THE INFLUENCE OF INTRAMUSCULAR GLYCOGEN CONCENTRATION ON PERFORMANCE DURING MAXIMAL INTERMITTENT EXERCISE

by

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ABSTRACT

It was the purpose of this study to evaluate the effects of muscle glycogen concentration on maximal intermittent exercise performance. Ten male subjects performed an intermittent exercise test (IET) consisting of ten bouts of eight maximal isokinetic leg extensions during each of two conditions. Each bout lasted approximately six seconds and there was a thirty second rest interval between each bout. The order in which subjects undertook the treatment conditions was randomised. On the morning of day 1 of the unrepleted (U) condition subjects performed strenous exercise designed to deplete the glycogen concentration of the right leg extensors. After consuming a low carbohydrate diet for approximately 48 hours the subjects performed the IET on the morning of day 3. The repleted (R) condition was identical to the U condition, except that a normal balanced diet, rich in carbohydrates was consumed during days 1 and 2. Biopsies from the vastus lateralis before the IET confirmed a difference in intramuscular glycogen between conditions (67.6±23.2 vs 113.1±27.7 mmol glucosyl units·kg^{-1} w.w. for U and R respectively, $p < 0.01$). A two-way analysis of variance showed significant ($p < 0.001$) decreases in performance with increasing bout number for both conditions, but no significant difference was found between the conditions. Nevertheless when the first three exercise bouts were considered alone, a significantly greater amount of work ($p < 0.05$) was produced during the first three bouts of the IET in the R condition. This was largely attributable to the eccentric component of contraction. Since a reduced eccentric performance in the IET is possibly due to the muscle soreness associated with prior eccentric contraction, it was not possible to attribute the impaired performance in the U condition solely to a reduced availability of glycogen for energy supply. However the significantly lower blood lactates ($p < 0.05$ - $p < 0.01$) and lower rates of muscle glycogen utilization ($p < 0.001$) observed in the U versus the R condition, indicated a reduced rate of glycolysis during the U condition. It was concluded that when muscle glycogen is available (i.e. in the R condition) it makes a substantial contribution to energy supply during high intensity intermittent work. Futhermore when glycogen stores are reduced (i.e. in the U

condition) then the reduced glycolytic contribution may be compensated for by increased use of PCr and ATP stores.

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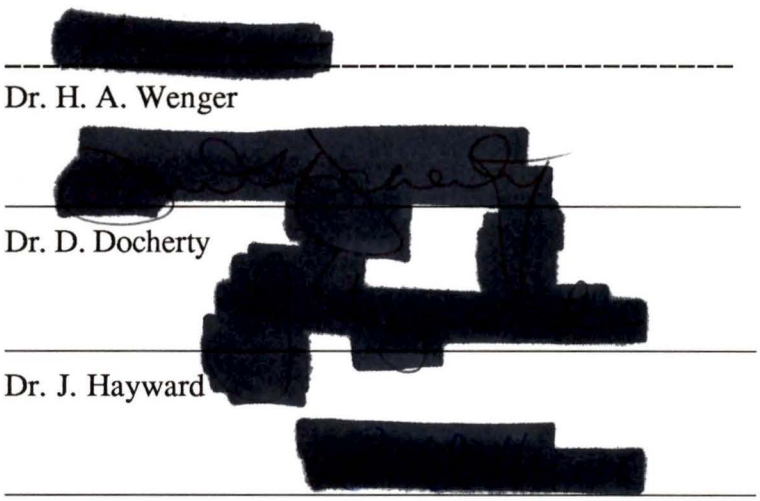

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CHAPTER I
INTRODUCTION

Many of the most popular sports, e.g football, hockey, rugby and the racquet games, involve brief periods of high intensity activity, separated by periods of rest or low intensity exercise (Reilly and Thomas, 1976; Mayhew and Wenger, 1985; Eckblom, 1986). Despite "multiple sprint" exercise being typical of many of the mass participatory sports, little research has addressed the factors which limit performance during this type of exercise.

1.1 Energy supply during high intensity exercise

The relationship between intramuscular glycogen stores and performance during sub-maximal exercise is well established. Bergstrom et al. (1967) demonstrated that time to exhaustion was extended following adherence to a high carbohydrate diet compared to a normal diet. Futhermore, the time to cover a certain distance has been shown to be decreased when intramuscular glycogen concentration is elevated prior to exercise (Karlsson and Saltin, 1971). However, there is much less evidence concerning energy supply and the causes of fatigue during high intensity activity.

Phosphocreatine (PCr) and glycogen are both available in the muscle to act as substrates for the rapid resynthesis of ATP during short-term, high intensity activity. Early studies of metabolism during maximal exercise reported that for brief bursts of intense exercise, energy is supplied exclusively by the breakdown of the high energy phosphates (ATP, PCr), glycogen degradation to lactate only making a significant contribution when this source was exhausted (Margaria et al., 1969; Cerretelli and Ambrosoli, 1973). However these authors were not investigating maximal exercise, employing instead exercise intensities that were related to maximal aerobic power.

The importance of glycogenolysis during high intensity activity was first suggested by Saltin and Karlsson (1971) who observed increased rates of glycogen utilisation as cycling intensity increased. More recent investigation of high intensity activity has reported marked decreases in muscle glycogen and increases in muscle lactate, indicating the importance of muscle glycogen as a substrate during short-term maximal exercise (Jacobs, 1981a; 1987; Jacobs et al., 1983b; Boobis et al., 1982; Hultman and Sjoholm, 1983). Conversely, some authors maintain that low muscle

glycogen should have no effect upon this type of exercise, since intramusclar levels remain high even at fatigue (Hermansen, 1981). It has been claimed for example that low muscular glycogen levels do not impair high intensity exercise per se, but decrements in performance are instead due to an alteration in blood acid-base status (Greenhaff et al., 1987a).

1.2 Energy supply during high intensity, intermittent exercise.

The study of energy supply during maximal intermittent activity is further complicated by the partial recovery of the potential energy systems during recovery periods, and is less well documented. Essen and Kaijser (1978), have suggested that during intermittent exercise (15 seconds on: 15 seconds off), glycolysis is inhibited by ATP,PCr and citrate which accumulate during recovery periods. However the exercise intensity used in this study was again related to aerobic power. Maximal exertion can generate power outputs 2-3.5 fold greater that those elicited at VO_2max (Wootton and Williams, 1983).

More recently, McCartney et al. (1986) used isokinetic cycling at high velocities to follow the changes in power output and the associated metabolic changes during repeated bouts of maximal exercise. They found evidence of the simultaneous breakdown of PCr and glycogen during four 30 second bouts of exercise, performed every four minutes. Fatigue, as evidenced by a decrease in power output from exercise bout to exercise bout, was accounted for by a change in the relative contributions to energy provision of the breakdown of PCr, glycogen and aerobic metabolism. Similarly, Lawerence et al. (1989) suggested that the high muscle lactates generated by the marked glycolytic flux during the initial bouts of isokinetic cycling, led to an inhibition of glycolysis during the subsequent bout. This, they suggested was due to an inhibitory effect of increased hydrogen ion concentration on the activity of phosphorylase.

The investigations of McCartney et al. (1986) and Spriet et al. (1989), used maximal intermittent exercise in order to examine the regulation of muscle force production and glycolysis. However the duration of the exercise and recovery periods used in the studies of McCartney and colleagues, bear little relation to those involved in many sports. Time motion analysis has shown that team games typically involve

intermittent bursts of high intensity exercise lasting an average of approximately 5 seconds, with intervening periods of rest or light activity (Mayhew and Wenger,1985; Ekblom, 1986). Energy supply and fatigue during this type of intermittent exercise may be very different from that associated with 30 second bouts of maximal activity repeated every four minutes.

Metabolism during repeated, 6 second bursts of exercise has been investigated by varying the recovery interval between exercise bouts. Greater blood lactates and performance decrements were observed when recovery durations were 30 seconds compared to 60 seconds (Wootton and Williams, 1983; Holmyard et al., 1987). In these studies, the principle effect of the different recovery durations was a manipulation of the PCr resynthesised during recoveries and therefore available in the muscle for energy supply prior to each exercise bout. The greater fatigue found with the shorter recovery durations implicates PCr as a major source of ATP resynthesis and incomplete PCr resynthesis as a major cause of fatigue for this type of exercise . However, as was previously mentioned, both PCr breakdown and glycolysis contribute to ATP resynthesis, during even the briefest periods of maximal exercise (Boobis et al., 1982; Hultman and Sjoholm, 1983). Therefore, it is additionally important to investigate the effects of altered intramuscular levels of glycogen on maximal intermittent exercise performance.

Thus it was the purpose of this study to examine the effects of different concentrations of intramuscular glycogen on the performance of maximal intermittent exercise and thereby contribute to an improved understanding of fatigue during this type of activity. To achieve different concentrations of intramuscular glycogen subjects underwent glycogen depleting exercise followed in one condition by a glycogen replenishing (repleting) diet and in the other condition, glycogen concentration was kept low (depleted) through dietary manipulation.

1.3 Research questions

The following research questions were addressed:

1. Will glycogen use be altered during the unrepleted glycogen condition versus the repleted condition ?

2. Will fatigue occur between exercise bouts 1 to 10 in either condition, as measured by a significant fall in work done or peak torque ?

3. Will performance decrements be greater in the unrepleted versus the repleted condition ?

4. Will there be a reduced performance during the first three exercise bouts in the unrepleted versus the repleted condition ?

1.4 Limitations of the study

The limitations of this study include the small sample size which may have masked the significance of any observed differences between conditions and this may limit the application of the findings to wider populations. Futhermore, while the mode of exercise employed in the study allowed for accurate measurement and eliminated many extraneous variables, it was not typical of the sporting situation. Therefore the findings may be, to a certain extent specific to single leg extension exercise.

The inability to exactly control the diet consumed by the subjects during the experiment may also be cited as a limitation. However, as intramuscular glycogen concentration not carbohydrate was the independent variable of interest, the direct measurement of glycogen in muscle samples overcomes this limitation.

CHAPTER II
REVIEW OF LITERATURE

This thesis examined the influence of intramuscular carbohydrate stores on the performance of high intensity intermittent exercise. The reason for examining performance under different conditions of muscle glycogen concentration is to further the understanding of the supply and utilisation of energy during brief bursts of maximal activity and to clarify the limitations of performance and the causes of fatigue during this type of exercise.

This review begins with an examination of the physical performance during maximal exercise and then the associated metabolic responses. In the third section, energy supply during maximal activity is discussed in the light of studies which have examined the influence of diet on performance. Thus major changes in power output associated with manipulation of dietary carbohydrate intake may implicate glycogen break down as an important source of energy supply during high intensity exercise. In the fourth and fifth sections current theories of energy supply and fatigue during maximal exercise are reviewed. The final section focuses on literature relating to intermittent exercise and examines the possible causes of fatigue during intermittent exercise, related to information on continuous maximal activity.

2.1 PHYSICAL PERFORMANCE DURING MAXIMAL EXERCISE

The responses to submaximal activity are well documented, but until recently there has been little information on the performance of maximal exercise of brief duration. This situation has arisen largely as a result of two methodological constraints. Firstly because of a lack of a suitable exercise test allowing the examination of high intensity exercise in the laboratory and secondly because of the difficulty in making physiological and metabolic measures during short duration exercise (Cheetham, 1987).

Some of the earliest research conducted into high intensity exercise was conducted by Saltin and Karlsson (1971). However, the exercise intensity used in this and similar studies was with respect to maximal aerobic power of the subjects, and was not maximal power output, which can be 2-3.5 times greater than the power output required to elicit maximum oxygen consumption (Wootton and Williams, 1983). The

development of the Wingate test in the late 1970's by Bar-Or and colleagues meant that for the first time it was possible to measure peak power and the time course of decline in power output during true maximal exercise (Bar-Or et al, 1977).

2.1.1 Performance during maximal cycling and sprinting

Mean power outputs of 747 and 539W have been reported for male subjects engaged in 10 and 30 seconds of cycling when the applied load was with respect to body weight (Jacobs et al., 1983b). With sprint trained male subjects, peak power outputs of 737W were recorded by Boobis et al. (1983b) during a 30 second maximal, cycle ergometer test.

Performance during sprint running has recently been investigated using non-motorised treadmills, instrumented to record instantaneous power output (Lakomy, 1984). Using such an ergometer, peak power outputs during one second, of 534W (Cheetham et al., 1986), 732W (Cheetham et al., 1985) and 830W (Holmyard et al., 1987) have been recorded for trained females, untrained males and male rugby backs respectively. Differences in peak power outputs between the exercise modalities may be partly due to the optimization of the resistive mass in cycle ergometry, (Lakomy and Wootton, 1981).

2.1.2 Performance during maximal isokinetic exercise

In isolated preparations, the force velocity behavior of a muscle can be empirically determined. However, in human movements the resistance offered to the muscle by an external load is modified by the properties of the limb lever system, such that the muscle tension varies throughout the range of motion. Consequently the work performed in an isotonic contraction, as with maximal cycling on a dynamically braked ergometer, is maximal for only a brief time (McCartney et al., 1983). To help overcome this problem, the isokinetic principle of accommodating resistive exercise was developed (Hislop and Perrine, 1967). In this technique the angular velocity of the instruments lever arm is maintained constant.

(i) Leg extension exercise

Peak torque has been demonstrated to have the familiar inverse relationship with velocity for leg extension exercise on an isokinetic device, with values of approximately 195 and 130 Nm at angular velocities of 15 and 180 °.s^{-1} respectively (Thorstensson, 1976). Similar findings have been reported by Jacobs et al. (1981). Unfortunately isokinetic devices such as the Cybex II (Lumex Inc., New York City), have been shown to be reliable only at velocities below 25% of the maximum voluntary contraction velocity (Thorstensson, 1976). Furthermore velocity overshoot and torque spikes, associated with lever arm over-speeding, have also been reported for isokinetic equipment, (Sapega et al., 1982).

(ii) Isokinetic cycling

The above considerations led to the development of a constant velocity cycle ergometer by McCartney and colleagues, for the study of dynamic muscle function (McCartney et al., 1983). Using this ergometer instantaneous peak power outputs of 1,626W have been recorded at 100 r.p.m (McCartney et al., 1986b). The parabolic power-velocity relationship has been demonstrated for isokinetic cycling (Sargeant et al., 1981). Peak power output has been found to be higher at 140 r.p.m. (1,473W) than at 60 r.p.m. (1,122W), but the decline in power during 30 seconds of maximal cycling is greater at the higher velocity (Jones et al., 1985). There is evidence that peak torque achieved at relatively high angular velocities is highly correlated with the percentage of fast twitch (FT) fibers (Tesch et al., 1983), and that males high in FT fibers produce higher muscle lactates and are more suseptable to fatigue than controls with a more heterogeneous muscle metabolic profile (Karlsson et al., 1981). However the close relationship between percent FT fibers and high intensity performance was found by Inbar et al. (1981) to exist only for well trained individuals.

(iii) Performance on the Kinetic Communicator exercise ergometer

The generation of power involves displacement associated with muscle shortening and lengthing. Green (1986) states that this imposes definite limits on the

duration of the contraction, since the muscle must relax and return to its original position once full extension or flexion is achieved if an additional contraction cycle is to be completed. This time delay represents a potential recovery period, for the possible restoration of sub-cellular processes critical for continued function. The Kinetic Communicator (KinCom; Med*Ex Diagnostics of Canada Inc.) is a computer controlled, isokinetic exercise system which can induce concentric contraction, followed immediately by eccentric contraction of the involved muscle group. This effectively eliminates the complication of duty cycle (defined as the ratio of the time of contraction to the time of relaxation plus recovery, Green, 1986) when investigating power generation during repetitive contraction cycles. The KinCom has the further advantage over similar isokinetic device in that the lever arm is driven by an internal motor, once the set minimum torque has been applied to the lever arm. Control is achieved through a series of feedback loops. This results in minimal periods of acceleration and decceleration to and from the target velocities, little or no overshoot of the target lever arm speed and the absence of a torque spike (Farrell and Richards, 1986), all of which are problems when using the Cybex II as a research device. Peak torques of 164Nm and 193Nm have been reported for male subjects performing concentric and eccentric contractions respectively, of the knee extensors at 180°/s on a KinCom isokinetic ergometer (Poulin et al., 1989).

2.1.3. Concentric versus eccentric muscle contraction

There are three types of muscle contraction: (1) Isometric, where the muscle develops tension but the muscle length remains constant; (2) Concentric, in which the muscle shortens while producing tension; (3) Eccentric, in which the muscle lengthens while producing tension. Normal human movements are usually performed by concentric and eccentric contractions (Komi, 1973).

The tension developed and force produced during maximal eccentric contractions have been demonstrated to be up to two times greater than during concentric contraction of the same muscle or muscle group (Komi, 1973; Seliger et al., 1980; Poulin et al., 1989). Eccentric muscle activity has also been shown to be a more efficient form of contraction than concentric contraction at the same tension or exercise intensity (Bonde-Peterson et al., 1972; Bonde-Peterson et al., 1973). Using a

specially applied cycling ergometer Bonde-Peterson et al. (1973), found a lower oxygen consumption for eccentric exercise compared to concentric exercise of the same intensity. These authors also found that eccentric exercise at 230 watts had a similar oxygen demand to concentric exercise at 48-115 watts.

(i) Electromyographic activity

The integrated electromyograph (IEMG) provides a composite measure of the number of active motor units and their frequency of firing, i.e. motor unit involvement. IEMG activity has typically been shown to be significantly less during eccentric contractions than concentric contractions at the same muscle tension (e.g. Bigland-Ritchie and Woods, 1976). However, no clear differences were found by Komi (1973) in the IEMG activity for eccentric and concentric contraction of the elbow flexors when muscle tension was expressed in percent of maximum voluntary contraction (MVC). Also, Rodgers and Berger (1974) found no difference in the motor unit involvement for maximal eccentric and maximal concentric contractions of the elbow flexors.

A more recent study by Moritani et al. (1988) obtained simultaneous surface and intramuscular IEMG data during both eccentric and concentric contractions of the human biceps briachii. Moritani found less motor unit activity during eccentric contractions than concentric contractions at a given muscle tension. The limited range of movement and the relatively slow velocities employed by Komi et al. (1973) and Rodgers and Berger (1974) may explain this discrepancy, since eccentric muscular force has been shown to increase with velocity (Nelson et al., 1973). The IEMG activity of the leg extensors during maximal contraction were investigated by Seliger et al. (1980). While these authors observed consistently lower IEMG activity during maximal eccentric contractions at all angles investigated, they were not significantly different from IEMG activities for the corresponding concentric contractions.

(ii) Contractile mechanisms

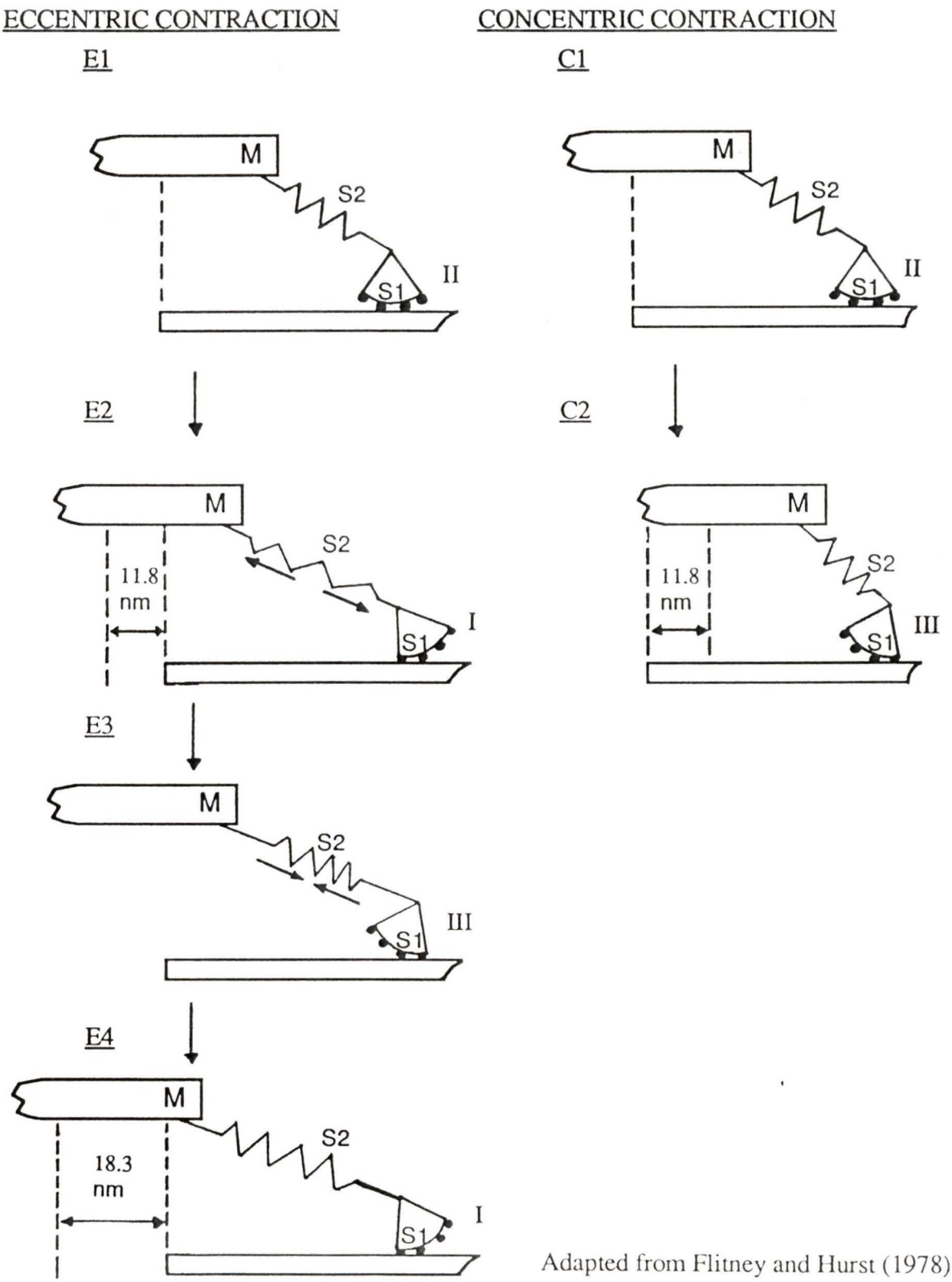
Thus, eccentric contractions are associated with greater forces, higher tensions in active fibres and a lower metabolic cost for a given tension than during concentric

contractions. However, the mechanical and/or metabolic factors responsible for these differences are not clearly established.

The potentiation of muscle contraction during or immediately following stretch has been explained on the basis of the combined effects of restitution of elastic energy and stretch reflex potentiation via the muscle spindles (Cavagna et al.,1968). During a sustained eccentric contraction, made at relatively low velocity there is no sudden stretch, therefore the effect of reflex potentiation is reduced (Moritani, 1988). Elastic energy is stored in the series elastic component (SEC) of muscle, located in part in the tendon (Komi, 1984). However, there is also some evidence to suggest the existence of a considerable elastic potential residing in the actin-myosin cross-bridges themselves during stretch (Flitney and Hurst, 1978a). It has been suggested that the differences between eccentric and concentric contractions may be mainly due to different internal events at cross-bridge level (Rodgers and Berger, 1974; Knuttgen and Klausen 1971). It seems plausible that with the actin and myosin filaments sliding together in concentric contractions and apart in eccentric contractions that the manner in which cross-bridges are established, maintained and released is specific to the type of contraction involved. Flitney and Hurst (1978b), investigated the effect of double cycles of stretch and release on the relative sliding movement of the actin and myosin filaments in active frog muscle. They found that the cross-bridges linking filaments together are able to accommodate a greater range of filament displacement before becoming detached, during a second stretch, providing it commences without delay following the preceding stretch and release. This was because the myosin S1 units or heads adopt different, 'preferred' positions in the isometric steady-state and at the end of a previous stretch and release. Figure 2.1 is a schematic representation of the events which may be occurring at cross-bridge level in concentric and eccentric muscle contractions. During a concentric contraction (C1-C2), the actin and myosin filaments slide towards each other when the S1 unit of the myosin filament changes its attachment site on the actin filament in a stepwise manner. This "power stroke" shortens the sarcomere by 11.8nm. The situation is quite different when the muscle is being stretched, as in an eccentric contraction (E1-E4). During a stretch, tension is initially generated by the extension of the S2 sub-unit, but at some point the attached head begins to be forced backwards against its natural tendency to move to the attachment site of lower potential energy. Tension continues to rise (E2), the length change now being shared between backward rotation of the S1 unit and

Figure 2.1

Possible mechanisms of eccentric and concentric muscle contraction.



Adapted from Flitney and Hurst (1978)

further extension of the S2 elastic linkage (E2). Cross-bridges are then progressively recycled, such that there is a net shift in the distribution of the re-attached heads to position III (E3). With the S1 head in the III position the cross-bridges are able to accommodate a sarcomere stretch of 18.3 nm (E4).

Thus concentric contractions involve a shortening of 11.5 nm per unit of ATP hydrolysed, whereas in eccentric contractions there is a lengthening of the 18.3 nm for the same amount of ATP hydrolysis. Therefore the amount of work done during the two muscle contractions per unit of ATP hydrolysed is in the ratio of 1:1.5 for concentric and eccentric contractions respectively. This finding is in close agreement with the relative efficiencies of the two forms of contraction measured by other methods.

(iii) Muscle damage and eccentric exercise

It has been shown that post-exercise pain and tenderness are associated with eccentric rather than concentric contractions (Davies and White, 1981). Furthermore the performance of eccentric exercise is associated with ultrastructural indications of muscle damage (Newham et al., 1983; Friden, 1984) and post exercise increases in the plasma activities of certain proteins normally found in skeletal muscle (Evans et al., 1986; Newham et al., 1986) to a much greater extent than in concentric exercise.

The morphological damage and metabolic responses to eccentric exercise have been described in humans between one and ten days following exercise. Futhermore, O'Reilly et al. (1987) have found evidence of delayed repair of ultrastructural damage and impaired muscle glycogen repletion ten days after exercise. The exact causes of the greater muscle damage following eccentric exercise are unclear but it may be due to the greater tension per active fibre for this type of contraction (Newham et al., 1983).

Several studies have attempted to establish if the marked metabolic and structural responses to eccentric exercise are ameliorated in subjects accustomed to eccentric exercise. Chronic increases in plasma creatine kinase, interleukin-1 and urinary 3 -methylhistidine have been observed in untrained men which were not observed in a group of trained men (Evans et al., 1986). The oxygen cost of eccentric exercise has also been shown to be reduced following a period of eccentric training by Bonde-Peterson et al., (1972), who hypothesised that this was as a result of an

increased utilisation of the mechanical energy received during eccentric contraction. Changes in the fine structure of the vastus lateralis were studied by Friden (1984) before and after a two month programme of eccentric training. Friden found evidence of a reorganisation of the muscle fibres induced by the high muscle tensions of eccentric contractions. He concluded that this adaption brought about an optimal overlap between actin and myosin filaments which results in a better ability of the muscle to develop tension under stretch and a reduced risk of mechanical damage.

2.2 METABOLIC RESPONSES TO MAXIMAL EXERCISE

An indirect means of assessing the limitations of performance and the causes of fatigue during high intensity exercise is to examine the metabolic responses to this activity.

2.2.1 Changes in PCr and ATP during maximal exercise

The principle energy-rich phosphagens in human muscle are adenosine triphosphate (ATP) and phosphocreatine (PCr). The resting concentrations of these phosphagens determined in acid extract biopsy samples are 24.0 ± 2.6 and 75.5 ± 7.6 mmol·kg^{-1} dry weight respectively (Hiroven et al., 1987).

The relative changes in ATP and PCr have been measured to be 7.1 and 37.7 mmol·kg^{-1} dry weight respectively for female subjects engaged in 30 seconds of maximal cycling (Jacobs et al., 1983a), and 10.3 and 56.5 mmol·kg^{-1} dry weight respectively for females after 30 seconds of treadmill sprinting (Cheetham et al., 1986). The greater changes observed in the latter study may be due to differences between the subjects, differences in the metabolic profile of the muscle fibers sampled, or may be as a result of the relative demands of the differing modes of exercise.

Changes in these metabolites have also been determined after 6 seconds of maximal cycling. In this study, Boobis et al. (1982) found changes in ATP and PCr of 2.2 and 29.5 mmol·kg^{-1} dry weight respectively.

2.2.2 Muscle and blood lactate concentrations after maximal exercise

The maximum concentrations of lactate that can accumulate in muscle and blood are unknown. It does appear however, that the highest levels occur following multiple bouts of dynamic exercise (Hermansen, 1971).

(i) Blood lactate

Blood lactate has been found to increase to approximately 15 $mmol \cdot L^{-1}$ in males following 30 seconds of isokinetic cycling (Jones et al., 1985). However significantly higher blood lactates, between 20 and 30 $mmol \cdot L^{-1}$, have been reported after intermittent maximal exercise (Hermansen and Osnes, 1972; Hermansen and Vaage, 1977). Snow et al. (1985) reported a mean blood lactate concentration of approximately 35 $mmol \cdot L^{-1}$ for three trained thoroughbred horses given four bouts of maximal exercise over 620m.

The blood lactates recorded after maximal, isokinetic leg extention exercise are lower than those recorded during cycling and sprint running. This is probably due to the larger mass of active muscle involved in the latter exercise. Peak blood lactate values of 8.7 ± 2.1 $mmol \cdot L^{-1}$ have been reported following 50 maximal leg extensions at 180 $^{\circ} \cdot s^{-1}$ (Symons and Jacobs, 1989).

(ii) Muscle lactate

The capacity of muscle to produce lactic acid during high intensity exercise is directly related to the proportion of FT fibers contained in that muscle (Karlsson et al., 1981). Thus, the post-exercise muscle lactate concentration depends on the fiber type distribution of the muscles involved and the training status of the individual. Muscle lactates between 72.9 and 108 $mmol \cdot kg^{-1}$ dry weight have been observed in the human quadriceps femoris following 30 seconds of high intensity exercise (Boobis et al., 1982; Jacobs et al., 1983a; 1983b; Cheetham et al., 1986; McCartney et al., 1986). Values have also been reported for shorter durations of exercise. After 6 and 10 seconds of maximal cycling muscle lactates of 28.4 and 46.1 $mmol \cdot kg^{-1}$ dry weight respectively were found (Boobis et al., 1983; Jacobs et al., 1983).

Lactate within active muscle is not in equilibrium with that of the extracellular space, and consequently of blood (Jeul, 1987). Therefore levels of blood and muscle lactate are affected by the rate of efflux of lactate from the muscle (Gollnick et al., 1986). Furthermore, concentrations of blood lactate are affected by lactate uptake by non-exercising muscle and other organs such as the liver and heart (Essen et al., 1975). Thus, the assumption that blood lactate concentration following exercise gives an estimate of the quantitative involvement of glycolytic energy supply is questionable. Nevertheless, good relationships have been observed between blood and muscle lactate soon after exercise has stopped (e.g. Hermansen and Osnes, 1972).

2.2.3 Blood and muscle pH following maximal exercise

Lactic acid dissociates into a proton (H^+) and lactate ion almost immediately upon production. The pH and H^+ concentration are directly related as follows:

$$pH = - \log^{10} [H^+]$$

It has been estimated that 94% of the fall in blood and muscle pH during high intensity exercise can be attributed to the increase in lactic acid (Hultman and Sahlin, 1980). Resting muscle pH has been determined to be between 7.0 and 7.1 by the homogenate technique and resting blood pH has been shown to lie between 7.35 and 7.4. These values have been reported to fall to 6.46 and 7.18 for muscle and blood respectively, following maximal cycling to exhaustion (Hermansen and Osnes, 1972). Muscle pH of 6.73 was observed following 30 seconds of maximal treadmill sprinting by Cheetham et al. (1986).

However considerable differences in blood and muscle buffering capacity have been found to exist between individuals (Sharp et al., 1986). As a consequence, pH cannot be regarded as a quantitative predictor of lactate production or glycolytic and glycogenolytic involvement during exercise.

2.2.4 Changes in muscle glycogen and glycolytic intermediates following maximal exercise.

A more accurate picture of the contribution of the glycolysis to energy supply

during maximal exercise may be obtained by monitoring changes in the concentrations of muscle glycogen and glycolytic intermediates. The rate of glycogen utilisation has been shown to increase exponentially as a function of exercise intensity (Saltin and Karlsson, 1971). These authors reported utilisation rates of 10-12 mmol glucosyl units·kg^{-1} dry weight·min^{-1} at an exercise intensity designed to elicit 150 % of VO_2max. Maximal cycling exercise lasting only 30 seconds has been reported to elicit rates of muscle glycogen utilisation of between 101-175 mmol glucosyl units·kg^{-1} dry weight·min^{-1} (Jacobs et al., 1982a; Boobis et al., 1982). In addition, marked increases in glycolytic intermediates have been observed after only 6 seconds of maximal cycling (Boobis et al., 1982). This evidence, in conjunction with the large decreases in muscle glycogen after brief, high intensity exercise, suggests that muscle glycogenolysis is initiated almost immediately with the onset of maximal exercise (Boobis et al., 1982).

2.2.5 Hormonal responses to maximal exercise

Little information is available on the hormonal responses to high intensity exercise. The catecholamine responses to 30 seconds of maximal cycling have been shown to be a 5-6 fold increase in epinephrine and a 6-7 fold increase in norepinephrine (MacDonald et al., 1983; Brooks et al., 1988). These responses have been shown to be greater in magnitude following a period of sprint training (Nevill et al. 1989). Increased blood concentrations of catecholamines serve to increase the glycogenolytic rate by increasing the mole fraction of phosphorylase a and decreasing the activity of glycogen synthetase. These changes have been observed after epinephrine infusion alone, however the glycogenolytic rate does not approach maximum (V_{max}) unless muscular contraction occurs (Chasiotis et al., 1983).

2.3 EFFECTS OF CARBOHYDRATE STORES ON THE PERFORMANCE OF MAXIMAL EXERCISE.

Skeletal muscle glycogen concentrations range between 50 to 120 mmol glucosyl units·kg^{-1} wet weight, (equivalent to approximately 220 to 520 mmol glucosyl units·kg^{-1} dry weight) (Bergstrom and Hultman,1967; Hultman, 1967). Fatigue during prolonged exercise is associated with the depletion of skeletal muscle glycogen stores

(Bergstrom et al., 1967). Fatigue during a brief period of exercise is associated with a decrease in muscle pH, rather than an exhaustion of intramuscular glycogen stores. Although, as has previously been pointed out, glycogenolysis occurs at a rapid rate during a single burst of high intensity exercise, it does not proceed to the point where glycogen stores are limiting (Hermansen, 1981).

A decrease in maximal isokinetic force generation of the leg extensors has been reported one hour after after exercise designed to deplete intramuscular glycogen (Jacobs et al., 1981; Jacobs et al; 1982b). In these studies subjects were required to perform a test, described by Thorstensson et al. (1976), consisting of 50 maximal leg extentions at 180 $^{\circ}\cdot s^{-1}$. The decrease in maximum voluntary force after glycogen depletion has been found to be more pronounced in individuals with a high proportion of FT fibers (Jacobs, 1981a). When different fiber pools were selectively depleted with appropiate exercise, Jacobs found strength reductions only when the FT fibers were depleted. However, following exercise designed to deplete the ST fibers, fatigue occurred with repeated contractions (Jacobs, 1981a). It has been speculated upon this evidence that depletion of intramuscular glycogen stores is more likely to impair the FT fibers, since the fibers are best suited to metabolise glycogen (Jacobs, 1987). However in these studies performance impairment cannot be attributed solely to the lack of glycogen per se, as the prior activity, designed to deplete glycogen, may be itself responsible for much of the decrease in performance (Symons and Jacobs, 1989).

Davies and Young (1983) had their subjects perform glycogen depleting exercise followed in one condition by a low carbohydrate diet, and in the second condition by a high carbohydrate diet. Changes in electrically evoked muscle forces and a single burst of maximum voluntary contraction were evaluated after each dietary manipulation, thereby focusing on the effects of glycogen depletion, while controlling for the effects of previous exercise. These investigators found that the 'exercise-induced muscle weakness was significantly greater following the low carbohydrate diet. Unfortunately, since muscle biopsies were not taken for glycogen concentration, interpretation of the results is complicated. Using a similar resarch design and the inclusion of direct measures of muscle glycogen, Symons and Jacobs (1989), evaluated the effect of glycogen depletion on one bout of high intensity exercise performance. Repeated isokinetic contractions, maximal isokinetic contractions and electrically evoked muscle contractions were not found to be affected by different levels of

intramuscular glycogen. The authors suggested that even though the glycogen levels were well below normal, they did not fall below the "threshold" value for the reduction of glycolytic flux. Indeed the Michaelis Menten constant (Km) of glycogen for phosphorylase in vitro is lower than the depleted levels of glycogen reported in the literature. However the in vivo relationship between glycogen and phosphorylase has yet to be established.

It has been suggested that there is no impairment of the ability of muscle to perform a short bout of contraction with reduced glycogen stores since the glycolytic rate can be kept high by the use of glucose from the blood even when the glycogenolytic rate is decreased due to shortage of available substrate (Hultman and Sjoholm, 1986). This theory is in contrast to studies which have demonstrated an attenuation of blood and muscle lactate when the glycogen concentration of active muscle falls below approximately 220 mmol glucosyl units·kg^{-1} dry weight (Jacobs 1981b). A possible explanation of this anomaly is that glycolytic flux may be undiminished when glycogen levels are low , but in this condition lactate uptake is increased by inactive fibers in the exercising muscle. However it seems unlikely that the high levels of lactic acid observed during maximal exercise could be metabolised so rapidly.

Some investigators maintain that the decrements in high intensity performance associated with low intramuscular levels of glycogen may be due to an alteration in the blood acid-base status, induced by the exercise and dietary manipulations (Maughan and Poole, 1981; Greenhaff et al., 1987a; 1987b; 1988a; 1988b). Greenhaff and his co-workers have postulated that the high intake of protein with a low carbohydrate diet can significantly influence the acid-base status of the blood prior to exercise, thereby altering glycolytic flux, H^+ efflux from muscle or buffering capacity. Symons and Jacobs (1989) found no differences in resting acid-base status or buffering capacity between individuals adhering to a high carbohydrate diet and a mixed diet. However this study did not include a low carbohydrate diet for comparison.

If high intensity exercise was limited by diet-induced reductions in pre-exercise pH and buffering capacity, then one might expect improved performance as a result of pre-exercise alkalosis and enhanced buffering capacity. Sodium citrate and sodium bicarbonate ingestion have been shown to significantly increase blood pH and buffering capacity above resting levels. However the change in resting blood acid-base status by

ingestion of these substances was not found to be associated with any significant improvements in the subsequent performance of a series of three Wingate tests (Parry-Billings and MacLaren, 1986).

The above literature concerns the effects of low muscle glycogen concentration on the performance a single period of maximal exercise. However the situation may be different during the repeated bursts of maximal exercise often involved in sports. The low concentrations of muscle glycogen observed in individuals following team games (Leatt and Jacobs, 1987), may have a greater role in the development of fatigue in this situation.

2.4 ENERGY SUPPLY DURING MAXIMAL EXERCISE

The amount of ATP in resting muscle is approximately 25 mmol·kg^{-1} dry weight (Tesch et al., 1989), which alone could sustain contraction for only about two seconds of maximal exercise. During maximal exercise PCr and glycogen are available in the muscle for the resynthesis of ATP. Levels of ATP remain relatively high during exercise initially at the expense of creatine phosphate (Hirvonen et al., 1987). However the integrative nature of energy metabolism is illustrated by the fact that ADP and Pi, the products of ATP hydrolysis, are potential stimulators of glycolysis (Newsholm and Leech, 1983).

2.4.1 The Phosphocreatine system

This system is essentially a reservoir of high potential phosphoryl groups in the form of creatine phosphate, which are used to resynthesis ATP by the following reaction:

$$ADP + PCr \xrightarrow[\text{Creatine kinase}]{} ATP + Cr$$

PCr itself is resynthesised by a reversal of the above reaction during recovery, when ATP is abundant. The ATP for this resynthesis is largely derived from aerobic metabolism and the recovery of PCr to resting levels following exercise has been shown to be dependent on the oxygen availability (Sahlin et al., 1979).

Some of the initial studies of high intensity performance proported the idea that the energy for contraction during this type of exercise is primarily met by the "alactic" phosphagen-splitting mechanism. Margaria and others suggested that only once this system was completely exhausted (after 10-15 seconds), was energy drawn from glycolytic mechanisms (Margaria et al., 1967; Cerretelli and Ambrosoli, 1971).

A common feature of more recent research employing maximal cycling, has been the finding that the rate of glycolysis, as calculated from muscle lactate concentrations, are highest during the first few seconds of high intensity exercise Boobis et al., 1982; Jones et al., 1985). Thus it would appear that both the rate of glycolysis as well as the rate of PCr breakdown are maximal during the initial stages of high intensity exercise. The utilisation of energy yielding substrates during only a few seconds of isometric contraction has been investigated by Hultman and Sjoholm (1986) using electrically stimulation of the human quadriceps femoris muscle. During 1.26 seconds of maximal contraction 80% of the ATP turnover was calculated to be derived from PCr breakdown and 20% from glycogen degradation to lactate. The contribution of glycogenolysis was found to increase to 50% of total ATP resynthesis during the second 1.26 seconds of contraction. If the rate of anaerobic glycolysis remains relatively constant during maximal exercise, the additional energy accounting for the high power outputs during the first few seconds of exercise must be supplied by PCr degradation (Cheetham, 1987). Reductions in PCr have been found to be greater in sprinters than controls following a 40m track sprint (Hiroven et al., 1987). Thus it may be that individuals who have the highest rates of PCr degradation are able to produce the highest initial levels of power output.

2.4.2 Anaerobic glycolysis

Anaerobic glycolysis has been estimated to account for 60% of the ATP resynthesised from anaerobic metabolism during a 30 second treadmill sprint (Nevill et al., 1989). It has been calulated that if these maximal rates of glycogen degradation were continued unabated during maximal exercise, that muscle glycogen depletion would occur in approximately two minutes (Newsholm and Leech, 1983). Clearly the rate of glycogen degradation must be controlled.

(i) Regulation of glycolysis and glycogenolysis during high intensity exercise

During high intensity exercise, regulation of glycogen metabolism and glycolytic control are known to involve allosterically acting inhibitors and activators, end product inhibition and covalent modification of certain key enzymes (Stryer, 1988). However the exact mechanisms by which this pathway is regulated during exercise remains unclear.

The glycolytic intermediates glucose 6-phosphate and fructose 6-phospate have been shown to increase from 2.2 to 29.5 and from 0.5 to 5.3 mmol·kg^{-1} dry weight respectively (McCartney et al., 1986). The accumulation of glycolytic intermediates during maximal exercise may be due to a greater activity of phosphorylase than the rate limiting enzyme phosphofructokinase (PFK). In vitro evidence suggests that phosphorylase is under the dual control of calcium (Ca^{2+}) and adenosine 3',5'-cyclic monophosphate (cAMP) (Stryer, 1988). The concentration of Pi has also been shown to influence the activity of the 'a' form of phosphorylase (Chasiotis et al., 1982).

The initial activation of phosphorylase from the 'b' to the 'a' form seems to occur via a Ca^{2+} mediated process. This activation has been found to be short lived in vitro, and the cAMP mediated system appears to be neccessary to maintain phosphorylase 'a' activation (Goldfarb et al., 1989). Small concentrations of the hormone epinephrine have a marked stimulatory effect on glycogen break down via a reaction cascade in which cAMP is the "secondary messenger" within the cell (Stryer, 1988). This cascade involves three enzyme-catalysed control stages. By this mechanism, very small amounts of epinephrine can stimulate significant glycogen breakdown. In vivo evidence suggests that there is depression of cAMP and phosphorylase b to a transformation at pH levels commonly found in exercising muscle at the point of fatigue (Chasiotis et al., 1982). These authors suggest that this is due to an acidotic inhibition of adenylate cyclase and phosphorylase b kinase.

That the glycolytic rate has been shown to decrease in exercising rats, while phosphorylase remains in the active 'a' form indicates that there may be other factors responsible for the regulation of glycolysis. PFK controls the rate limiting step of glycolysis (Stryer, 1988) and thus is itself finely controlled. During rest PFK is inhibited by high levels of ATP and citrate, a logical situation since an abundance of these metabolites negates the need for energy production. During intense activity the

accumulation of hexose phosphates and decreasing adenylate charge ([ATP]/[ADP]+[AMP]) overcomes this inhibition. However as exercise continues, the fall in pH and increased concentration of NH_4^+ (produced when NH_3 from the purine nucleotide cycle combines with the abundant H^+ in the muscle cell), are thought to inhibit PFK activity. Hence the rates of glycogenolysis and glycolysis may both be slowed as a result of a decrease in muscle pH during high intensity exercise.

2.5 CAUSES OF FATIGUE DURING MAXIMAL EXERCISE

Fatigue, the progressive reduction in the force generation of skeletal muscle during maximal voluntary exercise and electrically stimulated muscle contraction, has long been subject to experimental investigation. Muscle contraction is achieved through a complex series of events, starting with higher neural centers and extending down to the excitation-contraction process in the muscle cell. As a consequence, identifying the exact causes of fatigue during voluntary exercise is a difficult process (Green 1987).

2.5.1 Central causes of fatigue

The large disturbance to the resting homeostasis of the muscle cell during maximal exercise suggest that for highly motivated individuals peripheral rather than central factors are the major limits of performance (Cheetham, 1987). Furthermore methodological problems have prevented an examination of the the possible contribution of central factors to fatigue. Consequently the possibility that central factors limit high intensity exercise performance cannot be dismissed.

Excitation of the muscle occurs via the motor neurone which is unable to develop an action potential if (a) there is an inadequate supply of acetylcholine at the motor end plate, (b) excitation is lost at axon branch points, or (c) the motor neurone itself loses its responsiveness due to a reduction in membrane integrity. There is some evidence to suggest that firing rate declines during maximal isometric contractions and it has been proposed that this is a beneficial adaptation designed to exploit the full contractile capabilities of the different fiber pools of the fatiguing muscle (Bigland-Ritchie et al., 1983). Supraspinal inhibition of motor neurone excitability is thought to be stimulated either by neurally mediated afferent feedback or by the passage of blood-

borne substances across the blood-brain barrier, most notably ammonia and certain aromatic amino acids (Green, 1987).

2.5.2 Peripheral causes of fatigue

(i) Fatigue as a result of a decreased the rate of ATP resynthesis

It is generally accepted that the muscular contraction is directly connected with the breadown of ATP, and that ATP hydrolysis increases with contractile force (Dawson et al., 1978; Edwards, 1981). The amount of ATP stored in muscle is relatively small and therefore any ATP hydrolysis must be closely matched by an equivalent rate of ATP resynthesis if levels are to be maintained. If the rate of ATP resynthesis is reduced during maximal exercise the result will be a reduced supply of energy for contractile mechanisms and other cellular functions (Edwards, 1981).

The marked reductions in PCr following maximal exercise have been reviewed in section 2.2.1. Since the PCr system offers the most rapid form of ATP resynthesis, the large decreases in PCr after the first few seconds of maximal exercise may limit the rate of energy supply as maximal exercise continues. Not only does PCr provide the most rapid rate of ATP resynthesis, but it provides ATP and removes ADP at the contractile site. Thus newly synthesised ATP is immediately available at the site of cross bridge formation and ADP is removed, thereby preventing product inhibition of ATP hydrolysis (Bessman and Geiger, 1981).

Anaerobic glycolysis appears to be the major pathway of ATP resynthesis during high intensity exercise (section 2.4.2). Therefore if the rate of glycolysis were reduced during maximal exercise, ATP resynthesis and supply would be markedly reduced. The high rates of anaerobic glycolysis during maximal exercise leads to the accumulation of H^+ and a fall in muscular pH. Low pH has been shown to markedly reduce the activities of phosphorylase and PFK in vitro (section 2.4.2(i)), two key glycolytic enzymes. Indeed at a pH of 6.4, close to that in muscle following maximal exercise, the activity of both these enzymes is almost completely inhibited (Hermansen, 1981). However, it has been reported that the rate of glycolysis is not slowed during 25 seconds of electrically induced contraction (Hultman et al., 1981).

A possible explanation of the discrepency between the in vitro and in vivo

evidence is the recent suggestion that during exercise the pH inhibition of PFK is removed by positive modulators of glycolysis, e.g. fructose 2,6-bisphosphate (Dobson et al., 1986). A reduced rate of ATP hydrolysis would conveniently account for fatigue during maximal exercise if energy supply were the only limiting factor. However it may well be that there is also a failure of the contractile mechanism (i.e. ATP utilization) during maximal exercise.

(i) Fatigue as a result of a decrease in the rate of utilization of ATP

There are several possible mechanisms by which an accumulation of H^+ might exert a negative influence on the force generating mechanisms of muscle (Hermansen, 1981). The potential sites of mechanical failure have been identified by Green (1987) and include (1) loss of excitation coupling between the T-tubule and the sarcoplasmic reticulum, (2) depressed Ca^{2+} release from the sarcoplasmic reticulum, (3) a reduced binding affinity of troponin for Ca^{2+}, (4) a failure of cross bridge cycling , (5) delayed cross bridge dissociation and (6) depressed Ca^{2+} accumulation by the sarcoplasmic reticulum. The accumulation of H^+ may be associated with a number of these events (Hermansen 1981). Direct evidence exists for the negative influence of high H^+ concentration on the level of Ca^{2+} release from the sarcoplasmic reticulum (Nakamura and Schwartz, 1972) and the responsiveness of the contractile proteins to Ca^{2+} (Fuchs et al., 1970).

It has been observed that the recovery in muscular levels of PCr is dependent upon pH and that the return of both these variables to resting values following exercise proceeds at a similar rate (Sahlin et al., 1979). Nuclear magnetic resonance (NMR), has been used to investigate muscle metabolism during exercise (Dawson et al., 1978; 1980). It has been suggested, from NMR evidence, that the slowing of relaxation rate in fatiguing muscle is due to PCr depletion rather than the decrease in muscle pH (Dawson et al., 1978). Similarly Harris et al. (1976) established that the pattern of force recovery after fatiguing exercise follows a similar time course to the restoration of muscle PCr levels. The above evidence would suggest that energy supply has a causal role in muscular fatigue.

However, the fact that levels of ATP are relatively well maintained even after

repeated bouts of highly intense activity (McCartney et al., 1986) suggests that it is not a reduction in energy supply alone which causes fatigue in maximal activity. Alternatively, while the interference in the contractile mechanism by H^+ provides a convenient means to account for fatigue, it seems unlikely that the marked decreases in power output observed during maximal exercise of brief duration can be completely accounted for by a decreased ability of the contractile mechanism to utilize ATP. Hermansen (1981) has suggested that H^+ exerts a dual influence, impeding both the resynthesis of ATP and the rate of ATP utilisation by the contractile mechanism itself.

2.6 INTERMITTENT MAXIMAL EXERCISE

2.6.1 Physical performance during intermittent maximal exercise

It has been suggested that 10 second bouts of maximal cycling performed every 30 seconds could be sustained over long periods with no discernable decline in power output (Saltin and Essen, 1971). However while the exercise intensities employed in such studies are supramaximal with respect to the power output achieved at maximal aerobic capacity, they are considerably lower than those attained during truly maximal exercise (Wootton and Williams, 1983).

The changes in power output of male subjects engaged in four 30 second bouts of maximal isokinetic cycling performed every 4 minutes were observed by McCartney et al. (1986). The peak power output of one pedal revolution was approximately 990W and was achieved during the first few seconds of the first bout of exercise. This variable was reduced by 20% in the second bout and a further 21% in the third exercise bout. No further decline in peak power output was observed during the fourth period of exercise. The fatigue index however remained between 50 and 60% for all four periods. Peak powers were achieved 2 seconds from the start in the first and second periods, whereas in the fourth period, peak power was not generated until after approximately 10 seconds (McCartney et al., 1986). Similar responses during isokinetic cycling were observed by Spriet et al. (1989).

The influence of recovery duration on five 6-second bouts of maximal cycling was investigated by Wootton and Williams (1983). Initial peak power outputs of approximately 830W were found to decrease significantly by the fifth exercise bout

with 30 second recovery periods. This was in contrast to the situation with longer recoveries where no significant fall in peak power out put was observed. Using a similar research design, Holmyard et al. (1987) observed performance during a series of ten sprints on a non-motorised treadmill, each lasting 6 seconds. Peak power output for this mode of exercise was approximately 810W, and again significantly greater performance decrements were observed with shorter recoveries between exercise periods. Furthermore a statistically significant relationship was observed between the peak power output achieved and the degree of fatigue experience by subjects. In both the above investigations, the greatest falls in performance from the first to the last bout were found in the power output at the end of each bout (end power output). For example, decrements in performance of approximately 13 and 30% were observed for peak power output and end power output respectively.

2.6.2 Metabolic responses to maximal intermittent exercise

The metabolic responses of thoroughbred horses to a series of four gallops (each over 620m; mean duration 53 seconds),performed every four minutes have been described by Snow et al. (1985). This exercise resulted in a 40% decline of the glycogen content of the equine muscle. ATP was reduced by 59% and muscle lactates of up to 204 $mmol \cdot kg^{-1}$ dry weight were reported.

In human subjects evidence exists for the simultaneous break down of PCr and glycogen during repeated 30 seconds bouts of maximal isokinetic cycling (McCartney et al., 1986). It has been suggested that the high concentrations of muscle lactate generated by the large glycolytic flux during the initial bouts will lead to an inhibition of glycolysis during subsequent exercise bouts (Spriet et al., 1989).

It was previously noted that fatigue during a single burst of maximal activity is associated with a fall in pH rather than glycogen depletion (Boobis et al., 1982). However, during intermittent exercise at maximal intensity there is a much greater potential for the limited glycogen stores to become depleted. Team games such as soccer and hockey are typified by short periods of intense activity with intervening periods of rest or light activity (Reilly and Thomas, 1976; Green et al., 1978; Mayhew and Wenger, 1985; Bangsbo et al., in press b). Mean intramuscular glycogen levels of 46 $mmol \cdot kg^{-1}$ wet weight (approximately 50% of normal resting concentrations) have

been measured in professional soccer players following a match (Jacobs et al., 1982c). Moreover Karlsson (1969), observed muscle glycogen values well below resting values in soccer players at the half time interval of a match. In this study players with the lowest values of muscle glycogen at half time were observed to have the slowest average speed and to cover less ground during the second half (cited in Eckblom, 1986). Significant elevations in blood lactate and decreases in muscle glycogen content were observed in ice hockey players during and following a game (Green et al., 1978).

Marked elevations in blood lactate concentration (12-18mmol·l^{-1}) have been observed after repeated, 6 seconds bouts of exercise, suggesting the importance of anaerobic glycolysis for energy supply during exercise of this nature (Wootton and Williams, 1983; Holmyard et al., 1987).

2.6.3 Energy supply and fatigue during intermittent maximal exercise

The decline in power output during 30 seconds of maximal exercise is in the region of 50% (Jacobs et al., 1983b; Cheetham et al., 1986). However the decline in power output observed during five brief bouts of exercise is approximately 13% (Wootton and Williams, 1983), even though the total exercise time is identical in each case. Clearly the nature of fatigue is different between intermittent and continuous maximal exercise.

Boobis et al. (1982) calculated that PCr contributes approximately 50% of the ATP resynthesised during 6 seconds of maximal exercise. Over 30 seconds of high intensity activity Cheetham et al. (1986) calculated that 72% of the ATP supplied anaerobically was from glycolysis. With repeated bouts of 6 seconds duration, glycolysis may make the greatest contribution to energy supply when PCr supplies have been reduced or when the restoration of PCr during rest intervals is incomplete. The nature of energy supply and fatigue during a series of brief bursts of maximal exercise has been investigated by varying the recovery interval between sucessive exercise bouts (Wootton and Williams, 1983; Holmyard et al.,1987). The markedly greater performance decrements with shorter recover durations found in these studies could be due to a more limited PCr resynthesis during recoveries. Falling levels of PCr could account for the greater fatigue observed with shorter recovery durations in both these studies. However a greater muscular acidosis could also account for the greater

fatigue with the shorter recoveries since there would be less time for H^+ efflux from the muscle. This suggestion is to some extent supported by the higher post-exercise blood lactate concentrations found when the shorter recoveries were employed (Wootton and Williams, 1983; Holmyard et al.,1987).

The relative importance of the progressively lower PCr availability at the start of each exercise bout, and the increasingly acidotic state of the muscle, to the process of fatigue during intermittent exercise is yet to be established. However the major falls in end power output compared to peak power, which is relatively well maintained suggests that it is energy supply rather than energy utilization which is limiting for this type of exercise. Thus, if acidosis were a major contributing factor to fatigue during intermittent exercise, then negative influence it exerts would mainly be on the activities of phosphorylase, PFK and creatine kinase rather than on the contractile mechanism.

CHAPTER III
METHODS

3.1 SUBJECTS

Informed written consent was obtained from ten male students from the University of Victoria, who volunteered to take part in the study (Appendix C). Protocol approval had previously been obtained from the University Human Subjects committee. All subjects were accustomed to the high intensity exercise to be encountered in this study on at least five occassions.

In the week prior to testing, subjects received a medical screening and an explanation of the experimental protocol and possible accompanying risks and discomforts. During this week subjects also performed a maximal test on a cycle ergometer. Pedaling rate was 60 rpm in this test which was for the purpose of measuring maximal oxygen uptake and the exercise intensity at which the ratio of expired air to oxygen consumption (ventilatory equivalent) increased exponentially with the respiratory exchange ratio > 1.0. The latter exercise intensity was referred to as anaerobic threshold. The age, height and weight of the subjects were also recorded.

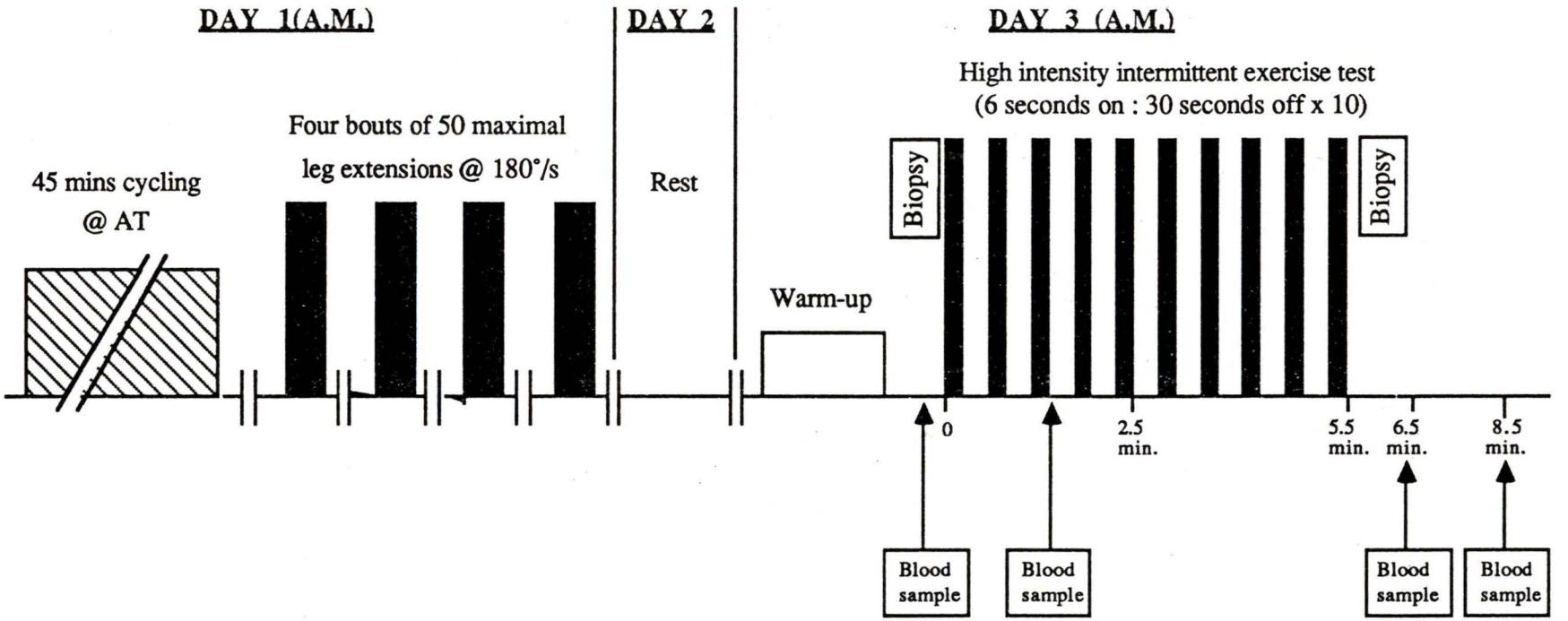
3.2 EXPERIMENTAL DESIGN

Each subject participated in two conditions (figure 3.1). On Day 1 of both conditions, following an overnight fast, subjects completed an exercise protocol designed to deplete glycogen from the extensor muscles of the right leg. The glycogen depletion exercise was as follows : A forty-five minute ride on a Monark cycle ergometer at 60 rpm, against a resistive mass at which anaerobic threshold was previously determined. After a short rest (approximately 15 minutes), subjects were asked to perform three or four bouts of 50 repetitions of maximal voluntary isokinetic contraction of the right leg extensors, at $180°\cdot s^{-1}$ on the Kinetic Communicator (KinCom) system. Subjects took short rests, of approximately five minutes between each bout.

For the remainder of Day 1 and Day 2 subjects consumed a strictly controlled diet. In the repleted condition (R) this was a high carbohydrate diet (Appendix C) and

Figure 3.1

Experimental design and sequence of glycogen depletion, diets and the maximal intermittent exercise test



in the unrepleted condition (U) this was a low carbohydrate diet (Appendix C). The order in which each subject undertook the conditions was randomly assigned, and there was at least one week between experimental conditions. During experimental days 1,2 and 3, subjects were instructed to avoid exercise except as required by the experimental protocol.

On the morning of Day 3 subjects completed a standardised warm-up consisting of three minutes exercise on a cycle ergometer at a power output of approximately 70 watts, 3-5 minutes of stretching and 3 submaximal right leg extensions on the KinCom at the experimental velocity. The exercise test consisted of ten bouts of maximal isokinetic contraction of the right leg extensors at $180°\cdot s^{-1}$. Each bout consisted of eight complete concentric/eccentric contractions through an angle of sixty-eight degrees from a standard starting angle. Exercise bouts lasted approximately 6.2 seconds. Subjects were allowed thirty seconds of passive recovery between bouts of exercise. Immediately before and approximately thirty seconds after the intermittent exercise had ceased, muscle biopsies were obtained from the musculus vastus lateralis of the right leg and subsequently analysed for glycogen (3.2.1(i)). Venous blood samples were taken pre-exercise, after three bouts, and one- and three minutes post exercise and were analysed for lactate (3.3.4).

3.3 EXPERIMENTAL PROCEDURES

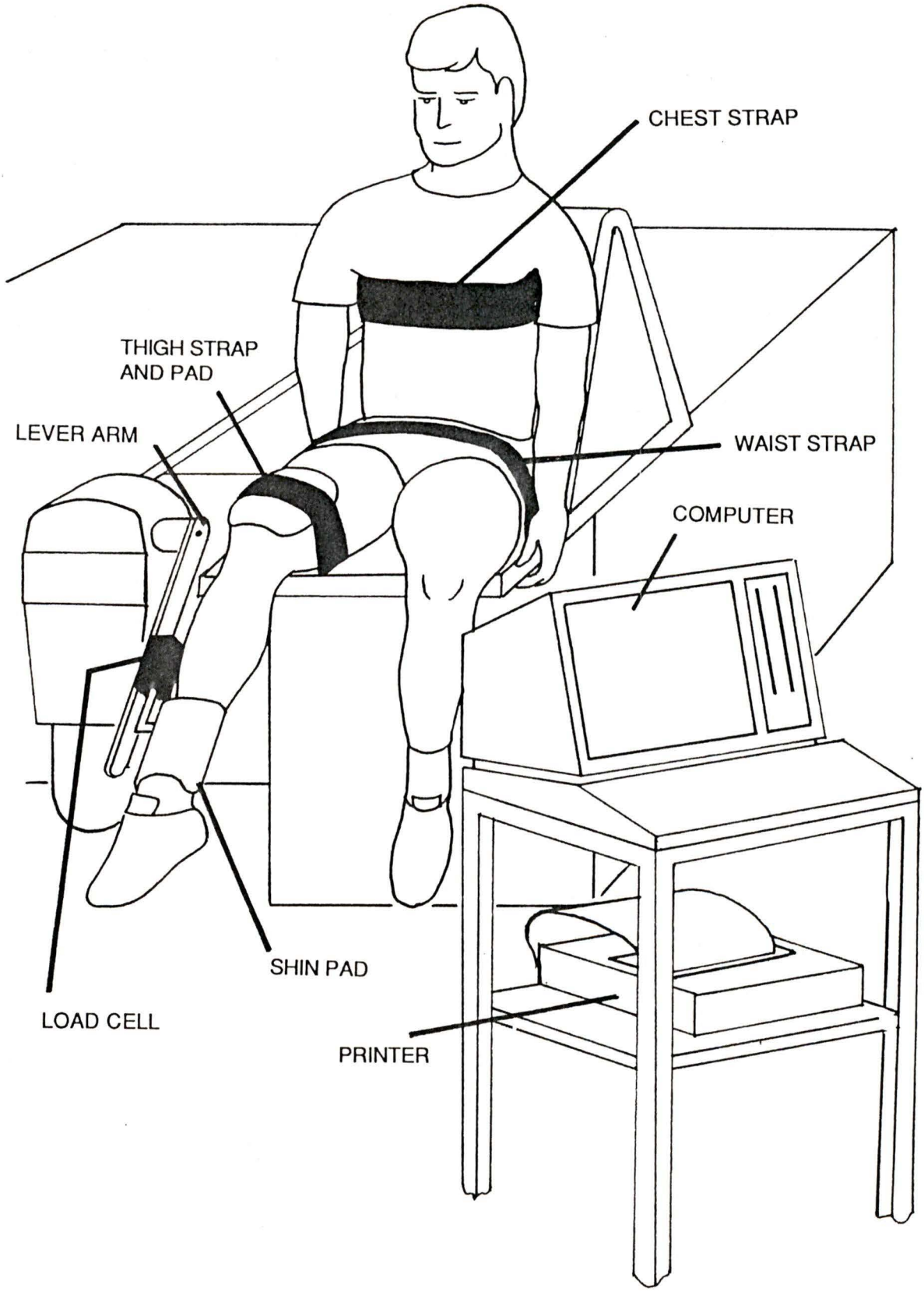
3.3.1 Isokinetic exercise set-up

The isokinetic exercise test was performed on the Kinetic Communicator (KinCom), computer controlled isokinetic exercise system, (Med*Ex Diagnostics of Canada Inc.)(See section 2.1.2). Version (I)3.13 software was used, which collects data on force, velocity and range of motion and calculates power, torque and work data which may be stored on disk for subsequent analysis. Farrell and Richards (1986), have reported that the measurement of lever arm position and velocity, and the measurement of force with the KinCom system are both valid and reliable.

Subjects exercised in a sitting position, with the back supported and hands clasped on the edge of the seat beneath the hips (figure 3.2). The back of the knees were against the edge of the seat and the hip and knee of the testing leg were in line

Figure 3.2

The arrangement of the subject on the Kin Com for exercise testing



with the plane of the lever arm, when viewed from above. Chest, waist and thigh straps ensured maintainence of the proper position. Prior to each test, the centre of the right knee was aligned with the pivotal axis of the lever arm. This was achieved by changing the position of the seat in relation to the lever arm head, in two planes using the KinCom motor driven controls. The centre of the knee was taken as being that point mid-way between the lateral condyles of the femur and the tibia.

Subjects exerted force against a shin pad attached to the lever arm via a load cell. The position of the pad on the lever arm was adjusted so that its bottom edge was approximately five centimeters above the level of the subjects right medial maleolus. This position was recorded for each subject during familiarization, and was kept constant throughout testing. Each bout of exercise was begun at the 'start' angle, which was with the leg in approximately ninety degrees of flexion. One repetition consisted of a concentric contraction of the knee extensors through sixty-eight degrees until the return angle was reached, followed immediately by an eccentric contraction, 'resisting' the movement of the lever arm back down to the start angle position.

This limited range was employed so that the pain associated with forceful knee hyper-extension would not interfere with maximal voluntary contraction. Also, weaknesses at the extreme of the range of movement can lead to force production which is insufficient to supply the minimum torque (in this case 50 Nm), required to drive the motor unit of the KinCom. This consequently brings exercise to a stop before fatigue has occurred in the main range of movement. Torque and work data were obtained at .01 second intervals throughout the range of leg extention. Data from the extreme 15 degrees of leg flexion and extention was not included in the analysis. This was to ensure that the data analysed was actually produced when the lever arm was moving at the target velocity of 180 $^{\circ}\cdot s^{-1}$.

Subjects were encouraged to exert maximum force from the beginning of each bout, in both the concentric and eccentric phases of the contraction.

3.3.2 Muscle Biopsies

Muscle samples (approximately 60 mg wet weight) were obtained from the vastus lateralis, post warm-up and post exercise, using a Bergstrom needle. Prior to the first biopsy a five mm incision was made in the skin and muscle fascia under local

anaesthetic (4% lidocaine). The post-exercise biopsy entered the leg through the same incision, but at a slightly different angle so that undamaged tissue was sampled. The muscle samples obtained were trimmed free of connective tissue and divided into two portions. The larger portion was immediately frozen in liquid nitrogen for subsequent biochemical analysis, while the second more intact portion, for histochemical analysis was frozen in a steel beaker of isopentane, cooled in liquid nitrogen. Both sets of samples were stored in a freezer at -60°C until they were analysed.

(i) Biochemical Analysis

Small sections of tissue were thawed, weighed and homogenised and the endogenous glycogen was hydrolysed to glucose by heating the samples in 2N HCL. Glucose was then quantitatively assayed using the Hexokinase method (Sigma Chemical Co.). (Appendix A).

(ii) Histochemical Analysis

Muscle samples for qualitative histochemical analysis were mounted in OCT embedding medium and freeze sectioned with a cryostat (American Instruments). Sections were stained for myofibrillar ATPase and for glycogen using the P.A.S. technique. (Appendix A).

3.3.4 Blood samples

After the warm-up, a catheter was inserted into the antecubital vein of the left arm of the subject. Four blood samples of approximately 2ml each were taken at the following times: pre-exercise, after three bouts of exercise, one minute post exercise and three minutes post exercise. Although venous samples from the left arm are not representative of blood in the arteries of the right leg, there was assumed to be no difference between the two conditions. Aliquots of each sample (0.5 ml) were deproteinised in ice-cold perchloric acid and centrifuged for fifteen minutes. The supernatent was decanted off, frozen and subsequently assayed for lactate by the spectrophotometric technique (Sigma Chemical Co. 1981).(Appendix A)

3.3.5.Controlled Diets

In the unrepleted condition (U), subjects were instructed to moderate their caloric intake and to keep to an 'allowable food list', on Day 1, Day 2 and until they were tested on Day 3. Subjects were also provided with meal suggestions and a food diary in which to record the type and quantity of food they had eaten during this period. During the repleted condition (R), caloric intake was unrestricted, but subjects were asked to minimise fats and maximise carbohydrate in their diet. Subjects were educated how to achieve this and were again provided with meal suggestions and a food diary. (Appendix C contains details of the allowable food list and meal suggestions for each condition). The recorded diets were analysed using the 'Nutrient' computer software program. (For details of the diets see section 4.1)

3.3.6.Treatment of Results and Statistical analysis

The KinCom provided a detailed summary of each exercise bout. From this, work output and peak torques for the concentric and eccentric phases of each exercise bout were obtained. Values in the text, tables and figures are shown as group means ± standard deviations or standard error of the mean. Total work output (concentric + eccentric), concentric work output, eccentric work output, concentric peak torque and eccentric peak torque for each of the 6 second exercise bouts were analysed using an analysis of variance with repeated measures on two factors. Subsequent comparisons between specific means were carried out using a student's t-test for correlated data.

Blood lactate and muscle glycogen values were compared using a student's t-test for correlated means. Significant difference between means was accepted at the 0.05 level.

CHAPTER IV
RESULTS

The mean physical chacteristics of the subjects are shown in table 4.1. Staining for myofibrillar ATPase indicated that the vastus lateralis of the subjects in the present study contained 55 ±10% Type I fibers and 45 ±10% Type II fibers (n = 7). The fiber type of the subjects in this study was similar to those previously found for groups of runners by Jacobs et al. (1981b) and Mackova et al. (1985).

4.1 DIETARY ANALYSIS

Table 4.2 gives an estimated nutritional breakdown of the meals subjects consumed during the controlled diets. Between the glycogen depleting exercise on Day 1 and the exercise test on Day 3 it was calculated that the subjects consumed 3301±669 kilocalories and 4001±1130 kilocalories for the U and R conditions respectively. The mean composition of this caloric intake was 16.7% carbohydrate, 25.7% protein and 55.6 % fat, in the U condition. In the R condition the composition was 57.0% carbohydrate, 13.4% protein and 27.1% fat.

4.2 METABOLIC DATA

4.2.1 Muscle glycogen concentration

(i) Biochemical analysis

Intramuscular glycogen concentrations prior to the intermittent exercise on day 3 were 40% lower in the unrepleted condition, (67.6 ± 23.2 and 113.1± 27.7 mmol glucosyl units·kg^{-1} w.w. for the U and R conditions respectively). (See table 4.3 and figure 4.1). Muscle glycogen 'use'in the U condition represented only 18% of glycogen use in the R condition, (6.1± 5.4 and 34.0 ± 30.2 mmol glucosyl units.kg^{-1} w.w.), as determined from glycogen concentration in biopsy samples taken pre- and post-exercise.

(i) Histochemical analysis

The results of the qualitative histochemistry support the biochemical finding of lower pre-exercise muscle glycogen in the U condition. Figure 4.2 shows photographs of PAS stains on pre-exercise muscle samples for two subjects in each condition. In both cases the PAS stain in the R condition is more intense than the corresponding stain on the sample from the U condition indicating a greater intramuscular concentration of glycogen prior to exercise in the R condition.

4.2.2 Blood lactate concentration

Blood lactate concentrations rose to a peak value of 4 - 5 $mmol.L^{-1}$ in both conditions at one minute post-exercise (Table 4.4). However, throughout exercise blood lactate concentration was approximately 1 $mmol.L^{-1}$ higher for the R when compared to the U condition ($p<0.05$).(Figure 4.3).

4.3 PHYSICAL PERFORMANCE

4.3.1 Work output

The amount of work subjects performed in the 10 exercise bouts, each consisting of 8 leg extensions is shown in Tables 4.5, 4.6 and 4.7. Values are given for concentric work, eccentric work and total work output (i.e. concentric + eccentric) for each bout in each respective table.

In both conditions there was a reduction in the total work output (TWO) in each bout, as the intermittent exercise progressed ($p< 0.001$). The two-way analysis of variance did not reveal any significant difference in total work output between conditions or any interaction effect. However, when the first three bouts of exercise are considered alone, there was a greater average TWO in these bouts in the R condition ($p< 0.05$). There was also a greater decline in TWO from bout one to bout ten in the R condition than was the case in the U condition (26% and 17% for the R and U conditions respectively) ($p< 0.05$). A significant correlation was found between the total work achieved by subjects in the first exercise bout and the decline in work done

in the U condition ($r = 0.80$; $p<0.01$). However, no such relationship was found in the R condition. (Figure 4.5)

A decline in concentric work output (CWO) occurred from exercise bouts 1 to 10 ($p< 0.001$) but no effect of condition or interaction was found. There was no difference in the magnitude of the decline in CWO between the R condition (23%), and the U condition (22%). (Figure 4.6).

A marked decline in eccentric work output (EWO) was also found ($p< 0.001$). Although there was no significant difference between conditions, an interaction effect between bout number and condition was observed ($p< 0.05$). This was largely due to the greater average EWO during the first three bouts of exercise in the R condition ($p< 0.05$). The fall in EWO from bout 1 to bout 10 was greater in the R condition (27%) compared to the U condition (13%), ($p< 0.05$). See figure 4.7.

4.3.2 Peak Torques

Concentric peak torques are smaller than the corresponding eccentric values for each subject (Table 4.8 and 4.9 respectively). Peak torques followed a similar pattern to the work output data. There was a marked decline in both concentric and eccentric peak torque as exercise continued ($p< 0.001$). The two-way analysis of variance showed no difference between the conditions or any interaction for either concentric or eccentric peak torques.

However when the peak eccentric torques achieved in individual exercise bouts were compared between conditions, they were greater in the R condition for exercise bouts one ($p< 0.01$), two, five, six and seven (all at $p< 0.05$).

Table 4.1

Physical chacteristics of the subjects. Means ± standard deviations

Variable		Value	n
Age (years)	Mean	23	10
	SD	2.8	
Weight (kg)	Mean	75.8	10
	SD	4.7	
Height (cm)	Mean	175.5	10
	SD	4.5	
$VO_2max.$ ($ml.kg^{-1}.min.^{-1}$)	Mean	51.8	10
	SD	6.1	
Muscle fiber type (% FT)	Mean	45	7
	SD	10	

Table 4.2

Nutritional composition of the controlled diets consumed during day 1 and day 2 (percentages of total energy intake and kcal.) Means ± standard deviations (n=10)

		Condition	
		UNREPLETED	REPLETED
Carbohydrate	(%)	16.7±5.9	57.0±8.3
	kcal.	534±184	2269±649
Protein	(%)	25.7±3.4	13.4±3.2
	kcal.	855±242	533±189
Fat	(%)	55.6±6.3	27.1±11.0
	kcal	1851±514	1118±597
Total Kcal		3301±669	4001±1130

Table 4.3

Intramuscular glycogen concentrations pre and post exercise (mmol glucosyl units.kg^{-1} w.w.). Means ± standard deviations (n=10).

Time of sample		Condition	
		UNREPLETED	REPLETED
Pre exercise	Mean	67.6	113.1 **
	SD	23.2	27.7
post exercise	Mean	61.5	79.1 **
	SD	21.8	24.9
			**

" *'s " in the body of the table indicates that the R condition value is significantly greater than the corresponding value in the U condition.

" *'s " at the bottom of the table indicates a significantly smaller post exercise value than pre exercise value

*	p< 0.05
**	p< 0.01
***	p< 0.001

Table 4.4

Blood lactate concentrations ($mmol \cdot L^{-1}$). Means ± standard deviations (n=10).

Time of sample		Condition	
		UNREPLETED	REPLETED
Pre exercise	Mean	**1.09**	**1.60** **
	SD	0.41	0.30
Mid exercise #	Mean	**2.77**	**3.29** **
	SD	0.50	0.80
1'-Post exercise	Mean	**4.09**	**5.54** **
	SD	0.71	1.95
3'-Post exercise	Mean	**4.01**	**4.81** *
	SD	1.12	1.84

* Significantly higher ($p<0.05$) in the R condition

** Significantly higher ($p<0.01$) in the R condition

#Sample taken immediately after the third exercise bout

Table 4.5

Total work output during the 8 maximal concentric and eccentric leg extensions of each exercise bout (Joules). Means ± standard deviations (n=10).

	Bout	Condition
	UNREPLETED	REPLETED
1	2746±794	3056±670
2	2570±621	2765±651
3	2546±591	2734±621
X_{123}	2621±655	2852±637 *
4	2515±644	2676±585
5	2408±681	2612±653
6	2355±651	2532±610
7	2310±702	2477±625
8	2297±608	2370±666
9	2300±631	2351±632
10	2271±601	2273±671
X_{8910}	2307±606	2331±646
	***	***

$X_{1,2,3}$ = Average mean work done in bouts 1,2 & 3

$X_{8,9,10}$ = Average mean work done in bouts 8,9 &10

" *'s " in the body of the Table indicates that the R condition value is significantly greater than the corresponding value in the U condition.

" *'s " at the bottom of the Table indicates a significant decline in performance between bouts 1 and 10

* $p< 0.05$

** $p< 0.01$

*** $p< 0.001$

Table 4.6

Concentric work output during each exercise bout (Joules).Means ± standard deviations (n=10).

Bout	Condition	
	UNREPLETED	REPLETED
1	1229±151	1280±148
2	1189±169	1178±171
3	1114±161	1160±140
X_{123}	1177±155	1206±146
4	1093±160	1123±147
5	1045±165	1099±150
6	1013±151	1064±178
7	992±192	1039±199
8	982±174	1011±208
9	969±183	998±194
10	961±166	980±241
X_{8910}	987±191	997±213
	***	***

$X_{1,2,3}$ = Average mean work done in bouts 1,2 & 3

$X_{8,9,10}$ = Average mean work done in bouts 8,9 &10

" *'s " in the body of the Table indicates that the R condition value is significantly greater than the corresponding value in the U condition.

" *'s " at the bottom of the Table indicates a significant decline in performance between bouts 1 and 10

*	$p< 0.05$
**	$p< 0.01$
***	$p< 0.001$

Table 4.7

Ecccentric work output in each exercise bout (Joules). Means ± standard deviations (n=10).

Bout	Condition	
	UNREPLETED	REPLETED
1	1517±716	1776±565
2	1381±582	1587±547
3	1432±560	1575±541
X_{123}	1443±606	1646±545 *
4	1421±600	1551±503
5	1363±623	1512±565
6	1343±576	1468±509
7	1326±614	1437±500
8	1315±547	1360±5358
9	1331±547	1353±521
10	1310±554	1292±507
X_{8910}	1320±507	1335±513
		***

$X_{1,2,3}$ = Average mean work done in bouts 1,2 & 3

X8,9,10 = Average mean work done in bouts 8,9 &10

" *'s " in the body of the Table indicates that the R condition value is significantly greater than the corresponding value in the U condition.

" *'s " at the bottom of the Table indicates a significant decline in performance between bouts 1 and 10

*	p< 0.05
**	p< 0.01
***	p< 0.001

Table 4.8

Peak concentric torques recorded during the eight maximal contractions of each exercise bout (Nm). Means ± standard deviations (n=10)

Bout Number	Condition	
	UNREPLETED	REPLETED
1	171±26	182±26
2	165±26	171±26
3	161±22	163±28
4	158±22	157±23
5	152±25	157±28
6	151±29	151±28
7	149±27	151±33
8	146±30	153±33
9	142±30	146±28
10	146±25	143±37
	***	***

" *'s " in the body of the Table indicates that the R condition value is significantly greater than the corresponding value in the U condition.

" *'s " at the bottom of the Table indicates a significant decline in performance between bouts 1 and 10

*	$p < 0.05$
**	$p < 0.01$
***	$p < 0.001$

Table 4.9

Peak eccentric torques recorded during the eight maximal contractions of each exercise bout (Nm).Means ± standard deviations (n=10)

Bout Number	Condition	
	UNREPLETED	REPLETED
1	244±92	271±71 **
2	211±81	249±74 *
3	244±81	232±72
4	218±90	233±60
5	205±82	241±65 *
6	206±77	234±61 *
7	205±83	232±61 *
8	209±72	221±62
9	210±73	212±71
10	198±79	212±67
	***	***

" *'s " in the body of the Table indicates that the R condition value is significantly greater than the corresponding value in the U condition.

*	p< 0.05
**	p< 0.01
***	p< 0.001

" *'s " at the bottom of the Table indicates a significant decline in performance between bouts 1 and 10

Figure 4.1

Intramuscular glycogen concentration pre- and post-exercise (mmol glucosyl units ·kg^{-1} w.w.) Means ± SEM (n=10).

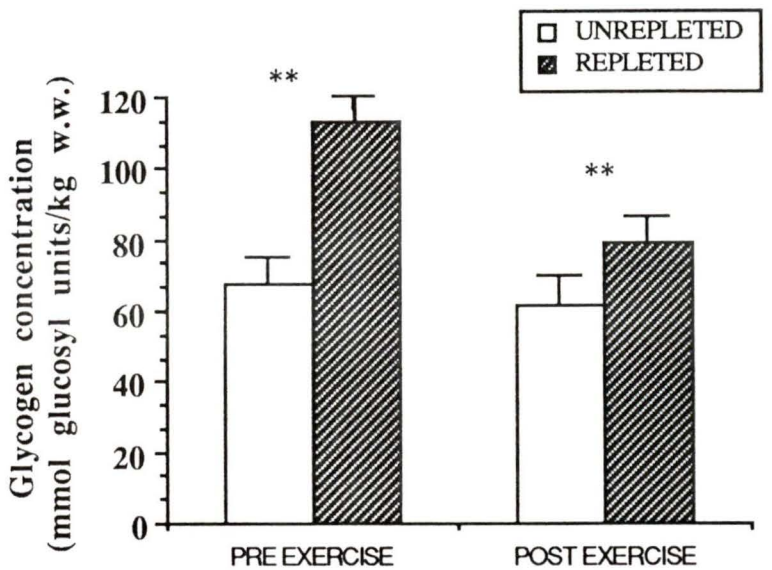
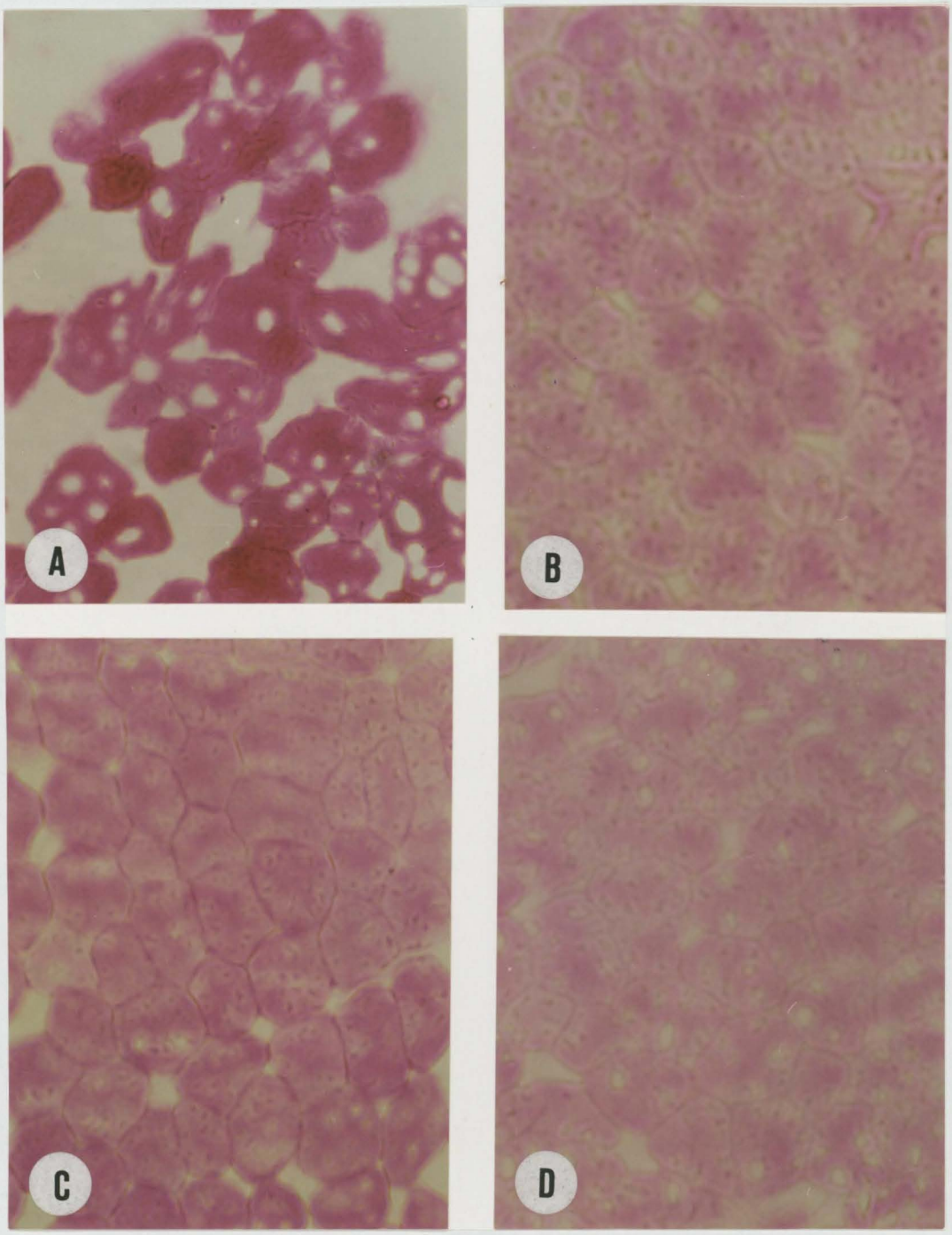


Figure 4.2

P.A.S. stained muscle sections from biopsy samples obtained from two subjects before each condition (original magnification = X 40).



A: subject 7 pre-exercise R condition, B: subject 7 pre-exercise U condition

C: subject 8 pre-exercise R condition, B: subject 8 pre-exercise U condition

Figure 4.3

Blood lactate concentrations (mmol.L^{-1}), Means ± SEM (n=10)

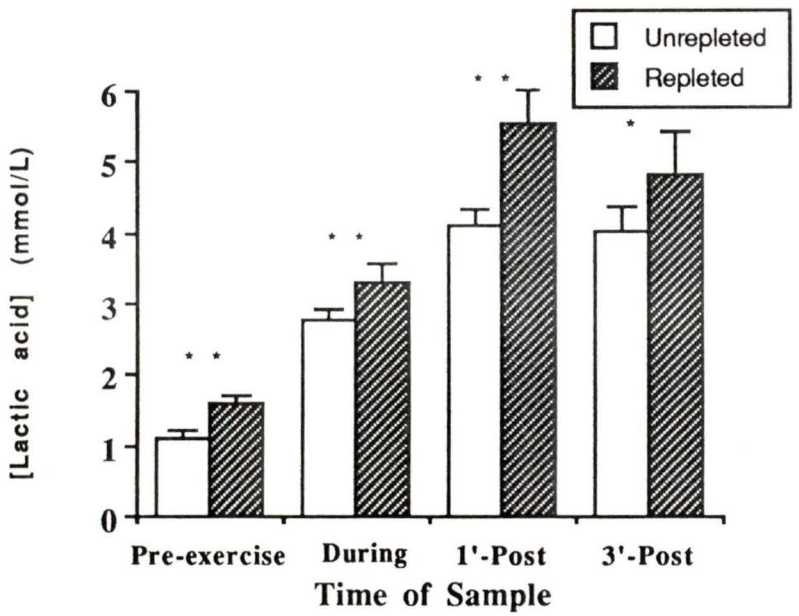


Figure 4.4

Total work output in each exercise bout (Joules). Means ± SEM (n=10).

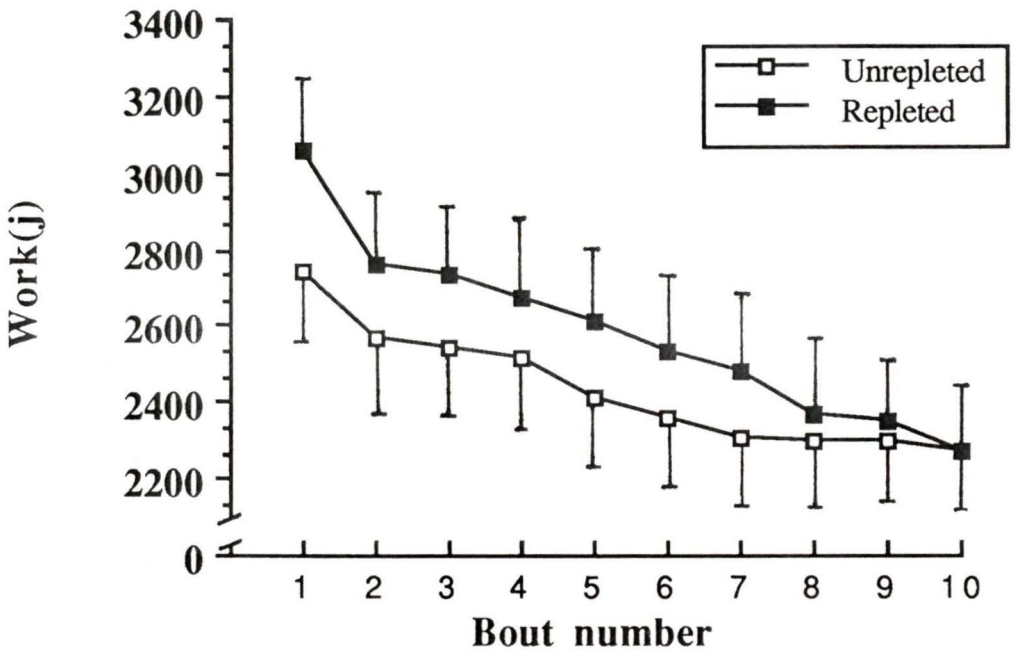


Figure 4.5

Concentric work output in each exercise bout (Joules). Means ± SEM (n=10).

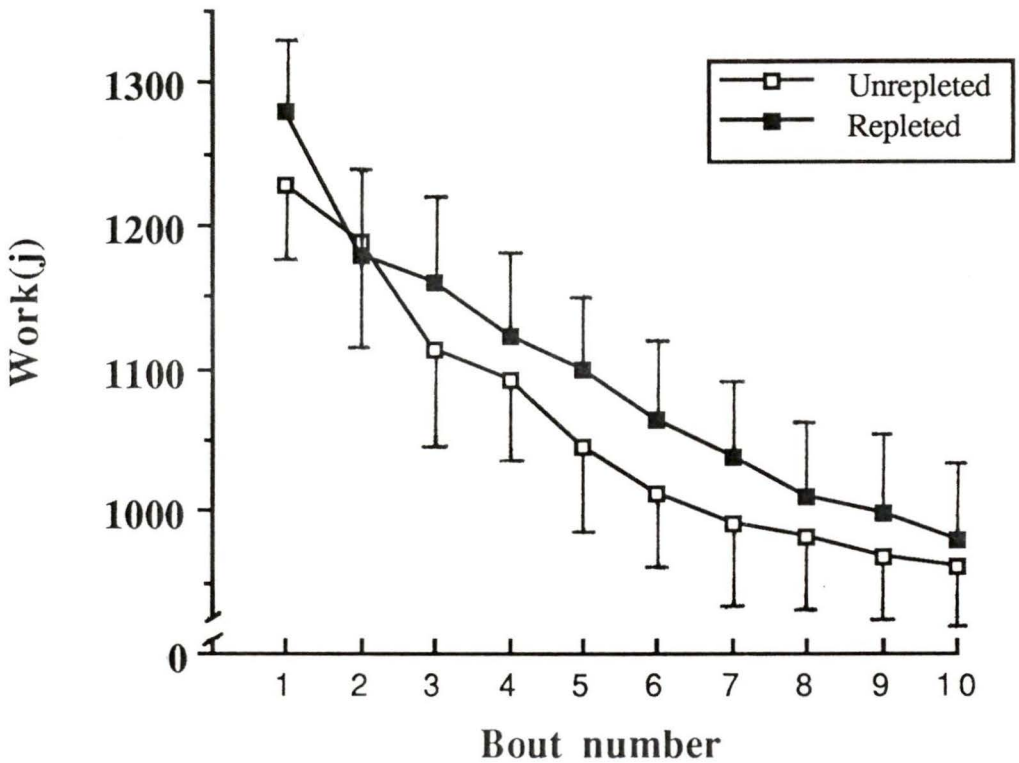
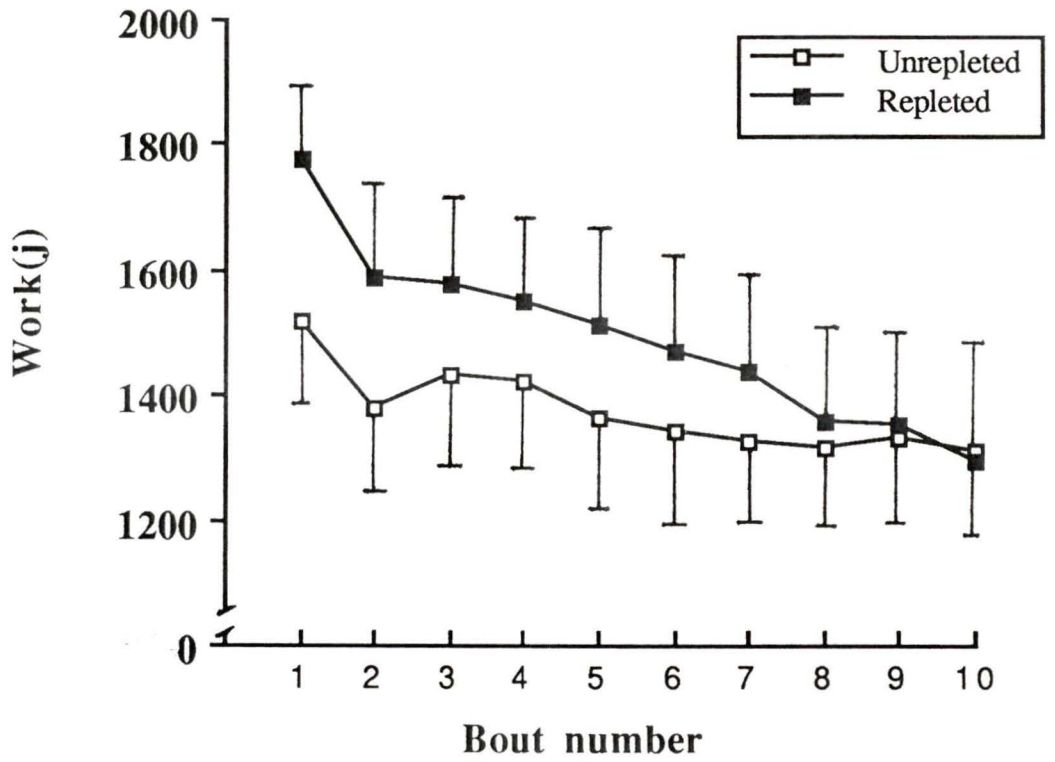


Figure 4.6

Eccentric work output in each exercise bout (Joules). Means ± SEM (n=10).



CHAPTER V
DISCUSSION

It was the purpose of this study to examine the effects of low intramuscular carbohydrate concentration on the performance of maximal intermittent exercise. It was reasoned that by monitoring the physical performance associated with both a normal and a reduced pre-exercise muscle glycogen concentration, that information could be acquired about the involvement of different energy systems (specifically PCr degradation and anaerobic glycolysis), throughout a series of brief maximal exertions.

Therefore the discussion will initially focus on the evidence which suggests that there was a decreased availability of energy from glycogenolysis when intramuscular glycogen stores are reduced. Next, the impact of the altered intracellular conditions on the physical performance will be assessed, with special attention given to the potentially different effects of the experimental conditions on concentric and eccentric muscle contractions.The final section of this chapter will discuss the probable nature of energy supply and the potential causes of fatigue during the type of intermittent maximal exercise investigated in this study.

In the present study, subjects performed ten bouts of maximal exercise, each lasting approximately 6 seconds, with a 30 second recovery period between bouts. The exercise in each bout consisted of eight repetitions of maximal leg extension and each repetition involved a concentric contraction followed immediately by an eccentric contraction. During maximal voluntary contractions the subjects did considerably more work in the eccentric phase of the movement than the concentric phase. This finding is in agreement with previous research which has examined both eccentric and concentric contraction (Komi, 1986; Seliger et al., 1980). The peak concentric torques are similar to those reported by Poulin et al. (1989) for adult male subjects. However the eccentric peak torques are, in both conditions, somewhat greater than those reported in the latter study. This may be as a result of methodological differences (e.g. the use of a shorter lever arm), or that the subjects were more accustomed to eccentric leg extension.

The highest blood lactates of 5.54±1.95 (group mean ± SD) $mmol \cdot L^{-1}$ recorded 1 minute post exercise in the R condition, are much lower than those previously reported for intermittent cycling (Wootton and Williams, 1983) and sprinting

(Holmyard et al. 1987) with the same work to rest intervals. This is probably due to the smaller muscle mass active in leg extension. The values are much more comparable to those obtained by Symons and Jacobs (1989) after a continuous period of 50 maximal leg extensions at $180°\cdot s^{-1}$, though again they are of somewhat smaller magnitude. Eighty maximal isokinetic leg extensions were performed in the present study. The lower lactate values may be due to an uptake of lactate by inactive muscle tissue during the recovery intervals in the intermittent exercise mode (Brooks, 1986), or a decreased involvement of glycolysis in the intermittent mode.

Although blood lactate concentrations may correlate well with muscle values, particularly in whole body exercise, the contribution of glycolysis to energy supply in the type of one-legged activity involved in this study is best investigated by examining the glycogen use in muscle itself. In the present study the concentrations of glycogen measured in muscle biopsy samples prior to exercise are within the range previously reported for human muscle tissue (Hultman, 1967), in both the U and R condition. However the mean value in the U condition is at the low extreme of the reported range and the R condition value is similar to those previously reported for endurance athletes. This suggests that there was some degree of glycogen supercompensation or at least a full return to resting values as a result of the depletion exercise and subsequent 'normal' diet. The mean pre-exercise value in the U condition of 67.6±23.2 mmol glucosyl units$\cdot kg^{-1}$ w.w.confirms the success of the low carbohydrate diet in maintaining a low intramuscular glycogen concentration. The histochemical analysis for glycogen lends further support to the biochemical evidence of a marked difference in the pre-exercise concentrations between the conditions.

Jacobs (1981a) has suggested that muscle lactate production will not be reduced when glycogen levels are low, unless they are below a threshold level of 220 mmol glucosyl units$\cdot kg^{-1}$ d.w. In the U condition the mean pre-exercise intramuscular glycogen level, expressed as dry tissue content was 290±99 mmol glucosyl units$\cdot kg^{-1}$ d.w. Therefore the energy supply from glycolysis may well not have been compromised. However, the significantly smaller glycogen utilisation ($p< 0.05$) and the significantly lower blood lactates ($p< 0.01$) observed during the intermittent exercise in the U condition relative to the R condition, suggests there was a reduced involvement of glycogenolysis when pre-exercise glycogen was depleted. Even if the rate of glycogenolysis was reduced in the U condition it may be as Hultman and

Sjoholm (1983) suggest, that the glycolytic rate is relatively well maintained through the uptake and use of blood glucose.

Because the average amount of work done during the first three bouts of exercise is significantly less ($p< 0.05$) in the U condition than the R condition, suggests a reduced energy supply during the early stages of the intermittent exercise. However, the difference in work done between the two conditions is almost entirely due to the eccentric phase of the exercise, with no significant differences in performance between the two conditions when the concentric contractions are considered alone. Similarly several significant differences exist between the peak eccentric torques between conditions, whereas the peak concentric torques are only significantly different between conditions for the first exercise bout. It is not clear why the manipulation of intramuscular levels of glycogen should have a greater effect on eccentric when compared to concentric contractions. No evidence exists to suggest that eccentric contractions have a greater sensitivity to low levels of glycogen than do concentric contractions. One possibility, suggested by electromyographic data (section 2.1.3), is that even within the same muscle the two types of contraction do not involve identical motor units. Ultrastructural damage, incurred by the eccentric contractions involved in the glycogen depleting exercise may have caused a delayed repletion of muscle glycogen in the muscle fibers involved in eccentric contraction (O'Reilly et al., 1987). This delayed repletion in conjunction with a low carbohydrate diet may have resulted in the performance decrements observed during eccentric contractions in the U condition.

However, irrespective of contraction type, the largest decreases in work output from exercise bouts 1 to 10 occurred in the R condition. A possible explanation for these findings is that there was a greater involvement of glycolysis during the R condition and as a consequence a greater muscular acidosis. During maximal exercise even small changes in pH as a result of lactic acid production, may exert a profound influence on metabolism by affecting the charge of certain ionisable groups on key enzymes and their substrates.

The rate of ATP utilization during 30 seconds of maximal exercise has been calculated to be approximately 6.10 $mmol \cdot kg^{-1} \cdot s^{-1}$ (Cheetham et al., 1986). Applying a similar calculation to the metabolic changes observed by Boobis et al. (1982) it is clear that the ATP turnover rate would be in excess of twice this figure during 6 seconds of maximal exercise. If, as Boobis suggests, PCr breakdown accounts for approximately

half of the ATP resynthesis during a 6 second bout of exercise, then a series of these bouts performed every 30 seconds would result in progressively lower and lower levels of PCr at the start of each exercise bout. It has been suggested that fatigue during maximal intermittent exercise could result from insufficient PCr resynthesis during recoveries (Wootton and Williams, 1983). Indeed in isolated muscle preparations where glycolysis is blocked with iodoacetic acid (IAA), the decrease in force production parallels the exhaustion of the PCr store (Hultman and Sjoholm, 1986).

However, if PCr degradation proceeded at the above calculated rate, then no more than 40 $mmol.kg^{-1}$ would be used during each exercise bout. Assuming resting levels of approximately 80 $mmol{\cdot}kg^{-1}$ and a half time of PCr resynthesis of 22 seconds (Harris et al., 1976), then energy supply from PCr degredation would not be compromised until the end of the third or fourth exercise bout, when the concentration of PCr may not be sufficient to fully saturate the active sites of creatine kinase. However, investigation of performance during brief maximal exercise commonly reports an almost immediate decline in peak power output after the first 2-4 seconds of exercise (Cheetham et al., 1986; Nevill et al, 1989), which is not explained by the above theory of energy supply.

The causes of fatigue in this case must also include factors other than a decreased availability of PCr. Fatigue during maximal exercise is most commonly associated with an accumulation of H^+ in the active muscle. There are several possible mechanisms by which H^+ might contribute to a reduction in performance during maximal intermittent exercise. Firstly by altering the equilibrium constant of creatine kinase (Sahlin et al., 1975) thereby further hindering the resynthesis of PCr during recovery periods. This effect might be particularly pronounced in FT fibers which have the greatest activity of creatine kinase and demonstrate the largest rate of lactate accumulation (Tesch et al., 1989). If this were the case, then levels of PCr in the fibers active during high intensity exercise would be lower than indicated by analysis of biopsy samples. Secondly an increase in H^+ has been shown to inhibit the activity of PFK in vitro. However in Chapter II the reversal of the pH dependent inhibition of PFK, by positive modulators of the enzyme has already been discussed. Thirdly evidence exists for an inhibitory influence of low pH on phosphorylase, which would lead to a reduced use of glycogen as a supply of substrate for glycolysis.

A possible explanation of energy supply during maximal bursts of exercise

similar to those employed in this study, which could account for the observed fatigue during high intensity intermittent exercise is as follows. During maximal muscle contractions ATP is hydrolysed and the product, ADP is almost immediately phosphorylated to ATP by PCr at the site of contraction. The products of ATP hydrolysis, Pi and ADP stimulate the activation of phosphorylase, leading to an accumulation of glycolytic intermediates. These metabolites lead in turn, to an activation of PFK, which governs the rate at which glycolysis proceedes. Peak power outputs are achieved during the first few seconds as the optimal limb velocity is reached and the rates of PCr breakdown and glycogenolysis achieve maximum velocity. As exercise continues rapid decreases in the concentration of PCr occur and H^+ (from glycolysis) accumulates. The initial decrements in performance may be due to the concentration of PCr falling below the level required for maximal turnover of creatine kinase and an acidotic inhibition of the maximal rate of glycolysis. That the initial reduction in power output occurs due to the limitation of glycolytic rate, would be supported by the increased initial power outputs observed in individuals after sprint training (Cheetham, 1987). Such a training response is associated with increases in glycolytic enzymes but no changes in pre-exercise PCr concentration.

After several bouts of exercise, the accumulation of H^+ may begin to exert a strong negative influence on the equilibrium constant of creatine kinase, thereby reducing further the amount of PCr available for ATP supply. The very high concentrations of H^+ accumulating during the latter bouts of exercise may, as Hermansen has suggested, have a dual role in the development of fatigue, affecting both energy supply and the ATP utilisation by the contractile mechanism itself (Hermansen, 1981). As ATP can be reduced to zero in IAA poisoned muscle, resulting in rigor and irreversible damage, the role of H^+ might be regarded as a vital protection mechanism, safeguarding against the complete exhaustion of energy yielding substrates and muscle damage.

The results of previous studies involving brief bursts of high intensity exercise and low glycogen availability would suggest that a reduction in glycogenolysis per se does not limit a single bursts of maximal exercise (Symons and Jacobs, 1989). Intermittent maximal exercise however, takes place over a longer duration than continuous maximal exercise, during which fatigue develops very rapidly and glycogen stores remain relatively undiminished. The low muscle glycogen concentrations found

by Jacobs et al. (1982c) following soccer, and the reduced intermittent exercise performance of subjects consuming a low carbohydrate diet (Bangsbo et al., in press b), suggest glycolysis is of primary importance for energy supply during intermittent maximal exercise.

In the present study the performance of intermittent maximal exercise was impaired, only to a limited extent by a reduced pre-exercise concentration of muscle glycogen. The main effects were lower initial levels of work output and peak torque, which was mainly accounted for by the eccentric phase of contraction, and a smaller decline in work output throughout the intermittent exercise. As was previously pointed out, the effects on eccentric contraction could have been due to muscle damage resulting from the prior exercise, designed to deplete muscle glycogen stores. No major differences in performance between the two conditions were found for concentric contractions despite the reduced rate of glycolysis, suggested by the lower glycogen utilization and lower blood lactates in the U condition. One possible conclusion from this evidence is that glycolysis is of secondary importance for energy supply during maximal exercise, and that it only makes a major contribution when PCr becomes depleted and exercise intensity declines. It must be remembered that team games involve a great deal of aerobic work and the low levels of muscle glycogen found by Jacobs et al. (1982c), after soccer may well reflect glycogen degradation occurring during the low and high intensity phases of the game.

However the marked accumulation of glycolytic intermediates and muscle lactate observed by Boobis et al. (1982) after 6 seconds of maximal cycling and Hultman and Sjoholm (1983) after 1.26 seconds of electrical stimulation is almost irrefutable evidence that glycolysis makes a major contribution to energy supply during brief bursts of maximal exercise. Despite these findings, reduced concentration of muscle glycogen, the immediate substrate for glycolytic energy supply, has not yet been conclusively demonstrated to impair the performance of high intensity exercise, or in this case, of brief bursts of intermittent maximal exercise. The explanation for this anomaly is unclear, but may be due to a combination of an inability to sufficiently deplete muscle glycogen stores in the experimental situation and/or a lack of sensitivity in the measurement of maximal exercise performance. However the consistency of these findings suggests they are not simply due to experimental artifact.

The possibility exists that a reduced energy supply from glycolysis can be compensated for by the hydrolysis of PCr until stores of PCr itself become depleted

It has been suggested that the ATP produced from glycolysis is used for the phosphorylation of creatine in the cytoplasm and that the formed PCr diffuses (or is shuttled via a translocase) with relative ease (when compared to ATP) to the site of utilisation on the myosin heads. The fact that an isoenzyme of creatine kinase has been found to be integral with the myofibrils lends some support to this theory (Bessman and Geiger, 1981). If this hypothesis were correct then the rate of energy utilization during maximal exercise would be compromised when muscle PCr became depleted and ATP from glycolysis were directly hydrolysed at the site of contraction. This hypothesis accounts for both the marked involvement of glycolytic energy supply during brief high intensity exercise, and the absence of an impairment of maximal force production with low muscle glycogen concentrations. If this were the case then a higher rate of ATP supply during the first few exercise bouts would lead to an early depletion of PCr and a rapid development of fatigue. This is to some extent supported by the relationship found between the work output in the first exercise bout and the degree of subsequent fatigue in the U condition ($r = .80$), when PCr breakdown would be (theoretically) the most important source of ATP supply. However the initial decrease in power output is difficult to account for, but may be due to reductions in the efficiency of certain key enzymes as a result of H^+ accumulation.

Thus the finding of little impairment of performance when muscle glycogen concentration is reduced may not reflect the unimportance of glycolytic energy supply during repeated bursts of maximal exercise. Instead it illustrates that the interrelationships between the two anaerobic energy yielding pathways and the site of utilisation of ATP, may not be as straightforward as has previously been assumed. Current knowledge is still unable to accurately account for the observed decline in power output during maximal exercise and there is a need for further research to clarify the events occurring at a cellular level concurrent with the development of fatigue during brief bursts of maximal exercise.

With reference to the original research questions, the following conclusions may be drawn from the experimental evidence and literature reviewed in this study:

1. The lower rate of glycogen use found in the U condition shows that glycogenolytic rate is reduced when intramuscular glycogen stores are lowered through dietary manipulation. The lower blood lactate concentrations in the U

condition suggest that the reduced glycogenolytic rate leads to a reduction in the glycolytic rate.

2. Significant decrements in work output and peak torque were found between exercise bouts 1-10 in both conditions. Previous investigation has suggested that, for highly motivated subjects, these decrements result from the combined effects of decreasing pH and a progressive depletion of PCr stores in active muscle.

3. Greater performance decrements and higher blood lactates were observed in the R condition which suggests that intramuscular pH was lower than in the U condition. Since PCr concentrations would be similar between conditions, or possibly lower (in the latter bouts) in the U condition, it would appear that muscular acidosis rather than PCr depletion has the more important role in the development of fatigue during high intensity intermittent exercise.

4. The lower eccentric work outputs observed during the first three bouts of exercise in the U condition suggests that glycolysis is crucial for the development of maximal force during muscular contraction. However, the finding of no significant difference in concentric performance between conditions suggests that the phosphocreatine system can, for certain types of muscular contraction, compensate for a reduced glycolytic energy supply when intramuscular glycogen concentration is reduced.

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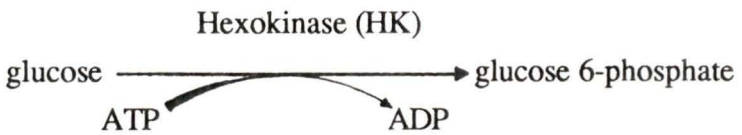
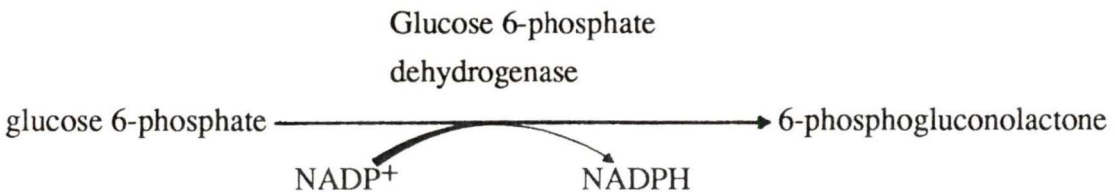
APPENDIX A.

Fluorometric method for the determination of glycogen in muscle

PRINCIPLE: Acid hydrolysis of the glycogen macromolecule yields free glucose which can be assayed through the following series of reactions:





The appearance of NADPH is used to quantify the concentration of glucose residues. This may be determined by spectrophotometric or fluorometric methods and is taken as the concentration of glycogen in the muscle.

EQUIPMENT: Double monochromator fluorometer (e.g. Aminco SPF 125).
Water bath 37° C
Fluorometer cuvette with all surfaces polished.
Oven.
100 µL and 1ml air displacement pipettes.
Glass homogenizer.

REAGENTS: Glucose (HK) reagent. Sigma catalog # 16-UV.
Distilled water.
Glucose standard solution (100mg/dL). Sigma catalog # 635-100.
4N HCl.
2N NaOH.

PROCEDURE: **A: Tissue preparation and hydrolysis**

1. Turn on oven to 100° C.
2. Remove muscle sample from freezer and cut off a piece of 5 - 15 mg. Avoid connective tissue.
3. Weigh and record.
4. Place muscle sample in a glass homogenizer containing 0.25 mL of distilled water and homogenize sample thoroughly. If obvious pieces of connective tissue remain, remove, pat dry and weigh them. Subtract their weight from the original value for the sample.
5. Wash the pestle with a futher 0.25 ml of distilled water and collect this in the homogenizer.
6. Carefully add 0.5 ml of 4N HCl to the homogenate.
7. Agitate the homogenizer to ensure complete mixing and pipette off 0.8 ml of the homogenate into a culture tube.
8. Weigh and record (both homogenate and tube to $\pm$ 1mg).
9. Cover tube with a glass marble and place it in an oven at 100° C for 2 hours. Gently agitate samples occasionally.
10. Reweigh and reconstitute the volume lost to evaporation, i.e. to the original weight, with distilled water using a 100 µL pipette.
11. Neutralise by adding 0.8 ml of 2N NaOH. Shake sample until all muscle is dissolved. Using pH paper check the neutrality of the sample.

B: Determination of glycogen concentration

12. Label five cuvettes 1 - 5 and add the following solutions to each:

Standard #	Glucose stock (mL)	Distilled H_2O (mL)	Glucose conc. (mg/dL)	(mM/L)
1	0.00	10.00	0	0
2	0.10	9.90	1.0	.055
3	0.25	9.75	2.5	.139
4	1.00	9.00	10.0	.555
5	1.50	8.50	15.0	.834

13. In duplicate, add 24 µL of each standard to a culture tube containing 1.2 mL of hexokinase reagent (i.e. a 1: 50 sample to reactant ratio).
14. Place the culture tubes in a water bath at 37° C, and record the fluorescence of each after 45 minutes.
15. Plot a calibration curve.
16. Repeat steps 13 and 14 with the samples instead of standards. Determine the glucose concentration (mM) of each sample using the calibration curve.
17. Calculate the concentation of glycogen originally present in the muscle using the following equation:

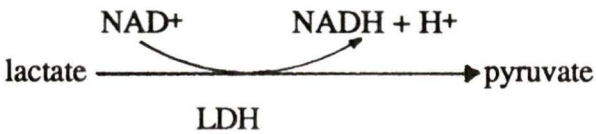
$$\textbf{Muscle glycogen concentration (mM/kg)} = \frac{\text{Glucose concentration (mM)} \times 2\text{ml}}{\text{weight of muscle sample (mg)}}$$

This procedure also measures muscle glucose and glucose 6-phosphate, but their concentrations, compared to that of glycogen are considered insignificant.

Spectrophotometric method for the determination of lactate in blood.

(Variation of Sigma Technique No. 826 U.V.).

PRINCIPLE:



EQUIPMENT:
1. Water bath 37 ° C
2. Spectrophotometer (Philips PU8600)
3. Cuvettes (matched set)
4. 5ml and 1ml air displacement pipettes

REAGENTS:
1. Distilled water at room temperature
2. Glycine buffer
3. Lactate dehydrogenase
4. NAD+
5. 4% Perchloric acid
6. Lactic acid standard

PROCEDURE:
1. Prepare reaction mixture

Glycine buffer	6.0 ml
NAD	30 mg
Distilled H_2O	0.7 ml
LDH	0.3 ml

2. Prepare standards

Cuvette No.	Reaction mixture (ml)	Distilled water (ml)	Lactic acid Solution (ml)	Concentration ($mmol.l^{-1}$)
1	1.0	2.0	0.0	0.00
2	1.0	1.9	0.1	2.22
3	1.0	1.8	0.2	4.44
4	1.0	1.7	0.3	6.67
5	1.0	1.6	0.4	8.89
6	1.0	1.5	0.5	11.11

3. Add 2.8 ml of reaction mixture to 0.2 ml aliquots of samples, standards and perchloric acid blanks in duplicate. Mix well.
4. Incubate at 37 ° C for 25 minutes.
5. Record the absorbance and determine the lactate concentration of samples by plotting a calibration curve.

Myosin ATPase stain

PRINCIPLE:

This histochemical reaction depends upon a complex series of reactions for the production of its end product. The tissue section is incubated in a solution containing ATP and calcium. The enzyme ATPase splits off the terminal phosphate from ATP and this phosphate reacts immediately with the calcium in the solution to form calcium phosphate. At an alkaline pH the calcium phosphate is insoluble and is deposited at the site of enzyme activity. The section is then exposed to cobalt chloride and the cobalt is exchanged for the calcium where calcium chloride was previously present. the section is then exposed to ammonium sulphide which results in the formation of a black, insoluble, cobaltous sulphide. Thus, the site of enzyme originally present is demonstrated.

Type II fibers, rich in myosin ATPase, stain more darkly than type I fibers with an alkaline preincubation. However, preincubation at pH 4.3 results in a reversal of the above staining pattern so that type II fibers no longer stain and type I fibers stain heavily.

REAGENT PREPARATION:

Alkaline stock solution

Add 2.253 g glycine, 2.40 g $CaCl_2$, 1.755 g NaCl, 1.08 G NaOH (25 ml of 1N NaOH) and bring to a final volume of 400ml with water. Adjust to pH 9.4. Store in freezer.

Alkaline preincubation solution

Remove 10 ml of the alkaline stock solution and readjust to pH 10.3 using 1N NaOH or 1N HCl as needed.

Acid preincubation stock solution

This is a 0.2 M stock barbital acetate buffer. Add 1.94 g NaOAc, 2.94 g sodium barbital (controlled substance) to 100 ml of water. Store in freezer.

Acid preincubation solution

For each jar of acid preincubation solution add 2.5 ml of 0.2 M barbital acetate buffer to 5.0 ml of 0.1 M HCl and 4.0 ml distilled water. Adjust to pH 4.3 using 1N NaOH or 1N HCl as needed.

Incubation solution

Add 17 mg ATP to 10 ml of the alkaline stock solution and adjust to pH 9.4 with 1N NaOH or 1N HCl as needed.

PROCEDURE:

1. Preincubation

   Acid

   Add approximately 10 ml of the acid preincubation solution to its a Columbia jar. Place labelled, glass microscope slides with attached, dehydrated muscle sections (2 microns thick) in the jar and agitate slightly. Incubate at room temperature for 5 minutes.

   Alkaline

   Add approximately 10 ml of the alkaline preincubation solution to its a Columbia jar. Place labelled, glass microscope slides with attached, dehydrated muscle sections (2 microns thick) in the jar and agitate slightly. Incubate at room temperature for 15 minutes.

2. Incubation.

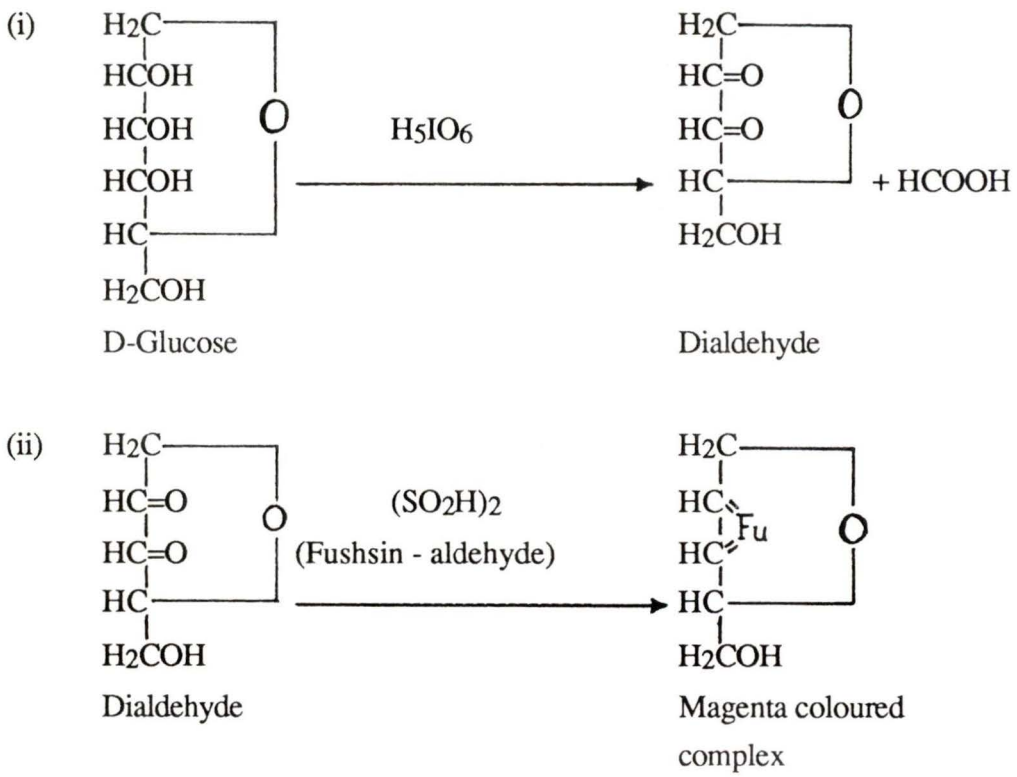
   Gently rinse preincubation solution from slides with water. Place slides in a new Columbia jar, containing 10 ml of the incubation solution and incubate in a shaker bath at 37 °C for 45 minutes.

3. Rinse twice with water.
4. Incubate in 1% $CaCl_2$ for 3 minutes. Rinse well with water.
5. Incubate in $CoCl_2$ for 3 minutes. Rinse well with water.
6. Incubate in 1% $(NH_4)_2S$ for 1 minute. Rinse well with water and allow to dry.
7. Dehydrate in ascending alcohols (80%, 95%, 95%, 100%, 100%).
8. Clear in Xylene.
9. Mount with "Histoclad" mounting medium.

Periodic acid schiff's stain

(Variation of Sigma Technique No. 395)

PRINCIPLE: The purpose of this staining technique is to visualize glycogen. This is accomplished by first oxidizing the glucose residues to dialdehydes by addition of periodic acid (i), and then visualizing these dialdehydes by the addition of Schiff's reagent (ii).



REAGENTS:
1. Periodic acid solution (0.5%)
2. Schiff's reagent
3. Carnoy's Fixative (ethanol, chloroform and glacial acetic in the ratio 6:3:1)
4. Distilled water

PROCEDURE:1. Place slide with muscle section adhering to it a Columbia jar and add Carnoy's fixative for 5 to 10 minutes. Rinse with distilled water.

2. Place the slides in a new Columbia jar containing 0.5% periodic acid for 4 minutes. Then rinse with distilled water.
3. Place in Schiff's reagent for 15 minutes.
4. Rinse with tap water for 10 - 15 minutes.
5. Dehydrate in ascending alcohols (80%, 95%, 95%, 100%, 100%).
6. Clear in Xylene.
9. Mount with "Histoclad" mounting medium.

APPENDIX B.

Physical chacteristics of the subjects

Subject	Age (years)	Height (cm.)	Weight (kg)	$\dot{V}O_2$max. (ml/kg/min.)
1	21	168.5	71.3	49.7
2	23	176.7	76.1	51.5
3	29	174.3	69.5	55.4
4	20	176.0	69.5	51.6
5	23	184.5	77.7	50.6
6	24	173.8	75.6	53.9
7	26	175.5	79.7	49.7
8	20	172.9	81.8	43.6
9	25	179.7	82.1	46.0
10	23	173.5	74.4	66.1
mean	23	175.5	75.8	51.8
S.D.	2.8	4.5	4.7	6.1

APPENDIX C.

Outline and informed consent form

PURPOSE: To investigate the influence of muscle glycogen concentration on the ability to perform repeated, brief bursts of maximal exercise.

PROCEDURE:

Week 1

You will be asked to exercise on a cycle ergometer with progressively increasing work loads to elicit maximal oxygen consumption. You will also undergo one refamiliarization session on the KinCom.

Week 2 & 3

After a standardized warm up you will be asked to perform ten bouts of maximal exercise of the right leg on an isokinetic ergometer at 180°/sec. Each bout will last for six seconds and there will be thirty seconds of passive recovery between bouts of exercise.

You will be asked to perform this exercise protocol on two occasions. Once in a "low glycogen" state (L.G.), and once in a "high glycogen" state (H.G.). The L.G. state will be achieved by glycogen depleting exercise followed by approximately 48 hours on a low carbohydrate diet. The H.G. state will be achieved by glycogen depleting exercise followed by approximately 48 hours on a high glycogen diet. In both cases the intermittent exercise described above, will take place immediately following the dietry manipulations. Subjects may experience some fatigue in the L.G. state.

The glycogen depleting exercise will consist of 45 minutes of sub-maximal cycling at anaerobic threshold followed by three bouts of maximal isokinetic exercise of the right leg at 180°/s. Each bout will continued for 50 repetitions. Short periods of rest (approximately 15 minutes), will be given between each activity.

BLOOD AND MUSCLE SAMPLES:

A small piece of muscle from the thigh (vastus lateralis) will be obtained by needle biopsy before and after the intermittent exercise test, on both occasions. There will be a total of four biopsies. Small amounts of

blood (2ml) will be obtained from a venous catheter pre-exercise after three bouts of exercise, and 1 minute and 3 minutes post exercise. For the muscle biopsy a 5mm incision is made in the skin and muscle fascia under local anaesthetic and a hollow needle inserted approximately 3 cm into the muscle. The needle will extract a small amount of tissue (approximately 60 mg). There will be little or no discomfort associated with the procedure, however, you will experience a sensation of pressure. Rarely you could experience some muscle spasm.

POSSIBLE RISKS AND DISCOMFORTS:

The needle biopsy will be performed by a skilled physician and the blood sample by certified personel. Both procedures are considered safe with little risk of complication, although as with any laceration there is some risk of infection. Several hours after sampling you may experience minor bruising at the biopsy site.

CONSENT:

I have read the above and agree to participate in this research project at my own risk. I am nineteen years of age or older and regularly take part in strenuous physical activity at least as intense as these tests. I realize that I may expect a thorough explanation and/or demonstration of any procedures and that I may terminate participation at any time in any or all procedures of my own volition.

Having voluntarily assumed participation and risks thereof in the project, I hereby disclaim and release the University of Victoria, its agents, servants or employees, including all personel involved in the research project, from any liability that might otherwise arise as a result of any participation as a research subject in this study.

NAME:_____

(please print)

DATE:_____ SIGNATURE:_____

Low carbohydrate diet

ALLOWABLE FOOD LIST

The following is a list of 'allowable foods' that can be consumed during meals. Please select those foods you wish to eat, **but only in moderation.** The quantity listed beside each item is the amount suggested per meal.

Bacon - (3 strips)
Cheese - cream, swiss, American
Eggs - (2)
Ham - canned (4-6 oz.)
Kidney, beef, lamb (4-6 oz.)
Lettuce
Margarine or butter (2 tbsp.)
Olives
Parsley
Green peppers
Mayonnaise (1 tbsp.)
Sauces - Hollandaise, Tartar
- low calorie salad dressing (1tbsp.)
Saussage - Frankfurter, Liverwurst, pork, salami, Vienna
Luncheon meat - pork (cured, canned, packaged)
Shrimp - (2-3 oz.)
Crab / tuna / salmon - (2-3 oz.)
Fish (unbreaded) - (3-4 oz.)
Chicken - (2-3 pieces)
Natural yogurt - (1 cup)
Avacado - (1)
Croissant - (1)

DOs		DON'Ts
√ Do eat food high in fat and protein	X	Don't have more than one piece of bread with each meal
√ Do drink low calorie/diet pop	X	Don't eat vegetables or fruits
√ Do eat mainly meat, eggs and cheese	X	Don't eat cereals, grains, pasta or pastry
	X	Don't eat candy or sweet foods
	X	Don't drink fruit juice
	X	Don't take sugar in coffee or tea

SUGGESTED MEALS

Breakfast

- Cheese, ham, eggs and sausage
- A croissant rather than bread
- An omlette (with cheese, ham, raw onion, or green pepper)

Lunch

- Cheese burger with bacon (Only half a bun)
- Fried chicken
- Tuna / shrimp and mayonaise with lettuce
- Open sandwich or melt (with cheese, ham, luncheon meat or avocado)
- Quiche (remove crust)

Dinner

- Fried chicken
- Steak in bernaise sause
- lamb or pork chops
- Ground beef with green peppers and onions

Snacks

- Celery and cream cheese
- Egg and mayonaise sandwich in a croissant
- Avocado and cream cheese

High carbohydrate diet

While the caloric intake of this diet is unrestricted, it may be necessary for you to reduce the amounts of fats and proteins you normally eat to ensure that the majority of the diet consists of carbohydrate.

	DOs		DON'Ts
√	Do eat pleanty of cereals, grain, pasta and bread etc.	X	Don't eat fried foods
√	Do eat lots of fruit and vegetables	X	Don't eat much added fat (e.g. butter, mayonaise gravy etc.)
√	Do reduce your intake of red meat and cheese	X	Don't drink alcoholic beverages
√	Do drink fat reduced milk rather than whole milk		

MEAL SUGGESTIONS

Breakfast

- Cereals, pancakes, waffles, toast, bagels
- syrup, honey, jam, marmalade etc.
- Fruit juice, coffee / tea with sugar if prefered
- Muffins, cinamon buns

Lunch

- Pasta, sandwiches, baked potato, vegtables
- Donuts, cookies, muffins etc.
- Fruit juice, full calorie soft drinks

Dinner

- Broiled chicken, potatos, pasta, rice, vegetables
- Corn on the cob
- Paistries, sweetened yoghurt, ice cream

Snacks

- Popcorn
- Candy bars
- Raisins

VITA

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Holmyard, D.J., Cheetham, M.E., Lakomy, H.K.A. and Williams, C. (1987). Effect of recovery duration on multiple treadmill sprint performance. In T. Reilly, A. Lees, K. Davids and W.J. Murphy (Eds.), Proceedings of the First World Congress of Science and Football, (pp 134-142), London, E. & F.N. Spon.

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Author 

DAVID JAMES HOLMYARD

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