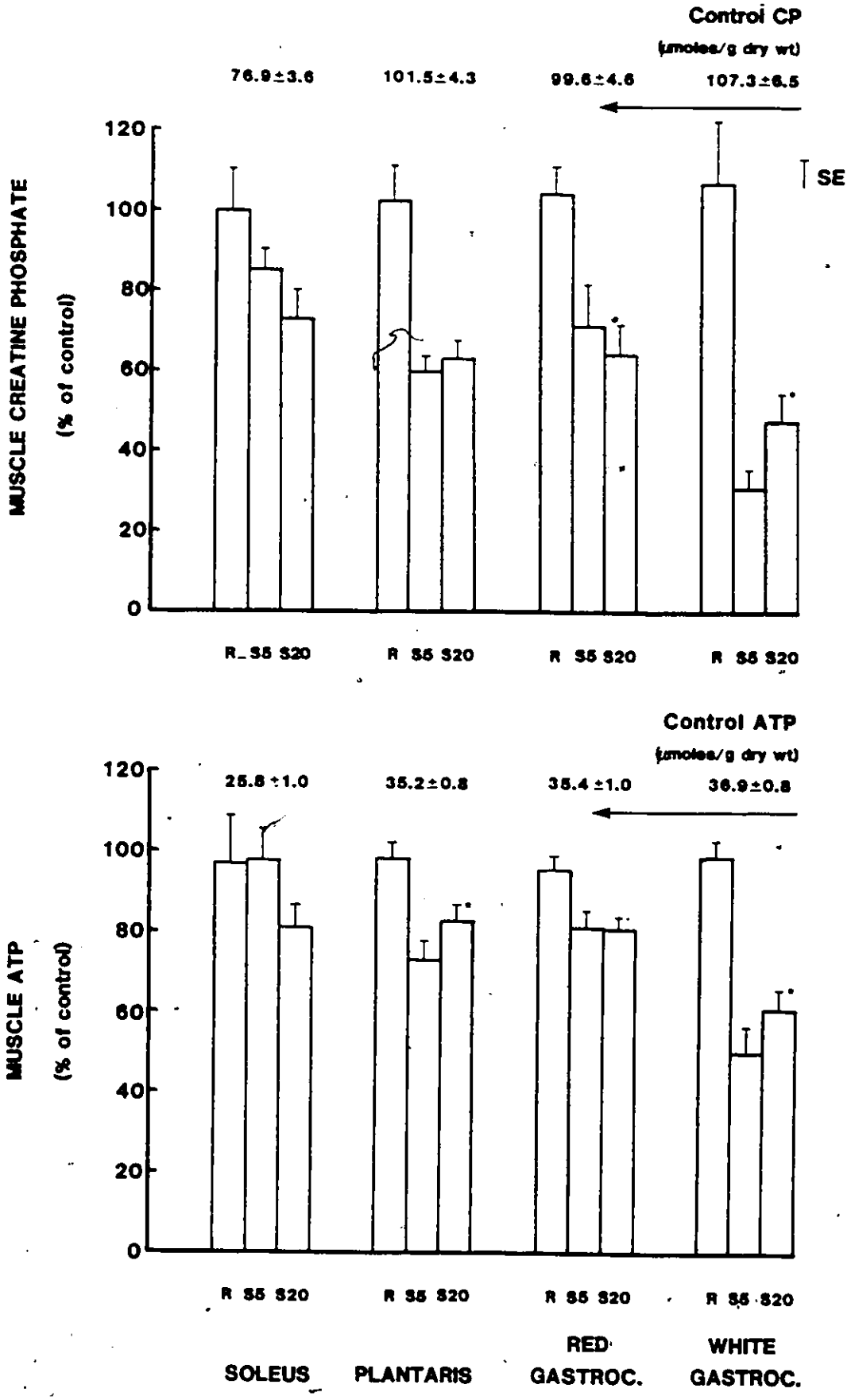


Fig. 9. Hindquarter free fatty acid and glycerol release at rest and during stimulation.

Fig. 10. Muscle glycogen and lactate concentrations following 30 min rest perfusion (R), 30 min rest perfusion and 5 min stimulation (S5) and 30 min rest perfusion and 20 min stimulation (S20). All S5 values significantly different from R except in soleus. All S20 values significantly different from R except for soleus lactate. * denotes significantly different from S5.

Fig. 11. Muscle creatine phosphate and ATP concentrations following the perfusions. Abbreviations as in Fig. 10. All S5 values significantly different from R except in soleus. All S20 values significantly different from R. * denotes significantly different from S5.


Control lactate
(μmoles/g dry wt)
7.0±1.0
6.7±0.5
9.0±1.0
9.3±0.8
MUSCLE LACTATE
(% of control)
SE
1100
1000
900
600
500
400
300
200
100
0
R S5 S20
Control glycogen
157.9±8.1
169.4±7.8
171.9±6.2
179.7±7.7
(μmoles glucosyl units/g dry wt)
MUSCLE GLYCOGEN
(% of control)
100
80
60
40
20
0
SOLEUS
PLANTARIS
RED GASTROC.
WHITE GASTROC.


Control CP
(μmoles/g dry wt)
76.9±3.6
101.5±4.3
99.6±4.6
107.3±6.5
SE
MUSCLE CREATINE PHOSPHATE
(% of control)
120
100
80
60
40
20
0
R S5 S20
R S5 S20
R S5 S20
R S5 S20
Control ATP
(μmoles/g dry wt)
25.8 ±1.0
35.2±0.8
35.4 ±1.0
36.9±0.8
MUSCLE ATP
(% of control)
120
100
80
60
40
20
0
R S5 S20
R S5 S20
R S5 S20
R S5 S20
SOLEUS
PLANTARIS
RED GASTROC.
WHITE GASTROC.

Tables 13-14) in the 4 muscle samples except in the RG muscle where 21.4% of the pre-perfusion glycogen was utilized. Thirty min of rest perfusion plus 5 min of stimulation (S5) resulted in large decreases in glycogen, CP, ATP and TA concentrations and ECP and an increase in lactate levels in the PL, RG and WG muscles (Fig. 10-11, Tables 13,15). The ADP and AMP levels increased significantly only in the RG muscle. The ATP/ADP ratio decreased significantly in the three muscles as did the CP/ATP ratio in the PL and WG muscles. No changes in any of the measured variables were found in the SOL muscle (Fig. 10-11, Tables 13,15).

The S20 measurements (following 30 min rest and 20 min stimulation) produced significant decreased in glycogen, CP, ATP and TA levels and in ATP/ADP and CP/ATP ratios and ECP in the PL, RG and WG muscles (Fig. 10-11, Appendix B, Tables 13,16). The lactate concentration increased significantly in the same three muscles. The ADP and AMP levels were unchanged in all muscles except the RG where AMP increased. The additional 15 min of stimulation in S20 significantly decreased glycogen, CP and ATP levels and ATP/ADP and CP/ATP ratios of the SOL muscle. No increase in lactate concentration was found in the SOL muscle (Fig. 10).

When comparing the S5 and S20 conditions, lactate concentrations were significantly higher after S5 in the PL, RG, WG muscles (Fig. 10). Glycogen depletion was greatest following S20 in all 4 muscles. However, in the PL, RG and

WG muscles, the absolute amount of glycogen utilized in the first 5 min of stimulation was far greater than during the subsequent 15 min. The CP and ATP levels decreased similarly following S5 and S20 in all muscles except the WG where both variables were lower following S5 (Fig. 11).

A second set of 50 min perfusions with biopsies at S20 were performed to examine muscle triacylglycerol content (Fig. 12). Soleus TG decreased by 15%, a non-significant amount. Significant decreases in TG were found in the PL and RG muscles while 20 min of stimulation had no effect on WG levels. The 13.5% decrease in PL muscle was significant since 7 of 8 animals showed decreases in TG content while the decrease in the RG muscle amounted to 31.6%.

3.4 Discussion

The present work refined the perfused rat hindquarter preparation, previously used to examine resting muscle metabolism (Lewis et al, 1977; Dietz et al, 1980; Ivy and Holloszy; 1981), to the point where the metabolism and performance of heavily contracting hindquarter muscles could be examined.

Many previous investigators did not quantify tension generation by the exercising hindquarter (Berger et al, 1975; Ruderman et al, 1980; Walker et al, 1982a), or used submaximal non-tetanic stimulation rates resulting in low tension production (Everts et al, 1981; Richter et al,

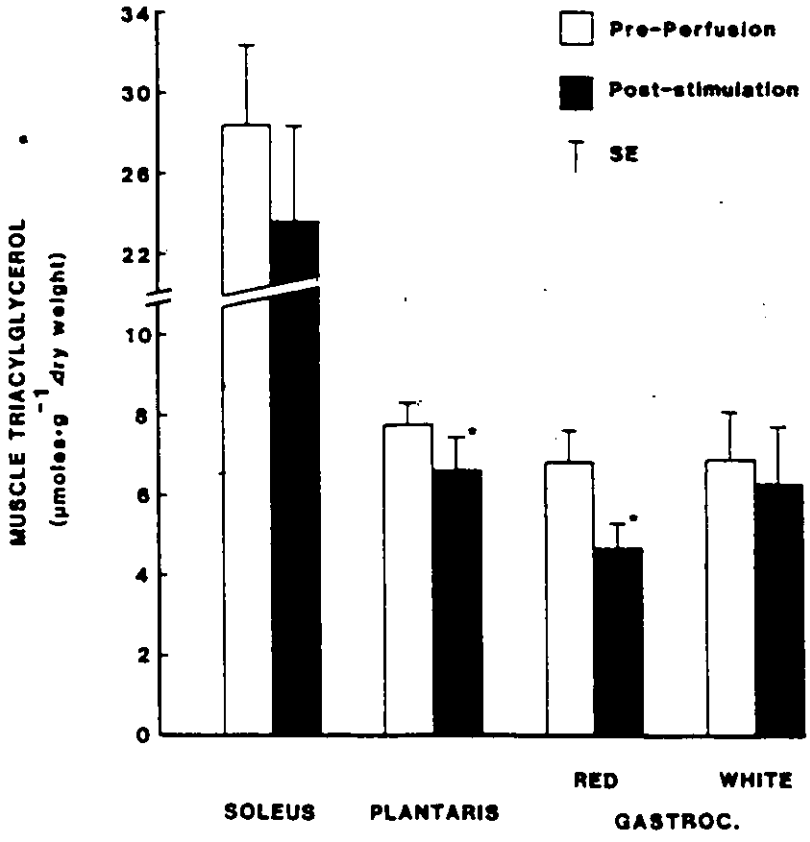


Fig. 12. Muscle triacylglycerol content pre-perfusion and following 20 min of stimulation. * denotes significantly different from pre-perfusion.

1982a). The generated isometric tension in the present study far exceeded most existing reports of tetanic tension produced during rat hindquarter stimulation. Rennie and Holloszy (1977) reported a peak tension of 1000 g and a subsequent decay to 40-50% in 10 min of stimulation while McLane et al (1981) reported, from the same laboratory 4 years later, a peak tension of approximately 1500 g falling to 800 g after 10 min of stimulation. Rennie and Holloszy (1977) used a stimulation rate identical to ours but McLane et al (1981) stimulated with 100 msec trains at 50 Hz (5 impulses per train vs 10 per train in the present study). Only Hood et al (1983) have recently reported similar peak tetanic tensions to the present study (3590 $\pm$ 141 g at a stimulation frequency of 90 tetani per min and with larger rats).

There appear to be several factors which may be responsible for the higher tension in the present investigation. First, perfusion with less fragile, more normally sized fresh bovine cells and an increased Hb concentration (14 vs 12 $g.dl^{-1}$) may have contributed to an enhanced tissue perfusion and O_2 delivery during stimulation. This is unlikely to be the whole explanation as the exercising hindquarter O_2 uptake values were similar in all studies (Rennie and Holloszy, 1977; McLane et al, 1981; Hood et al, 1983). Secondly, improved joint stabilization and transducer attachment allowed large tensions to be developed

without fraying of the tendon or destabilization of the joint (Fig. 3). Care was taken to ensure that the metal screws were placed into bone at the knee to avoid disruption of blood flow through the popliteal fossa to the lower leg. Thirdly, rats were perfused on their dorsal surface in the present study while stabilization and stimulation occurred with the rat on its ventral surface in the previous studies perhaps accounting for the tension discrepancy (Rennie and Holloszy, 1977; McLane et al, 1981). Lastly, McLane et al (1981) used a recirculating perfusion system unlike the one-pass system in this study.

The lactate release by the working muscles increased approximately 30 fold within 3 min of stimulation and reached a plateau at 10 13 fold greater than rest during the final 10 min (Fig. 8). Most investigators measured lactate release only every 5 or 10 min and failed to demonstrate the dynamic changes in lactate release (Ruderman et al, 1971; Berger et al, 1976; Rennie and Holloszy, 1977; McLane et al, 1981). Walker et al (1982b) reported lactate release data obtained at 10 min intervals but during the low intensity stimulation protocol used by these workers frequent sampling may not be important.

Hindquarter glucose uptake was considerably lower both at rest and during exercise compared to experiments that included insulin in the perfusate (Berger et al, 1976; Richter et al, 1982b; Walker et al, 1982a). Ivy and Holloszy

(1981). reported a resting glucose uptake of 0.027 umol.min^{-1}.g^{-1} perfused muscle in the absence of insulin which agrees closely with our value of 0.026 umol.min^{-1}.g^{-1} perfused muscle. In the present study the glucose uptake increase with stimulation was approximately 7 times that at rest and much larger than the 3 fold increase in glucose delivery due to the increased flow rate.

The advantages of the perfused rat hindquarter model in the study of muscle metabolism were recognized by Houghton, Ruderman and Hems in their initial studies (Houghton, 1971; Ruderman et al, 1971). We have extended the use of this model to examine both the performance and metabolism of skeletal muscle during heavy contraction. The reported measurements enable estimates to be made of the relative contributions of individual muscles and individual fuels during the course of heavy stimulation.

During the first 5 min of stimulation an average tension of 66,510 g.min^{-1} (2217 g x 30 contractions) was produced by the GPS muscles. Since this muscle group weighed 2.196 g, the average tension production was 30,287 g.min^{-1}.g^{-1} active muscle. Although O_2 uptake increased promptly to a steady state level (Fig. 7) glycolytic metabolic processes resulted in high lactate production and glycogen utilization in fast twitch fibers (Fig. 10) accompanied by falls in CP and ATP (Fig. 11). Little change occurred in slow twitch muscles. From the measured amounts

of O_2 and CP utilized and lactate produced we calculated the absolute amounts of energy produced from the major energy releasing pathways of oxidative metabolism and the "lactic" and "alactic" components of anaerobic metabolism as outlined by Margaria (1976)(Appendix A). During the first 5 min of stimulation 71.7% of the total energy produced was derived aerobically, 22.8% from glycolysis and 5.5% through CP breakdown (Table 5). The heavy glycolytic involvement attested to the severity of the stimulation and was associated with a large amount of lactate released from the hindquarter and a rapid tension decline during the initial 5 min. The decreased ECP found in the fast twitch muscles was also indicative of the heavy metabolic load (Atkinson, 1968).

During the first 5 min of stimulation carbohydrate was rapidly utilized and 94% of the total glucose used was derived from muscle glycogen (Table 6). Lactate production (87 umoles released from the muscle and 54 umoles remaining in the muscle) accounted for 45% of the metabolized glucose, aerobic metabolism accounting for the remainder.

The perfusion system allowed O_2 delivery to the exercising muscle to increase without delay and consequently O_2 extraction by the muscle increased rapidly (Fig. 7). To reconcile these changes with the extensive lactate production, it appeared that activation of pyruvate dehydrogenase, the non equilibrium enzyme controlling pyruvate entry into the citric acid cycle, was delayed

Table 5. Energy released during 20 min of stimulation.

	O_2 Utilized	Lactate Produced	CP Utilized	Total Energy Released
		energy released, joules		
min 30-35	40.3	12.8	3.1	56.2
	(71.7)	(22.8)	(5.5)	
min 35-50	104.8	12.1	-	116.9
	(89.7)	(10.3)		

Values are per working hindquarter. Bracketed values are percent of total energy released. Calculations based on 1.0 ml O_2 = 20.9 joules, 1.0 mg lactate = 1.0 joules and 1.0 umole CP = 0.05 joules as outlined by Margaria (1976). Assumptions; calculations based on O_2 and CP utilized and lactate produced above resting values, O_2 uptake reaches maximal value instantly, plantaris muscle represents non-sampled working muscle, non-working metabolism remains constant with stimulation, lactate produced not metabolized to any great extent due to one-pass perfusion system. Also, alternate fates of glucose such as expansion of intracellular hexosephosphate and glucose pools and alanine production were not assessed and assumed to be minor.

Table 6. Sources and fates of glucose during 20 min of stimulation.

	Sources of Glucose			Fates of Glucose	
	Muscle Glycogen	Exogenous Perfusate	Total	Lactate	Aerobic Metabolism
			umoles		
min 30-35	149.0	9.5	158.5	70.5	88.0
	(94.0)	(6.0)		(44.5)	(55.5)
min 35-50	46.9	29.5	76.4	66.7	9.7
	(61.4)	(38.6)		(87.3)	(12.7)

Values are per working hindquarter. Bracketed values are percent of total glucose.
Assumptions; as in Table 5; also, all glucose not metabolized to lactate was assumed to be used aerobically.

relative to the rate limiting glycolytic enzymes phosphorylase and phospofructokinase. Ward et al (1983) showed that activation of this enzyme is incomplete in heavy short term exercise. Thus in the type of exercise studied here it seems likely that increased O_2 extraction may follow the mobilization of appropriate aerobic substrates, rather than the classical alternate view that the use of anaerobic pathways follows an inadequate O_2 delivery.

During the final 15 min of stimulation an average tension of 21,175 $g.min^{-1}.g^{-1}$ active tissue was maintained, the total tension production during this time being about 2.1 fold larger than in the initial 5 min. Reductions in glycogen were small and similar in all muscles and CP and ATP increased only in the WG muscle which contains predominantly FG fibers. Fast twitch muscles continued to produce lactate but at a much lower rate leading to a net loss of muscle lactate in the final 15 min and a steady state in muscle lactate release. Oxygen uptake was only slightly lower than peak values and constant, paralleling the tension values. Energy calculations revealed that oxidative metabolism accounted for 90% of the energy produced and the total energy produced in the final 15 min was 2.1 fold greater than in the initial 5 min of stimulation, precisely matching the increase in total tension production (Table 5). Approximately 60% of peak tension was held in the final 15 min most likely by the hindquarter FOG fibers which make up 50% of the total fiber

composition (Ivy and Holloszy, 1981). Of the 71 umoles of glucose utilized, 61% was derived from muscle glycogen and 39% was taken up from the perfusate. The produced lactate accounted for 87% of the utilized glucose leaving only 10 umoles or 13% for aerobic metabolism (Table 6). If all the available glucose was oxidized, about 75% of the aerobic metabolic processes remained unaccounted for. These data suggested that exogenous FFA and/or intramuscular TG were used extensively during the last 15 min of stimulation.

Direct measurements revealed that intramuscular TG (TGm) was utilized during 20 min of stimulation (Fig. 12). It seems likely that the TGm was used mainly in the final 15 min of stimulation since glycogen degradation was rapid at the onset of stimulation and glycogen depletion was nearly complete after only 5 min of stimulation. Calculations based upon the glucose available for aerobic metabolism (Table 6) and the TGm metabolized (Fig. 12) suggested that endogenous TG metabolism may have been the major source of energy in the final 15 min (Table 7, Appendix A). Glucose and TG metabolism accounted for 90% of the measured O_2 uptake, and the remaining 10% was assumed used for the oxidation of exogenous FFA (Table 7). These findings demonstrate that muscles containing high aerobic capacities will metabolize FFA from endogenous TG stores during heavy electrical stimulation when alternate fuels are unavailable.

The rat hindquarter released FFA during perfusions at

Table 7. The contribution of energy substrates to aerobic metabolism during the final 15 min of stimulation.

Substrate, and Source	Energy Released	
	Joules	Percent of Total
GLUCOSE, muscle glcogen and exogenous glucose	30.5*	28.1
FFA, from intramuscular triacylglycerol stores	67.5+	62.2
EXOGENOUS FFA, perfusate and adipose tissue	10.5++	9.7
total	108.5	100.0

Assumptions as in Table 5. Also assumes; * all glucose not used anaerobically was used aerobically; + assumes all FFA from TGm were metabolized by the muscles during the final 15 min of stimulation; ++ calculated from the unaccounted portion of O_2 utilized.

rest and during stimulation (Fig. 9). The released FFA may have originated from hindquarter adipose tissue or from the hydrolysis of intramuscular triacylglycerol (TGm). It is unlikely that TGm was metabolized to any large extent at rest since carbohydrate fuels were readily available. If the FFA originated from adipose tissue lipolysis and all of the FFA and glycerol were released into the plasma, the expected measured FFA/glycerol release ratio would be 3. This assumes that adipose tissue (Vaughan and Steinberg, 1963) and skeletal muscle (Garland and Randle, 1964) do not metabolize glycerol. In vivo, catecholamines are primarily responsible for TG lipolysis in adipose tissue while the presence of glucose and insulin reesterifies much of the released FFA. All of the released glycerol leaves the adipose tissue along with the FFA not reesterified leading to a FFA/glycerol release ratio of less than 3 (Havel and Carlson, 1962).

In this hindquarter preparation at rest, in the absence of catecholamines and insulin and in the presence of glucose, the measured FFA/glycerol release ratio was 0.37. This suggested that most of the FFA accumulated during lipolysis were immediately reesterified within adipose tissue or released from adipose tissue and metabolized by the muscles. However, at rest only a small amount of exogenous FFA would be required for muscle metabolism thereby not altering the FFA/glycerol release ratio significantly. Consequently, it appears that TG-fatty acid cycling in

adipose tissue determined the release of glycerol and FFA into the perfusate. Additionally, other rat hindquarter preparations containing only glucose in the perfusate also reported FFA release at rest (Van Hardeveld and Kassenaar, 1977; Goodman et al, 1983). When insulin was included FFA release was non-existent and glycerol release decreased suggesting that insulin may enhance FFA reesterification and directly inhibit TG lipolysis to prevent FFA release (Ruderman et al, 1971; Goodman et al, 1983). In preparations with no insulin, TG lipolysis appeared to proceed even in the absence of stimulatory catecholamines while glucose taken up from the perfusate provides alpha-glycerophosphate, a precursor for TG reesterification.

Upon stimulation both FFA and glycerol release increased immediately by approximately 2 fold leaving the FFA/glycerol release ratio unchanged at 0.36, suggesting that adipose tissue dynamics also dominated the release of FFA and glycerol during stimulation (Fig. 9).

During the final 15 min of stimulation aerobic metabolism was the dominant pathway of energy production and TGm metabolism provided 62% of the aerobic substrate. An expected increase in glycerol release resulting from the TGm hydrolysis was not seen (Fig. 9). It is possible that skeletal muscle may contain sufficient glycerol kinase or a glycerol dehydrogenase to metabolize significant amounts of glycerol (Toews, 1966b; Robinson and Newsholme, 1967).

4. EFFECTS OF ACIDOSIS ON RAT MUSCLE METABOLISM AND PERFORMANCE DURING HEAVY EXERCISE

4.1 Introduction

During heavy muscular contraction the energy required to generate force is greater than the amount produced through aerobic metabolism. Anaerobic metabolism contributes the remaining portion of the required energy needed to maintain the workload. Since local phosphagen stores are depleted rapidly anaerobic glycolysis (glycolysis ending in lactic acid formation) dominates the anaerobic energy involvement. The byproduct of this anaerobiosis is the buildup of lactate and hydrogen ions, the latter contributing to a decreased local pH. This local acidosis has been implicated in the direct metabolic inhibition of glycolytic flux (Trivedi and Danforth, 1966; Chasiotis et al, 1983) and in the genesis of fatigue leading to decreased performance (Karlsson, 1971a; Fretthold and Garg, 1978). Additionally it may also interfere with lactate removal from the muscle cell (Mainwood and Worsley-Brown, 1975; Jones et al, 1977).

Although it is generally accepted that increased H^+ concentration adversely affects glycolysis several aspects concerning the metabolic effects of acidosis remain unclear. Respiratory and metabolic acidosis may exert different

metabolic effects (Mainwood and Worsley-Brown, 1975; Ehrsam et al, 1982). Muscle O_2 uptake may be depressed by acidosis during stimulation through a decrease in muscle blood flow and O_2 delivery (Hirche et al, 1975; Steinhagen et al, 1976) or through reduced substrate provision. Acidosis may produce fatigue indirectly through depletion of the high energy phosphates (Sahlin et al, 1983). It also remains possible that the predominant effect of acidosis is not on metabolism directly but mediated through changes in Ca^{2+} dynamics and excitation-contraction coupling (Portzehl, 1969; Fabiato and Fabiato, 1978).

The work described in this chapter attempted to comprehensively examine the major energy releasing pathways in skeletal muscle during induced acidosis and contribute to a more complete understanding of the metabolic effects of acidosis. The rat hindquarter preparation is well suited for this work since it permits a tight control over all aspects of the muscle's environment. In this study exogenous fuel sources were limited such that fuel for energy production was derived mainly from intramuscular sources during conditions of normal acid-base status and extracellular acidosis.

4.2 Methods

Fed male Sprague-Dawley rats weighing 283 $\pm$ 16 g ($\bar{x}$ $\pm$ SD, n=36) were used in the study.

An experimental protocol identical to that described

in Chapter 3 (Fig. 5) was used to examine the effects of three experimental conditions; control (C), metabolic acidosis (MA) and respiratory acidosis (RA), on the performance and metabolism of the isolated perfused rat hindquarter.

The perfusion medium and apparatus were as described in Chapter 3. The initial lactate concentration in the arterial perfusate was 0.99 $\pm$ 0.05 mM (n=36). The erythrocyte lactate production rate in C was 0.24 $\pm$ 0.04 $mM.hr^{-1}$ and significantly higher in MA (0.43 $\pm$ 0.06 $mM.hr^{-1}$) and RA (0.40 $\pm$ 0.02 $mM.hr^{-1}$).

During C the gassing of the perfusate resulted in arterial PO_2, PCO_2, pH and plasma HCO_3^- values of 390 $\pm$ 11 mmHg, 37.7 $\pm$ 0.7 mmHg, 7.406 $\pm$ 0.008 and 23.6 $\pm$ 0.05 mM, respectively (Table 8). Metabolic acidosis was induced by decreasing the perfusate HCO_3^- to 12.9 $\pm$ 0.4 mM resulting in a pH of 7.148 $\pm$ 0.009 at a PCO_2 of 38.2 $\pm$ 1.2 mmHg. Respiratory acidosis was achieved by increasing the PCO_2 to 63.0 $\pm$ 1.6 mmHg producing a pH of 7.152 $\pm$ 0.009 at a plasma HCO_3^- of 21.3 $\pm$ 0.8 mmHg (Table 8).

The surgical preparation of the hindquarter, stimulation and sampling of the perfused skeletal muscle and analytical methods were as described in Chapter 3.

A paried t-test was used to compare measurements made on muscle biopsies taken pre and post 30 min of rest perfusion in each of the three conditions. In perfusions of

Table 8. Characteristics of the perfusion mediums under control and acidotic conditions (mean ± SE).

	PaO_2, mmHg	$PaCO_2$, mmHg	pH	HCO_3^-, mmol/l	P_{50}, mmHg	[Hb], g/dl	Hct, %	Protein, g/dl
Control (n = 15)	390 ±11	37.7 ±0.7	7.406* ±0.008	23.6 ±0.5	27.0** ±0.5	13.7 ±0.2	40.1 ±0.4	3.9 ±0.1
Metabolic Acidosis (n = 10)	388 ±11	38.2 ±1.2	7.148 ±0.009	12.9++ ±0.4	26.1 ±0.7	14.1 ±0.2	41.1 ±0.5	3.9 ±0.1
Respiratory Acidosis (n = 12)	374 ±10	63.0+ ±1.6	7.182 ±0.009	21.3 ±0.8	23.9 ±0.9	13.5 ±0.3	40.3 ±0.7	4.1 ±0.1

* significantly different from MA and RA; + significantly different from C and MA; ++ significantly different from C and RA; ** significantly different from RA.

S5 and S20 durations the difference between pre and post muscle measurements were taken and the effects of the conditions (C, MA and RA) on the muscle variables were assessed by a series of oneway analyses of variance. All other dependent variables were also assessed by oneway analyses of variance (dependent variable x conditions).

4.3 Results

Perfusate electrolyte values were stable throughout all perfusions in each condition (Table 9).

Resting arterial perfusion pressures were not different in C, MA and RA at similar flow rates of 5.9 ± 0.1, 6.0 ± 0.1 and 6.0 ± 0.1 $ml.min^{-1}$, respectively (Fig. 13). During stimulation the respective flow rates were increased to 18.0 ± 0.6, 19.4 ± 0.5, and 18.0 ± 0.3 $ml.min^{-1}$. Perfusion pressure was greatest in C, least in RA and intermediate in MA.

The peak isometric tension generated by the GPS muscle group during C was 2648 ± 55 g. decreasing to 69.3, 59.7, and 51.6% of peak tension following 5, 10 and 20 min of stimulation, respectively (Fig. 14). Peak tensions during acidosis were not different from C although RA peak tension (2737 ± 57 g) was significantly greater than MA (2497 ± 77 g). At all time points in the subsequent 20 min of stimulation MA and RA tensions were not different but were consistently lower than C. The tension decay in the initial

Table 9. Electrolyte data at rest and during stimulation during control and acidosis perfusions (mean ± SE; A, arterial; V, venous).

		Na^+	K^+	Cl^-	Ca^{2+}
		mmol/l			
Control	20 min A	148 ± 1	5.8 ± 0.1	121 ± 1	2.4 ± 0.1
(n = 15)	40 min A	149 ± 1	5.7 ± 0.1	122 ± 1	2.4 ± 0.1
	40 min V	149 ± 1	6.0 ± 0.1	121 ± 1	2.4 ± 0.1
Metabolic	20 min A	141 ± 1	6.6 ± 0.1	125 ± 1	2.6 ± 0.1
Acidosis	40 min A	141 ± 1	7.1 ± 0.1	123 ± 1	2.7 ± 0.1
(n = 10)	40 min V	142 ± 1	7.4 ± 0.1	122 ± 1	2.7 ± 0.1
Respiratory	20 min A	140 ± 1	6.5 ± 0.1	118 ± 1	2.6 ± 0.1
Acidosis	40 min A	140 ± 3	6.9 ± 0.2	118 ± 3	2.6 ± 0.1
(n = 12)	40 min V	140 ± 2	7.1 ± 0.2	118 ± 2	2.7 ± 0.1

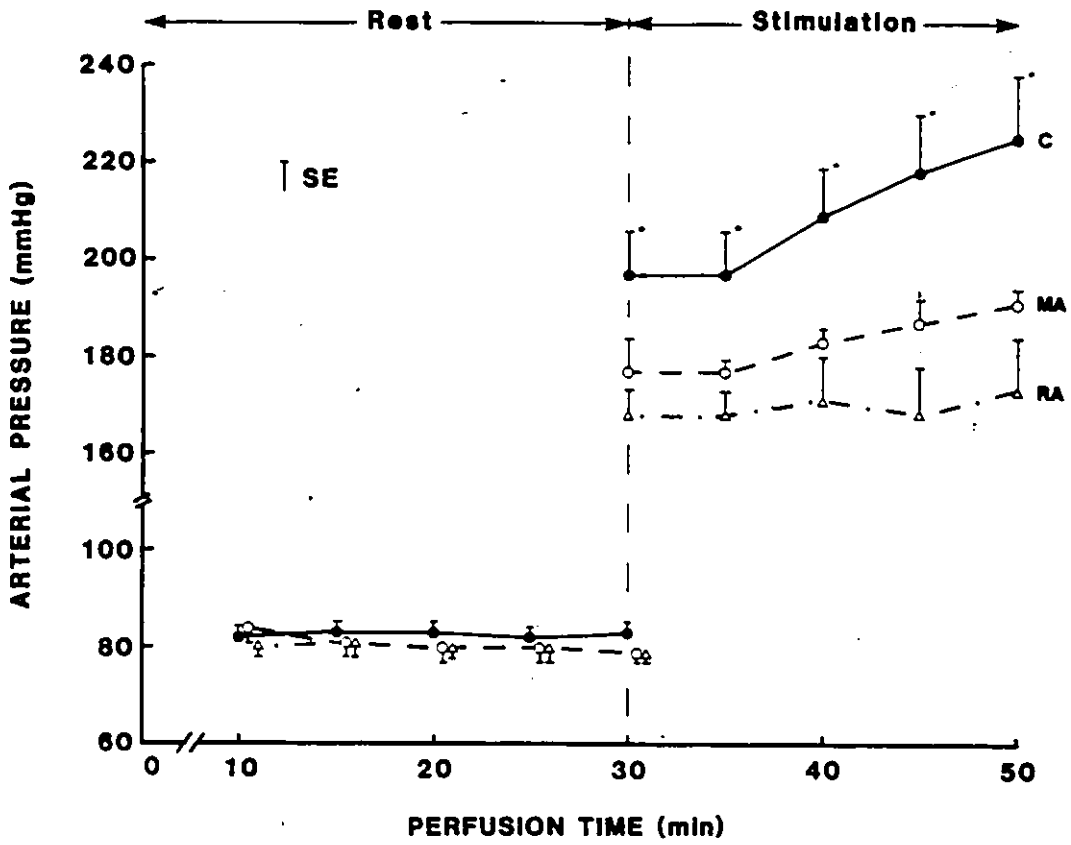


Fig. 13. Perfusion pressure during acidosis. C, control; MA, metabolic acidosis; RA, respiratory acidosis. * significantly different from MA and RA.

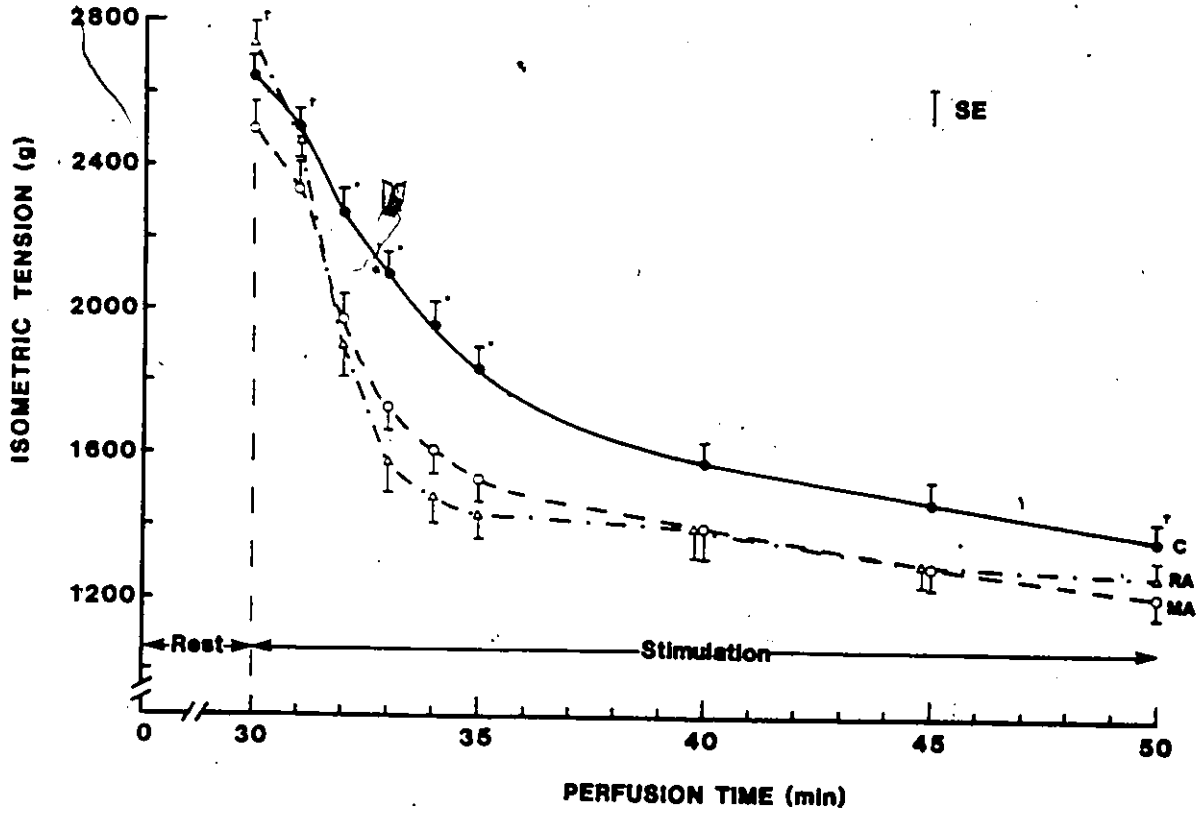


Fig. 14. Isometric tension generated by the gastrocnemius-plantaris-soleus muscle group during acidosis (100 msec trains (100Hz), 5 volts, 0.5 Hz). Abbreviations as in Fig. 13. * significantly different from MA and RA, † significantly different from MA.

5 min was greatest in RA (47.6%), intermediate in MA (38.6%) and least in C (30.7%) (Fig. 14). During min 35-40 tension decay was greatest during C and in the final 10 min of stimulation tension decayed less than 10% in all conditions. The decay in tension during the 20 min of stimulation was 48.5% in C, 51.3% in MA and 53.7% in RA.

Resting O_2 uptake tended to be lower in acidosis but was not significantly different from C (Fig. 15). This trend continued during stimulation with control O_2 uptake being the highest at all time points, RA the lowest and MA intermediate. During the final 10 min of stimulaton the O_2 uptake decreased gradually in all conditions in parallel with the decrease in tension.

At rest, hindquarter lactate release during C was significantly greater than MA, while RA was intermediate (Fig. 16). During stimulation the hindquarter lactate release increased in all conditions; the increase was greatest in C, least in MA and intermediate in RA. During the first 7 min of stimulation, control lactate release was significantly greater than both acidotic conditons and remained significantly greater than MA throughout the stimulaton. Acidosis produced a slight delay in peak lactate release as compared to C (Fig. 16).

Hindquarter glucose uptake at rest was smilar in all conditions (Fig. 17). During the initial 10 min of stimulation glucose uptake was slightly higher during

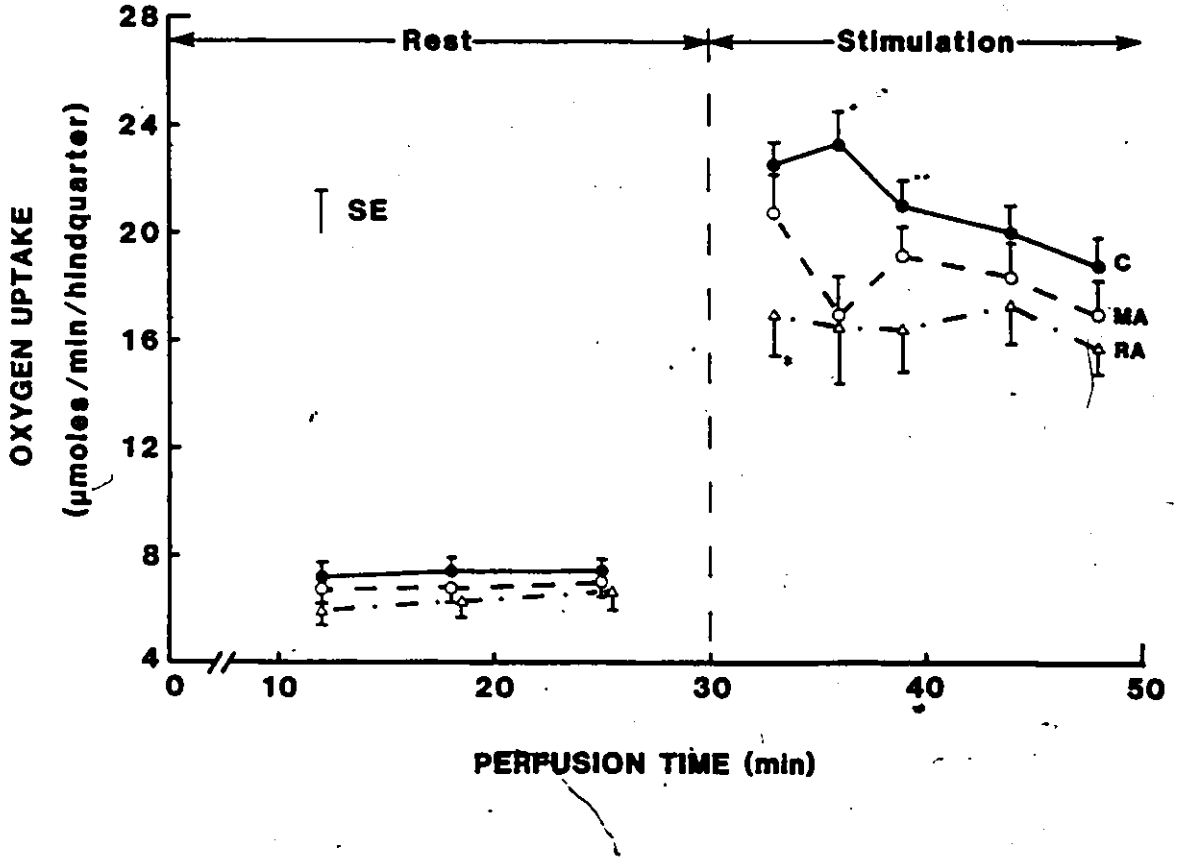


Fig. 15. Oxygen uptake during acidosis. Abbreviations as in Fig. 13. * significantly different from MA and RA, ** significantly different from RA, ‡ significantly different from C and MA.

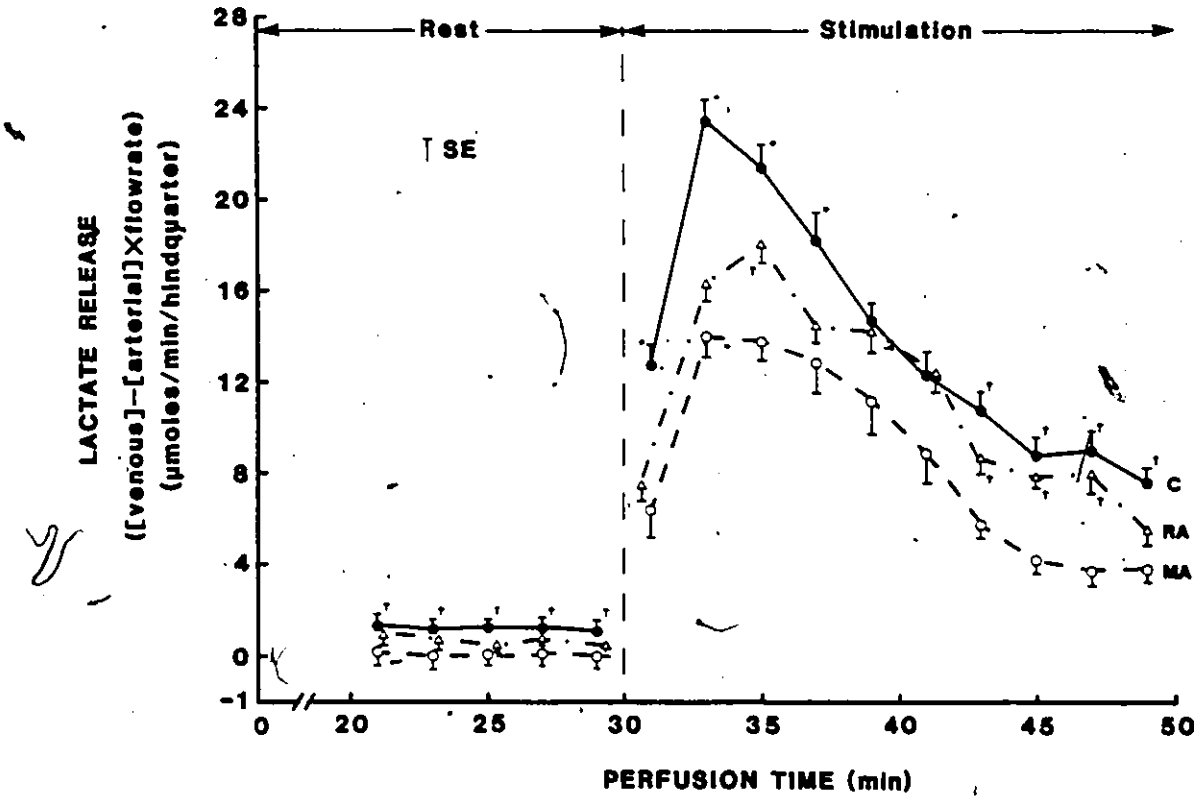


Fig. 16. Lactate release during acidosis. Abbreviations as in Fig. 13. * significantly different from MA and RA, † significantly different from MA.

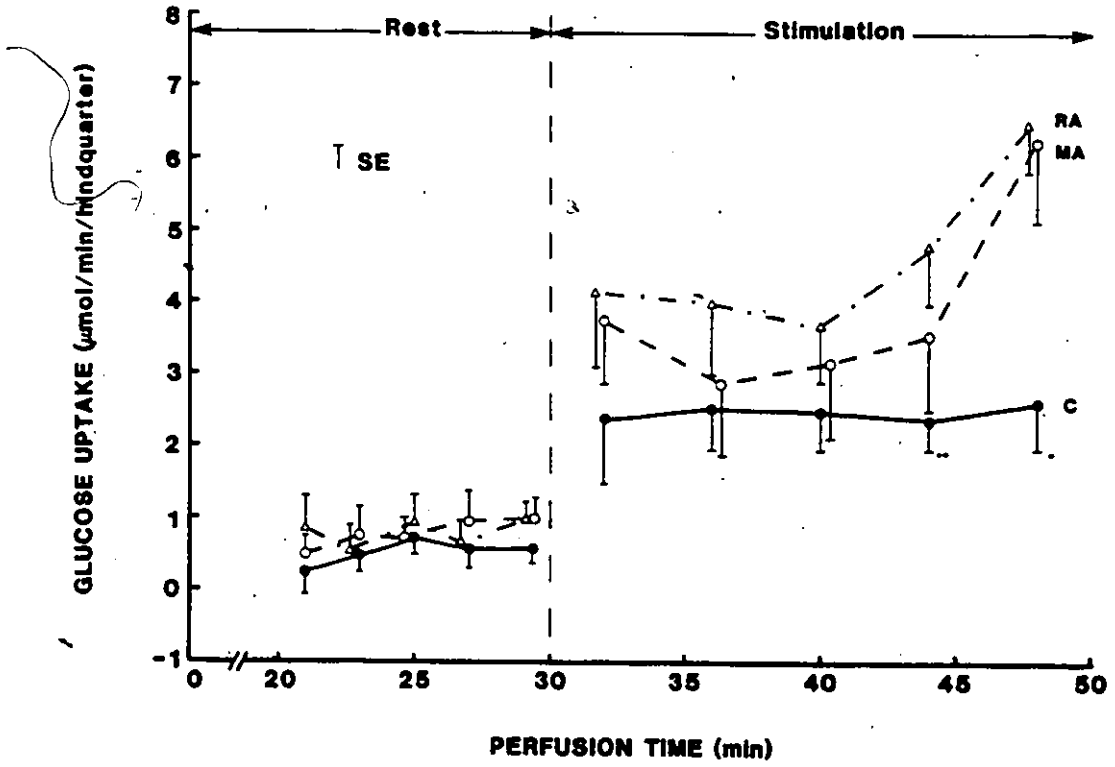


Fig. 17. Glucose uptake during acidosis. Abbreviations as in Fig. 13. * significantly different from MA and RA, ** significantly different from RA.

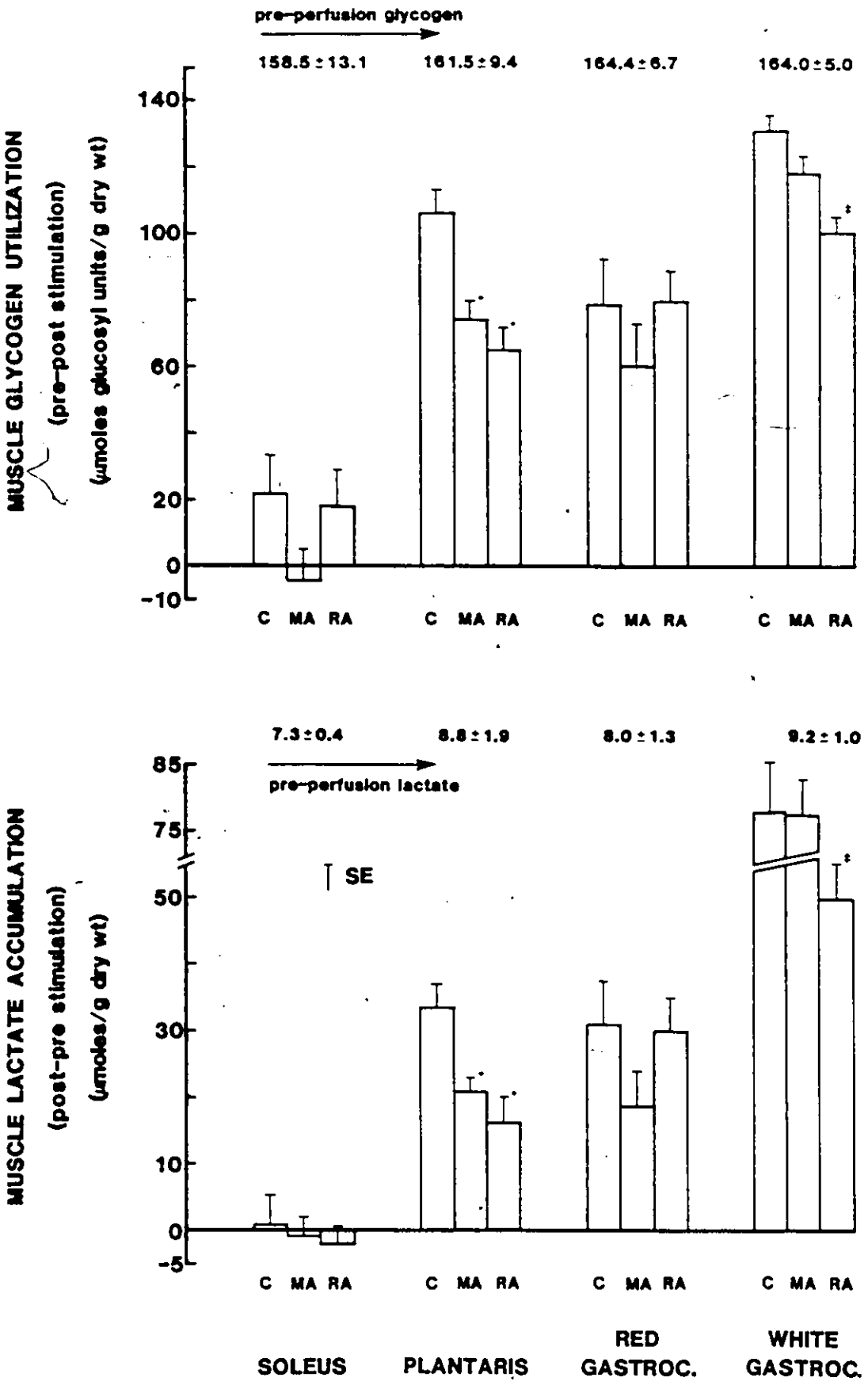
acidosis than control (NS) and constant in all conditions. In the final 10 min glucose uptake increased dramatically in acidosis while remaining constant in C.

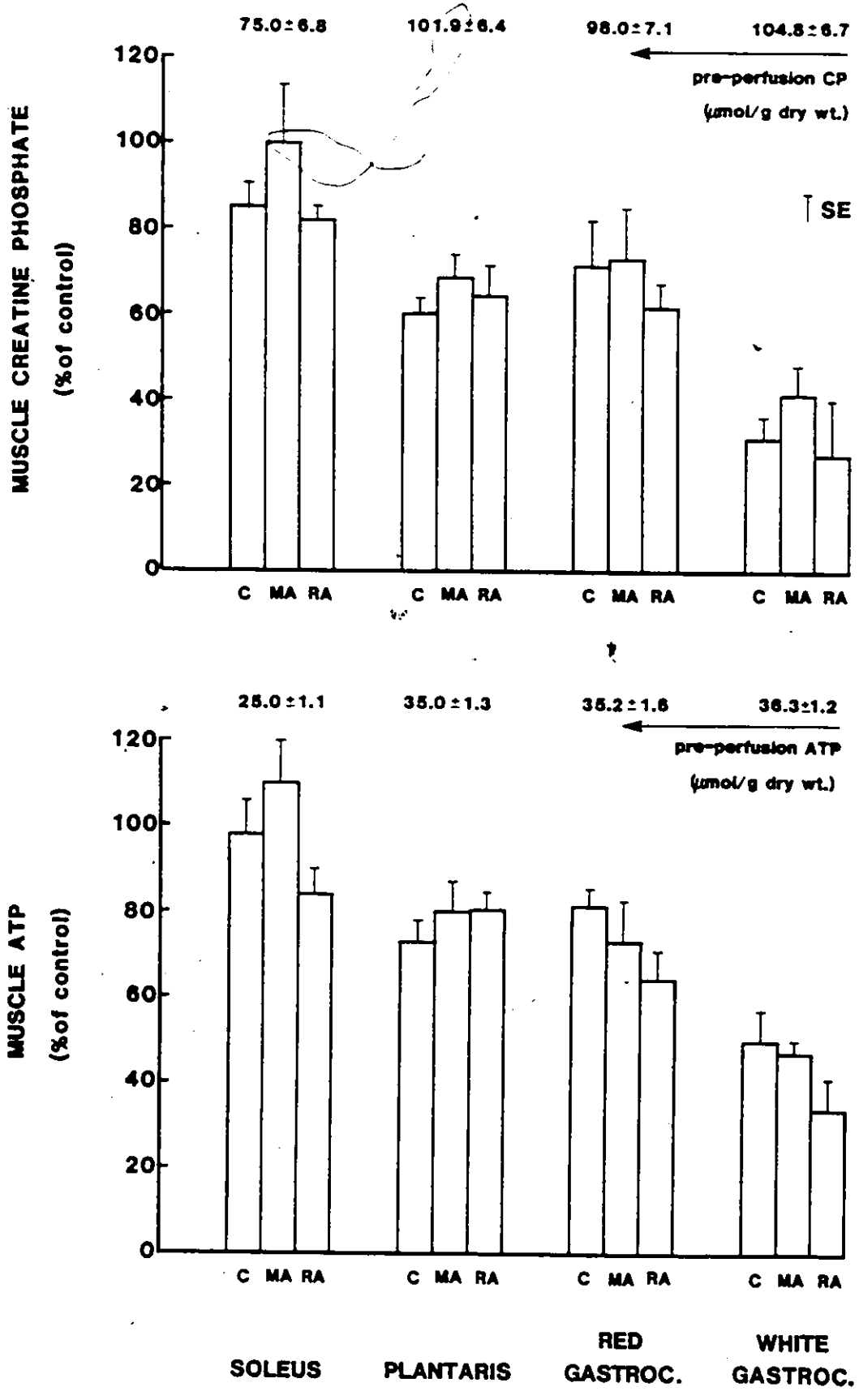
Muscle biopsies were taken following 30 min of perfusion at rest to examine the effect of rest perfusion only. In all conditions the 30 min perfusion had no effect on the muscle concentrations of glycogen, lactate, CP and ATP (Appendix D, Table 38). For this reason, biopsies sampled during stimulation were compared to pre-perfusion biopsies.

Thirty min of rest and 5 min of stimulation (S5) had little effect on glycogen or lactate levels in the SOL muscle (Fig. 18) but marked changes occurred in the other muscles. In the PL muscle, both acidotic conditions resulted in less glycogen utilization and lactate accumulation than in C. In the RG muscle a trend towards lower glycogen utilization and lactate accumulation was apparent in MA whereas in the WG muscle this trend was most pronounced in RA. In all S5 samples there was broad agreement between reductions in glycogen concentration and increases in lactate concentration. Creatine phosphate and ATP concentrations in the SOL muscle were similar in all conditions and virtually unchanged from pre-perfusion levels (Fig. 19). In the PL and RG muscles, CP and ATP levels decreased to 60-70% and 65-80% of pre-perfusion values, respectively, in all conditions (Fig. 19). Creatine phosphate decreased to 30-40% of pre-perfusion and ATP to 35-50% of pre-perfusion levels in

Fig. 18. Muscle glycogen utilization and lactate accumulation following 5 min of stimulation during acidosis. C, control; MA, metabolic acidosis; RA, respiratory acidosis. * significantly different from C, ‡ significantly different from C and MA.

Fig. 19. Muscle creatine phosphate and ATP concentrations following 5 min of stimulation during acidosis. Abbreviations and symbols as in Fig. 18.


pre-perfusion glycogen
158.5 ± 13.1
161.5 ± 9.4
164.4 ± 6.7
164.0 ± 5.0
MUSCLE GLYCOGEN UTILIZATION
(pre-post stimulation)
(μmoles glucosyl units/g dry wt)
140
100
60
20
0
-10
C MA RA
C MA RA
C MA RA
C MA RA
7.3 ± 0.4
8.8 ± 1.9
8.0 ± 1.3
9.2 ± 1.0
pre-perfusion lactate
SE
MUSCLE LACTATE ACCUMULATION
(post-pre stimulation)
(μmoles/g dry wt)
85
75
50
30
10
0
-5
C MA RA
C MA RA
C MA RA
C MA RA
SOLEUS
PLANTARIS
RED GASTROC.
WHITE GASTROC.


75.0±6.8
101.9±6.4
98.0±7.1
104.8±6.7
pre-perfusion CP
(μmol/g dry wt.)
SE
MUSCLE CREATINE PHOSPHATE
(%of control)
120
100
80
60
40
20
0
C MA RA
C MA RA
C MA RA
C MA RA
25.0±1.1
35.0±1.3
35.2±1.6
36.3±1.2
pre-perfusion ATP
(μmol/g dry wt.)
MUSCLE ATP
(%of control)
SOLEUS
PLANTARIS
RED GASTROC.
WHITE GASTROC.

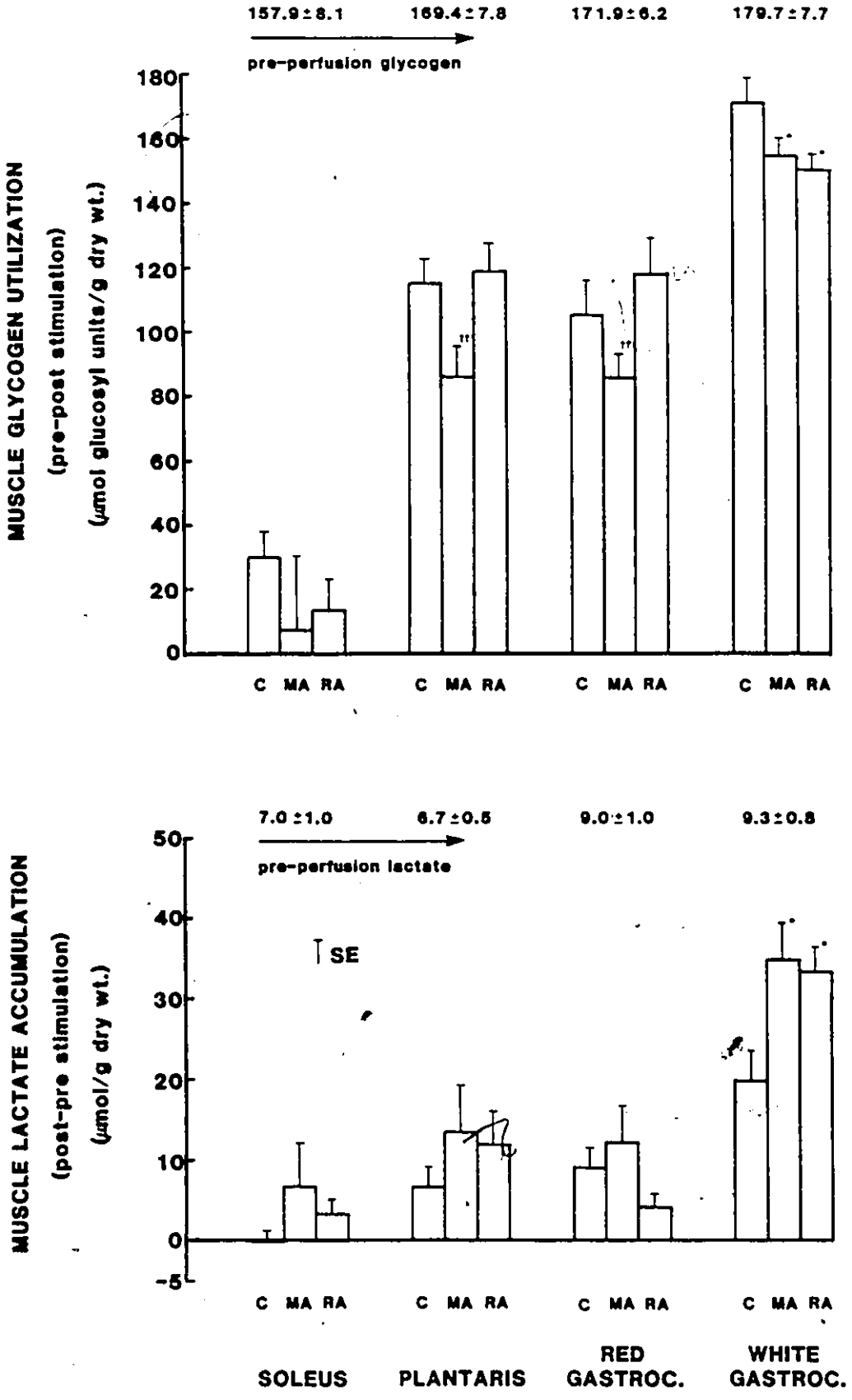
all conditions in the WG muscle. The greatest reductions in CP and ATP occurred in the WG muscle in which the greatest glycogen depletion and lactate accumulation were found.

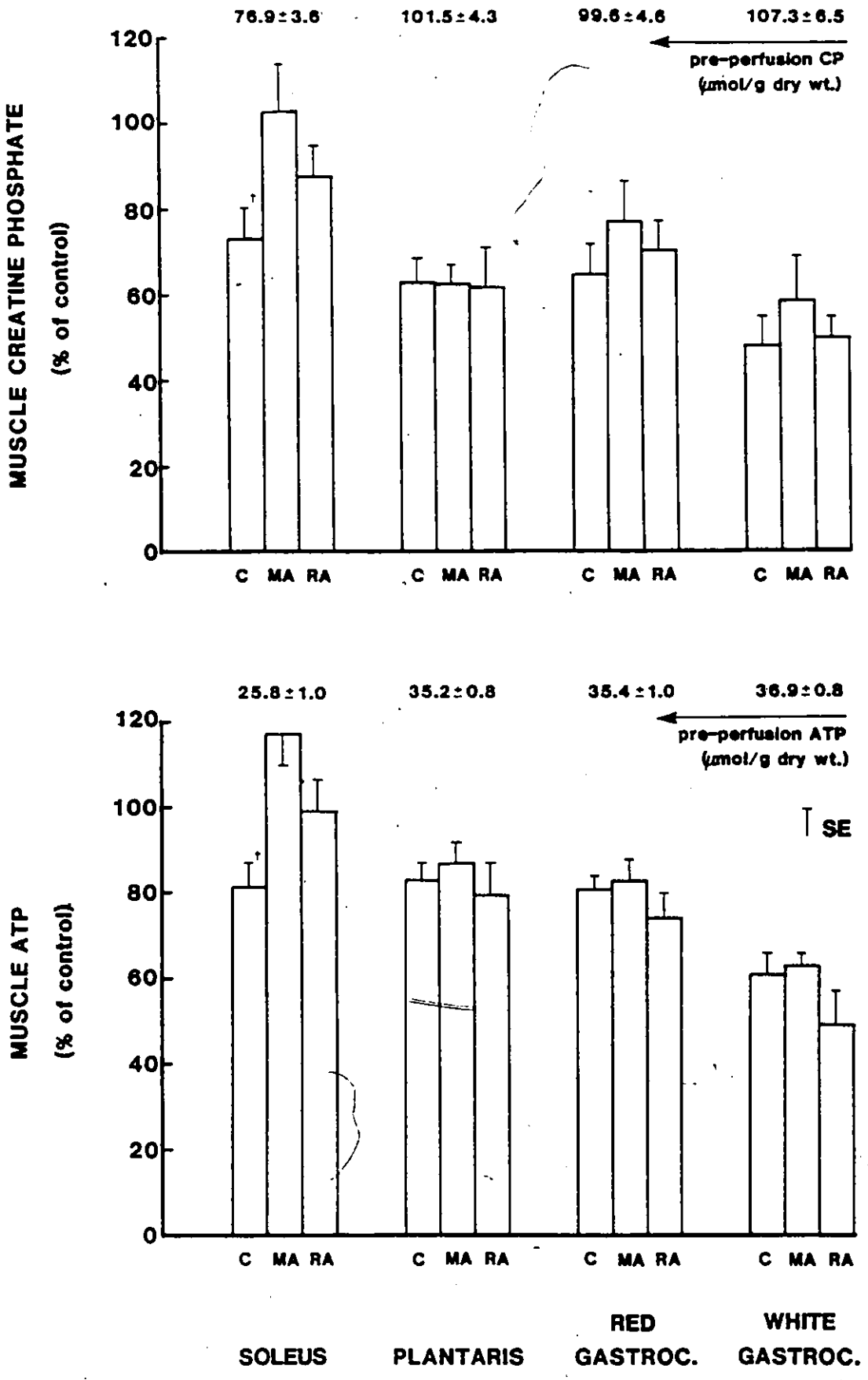
The amount of glycogen utilized and lactate accumulated by the SOL muscle was small in all conditions following 20 min of stimulation (S20) (Fig. 20). In the PL and RG muscles glycogen utilization was significantly lower in MA versus C and RA while the respective lactate accumulations were not different and only 2-3 fold greater than pre-perfusion levels. Following S20 in the WG muscle, glycogen utilization was lower and lactate accumulation greater in acidosis. Soleus muscle CP and ATP concentrations were significantly lower than pre-perfusion levels only in the C condition following S20 (Fig. 21). In all conditions the CP and ATP levels in the PL and RG muscles fell to 60-75% and 75-85% of pre-perfusion concentrations, respectively. Creatine phosphate and ATP levels in the WG muscle decreased to 50-60% of pre-perfusion levels, respectively in all conditions. The WG was the only muscle to show any recovery in CP and ATP levels during the last 15 min of stimulation.

Thus, between S5 and S20 in the C condition, no significant reduction in glycogen occurred in the SOL, PL and RG muscles but the WG showed continued glycogen utilization. Between S5 and S20 in RA glycogen continued to be used such that total glycogen utilization was less than C only in the WG muscle. Glycogen utilization in MA was less, leaving

Fig. 20. Muscle glycogen utilization and lactate accumulation following 20 min of stimulation during acidosis. C, control; MA, metabolic acidosis; RA, respiratory acidosis. * significantly different from C, †† significantly different from C and RA.

Fig. 21. Muscle creatine phosphate and ATP concentrations following 20 min of stimulation during acidosis. Abbreviations and symbols as in Fig. 20. † significantly different from MA.


157.9±8.1
169.4±7.8
171.9±6.2
179.7±7.7
pre-perfusion glycogen
MUSCLE GLYCOGEN UTILIZATION
(pre-post stimulation)
(μmol glucosyl units/g dry wt.)
180
160
140
120
100
80
60
40
20
0
C MA RA
C MA RA
C MA RA
C MA RA
7.0±1.0
6.7±0.5
9.0±1.0
9.3±0.8
pre-perfusion lactate
MUSCLE LACTATE ACCUMULATION
(post-pre stimulation)
(μmol/g dry wt.)
SE
50
40
30
20
10
0
-5
C MA RA
C MA RA
C MA RA
C MA RA
SOLEUS
PLANTARIS
RED GASTROC.
WHITE GASTROC.


76.9±3.6
101.5±4.3
99.6±4.6
107.3±6.5
pre-perfusion CP
(μmol/g dry wt.)
MUSCLE CREATINE PHOSPHATE
(% of control)
120
100
80
60
40
20
0
C MA RA
C MA RA
C MA RA
C MA RA
25.8±1.0
35.2±0.8
35.4±1.0
36.9±0.8
pre-perfusion ATP
(μmol/g dry wt.)
SE
MUSCLE ATP
(% of control)
SOLEUS
PLANTARIS
RED GASTROC.
WHITE GASTROC.

total utilization after S20 less than the C condition in the PL, RG and WG muscles. Lactate concentration fell between S5 and S20 in all muscles except the SOL. Little or no change occurred in the high energy phosphate concentrations between S5 and S20 in all muscles and all conditions.

Acidosis had no effect on the concentrations of ADP and AMP and the CP/ATP and ATP/ADP ratios in any of the muscles sampled both at S5 and S20 (Tables 14-16, 41-44).

4.4 Discussion

This study examined the effects of metabolic and respiratory acidosis on the performance and metabolism of an isolated perfused rat hindquarter during heavy exercise. The model permitted precise manipulation of the blood gases and acid-base status of the arterial perfusate to produce experimental conditions of pure metabolic acidosis and pure respiratory acidosis both at a perfusate pH of 7.15 (Table 8).

During rest and stimulation, hindquarter flow rates were held constant to ensure equal oxygenation in all conditions. Perfusion pressures were lower in acidosis than control with the lowest pressures observed in RA. All experiments employed a hyperoxic perfusate which exerted a constricting effect on the vascular bed. As a result, with a rest perfusion of 6 $ml.min^{-1}$ the arterial pressure was high enough to open and oxygenate all vascular beds and maintain

tissue integrity. In MA the increased H^+ concentration counteracted the hyperoxic effect to a small extent and in RA both an increased H^+ and PCO_2 further reduced the hindquarter's vascular resistance. However, in all conditions, increasing the flow rate above the reported stimulation values produced no increases in O_2 uptake or generated tension, suggesting that O_2 uptake was maximal and not limited by perfusion flow. Also, to prevent catecholamines from altering the hindquarter resistance, the integrity of the sympathetic nervous system was not maintained during the perfusions and no catecholamines were added to the perfusate.

The high isometric tensions generated by the hindquarter muscles suggested that stimulation with supramaximal impulses produced contaction of all innervated motorunits. The high rate of tension decay in all conditions during the first 5 min of stimulation (S5) was attributed to the fatiguability of the fast twitch, glycolytic (FG) motor units (Fig. 14). In the WG muscle, containing predominantly FG fibers, the largest glycogen utilization, lactate accumulaton and reductions in CP and ATP levels were seen (Fig. 18-19). Similar changes were found in the PL muscle, which contains 50% FG fibers. During acidosis less glycogen utilization and lactate accumulation was found in these muscles and isometric tension decay was greater. Hindquarter O_2 uptake was also reduced during stimulation in acidosis

(Fig. 15).

In an attempt to explain these performance and metabolic changes during the first 5 min of stimulation (S5), the amount of energy produced by the major energy releasing pathways was calculated from the measured amounts of O_2 utilized (oxidative metabolism), lactate produced (anaerobic glycolysis) and CP utilized as outlined by Margaria (1976) (Appendix A). The total amount of energy released was greatest in C, intermediate in MA and least in RA (Table 10). These findings correlated with the rates of tension decay; in RA the decay rate was highest and energy release was lowest; in C the tension decay was lowest while the energy released was highest; and in MA both were intermediate. The relative contributions of the three major sources of energy shifted slightly towards a lesser dependance on anaerobic glycolytic metabolism in acidosis; in MA and RA, 76.6 and 73.3% of the produced energy was derived from oxidative metabolism and 18.0 and 21.6% from anaerobic glycolysis, respectively. In C, 71.7% was derived aerobically and 22.7% from anaerobic glycolysis. Acidosis was associated with a reduction in the absolute amount of energy derived from CP stores such that its relative contribution was unchanged.

Further calculations were made to estimate the total amount of glucose made available to the muscles from glycogen breakdown and glucose taken up from the perfusate. Measurement of the total lactate production made it possible

to calculate what percentage of the available carbohydrates (the major source of energy) were metabolized through glycolysis, leaving the remainder for aerobic use. The total amount of available glucose during S5 was greatest in C, lower in MA and lowest in RA (Table 11). Both a greater absolute amount and a greater percentage of the available glucose was used glycolytically in C. Additionally, a smaller percentage of the available glucose was derived exogenously in C (6.0%) versus MA (11.4%) and RA (14.2%). The absolute amount of glucose made available for aerobic use was consistent with the amount of O_2 utilized; the greatest available glucose corresponded to the highest O_2 uptake (C) and the lowest available glucose corresponded to the lowest O_2 uptake (RA) with MA again intermediate.

These results collectively indicate that glycolytic metabolism and to a lesser extent oxidative metabolism decreased during 5 min of heavy electrical stimulation in acidosis The energy derived from oxidative metabolism represented 88.1 and 69.2% of C in MA and RA, respectively. Similarly, the energy derived from glycolysis represented 64.8% of C in both MA and RA (Table 10). It seems likely that these effects were mediated in large part by a fall in muscle pH. As the PCO_2 was high in RA, it is likely that the muscle pH was lower than in C or MA due in part to a higher muscle PCO_2 and in part to impaired removal of CO_2 generated from carbonic acid early in the stimulation period.

Table 10. Energy released during 20 min of stimulation under control and acidosis conditions.

	O_2 Utilized	Lactate Produced	CP Utilized	Total Energy Released	Percent of C Energy
			Energy released during min 30-35, joules		
C	40.3	12.8	3.1	56.2	-
	(71.7)	(22.8)	(5.5)		
MA	35.5	8.3	2.5	46.3	82.4
	(76.7)	(17.9	(5.4)		
RA	27.9	8.3	2.0	38.2	68.0
	(73.0)	(21.7)	(5.3)		
			Energy released during min 35-50, joules		
C	104.8	12.1	-	116.9	-
	(89.7)	(10.3)			
MA	87.0	9.6	-	96.6	82.6
	(90.1)	(9.9)			
RA	80.4	14.2	-	94.6	80.9
	(85.0)	(15.0)			

C, control; MA, metabolic acidosis; RA, respiratory acidosis; CP, creatine phosphate. Values are per working hindquarter. Bracketed values are percent of total energy released in each condition. Calculations based on 1.0 ml O_2 = 20.9 joules, 1.0 mg lactate = 1.0 joules and 1.0 umole CP = 0.05 joules as outlined by Margaria (1976). Assumptions; as in Table 5.

Table 11. Sources and fates of glucose during 20 min of stimulation under control and acidosis conditions.

	Sources of Glucose			Fates of Glucose	
	Muscle Glycogen	Exogenous Perfusate	Total	Lactate Produced	Aerobic Metabolism
min 30-35			umoles		
C	149.0	9.5	158.5	70.5	88.0
	(94.0)	(6.0)		(44.5)	(55.5)
MA	109.1	14.0	123.1	42.1	81.0
	(88.6)	(11.4)		(34.2)	(65.8)
RA	100.0	16.6	116.6	45.5	71.1
	(85.8)	(14.2)		(39.0)	(61.0)
min 35-50					
C	46.9	29.5	76.4	66.6	9.8
	(61.4)	(38.6)		(87.2)	(12.8)
MA	22.5	48.7	71.2	53.3	17.9
	(31.6)	(68.4)		(74.9)	(25.1)
RA	72.1	60.1	132.2	78.9	53.3
	(54.5)	(45.5)		(59.7)	(40.3)

Abbreviations as in Table 10. Values are per working hindquarter. Bracketed values are percent of total glucose provided or metabolized. Assumptions; as in Table 5. Also, glucose not metabolized to lactate burned aerobically, exogenous glucose not used for anabolic purposes.

Performance and metabolism in the final 15 min of stimulation differed markedly from the first 5 min. The rate of tension decay was low in all conditions, remaining virtually constant in the final 5-10 min. The generated tension and O_2 uptake were consistently higher in C than in acidosis. Following S20 muscle glycogen depletion remained less than C only in MA in the PL and RG muscles and in the WG muscle in both MA and RA (Fig. 20) (glycogen depletion in C was essentially complete following S5). Creatine phosphate and ATP levels did not change appreciably from S5 except in the WG muscle, in which resynthesis of CP and ATP occurred (Fig. 21). The lactate production in this time period was lower than the first 5 min, and similar in C and RA, but reduced in MA (Table 10). Glucose uptake increased dramatically during the final 10 min of stimulation in acidosis only.

The total energy released in the final 15 min of stimulation was greater in C than MA and RA (80% of C) correlating with the greater tension generated in C (Fig. 14, Table 10). In all conditions there was a major shift towards a greater reliance on oxidative metabolism, between 85.0 and 90.0% of the energy being derived aerobically. The total amounts of available glucose were not different in C or MA but higher in RA and the amount of glucose accounted for by lactate production was relatively smaller in acidosis, more being available for aerobic metabolism (Table 11). The

amount of glucose obtained exogenously was 68.4% and 45.5% of the total in MA and RA, respectively and 38.6% in C. These calculations reveal that during the final 15 min of stimulation, oxidative metabolism in MA and RA (83.0 and 76.7% of C, respectively) and glycolytic metabolism only in MA (79.3% of C) were decreased.

Intramuscular TG utilization during the final 15 min of stimulation was not assessed during acidosis. However, more carbohydrate was available for aerobic use in acidosis suggesting a smaller role for TGm metabolism as compared to the C condition (Chapter 3).

The decreased glycogen breakdown and lactic acid production during acidosis agrees with earlier findings with RA in an in situ rat preparation (Gimenez and Florentz, 1979) and in MA with exercising humans (Sutton et al, 1981) and appears to be mediated through changes in the activity of regulatory enzymes in the glycolytic pathway. A decrease in pH inhibits the regulatory enzyme phosphofructokinase but the subsequent increase in fructose-6-phosphate tends to lift the inhibition since it acts as an allosteric activator of the enzyme (Trivedi and Danforth, 1966). The result is an increased glucose-6-phosphate concentration which will inhibit phosphorylase and possibly hexokinase, thereby reducing glycolytic flux (Toews, 1966a).

A decreased pH inhibits the conversion of phosphorylase b (inactive) to phosphorylase a (active),

epinephrine partially counteracting this effect (Danforth, 1965; Chasiotis et al, 1983). In our preparation the lack of epinephrine may have enhanced the pH inhibition of phosphorylase and resulted in decreased glycogen breakdown in acidosis during the initial 5 min of stimulation. Epinephrine and muscular contraction exert a dual control on muscle glycogenolysis during exercise, contraction stimulating glycogenolysis early and epinephrine stimulating glycogenolysis later (Richter et al, 1982a).

Acidosis may increase the activity of pyruvate dehydrogenase acting to increase the incorporation of pyruvate into the tricarboxylic cycle. As the pyruvate dehydrogenase reaction is downstream to both phosphorylase and phosphofructokinse, evidence of enhanced activity of this enzyme may be hard to obtain, but this may in part contribute to lower lactate production and maintained aerobic metabolism of glucose in acidosis.

The decreased isometric tension in acidosis may have been due to a number of factors in addition to the reduced energy production. The locus of fatigue could be neuromuscular but this is unlikely since tension development was unchanged by direct muscle stimulation. An increased H^+ concentration may exert a direct negative effect on the excitation-contraction coupling mechanism by inhibiting the release, binding or uptake of Ca^{2+} by the sarcoplasmic reticulum (Nakamaru and Schwartz, 1972; Fabiato and Fabiato,

1978) or by altering the Ca^{2+} sensitivity of contractile elements (Portzehl et al, 1969). Alternately, the increased fatiguability associated with acidosis may result from an increased K^+ conductance of the muscle membrane again interfering with the role of Ca^{2+} in the excitation-contraction coupling mechanism (Fink et al, 1982).

Muscle high-energy phosphate stores were unaffected by acidosis in this preparation unlike the findings of Sahlin et al (1983) who reported a 66% decrease in CP stores in isolated muscle following a 90 min incubation with 30% CO_2. The authors explained their findings on the basis of an altered equilibrium constant for the CP kinase reaction due to the enhanced intramuscular H^+ concentration. While it is possible that the intramuscular pH was unaltered prior to stimulation in the present study (arterial blood gassed with 5-9% CO_2), our results reveal that CP fell to similar levels in all conditions following 5 and 20 min of stimulation, when the intramuscular pH must have fallen. The discrepancy between the two studies may be due to differences in the severity or duration of acidosis or in the experimental preparations used.

Hindquarter lactate release was reduced during stimulation in metabolic and respiratory acidosis as reported in isolated muscle preparations (Hirche et al, 1975; Mainwood and Worsley-Brown, 1975; Steinagen et al, 1976) and with exercising humans (Jones et al, 1977; Sutton et al, 1981;

Ehrsam et al, 1982). Two major factors may account for the lower lactate release in acidosis. First, a reduction in muscle lactate formation due to the inhibition of glycolysis in the first 5 min of exercise occurred, when anaerobic metabolism played an important role in energy production (Table 10). During the final 15 min of stimulation lactate production was lower in MA than C but greater in RA (Table 11) and anaerobic metabolism contributed less to the overall energy supply in all conditions. Second, lactate release across the muscle cell membrane may have been reduced by acidosis. To establish if lactate release was hampered by acidosis independent of the decreased production rate, the total amount of lactate produced (stored and released) in each condition was calculated for the first 5 min and final 15 min of stimulation (Table 12). Although the total amount of lactate produced was significantly reduced in acidosis (65% of C) the relative amounts of lactate which left the muscle during the initial 5 min were very similar (C, 61.6% vs 58.5% and 66.5% for MA and RA) (Fig. 22). The greater amount of lactate leaving muscle during RA compared to MA supports the view that external HCO_3^- concentration may be an important factor regulating lactate efflux (Mainwood and Worsley-Brown, 1975). Also, when lactate release rates were normalized for the isometric tension produced, the lowest values were found in MA while RA release rates closely resembled these in C (Fig. 23).

Table 12. Muscle lactate, lactate released from muscle and total lactate produced during 20 min of stimulation under control and acidosis conditions.

	Lactate Released	Muscle Lactate	Total Lactate Produced	Percent of C Lactate
min 30-35		umoles		
C	86.9	54.1	141.0	-
	(61.6)	(38.4)		
MA	54.0	38.3	92.3	65.5
	(58.5)	(41.5)		
RA	60.5	30.5	91.0	64.5
	(66.5)	(33.5)		
min 35-50				
C	163.3	12.1	133.3*	-
MA	99.7	22.6	106.6*	80.0
RA	149.6	19.2	157.5*	118.2

Abbreviations as in Table 10. Values are per working hindquarter. Bracketed values are percent of total lactate produced in each condition. * Calculation of total lactate produced = lactate released + muscle lactate - loss in muscle lactate from min 35 to 50 (upper muscle lactate - lower muscle lactate). Assumptions; as in Table 5. Also, lactate metabolized was minimal due to one-pass perfusion system.

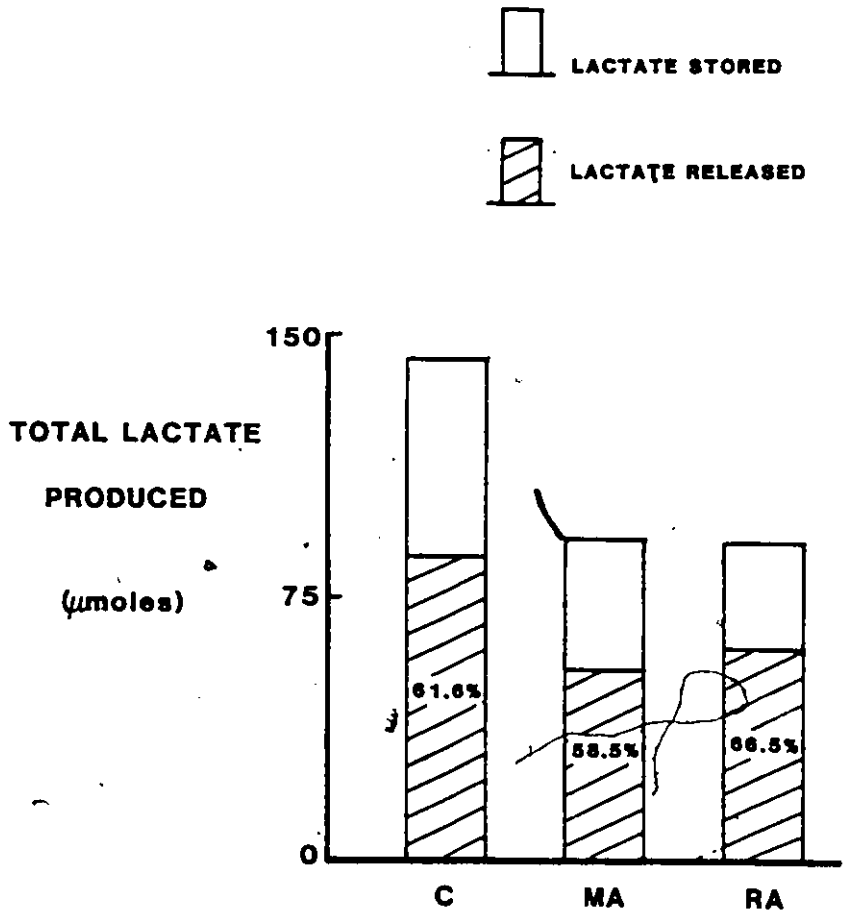


Fig. 22. Relative lactate release from working muscle during 5 min of stimulation under control (C), metabolic acidosis (MA) and respiratory acidosis (RA) conditions.

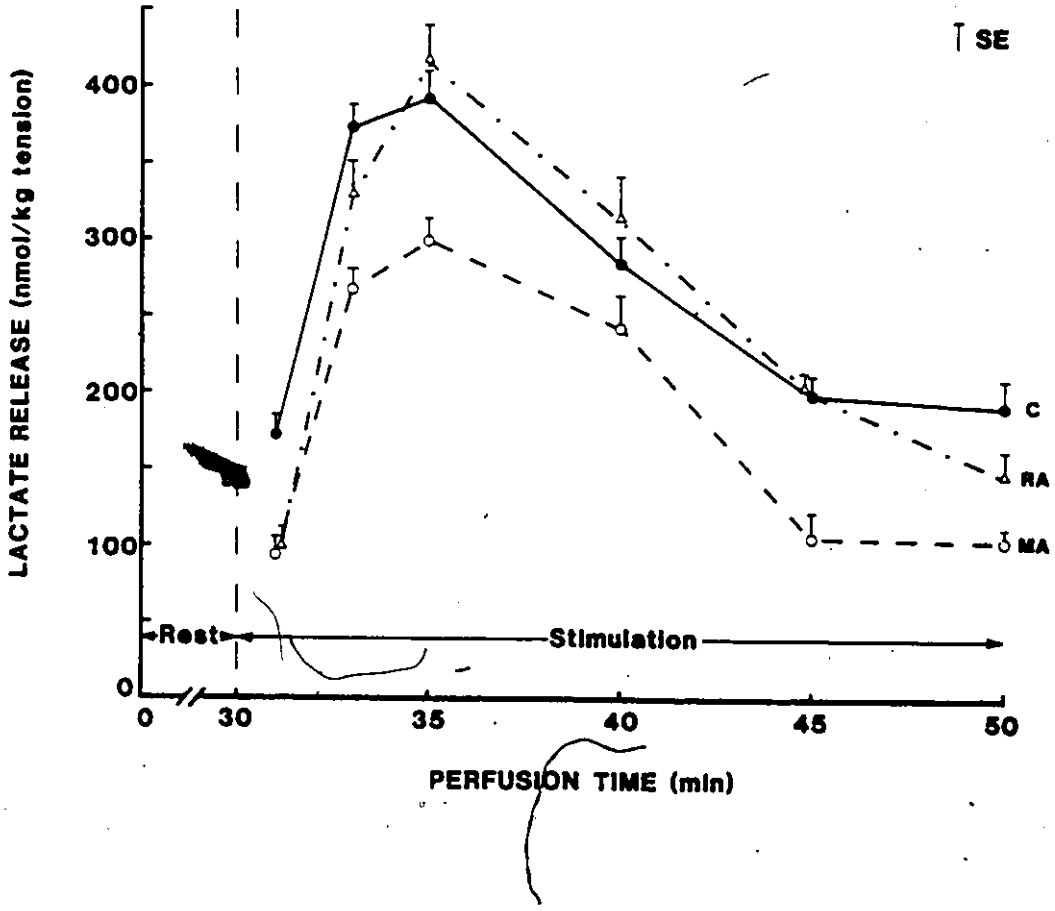


Fig. 23. Lactate release normalized for isometric tension generation during stimulation under acidosis. C, control; MA, metabolic acidosis; RA, respiratory acidosis.

If there is a maximum lactate release rate from rat skeletal muscle, as suggested by Jorfeldt et al (1978) in human muscle, which is not simply a function of the muscle to plasma concentration gradient, it may not be correct to compare acidosis and control lactate release rates because of the different amounts of lactate produced in the different conditions. If muscle lactate concentration reached a level above which lactate release cannot increase early in the C condition, only a limited amount of lactate might leave in the first 5 min, whereas in acidosis this level may have been reached later or not al all.

The lactate release data for the final 15 min of stimulation are more difficult to interpret, for muscle lactate concentrations were different at the start of the period in the three conditions. More lactate left the muscle in the control study, less in RA and even less in MA (Table 12). These findings are in keeping with the hypothesis that muscle lactate efflux is decreased during exercise in acidosis with MA being most affected due to the decreased perfusate HCO_3^- concentration (Fig. 23).

Finally, in applying the present results to exercise in the whole organism it must be remembered that the perfusing lactate concentration was artificially low (0.5-1.5 mM) due to the one-pass perfusion system. In the exercising animal arterial lactate concentration increases rapidly and may influence the rate of muscle lactate release.

5. GENERAL SUMMARY

5.1 Introduction

This chapter is designed to briefly summarize the work of this thesis, highlighting the major observations and conclusions. Some thoughts concerning future research are also discussed.

5.2 Observations and Conclusions of the Thesis

The purpose of this thesis was to directly examine the relative contributions of the major energy releasing pathways in skeletal muscle during heavy electrical stimulation. This required direct and simultaneous measurements of muscle endogenous and exogenous fuel utilization, O_2 uptake, lactate production and phosphagen hydrolysis. To obtain these measurements from intact contracting human muscle would have required an unacceptable level of invasiveness. Therefore, an isolated perfused rat hindquarter model previously used to examine resting muscle metabolism was extended for use during muscular contraction.

A number of advantages make this model particularly suitable for this work. The hindquarter consists mainly of skeletal muscle with very little adipose tissue. The muscle receives exogenous substrates through normal vascular

channels and the normal bone, muscle and nerve relationships are maintained. All components of the arterial perfusate may be tightly controlled and manipulated. Simultaneous sampling of arterial and venous perfusates permit the measurement of uptake and release of energy substrates and metabolites. Finally, pre and post-perfusion muscle biopsies are readily obtained for the measurement of intramuscular energy stores and metabolites.

Chapter 2 dealt with the development of the model for use during muscular contraction. There were two major problems associated with extending the model from rest to exercise. Firstly, to ensure optimal muscular performance, O_2 delivery to the working muscles needed to be greatly enhanced. This was achieved by perfusing with carefully prepared fresh RBCs at a normal rat Hb concentration and a sufficiently high flow rate. Secondly, the heavy electrical stimulation rate used made it difficult to quantify the isometric tension produced. Adequate stabilization of the knee and ankle joints alleviated this problem and permitted accurate tension measurement.

The exercising preparation was carefully characterized and used to examine numerous uncertainties regarding the metabolism and performance of skeletal muscle during heavy contraction. This study as outlined in Chapter 3 demonstrated the improvement in performance of this model over previous attempts to stimulate the hindquarter (Rennie,

and Hollosźy, 1977; McLane et al, 1981).

The working muscles of the hindquarter containing approximately 50% FG and 50% FOG fibers with very few SO fibers were stimulated to contract for 20 min. Energy calculations based upon the measurements of O_2 uptake (aerobic metabolism), lactate production (anaerobic glycolysis) and CP hydrolysis (alactic anaerobiosis) were made for the initial 5 min of stimulation when isometric tension production was high but rapidly fatiguing and for the final 15 min when tension production was essentially constant. The initial 5 min of stimulation were characterized metabolically by a rapid increase in O_2 uptake with large amounts of CP hydrolysis and lactate production in all fast-twitch fibers. Total anaerobic energy production accounted for nearly 30% of the total energy release with the greatest anaerobic involvement in muscles containing predominantly FG fibers. Muscle glycogenolysis provided the major substrate for both aerobic metabolism and anaerobic glycolysis. The rapid decay in tension production appeared related to the fatigue of FG motorunits and the glycogen depletion associated with these fibers.

During the final 15 min of stimulation approximately 60% of peak tensions were held. Tension decay was minimal suggesting force production was maintained primarily by FOG fibers. In support of this O_2 uptake remained constant and aerobic metabolism provided 90% of the total energy release.

Glycogen utilization was minimal in all fibers and intramuscular triacylglycerol became the predominant fuel for the oxidative fibers (FOG) of the working muscle. During this period approximately 62% of the produced energy was derived from TGm, demonstrating that endogenous fat stores can be metabolized during heavy muscular contraction.

This study demonstrated the future potential of the hindquarter preparation and emphasized the importance of tension measurements in addition to O_2 uptake, fuel and metabolite changes. The similarity between tension and O_2 uptake changes supported the validity of the metabolic changes as indicating the relative changes in fuel sources.

These results indicated that the model could be used to examine the effects of imposed extracellular acidosis on muscle metabolism and performance. Acidosis has been implicated in the direct inhibition of glycolytic metabolism (Trivedi and Danforth, 1966; Chasiotis et al, 1983) and in contributing to fatigue and decreased performance n humans (Karlsson, 1971a).

During both respiratory and metabolic acidosis muscle glycogenolysis and lactate production were significantly reduced during the initial 5 min of stimulation (65% of control). There was a decrease in glycolytic flux and in O_2 consumption. The reduction in energy provision from both aerobic and anaerobic sources was associated with an increased rate of isometric tension decay in acidosis. The

reduction in glycolytic flux may have been due to an earlier fall in muscle pH during acidosis and resultant inhibition of the regulatory enzyme PFK and of phosphorylase b to a conversion.

Total energy from anaerobic and aerobic sources were again reduced during the final 15 min of stimulation in acidosis (80% of control). Tension production was correspondingly lower during acidosis. Lactate release from the muscle was reduced during metabolic acidosis presumably due to the reduced extracellular HCO_3^- concentration (Mainwood and Worsley-Brown, 1975).

Although a causal relationship between metabolic inhibition and enhanced fatigue is implicated in acidosis, additional and/or alternate sites of acidotic inhibition may have occurred. Acidosis may exert a direct negative effect on the excitation-contraction coupling mechanism by inhibiting Ca^{2+} dynamics (Fabiato and Fabiato, 1978), altering the Ca^{2+} sensitivity of contractile elements (Portzehl et al, 1969) or increasing K^+ conductance of the muscle membrane (Fink et al, 1982).

5.3 Questions for Future Research

The advantages of the perfused rat hindquarter model for the study of muscle metabolism during exercise were listed in the previous section. Since all constituents of the perfusate medium (fuels, metabolites, hormones and

acidity) are easily manipulated, the muscle's environment can be altered to simulate any number of physiological and clinical conditions. Therefore, in concert with direct muscle sampling several aspects of muscle metabolism during exercise may be examined as has occurred for resting muscle metabolism. During the course of this work several topics have been examined with similar models; glucose uptake (Walker et al, 1982a), muscle glycogenolysis (Richter et al, 1982a, c), reduced blood flow (Walker et al, 1982b), iron deficiency (McLane et al, 1981) and hypo and hyperthyroidism (Everts et al, 1981, 1983).

The results of this thesis demonstrated that intramuscular fat stores were metabolized when alternate fuel sources were depleted or artificially restricted during heavy muscular contraction. Little is known regarding both the biochemical control of intramuscular lipolysis and the mechanism by which TG hydrolysis is regulated and integrated with the release and uptake of alternate substrates, which are ultimately metabolized by common energy releasing pathways in muscle. The concentrations of high energy phosphates, H^+, Ca^{2+} and key metabolic intermediates such as citrate, acetyl CoA and glucose-6-phosphate are thought to regulate the level of energy metabolism and the relative rates of glycolysis and fat or pyruvate oxidation. The preparation described in this work could be used to examine some of these unknowns concerning intramuscular TG

metabolism.

No assessment of intramuscular fat utilization during acidosis was made in this thesis. It is unknown whether enhanced cellular acidity adversely affects TGm metabolism. During the predominantly aerobic portion of the stimulation (final 15 min), O_2 uptake was reduced in acidosis yet more glucose was available for aerobic metabolism than in the control condition. In the control condition the major substrate for aerobic metabolism was TGm, accounting for the higher O_2 uptake. The suggestion is that during acidosis TGm metabolism did not make a major contribution to the total energy production. The reason for this is unknown and may be linked to the cellular acidity. Clarifying this postulate would help elucidate the control of TGm metabolism as discussed in the previous paragraph.

During acidosis total energy production by the working muscles was reduced. Both anaerobic glycolysis and aerobic metabolism were reduced, most severely early in the stimulation. Presumably the enhanced local acidosis exerted a direct negative effect on glycolysis resulting in reduced energy production and concomitant decreases in performance. However the direct negative effect of acidosis may be an uncoupling of the excitation-contraction process as discussed in Chapter 4. Such a problem would result in a decreased energy requirement and consequently reduced metabolism. The fact that muscle CP and ATP stores were depleted to similar

levels in all conditions supports this premise. Muscular performance would be reduced in concert with the extent of the excitation-contraction interference. Further investigation of this hypothesis would require the use of both the present preparation and a totally isolated muscle preparation as described by Jones et al (1982). In an isolated preparation factors affecting excitation-contraction coupling may be added directly to the bathing medium while performance is quantitated.

Artificially induced alkalosis has been shown to increase endurance time, muscle glycogenolysis and lactate efflux from muscle (Hill and Lupton, 1923; Jeryell, 1928; Jones et al, 1977; Sutton et al, 1981). It is believed that the enhanced alkali delays the buildup of muscular acidosis during heavy work requiring a large anaerobic component. An examination of artificially induced alkalosis using this model could encompass measurements of muscle metabolites and fuels, performance and intramuscular pH to conclusively investigate the relationships between these variables.

APPENDIX A

Calculations

1. Oxygen Uptake Calculation

$$VO_2 \text{ (ml)} = ([Hb] \times 1.34 \times \% O_2Sat) + (PO_2 \times 0.0031)$$

2. Energy Release Calculations

Calculations represent values for normal acid-base status perfusions for the entire hindquarter. Perfused hindquarter muscles weighed 13.74 g. The energy conversions used in these calculations were as outlined by Margaria (1976);

1.0 ml O_2 = 20.9 joules

1.0 mg lactate = 1.0 joules

1.0 umole CP = 0.05 joules

a) Total O_2 Utilized

Average resting O_2 uptake (VO_2) = 7.47 $umol.min^{-1}$ (1.0 umol = 0.02545 ml) or 0.1901 $ml.min^{-1}$.

Total O_2 utilized above rest: During the initial 5 min of stimulation; 2.8746 ml - resting VO_2 (0.9505)= 1.9241 ml. During the last 15 min of stimulation; 7.8577 - resting VO_2 (2.8515) = 5.0062 ml.

Energy released from combustion of O_2 utilized above rest: Initial 5 min of stimulation; 1.9241 ml x 20.9 joules.ml^{-1} = 40.3 joules. Final 5 min of stimulation; 5.0062 ml x 20.9 joules.ml^{-1} = 104.8 joules.

b) Lactate Produced

Average resting lactate release = 1.37 umol.min^{-1}.

Total lactate production above rest: During the initial 5 min of stimulation; Measured lactate release = total lactate release (93.8) - resting lactate release (6.9) = 86.9 umoles. The lactate stored in the muscles was estimated from the measured lactate in the sampled muscles. Total muscle mass stimulated was 7.72 g. Wet weights and wet/dry wet ratios of sampled muscle were measured;

	wet wt (g)	dry wt (g)
Soleus	0.117	0.020
Plantaris	0.294	0.054
Gastrocnemius	1.785	0.336
Remainder	5.524	1.015

Total lactate stored was calculated from the lactate concentration in each muscle x its dry weight to give the total umoles in each muscle. The average of the red and white gastrocnemius lactate concentrations was used to estimate total gastrocnemius lactate storage. The plantaris lactate concentration was used to estimate the lactate stored in the non-sampled stimulated muscle since it best represents

the rat hindquarter musculature, being composed of roughly equivalent numbers of FG and FOG fibers (Ivy and Holloszy, 1981).

The total lactate stored in muscle at the end of 5 min of stimulation was 54.1 umoles.

Total lactate produced during first 5 min of stimulation = lactate effluxed (86.9) + muscle lactate (54.1) = 141.0 umoles.

During the last 5 min of stimulation; Measured lactate release = total lactate release (183.9) - resting lactate release (20.6) = 163.3 umoles. Muscle lactate = 12.1 umoles (actually 42.0 umoles less than after 5 min of stimulation). Total lactate produced during last 15 min of stmulation = lactate effluxed (163.3) + muscle lactate (12.1) - (loss in muscle lactate from min 5 to min 20 [54.1 - 12.1 = 42.0]) = 133.3 umoles.

Energy released through lactate production above rest: Initial 5 min of stimulation; 141.0 umoles or 12.8 mg (1 umole lactate = 90 ug) x 1 joule.mg^{-1} = 12.8 joules. Final 5 min of stimulation; 133.3 umoles or 12.1 mg x 1 joule.mg^{-1} = 12.1 joules.

c) Creatine Phosphate Utilized

During the initial 5 min of stimulation CP utilized was 62.0 umoles (estimations from tissue levels as in previous section), therefore total energy released was 62.0 umoles x 0.05 joules.mg^{-1} = 3.1 joules.

During the final 15 min of stimulation no further CP hydrolysis occurred.

d) Glycogen Utilization and Glucose Uptake

During the initial 5 min of stimulation 149.0 umoles of glucose were released from glycogen (estimated from tissue levels as in previous section) and 9.5 umoles were taken up from the perfusate giving 158.5 umoles of glucose provided.

Total lactate production was 141.0 umoles, accounting for 70.5 umoles of glucose and leaving 78.5 unaccounted for or available for aerobic metabolism (assumption; anaerobic metabolism of 1.0 mole of glucose produced 2.0 moles of lactate).

Similarly during the final 15 min of stmulation 46.9 umoles of glucose were released from glycogen and 29.5 were extracted from the perfusate for a total of 76.4 umoles. Lactate production (133.3 umoles) accounted for 66.7 umoles leaving only 9.7 umoles unaccounted for or available for aerobic use.

e) Summary

These calculations produced the data in Tables 5 and 6. All assumptions inherent in the calculatons appear in Table 5.

Similar calculations were made with the data generated in the acidosis study and appear in Tables 10-12.

3. Calculation of Contribution of Energy Substrates for Aerobic Metabolism During the Final 15 min of Stimulation

From the triacylglycerol (TG) utilization in the sampled muscles, the total TG utilization was 1.926 umole, calculated as described in a previous section. Since 1.0 umole TG = 0.8854 mg, 1.926 umoles = 1.705 mg TG. Each mg of TG releases 39.6 joules when fully combusted (9.46 cal x 4.184 joules) and therefore 1.705 mg TG releases 67.5 joules. This assumes that all the FFA released from the intramuscular TG was metabolized locally and the plantaris represents the non-sampled working muscle.

From section 2 of this Appendix, calculations revealed 9.7 umoles of carbohydrate fuel were available for aerobic metabolism during the final 15 min of stimulaton. If it was all burned, 9.7 umoles or 1.746 mg (1.0 umole glucose = 0.18 mg) would release 30.5 joules of energy since 1 mg of glucose fully oxidized releases 17.49 joules (4.18 cal x 4.184 joules/cal).

Burning 1.0 ml of O_2 to metabolize only fat releases

19.62 joules (4.69 cal x 4.184 joules/cal). Therefore the release of 67.5 joules (see above) would require 3.435 ml O_2. Similarly, burning 1.0 ml O_2 to metabolize solely carbohydrate releases 21.13 joules (5.05 cal x 4.184 joules). Therefore the release of 30.5 joules would require 1.444 ml O_2.

The average O_2 uptake over the final 15 min of stimulation in the two experiments where stimulation persisted for 20 min was 5.415 ml. Therefore 5.415 - (3.435 + 1.444) = 0.536 ml of O_2 which was used to burn some additional fuel.

The only alternative appears to be FFA derived from the perfusate or from hindquarter adipose tissue. Using 0.536 ml of O_2 to burn fat would produce 10.49 joules of energy (1.0 ml fat metabolized produces 19.62 joules).

These calculations are summarized in Table 7. The estimations of O_2 utilized and energy released from endogenous TG and carbohydrates may be slight overestimations due to the assumption of complete combustion. Therefore the contribution from circulating FFA is underestimated.

APPENDIX B

Characterization Study Mean Muscle Metabolite Data

Mean muscle metabolite data and calculations are listed for perfusions of varying lengths. The tables include data obtained from perfusions employing perfusate of normal acid-base status as found in Chapter 3.

Table 13.
MEAN LACATE, GLYCOGEN, CREATINE PHOSPHATE AND ATP DATA PRE AND POST THE PERFUSIONS (mean ± SE; PP, pre-perfusion; R, post rest perfusions, n = 8; S5, post 5 min stimulation, n = 6; S20, post 20 min stimulation, n = 9; *, significantly different from PP.

		Lactate	glycogen	CP	ATP
		umol.g^{-1} dry weight			
SOLEUS	PP	7.3 ± 1.3	153.4 ± 12.1	71.6 ± 2.8	27.4 ± 2.6
	R	6.2 ± 0.8	153.3 ± 8.6	70.7 ± 7.5	26.6 ± 3.3
	PP	10.0 ± 2.6	191.5 ± 19.3	86.1 ± 10.8	26.4 ± 2.1
	S5	9.8 ± 2.9	169.4 ± 12.4	73.5 ± 4.2	25.8 ± 2.2
	PP	6.0 ± 1.0	139.5 ± 7.2	71.7 ± 3.2	24.2 ± 1.3
	S20	5.8 ± 1.0	109.4 ± 8.0*	52.2 ± 5.4*	19.7 ± 1.4*
PLANTARIS	PP	6.7 ± 0.8	152.8 ± 18.1	93.0 ± 8.5	34.5 ± 1.6
	R	7.8 ± 2.1	145.3 ± 11.1	95.5 ± 8.4	34.0 ± 1.4
	PP	7.3 ± 0.5	187.7 ± 13.8	114.5 ±10.0	38.1 ± 1.9
	S5	40.7 ± 4.7*	81.7 ± 6.3*	68.6 ± 4.6*	27.9 ± 1.8*
	PP	6.4 ± 1.1	171.8 ± 5.5	100.3 ± 3.9	33.8 ± 0.8
	S20	13.1 ± 1.7*	57.0 ± 5.6*	62.9 ± 5.5*	27.9 ± 1.4*
RED GAST.	PP	10.8 ± 1.0	159.5 ± 12.5	92.9 ± 6.6	35.2 ± 1.2
	R	8.4 ± 2.0	125.3 ± 11.0*	97.1 ± 6.4	33.8 ± 1.0
	PP	8.9 ± 2.9	177.2 ± 10.8	106.1 ±10.0	36.6 ± 2.9
	S5	39.6 ± 9.1*	98.1 ± 14.5*	75.7 ±11.3*	29.8 ± 1.5*
	PP	7.4 ± 1.3	178.3 ± 9.3	101.5 ± 7.9	35.0 ± 0.8
	S20	16.5 ± 1.9*	71.8 ± 5.3*	65.3 ± 7.9*	28.4 ± 1.0*
WHITE GAST.	PP	9.8 ± 2.5	163.1 ± 18.1	101.9 ±16.1	36.9 ± 1.6
	R	10.9 ± 2.8	139.6 ± 16.3	109.4 ±15.8	36.5 ± 1.6
	PP	8.0 ± 1.1	178.9 ± 6.2	117.3 ±11.2	37.9 ± 1.4
	S5	82.8 ±12.7*	48.3 ± 7.1*	36.4 ± 5.5*	18.9 ± 2.6*
	PP	7.9 ± 0.8	195.1 ± 9.5	106.5 ± 4.5	36.2 ± 1.4
	S20	27.9 ± 3.3*	23.7 ± 4.1*	51.0 ± 7.6*	22.0 ± 1.8*

Table 14.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 30 MIN REST PERFUSION (mean ± SE, n = 8; PP, pre-perfusion; R, post 30 min rest perfusion; No significant differences between any corresponding PP and R values. TA, total adenine; ECP, energy charge potential = ATP + 1/2 ADP/ATP + ADP + AMP).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		umol.g^{-1} dry weight					
SOLEUS	PP	5.9 ± 0.9	0.55 ± 0.06	33.9 ± 3.3	4.6 ± 0.6	2.6 ± 0.3	0.90 ± 0.01
	R	5.1 ± 0.1	0.61 ± 0.10	32.3 ± 3.5	5.2 ± 0.7	2.7 ± 0.4	0.90 ± 0.01
PLANT.	PP	4.5 ± 0.3	0.44 ± 0.07	39.4 ± 1.6	7.7 ± 0.6	2.7 ± 0.3	0.93 ± 0.01
	R	4.3 ± 0.3	0.46 ± 0.06	38.8 ± 1.4	7.9 ± 0.6	2.8 ± 0.3	0.93 ± 0.01
RED GAST.	PP	4.2 ± 0.4	0.41 ± 0.02	39.8 ± 1.4	8.4 ± 0.6	2.6 ± 0.2	0.94 ± 0.01
	R	4.2 ± 0.3	0.45 ± 0.08	38.5 ± 1.0	8.1 ± 0.5	2.9 ± 0.2	0.94 ± 0.01
WHITE GAST.	PP	4.3 ± 0.3	0.45 ± 0.07	41.7 ± 2.0	8.6 ± 0.7	2.8 ± 0.7	0.94 ± 0.01
	R	4.3 ± 0.2	0.43 ± 0.06	41.2 ± 1.5	8.5 ± 0.5	3.0 ± 0.5	0.94 ± 0.01

Table 15.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 30 MIN REST PERFUSION AND 5 MIN STIMULATION (mean ± SE, n = 6; PP, pre-perfusion; S5, post 5 min stimulation; *, significantly different from PP; Abbreviations as in Table 14).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		umol.g^{-1} dry weight					
SOLEUS	PP	4.7 ± 0.4	0.67 ± 0.11	31.8 ± 2.3	5.6 ± 0.6	3.3 ± 0.6	0.90 ± 0.01
	R	4.9 ± 0.2	0.57 ± 0.08	31.3 ± 1.8	5.3 ± 0.6	2.9 ± 0.2	0.90 ± 0.01
PLANT.	PP	4.6 ± 0.1	0.43 ± 0.04	43.1 ± 1.8	8.3 ± 0.4	3.0 ± 0.1	0.94 ± 0.00
	R	4.2 ± 0.4	0.52 ± 0.11	32.6 ± 1.7*	6.6 ± 0.7*	2.5 ± 0.1*	0.92 ± 0.01*
RED GAST.	PP	4.3 ± 0.5	0.49 ± 0.03	41.4 ± 3.0	8.5 ± 0.5	2.9 ± 0.3	0.94 ± 0.01
	R	5.3 ± 0.4*	0.70 ± 0.11*	35.8 ± 1.5*	5.6 ± 0.5*	2.5 ± 0.3	0.91 ± 0.01*
WHITE GAST.	PP	4.4 ± 0.3	0.45 ± 0.06	42.8 ± 1.5	8.6 ± 0.4	3.1 ± 0.2	0.94 ± 0.00
	R	4.9 ± 0.6	0.78 ± 0.22	24.6 ± 3.0*	3.6 ± 1.3*	1.9 ± 0.2*	0.87 ± 0.02*

Table 16.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 30 MIN REST PERFUSION AND 20 MIN STIMULATION (mean ± SE, n = 9; PP, pre-perfusion; S20, post 30 min stimulation; *, significantly different PP; Abbreviations as in Table 14).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		umol.g^{-1} dry weight					
SOLEUS	PP	3.9 ± 0.3	0.53 ± 0.08	28.6 ± 1.6	6.2 ± 0.5	3.0 ± 0.1	0.91 ± 0.01
	R	4.1 ± 0.4	0.49 ± 0.15	24.3 ± 1.4*	4.8 ± 0.5*	2.7 ± 0.1*	0.90 ± 0.01
PLANT.	PP	4.2 ± 0.2	0.33 ± 0.04	38.3 ± 0.9	8.1 ± 0.3	3.0 ± 0.1	0.94 ± 0.00
	R	4.2 ± 0.4	0.42 ± 0.06	32.2 ± 1.5*	7.2 ± 0.5*	2.3 ± 0.2*	0.93 ± 0.01*
RED GAST.	PP	3.9 ± 0.1	0.38 ± 0.06	39.3 ± 2.4	9.0 ± 0.5	2.9 ± 0.2	0.94 ± 0.01
	R	4.2 ± 0.2	0.58 ± 0.07*	33.2 ± 1.1*	6.9 ± 0.5*	2.3 ± 0.3*	0.92 ± 0.01*
WHITE GAST.	PP	4.4 ± 0.3	0.45 ± 0.08	41.0 ± 2.2	8.4 ± 0.7	2.9 ± 0.1	0.94 ± 0.01
	R	4.2 ± 0.3	0.56 ± 0.09	26.8 ± 1.9*	5.2 ± 0.6*	2.3 ± 0.2*	0.90 ± 0.01*

APPENDIX C

Characterization Study Individual Data

All individual data pertaining to the characterization study in Chapter 3 are presented in tabular form.

Table 17.
CHARACTERISTICS OF THE PERFUSATE, INDIVIDUAL DATA FOR PERFUSIONS OF NORMAL ACID-BASE STATUS (Chapter 3; Rats 1-8, Rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23. 20 min stim perfusions).

Rat #	PaO_2, mmHg	$PaCO_2$, mmHg	pH	HCO_3^-, mmol.l^{-1}	P_{50}, mmHg	Hb, g.dl^{-1}	Hct, %	protein, g.dl^{-1}
1	324	37.5	7.486	28.3	-	17.3	51.2	4.1
2	381	38.4	7.462	27.7	-	16.2	-	-
3	334	50.4	7.435	34.0	-	16.4	-	-
4	281	43.3	7.404	27.0	-	16.4	-	4.0
5	320	44.1	7.364	25.0	-	14.5	43.2	3.6
6	300	47.1	7.423	30.8	-	14.1	40.7	3.2
7	250	46.2	7.357	25.7	-	14.0	40.3	3.3
8	246	40.2	7.453	28.1	-	14.5	41.9	3.4
9	433	36.8	7.386	21.9	29.8	14.1	40.0	4.3
10	366	32.5	7.448	22.5	26.5	13.4	40.0	4.0
11	333	33.2	7.441	22.5	26.5	13.6	40.0	4.0
12	444	41.1	7.397	25.0	29.0	12.4	38.0	4.1
13	420	36.6	7.393	22.2	26.5	14.1	41.0	3.4
14	419	40.4	7.398	25.0	26.0	12.9	37.5	3.9
15	362	38.3	7.342	20.7	25.0	13.6	40.0	4.3
16	320	38.0	7.452	26.5	28.0	14.3	41.0	4.6
17	321	37.0	7.454	25.8	22.5	13.8	38.5	3.9
18	396	36.2	7.392	21.8	27.0	14.3	42.5	4.4
19	428	37.9	7.416	24.2	28.0	13.7	39.5	3.9
20	399	38.4	7.388	23.0	29.0	13.0	39.0	4.2
21	405	37.7	7.377	21.9	26.7	14.7	44.0	3.4
22	390	38.2	7.408	24.0	27.0	13.6	39.5	3.1
23	413	42.8	7.398	26.2	27.0	14.0	41.0	3.6
$\bar{X}$ 1-23	360	39.7	7.412	25.2	27.0	14.3	40.9	3.8
	12	0.9	0.008	0.7	0.5	0.3	0.7	0.1
$\bar{X}$ 9-23	390	37.7	7.406	23.6	27.0	13.7	40.1	3.9
	11	0.7	0.008	0.5	0.5	0.2	0.4	0.1

Table 18.
ADDITIONAL PERFUSATE AND PERFUSION VARIABLES, INDIVIDUAL DATA FOR PERFUSIONS OF NORMAL ACID-BASE STATUS (Chapter 3; Rats 1-8, rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	Rat wt (g)	Perfusate [Lactate], Zero Time (mM)	Perfusate Lactate Prod. Rate ($mM.hr^{-1}$)	Flow Rate Rest ($ml.min^{-1}$)	Flow Rate Stimulation ($ml.min^{-1}$)
1	266	1.65	0.16	6.0	-
2	265	1.44	0.26	5.9	-
3	264	1.64	0.37	6.1	-
4	264	1.64	0.10	5.8	-
5	276	0.76	0.26	5.6	-
6	267	0.94	0.36	6.3	-
7	288	0.88	0.52	5.7	-
8	419	0.93	0.20	6.2	-
9	282	0.95	0.34	6.6	17.8
10	304	0.79	0.37	5.4	14.5
11	279	1.54	-0.20	5.3	14.6
12	284	0.70	0.20	6.0	19.2
13	278	1.30	0.11	6.2	18.0
14	254	0.62	0.27	5.7	20.0
15	283	0.98	0.14	6.3	17.0
16	292	0.85	0.26	5.7	15.8
17	279	0.71	0.13	5.8	16.0
18	290	0.91	0.34	5.8	21.5
19	302	1.12	0.24	6.1	17.8
20	293	0.86	0.19	6.0	16.5
21	254	0.98	0.46	5.9	16.5
22	252	0.58	0.31	5.7	19.8
23	284	0.75	0.46	6.0	21.6
$\bar{X}$ 1-23	283	1.02	0.25	5.9	18.0
$\pm$SE	7	0.07	0.03	0.1	0.6
$\bar{X}$	280	0.91	0.24	5.9	18.0
$\pm$SE	4	0.07	0.04	0.1	0.6

Table 19.
INDIVIDUAL PERFUSATE ELECTROLYTE DATA DURING THE PERFUSIONS (Characterization study, Chapter 3; Rats 1-4, Rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions; A, arterial; V, venous).

	Rat #	Na^+	K^+	Cl^-	Ca^{2+}
		$mmol.l^{-1}$			
20 MIN ARTERIAL REST	1	151	6.0	30	2.9
	2	152	6.0	117	2.7
	3	151	6.0	117	2.6
	4	148	5.7	115	2.5
	9	143	5.9	120	2.3
	10	145	5.6	118	2.3
	11	147	5.6	120	2.3
	12	144	5.8	118	2.4
	13	150	5.8	123	2.2
	14	139	5.7	120	2.0
	15	135	5.8	114	2.5
	16	134	5.0	110	2.4
	17	156	5.7	124	2.5
	18	145	5.7	121	2.5
	19	145	5.8	121	2.2
	20	143	5.7	117	2.2
	21	152	5.7	124	2.5
	22	150	5.7	123	2.1
	23	152	5.8	124	2.2
	$\bar{X}$	148	5.8	121	2.4
	$\pm$SE	1	0.1	1	0.1

40 MIN STIMULATION		A	V	A	V	A	V	A	V
	15	-	-	-	-	-	-	2.5	2.5
	16	149	149	5.5	6.0	120	121	2.7	2.7
	17	-	-	-	-	-	-	2.6	2.7
	18	147	146	5.7	5.9	122	120	2.6	2.5
	19	144	147	5.7	6.2	120	121	2.3	2.3
	20	145	145	5.8	6.1	119	118	2.2	2.2
	21	154	154	5.7	6.1	126	124	2.5	2.5
	22	150	150	5.7	6.0	124	122	2.1	2.1
	23	152	150	5.8	6.0	124	122	2.1	2.4
	$\bar{X}$	149	149	5.7	6.0	122	121	2.4	2.4
	$\pm$SE	1	1	0.1	0.1	1	1	0.1	0.1

Table 20.
INDIVIDUAL ISOMETRIC TENSION DATA DURING 20 MIN OF STIMULATION (Characterization study, Chapter 3; Rats 1-6, 5 min stim perfusions; Rats 7-15, 20 min stim perfusions).

Rat #	Isometric Tension (g)								
	peak	31	32	33	34	35	40	45	50
	perfusion time (min)								
1	2530	2400	2130	1930	1650	1540	-	-	-
2	2780	2600	2100	2030	1820	1720	-	-	-
3	2550	2330	2100	1850	1740	1640	-	-	-
4	2700	-	-	-	-	2040	-	-	-
5	2375	2280	2000	1880	1680	1520	-	-	-
6	2370	2290	1920	1700	1570	1480	-	-	-
7	2820	2690	2570	2330	2200	2120	1740	1590	1480
8	2850	2650	2500	2240	2130	1950	1600	1520	1410
9	2950	2720	2600	2470	2280	2100	1610	-	-
10	2550	2500	2200	2275	1975	1810	1550	1420	1300
11	2800	2700	2500	2250	2100	1950	1640	1500	1400
12	3030	2870	2500	2250	2300	2270	1840	1780	1610
13	2370	2220	2030	1860	1730	1590	1220	1140	1100
14	2550	2525	2400	2200	2000	1940	1510	1360	1240
15	2500	2420	2270	2100	2000	1850	1530	1440	1380
$\bar{X}$	2648	2511	2273	2098	1960	1835	1582	1469	1365
±SE	55	49	63	60	65	63	57	65	55

Table 21.
INDIVIDUAL OXYGEN UPTAKE DATA (Characterization study, Chapter 3; Rats 1-8, Rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	O_2 Uptake (umol.min^{-1}.hindquarter).							
	Rest			Stimulation				
	12	18	25	33	36	39	44	48
	perfusion time (min)							
1	6.7	6.4	7.5	-	-	-	-	-
2	7.4	8.5	7.9	-	-	-	-	-
3	7.0	8.0	8.4	-	-	-	-	-
4	8.3	8.6	8.5	-	-	-	-	-
5	9.2	8.5	7.6	-	-	-	-	-
6	7.1	6.7	6.1	-	-	-	-	-
7	10.4	9.4	9.6	-	-	-	-	-
8	10.8	10.2	10.6	-	-	-	-	-
9	7.4	6.7	7.0	18.7	-	-	-	-
10	10.9	10.3	8.0	24.3	-	-	-	-
11	8.9	8.2	7.4	16.3	-	-	-	-
12	7.0	7.2	6.7	23.4	-	-	-	-
13	8.5	7.7	8.8	23.9	-	-	-	-
14	6.2	7.7	7.2	23.9	-	-	-	-
15	6.1	6.3	6.6	21.1	23.6	23.9	20.9	22.2
16	7.0	7.6	7.9	22.9	25.6	23.5	23.4	21.8
17	6.8	7.6	9.3	24.6	25.4	23.0	18.4	17.2
18	7.4	7.0	7.4	25.0	22.7	20.4	18.7	15.5
19	6.4	7.0	6.9	19.2	15.9	15.6	15.2	14.4
20	8.1	8.2	7.0	24.7	28.4	21.3	22.0	18.1
21	5.5	6.5	8.3	21.0	20.2	18.5	17.5	16.2
22	6.4	7.0	7.6	22.4	22.2	18.9	19.5	20.2
23	6.8	7.4	7.6	26.2	27.0	25.5	24.2	23.2
$\bar{X}$ 1-23	7.7	7.8	7.8	22.5	23.4	21.2	20.0	18.8
$\pm$ SE	0.3	0.2	0.2	0.7	1.3	1.0	1.0	1.1
$\bar{X}$ 9-23	7.3	7.5	7.6					
$\pm$ SE	0.4	0.3	0.2					

Table 22.
INDIVIDUAL ARTERIAL PRESSURE DATA (Characterization study, Chapter 3; Rats 1-8, Rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	Arterial Pressure (mmHg)									
	Rest					Stimulation				
	10	15	20	25	30	30	35	40	45	50
	perfusion time (min)									
1	100	100	100	105	105	-	-	-	-	-
2	80	85	84	82	85	-	-	-	-	-
3	90	98	100	102	104	-	-	-	-	-
4	90	90	84	84	86	-	-	-	-	-
5	74	72	73	74	76	-	-	-	-	-
6	80	80	80	80	80	-	-	-	-	-
7	72	70	69	71	74	-	-	-	-	-
8	72	76	78	78	76	-	-	-	-	-
9	86	88	86	89	90	200	204	-	-	-
10	75	72	76	74	72	136	134	-	-	-
11	82	82	84	83	83	230	230	-	-	-
12	72	75	74	74	75	192	180	-	-	-
13	84	84	80	82	84	182	181	-	-	-
14	80	86	82	85	85	210	216	-	-	-
15	74	74	76	80	82	160	160	160	162	162
16	94	101	94	95	98	182	182	184	190	196
17	92	96	104	-	-	210	204	220	232	240
18	72	76	74	76	76	208	210	220	236	248
19	84	87	88	90	94	220	232	250	264	276
20	76	70	72	72	74	210	192	194	194	200
21	86	86	84	86	86	180	186	192	195	200
22	98	96	90	88	88	224	236	252	266	280
23	78	80	80	80	80	214	206	212	216	220
$\bar{X}$ 1-23	82	84	83	85	84	197	197	209	218	225
±SE	2	2	2	3	2	7	7	10	12	13
$\bar{X}$ 9-23	82	83	83	82	83					
	2	2	2	2	3					

Table 23.

INDIVIDUAL RESTING LACTATE RELEASE DATA (Characterization study, Chapter 3; Rats 1-8, rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	Lactate Release ($umol.min^{-1}.hindquarter^{-1}$)				
	21	23	25	27	29
	perfusion time (min)				
1	2.16	1.56	1.14	1.85	2.10
2	0.71	1.18	0.94	0.53	0.47
3	1.89	1.04	0.94	1.22	1.71
4	1.04	0.29	0.78	0.75	-
5	1.85	1.62	1.40	1.57	1.62
6	2.02	2.52	1.89	0.95	1.89
7	0.91	1.03	0.86	0.68	0.57
8	-0.43	-0.68	-0.74	-0.37	-0.47
9	1.06	1.39	1.21	1.42	1.12
10	0.97	0.32	1.00	0.97	0.54
11	0.48	1.19	0.95	0.58	0.93
12	3.36	1.92	1.53	2.28	2.55
13	0.74	0.90	1.30	1.18	1.15
14	3.88	-0.22	1.95	1.88	2.22
15	0.70	0.73	0.52	1.32	0.90
16	0.92	1.10	1.53	1.48	1.07
17	0.90	1.42	3.13	1.20	1.05
18	0.77	0.76	0.68	0.63	0.57
19	0.51	0.63	0.60	0.97	0.74
20	0.42	0.32	0.35	0.00	0.26
21	0.50	0.75	0.63	0.50	0.44
22	4.15	4.32	3.13	3.41	3.07
23	2.36	2.34	2.88	2.47	2.19
$\bar{X}$ 1-23	1.39	1.19	1.24	1.19	1.21
±SE	0.24	0.32	0.19	0.17	0.18
$\bar{X}$ 9-23	1.45	1.26	1.43	1.34	1.25
±SE	0.34	0.31	0.24	0.23	0.22

Table 24.
INDIVIDUAL LACTATE RELEASE DATA DURING STIMULATION (Characterization study, Chapter 3; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	Lactate Release ($umol.min^{-1}.hindquarter^{-1}$)									
	31	33	35	37	39	41	43	45	47	49
	perfusion time (min)									
9	13.7	21.0	18.3	-	-	-	-	-	-	-
10	8.9	19.9	18.4	-	-	-	-	-	-	-
11	7.1	25.7	24.0	-	-	-	-	-	-	-
12	20.7	27.8	24.7	-	-	-	-	-	-	-
13	7.8	14.9	12.9	-	-	-	-	-	-	-
14	13.9	22.3	19.2	-	-	-	-	-	-	-
15	12.7	26.0	26.6	17.0	14.8	13.5	12.8	7.3	10.1	5.6
16	12.0	22.4	20.7	17.1	13.7	10.5	11.3	3.8	4.2	8.7
17	17.8	24.5	22.1	16.8	16.9	12.9	13.0	10.9	8.7	9.2
18	8.9	16.6	15.7	12.2	10.4	9.8	6.9	6.9	6.6	5.3
19	7.9	17.3	15.9	13.7	10.1	8.6	4.9	7.7	9.5	9.4
20	12.2	20.6	19.8	14.7	11.7	6.9	8.0	7.2	8.2	4.6
21	13.1	26.2	21.2	20.0	16.0	13.1	10.3	8.6	6.1	5.9
22	16.2	33.1	28.3	23.4	16.8	13.6	13.9	13.0	12.8	9.5
23	17.8	33.0	35.4	28.5	21.6	21.3	16.3	13.1	12.8	12.2
$\bar{X}$	12.7	23.4	21.5	18.1	14.7	12.2	10.8	8.7	8.8	7.8
$\pm$SE	1.1	1.4	1.5	1.7	1.2	1.4	1.2	1.0	1.0	0.9

Table 25.
INDIVIDUAL RESTING GLUCOSE UPTAKE DATA (Characterization Study, Chapter 3; Rats 1-8, rest perfusions; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	Glucose Uptake ($\mu mol \cdot min^{-1} \cdot hindquarter^{-1}$)				
	21	23	25	27	29
	perfusion time (min)				
1	0.30	1.41	0.84	-	0.90
2	0.74	1.12	1.06	1.00	1.77
3	0.52	-0.40	0.46	-2.01	-4.09
4	-0.70	0.58	1.04	0.87	-
5	-0.08	0.34	-0.25	0.53	0.31
6	0.13	-0.61	1.06	0.70	0.22
7	0.17	0.40	-0.06	0.03	-0.20
8	1.43	-0.16	0.87	0.43	0.37
9	0.63	0.18	-0.15	0.65	0.13
10	0.46	0.51	-	0.00	-0.25
11	0.43	0.58	1.26	1.78	0.39
12	0.00	0.34	1.22	0.06	0.70
13	0.58	0.51	0.12	0.67	1.15
14	-3.40	0.76	0.41	0.64	0.41
15	-0.04	1.06	2.79	2.45	2.08
16	0.45	0.77	-1.16	-	-0.14
17	1.55	-0.40	0.53	0.26	0.64
18	0.86	1.23	0.77	0.00	0.39
19	0.89	0.39	0.41	0.48	0.79
20	0.31	0.42	1.26	-0.24	0.23
21	1.59	0.24	0.98	0.77	1.63
22	-1.56	-2.00	1.20	-1.69	2.14
23	-1.31	-1.52	0.41	-1.26	-1.30
$\bar{X}$	0.17	0.25	0.69	0.29	0.37
$\pm$SE	0.23	0.17	0.16	0.22	0.27

Table 26.
INDIVDUAL GLUCOSE UPTAKE DATA DURING STIMULATION (Characterization study, Chapter 3; Rats 9-14, 5 min stim perfusions; Rats 15-23, 20 min stim perfusions).

Rat #	Glucose Uptake (umol.min^{-1}.hindquarter^{-1})					
	31	35	39	43	47	49
	perfusion time (min)					
9	3.05	2.27	-	-	-	-
10	1.66	3.47	-	-	-	-
11	1.62	3.60	-	-	-	-
12	2.19	1.18	-	-	-	-
13	3.63	1.54	-	-	-	-
14	3.04	4.48	-	-	-	-
15	1.22	-0.34	3.26	0.00	-	4.19
16	4.82	5.83	-	2.52	5.32	4.13
17	-	-0.17	-	-	0.56	-
18	4.36	1.89	4.44	2.55	0.82	4.16
19	1.93	2.51	-0.35	-	-	-0.65
20	1.48	4.04	1.21	2.48	-0.57	6.11
21	4.00	3.72	-	-	4.40	-
22	2.96	2.56	-0.28	-	-	1.08
23	-1.00	-	5.41	3.09	-	1.77
$\bar{X}$	2.50	2.61	2.28	2.13	2.11	2.97
$\pm$SE	0.41	0.46	1.00	0.54	1.16	0.87

Table 27.
INDIVIDUAL FREE FATTY ACID RELEASE DATA DURING 30 MIN REST PERFUSION AND 20 MIN STIMULATION (Characterization study, Chapter 3).

Rat #	FFA Release ($nmol.min^{-1}.hindquarter^{-1}$)					
	19	21	23	25	27	29
	perfusion time (min)					
REST						
1	105	-	115	97	132	146
2	58	12	54	44	19	33
3	97	87	52	52	49	45
4	52	15	10	38	6	-49
5	304	153	210	358	326	270
6	30	7	-2	0	11	-16
7	160	110	127	195	184	158
8	115	34	77	0	46	54
$\bar{X}$	89		89		87	
$\pm$SE	20		24		27	

	31	33	35	37	39	41	43	45	47	49
	perfusion time (min)									
STIMULATION										
1	374	206	15	108	-	108	275	152	-	-
2	0	53	24	-22	17	-11	-159	-145	14	-
3	-91	-175	-106	-160	-233	250	16	-160	32	138
4	166	0	157	0	41	132	88	79	-	-
5	752	566	545	534	711	612	757	695	446	425
6	15	170	67	131	181	81	140	53	50	53
7	469	457	368	499	592	475	625	670	314	425
8	294	259	345	185	312	335	189	0	146	172
$\bar{X}$	220		168		207		205		201	
$\pm$SE	64		56		76		78		51	

Table 28.
INDIVIDUAL GLYCEROL RELEASE DATA DURING 30 MIN REST PERFUSION AND 20 MIN STIMULATION (Characterization study, Chapter 3).

Rat #	Glycerol Release ($nmol.min^{-1}.hindquarter^{-1}$)					
	19	21	23	25	27	29
	perfusion time (min)					
REST						
1	364	363	276	260	315	301
2	375	375	321	284	268	277
3	127	167	238	198	182	167
4	573	498	383	505	485	390
5	209	233	184	277	301	293
6	235	196	192	209	196	209
7	212	139	95	175	139	139
8	54	37	50	19	41	37
$\bar{X}$	260		229		234	
±SE	38		30		30	

	31	33	35	37	39	41	43	45	47	49
	perfusion time (min)									
STIMULATION										
1	701	655	423	695	879	370	598	365	-	
2	751	563	778	760	853	778	778	859	859	-
3	469	446	350	182	314	365	410	182	[illegible]	350
4	1151	802	802	755	825	917	778	685	[illegible]	708
5	670	495	647	428	560	560	543	630	630	675
6	425	461	301	215	253	339	511	326	436	326
7	338	249	338	293	238	239	271	239	338	150
8	121	62	121	74	121	39	85	74	121	91
$\bar{X}$	522		448		478		458		435	
±SE	68		63		73		63		72	

Table 29.

INDIVIDUAL MUSCLE GLYCOGEN AND LACTATE CONCENTRATIONS PRE AND POST 30 MIN OF REST PERFUSION (Characterization study, Chapter 3; PP, pre-perfusion; R, post 30 min rest perfusion).

Rat #	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
	PP	R	PP	R	PP	R	PP	R
GLYCOGEN								
1	90.6	124.7	145.4	76.6	134.7	105.0	182.3	138.9
2	174.1	125.5	146.4	166.3	158.3	112.1	188.2	136.8
3	159.9	176.7	179.7	153.8	191.7	140.9	207.3	176.7
4	138.7	130.6	251.9	178.3	-	173.7	245.2	231.4
5	170.5	165.5	133.0	151.7	177.7	122.8	106.0	81.3
6	181.7	159.6	162.8	165.0	195.3	138.5	150.0	117.8
7	120.9	154.6	133.3	154.4	156.5	-	123.1	105.8
8	190.5	188.8	69.8	116.5	102.1	83.8	102.9	127.8
$\bar{X}$	153.4	153.3	152.8	145.3	159.5	125.3	163.1	139.6
±SE	12.1	8.6	18.1	11.7	12.5	11.0	18.1	16.3
LACTATE								
1	6.1	9.6	7.8	18.5	9.7	21.6	6.1	26.9
2	7.5	8.7	7.1	16.4	16.4	8.1	10.0	11.3
3	8.7	6.5	6.3	4.7	12.2	6.3	10.2	7.2
4	-	7.8	9.9	5.0	10.1	10.1	8.6	8.7
5	2.9	4.3	9.7	5.1	12.0	3.6	18.5	4.9
6	10.1	5.1	3.7	3.3	7.4	4.9	18.6	7.7
7	12.2	4.7	4.2	5.1	10.8	4.7	10.4	10.7
8	3.6	2.9	4.6	4.0	8.1	7.7	12.7	9.6
$\bar{X}$	7.3	6.2	6.7	7.8	10.8	8.4	9.8	10.9
±SE	1.3	0.8	0.8	2.1	1.0	2.0	2.5	2.8

Table 30.
INDIVIDUAL MUSCLE CREATINE PHOSPHATE AND ATP CONCENTRATIONS PRE AND POST 30 MIN OF REST PERFUSION (Characterization study, Chapter 3; PP, pre-perfusion; R, post 30 min rest perfusion).

Rat #	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
	PP	R	PP	R	PP	R	PP	R
CP								
1	70.6	86.2	76.9	74.2	86.8	81.8	87.9	53.6
2	73.7	44.5	77.0	71.7	70.5	83.7	78.7	81.6
3	61.9	76.1	71.2	78.6	90.6	74.4	82.1	81.6
4	-	-	66.6	69.6	72.6	82.2	86.1	88.0
5	65.0	52.5	100.9	117.3	106.2	110.9	110.4	127.1
6	68.6	76.9	126.6	121.2	128.4	112.0	55.9	131.1
7	78.4	58.1	127.4	120.5	97.9	117.3	206.6	113.1
8	82.8	100.4	97.7	110.8	90.5	114.5	107.7	199.2
$\bar{X}$	71.6	70.7	93.0	95.5	92.9	97.1	101.9	109.4
±SE	2.8	7.5	8.5	8.4	6.6	6.4	16.1	15.8
ATP								
1	32.6	37.0	31.1	36.0	39.8	30.7	38.9	31.1
2	28.3	24.0	37.7	34.3	34.6	37.9	39.3	37.2
3	33.4	32.3	36.4	35.9	39.3	35.7	41.0	38.3
4	-	-	29.5	26.4	32.4	31.3	39.8	33.2
5	16.3	13.6	43.2	35.0	35.3	34.2	-	-
6	-	-	34.7	34.6	37.2	37.1	37.4	44.1
7	24.9	25.8	31.5	30.4	32.1	32.6	31.6	34.4
8	29.0	27.0	32.1	39.2	30.4	30.9	30.4	37.5
$\bar{X}$	27.4	26.6	34.5	34.0	35.2	33.8	36.9	36.5
±SE	2.6	3.3	1.6	1.4	1.2	1.0	1.6	1.6

Table 31.
INDIVIDUAL MUSCLE ADP AND AMP CONCENTRATIONS PRE AND POST #) MIN OF REST PERFUSION (Characterization study, Chapter 3; PP, pre-perfusion; R, post 30 min rest perfusion).

Rat #	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
	PP	R	PP	R	PP	R	PP	R
ADP								
1	10.3	6.9	5.3	5.9	4.6	5.7	4.5	4.5
2	5.8	6.3	6.4	4.8	6.3	4.3	5.5	4.4
3	5.4	5.1	4.6	4.3	4.4	4.8	4.7	4.8
4	9.3	4.7	4.2	4.3	4.2	4.6	4.8	4.8
5	4.0	4.8	4.0	3.9	3.3	3.8	4.2	4.2
6	4.2	5.1	3.6	3.7	3.7	3.7	4.0	4.3
7	3.8	3.8	3.8	3.9	3.6	3.5	3.5	3.6
8	4.6	3.9	4.3	3.5	3.3	3.7	3.2	3.9
$\bar{X}$	5.9	5.1	4.5	4.3	4.2	4.2	4.3	4.3
±SE	0.9	0.4	0.3	0.3	0.4	0.3	0.3	0.2
AMP								
1	0.79	1.16	0.91	0.79	0.51	1.01	0.61	0.74
2	0.53	0.81	0.45	0.59	0.37	0.33	0.68	0.46
3	0.72	0.47	0.34	0.42	0.46	0.45	0.63	0.35
4	-	0.53	0.46	0.39	0.36	0.43	0.48	0.55
5	0.49	0.76	0.26	0.37	0.37	0.30	0.28	0.33
6	0.54	0.30	0.26	0.30	0.45	0.34	0.49	0.42
7	0.43	0.41	0.45	0.46	0.41	0.37	0.06	0.42
8	0.39	0.46	0.39	0.35	0.37	0.40	0.35	0.27
$\bar{X}$	0.55	0.61	0.44	0.46	0.41	0.45	0.45	0.43
±SE	0.06	0.10	0.07	0.06	0.02	0.08	0.07	0.06

Table 32.
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CREATINE PHOSPHATE CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION AND 5MIN STIMULATION (Characterization study, Chapter 3; PP, pre-perfusion; S5, post 5 min stimulation).

Rat #	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
	PP	S5	PP	S5	PP	S5	PP	S5
GLYCOGEN								
1	266.5	195.8	165.0	82.7	170.0	92.1	176.2	43.3
2	209.9	191.8	247.9	97.6	202.2	107.3	192.9	58.1
3	205.2	202.1	188.5	102.5	202.4	139.9	199.6	45.9
4	156.1	135.0	184.0	73.1	179.0	47.6	178.9	75.3
5	131.5	140.0	149.1	66.1	131.7	70.5	162.9	23.2
6	179.5	151.6	192.4	68.4	175.9	131.5	162.7	44.0
$\bar{X}$	191.5	169.4	187.8	81.7	177.2	98.1	178.9	48.3
±SE	19.3	12.4	13.8	6.3	10.8	14.5	6.2	7.1
LACTATE								
1	20.8	8.1	7.2	32.0	6.1	33.8	6.1	101.6
2	4.5	6.7	8.4	42.1	22.7	24.0	12.2	78.1
3	4.8	6.5	5.2	59.1	6.1	52.1	9.2	120.3
4	7.3	24.4	7.8	48.4	2.8	79.1	5.6	33.6
5	6.8	7.3	6.8	30.0	7.2	24.2	5.4	83.4
6	3.8	6.0	8.1	32.9	8.9	24.2	9.3	100.1
$\bar{X}$	8.0	9.8	7.3	40.7	8.9	39.6	8.0	82.8
CP								
1	75.6	82.6	100.7	65.6	99.4	75.5	122.6	51.7
2	133.4	89.7	153.5	85.6	114.2	112.2	158.3	34.2
3	94.5	70.0	120.7	71.9	73.2	32.2	-	-
4	81.4	64.6	124.8	73.0	138.4	82.5	98.2	41.0
5	55.9	66.3	90.0	63.3	86.0	59.2	97.2	17.8
6	75.5	68.1	97.1	52.2	125.2	92.6	110.3	37.2
$\bar{X}$	86.1	73.5	114.5	68.6	106.1	75.7	117.3	36.4
±SE	10.8	4.2	9.6	4.6	10.0	11.3	11.2	5.5

Table 33.
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION AND 5 MIN STIMULATION (Characterization study, Chapter 3; PP, pre-perfusion; S5, post 5 min stimulation).

Rat #	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
	PP	S5	PP	S5	PP	S5	PP	S5
ATP								
1	32.7	28.2	36.0	27.8	34.4	29.6	38.7	22.0
2	31.2	33.3	43.8	28.9	42.2	33.8	41.9	19.1
3	24.9	18.6	37.3	29.2	-	25.5	40.8	29.5
4	27.5	29.2	41.9	33.9	44.8	34.1	35.8	12.7
5	19.2	22.2	30.9	24.9	31.6	26.3	32.3	12.2
6	23.1	23.5	38.8	20.5	30.0	29.4	37.8	17.8
$\bar{X}$	26.4	25.8	38.1	27.9	36.6	29.8	37.8	18.9
±SE	2.1	2.2	1.9	1.8	2.9	1.5	1.4	2.6
ADP								
1	5.9	5.7	4.1	3.2	4.0	3.9	3.9	5.2
2	4.4	4.5	-	3.6	4.8	4.6	4.5	3.8
3	5.3	-	4.4	4.4	2.2	5.1	5.1	2.5
4	4.0	4.3	4.8	4.6	5.3	6.4	5.2	2.7
5	3.5	5.2	4.6	3.7	4.3	5.8	3.7	4.0
6	4.9	4.9	4.8	5.7	5.1	6.0	4.3	6.3
$\bar{X}$	4.7	4.9	4.6	4.2	4.3	5.3	4.4	4.1
±SE	0.3	0.2	0.1	0.4	0.5	0.4	0.3	0.6
AMP								
1	0.56	0.61	0.47	0.21	0.43	0.50	0.32	-
2	0.48	0.30	-	0.30	0.48	0.42	0.36	0.49
3	0.99	0.57	0.44	0.90	0.60	0.91	0.46	0.33
4	0.37	0.60	0.27	0.47	0.38	0.48	0.40	0.67
5	0.68	-	0.51	0.43	0.50	0.78	0.45	0.78
6	0.96	0.77	0.48	0.79	0.58	1.11	0.72	1.68
$\bar{X}$	0.67	0.57	0.43	0.52	0.49	0.70	0.45	0.78
±SE	0.11	0.08	0.04	0.11	0.03	0.11	0.06	0.22

Table 34.
INDIVIDUAL MUSCLE GLYCOGEN AND LACTATE CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION AND 20 MIN STIMULATION (Characterization study, Chapter 3; PP, pre-perfusion; S20, post 20 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
#	PP	S20	PP	S20	PP	S20	PP	S20
GLYCOGEN								
1	154.8	118.0	171.7	64.0	179.3	81.8	243.1	43.1
2	169.9	139.3	150.5	60.4	159.5	67.8	190.1	9.7
3	123.0	95.4	178.2	31.7	216.3	46.4	189.8	17.8
4	138.1	116.7	183.1	51.5	168.7	62.6	181.7	-
5	156.1	68.3	199.5	41.5	223.1	97.2	191.3	34.4
6	160.3	142.3	185.4	89.2	196.6	85.0	233.4	29.4
7	125.8	109.8	158.8	70.3	151.7	75.0	201.3	19.5
8	119.9	109.1	166.5	50.8	146.1	54.8	174.8	10.1
9	107.1	85.7	152.2	53.5	163.5	75.6	150.4	25.2
$\bar{X}$	139.5	109.4	171.8	57.0	178.3	71.8	195.1	23.7
$\pm$SE	7.2	8.0	5.5	5.6	9.3	5.3	9.5	4.1
LACTATE								
1	10.8	3.4	5.4	18.2	5.0	21.4	7.5	18.3
2	3.8	4.4	5.9	12.0	5.6	13.0	12.4	31.7
3	2.7	3.3	3.9	18.5	6.8	17.2	5.6	32.0
4	4.4	4.5	14.7	6.0	15.4	20.3	10.7	16.4
5	7.7	8.3	7.2	19.0	10.9	10.4	7.4	19.7
6	9.8	4.2	6.0	17.1	8.9	19.3	7.8	46.1
7	5.0	7.9	4.0	8.9	3.7	25.4	5.0	38.0
8	6.3	12.0	6.3	10.3	2.7	14.3	6.0	26.2
9	3.8	4.7	4.6	8.4	7.5	7.0	9.0	22.6
$\bar{X}$	6.0	5.8	6.4	13.1	7.4	16.5	7.9	27.9
$\pm$SE	1.0	1.0	1.1	1.7	1.3	1.9	0.8	3.3

Table 35.
INDIVIDUAL MUSCLE CREATINE PHOSPHATE AND ATP CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION AND 20 MIN STIMULATION (Characterization study, Chapter 3; PP, pre-perfusion; S20, post 20 min stimulation).

Rat #	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	PP	S20	PP	S20	PP	S20	PP	S20
CP								
1	74.9	60.7	87.5	57.7	83.0	62.0	92.8	35.5
2	72.0	65.3	80.7	34.5	92.9	47.7	81.2	46.6
3	73.6	57.6	107.6	50.9	153.4	81.6	108.4	78.9
4	51.1	31.5	95.0	88.0	-	69.5	120.9	97.3
5	76.8	35.9	105.6	78.4	105.4	101.6	126.1	33.3
6	81.8	70.9	116.8	52.1	104.6	24.6	107.8	29.7
7	73.1	26.6	96.7	70.6	97.2	42.9	105.1	39.4
8	62.3	63.6	99.3	60.2	86.5	81.5	104.0	48.3
9	79.2	57.8	113.6	73.5	89.0	76.4	111.9	49.8
$\bar{X}$	74.7	52.5	100.3	62.9	101.5	65.3	106.5	51.0
±SE	3.2	5.4	3.9	5.5	7.9	7.9	4.5	7.6
ATP								
1	25.8	23.9	32.4	26.5	33.2	30.6	35.3	29.4
2	24.6	24.9	30.3	26.6	37.0	30.5	34.5	19.5
3	26.4	20.9	33.5	24.4	39.0	26.0	38.5	24.8
4	16.4	16.3	35.5	34.4	-	26.8	38.2	30.9
5	28.2	12.6	37.2	31.6	40.5	33.3	41.3	20.1
6	25.9	22.0	36.7	31.6	37.4	24.3	36.9	18.0
7	25.3	15.6	31.9	25.5	31.2	27.6	32.0	15.9
8	19.2	19.0	32.5	21.5	28.4	25.2	28.8	16.2
9	26.1	21.8	34.1	29.4	33.5	31.2	40.6	23.3
$\bar{X}$	24.2	19.7	33.8	27.9	35.0	28.4	36.2	22.0
±SE	1.3	1.4	0.8	1.4	1.5	1.0	1.4	1.0

Table 36.
INDIVIDUAL MUSCLE ADP AND AMP CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION AND 20 MIN STIMULATION (Characterization study, Chapter 3; PP, pre-perfusion; S20, post 20 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
#	PP	S20	PP	S20	PP	S20	PP	S20
ADP								
1	5.0	3.8	3.7	3.7	3.8	3.9	4.2	4.9
2	2.6	3.5	3.4	4.4	4.0	3.2	3.9	3.9
3	3.4	4.1	3.6	2.7	4.0	3.6	3.5	4.3
4	2.0	5.4	4.5	4.1	3.4	3.8	3.9	3.6
5	4.3	2.0	4.4	3.9	4.5	4.4	3.8	3.1
6	4.9	4.4	4.8	6.8	-	5.2	-	-
7	4.3	5.9	4.3	4.4	4.3	4.9	5.4	5.3
8	4.4	3.8	4.6	3.7	4.1	4.1	4.1	4.2
9	4.2	4.2	4.1	4.3	3.4	4.8	6.0	4.8
$\bar{X}$	3.9	4.1	4.2	4.2	3.9	4.2	4.4	4.2
±SE	0.3	0.4	0.2	0.4	0.1	0.2	0.3	0.3
AMP								
1	0.71	0.14	0.15	0.20	0.18	0.17	0.40	0.27
2	0.26	0.31	0.25	0.36	0.28	0.52	0.18	0.65
3	0.23	0.12	0.20	0.40	0.16	0.50	0.30	0.57
4	0.60	0.27	0.25	0.22	0.33	-	0.43	0.17
5	0.34	0.25	0.33	0.38	0.48	0.51	0.30	0.38
6	0.52	0.47	0.40	0.79	-	0.78	-	-
7	-	1.54	0.49	0.45	0.49	0.80	0.52	0.63
8	0.88	0.58	0.47	0.42	0.47	0.61	0.47	0.89
9	0.67	0.75	0.49	0.56	0.69	0.76	0.98	0.92
$\bar{X}$	0.53	0.49	0.33	0.42	0.38	0.58	0.45	0.56
	0.08	0.15	0.04	0.06	0.06	0.07	0.08	0.09

Table 37.
INDIVIDUAL MUSCLE TRIACYLGLYCEROL CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION AND 20 MIN STIMULATION (Characterization study, Chapter 3; PP, pre-perfusion; S20, post 20 min stimulation; *, Rats 1 and 7 for white gast. not included in mean due to adipose tissue contamination).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
	PP	S20	PP	S20	PP	S20	PP	S20
1	23.27	42.88	7.06	5.96	6.31	3.28	5.87	79.32*
2	38.54	16.78	5.65	2.31	-	-	9.75	11.61
3	12.43	20.82	7.10	6.88	4.80	2.64	10.57	3.71
4	46.66	17.28	10.25	9.28	10.01	4.59	6.57	8.71
5	10.56	12.77	8.10	8.46	5.88	5.31	6.97	5.33
6	23.32	10.24	5.31	4.90	8.92	7.09	4.18	2.08
7	45.74	29.25	9.29	7.57	7.71	6.39	6.01	80.72*
8	25.15	39.94	8.84	7.95	3.86	3.21	2.98	6.07
$\bar{X}$	28.21	23.75	7.70	6.66	6.78	4.64	6.84	6.25
±SE	4.95	4.35	0.61	0.80	0.84	0.64	1.22	1.41

APPENDIX D

Acidosis Study Mean Muscle Metabolite Data

Mean muscle metabolite data and calculations are listed for perfusions of varying lengths. The tables include data obtained from perfusions employing acidotic perfusates as found in Chapter 4.

Table 38.
MEAN MUSCLE LACTATE, GLYCOGEN, CREATINE PHOSPHATE, ATP, ADP AND AMP DATA PRE AND POST 30 MIN REST PERFUSION DURING ACIDOSIS (mean ± SE; MA, metabolic acidosis, n = 5; RA, respiratory acidosis, n = 5; PP, pre-perfusion; R. post 30 min rest perfusion; No significant differences between corresponding PP and R values).

			Glycogen	Lactate	CP	ATP	ADP	AMP
			umol.g^{-1} dry weight					
SOLEUS	MA	PP	95.6 ± 12.7	7.1 ± 2.3	68.3 ± 8.4	19.5 ± 1.1	4.3 ± 0.6	0.46 ± 0.11
		R	97.3 ± 10.6	7.8 ± 1.4	77.8 ± 6.0	21.5 ± 1.6	3.8 ± 0.3	0.52 ± 0.06
	RA	PP	96.4 ± 3.6	6.6 ± 0.9	71.7 ± 2.4	24.1 ± 1.5	4.0 ± 0.2	0.54 ± 0.06
		R	98.0 ± 3.1	7.3 ± 0.9	69.9 ± 4.4	24.0 ± 1.7	4.1 ± 0.2	0.56 ± 0.03
PLANT.	MA	PP	126.3 ±10.0	7.7 ± 1.3	103.9 ±20.3	28.0 ± 0.8	3.4 ± 0.4	0.36 ± 0.03
		R	135.2 ± 1.5	7.4 ± 1.7	105.7 ± 9.9	30.1 ± 2.1	3.9 ± 0.2	0.36 ± 0.03
	RA	PP	145.6 ± 9.5	7.1 ± 1.1	97.4 ± 8.2	31.2 ± 2.4	3.8 ± 0.2	0.39 ± 0.04
		R	143.7 ± 5.5	7.2 ± 0.5	97.7 ± 7.3	30.2 ± 1.8	4.0 ± 0.2	0.42 ± 0.03
R GAST.	MA	PP	152.1 ± 5.6	10.5 ± 2.8	99.0 ±10.4	29.0 ± 1.2	3.6 ± 0.3	0.35 ± 0.03
		R	146.2 ± 8.0	10.6 ± 2.8	93.4 ± 5.1	29.0 ± 2.6	4.3 ± 0.3	0.35 ± 0.04
	RA	PP	154.8 ± 7.0	11.3 ± 1.3	93.9 ± 6.0	31.2 ± 1.7	4.2 ± 0.5	0.41 ± 0.04
		R	150.5 ± 7.6	9.7 ± 1.1	96.5 ± 4.8	31.7 ± 1.2	4.0 ± 0.3	0.39 ± 0.05
W GAST.	MA	PP	130.3 ±8.3	8.3 ± 2.3	84.2 ± 4.1	29.5 ± 1.2	3.6 ± 0.2	0.36 ± 0.04
		R	127.9 ± 9.3	14.7 ± 3.8	91.3 ± 8.0	31.5 ± 2.9	4.0 ± 0.3	0.39 ± 0.05
	RA	PP	165.8 ± 7.9	9.3 ± 1.0	96.8 ± 4.9	31.1 ± 1.2	4.0 ± 0.3	0.39 ± 0.04
		R	162.3 ± 6.5	10.1 ± 1.7	96.5 ± 5.0	30.8 ± 1.1	4.0 ± 0.1	0.45 ± 0.08

Table 39.
MEAN MUSCLE LACTATE, GLYCOGEN, CREATINE PHOSPHATE AND ATP DATA PRE AND POST METABOLIC ACIDOSIS PERFUSIONS (mean ± SE; PP, pre-perfusion; S5, post 5 min stimulation, n = 4; S20, post 20 min stimulation, n = 6).

		Lactate	Glycogen	CP	ATP
		umol.g^{-1} dry weight			
SOLEUS	PP	8.9 ± 2.2	118.3 ± 16.5	85.2 ± 6.2	25.3 ± 2.1
	S5	7.9 ± 1.4	123.1 ± 15.8	85.1 ± 11.5	27.8 ± 2.5
	PP	8.4 ± 1.0	123.6 ± 7.7	69.5 ± 5.6	18.9 ± 0.6
	S20	15.1 ± 5.6	116.0 ± 17.6	71.3 ± 7.9	22.1 ± 1.3
PLANT.	PP	5.0 ± 0.9	152.2 ± 11.9	111.6 ± 3.0	35.8 ± 1.1
	S5	25.8 ± 2.6	78.2 ± 10.2	76.5 ± 6.1	28.7 ± 2.4
	PP	10.2 ± 1.3	153.0 ± 16.4	102.7 ± 9.9	28.9 ± 1.5
	S20	23.8 ± 5.0	66.9 ± 3.4	64.8 ± 4.7	25.2 ± 1.4
RED GAST.	PP	7.4 ± 1.5	146.2 ± 10.1	113.7 ± 8.4	38.6 ± 1.5
	S5	25.9 ± 6.5	85.7 ± 11.5	82.7 ± 13.4	28.2 ± 3.6
	PP	10.8 ± 3.4	160.8 ± 23.1	98.4 ± 5.0	30.8 ± 1.2
	S20	23.0 ± 3.4	74.5 ± 4.6	75.7 ± 11.3	25.6 ± 1.5
WHITE GAST.	PP	6.5 ± 1.4	160.7 ± 4.5	115.2 ± 7.0	38.2 ± 2.6
	S5	84.0 ± 11.5	42.2 ± 4.4	47.4 ± 7.9	17.9 ± 1.2
	PP	9.4 ± 1.0	178.9 ± 19.1	103.3 ± 5.6	31.7 ± 1.3
	S20	44.5 ± 5.2	31.5 ± 5.5	60.4 ± 11.0	20.0 ± 0.9

Table 40.
MEAN MUSCLE LACTATE, GLYCOGEN, CREATINE PHOSPHATE AND ATP DATA PRE AND POST RESPIRATORY ACIDOSIS PERFUSIONS (mean ± SE; PP, pre-perfusion; S5, post 5 min stimulation, n = =4; S20, post 20 min stimulation, n = 7).

		Lactate	Glycogen	CP	ATP
		$\mu mol.g^{-1}$ dry weight			
SOLEUS	PP	4.8 ± 2.5	149.1 ± 17.4	50.9 ± 4.0	22.7 ± 0.3
	S5	2.5 ± 0.8	101.2 ± 6.2	41.7 ± 1.5	19.1 ± 1.3
	PP	5.7 ± 0.5	151.4 ± 13.5	68.7 ± 3.9	23.2 ± 0.7
	S20	9.0 ± 2.1	137.7 ± 17.5	60.3 ± 5.0	23.1 ± 1.6
PLANT.	PP	7.5 ± 1.5	131.2 ± 5.8	73.5 ± 2.3	29.6 ± 0.7
	S5	23.7 ± 9.4	90.4 ± 1.6	47.3 ± 5.2	38.8 ± 1.2
	PP	7.0 ± 0.5	181.0 ± 4.4	98.0 ± 5.3	32.0 ± 1.5
	S20	19.0 ± 4.4	61.7 ± 6.2	60.5 ± 8.9	25.5 ± 2.2
RED GAST.	PP	7.3 ± 1.4	164.0 ± 10.5	70.3 ± 8.8	30.1 ± 1.2
	S5	37.2 ± 3.9	84.4 ± 16.0	43.4 ± 3.9	19.4 ± 0.8
	PP	12.9 ± 3.8	192.8 ± 20.7	88.2 ± 7.1	34.0 ± 2.0
	S20	17.1 ± 3.3	74.3 ± 11.8	62.2 ± 6.1	25.3 ± 1.9
WHITE GAST.	PP	13.8 ± 1.2	143.9 ± 4.0	78.7 ± 1.9	32.2 ± 0.9
	S5	62.7 ± 7.1	23.7 ± 7.3	21.5 ± 9.6	10.9 ± 2.2
	PP	13.1 ± 2.0	187.6 ± 18.3	106.0 ± 6.2	35.0 ± 1.4
	S20	46.7 ± 4.9	42.2 ± 4.9	52.7 ± 8.0	17.4 ± 2.6

Table 41.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 5 MIN STIMULATION DURING METABOLIC ACIDOSIS (mean ± SE, n = 4; PP, pre-perfusion; S5, post 5 min stimulation; TA, total adenine; ECP, energy charge potential = ATP + 1/2 ADP/ ATP + ADP + AMP).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		$umol.g^{-1}$ dry weight					
SOLEUS	PP	5.4 ± 0.2	0.94 ± 0.06	31.4 ± 2.2	4.8 ± 0.3	3.4 ± 0.2	0.89 ± 0.01
	S5	6.4 ± 1.0	0.92 ± 0.11	35.0 ± 3.3	4.6 ± 0.5	3.0 ± 0.1	0.88 ± 0.01
PLANTARIS	PP	4.7 ± 0.2	0.54 ± 0.04	41.0 ± 1.0	7.6 ± 0.3	3.1 ± 0.1	0.93 ± 0.00
	S5	4.8 ± 0.2	0.57 ± 0.03	35.1 ± 2.5	5.9 ± 0.3	2.4 ± 0.3	0.91 ± 0.00
RED GAST.	PP	5.0 ± 0.3	0.60 ± 0.05	44.1 ± 1.5	7.9 ± 0.6	3.0 ± 0.2	0.93 ± 0.00
	S5	4.3 ± 0.5	0.59 ± 0.08	33.1 ± 3.9	6.9 ± 0.8	2.9 ± 0.1	0.92 ± 0.01
WHITE GAST.	PP	4.9 ± 0.8	0.58 ± 0.10	43.7 ± 3.4	8.0 ± 0.6	3.1 ± 0.2	0.93 ± 0.01
	S5	4.6 ± 1.0	0.84 ± 0.06	23.3 ± 0.9	4.1 ± 0.7	2.6 ± 0.3	0.86 ± 0.01

Table 42.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 5 MIN STIMULATION DURING RESPIRATORY ACIDOSIS (mean ± SE, n = 4; Abbreviations as in Table 41).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		umol.g^{-1} dry weight					
SOLEUS	PP	5.0 ± 0.3	0.60 ± 0.07	28.3 ± 0.4	4.6 ± 0.2	2.2 ± 0.2	0.89 ± 0.00
	S5	5.1 ± 0.2	0.60 ± 0.06	24.8 ± 1.3	3.8 ± 0.3	2.2 ± 0.1	0.87 ± 0.01
PLANTARIS	PP	4.6 ± 0.3	0.55 ± 0.05	34.8 ± 0.7	6.7 ± 0.5	2.5 ± 0.1	0.92 ± 0.00
	S5	4.9 ± 0.3	0.57 ± 0.05	29.3 ± 1.5	4.9 ± 0.2	2.0 ± 0.2	0.90 ± 0.00
RED GAST.	PP	4.8 ± 0.2	0.56 ± 0.06	35.5 ± 1.2	6.3 ± 0.3	2.3 ± 0.3	0.92 ± 0.00
	S5	4.9 ± 0.3	0.63 ± 0.04	24.9 ± 0.8	4.0 ± 0.3	2.3 ± 0.3	0.87 ± 0.01
WHITE GAST.	PP	4.8 ± 0.6	0.52 ± 0.03	37.4 ± 0.6	7.2 ± 1.0	2.5 ± 0.1	0.92 ± 0.01
	S5	5.0 ± 0.4	0.82 ± 0.03	16.7 ± 1.8	2.4 ± 0.7	1.8 ± 0.7	0.79 ± 0.03

Table 43.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 20 MIN STIMULATION DURING METABOLIC ACIDOSIS (mean ± SE, n = 6; S20, post 20 min stimulation; Additional abbreviations as in Table 41).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		$umol.g^{-1}$ dry weight					
SOLEUS	PP	3.9 ± 0.4	0.47 ± 0.09	22.9 ± 0.7	5.3 ± 0.3	3.7 ± 0.4	0.92 ± 0.01
	S20	3.9 ± 0.3	0.37 ± 0.04	26.0 ± 1.6	6.1 ± 0.3	3.2 ± 0.3	0.92 ± 0.00
PLANTARIS	PP	4.1 ± 0.2	0.34 ± 0.02	33.3 ± 1.4	7.3 ± 0.6	3.6 ± 0.5	0.93 ± 0.01
	S20	4.0 ± 0.3	0.52 ± 0.11	29.3 ± 1.3	6.9 ± 0.5	2.6 ± 0.3	0.92 ± 0.01
RED GAST.	PP	3.9 ± 0.1	0.38 ± 0.03	35.6 ± 1.6	8.1 ± 0.5	3.2 ± 0.1	0.92 ± 0.01
	S20	4.1 ± 0.3	0.47 ± 0.08	29.0 ± 1.5	6.6 ± 0.5	3.0 ± 0.4	0.92 ± 0.01
WHITE GAST.	PP	3.6 ± 0.2	0.31 ± 0.03	35.5 ± 1.4	9.2 ± 0.3	3.3 ± 0.2	0.94 ± 0.00
	S20	4.2 ± 0.3	0.51 ± 0.09	24.4 ± 1.1	5.1 ± 0.3	3.2 ± 0.7	0.90 ± 0.01

Table 44.
ADDITIONAL MEAN MUSCLE METABOLITE DATA AND CALCULATIONS PRE AND POST 20 MIN STIMULATION DURING RESPIRATORY ACIDOSIS (mean ± SE, n = 7; Abbreviations as in Tables 41 and 43).

		ADP	AMP	TA	ATP/ADP	CP/ATP	ECP
		$umol.g^{-1}$ dry weight					
SOLEUS	PP	4.9 ± 0.3	0.37 ± 0.04	28.5 ± 0.9	4.8 ± 0.2	2.9 ± 0.1	0.90 ± 0.00
	S20	4.3 ± 0.3	0.33 ± 0.02	27.3 ± 2.0	5.3 ± 0.3	2.6 ± 0.2	0.91 ± 0.01
PLANTARIS	PP	4.4 ± 0.2	0.25 ± 0.03	36.7 ± 1.4	7.3 ± 0.5	3.0 ± 0.1	0.93 ± 0.01
	S20	4.1 ± 0.2	0.33 ± 0.02	30.0 ± 2.3	6.2 ± 0.5	2.3 ± 0.3	0.92 ± 0.01
RED GAST.	PP	4.6 ± 0.2	0.36 ± 0.03	38.9 ± 2.0	7.5 ± 0.4	2.6 ± 0.2	0.93 ± 0.01
	S20	4.5 ± 0.6	0.43 ± 0.13	30.6 ± 2.4	5.9 ± 0.6	2.5 ± 0.3	0.90 ± 0.01
WHITE GAST.	PP	4.4 ± 0.2	0.31 ± 0.02	39.8 ± 2.0	8.1 ± 0.4	3.0 ± 0.1	0.94 ± 0.00
	S20	5.3 ± 1.1	0.83 ± 0.26	22.2 ± 2.7	4.2 ± 0.6	3.3 ± 0.7	0.87 ± 0.02

APPENDIX E

Acidosis Study Individual Data

All individual data pertaining to the acidosis study in Chapter 4 are presented in tabular form.

Table 45.
CHARACTERISTICS OF THE PERFUSION MEDIUMS. INDIVIDUAL DATA FOR METABOLIC ACIDOSIS AND RESPIRATORY ACIDOSIS PERFUSIONS (Equivalent data for Control perfusions found in Table 17, rats 9-23).

Rat #	PaO_2, mmHg	$PaCO_2$, mmHg	pH	HCO_3^-, mmol.l^{-1}	P_{50}, mmHg	Hb, g.dl^{-1}	Hct, %	protein, g.dl^{-1}
METABOLIC ACIDOSIS								
1	439	41.9	7.122	13.3	-	13.8	42.0	3.2
2	442	37.2	7.149	11.2	-	14.1	42.0	3.6
3	412	36.4	7.155	12.5	-	13.3	40.0	3.6
4	409	38.0	7.150	12.8	-	13.0	40.0	-
5	360	34.4	7.118	11.2	23.5	14.1	39.8	4.4
6	388	36.3	7.209	14.2	25.5	14.1	39.3	4.1
7	337	35.8	7.178	12.8	25.0	14.1	41.0	3.9
8	367	43.5	7.143	14.5	26.5	15.4	43.0	4.0
9	384	43.7	7.131	14.2	28.5	14.4	40.0	4.0
10	346	38.3	7.129	12.2	27.5	14.3	44.0	4.0
$\bar{X}$	388	38.2	7.148	12.9	26.1	14.1	41.1	3.9
$\pm$SE	11	1.2	0.009	0.4	0.7	0.2	0.5	0.1
RESPIRATORY ACIDOSIS								
1	353	73.8	7.207	28.5	-	14.1	45.0	4.0
2	374	72.9	7.155	25.0	-	13.9	44.0	4.0
3	463	58.3	7.117	18.2	-	11.9	37.0	4.0
4	423	63.2	7.083	18.2	-	12.9	38.0	4.0
5	358	58.0	7.157	20.0	27.3	12.9	38.0	3.7
6	345	59.1	7.158	20.5	27.5	13.2	40.0	4.1
7	359	64.5	7.144	21.5	21.2	13.4	42.0	4.3
8	381	64.0	7.103	19.3	24.0	13.9	28.5	3.1
9	345	56.7	7.171	20.5	23.0	13.6	39.0	3.6
10	380	58.8	7.178	21.3	23.4	12.7	39.5	4.3
11	342	62.7	7.153	21.3	21.5	14.8	42.9	5.1
12	361	64.1	7.154	21.7	22.0	13.5	40.0	4.5
$\bar{X}$	374	63.0	7.152	21.3	23.9	13.5	40.3	4.1
$\pm$SE	10	1.6	0.009	0.8	0.9	0.3	0.7	0.1

Table 46.
ADDITIONAL PERFUSATE AND PERFUSION VARIABLES; INDIVIDUAL DATA FOR METABOLIC AND RESPIRATORY ACIDOSIS PERFUSIONS.

Rat #	Rat wt (g)	Perfusate [Lactate], Zero Time (mM)	Perfusate Lactate Prod. Rate ($mM.hr^{-1}$)	Flow Rate Rest ($ml.min^{-1}$)	Flow Rate Stimulation ($ml.min^{-1}$)
METABOLIC ACIDOSIS					
1	293	0.62	0.06	5.7	18.0
2	316	0.65	0.15	5.7	18.4
3	290	0.55	0.35	6.3	17.7
4	282	0.53	0.22	6.0	23.4
5	258	1.47	0.42	5.5	19.5
6	258	1.54	0.65	5.8	19.5
7	286	1.46	0.31	5.6	20.5
8	288	1.40	0.44	6.5	18.5
9	295	1.96	0.23	6.2	18.5
10	294	1.06	0.50	6.2	20.0
$\bar{X}$	286	1.12	0.33	6.0	19.4
$\pm$SE	6	0.16	0.06	0.1	0.5
RESPIRATORY ACIDOSIS					
1	256	1.11	0.48	5.6	17.0
2	265	1.12	0.40	6.0	18.0
3	270	0.76	0.20	5.8	18.0
4	276	0.83	0.26	6.4	18.2
5	295	0.79	0.33	6.1	16.5
6	303	1.12	0.48	6.2	18.0
7	315	1.03	0.32	6.0	20.0
8	297	1.05	0.46	6.2	19.0
9	292	1.01	0.36	6.1	18.0
10	340	0.97	0.43	6.2	17.5
11	282	0.91	0.42	6.3	17.5
$\bar{X}$	290	0.97	0.38	6.1	18.0
$\pm$SE	7	0.04	0.03	0.1	0.3

Table 47.
INDIVIDUAL PERFUSATE ELECTROLYTE DATA DURING METABOLIC ACIDOSIS PERFUSIONS (A, arterial; V, venous).

	Rat	Na^+		K^+		Cl^-		Ca^{2+}	
	#				mmol.l^{-1}				
20 MIN ARTERIAL REST									
	1	140		6.3		124		2.4	
	2	142		6.6		128		2.4	
	3	143		6.1		126		2.8	
	4	141		6.5		125		2.2	
	5	136		6.7		124		2.6	
	6	140		6.8		122		2.5	
	7	143		6.8		126		2.6	
	8	-		-		-		-	
	9	-		-		-		-	
	10	140		6.6		123		3.1	
	$\bar{X}$	141		6.6		125		2.6	
	$\pm$SE	1		0.1		1		0.1	
40 MIN STIMULATION		A	V	A	V	A	V	A	V
	5	138	139	7.1	7.2	124	123	2.6	2.6
	6	143	142	7.1	7.3	124	123	2.5	2.5
	7	145	143	6.8	7.1	125	126	2.5	2.6
	8	141	145	6.8	7.3	122	120	2.6	2.6
	9	139	140	7.7	7.9	121	119	2.8	2.7
	10	141	143	7.0	7.4	122	123	3.2	3.1
	$\bar{X}$	141	142	7.1	7.4	123	122	2.7	2.7
	$\pm$SE	1	1	0.1	0.1	1	1	0.1	0.1

Table 48.
INDIVIDUAL PERFUSATE ELECTROLYTE DATA DURING RESPIRATORY ACIDOSIS PERFUSIONS (A, arterial; V, venous).

	Rat #	Na^+	K^+	Cl^-	Ca^{2+}
		$mmol.l^{-1}$			
20 MIN ARTERIAL REST					
	1	145	6.4	123	2.4
	2	145	6.4	123	2.4
	3	144	6.3	123	3.0
	4	144	6.3	123	3.0
	5	140	6.2	115	2.5
	6	143	6.5	118	2.3
	7	138	6.2	114	2.9
	8	143	7.2	122	2.5
	9	141	7.0	121	2.4
	10	131	6.4	113	2.5
	11	135	6.6	113	2.4
	12	134	6.0	112	2.6
	$\bar{X}$	140	6.5	118	2.6
	$\pm$SE	1	0.1	1	0.1

		A	V	A	V	A	V	A	V
40 MIN STIMULATION									
	5	136	133	6.3	6.4	112	109	2.6	2.6
	6	143	-	6.7	-	119	-	2.6	-
	7	149	142	7.0	7.0	122	116	2.8	2.9
	8	150	142	7.8	7.5	127	123	2.4	2.8
	9	144	140	7.5	7.2	124	120	2.3	2.6
	10	137	144	7.0	7.5	118	121	2.5	2.5
	11	126	141	6.1	7.1	105	116	3.0	2.8
	12	137	-	6.4	-	115	-	2.6	2.5
	$\bar{X}$	140	140	6.9	7.1	118	118	2.6	2.7
	$\pm$SE	3	2	0.2	0.2	3	2	0.1	0.1

Table 49.
INDIVIDUAL ISOMETRIC TENSION DATA DURING STIMULATION UNDER ACIDOSIS.

Rat #	Isometric Tension (g)								
	peak	31	32	33	34	35	40	45	50
	perfusion time (min)								
METABOLIC ACIDOSIS									
1	2450	2375	2040	1760	1620	1560	-	-	-
2	2300	2200	1875	1650	1550	1450	-	-	-
3	2500	2275	1975	1710	1570	1460	-	-	-
4	2580	2280	1930	1660	1540	1400	-	-	-
5	2150	1980	1440	1260	1160	1150	1110	1070	1120
6	2250	2080	1860	1580	1480	1400	1210	1190	1170
7	2370	2370	2200	1980	1810	1690	1400	1260	1160
8	2650	2350	2100	1860	1740	1680	1540	1400	1240
9	2850	2830	2150	1900	1820	1790	1640	1490	1440
10	2870	2600	2125	1975	1820	1760	1470	1340	1260
$\bar{X}$	2497	2334	1970	1734	1611	1534	1395	1292	1215
$\pm$SE	77	77	70	69	65	63	82	62	54
RESPIRATORY ACIDOSIS									
1	2550	-	-	-	-	-	-	-	-
2	3080	2652	2142	1703	1596	1518	-	-	-
3	2550	2346	1816	1485	1320	1237	-	-	-
4	2754	2550	2193	1703	1485	1394	-	-	-
5	-	-	-	-	-	-	-	-	-
6	2900	2625	2225	1975	1840	1750	1600	1430	1390
7	2650	2425	1600	1120	1110	1140	1070	1050	1050
8	2600	2350	1900	1320	1360	1400	1260	1260	1320
9	2670	2350	1400	-	-	1190	1540	1400	1380
10	2950	2500	1900	1620	1520	1690	1480	1350	1200
11	2670	2400	1920	1720	1620	1590	1410	1310	1270
$\bar{X}$	2737	2466	1900	1581	1481	1434	1393	1300	1268
$\pm$SE	57	40	91	94	78	73	80	56	52

Table 50.
INDIVDUAL OXYGEN UPTAKE DATA DURING ACIDOSIS.

Rat	O_2 Uptake ($\mu mol.min^{-1}.hindquarter^{-1}$)							
	Rest				Stimulation			
	12	18	25	33	36	39	44	48
	perfusion time (min)							
METABOLIC ACIDOSIS								
1	6.8	6.9	7.0	25.5	-	-	-	-
2	9.3	9.0	9.6	28.6	-	-	-	-
3	4.7	5.7	6.2	21.5	-	-	-	-
4	7.6	8.1	8.5	26.5	-	-	-	-
5	8.1	7.6	7.9	16.2	12.9	17.2	12.2	13.0
6	5.6	5.3	5.1	16.1	13.9	17.0	16.6	15.7
7	6.0	5.9	6.3	16.3	15.0	20.8	19.5	14.6
8	9.5	8.2	8.1	18.5	19.2	18.3	19.1	16.7
9	6.9	7.4	8.2	22.1	20.0	19.8	23.7	21.8
10	5.6	5.0	7.0	17.1	20.8	21.9	19.5	19.4
$\bar{X}$	7.0	6.9	7.4	20.8	17.0	19.2	18.4	16.9
$\pm$SE	0.5	0.4	0.4	1.5	1.4	0.8	1.6	1.3
RESPIRATORY ACIDOSIS								
1	-	8.0	7.4	18.7	-	-	-	-
2	-	10.4	9.8	26.9	-	-	-	-
3	-	7.2	6.9	17.9	-	-	-	-
4	-	5.5	5.3	19.5	-	-	-	-
5	6.0	6.5	8.3	-	20.6	20.6	18.8	19.0
6	5.2	4.9	6.3	15.3	15.9	16.7	14.8	13.5
7	5.7	5.3	6.1	10.2	10.5	11.7	14.0	13.0
8	5.5	4.9	5.1	8.4	11.3	14.2	14.6	16.9
9	5.0	4.5	4.7	10.9	12.6	14.0	16.6	18.4
10	9.4	8.8	8.3	24.0	29.4	26.0	24.0	-
11	5.5	6.1	6.3	18.8	19.7	19.4	19.2	15.8
$\bar{X}$	6.0	6.4	6.7	16.9	16.4	16.3	17.2	15.7
$\pm$SE	0.5	0.5	0.4	1.6	2.0	1.6	1.2	1.0

Table 51
INDIVIDUAL ARTERIAL PRESSURE DATA DURING ACIDOSIS.

Rat #	Arterial Pressure (mmHg)									
	Rest					Stimulation				
	10	15	20	25	30	30	35	40	45	50
	perfusion time (min)									
METABOLIC ACIDOSIS										
1	96	100	94	94	94	176	172	-	-	-
2	108	100	98	98	98	180	180	-	-	-
3	88	86	88	86	84	166	164	-	-	-
4	74	74	74	72	72	180	174	-	-	-
5	75	70	68	68	70	180	184	188	195	196
6	70	74	76	72	66	180	180	182	186	194
7	68	70	70	66	63	184	188	190	196	202
8	92	75	75	75	80	165	170	178	184	190
9	80	75	75	78	75	178	179	179	180	180
10	88	85	85	85	85	180	177	179	180	185
$\bar{X}$	84	81	80	79	79	177	177	183	187	191
$\pm$SE	4	4	3	4	4	2	2	2	3	3
RESPIRATORY ACIDOSIS										
1	75	75	75	75	75	169	176	-	-	-
2	86	86	83	83	83	162	162	-	-	-
3	90	72	72	72	72	163	162	-	-	-
4	81	83	86	86	86	180	176	-	-	-
5	90	95	90	88	88	190	184	202	215	228
6	88	94	90	92	92	180	177	182	192	200
7	74	76	74	72	70	150	142	142	145	145
8	75	85	80	77	77	190	178	165	160	160
9	75	75	72	70	70	150	150	147	147	150
10	70	70	75	73	73	150	156	158	160	170
11	75	70	75	73	70	147	150	155	156	159
$\bar{X}$	80	81	80	80	79	167	165	164	170	173
$\pm$SE	2	3	2	3	2	5	4	8	9	11

Table 52.
INDIVIDUAL RESTING LACTATE RELEASE DATA DURING ACIDOSIS.

Rat #	Lactate Release ($\mu mol.min^{-1}.hindquarter^{-1}$)				
	21	23	25	27	29
	perfusion time (min)				
METABOLIC ACIDOSIS					
1	0.91	0.91	0.71	0.71	0.17
2	1.51	1.37	1.85	1.60	1.23
3	0.57	0.55	0.66	0.20	0.19
4	2.49	2.34	1.92	1.89	1.62
5	-1.46	-2.07	-1.89	-1.77	-2.32
6	-0.54	0.00	-0.16	0.16	-0.32
7	-0.12	-0.52	0.40	0.35	0.12
8	-0.37	-1.74	-1.55	-0.50	-0.43
9	-0.41	-0.06	0.17	-0.58	0.23
10	0.00	-0.06	-0.12	0.37	0.17
$\bar{X}$	0.26	0.07	0.20	0.24	0.07
$\pm$SE	0.36	0.42	0.39	0.34	0.33
RESPIRATORY ACIDOSIS					
1	2.35	2.05	2.07	1.99	1.62
2	4.47	3.20	2.70	2.63	2.20
3	1.76	1.62	1.33	1.16	1.41
4	1.44	1.52	1.47	1.28	1.49
5	1.08	0.20	0.20	-0.41	0.00
6	-0.19	-0.63	-0.89	-0.32	-1.08
7	0.62	-0.37	0.62	0.56	0.62
8	1.00	1.00	1.00	-0.24	0.49
9	0.31	-0.31	-0.38	-	-0.75
10	1.29	1.47	0.68	1.23	-
11	-1.44	-0.94	-0.31	-	-0.31
$\bar{X}$	1.15	0.86	0.77	0.88	0.57
$\pm$SE	0.45	0.38	0.33	0.36	0.35

Table 53.
INDIVIDUAL LACTATE RELEASE DATA DURING STIMULATION UNDER ACIDOSIS.

Rat #	Lactate Release ($umol.min^{-1}.hindquarter^{-1}$)									
	31	33	35	37	39	41	43	45	47	49
	perfusion time (min)									
METABOLIC ACIDOSIS										
1	7.4	15.1	13.3	-	-	-	-	-	-	-
2	8.7	14.6	13.4	-	-	-	-	-	-	-
3	3.4	13.6	11.2	-	-	-	-	-	-	-
4	16.4	20.9	19.7	-	-	-	-	-	-	-
5	4.9	14.6	12.5	13.1	12.3	7.3	6.0	2.4	5.1	3.5
6	3.9	12.4	14.7	14.4	12.0	7.2	4.9	5.2	4.9	3.9
7	-	15.3	17.5	18.1	10.8	8.0	7.8	3.4	4.2	4.1
8	1.5	14.1	13.7	13.7	9.6	9.4	4.9	4.9	2.4	3.8
9	6.7	11.7	11.7	7.9	12.3	13.2	5.9	4.4	5.2	5.6
10	1.1	9.9	12.3	9.1	4.3	3.3	3.0	5.5	0.7	2.3
$\bar{X}$	6.0	14.2	14.0	12.7	11.2	8.9	5.8	4.3	3.8	3.9
$\pm$SE	1.6	0.9	0.9	1.5	1.8	1.5	0.7	0.5	0.8	0.4
RESPIRATORY ACIDOSIS										
1	10.6	11.2	15.1	-	-	-	-	-	-	-
2	11.6	19.1	18.0	-	-	-	-	-	-	-
3	9.3	13.2	14.3	-	-	-	-	-	-	-
4	10.6	16.3	14.7	-	-	-	-	-	-	-
5	3.4	13.6	13.8	11.8	12.6	10.6	9.2	8.2	10.0	7.2
6	5.3	17.5	16.3	14.2	11.2	8.8	6.0	5.4	5.3	3.0
7	6.0	19.8	21.7	17.0	15.2	12.6	10.9	8.9	-	5.6
8	2.2	13.1	20.2	17.1	13.1	13.0	10.4	8.1	7.7	7.2
9	7.2	20.1	22.6	10.8	11.2	11.8	8.0	8.6	10.8	6.8
10	9.9	22.8	22.1	12.5	18.7	13.9	10.2	7.6	-	3.6
11	2.2	11.7	19.6	18.2	18.0	16.2	11.5	8.8	6.1	-
$\bar{X}$	7.1	16.2	18.0	14.5	14.3	12.4	9.5	7.9	8.0	5.6
$\pm$SE	1.1	1.2	1.0	1.1	1.2	0.9	0.7	0.5	1.1	0.8

Table 54.
INDIVIDUAL RESTING GLUCOSE UPTAKE DATA DURING ACIDOSIS.

Rat #	Glucose Uptake ($umol.min^{-1}.hindquarter^{-1}$)				
	21	23	25	27	29
	perfusion time (min)				
METABOLIC ACIDOSIS					
1	0.29	0.29	0.00	-0.29	-0.29
2	0.80	1.03	1.03	1.71	0.68
3	-0.50	-0.25	0.32	0.38	0.00
4	0.84	1.08	0.24	1.80	0.72
5	1.82	1.93	1.38	0.83	1.82
6	0.70	-1.10	0.64	0.46	0.35
7	-0.11	0.90	1.29	2.07	0.67
8	-	2.86	-0.07	3.77	1.76
9	0.25	0.19	0.06	-0.06	3.10
10	-	-	2.36	-1.30	-
$\bar{X}$	0.51	0.77	0.73	0.94	0.98
$\pm$SE	0.25	0.39	0.25	0.46	0.35
RESPIRATORY ACIDOSIS					
1	0.28	0.39	0.22	0.28	0.50
2	-1.02	-1.02	-0.18	0.00	0.18
3	-0.17	0.12	0.00	-0.29	0.64
4	-0.19	0.32	0.13	0.77	0.32
5	0.18	0.16	0.24	0.79	-0.31
6	3.41	1.18	1.19	1.36	2.54
7	-0.54	-0.61	1.22	1.02	-0.48
8	2.67	1.49	3.04	1.43	2.91
9	0.49	0.67	1.16	0.61	0.98
10	3.91	0.74	2.54	2.42	1.67
11	0.76	2.71	-	-0.82	2.27
$\bar{X}$	0.89	0.56	0.96	0.69	1.02
$\pm$SE	0.50	0.30	0.35	0.27	0.35

Table 55.
INDIVIDUAL GLUCOSE UPTAKE DATA DURING STIMULATION UNDER ACIDOSIS.

Rat #	Glucose Uptake (umol.min^{-1}.hindquarter^{-1})				
	32	36	40	44	48
	perfusion time (min)				
METABOLIC ACIDOSIS					
1	1.37	2.24	-	-	-
2	6.05	4.12	-	-	-
3	4.32	0.83	-	-	-
4	3.88	4.59	-	-	-
5	1.85	0.74	1.21	1.84	3.98
6	0.90	4.07	5.69	6.29	8.07
7	0.36	-3.30	-0.18	-0.78	3.02
8	6.60	5.89	6.06	5.22	8.97
9	4.67	7.49	3.61	3.60	8.40
10	7.72	2.11	2.28	5.07	4.92
$\bar{X}$	3.77	2.88	3.11	3.54	6.23
$\pm$SE	0.81	0.97	1.11	1.17	1.14
RESPIRATORY ACIDOSIS					
1	4.97	5.22	-	-	-
2	2.84	0.23	-	-	-
3	8.58	9.08	-	-	-
4	8.31	6.83	-	-	-
5	3.15	2.98	2.70	4.20	4.80
6	0.40	1.81	3.15	5.33	5.52
7	1.53	2.52	3.40	4.90	6.78
8	4.68	5.95	5.58	6.60	7.00
9	-2.19	-2.01	-0.36	-0.04	3.70
10	4.42	4.52	4.79	5.01	9.74
11	8.08	6.93	6.62	7.64	7.98
$\bar{X}$	4.07	4.01	3.70	4.81	6.50
$\pm$SE	1.08	0.98	0.86	0.92	0.77

Table 56
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CP CONCENTRATIONS PRE AND POST 30 MIN OF REST PERFUSION DURING METABOLIC ACIDOSIS (PP, pre-perfusion; R, post 30 min rest).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	R	PP	R	PP	R	PP	R
GLYCOGEN								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	92.4	97.9	114.4	138.1	151.2	140.7	166.7	146.4
4	75.3	78.6	118.6	134.1	142.9	124.4	128.9	119.3
5	119.0	115.3	146.1	133.4	162.3	113.4	145.2	117.8
$\bar{X}$	95.6	97.3	126.4	135.2	152.1	126.2	130.3	127.9
±SE	12.7	10.6	10.0	1.5	5.6	8.0	8.3	9.3
LACTATE								
1	12.7	12.1	9.4	8.1	8.4	10.6	-	9.3
2	8.9	9.9	11.5	11.2	14.1	13.2	15.1	16.7
3	3.4	3.3	3.7	3.0	19.6	3.5	5.3	4.0
4	3.4	7.7	6.5	7.3	4.5	6.4	6.9	17.3
5	-	7.9	7.5	-	5.9	19.5	6.0	26.0
$\bar{X}$	7.1	7.8	7.7	7.4	10.5	10.6	8.3	14.7
±SE	2.3	1.4	1.3	1.7	2.8	2.8	2.3	3.8
CP								
1	44.6	83.3	80.5	128.0	83.1	112.7	97.7	118.2
2	87.8	61.4	75.0	96.4	90.9	92.7	81.5	90.3
3	63.4	65.4	84.4	123.5	93.7	85.9	78.4	97.2
4	58.3	90.1	96.0	73.4	140.0	83.9	89.0	79.3
5	87.4	88.8	183.7	107.4	87.1	92.0	74.4	71.5
$\bar{X}$	68.3	77.8	103.9	105.7	99.0	93.4	84.2	91.3
±SE	8.4	6.0	20.3	9.9	10.4	5.1	4.1	8.0

Table 57
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 30 MIN REST PERFUSION DURING METABOLIC ACIDOSIS (PP, pre-perfusion; R, post 30 min rest).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	R	PP	R	PP	R	PP	R
ATP								
1	18.4	27.2	29.6	35.0	29.6	35.7	28.5	39.1
2	22.8	20.0	29.2	33.7	29.7	33.0	30.6	34.6
3	21.3	19.3	27.7	31.0	32.6	29.1	29.4	33.6
4	17.2	22.5	28.5	23.9	25.1	26.6	25.8	22.5
5	17.8	18.4	25.2	27.1	28.1	20.7	33.2	27.7
$\bar{X}$	19.5	21.5	28.0	30.1	29.0	29.0	29.5	31.5
±SE	1.1	1.6	0.8	2.1	1.2	2.6	1.2	2.9
ADP								
1	4.0	4.9	4.0	4.3	3.6	5.3	3.4	5.2
2	3.9	4.1	3.9	4.2	2.7	3.8	4.1	3.8
3	3.9	3.3	3.8	3.6	4.4	4.3	3.9	3.9
4	3.1	3.8	3.7	3.4	3.4	3.5	2.9	3.7
5	6.5	3.2	1.9	3.8	4.2	4.3	3.7	3.5
$\bar{X}$	4.3	3.8	3.4	3.9	3.6	4.3	3.6	4.0
±SE	0.6	0.3	0.4	0.2	0.3	0.3	0.2	0.3
AMP								
1	0.80	0.67	0.39	0.33	0.29	0.22	0.34	0.36
2	0.35	0.53	0.27	0.41	0.40	0.39	0.30	0.47
3	0.61	0.43	0.37	0.43	0.31	0.39	0.53	0.51
4	0.26	0.66	0.46	0.34	0.43	0.42	0.32	0.35
5	0.26	0.34	0.33	0.30	0.35	0.34	0.30	0.24
$\bar{X}$	0.46	0.52	0.36	0.36	0.35	0.35	0.36	0.39
±SE	0.11	0.06	0.03	0.03	0.03	0.04	0.04	0.05

Table 58
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CP CONCENTRATIONS PRE AND POST 30 MIN OF REST PERFUSION DURING RESPIRATORY ACIDOSIS (PP, pre-perfusion; R, post 30 min rest).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	R	PP	R	PP	R	PP	R
GLYCOGEN								
1	96.5	92.4	150.6	139.2	137.8	125.3	175.6	162.3
2	105.4	98.1	122.7	131.4	175.6	169.4	190.7	185.0
3	84.6	90.5	173.6	159.7	154.3	162.5	145.6	147.3
4	93.4	101.6	125.8	134.5	162.5	149.8	155.8	152.0
5	101.9	107.3	155.4	153.9	143.9	145.7	161.1	164.8
$\bar{X}$	96.4	98.0	145.6	143.7	154.8	150.5	165.8	162.3
±SE	3.6	3.1	9.5	5.5	7.0	7.6	7.9	6.5
LACTATE								
1	4.3	4.9	4.0	7.3	12.4	11.7	11.6	15.4
2	9.7	8.3	7.8	8.2	9.8	6.3	9.3	5.2
3	6.0	7.1	10.4	5.6	11.6	9.0	6.0	10.3
4	7.2	6.4	7.9	8.3	7.4	12.4	10.8	8.4
5	5.9	10.0	5.6	6.4	15.4	9.1	8.9	11.3
$\bar{X}$	6.6	7.3	7.1	7.2	11.3	9.7	9.3	10.1
±SE	0.9	0.9	1.1	0.5	1.3	1.1	1.0	1.7
CP								
1	75.4	84.3	101.5	98.4	111.5	106.7	95.9	103.2
2	63.7	75.2	117.9	118.6	101.6	98.4	93.5	94.1
3	74.9	64.6	94.8	90.4	82.4	96.5	89.2	77.8
4	68.7	60.0	104.6	105.9	79.3	78.6	106.0	105.3
5	76.0	65.6	68.2	75.3	94.7	102.1	109.3	101.9
$\bar{X}$	71.7	69.9	97.4	97.7	93.9	96.5	96.8	96.5
±SE	2.4	4.4	8.2	7.3	6.0	4.8	4.9	5.0

Table 59
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 30 MIN OF REST PERFUSION DURING RESPIRATORY ACIDOSIS (OO, pre-perfusion; R, post 30 min rest).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	R	PP	R	PP	R	PP	R
ATP								
1	24.5	26.4	29.4	27.7	28.7	29.5	34.7	30.4
2	28.9	24.3	35.6	30.8	33.8	31.7	31.3	29.4
3	19.7	17.3	38.0	36.5	35.2	36.0	27.8	27.9
4	23.6	25.9	25.2	29.7	26.0	29.5	29.4	31.6
5	24.0	26.2	27.6	26.3	32.4	31.6	32.4	34.6
$\bar{X}$	24.1	24.0	31.2	30.2	31.2	31.7	31.1	30.8
±SE	1.5	1.7	2.4	1.8	1.7	1.2	1.2	1.1
ADP								
1	4.3	4.1	4.4	4.1	3.1	4.1	2.9	4.0
2	3.7	4.1	3.6	3.2	6.2	5.2	4.7	4.1
3	4.0	3.4	3.2	4.3	4.5	3.7	4.2	3.7
4	3.4	4.6	4.1	4.0	3.7	3.8	3.9	3.6
5	4.7	4.2	3.9	4.2	3.6	3.4	4.1	4.4
$\bar{X}$	4.0	4.1	3.8	4.0	4.2	4.0	4.0	4.0
±SE	0.2	0.2	0.2	0.2	0.5	0.3	0.3	0.1
AMP								
1	0.39	0.52	0.36	0.43	0.47	0.63	0.43	0.56
2	0.45	0.55	0.29	0.41	0.54	0.49	0.24	0.35
3	0.72	0.63	0.34	0.33	0.29	0.35	0.34	0.21
4	0.63	0.47	0.49	0.51	0.36	0.39	0.45	0.52
5	0.49	0.61	0.47	0.44	0.41	0.51	0.47	0.63
$\bar{X}$	0.54	0.56	0.39	0.42	0.41	0.47	0.39	0.45
±SE	0.06	0.03	0.04	0.03	0.04	0.05	0.04	0.08

Table 60
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CP CONCENTRATIONS PRE AND POST 5 MIN STIMULATION DURING METABOLIC ACIDOSIS (PP, pre-perfusion, S5, post 5 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S5	PP	S5	PP	S5	PP	S5
GLYCOGEN								
1	123.3	149.6	160.8	104.1	160.7	62.6	152.7	40.0
2	163.0	148.5	180.8	80.1	166.4	117.4	171.5	51.3
3	90.7	84.7	126.3	54.3	130.9	78.6	164.4	31.0
4	96.3	109.5	141.0	74.2	126.7	84.1	154.3	46.5
$\bar{X}$	118.3	123.1	152.2	78.2	146.2	85.7	160.7	42.2
$\pm$SE	16.5	15.8	11.9	10.2	10.1	11.5	4.5	4.4
LACTATE								
1	14.3	10.0	4.6	26.2	8.1	42.6	5.2	93.2
2	10.7	5.1	7.0	33.0	11.3	29.6	9.8	63.0
3	5.0	10.9	3.0	21.8	5.4	16.6	7.6	112.3
4	5.5	6.0	5.6	22.0	4.8	14.7	3.5	67.8
$\bar{X}$	8.9	7.9	5.0	25.8	7.4	25.9	6.5	84.0
$\pm$SE	2.2	1.4	0.9	2.6	1.5	6.5	1.4	1.5
CP								
1	-	63.7	107.2	93.2	93.9	47.3	116.6	30.3
2	91.6	72.6	105.8	78.1	117.4	79.5	131.2	55.3
3	72.8	88.4	117.1	67.6	109.5	110.7	96.9	65.3
4	91.1	115.9	116.7	67.1	134.2	93.4	116.3	38.6
$\bar{X}$	85.2	85.1	111.6	76.5	113.7	82.7	115.2	47.4
$\pm$SE	6.2	11.5	3.0	6.1	8.4	13.4	7.0	7.9

Table 61
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 5 MIN STIMULATION DURING METABOLIC ACIDOSIS (PP, pre-perfusion; S5, post 5 min stimulation).

Rat	SOLEUS		PLANTARIS		RAD GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S5	PP	S5	PP	S5	PP	S5
ATP								
1	-	23.9	33.4	31.4	39.3	18.4	33.4	15.6
2	29.5	24.1	34.9	24.2	36.9	27.5	45.1	18.6
3	22.5	28.6	36.5	25.2	35.7	35.4	35.0	21.0
4	23.8	34.5	38.3	38.9	42.4	31.5	39.2	16.2
$\bar{X}$	25.3	27.8	35.8	28.7	38.6	28.2	38.2	17.9
±SE	2.1	2.5	1.1	2.4	1.5	3.6	2.6	1.2
ADP								
1	5.9	4.9	4.3	5.3	4.4	3.2	4.0	5.8
2	5.5	6.7	5.2	4.6	5.9	5.7	7.2	3.4
3	5.1	4.9	4.7	4.3	4.8	4.3	4.3	4.2
4	5.1	9.0	4.7	5.0	4.9	3.9	4.2	5.0
$\bar{X}$	5.4	6.4	4.7	4.8	5.0	4.3	4.9	4.6
±SE	0.2	1.0	0.2	0.2	0.3	0.5	0.8	1.0
AMP								
1	1.04	1.06	0.43	0.64	0.60	0.45	0.53	0.92
2	0.84	0.84	0.55	0.61	0.62	0.82	0.87	0.95
3	1.02	0.65	0.55	0.51	0.49	0.54	0.42	0.72
4	0.83	1.12	0.62	0.51	0.71	0.56	0.51	0.78
$\bar{X}$	0.94	0.92	0.54	0.57	0.60	0.59	0.58	0.84
±SE	0.06	0.11	0.04	0.03	0.05	0.08	0.10	0.06

Table 62
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CP CONCENTRATIONS PRE AND POST 5 MIN STIMULATION DURING RESPIRATORY ACIDOSIS (PP, pre-perfusion; S5, post 5 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S5	PP	S5	PP	S5	PP	S5
GLYCOGEN								
1	160.8	104.1	146.5	87.5	175.1	122.1	155.1	45.1
2	150.7	104.0	130.2	93.5	162.5	75.2	136.5	36.0
3	101.2	83.7	118.6	-	135.3	46.0	140.7	43.6
4	183.8	113.1	129.4	90.2	183.2	94.1	143.4	50.2
$\bar{X}$	149.1	101.2	131.2	90.4	164.0	84.4	143.9	43.7
±SE	17.4	6.2	5.8	1.6	10.5	16.0	4.0	7.3
LACTATE								
1	12.2	4.6	8.6	17.4	7.7	36.0	13.7	56.5
2	1.8	2.8	9.9	13.0	7.5	31.2	11.8	62.3
3	2.3	1.8	3.2	51.8	3.7	48.5	12.5	49.6
4	3.0	0.8	8.2	12.7	10.2	33.2	17.1	82.4
$\bar{X}$	4.8	2.5	7.5	23.7	7.3	37.2	13.8	62.7
±SE	2.5	0.8	1.5	9.4	1.4	3.9	1.2	7.1
CP								
1	59.3	37.2	77.8	52.5	47.6	31.9	77.6	43.9
2	53.2	43.4	67.1	42.4	67.9	48.5	78.5	30.0
3	50.9	42.4	75.3	35.3	75.9	47.0	74.8	11.0
4	40.4	43.8	73.7	58.8	89.9	46.2	83.9	1.0
$\bar{X}$	50.9	41.7	73.5	47.3	70.3	43.4	78.7	21.5
±SE	4.0	1.5	2.9	5.2	8.8	3.9	1.9	9.6

Table 63
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 5 MIN STIMULATION DURING RESPIRATORY ACIDOSIS (PP, pre-perfusion; S5, post 5 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S5	PP	S5	PP	S5	PP	S5
ATP								
1	23.2	15.4	29.3	23.0	28.2	19.6	32.3	17.4
2	23.2	19.7	27.7	24.8	28.1	17.1	29.7	8.8
3	22.3	20.6	30.8	20.9	32.9	19.7	32.8	8.8
4	22.3	20.7	30.8	26.6	31.0	21.1	33.9	8.5
$\bar{X}$	22.7	19.1	29.6	23.8	30.1	19.4	32.2	10.9
±SE	0.3	1.3	0.7	1.2	1.8	0.8	0.9	2.2
ADP								
1	4.9	5.1	4.2	4.9	4.3	5.0	3.4	3.9
2	5.5	5.0	5.1	5.7	5.0	4.7	6.3	5.2
3	4.3	4.6	4.9	4.1	4.9	5.6	4.9	5.6
4	5.2	5.6	4.0	4.8	5.1	4.3	4.4	5.2
$\bar{X}$	5.0	5.1	4.6	4.9	4.8	4.9	4.8	5.0
±SE	0.3	0.2	0.3	0.3	0.2	0.3	0.6	0.4
AMP								
1	0.43	0.51	0.46	0.49	0.64	0.72	0.55	0.79
2	0.59	0.49	0.47	0.55	0.69	0.65	0.60	0.89
3	0.75	0.70	0.56	0.52	0.43	0.57	0.45	0.76
4	0.62	0.68	0.69	0.71	0.49	0.58	0.49	0.85
$\bar{X}$	0.60	0.60	0.55	0.57	0.56	0.63	0.52	0.82
±SE	0.07	0.06	0.05	0.05	0.06	0.04	0.03	0.03

Table 64
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CP CONCENTRATIONS PRE AND POST 20 MIN STIMULATION DURING METABOLIC ACIDOSIS (PP, pre-perfusion; S5, post 5 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S20	PP	S20	PP	S20	PP	S20
GLYCOGEN								
1	144.4	95.8	144.4	52.4	129.8	55.8	228.2	46.1
2	136.6	43.9	128.5	73.2	126.1	76.1	131.8	28.1
3	103.7	166.5	97.9	66.2	111.1	70.4	115.9	25.6
4	137.3	148.5	208.8	69.7	267.3	82.1	226.3	34.2
5	129.6	117.1	187.9	75.6	172.6	88.6	187.7	45.2
6	90.2	124.4	150.7	64.6	150.7	74.0	183.8	9.7
$\bar{X}$	123.6	166.1	153.0	67.0	159.6	74.5	179.0	31.5
$\pm$SE	8.8	17.6	16.4	3.4	23.2	4.6	19.1	5.6
LACTATE								
1	7.7	40.8	7.2	46.0	10.4	21.2	11.9	61.6
2	12.9	18.3	10.8	28.9	19.8	20.1	7.3	46.6
3	8.1	10.0	14.8	12.2	11.9	16.5	7.7	31.5
4	8.8	5.7	7.0	18.2	9.7	42.4	12.6	57.5
5	7.5	7.3	13.0	15.3	6.0	16.5	10.4	35.2
6	5.4	8.6	8.3	22.1	6.8	21.1	6.8	34.6
$\bar{X}$	8.4	15.1	10.2	23.8	10.8	23.0	9.4	44.5
$\pm$SE	1.0	5.5	1.3	5.0	2.0	3.4	1.0	5.2
CP								
1	74.7	80.9	131.8	75.9	107.2	57.0	100.9	107.0
2	79.8	96.5	118.4	57.3	98.6	93.4	94.9	50.3
3	87.4	79.9	115.6	76.4	86.4	111.3	124.3	69.2
4	51.9	40.4	102.9	46.4	84.8	33.0	85.6	36.2
5	55.9	61.7	71.8	62.0	116.8	78.1	113.6	34.5
6	67.0	68.5	75.7	66.4	96.4	71.3	100.6	64.9
$\bar{X}$	69.5	71.3	102.7	64.1	98.4	75.7	103.3	60.4
$\pm$SE	5.6	7.9	9.9	4.7	5.0	11.3	5.6	11.0

Table 65
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 20 MIN STIMULATION DURING METABOLIC ACIDOSIS (PP, pre-perfusion; S5, post 5 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S20	PP	S20	PP	S20	PP	S20
ATP								
1	20.9	20.6	23.2	19.7	32.5	19.5	30.0	16.5
2	17.0	27.1	30.5	25.7	28.6	28.7	26.7	21.0
3	17.6	21.9	29.5	27.4	27.0	25.8	35.8	23.0
4	18.5	17.7	34.3	23.4	31.3	24.3	32.3	19.8
5	19.9	24.1	28.2	29.1	34.9	30.0	32.3	21.0
6	19.4	20.9	28.2	25.7	30.2	25.0	33.3	18.7
$\bar{X}$	18.9	22.1	28.9	25.2	30.8	25.6	31.7	20.0
±SE	0.6	1.3	1.5	1.4	1.2	1.5	1.3	0.9
ADP								
1	4.2	3.7	4.7	3.9	3.8	4.2	3.5	3.7
2	3.6	4.9	4.4	3.2	4.0	4.5	3.1	5.2
3	3.6	3.0	4.2	3.6	4.2	3.6	4.1	4.1
4	3.0	2.9	3.6	3.6	4.0	4.2	3.7	4.0
5	3.8	3.7	3.4	3.7	3.5	3.7	3.1	4.0
6	3.2	3.6	4.0	3.9	3.6	3.3	3.4	3.1
$\bar{X}$	3.9	3.9	4.1	4.0	3.9	4.1	3.6	4.2
±SE	0.4	0.3	0.2	0.3	0.1	0.3	0.2	0.3
AMP								
1	0.53	0.38	0.33	0.71	0.28	0.45	0.30	0.50
2	0.54	0.46	0.34	0.74	0.41	0.53	0.25	0.40
3	0.39	0.44	0.36	0.29	0.46	0.41	0.48	0.37
4	0.27	0.27	0.24	0.29	0.37	0.34	0.23	0.30
5	0.38	0.25	0.35	0.31	0.42	-	0.28	0.70
6	0.24	0.29	0.33	0.31	0.25	0.25	0.23	0.30
$\bar{X}$	0.47	0.37	0.34	0.52	0.38	0.49	0.31	0.51
±SE	0.09	0.04	0.02	0.11	0.03	0.08	0.03	0.09

Table 66
INDIVIDUAL MUSCLE GLYCOGEN, LACTATE AND CP CONCENTRATIONS PRE AND POST 20 MIN STIMULATION DURING RESPIRATORY ACIDOSIS (PP, pre-perfusion; S20, post 20 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S20	PP	S20	PP	S20	PP	S20
GLYCOGEN								
1	154.2	110.8	173.6	79.1	186.5	88.6	170.0	123.8
2	160.2	127.2	193.3	72.6	178.9	50.2	140.4	17.4
3	149.1	118.2	164.2	71.2	179.1	76.7	178.9	50.3
4	109.2	91.5	169.7	53.1	149.0	54.6	179.9	32.9
5	108.8	118.7	190.8	46.7	147.5	43.5	160.7	7.9
6	212.5	228.3	185.0	35.7	309.3	135.1	290.6	35.0
7	165.7	169.2	190.7	74.1	199.4	71.7	192.4	28.2
$\bar{X}$	151.4	137.7	181.0	61.8	192.8	74.3	187.6	42.2
$\pm$SE	13.5	17.5	4.4	6.2	20.7	11.8	18.3	14.5
LACTATE								
1	6.9	20.9	9.3	34.7	20.8	26.9	11.7	44.3
2	8.2	6.4	7.8	13.2	5.0	8.5	9.9	33.1
3	5.0	7.3	7.4	11.0	10.5	17.3	20.4	66.8
4	5.9	10.2	7.0	16.4	8.8	20.0	11.0	43.8
5	4.2	5.9	5.9	12.1	6.3	8.9	9.4	37.4
6	4.9	7.0	6.5	36.6	32.3	29.0	20.7	62.5
7	4.8	5.7	5.0	8.9	6.7	9.4	8.4	38.7
$\bar{X}$	5.7	9.0	7.0	19.0	12.9	17.1	13.1	46.7
$\pm$SE	0.5	2.1	0.5	4.4	3.8	3.3	2.0	4.9
CP								
1	60.7	45.6	80.7	40.0	54.5	39.6	121.0	36.7
2	83.5	44.8	114.2	64.5	86.8	50.7	121.9	21.2
3	74.2	58.3	92.6	85.0	82.1	73.6	109.3	68.7
4	69.2	65.3	107.1	63.8	76.9	73.2	95.5	47.9
5	50.7	72.5	79.0	74.8	88.5	68.7	84.4	66.8
6	71.6	78.3	105.8	18.3	113.3	47.3	121.6	-
7	70.9	57.3	106.7	77.0	110.5	82.5	88.4	74.8
$\bar{X}$	68.7	60.3	98.0	60.5	88.2	62.2	106.0	52.7
$\pm$SE	3.9	4.8	5.3	8.9	7.1	6.1	6.2	8.0

Table 67
INDIVIDUAL MUSCLE ATP, ADP AND AMP CONCENTRATIONS PRE AND POST 20 MIN STIMULATION DURING RESPIRATORY ACIDOSIS (PP, pre-perfusion; S20, post 20 min stimulation).

Rat	SOLEUS		PLANTARIS		RED GAST.		WHITE GAST.	
	umol.g^{-1} dry weight							
#	PP	S20	PP	S20	PP	S20	PP	S20
ATP								
1	21.7	20.8	30.9	26.9	41.9	27.4	38.4	28.2
2	25.6	19.8	36.0	29.2	34.0	14.8	38.2	13.1
3	23.6	18.5	30.9	26.2	30.1	24.4	32.8	10.1
4	22.4	21.0	28.1	27.5	27.9	28.9	29.2	16.4
5	20.6	25.6	27.0	27.4	29.3	24.6	32.5	15.3
6	24.1	30.3	35.0	12.3	38.3	28.1	39.1	-
7	24.5	25.7	36.5	29.3	36.4	28.9	35.5	21.2
$\bar{X}$	23.2	23.1	32.0	25.5	34.0	25.3	35.1	17.4
±SE	0.6	1.6	1.5	2.2	2.0	1.9	1.4	2.6
ADP								
1	4.2	3.4	5.1	4.2	5.1	4.5	4.5	4.3
2	6.0	4.1	4.3	4.0	4.1	2.9	3.9	4.3
3	4.4	4.3	5.1	4.6	5.0	4.7	5.4	3.7
4	4.1	4.7	4.0	4.5	4.3	3.8	3.9	5.6
5	4.5	4.0	4.2	4.2	4.1	4.2	3.8	3.3
6	5.9	5.3	4.4	3.3	4.4	7.6	4.9	12.0
7	5.3	-	3.9	3.8	4.9	3.7	4.2	3.9
$\bar{X}$	4.9	4.3	4.4	4.1	4.6	4.5	4.4	5.3
±SE	0.3	0.3	0.2	0.2	0.2	0.6	0.2	1.1
AMP								
1	0.38	0.33	0.16	0.27	0.32	0.31	0.25	0.36
2	0.44	0.34	0.26	0.28	0.23	0.21	0.30	0.72
3	0.22	0.27	0.36	0.38	0.44	0.41	0.39	0.43
4	0.22	0.43	0.17	0.36	0.29	0.29	0.36	1.15
5	0.45	0.26	0.34	0.28	0.48	0.39	0.33	0.50
6	0.37	0.34	0.25	0.40	0.39	1.18	0.33	2.26
7	0.51	-	0.23	0.36	0.34	0.25	0.21	0.38
$\bar{X}$	0.37	0.33	0.25	0.33	0.36	0.43	0.31	0.83
±SE	0.04	0.02	0.03	0.02	0.03	0.13	0.02	0.26

APPENDIX F

Rat Hindquarter Dye Perfusion Data

All individual data pertaining to the dye perfusions of the rat hindquarter as referenced in Chapter 3 are presented in tabular form.

Table 68.
INDIVIDUAL HINDQUARTER DYE PERFUSIONS AND DISSECTIONS (Dye perfusions determined the total perfused muscle mass. Muscles were seperated into stimulated and non-stimulated groups according to their innervation and weighed).

Rat #	Rat weight (g)	Soleus (mg)	Plantaris (mg)	Gastroc. (g)	Total Post. Super. Crural Group (g)	Total Muscles Perfused (g)	Stimulated Muscles (g)	Non-Stimulated Muscles (g)
1	312	135	334	1.998	2.467	16.60	9.10	7.50
2	262	108	276	1.592	1.976	11.26	6.79	4.47
3	302	089	277	1.980	2.346	15.63	8.21	7.42
4	268	124	297	1.703	2.124	12.91	7.44	5.47
5	284	147	328	1.745	2.220	14.42	8.19	6.23
6	273	101	249	1.694	2.044	11.62	6.58	5.04
$\bar{X}$	284	117	294	1.785	2.196	13.74	7.72	6.02
$\pm$SE	8	9	13	0.068	0.076	0.89	0.39	0.51

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