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THE EFFECTS OF SELECTED INTENSITIES OF REPEATED ISOMETRIC KNEE EXTENSION EXERCISES UPON SUBSEQUENT TOTAL PLASMA CREATINE KINASE ACTIVITIES

by

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Submitted to the School of Graduate Studies
and the Department of Kinanthropology
in partial fulfillment of the requirements
for the degree of Master of Science in Kinanthropology

School of Human Kinetics
University of Ottawa

1987

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ISBN 0-315-40694-1

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DEDICATION

I am dedicating this thesis to my grandmother, Rose Chin. Throughout my life, she has encouraged me to seek happiness through the acquirement of knowledge and the love of God. I would not have met success without such loving inspiration and support.

ACKNOWLEDGEMENTS

In completing this thesis, I found myself deeply indebted to many of my friends who, at the very least, deserve to be mentioned here.

Sincere thanks must first be extended to my advisor, Dr. Alfred Reed, for his interest and critical eye for details. I must also mention that he not only provided me with financial support for my study, but also more than my fair share of his valuable time.

Many thanks must also go to Drs. Frank Reardon, James Thoden, Michael Booth, and Donald Dickie for their helpful suggestions and their friendship.

Thanks go to all of the subjects who volunteered many hours of testing and endured the pain of countless blood samples. However, in my next study I may consider using rats.

As well, a word of appreciation must go to Pearl Lok, Christine Hakim, and Ralph Fournier for their technical assistance and good nature in the face of my many demands. Bill Montelpare and Ryan Stark also provided me with technical assistance in the area of computer word processing and graphics.

Special thanks must go to Glenn Garneys, Pearl Dixon, Nancy and Bill Montelpare, Anne and Pete Saar, and Deb Hutchinson. Although many miles now separate us, I will always remember and cherish their friendship, support, and love.

I also owe a great deal of thanks to Gladys and Morley Chin, as well as Karen and Jeffrey Chin. They always kept me well fed and in good spirits.

My deepest appreciation and love go to my parents for providing me with the opportunity to continue my education and for their loving support and patience for all of these years.

And finally, I must thank Eva for cheering for me at the finish line. It would not have been the same without her there.

ABSTRACT

A large amount of research attention has been given to the observation that serum or plasma activity levels of creatine kinase (CK; E.C. 2.7.3.2) increase following exercise. However, an accurate picture of this relationship is still not complete. The purpose of this investigation was to observe the effect of controlled isometric exercise, at three selected intensities, upon the post-exercise pattern of total plasma CK activity.

Nine sedentary male volunteers, between the ages of 21 and 31 years, participated in this study. On three separate exercise days, each subject was required to repeat a series of two-legged isometric knee extensions, under controlled conditions, at 10%, 30%, and 50% of his pre-measured maximal voluntary contraction (MVC). In order that the total impulse among each of the exercises was constant, the total number of contractions varied, depending upon the amount of isometric force to be exerted (i.e. 10% MVC x 105 contractions, 30% MVC x 35 contractions, and 50% MVC x 21 contractions). In all cases, contractions lasted for 10 seconds each, followed by a 20 second rest interval.

Blood samples were taken pre-exercise, post-exercise, 2, 4, 6, 8, 10, 12, 14, and 24 hours post-exercise. Total plasma CK activity was assayed in these samples by a photometric method.

The following was observed in this study:

1. Significant increases above pre-exercise levels in the total plasma CK activity appeared following only the treatment intensity of 50% MVC.

2. No significant changes in total plasma CK activity were found following or between the treatment intensities of 10% MVC and 30% MVC.

3. The levels of total plasma CK activity rose significantly above pre-exercise levels as early as 4 hours following the 50% MVC trials. A further significant increase, beyond the level of activity at 4 hours post-exercise, occurred at the 12 hour point, and remained so for the subsequent 14 hour sample.

4. A significant interaction exists between the treatment intensity and the time of sampling. Differences between the 50% MVC treatment and the 30% or 10% MVC treatment were not evident until 12 hours post-exercise.

In addition, it was demonstrated that a small amount of exercise, such as that used in the present study, would evoke significant changes in the measured total plasma CK activity, lasting for as long as 24 hours post-exercise. Also, the outlined experimental pre-test conditions and screening procedure produced exceptionally good estimates of, what appear to be, basal levels of total plasma CK activity, in each individual. Such procedures would be recommended for similar research which monitors total plasma CK activity.

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Chapter I

THE PROBLEM

1.1 Introduction

Creatine kinase (CK; EC 2.7.3.2.) is an enzyme which increases the supply of high energy phosphate, as it reversibly catalyzes the production of phosphocreatine and ADP from creatine and ATP. There are three main CK isoenzymes, each of which is principally, but not exclusively, found in different types of tissues; skeletal muscle (the "MM" form), cardiac muscle (the "MB" form), or brain tissue (the "BB" form). However, it has been reported that variable amounts of CK also normally exist in the blood (Griffiths, 1966; McCormick, 1976; Priest et al., 1982; Bais and Edwards, 1982).

A large amount of research attention has been given to the observation that total serum activity levels of CK increase following exercise. Unfortunately, the precise mechanisms by which CK isoenzymes are released into the blood have not yet been firmly established.

A variety of approaches have been taken in past research in attempts to determine the quantitative and qualitative factors which result in, or affect, high levels of serum CK activity. Although Sanders and Bloor (1975) suggest that the measurement of CK activity is one of the most sensitive indices of acute exercise stress, research findings, in several areas, are yet far from conclusive.

Experimental studies have been done which support the idea that exercise intensity is a major influence on increases in total serum CK activity (Shapiro et al., 1973; Schwartz et al., 1971; Cerny and Haralambie, 1983; Tiidus and Ianuzzo, 1983). These studies have indicated that post-exercise elevations in total serum CK activity have been higher after high intensity exercise, as opposed to relatively lower intensity exercise.

Likewise, the effect of exercise duration upon total serum CK activity has also been demonstrated (Griffiths, 1966; Schnohr, 1974; Riley et al., 1975; Berg and Haralambie, 1978). In the study by Berg and Haralambie (1978), serum enzyme activity increased in proportion to the exercise duration, under particular conditions, and according to the type of exercise.

Studies which have been concerned with the possible effects of the total work requirement on the post-exercise total serum CK activities are few (Cerny and Haralambie, 1983; Tiidus and Ianuzzo, 1983). So far, a strong relationship has not been shown between the total work done and the subsequent post-exercise total serum CK activities measured.

A study on the effects of physical conditioning was conducted by Nuttall and Jones in 1968. Studies by other researchers have followed (Burke et al., 1982; Buyze et al., 1976; Hunter & Critz, 1971; Sanders & Bloor, 1975; Shapiro et al., 1973). It has been generally found that the increases in post-exercise total serum CK activity is less for a well-conditioned group, as opposed to an unconditioned group, after both have been subjected to the same absolute workloads. Thus, it was the

suggestion of Nuttall and Jones (1968) that "an index of the degree of physical conditioning attained by an individual in a physical training program could be developed with the rise in serum CK activity following a standardized type and duration of exercise". A number of other studies (Hunter and Critz, 1971; Shapiro et al., 1973) have supported the work of Nuttall and Jones (1968) but no specific enzymatic "indices" of physical condition have yet been established. Schwartz et al. (1971) suggested that" the serum enzyme responses to exercise may be more closely related to the training of specific muscle groups than to general physical condition", (represented by a measure of maximal oxygen consumption).

In the light of this suggestion by Schwartz et al. (1971), the type or nature of an exercise also seems likely to have some effect on the total serum CK activity response. Berg and Haralambie (1978) showed that total serum CK activity varied in each of three types of exercise examined. These were categorized according to the amount of impact-type stress which seemed to be applied to the muscles. The increases in total serum CK activity were found the greatest in the highest of impact-type exertions per unit of time. Clarkson et al. (1982) and Schwane et al. (1983) have given support for the idea that it is the amount of tension developed within the muscle fibres, which affects the changes in total serum CK activity. Eccentric-type exercise, in particular, has been identified as a type of activity which produces large elevations in total plasma CK activity (Schwane et al., 1983; Armstrong et al., 1983).

In one aspect of the study by Tiidus and Ianuzzo (1983), subjects performed a dynamic leg exercise at percentages of their ten repetition maximum (10RM); that is, the maximum weight which could be lifted ten times. The results showed that the total serum CK activity was not significantly higher than the pre-exercise levels following 150 repetitions at 35 percent 10RM, but were significantly higher at 70 percent 10RM and 90 percent 10RM. This suggests that a threshold intensity may exist above which significant increases in total serum CK activity are seen. In view of the previous suggestions by Clarkson et al. (1982) and Schwane et al. (1983), this threshold, if it does indeed exist, might be more aptly a function of tension or actual force development than of repetition maximums. This remains to be investigated.

The course of total serum CK activity changes, over a post-exercise period of time, has been shown to vary among studies. It has been found that the rise in serum CK activity levels begins either some time during the exercise (Griffiths, 1966; Critz and Cunningham, 1972; Shapiro et al., 1973; Schnohr, 1974; Sanders and Bloor, 1975; Chahine et al., 1976; Berg and Haralambie, 1978; Steele et al., 1978; Schnohr et al., 1980), or briefly afterwards (Nuttall and Jones, 1968; King et al., 1976; Clarkson et al., 1982).

The time at which peak levels in serum CK activity are reached have also varied among studies, from less than one hour (Block et al., 1969), to six hours (Clarkson et al., 1982), to eight to sixteen hours (Nuttall and Jones, 1968), to eleven to nineteen hours (King et al., 1976), to twenty-five and one-half hours (Buyze et al., 1976), and to even four or

five days post-exercise (Newham et al., 1983). Unfortunately, comparisons among many of these studies are difficult to make because of the several factors which appear to affect CK efflux and because of differences among the protocols used in each experiment.

One of the major problems in achieving accuracy in the enzyme analyses has been in adequately controlling for the several other factors which may affect the total serum CK activity. Also, the determination of the actual peak level of total serum CK activity is difficult to determine because of its delayed increases after exercise and the apparent variance between individuals.

In the light of the various research which has been done, a number of determinants of CK efflux into the serum seems likely, although one may not be exclusive of the others. Firstly, as earlier suggested by Sweetin and Thomson (1973), intracellular enzymes may be retained through some role of ATP, and not simply the cell membrane. Furthermore, a reduction in the intracellular levels of ATP may lead to ionic imbalances and, subsequently, conditions which render the cell susceptible to injury.

Another possible determinant of increases in total serum CK activity which seems likely, is injury to the cell as a result of trauma, and the subsequent leakage of the enzyme into the blood. In consideration of these two possible determinants of CK efflux, it would appear that many studies have indicated that either or both may play a significant role. However, investigations have primarily observed the effects of high levels of intensity or duration of physical exertion upon changes in total serum CK activity.

It may be interesting to observe the patterns of change in total serum CK activity as a result of relatively low intensities of exercise. Through such an investigation, a threshold intensity may be found, below which total serum CK activity does not change post-exercise.

1.2 Rationale

In spite of the extensive research into the relationship between total serum CK activity levels and exercise, an accurate picture of this relationship is still not complete. In the interest of using total serum CK activity as a measure of an individual's specific level of conditioning in a particular activity, as earlier suggested (Nuttall and Jones, 1968), studies must better establish the patterns, if they exist, in the total serum CK activity responses to an exercise stress.

It would be interesting to determine if particularly low selected intensities of an exercise, in an individual, will result in different patterns of change in total plasma CK activity if there is no change in his state of physical conditioning. This includes comparing the measures of maximum total plasma CK activity and the times required to reach these peak levels.

1.3 Statement of the Problem

The purpose of this investigation was to observe the effect of controlled isometric exercise at three selected intensities upon the post-exercise pattern of total plasma CK activity. In the analyses of the resulting total plasma CK activities, particular attention was given to the times at which peak total plasma CK activity occurred and the differences in the measured peak total plasma CK activity among the selected intensities.

1.4 Limitations

In the present study, the subjects tested were sedentary, male volunteers. They were, however, not randomly selected from a larger pool of sedentary males because the number of qualified subjects, willing to devote the necessary time to this study, was small. Thus, caution is advised, if the results are applied to other, differently selected, sedentary, male groups. The results of this study cannot be applied to regularly physically active subjects or to female subject groups.

1.5 Abbreviations

ADP : Adenosine Diphophate

ATP : Adenosine triphosphate

CK : Creatine kinase

CK-BB : "Brain" isozymic form of creatine kinase

CK-MB : "Hybrid" isozymic form of creatine kinase

CK-MM : "Muscle" isozymic form of creatine kinase

E.C. : Enzyme Commission

FT : Fast twitch

IU/l : International Units per litre (also U/l)

MVC : Maximal voluntary contraction

MVO2 : Maximal rate of oxygen consumption

ST : Slow twitch

U/l : International Units per litre (also IU/l)

VO2 : Rate of oxygen consumption

Chapter II

REVIEW OF RELATED LITERATURE

2.1 Introduction

This review of literature regarding creatine kinase shall be presented in three sections. The first section shall deal with the enzyme, creatine kinase, the isozymic forms which exist, the functional role of creatine kinase, and the distribution of creatine kinase activity in human tissues and sera. The second section shall deal with the effects of exercise upon total serum creatine kinase activity. The relevant factors of concern which have been studied are: exercise intensity, exercise duration, the total work requirement, physical conditioning, the type of exercise, the time course changes in total serum creatine kinase activity, and particular characteristics of the muscle. The third section is a brief review of the theoretical processes put forth by previous research to explain the occurrence of CK efflux.

2.1.1 Creatine Kinase Isoenzymes

Structurally, creatine kinase is a dimeric enzyme and is usually distinguished as one of three forms when separated by electrophoresis. These are commonly referred to as "muscle" type (CK-MM), "hybrid" type (CK-MB), and "brain" type (CK-BB) isoenzymes, based on an increasing mobility towards the anode and the tissue in which each is predominant (Bais and Edwards, 1982).

In addition to these major isoenzymes, other variant types have been recognized on the basis of atypical electrophoretic bands, (Madsen, 1972; Lim, 1975; Sax et al., 1976; Leroux et al., 1977; Ljungdahl and Gerhardt, 1978). For example, Lim (1975) discovered two atypical electrophoretic bands in the analysis of human serum. One band appeared between the MB and BB bands and the other appeared between the MM and MB bands. The more anodic band, between the MB and BB bands, has been referred to as CKI-B (Lim, 1975) or the "X" band (Madsen, 1972). The other band, between the MM and MB bands, has been referred to as CKII-B (Lim, 1975) or CK-Z (Leroux et al., 1977).

Only theories exist to explain the presence of the atypical CK isoenzymes. Sax et al. (1976) suggested that a prolonged ischemia of myocardial and brain tissues could result in conformational changes in their isoenzymes such that their electrophoretic mobilities are altered.

Other investigations have identified high molecular weight complexes with creatine kinase activity, such as the CK-BB isoenzyme and immuno-

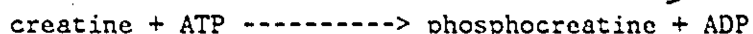
globulin, and referred to it as "macrocreatine kinase" (Urdal and Landaas, 1979; Jockers-Wretou and Plessing, 1979). This name also includes other types of large complexes, such as polymers of creatine kinase caused by ultraviolet radiation (Yuu et al., 1980). Yuu et al. (1980) showed that in the complexing process, variations in the immunoglobulin or the ratio of immunoglobulin to a CK isoenzyme occurs to create the variations seen.

Mitochondrial CK is another distinct isoenzyme which is bound to the exterior of the inner mitochondrial membrane (Saks et al., 1978). It exists in two interconvertible forms, M1 and M2, which separate as they migrate towards the cathode during electrophoresis (Hall et al., 1977), as opposed to the major isoenzymes which migrate towards the anode. However, the detection of this isoenzyme requires a very sensitive assay technique.

The relevance of this isoenzyme in the diagnoses of human heart diseases, skeletal muscle disorders, and nervous system disorders, as with the other isoenzymes, are still under investigation (Blum et al., 1981). Its functional role will be mentioned in the next section.

2.1.2 The Functional Role of Creatine Kinase

Creatine kinase (CK; EC 2.7.3.2) is an enzyme which reversibly catalyzes the production of phosphocreatine and ADP from creatine and ATP. This is achieved through the direct transfer of the terminal phosphoryl group of ATP to creatine (Jacobus, 1980).



CK

creatine + ATP ----------> phosphocreatine + ADP

It is presently held that creatine kinase plays a major role in the channeling of energy from the mitochondria to the various other cellular sites of utilization (Saks et al., 1978; Jacobus, 1980). In the general model, reviewed by Saks et al. (1978), it is proposed that the mitochondrial isoenzyme of CK, which is bound to the outer surface of the inner mitochondrial membrane, is in close proximity to adenine nucleotide translocase, which is found within the inner mitochondrial membrane. See figure 1.1. The main function of the adenine nucleotide translocase is to transfer the ATP, generated in the mitochondrion by oxidative phosphorylation, across the inner membrane in exchange for ADP from the cytoplasm. Saks et al. (1976) proposed that the translocase brings the ATP molecules directly to the active sites of mitochondrial CK on the outside of the membrane.

The formation of phosphocreatine is kinetically less favourable than the formation of ATP. However, if a high rate of translocation of ATP across the inner membrane occurs, the concentration of ATP at the active

sites of CK will be higher than the extramitochondrial ATP concentration. Therefore, the CK reaction would proceed forward to efficiently produce phosphocreatine from the translocated ATP and supplies of creatine from the medium (Saks et al., 1978).

Also, the adenine nucleotide translocase has a high affinity for ADP (Vignais, 1976). Thus, the ADP, which is produced along with the phosphocreatine in the CK reaction, is brought back into the mitochondrial matrix for rephosphorylation. Therefore, the translocase maintains the CK reaction in favour of phosphocreatine production in two ways; i) by increasing the local ATP concentration and thereby increasing the rate of the CK reaction in the forward direction; and ii) by decreasing the local ADP concentration and thereby decreasing the rate of the CK reaction in the reverse direction (Saks et al., 1978).

With a high phosphocreatine : creatine ratio in the cytoplasm, the preference of the reverse CK reaction towards ATP production becomes advantageous at the sites where ATP is utilized. In the presence of the cytoplasmic isoenzymes of creatine kinase, ADP is quickly rephosphorylated to regenerate and maintain ATP levels. Saks et al. (1978) have presented evidence which indicates that CK is bound to the myofibrils, sarcoplasmic reticulum, and plasma membrane of muscle. Such binding of CK to specific areas ensures that the energy of the system is distributed efficiently.

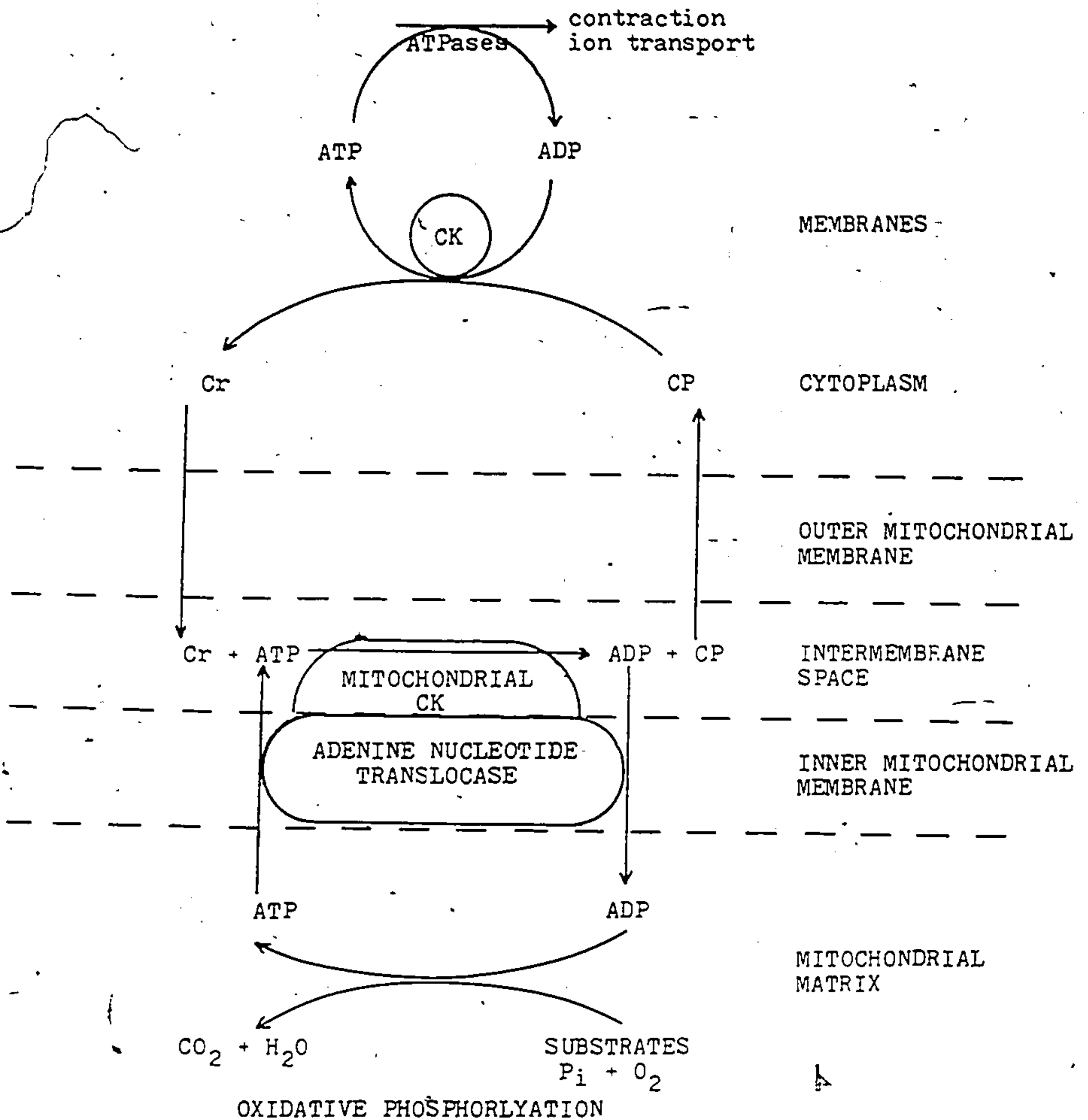


Figure 1.1: A schematic diagram of the creatine phosphate (CP) pathway for energy transport (adapted from Saks et al. (1978)). The CK, in the small circle, is representative of creatine kinase bound to myofibrils and plasma membranes.

2.1.3 The Distribution of Creatine Kinase

If it is assumed that CK plays a key role in the transport of energy from the source to the site of utilization within the cell (see the previous section), it would follow that CK is distributed throughout all tissues to some degree. This premise is given support through published data on CK isoenzyme patterns in various human tissues (reviews by Neumeier, 1981; Bais and Edwards, 1982).

It is apparent from the data that skeletal muscle shows the highest total CK activity relative to other tissues. Neumeier (1981) stated that "depending on muscle type, sex, and methodology used the total CK activity measured is between 225 and 12,000 U/g fresh tissue". With regard to isoenzyme proportions, skeletal muscle usually contains CK-MM activity in amounts greater than 90 percent of the total CK activity. CK-MB activity is correspondingly found to be smaller, but has accounted for as much as 30 percent of the total CK activity. CK-BB has been found in only a few studies in trace amounts (Neumeier, 1981; Bais and Edwards, 1982).

Myocardial tissue generally contains the next highest amount of total CK activity in the range of 100 to 2,200 U/g tissue. CK-MM is still predominant in this tissue; however, CK-MB activity is also consistently found, measuring up to as much as 50 percent of the total CK activity. The CK-BB isoenzyme can account for 0.9 to 14 percent of the total CK activity. (Neumeier, 1981; Bais and Edwards, 1982).

Brain tissue has been indicated, by most studies (reviewed by Neumei-

er, 1981; Bais and Edwards, 1982) to contain exclusively CK-BB activity in a range of total activity from 0 to 220 U/g tissue. CK-MM, CK-MB, and variant CK isoenzyme activities have been found, to small degrees in individual, otherwise unconfirmed, studies.

The CK activity of organ tissues has been measured relatively low, when compared to those activities of brain, heart, and skeletal muscle tissue. In terms of isoenzyme distribution, variable results exist. Many investigative studies have shown that CK-BB activity predominates in the gastrointestinal tract, lung, liver and kidney tissue, as well as in endocrine and exocrine glands. However, contrastingly, other studies have indicated that CK-MM activity predominates, or is in large proportions, in the lung, kidney, and liver tissues, and prostate, thyroid, adrenal, and parotid gland tissues. (Neumeier, 1981; Bais and Edwards, 1982).

2.1.4 Creatine Kinase Activity in Human Sera

CK-MM is the major isoenzyme present in the sera of normal human subjects (Bais and Edwards, 1982). The isoenzymes CK-MB and CK-BB may be present in only small or trace amounts (Varat and Mercer, 1975; Jung et al., 1979). The reviews of the literature, which have assessed the distributions of the CK isoenzymes in the tissues, suggest that skeletal muscle is a major source of serum enzyme activity (Neumeier, 1981; Jockers-Wretou and Pfleiderer, 1975; Tsung, 1981).

Studies have shown that the fraction of CK-MB does not usually account for a large proportion of the total serum CK activity levels at

rest or post-exercise (Varat and Mercer, 1975; Siegel et al., 1981; Apple et al., 1984). Varat and Mercer (1975) rationalized that at least some CK-MB should be present in normal serum, even if the total serum CK pool in normal subjects is derived primarily from skeletal muscle. Furthermore, if serum samples contain a greater total serum CK activity due to muscle damage, then the percentage portion of activity due to CK-MB should not increase.

In their study, CK-MB activity accounted for less than 2.0 percent of the total serum CK activity in all 24 of the normal, non-exercising control subjects, although the absolute values of the peak serum CK-MB activity varied over a wide range. Their finding, that the percentage serum CK-MB activity in acute myocardial infarction patients ranged from no less than 4.0 percent to 19.0 percent of the total serum CK activity, supports the theory that only larger proportions of serum CK-MB activity are resultant of a myocardial source.

Steele et al. (1978) found that exercise-induced myocardial hypoxia or uncomplicated cardiac catheterizations did not result in significant increases in the serum CK-MB isoenzyme. They then suggested that an increase in the activity of the CK-MB form would only occur after damage to the myocardium, which contains more of this enzyme. This supports the earlier findings of Varat and Mercer (1975).

Recently, Siegel et al. (1981) confirmed that although the serum CK-MB activity increased, after fifteen runners particiated in a marathon, the percentage of the CK-MB activity of the total serum CK activity remained at less than four percent. However,in another group of twelve marathon runners studied by them, it was revealed that the mean

percentage of CK-MB activity was 13 +/- 6.1 percent after the race. Yet, with the use of infarct-avid myocardial scintigraphy in these runners, to detect myocardial abnormalities, all results of scanning were within normal limits. Thus, it was suggested that the increases in CK-MB resulted from skeletal muscle or a non-cardiac source. This is contrary to the suggestion by Varat and Mercer (1975) that higher percentages of CK-MB activity are indicative of a myocardial source. The possibility of non-cardiac sources of CK-MB (ie. organ tissues) has not been directly investigated with respect to exercise.

In the analysis of the isoenzyme contents of the gastrocnemius muscles, through biopsy techniques, Apple et al. (1984) found a greater content of CK-MB in five marathon runners (7.7 +/- 2.4 percent) than in five non-runners (less than or equal to one percent). No substantial differences were found in the total CK activities per gram of tissue between the running and non-running subjects. This observation confirms the slightly higher than normal serum CK-MB activities found in marathon runners by Siegel et al. (1981), and supports the likelihood of skeletal muscle as the source of increased CK-MB activity. Thus, the earlier suggestion by Steele et al. (1978), that increases in the serum CK-MB activity are only likely to result from myocardial damage, is contradicted.

Based upon a number of more recent studies, serum CK-MB activity levels will be seen to increase after exercise; particularly in well-trained individuals and usually as an artifact of higher total serum CK levels (Schnohr et al., 1980; Robinson et al., 1982; Noakes et al., 1983; Diamond et al., 1983; Siegel et al., 1981; Strauss et al., 1982;

Stansbie et al., 1982; Jaffe et al., 1984). Some of the abnormally higher proportions of CK-MB activity seen (ie. greater than 4 to 6 percent (Siegel et al., 1981; Stansbie et al. 1982; Noakes et al., 1983; Diamond et al., 1983), might be simply explained by a higher CK-MB content within the more trained skeletal muscle, as suggested by Apple et al. (1984). Meanwhile, these elevated or abnormal levels of CK-MB activity have not been seen in training athletes of other studies (Symanski et al., 1983).

Research by Phillips et al. (1982) has indicated that serum CK-BB activity had increased beyond the upper limits of normal (ie. greater than 3 ng/ml) in seven marathon runners after a 42 kilometer race. However, Kaste and Sherman (1982) stated that, although CK-BB is the principle isoenzyme in the brain, these findings may not be indicative of brain damage. They suggest that other organ tissues may be possible sources. Furthermore, because significant concurrent increases in serum CK-MB activity were seen, Phillips et al. (1982) admit that some degree of cross-reaction with the CK-MB fraction likely occurred in their assay. This may have caused an overestimate of the CK-BB activity.

Nevertheless, in comparison to the total serum CK activity, the amount of serum CK-BB activity has been relatively small. The upper limit (95th percentile) in normal adults, corresponds to about 3 U/l, by a CK-BB specific radioimmunoassay technique developed by Roberts et al. (1977), and is thus below the detection limits of most other methods (Lang, 1981). In a qualitative analysis, by electrophoresis, of the sera of 31 runners of an ultra-marathon, CK-BB bands were visible in 21 (68 %) of the subjects (Noakes et al., 1983). However, the practical

significance of this and other findings, of raised serum CK-BB activity levels post-exercise, remains to be determined.

The normal range for total serum CK activity was investigated recently by McCormick (1976). It was found in this study that the distribution of total serum CK values among males and females did not conform to Gaussian distributions, but rather, exhibited skewness to the right. When serum samples were assayed at a temperature of 25 C degrees, males showed a mean total serum CK activity of 29.9 U/l , in a range from 4.2 to 75.0 U/l, while females showed a mean total serum CK activity of 16.3 U/l, in a range from 1.7 to 37.5 U/l. These ranges represent the values between which 95 percent of the male and female subjects had fallen.

In a more recent study by Nicholson et al. (1985a), the inter- and intra-individual variations in total serum CK activity were examined in a group of female subjects. On a minimum of three occasions, blood samples were collected, at rest, and under the condition that all forms of recreational exercise were avoided within three days prior to the collection.

Under this protocol, designed to minimize the effects of exercise, it was found that individuals tended to have consistent levels of total serum CK activities. The range of intra-individual variation, among the test days, was small, as indicated by a mean coefficient of variation (C.V.) of 19%. On the other hand, the inter-individual variation was found to be relatively larger, with a mean coefficient of variation of 47%. Nicholson et al. (1985a) concluded that individuals tend to have a basal serum CK activity level, which can be quite accurately measured, provided that the collection conditions are controlled. The primary

control condition of these authors, in this determination, was the exclusion of exercise prior to measurement.

Among the subjects, who recorded in a questionnaire that they had engaged in only minor levels of activity, within the three days preceding blood collection, significantly higher levels of total serum CK activity were measured. Thus, even though the subjects had originally perceived it as insignificant, minor exercise had an effect on the subsequent total serum CK activity and the group distribution.

In another study by Nicholson et al. (1985b), evidence was presented to suggest the appearance of non-Gaussian or skewed distributions of measured total serum CK activity in populations, is largely due to the effect of exercise. Gaussian distributions appeared when none of the subjects engaged in physical activity within three days prior to exercise. However, in another distribution, where exercise was not controlled, skewness resulted, with a few high total serum CK activity measurements. Furthermore, it was determined that these were, in fact, due to recent strenuous physical activity. This would explain the finding of a non-Gaussian distribution by McCormick (1976), since the control of exercise activity was not reported to have been done.

2.2.1 Total Serum Creatine Kinase Activity and Exercise

It has been shown in many studies that total serum activity levels of CK increase following physical exertion (Nuttall and Jones, 1968; Block et al., 1969; Schwartz et al., 1971; Critz and Cunningham, 1972; Misner et al., 1973; Shapiro et al., 1973; Schnohr, 1974; Riley et al., 1975; Sanders and Bloor, 1975; Buyze et al., 1976; Chahine et al., 1976; Goode and Meltzer, 1976; King et al., 1976; Berg and Haralambie, 1978; Yakovleva, 1979; Schnohr et al., 1980; Bornheimer and Lau, 1981; Clarkson et al., 1982; Priest et al., 1982; Cerny and Haralambie, 1983; Newham et al., 1983; Schwane et al., 1983). Unfortunately, the mechanisms by which CK is released into the blood has not yet been elucidated.

Thus far, the approach by researchers has been to attempt to determine the qualitative and quantitative factors which result in high levels of total serum CK activity. Although Sanders and Bloor (1975) suggest that the measurement of CK activity is one of the most sensitive indices of acute exercise stress, research findings, in several areas, are yet far from conclusive. The following sections shall deal with the factors affecting total serum CK activity which have been given the most attention in the past with respect to exercise.

2.2.2 Exercise Intensity and Total Serum CK Activity

A number of experimental studies have been done which support the idea that exercise intensity is a major influence on increases in total serum CK activity (Cerny and Haralambie, 1983; Shapiro et al., 1973; Tiidus and Ianuzzo, 1983).

Shapiro et al. (1973) examined the effects of prolonged marching, as an exercise stress, upon total serum CK activity. It was shown that at a given intensity, those subjects with a high maximal VO2 had smaller enzyme activity elevations than those subjects with a low maximal VO2. Additionally, in another group of subjects, in which work intensity was adjusted to each individual's maximal VO2, the enzyme activities were similar. Thus it would seem that total serum CK activity is more dependent upon the relative intensity of the exercise, as in an expression such as a percentage of an individual's aerobic capacity, rather than the absolute workload.

Cerny and Haralambie (1983) gave support to this idea as subjects, performing a bicycle ergometer test at 80 percent of maximal VO2 for 60 minutes, had higher elevations in total serum CK activity (at 24 hours post-exercise), than when they performed the same test at 50 percent of maximal VO2. However, significant differences were not found between the trials during which the subjects cycled at 75 percent and 50 percent of maximal VO2 for 100 minutes. It may be that a threshold intensity exists somewhere between 75.6 percent and 82.0 percent of maximal VO2 (the actual mean intensities applied), since the trials at 50 percent and 75 percent of maximal VO2 themselves did not raise the total serum

CK activities significantly above pre-exercise levels. However, this suggestion remains to be confirmed. Cerny and Haralambie (1983) specifically proposed that cell integrity is subject to metabolic control, and as the working muscle decreases the energy available, the loss of cellular enzymes may result. This was also earlier suggested by Thomson et al. (1975).

In a study by Clarkson et al. (1982), the intensity of exercise, with respect to strength loss in an isometric exercise, was examined. In eleven untrained subjects, two isometric knee extension exercise regimens were administered on different occasions. One regimen required 35 ten second maximal isometric contractions, separated by 20 second rest intervals. This resulted in a mean strength decrement of 23.4 percent of the MVC strength. The other regimen required the same type and number of contractions, but these were separated by only five second rest intervals. This resulted in a mean strength decrement of 54.6 percent of the MVC strength. Although the absolute tensions were not constant throughout either exercise regimen, a significantly greater post-exercise increase in the total serum CK activity was found in the regimen with the longer rest interval (ie. 10:20 work:rest condition) and consequently that which was able to allow a greater intensity of exercise, represented by the level of tension.

Tiidus and Ianuzzo (1983) have also studied the effect of exercise intensity on increases in total serum CK activity. Subjects performed a dynamic leg extension exercise at percentages of his 10 repetition maximum (10RM); that is, the maximum weight that could be raised ten times. The results showed that total serum CK activity was not significantly

above pre-exercise levels following 150 repetitions at 35 percent 10RM, but were significantly higher at 70 percent and 90 percent 10RM. Furthermore, the 90 percent 10RM elevations were significantly greater than those induced by the 70 percent 10RM load. This represents the most recent evidence of exercise intensity as being a major influence on increases in total serum CK activity. Additionally, this part of their study again points out the possibility of an existing threshold intensity, above which total serum CK activity is seen to significantly rise post-exercise.

2.2.3 Exercise Duration and Total Serum CK Activity

The effect of exercise duration upon the total serum CK activity has been demonstrated in a number of studies. Griffiths (1966) showed that prolonged low intensity exercise (ie. walking at an average speed of 3 1/2 mph) could elicit very large increases in total serum CK activity, after subjects covered distances of 25 and 53 miles. Running "marathons" and "ultra-marathons" have also been used in studies, demonstrating significant elevations in total serum CK activity after prolonged running (Riley et al., 1975; Schnohr, 1974; Schnohr et al., 1980).

Griffiths (1966) also found that an exercise of short duration (ie. climbing and descending 70 stairs) would not elicit significant elevations in total serum CK activity. However, this may have been because the intensity or rate of climbing was not high enough or because blood samples were only taken immediately post-exercise and not further.

Chahine et al. (1976) demonstrated significant total serum CK activity elevations in subjects performing an incremental work test on the treadmill for a mean time of 8 minutes and 42 seconds. Subjects working for less than six minutes on the treadmill, on the other hand, did not exhibit significant elevations. However, since an incremental test (ie. the Bruce protocol) was used, those who exercised for shorter periods of time also exercised at lower absolute intensities. Thus, precise comparisons, in this case, are difficult to make.

In a study by Cerny and Haralambie (1983), subjects performed bicycle ergometer tests, on four different occasions, at approximately 50 percent and 80 percent of maximal VO2, for durations of 60 and 100 minutes. No significant differences in total serum CK activity were found post-exercise as a result of the differences in exercise durations.

In an earlier study by Berg and Haralambie (1978), data was compiled to demonstrate that above 60 to 70 percent of the maximal aerobic capacity, total serum CK activity increased in proportion to exercise duration for up to 5 1/2 hours. Furthermore, these relationships were found to have a different slope in each of three categories of exercise (ie. bicycle ergometer testing; ski racing; impact type exertions, such as running, skating, and walking). Unfortunately, in this study, samples were only taken 6 to 10 minutes post-exercise and not serially for any length of time. On the basis of studies which observe total serum CK activity for periods of time post-exercise (see section on "The Time Course of Total Serum CK Activity Changes"), the true peak total serum CK activity is not achieved immediately post-exercise. To assume that the differences in total serum CK activity immediately post-exercise

would be proportional to the differences in peak total serum CK activity might be erroneous.

Nevertheless, the effects of duration on post-exercise total serum CK activity has been aptly demonstrated by Tiidus and Ianuzzo (1983). Although changes in serum CK activity were not demonstrated following 100 and 200 repetitions of a dynamic leg extension exercise at a given intensity, significant elevation of total serum CK activity did appear after 300 repetitions at the same intensity. The effects of exercise duration, at different intensities, and in different types of exercise, upon total serum CK activity post-exercise have yet to be further quantified.

2.2.4 Total Work Requirements and Total Serum CK Activity

The view of the total work requirement of an exercise as a factor which affects total serum CK activity, might seem reasonable when it is considered that both low intensity, long duration exercises and high intensity, short duration exercises result in significant enzyme activity elevations. This was investigated in a recent study by Cerny and Haralambie (1983).

On four separate occasions, subjects performed bicycle ergometer tests, in random order, under conditions: a) 50 percent MVO2 for 60 minutes, and b) for 100 minutes; and c) 80 percent MVO2 for 60 minutes, and d) 100 minutes. Thus, under conditions b) and c), the total work requirements were approximately equal. However, under condition c), sig-

nificantly higher elevations in total serum CK activity occurred than in condition b). Conversely, although larger differences in the total work required existed among other conditions (ie. a) vs. b), c) vs. d), a) vs. d), b) vs. d)), no significant differences were found in the elevations of total serum CK activity.

Supportive findings were documented by Tiidus and Ianuzzo (1983). In separate bouts of a dynamic leg extension exercise, intensity was adjusted inversely proportional to the number of repetitions in order to keep the total work requirement constant. As a result, significantly greater elevations in total serum CK activities occurred following the highest intensity, short duration exercise, than following the longer duration, lower intensity exercise.

Other studies which have investigated the effects of the total work requirement upon the total serum CK activity post-exercise have not been found by this author. These recent preliminary investigations, however, suggest that the effects are not as great as those of other factors, such as intensity and duration.

2.2.5 Physical Conditioning and Total Serum CK Activity

The effects of physical conditioning on total serum CK activity levels have been noted by a number of studies (Nuttall and Jones, 1968; Hunter and Critz, 1971; Shapiro et al., 1973; Sanders and Bloor, 1975; Buyze et al., 1976; Burke et al., 1982). It was demonstrated by Nuttall and Jones (1968) that the total serum CK activity response to a given

workload (ie. weightlifting a percentage of body weight) would not rise significantly in the female subjects and very little in the male subjects, after a three to five week period of physical conditioning. Thus, it was their suggestion that "an index of the degree of physical conditioning attained by an individual in a physical training program could be developed with the rise in serum CK activity following a standardized type and duration of exercise".

Similarly, Hunter and Critz (1971) demonstrated that as a ten week training program increased each subject's maximal VO2, the total serum CK activity response to maximal and submaximal exercise tests were significantly lower than pre-training exercise test results.

Shapiro et al. (1973), in a study previously mentioned, gives further support to the findings by Nuttall and Jones (1968). In their study, which involved prolonged marching as an exercise stress, it was shown that those subjects with a high maximal VO2 had smaller enzyme activity elevations than those with a low maximal VO2 working at similar intensities. Additionally, in another group of subjects, in which work intensity was adjusted to each individual's maximal VO2, the enzyme activities were similar.

Schwartz et al. (1971) proposed that a high degree of general cardiovascular fitness, represented by a high maximal VO2, might be important in modifying the response of total serum CK activity to exercise. This might be accomplished by better provision of the supply of energy necessary to preserve the impermeability of the cell membranes to protein; an idea also supported by Thomson et al. (1975) and Cerny and Haralambie (1983).

Contrary to the findings of Shapiro et al. (1973), Schwartz et al. (1971) found no correlation between enzyme activity levels, at different workloads, and maximal VO2. This may have occurred because blood samples were drawn only immediately post-exercise and one hour post-exercise. Also, it is explained that there may have been too small a difference between the two workloads studied. Nevertheless, it was their suggestion that the "serum enzyme responses to exercise may be more closely related to training of specific muscle groups than to general cardiovascular condition".

This specificity of response has been shown by Schwane and Armstrong (1983). Among their findings, they found that prior eccentric training of rats, through one 30 minute session of downhill or level running, was found to prevent the elevation of plasma CK activity, following a 90 minute downhill run. However, a training session of uphill running, which is more concentric in nature, had no such effect.

In a study on human runners, Byrnes et al. (1985) also found that one 30 minute session of downhill running resulted in significantly lower plasma CK activity and perceived muscular soreness, following a second running session. Particularly interesting, however, is the fact that the second sessions took place three or six weeks after the initial one. When the two sessions were separated by nine weeks, similar changes in stotal serum CK activity and perceived levels of soreness were found post-exercise. The authors suggested that the apparently prophylactic effect of the first session may be a result of a reduction in the pool of fragile muscle fibres. This would be a temporary effect lasting at least until the sixth week after the initial session.

Study into the area of training effects has also been done by Vihko et al. (1979), in a study of the effects of endurance training upon the acid hydrolase activity measured in the skeletal muscle of experimental mice. The presence of acid hydrolases are indicative of a degenerative process within the muscle cell, and will be reviewed in a later section. Vihko et al. (1979) demonstrated that after approximately four weeks of training, the skeletal muscle exhibited a greater concentration of most acid hydrolases measured, than in untrained control animals. However, five days following a session of exhaustive exercise, the acid hydrolase activity of the untrained runners significantly exceeded that of the trained runners.

Vihko et al. (1979) explain that the above-normal acid hydrolytic activity in endurance-trained muscle may be reflective of increased muscle breakdown. This is compensated for by an increase in the biosynthetic processes, which occur in muscle, in response to the training stimulus. The authors suggest that regular training furthermore adapts the muscle fibres in such a way that the resistance to the damaging effects of exercise is increased. If not by strengthening the fibres, it is also possible that training eliminates the fibres which are fragile and more susceptible to damage. Additionally, the adaptations of training may include a more rapid recovery of energy and ion balance after exhaustion, which may reduce the effects of damaging factors.

If it is recognized that total plasma CK activity increases as muscle damage occurs, these explanations help to make it apparent that the increases in enzyme activity may be less for a well-conditioned group, as opposed to an unconditioned group, when both are subjected to the same

absolute workloads. Furthermore, after physical training, resting values are either unchanged (Nuttall and Jones, 1968; Hunter and Critz, 1971) or higher (Misner et al., 1973), Raimondi et al., 1975; Buyze et al., 1976) in the conditioned subjects, possibly as an artifact of their higher levels of physical activity.

2.2.6 Type of Exercise and Total Serum CK Activity

The suggestion by Schwartz et al. (1971) that "serum enzyme responses to exercise may be more closely related to the training of specific muscle groups than to general cardiovascular condition", leads to the consideration of the type or nature of the exercise as a factor which influences total serum CK activity.

Berg and Haralambie (1978), in demonstrating the influence of exercise duration upon total serum CK activity, also showed that this relationship varied in each of three types of exercise examined. These were i) a bicycle ergometer test, ii) ski racing, and iii) impact-type exertions, such as running, skating, and walking. As the increases in total serum CK activity were found the greatest in the impact-type exertions per unit of time, the influence of mechanical factors in the loss of intracellular enzymes becomes a possible cause.

Clarkson et al.(1982) supported the suggestion that mechanical factors associated with tension levels within the muscle may affect the amount of CK efflux, through their study which applied isometric exercise as the experimental treatment. The exercise regimens in the study

called for 35 maximal isometric contractions, each ten seconds in duration. In two separate trials, the length of the rest period between contractions differed (ie. 5 seconds rest in one trial; 20 seconds rest in the other trial). The 10:5 second work:rest regimen resulted in a 54.6 percent strength loss, whereas the 10:20 second work:rest regimen only resulted in a 23.4 percent strength loss. But furthermore, the levels of total serum CK activity did not increase as high in the 10:5 second regimen, where tensions were not maintained high. Significant covariance was found ($p<0.01$) with total serum CK activity as the dependent variable and muscle tension as the covariate. Thus, there is an apparent contradiction to the theory that the levels of high energy phosphates affect the membrane permeability of the working cells. Clarkson et al. (1982) do not support this hypothesis, since they suggest a greater deficit in the phosphocreatine stores would have occurred in the 10:5 second work:rest regimen, where actually lower total serum CK activity resulted.

Schwane et al. (1983) observed the effects of eccentric muscular contractions, in a study in which plasma enzyme activity levels were determined during level and downhill running on a treadmill for forty-five minutes. Although the mean VO2 was lower during the downhill run (ie. 57 percent of MVO2) than during the level run (ie. 78 percent of MVO2), plasma CK activity levels were elevated by 351 percent at 24 hours after the downhill run. Plasma CK levels were not significantly elevated after the level runs.

Similar responses of total plasma CK activity to eccentric work has also been noted in rats, by Armstrong et al. (1983). This study showed

that eccentrically-based exercise resulted in greater increases in the subsequent total plasma CK activity, than in the concentrically-based exercise. Furthermore, histological evidence of muscular damage was found to be greater, following the eccentric exercise.

This might be explained by the greater stress on the muscle during downhill running. According to Komi and Viitasalo (1977), there is more tension per active muscle fibre in eccentric contractions (used to a greater extent in downhill running) than in concentric contractions. Again, this also emphasizes the relationship of serum enzyme responses to the training of specific muscle groups or for specific types of activities, as suggested by Schwartz et al. (1971).

2.2.7 The Time Course of Serum CK Activity Changes

The course of total serum CK activity changes over a period of time post-exercise has been shown to vary among studies done. Some researchers have shown that the rise in total serum CK activity is delayed until after the application of the exercise bout (Nuttall and Jones, 1968; King et al., 1976; Clarkson et al., 1982). Meanwhile, some studies, which only collected pre- and post-exercise blood samples, seem to indicate that total serum CK activity begins to increase at some time during the exercise bout (Griffiths, 1965; Critz and Cunningham, 1972; Shapiro et al., 1973; Schnohr, 1974; Sanders and Bloor, 1975; Chahine et al., 1976; Berg and Haralambie, 1978; Steele et al., 1978; Schnohr et al., 1980).

The point in time at which peak total serum CK activity is reached has also varied among studies; from less than one hour (Block et al., 1969), to eight to sixteen hours (Nuttall and Jones, 1968), to eleven to nineteen hours (King et al., 1976), to twenty four hours (Tiidus and Ianuzzo, 1983), to twenty five and one half hours post-exercise (Buyze et al., 1976), and to even four or five days post-exercise (Newham et al., 1983). These differences could, in some cases, be due to the large amount of time allowed between sampling points, thereby allowing a greater possibility of missing the true peak in the total serum CK activity. Roberts (1979) states that an appropriate frequency of sampling is an important consideration in order to obtain the meaningful results from enzyme analyses.

Tiidus and Ianuzzo (1983) have suggested that following workloads of various intensities and durations, that the time course of total serum CK activity will be similar in each case. In contrast, Priest et al. (1982) have suggested that lesser degrees of exercise have resulted in shorter intervals to peak values and the duration of the rise.

Meanwhile, the results of Buyze et al. (1976) illustrate the large variations seen between individuals in time course data. It is at least shown here that a bout of exercise will elicit peak total serum CK activity at different times in different individuals. King et al. (1976) have also shown that although peak total serum CK activity occurred at eleven hours in three subjects, the peak occurred after 19 hours in one other subject. Thus, it is apparent that more than one sampling time would be required to locate the true peak total serum CK activity levels in a group of subjects.

A study of the time course of CK activity elevations has been done in experimental animals by Esaki (1971). Exercise in the experiment was applied to rabbits by electrical stimulation of the lower limb muscles. After serial blood samples were taken post-exercise, two important observations were that: i) the maximum CK activity value increased depending on the frequency of the stimulus, and ii) the time required to reach the optimum was from six to ten hours at all of the frequencies examined. In a separate experiment, when the effect of CK injected into the muscle was observed, it was found that the time required to reach maximum total serum CK activity and that to recover to the pre-injection level remained in the same range regardless of the amount of CK injected. Although this supports the suggestions by Tiidus and Ianuzzo (1983), the presence of these properties in the human, in response to exercise have not yet been established.

With regard to how these delayed increases in total plasma CK activity might occur, it is most conceivable that a delay in the process of the degeneration of the injured cells are responsible for the delays in the enzyme release. As the damaged muscle complex prepares itself for regeneration, a degeneration and subsequent removal of the injured components must first take place (Carlson and Faulkner, 1983), possibly corresponding to the point in time at which CK is released to the greatest extent. This process will be discussed further in section 2.3.3 of this chapter.

2.2.8 Muscle Characteristics and Serum CK Activity

Since skeletal muscle has been identified as a major source of CK in the serum, determined by its activity, Garcia (1974) suggested that higher serum CK activity levels might be associated with persons having a larger muscle mass. Novak and Tillery (1977) were able to correlate total serum CK activity level to the percentage of lean body mass, but not the absolute values of lean body mass. Perhaps this is because the absolute values of lean body mass may not be well associated with the volume of the fluid compartment, into which the enzyme would be released. A percentage of the lean body mass, with respect to the total body mass, may be, to some degree, indicative of this fluid compartment. However, this was not discussed.

The relationship of the resting serum CK activity levels to percentage of lean body mass might be relevant when race, sex, and age are considered as possible influential factors. Meltzer (1971) found greater total serum CK activity levels in Blacks than in Caucasians, in both men and women. It was suggested that this may be due to the generally greater muscle mass in Blacks, although other factors which are genetic, affecting CK efflux and clearance, must also be considered. No interpretable trends in the total serum CK activity, with repect to age, was shown by Meltzer (1971).

The differences in the normal range of total CK activity between men and women have been shown in data collected by McCormick (1976). Total serum CK activity for males was determined to range from 4.2 to 75.0 U/l and 1.7 to 37.5 U/l for females (when assayed at 25 degrees C.).

Shumate et al. (1979) found that differences in total serum CK activity were significantly more pronounced between men and women, after performing at relatively equivalent exercise loads on the bicycle ergometer. At 24 hours post-exercise, the men had a mean increase of 541 U/l of activity, as opposed to 81 U/l for the women. Some of the difference might be attributable to differences in lean body mass between men and women. However, Schumate et al. (1979) have suggested that the muscle of females is less susceptible to damage by adverse factors.

Sherwin et al. (1969) observed, with an immunohistochemical technique, that fast, type II, skeletal muscle fibres contain higher concentrations of CK than do slow, type I, skeletal muscle fibres. They suggested that a readily available source of energy in the form of creatine phosphate would be more important in the type II fibre contractions, requiring rapid ATP replenishment.

In the analysis of enzyme activities within muscle biopsy samples in normal human subjects, Gollnick et al. (1974b) could not find significant differences between the soleus, a predominantly ST muscle, and either the gastrocnemius or vastis lateralis muscle, which is more of a mixture of ST and FT fibres ($p < .05$). No comparisons were made with a muscle which is classified as predominantly FT. It was suggested that an enzyme activity profile within single fibres might demonstrate a significant difference between fibre types.

Lowry et al. (1978) later carried out such single fibre analyses for a number of different enzymes. A total of twenty fibres were analyzed and compared. Yet, no significant correlation was found between CK actvity and fibre type.

Thorstensson et al. (1977), on the other hand, dissected 3,622 vastis lateralis muscle fibres, after biopsies were taken from two healthy males. 1,818 FT fibres and 1,804 ST fibres were pooled, as shown by ATPase staining, and then analyzed together for CK activity. The ratio of CK activity as FT:ST was found to be 1.3:1. It was noted that this represents a small difference,which might explain the difficulty in obtaining a correlation enzyme activity and fibre type in experiments on whole muscle homogenates (Gollnick et al. (1974b)) or in those with small sample sizes (Lowry et al. (1978)).

Clarkson et al. (1982) recently gave some support for the findings of Thorstensson et al. (1977) in their determination that resting serum CK values correlated significantly with the FT:ST area ratio ($r=0.70$, $p<0.05$). The FT:ST area ratio was defined as the mean area (cross-sectional) of the FT fibres divided by the mean area of the ST fibres. The percentage of the FT fibre area of the total cross-sectional area, however, did not correlate to the resting serum CK levels ($r=0.12$). Thus, it was concluded that the size, not the number of FT fibres, is a factor which may be important in determining the resting serum CK activity level.

2.3 Possible CK Efflux Mechanisms

It is apparent that sufficient damage or injury to the skeletal muscle fibre will result in the release of CK into the circulation. This may be a result of disease (e.g. muscular dystrophy), direct trauma, or ischemic or hypoxic conditions, which result in reduced intracellular ATP levels. Also, a subsequent regenerative response of the muscle fibre to injury has been typified by researchers (Carlson and Faulkner, 1983), and may be of significance in consideration of possible CK efflux mechanisms. Following is a review of the possible factors which may affect the integrity of the muscle cell, and the role of regenerating skeletal muscle in the observed post-exercise changes in total plasma CK activity. The complex muscular disease processes, which result in enzyme efflux, will not be discussed in this review.

2.3.1 The Process of Cell Injury Resulting from Reduced Intracellular ATP

Some research has emphasized that intracellular ATP levels bear a strong relationship to the release of intracellular enzymes. It was suggested by Sweetin and Thomson (1973) that intracellular enzymes may be retained through some role of ATP, and not simply the cell membrane, as an inert barrier. They had observed that human erythrocytes did not release significant amounts of enzymes until the cells had metabolized all of the glucose in the medium.

As a cell is subjected to ischemic conditions (i.e. partially or com-

pletely deprived of blood flow), hypoxia develops and the perfusion of substrates is restricted. Thus, energy production via oxidative phosphorylation becomes inhibited. If the cell has some capacity to produce ATP via anaerobic glycolysis, energy levels will be temporarily sustained. However, under conditions which will require more energy, this cannot continue long.

Robbins (1974) has outlined possible subsequent events which may occur in the pathogenesis of cell injury and death. Initially, with less energy available, the ion pumps of the cell membrane will function insufficiently to prevent a net influx of sodium ions and an efflux of potassium ions. The increase in solute within the cell results in a gain of water, and consequently, the cell begins to swell. This has been observed by Kloner et al. (1974). During an induced transient period of ischemia, swelling occurred "with the formation of large bleb-like fluid spaces beneath the sarcolemma". Kloner et al. (1974) further suggest that the lowered cellular pH, due to the production of lactic acid and hydrogen ions could also inhibit the sodium-potassium pumps, to facilitate cellular swelling. In the swollen state, the cell may be more fragile, and thus, more susceptible to rupture when traumatized (Kloner et al., 1974).

Robbins (1974) suggests that the decreased intracellular pH or the change in the ionic composition of the intracellular environment somehow leads to the release and activation of acid hydrolases from the lysosomes. This would ultimately destroy the cell and it's organelles, releasing enzymes, among other cellular contents into the interstitium. However, Ricciutti (1972) has shown that at least one hour of ischemia

was required to demonstrate a release of enzymes from the lysosomes of papillary muscle cells. Kloner et al. (1974) found that even after forty to sixty minutes of ischemia, no morphological differences existed between the experimental and control myocardial lysosomes.

These observations would thus seem to exclude the possibility that lysosomal activity produces the initial insult to the cell, as a result of reduced energy supply to the cell, in exercise. Karlson and Saltin (1970) indicated that intracellular ATP would decrease to about 70 to 80 percent and CP would decrease to about 20 to 30 percent of their respective resting levels, in brief exhaustive exercise, lasting from two to sixteen minutes. Thus, although the amount of energy within some muscle cells might be sufficiently reduced, in heavy exercise, the length of time during which their energy levels remain low, would likely be too brief to invoke lysosomal enzyme release, as seen by Kloner et al. (1974) and Ricciutti (1972).

Thomson et al. (1975) concurred that considerable physical exertion must take place to markedly lower the intracellular ATP levels, afterwhich CK efflux occurs. They documented that, after extensive stimulation of the gastrocnemius muscle in the cat, for greater than three hours, increases in serum CK activity occurred. However, no apparent fibre damage was evident in the micrographs of the muscle. A detailed explanation of a mechanism, such as in this case, which would alter the cell membrane's permeability to CK, without evident damage to the cell, has not been established.

In another study, Wilkinson and Robinson (1974) provided some evidence, pointing out that, in addition to being a reserve of energy, ATP

may have a direct effect in maintaining the integrity of the cell membrane. In their study, rat lymphocyte suspensions were incubated with phospholipase and variable amounts of either ADP or ATP. It was hypothesized that ATP would protect the cell membrane from the degradative effects of phospholipase, as indicated by a reduction in the leakage of intracellular lactate dehydrogenase.

The results indicated that, at concentrations greater than 5 mmol/l, ATP reduced the amount of lactate dehydrogenase released from the suspended lymphocytes, when in the presence of phospholipases. No such effect was found when ADP was added to the suspensions in similar concentrations. The apparent protective effect of ATP, defined by a reduction in enzymes released, was found to be proportional to the concentration of ATP in the suspension. These results bear some consistency with the view that the maintenance of cell integrity is, to some extent, dependent upon the energy level of the cell. Yet, the mechanism of this phenomenon, and whether it occurs in muscle cells in vivo, remains unclear.

In a recent study, involving isometric exercise, Mayer and Clarkson (1984) administered regimens on three separate occasions which, in each case, required a tension of 40 percent of the subject's MVC strength. However, variations were made in the number of seconds required in the work:rest ratios. Each subject completed a session of contractions wherein the work:rest ratios were 60:60 sec, 30:30 sec, and 15:15 sec. The results showed that the greatest increases in the total serum CK occurred after the 60:60 sec work:rest condition (the longest duration of the three regimens). Since, in all regimens, the flow of blood to the active muscles is restricted to a large degree at 40 percent of MVC

(Gollnick et al., 1974a), greater intracellular ATP depletion within the longer work intervals could be one explanation for the increases in the post-exercise serum CK activities, in spite of the correspondingly longer rest intervals. This supports the ATP depletion theory of Thomson et al. (1975).

A study of glycogen depletion patterns between FT and ST muscle fibres, following sustained isometric contractions was done by Gollnick et al. (1974a): Through biopsy and histochemical techniques, it was revealed that at intensities of less than 20 percent MVC, there is a major use of ST fibres and, above this critical tension, there is a selective use of FT fibres. It was postulated that the relative hypoxia present in the muscle, during such isometric efforts when blood flow is restricted, may be an important factor in the recruitment of FT fibres (Gollnick et al., 1974a).

It might be hypothesized that during low intensity exercise, the energy demands of the cell are such that they can be met by the more aerobic ST fibres. Hence, if they are not fatigued by the duration of the bout and intracellular levels of ATP remain high, the impermeability of the cell membrane to enzyme leakage will remain intact. However, at higher intensities, a greater output and turnover of ATP is required such that the more anaerobic FT fibres are recruited and the level of intracellular ATP, in these fibres, at equilibrium, is decreased. It may be that this level of ATP depletion is sufficient to increase the cell membrane permeability to enzyme leakage.

2.3.2 Cell Injury Resulting from the Trauma of Exercise

Whether or not significant elevations in total plasma CK activity can be evoked, without irreversibly damaging the muscle cells, remains questionable. However, it is apparent from a number of studies that exercise can result in significant damage to muscle fibres.

In contrast to Thomson et al. (1975), who found no apparent fibre damage after stimulating the hindlimbs of cats, Kuipers et al. (1983) found that an acute non-exhaustive treadmill exercise, given to rats, can cause transient focal degenerative changes, which are visible under a microscope. Interestingly, these changes were observed to be most pronounced 24 to 48 hours after exercise, demonstrating a delayed effect which might correlate to enzyme efflux. Friden et al. (1981) have also noticed myofibrillar disruptions at the z-bands in muscle biopsy samples, from human subjects experiencing an exercise-induced delayed muscle soreness.

An estimation of muscle damage in runners, following 50 and 100 mile ultra-marathons, has been accomplished by Matin et al. (1983), utilizing a radioisotope perfusion and scintigraphy method. Subjects were injected with technetium 99m pyrophosphate (Tc-PP), at a selected time before and/or at a particular time after the race. Other research has indicated that, through a number of possible mechanisms, described by Buja et al. (1977) and Brill (1981), the injected Tc-PP would be selectively and rapidly taken up by necrosing cells. Thus, a scintillation camera could provide images of the total body and selected views of the lower extremities, to locate the areas of muscle damage.

The results showed that, in nine of ten cases, abnormal amounts of Tc-PP were taken up by specific leg muscle groups, in the time period 24 to 48 hours after the race. In similar follow-up analyses, the degree of abnormality in Tc-PP levels were reduced, in a time period within five days of the race, and normal, in all cases, by seven and eight days after the race.

Another interesting finding was that subjective ratings of pain, by the subjects, on a five point scale, correlated positively with a similar five point rating of their respective scintigrams, indicating increasing degrees of muscle uptake of Tc-PP, by the experimenters. Although total serum CK activity was evaluated only once, immediately after the race, it was very elevated in all runners, ranging from 485 to 34,130 IU/l, with a mean of 8,640 IU/l. CK-MB activity was elevated in eight of the ten runners, in a range from 37 to 2,049 IU/l, with a mean of 279 IU/l. However, no evidence of myocardial damage was indicated in the scintigrams. The significance of this study is that it associates further evidence of the occurrence of muscle damage with the observation of elevated serum CK activity, as well as the phenomenon of delayed-onset muscular soreness.

Few other recent studies have investigated ~~the~~ relationship of delayed-onset muscle soreness to the levels of increased total serum CK activity. Following dynamic leg extension exercises, Tiidus and Ianuzzo (1983) found a correlation coefficient of 0.80 (significant at $p<0.005$), between muscular soreness perceived and the changes in total serum CK activity. Soreness was evaluated on a ten-point scale, at fifteen sites on the exercised muscle.

Also, in a recent study comparing responses to downhill and level running, Schwane et al. (1983) found extensively greater levels of soreness after downhill running than after level running. This appeared to correspond to the increased levels of plasma CK activity found after the downhill running. However, this was not tested statistically.

It was also found that although the soreness persisted in some of the runners for 24 and 48 hours after level running, soreness was found in all subjects after the downhill run, and persisted for 24 to 72 hours later. This, again might be indicative of the greater damaging potential of eccentric types of exercise (as discussed in section 2.2.6).

Byrnes et al. (1985) reported some similar findings following repeated bouts of downhill running, as total serum CK activity became significantly elevated. However, the peak levels recorded did not significantly correlate with the peak levels of soreness perceived, on a ten-point scale. In spite of the findings by Tiidus and Ianuzzo (1983), the results of Byrnes et al. (1985), at least demonstrates the possible difficulty to be encountered when using perceived soreness as an indicator of muscle damage.

2.1.3 The Process of Muscle Regeneration

Carlson and Faülkner (1983) have noted, in a review, that after skeletal muscle is injured, it will repair itself, while utilizing the remains of the intact fibre complex. Initially, the damaged muscle fibre will begin to degenerate itself. The myofibrils break into individual sarcomeric units and the mitochondria, the sarcoplasmic reticulum, and the sarcolemma begin to show disruption. Satellite cells, which are also located beneath the basal lamina, undergo an activation reaction, which enlarge the nuclei and increase the rates of DNA synthesis.

Next to occur is the breakdown and removal of the damaged components of the muscle cell (Carlson and Faulkner, 1983). Macrophages, neutrophils, and some other phagocytic cells enter the area, through the local circulation, to accomplish this function.

Once the myoplasmic debris has been removed, the regeneration of a new fibre begins. Spindle-shaped myoblast cells fuse together to form multinucleated myotubes. The myofibrils, in turn, intensively synthesize contractile proteins and arrange them into regular arrays of myofilaments. As the myotube matures, a greater proportion of it's volume is represented by contractile proteins. The nuclei become more compact, as they migrate to the periphery, at which point the myotube is then classified as a muscle fibre (Carlson and Faulkner, 1983).

In a histochemical study by Vihko et al. (1978a), evidence indicating that degeneration and regeneration of muscle occurs, following exhaustive exercise, was shown in experimental mice. In particular, hindlimb muscle tissue samples were removed from untrained mice, 1, 2, 3, 5, 7,

or 15 days following an exhaustive run on a motor-driven treadmill. Serial sections, stained for particular hydrolases, indicative of muscle lysosomal activity, revealed that the strongest activity was recorded following at least two days following exhaustion.

An increase in the activity of B-N-acetylglucosaminidase was two and three days after exercise, in red oxidative fibres, with the less oxidative white fibres demonstrating a smaller degree of activity. By the fifth and seventh days post-exercise, the predominance of activity granules increased further. However, the B-N-acetylglucosaminidase activity was again only slight by fifteen days after exhaustion.

Further histological analyses revealed that changes in B-glucuronidase activity were even more clearly evident from three to seven days after exercise exhaustion. In a similar manner to the analysis of B-N-acetylglucosaminidase activity, the strongest activity occurred in the red fibres, as opposed to the white fibres, or in the interfibrillar area. Vihko et al. (1978b), has suggested that this may occur as a result of greater recruitment of the oxidative red fibres during the exercise period.

The animal study by Vihko et al. (1978a), also showed that degenerating and necrotic fibres, as evidenced by the presence of mononuclear, phagocytic cells, were observed to be greatest at two and three days after exercise. The observation of regenerating fibres, as indicated by the the presence of centrally located nuclei, did not occur until the fifth and seventh days after exercise. By the fifteenth day post-exercise, there was no remaining evidence of degenerating fibres. However, regenerative changes were still seen in every sample at this time,

although to a lesser degree than seven days post-exercise. These observations thus correspond to the sequence of events in muscle regeneration, as outlined by Carlson and Faulkner (1983).

In the study by Kuipers et al. (1983), on untrained rats, running for one hour, at a submaximal intensity, a similar time course for these changes was observed. Some focal disorganization of myofibrils and minor signs of degeneration were observed immediately post-exercise. However, further degenerative changes were clearly visible after two to three hours, and were most pronounced within the 24 to 48 hour period. Similar recent histological evidence of skeletal muscle injury has been also found, in rats, resulting from eccentrically-based exercise, by Armstrong et al. (1983).

It would seem reasonable to assume that similar processes would occur as a result of exercise in human skeletal muscle. The factors which determine the time course of such muscle degeneration and subsequent regeneration, after exercise-induced damage, may represent the same cause of delayed increases seen in total plasma CK activity. If this is so, it would then seem likely that the release of intracellular enzymes would be greatest during the period of muscle degeneration.

Summary

In consideration of the possible mechanisms of CK efflux, it would appear that studies have indicated that intracellular ATP depletion, traumatic cell injury, and the process of muscle cell regeneration may play significant roles. Additionally, a review of past research has indicated that there are several other indirect, but important, factors which complicate the relationship between total plasma CK activity and exercise. However, investigations have primarily observed the effects of high levels of intensity or duration of physical exertion upon changes in total serum CK activity.

It would be interesting to observe the patterns of change in total plasma CK activity as a result of relatively low intensities of exercise. Through such an investigation, a threshold intensity may be found, below which total serum CK activity does not change post-exercise. This hypothetical threshold may be representative of an energy threshold, whereby greater energy output results in large enough depletions in intracellular ATP to result in membrane permeability changes. On the other hand, this theoretical threshold may also be representative of a particular level of muscular force exerted, above which, disruption of the myofibrils begins to take place, resulting in enzyme leakage into the blood. Nevertheless, it is possible that such an investigation, at lower intensities of exercise, which controls the factors which have been deemed influential, may help in further describing the general response of the total plasma CK activity levels to exercise.

Chapter III

METHODOLOGY

3.1 Subjects

Nine sedentary male subjects, between the ages of 21 and 31 years of age, participated in this study. In accordance with the Department of Kinanthropology policy regarding the use of human subjects, each individual was given a written general description of the study and its objectives. Also included in this document was a description of the experimental procedure in which each subject would participate and a description of the risks involved. Further questions were given reasonable explanations and each subject signed an Informed Consent Form (see Appendix A) to attest that this was done to his satisfaction and that consent had been given to participate under the conditions described.

3.2 General Experimental Procedures

Each subject volunteered to participate in this study for a period of about two weeks. On three separate exercise days, each subject was required to repeat a given exercise isometrically, under controlled conditions, at 10%, 30%, and 50% of his premeasured MVC strength. The order in which each of the separate exercises were done, with respect to intensity, was randomized for each subject prior to the start of the study.

The following schedule explains the procedures which took place on each day of a fourteen day testing period.

DAY 1: Screening Day

After questioning the subject about his current physical activities, and under the condition that he had not engaged in any strenuous, new, or prolonged activities in the two days prior to this day, a small blood sample was taken, with a lancet, from a fingertip and collected in heparinized capillary tubes (approximately 300 to 360 ul.) The tubes were then centrifuged at 11,500 rpm for ten minutes and the plasma was then taken and stored in the refrigerator at a temperature of three to five degrees Celsius. All subsequent blood samples were also taken and stored in this manner.

A determination of the total plasma CK activity for that blood sample was made shortly after collection. If the total plasma CK activity level was above a criterion level of 100.0 U/l, another day of rest was indicated to allow the activity level to decrease further.

DAY 2: MVC Strength Test Day

A determination of the subject's maximal voluntary contraction (MVC) strength for an isometric, two-legged, knee extension was made (see Measurement of MVC Strength).

DAY 3: Rest Day

No testing took place on this day. However, the subject was reminded not to engage in any strenuous, new or excessively prolonged physical activities during this period and throughout the study period.

DAY 4: Screening Day

No testing took place on this day. However, as on DAY 1, one blood sample was taken and the total plasma CK activity level was measured to determine if another day of rest was required.

DAY 5: Exercise Day

Prior to the start of exercise, a resting blood sample was taken.

After preparation (as in the MVC Strength Test preparations), the subject performed isometric leg extensions at one of the selected intensities (ie. 10% MVC, 30% MVC, or 50% MVC) for periods of ten seconds in duration, between which rest

periods of twenty seconds were allowed. While observing a feedback monitor, which responded to the tension developed across the load cell, the subject attempted to maintain the isometric tension output at the appropriate selected percentage of MVC strength. Measurements of the actual force exerted, during each of the contractions, were recorded by a microcomputer system (see section 3.4 The Exercise Session Apparatus).

In order that the total impulse (force exerted over time) among the three separate exercise days, was constant, the total number of contractions to be done varied depending upon the amount of isometric force to be exerted. These were done as follows:

(i) For the 10% MVC contractions, a total of 105 contractions were performed.

(ii) For the 30% MVC contractions, a total of 35 contractions were performed.

(iii) For the 50% MVC contractions, a total of 21 contractions were performed.

At the completion of the exercise period, the time was noted and a post-exercise blood sample was taken within five minutes. Thereafter, blood samples were similarly taken at 2, 4, 6, 8, 10, 12 and 14 hours post-exercise. After centrifugation and separation of the plasma, at each sampling time, each of the plasma samples was stored in the refrigerator, at three to five degrees Celsius, to be analyzed together at a later time.

DAY 6: 24 hr Post-Exercise Sample and Second MVC Test

On this day, a 24 hr post-exercise blood sample was taken and stored,and a second determination of the subject's MVC strength was made.

DAY 7: Rest Day

On this day, the subjects were reminded to not engage in any strenuous, new, or excessively prolonged activities. As on DAY 3, no exercise testing took place.

DAY 8: Screening Day

No exercise testing took place on this day. However, as in DAYS 1 and 4, one blood sample was taken and the total plasma CK activity level was measured to determine if another day of rest was required.

DAY 9: Second Exercise Day

As in DAY 5, the procedures followed were the same, except the exercise was done at one of the two other selected intensities, and for the appropriate total number of contractions.

DAY 10: 24 Hour Post-Exercise Sample & Third MVC Test

On this day, one 24 hour post-exercise blood sample was taken and, as on DAYS 2 and 6, a determination of maximum strength was again made.

DAY 11: Rest Day

On this day, the subject was again reminded to refrain from any strenuous, new, or excessively prolonged activities. As on previous rest days, no exercise testing took place.

DAY 12: Screening Day

No exercise testing took place on this day. However, as in DAYS 1, 4, and 8, one blood sample was taken and the total plasma CK activity level was measured to determine if another day of rest was required.

DAY 13: Third Exercise Day

As in DAYS 5 and 9, the procedures followed were the same, except the exercise was done at the third selected intensity, for the appropriate number of contractions.

DAY 14: 24 Hour Blood Sample

Only the 24 hour blood sample was taken on this day.

3.3 Measurement of Maximal Voluntary Contraction Strength

Maximum voluntary contraction (MVC) strength is defined, for this study, as the greatest amount of force which can be developed against an unyielding resistance and maintained in a single contraction for a duration of one second. Each subject performed 20 maximal voluntary isometric contractions, which were maintained for two to three seconds. Each MVC was separated by a one minute rest interval. The highest of the 20 measures was accepted as the MVC strength for the subject on that particular day.

This test for MVC strength of the knee extensors was done with the subject in a sitting position on a table, with the knees extended such that the acute angle at the lateral epicondyle of the knee, between the greater trochanter of the femur and the lateral malleolus of the fibula, was 120 degrees. Stabilization during each contraction was provided by: (i) the weight of the upper body, (ii) the table surface and a back rest at a right angle, (iii) a seat belt, which crossed over the thighs, near the hips, and (iv) the hands, which gripped the sides of the table (see figure 3.1). A previous study of videotaped trials revealed that, in such a stabilized position, the knee angle of 120 degrees would be maintained between trials within a +/- 5 degree range.

The extensions were done with both legs against a padded board, which was placed in position above the ankles. The exact position of the board was determined by first measuring the distance from the lateral epicondyle of the femur to the lateral malleolus of the fibula. A land-

mark was then made with a marking pen at a point, 75 percent of this length, from the lateral malleolus. This would ensure a consistent lever arm length, for each contraction, as the board was centred at this landmark.

The padded board was connected to a load cell (Lebow Assoc. Inc., model 3132), which, in turn, was connected to the table beneath the subject with a chain (see figure 3.1). The chain length was adjusted such that the limit of extension of the knee occurred at an angle of approximately 120 degrees. This was facilitated by the use of a protractor device. The voltage changes in the load cell were amplified in a bridge amplifier (Honeywell Inc., Accudata 218) and the signals were digitized using an Applescope Analog to Digital Converter (RC Electronics Inc., APL-D2 system), in conjunction with an Apple micro-computer system (see figure 3.2). A visual output of the voltage changes could be seen on the cathode ray tube (CRT) monitor of the system. The recorded data were subsequently stored on 5 1/4 inch diskettes, for later analysis.

Calibrations of the exercise apparatus was done prior to each of the testing sessions with known weights. Subsequent force determinations for each of the trial measurements were calculated from the linear regression equation of the given calibration forces on the recorded millivolt deflections.

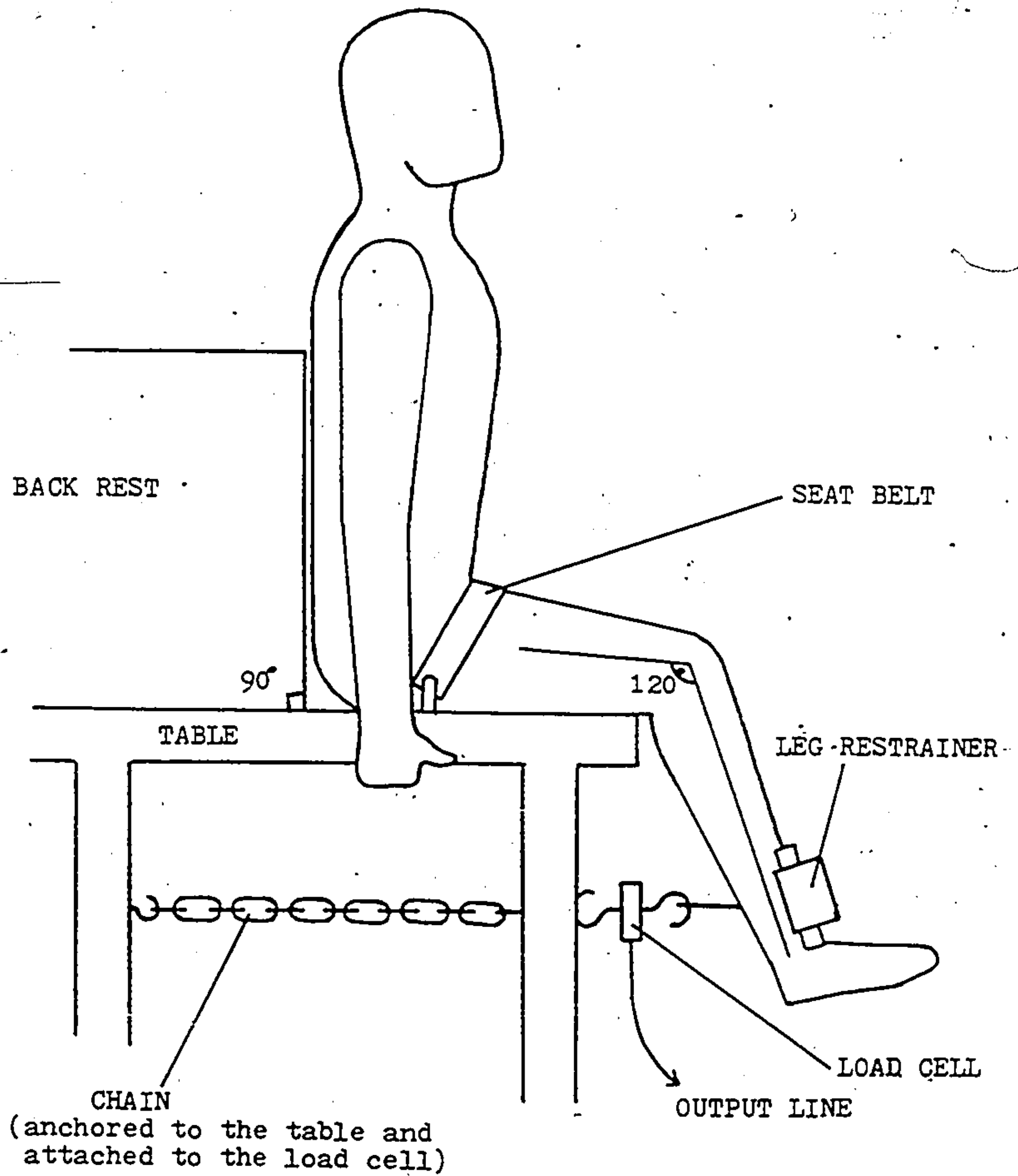


Figure 3.1: Diagram of the experimental apparatus (excluding the recording apparatus).

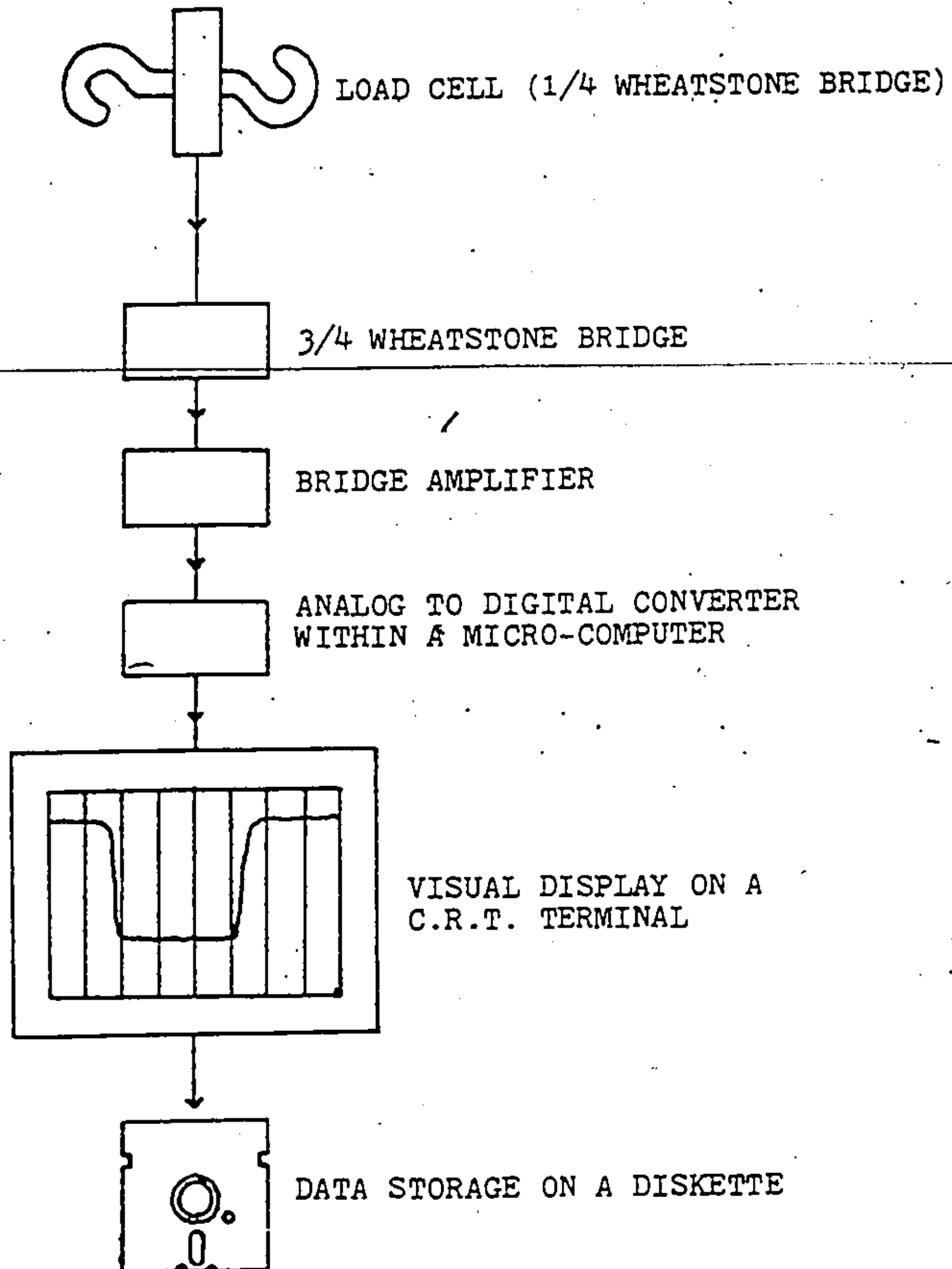


Figure 3.2: A schematic diagram of experimental recording system.

3.4 The Exercise Session Apparatus

The exercise session, as outlined in the General Experimental Procedures previously, took place on the same apparatus as for the MVC strength test. However, in each of these sessions, the subject was able to see an instantaneous measure of his force output on a CRT monitor, placed in front of him. The micro-computer screen displayed the output of the load cell, as digitized using an Applescope Analog to Digital Converter (RC Electronics Inc., APL-D2 system).

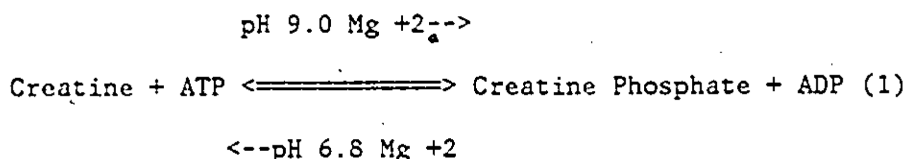
Prior to the session, the oscilloscope beam was set and marked on a line, on the monitor, by adding a weight to the load cell which resulted in a force equal to the appropriate preselected tension. Consequently, with the feedback on the monitor, the subject was able to maintain the tension exerted at the same preselected level.

3.5 The Determination of Total Plasma CK Activity

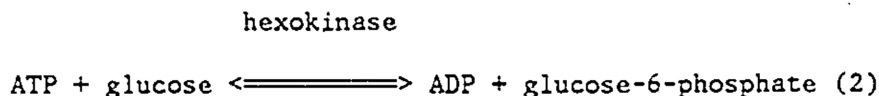
The samples of plasma were assayed for their total creatine kinase activity in a method originally used by Oliver (1955), and since modified by Rosalki (1967) and Szasz et al. (1976). The assay reagents were provided in kits by Boehringer Mannheim Ltd. (Cat. No. 126357).

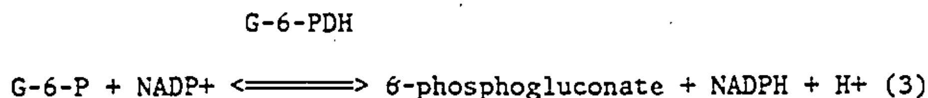
3.5.1 Assay Principles

Creatine kinase reversibly catalyzes the phosphorylation of creatine by ATP in the following reaction equation:



The estimation of total creatine kinase activity in this assay was based upon the production of creatine and ATP, through the "reverse" reaction. The formation of ATP in reaction (1) was then coupled with the following two reactions:





The conditions were fixed in the reagent solutions, such that the auxiliary enzymes, substrates, and cofactors, other than ATP, were not rate limiting in the above reactions. Thus, the formation of NADPH proceeds at the same rate, in reaction (3), as the formation of ATP, in equation (1), in equimolar amounts. The rate of NADPH formation can be measured photometrically, based on the principle that the reduced coenzyme, NADPH, absorbs ultraviolet light at a wavelength of 340 nm, whereas the oxidized form, NADP, shows no absorption (Adolph and Lorenz, 1982). Therefore, the total creatine kinase activity is directly proportional to the rate of change in the absorbance measures at 340 nm.

3.5.2 Assay Reagents

The reagents for this assay were prepared as outlined in the guide for CK analysis by Boehringer and Mannheim (Cat. no. 126357 and 181188). The working solution was prepared to contain the following concentrations of reagents:

Imidazole buffer: 0.11 mol/l, pH 6.7
Glucose: 21 mmol/l
Mg-acetate: 11 mmol/l
EDTA: 2.1 mmol/l
ADP: 2.1 mmol/l
AMP; 5.2 mmol/l
Diadenosine pentaphosphate: 10.5 umol/l
NADP: 2.1 mmol/l
Hexokinase: >=2.5 U/ml
Glucose-6-phosphate deydrogenase: >=1.6 U/ml
N-acetylcysteine: 21 mmol/l
creatine phosphate: 31 mmol/l

These represent the optimum reaction conditions for CK activity measurement as outlined by Szasz (1976).

Szasz (1976) also reports that creatine kinase is rapidly inactivated as a result of the oxidation of the sulfhydryl groups in its active sites. Thus, in order to reactivate the enzyme, thiol compounds may be added to the reagents used. In the Boehringer Mannheim assay kit, N-acetylcysteine (NAC) was used as the enzyme activator, in a concentration of 21 mmol/l.

Prior to analysis, the plasma samples had been stored in the refrigerator, at a temperature between three and five Celsius degrees. It has been reported that creatine kinase could be reactivated to within two percent of the original activity measurement, if the sample is stored at four degrees Celsius for up to seven days (Szasz, 1976; Boehringer Mannheim Guide for CK Assays). This was confirmed in a pilot study prior to this project.

3.5.3 Assay Procedures

The procedures for this assay were done as outlined in the guide for CK analysis by Boehringer and Mannheim (Cat. no. 126357 and 181188). Prior to the addition of the plasma sample, 1000 ul of the reagent solution was pipetted into a semi-micro cuvette (Spectrovette semi-micro cuvette UV #119). Subsequently, the cuvette was covered and placed into a temperature controlled water bath (American Dade, Tek-Bath B6990) to bring the solution to a reaction temperature of 37 degrees Celsius. The plasma sample, which had been stored in a sealed capillary tube in the refrigerator, was transferred into another capillary tube, and left open, to facilitate pipetting directly from the tube at the appropriate time.

To begin the assay, 20 ul of the plasma sample was pipetted into the pre-incubated reagent solution in the cuvette. The solution was mixed by covering and then gently inverting the cuvette several times. At this point, the cuvette was replaced into the water bath, to maintain the reaction temperature, and a stopwatch was started. Readings of the absorbance of ultraviolet light, at a wavelength of 340 nm, were made through the sample on a spectrophotometer (LKB Biochrom Ltd., LKB Ultrospec 4050). At exactly 5:00 min, the first reading of absorbance of the sample, was made. The sample was again replaced in the water bath, and subsequent readings of absorbance were made at exactly 10:00, 15:00, 20:00, 25:00, and 30:00 minutes.

3.5.4 Calculation of the Total Plasma CK Activity

Enzymes are not measured in terms of weight because many of them cannot be conveniently isolated in pure form. Thus, they are conventionally quantified in terms of their respective activities; that is, the amount of substrate they convert per unit of time. The International Unit (U) of enzyme activity was defined by the International Commission on Biochemical Nomenclature (1972) as the amount of an enzyme that will convert one micromole of substrate per minute under standard conditions.

In clinical chemistry, enzyme activities are usually measured in reference to the volume of the enzyme solution, whether it be serum, plasma, or other body fluid. Thus, with one litre as the reference volume, enzyme activity is often measured in International Units per litre (U/l).

A factor for the determination of the total plasma CK activity was derived from the following formula:

$$\text{CK activity} = \frac{\text{d(abs)/min} \times \text{total assay volume} \times 1000 \text{ ml/l}}{\text{M.E. coefficient} \times \text{light path} \times \text{sample volume}}$$

where:

d(abs)/min = the calculated rate of change in absorbance per minute

total assay volume = volume of the reagent solution and the sample = 1.020 ml

1000 ml/l = a factor for the conversion of the units to U/l

M.E. coefficient = the molar extinction coefficient for NADPH at 340 nm = 6.3 cm2/umole

light path = the width of the cuvette = 1 cm

sample volume = volume of the enzyme containing plasma sample = 0.020 ml

Therefore, the calculation for total plasma CK activity, at 37 degrees Celsius, becomes:

$$\frac{\text{d(abs)/min} \times 1.020 \text{ ml} \times 1000 \text{ ml/l}}{6.3 \text{ cm2/umole} \times 1 \text{ cm} \times 0.020 \text{ ml}} = \text{d(abs)/min} \times 8095$$

= total plasma CK activity in U/l

The determination of the rate of change in absorbance, d(abs)/min, required to calculate the total plasma CK activity, was done through a linear regression. A least square estimate of the slope was obtained from the regression of the absorbance values on time.

3.6 Statistical Analyses

Initially, a two-way analysis of variance with repeated measures was used to analyze the collected data. The intensity of the exercise and the time of the blood sample were the two independent variables in the analysis, while the measured total plasma CK activity, served as the dependent variable.

Significant changes in the total plasma CK activity were determined post-hoc with a Tukey test, modified for repeated measures. Thus, comparisons were made among the peak total plasma CK activities at each selected intensity. Also, attention was given to when significant increases in total plasma CK activity were seen, and when peak activity occurred, within each intensity level.

Chapter IV

RESULTS AND DISCUSSION

4.1 Results

This chapter will begin with a brief section which describes the subject group, followed by a section on the results of the responses to the separate MVC tests.

Following these results will be a section on the responses of the total plasma CK activity to each of the given treatment intensities of exercise. The analysis of these responses represented the main area of investigation.

4.1.1 The Subject Group

Nine male sedentary subjects were tested in this study. Prior to the start, it was determined that none of the individuals were currently involved in a program of regular physical activity, or had been involved in one within the past month. As agreed, they did not participate in any strenuous, new, or prolonged physical activities for the duration of the study.

The ages of the subjects were between 21 and 31 years, the mean age being 25.5 years (S.D. = 3.4 years).

4.1.2 The Maximal Voluntary Contraction Measurements

The mean maximal voluntary contraction (MVC), measured in newtons (N) of force, have been recorded for each of the test days in Table 4.1. The individual results, regarding the measured MVCs may be found, in a table, in Appendix B. Also included in Appendix B, are the order in which the treatment intensities were administered, on each separate testing day, and the absolute forces, which these intensities represented.

Table 4.1

Mean MVC Test Results for Each of the Three Test Days

Test Day	n	Mean MVC (Newtons)	Standard Deviation
1	9	1593	357
2	9	1748	408
3	9	1724	375

A one-way ANOVA for repeated measures on the recorded MVCs has been summarized in Table 4.2. Following this, in Table 4.3, are the results of the Tukey post-hoc analysis.

Table 4.2

Summary of ANOVA with repeated measures, examining the recorded MVCs over the three test days.

Source	Sum of squares	df	Mean square	F
Day of Test	125458.72	2	62729.36	7.52 *
Subjects	3342512.41	8	417814.05	50.09 *
Error	133453.00	16	8340.81	

* significant $p<0.05$

Table 4.3

Results of the Tukey post-hoc comparisons for the effects of the given day of the MVC test upon the measured MVC. Tabulated are the differences in mean MVCs, in newtons, between two given test days. The calculated shortest significant range = 111 N.

	Mean	Day 1 1593 N	Day 2 1748 N	Day 3 1724 N
Day 1	1593 N	------	155 *	131 *
Day 2	1748 N		------	24
Day 3	1724 N			------

* significant $p<0.05$

4.1.3 The Measurements of Total Plasma Creatine Kinase

The mean values of the measured responses of the total plasma CK activity, to each of the given treatment intensities, over time, have been recorded in Table 4.4, and are illustrated graphically in Figure 4.1. The responses of the total plasma CK activity, for each subject, may be found in Appendix C.

Table 4.4: Means of the measured responses of total plasma CK activity (U/l at 37 deg. C.), to each of the given treatment intensities, over time (sample n of 9). Standard deviations for each mean are in parentheses.

INTENSITY	TIME OF SAMPLE									
	PRE-EX	POST-EX	2 HR	4 HR	6 HR	8 HR	10 HR	12 HR	14 HR	24 HR
10% MVC	75.0	75.9	77.5	78.0	83.2	85.3	85.1	82.2	81.8	74.6
	(18.2)	(16.4)	(17.0)	(20.2)	(18.0)	(18.2)	(24.5)	(18.7)	(18.0)	(15.2)
30% MVC	73.0	74.8	76.5	80.1	83.0	86.9	86.6	88.4	85.3	81.0
	(15.6)	(16.1)	(18.0)	(20.4)	(21.4)	(21.1)	(24.0)	(23.2)	(24.1)	(26.8)
50% MVC	75.0	80.0	86.3	94.3	100.3	107.1	109.3	115.4	119.8	108.6
	(14.2)	(15.8)	(18.1)	(18.9)	(19.8)	(26.0)	(26.6)	(30.1)	(50.2)	(46.7)

Figure 4.1: Mean total plasma CK activity levels vs. time (assayed at 37 degrees Celsius, N=9)

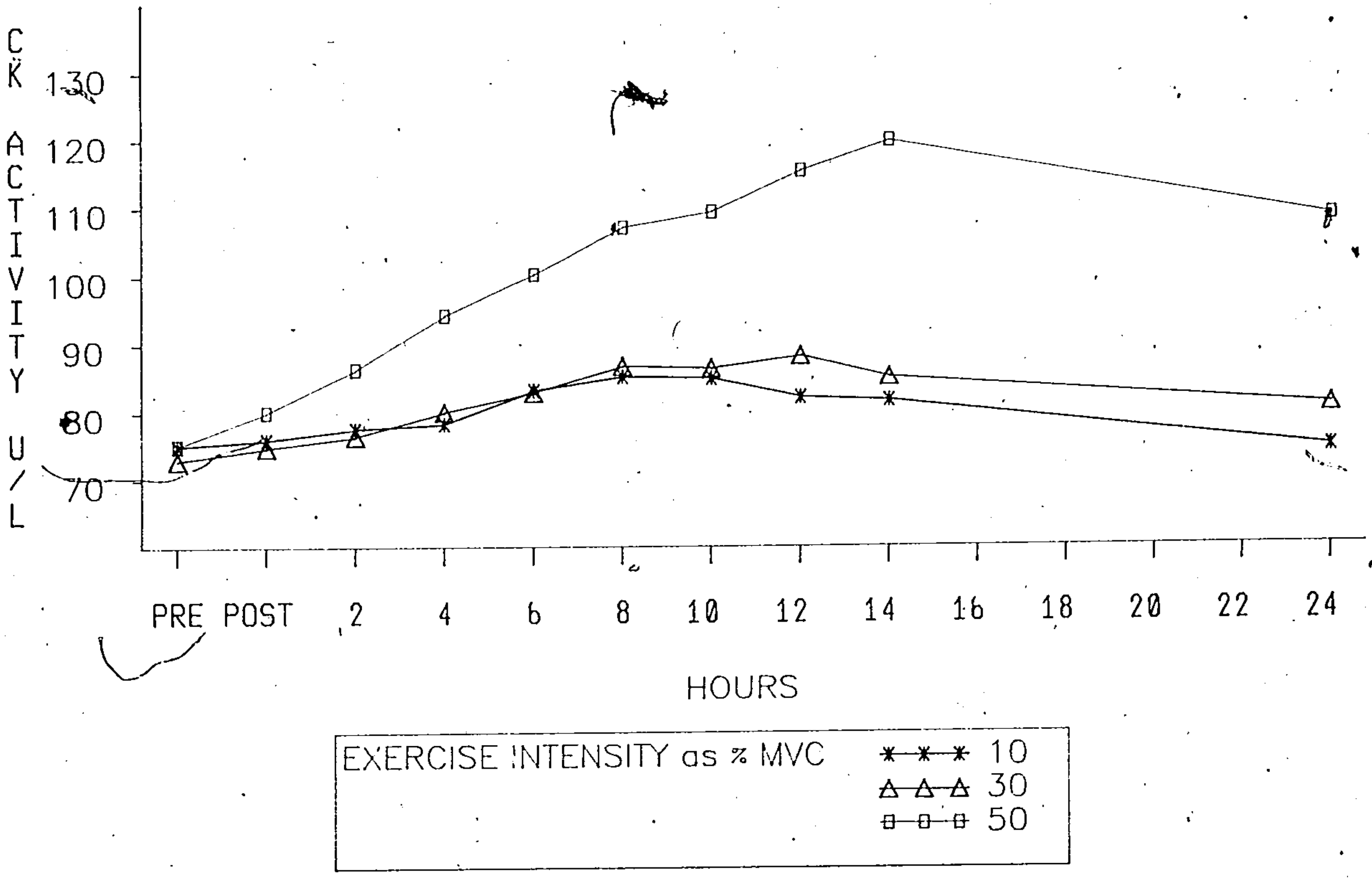


Table 4.5

Isolation of the pre-exercise total plasma CK activity values (U/l at 37 deg. C.), from each of the three test days, to illustrate the inter- and intra-individual variation (S.D.= standard deviation, C.V.= coefficient of variation).

Subject	DAY1	DAY2	DAY3	Mean	S.D.	C.V.
JW	78.1	64.9	76.7	73.4	7.5	10.2%
PS	82.2	73.4	92.6	82.7	9.6	11.6%
KM	67.2	66.4	74.6	69.4	4.5	6.5%
PT	91.6	90.0	89.2	90.3	1.2	1.4%
PM	88.8	82.3	90.5	87.2	4.3	5.0%
BH	31.4	38.4	52.4	40.7	10.7	26.2%
TM	69.4	72.7	60.4	67.5	6.4	9.4%
MD	82.7	86.6	76.3	81.9	5.2	6.4%
AG	83.4	82.4	62.9	76.2	11.6	15.2%
Mean	75.0	73.0	75.1			
S.D.	18.2	15.6	14.3			
C.V.	24.3%	21.4%	19.0%			

Mean inter-individual coefficient of variation= 21.6%
Mean intra-individual coefficient of variation= 10.2%

Table 4.6

Summary of an ANOVA with repeated measures on two factors, examining the effects of exercise intensity and the time of sampling upon the measured total plasma CK activity.

Source	Sum of squares	df	Mean square	F
Intensity	21544.72	2	10772.28	5.79 *
Error	29757.17	16	1859.82	
Time	14764.38	9	1640.49	13.01 *
Error	9077.56	62	126.08	
Int. X Time	7712.09	18	428.45	3.35 *
Error	18396.62	144	127.75	

* significant $p<0.05$

Table 4.7

ANOVA summary table examining the simple main effects of exercise intensity and the time of sampling upon the measured total plasma CK activity.

Source	Sum of squares	df	Mean square	F obs.
Intensity				
Int. at Pre	25.28	2	12.64	0.04
Int. at Post	135.57	2	67.79	0.22
Int. at 2 hr	526.56	2	263.28	0.88
Int. at 4 hr	1377.40	2	688.70	2.29
Int. at 6 hr	1768.98	2	884.49	2.94
Int. at 8 hr	2648.54	2	1324.27	4.40 *
Int. at 10 hr	3330.14	2	1665.07	5.53 *
Int. at 12 hr	5597.33	2	2798.66	9.30 *
Int. at 14 hr	7959.76	2	3979.88	13.22 *
Int. at 24 hr	5887.10	2	2943.55	9.78 *
Error	48153.79	160	300.96	
Time				
Time at 10%	1364.73	9	151.64	1.19
Time at 30%	2368.39	9	263.16	2.07 *
Time at 50%	18743.31	9	2082.59	16.37 *
Error	27474.18	216	127.20	

* significant $p<0.05$

Table 4.8

Results of the Tukey post-hoc comparisons for the simple main effects of intensity at the 12 hour sample time. Tabulated are the differences in mean total plasma CK activity levels, between given intensities. The calculated shortest significant range = 26.3 U/l ($p<0.05$).

Intensity	mean U/l	10% MVC	30% MVC	50% MVC
		82.2	88.4	115.4
10% MVC	82.2	------	6.1	33.2 *
30% MVC	88.4		------	27.0 *
50% MVC	115.4			------

* significant $p<0.05$

Table 4.9

Results of the Tukey post-hoc comparisons for the simple main effects of intensity at the 14 hour sample time. Tabulated are the differences in mean total plasma CK activity levels, between given intensities. The calculated shortest significant range = 26.3 U/l ($p<0.05$).

Intensity		10% MVC	30% MVC	50% MVC
	mean U/l	81.8	85.3	119.8
10% MVC	81.8	------	3.5	38.0 *
30% MVC	85.3		------	34.5 *
50% MVC	119.8			------

* significant $p<0.05$

Table 4.10

Results of the Tukey post-hoc comparisons for the simple main effects of intensity at the 24 hour sample time. Tabulated are the differences in mean total plasma CK activity levels, between given intensities. The calculated shortest significant range = 26.3 U/l ($p<0.05$).

Intensity		10% MVC	30% MVC	50% MVC
	mean U/l	74.6	81.0	108.6
10% MVC	74.6	------	6.4	34.0 *
30% MVC	81.0		------	27.6 *
50% MVC	108.6			------

* significant $p<0.05$

Table 4.11: Results of the Tukey post-hoc comparisons for the simple main effects of time at the 50% MVC intensity. Tabulated are the differences in mean total plasma CK activity levels (in U/l at 37 deg. C.), between given times. The calculated shortest significant range is 17.0 U/l ($p<0.05$).

TIME OF SAMPLE		PRE-EX	POST	2 HR	4 HR	6 HR	8 HR	10 HR	12 HR	14 HR	24 HR
	MEAN	75.0	80.0	86.3	94.3	100.3	107.1	109.3	115.4	119.8	108.6
PRE-EX	75.0	-----	5.0	11.3	19.3*	25.3*	32.1*	34.3*	40.4*	44.8*	33.6*
POST-EX	80.0		-----	6.3	14.3	20.3*	27.1*	29.3*	35.4*	39.8*	28.6*
2 HR	86.3			-----	8.0	14.0	20.8*	23.0*	29.1*	33.5*	22.3*
4 HR	94.3				-----	6.0	12.8	15.0	21.1*	25.5*	14.3
6 HR	100.3					-----	6.8	9.0	15.1	19.5*	8.3
8 HR	107.1						-----	2.2	8.3	12.7	1.5
10 HR	109.3							-----	6.1	10.5	0.7
12 HR	115.4								-----	4.4	6.8
14 HR	119.8									-----	11.2
24 HR	108.6										-----

* significant at the $p<0.05$ level.

4.2 Discussion

The finding that the mean measures of MVC increased significantly for the group, as a whole, after the first MVC test day, would seem to represent an unforeseen adaptation towards a more efficient technique in force production. This is in spite of allowing each subject twenty trials to obtain his MVC.

It might be suspected that the MVC test represented a sufficient impulse to produce strength gains, within the knee extensors, particularly in sedentary subjects. However, this idea is somewhat contradicted by the fact that no further significant increase was found between the second and third MVC tests.

In any case, the protocol was designed so that each individual's absolute exercise treatment intensity was determined relative to his most recently determined MVC. Thus, the possibility of incorrectly applying a particular intensity, relative to an MVC test, which was done two weeks earlier, was controlled for. Furthermore, the order of the given treatment intensities were varied, to control for any biasing factor, which may occur as a result of the treatment order.

Although not in the main focus of the study, the isolation of the pre-exercise total plasma CK activity values, from each of the test days (in Table 4.5), indicate that the experimental pre-test conditions and screening procedures produced exceptionally good estimates of basal total plasma CK activity levels. As found by Nicholson et al. (1985a), the data of this study showed that the inter-individual variation (mean

C.V.= 21.6%) was much greater than the calculated intra-individual variaition (mean C.V.= 10.2%). In comparison, Nicholson et al. (1985a) measured a mean inter-individual coefficient of variation (C.V.) of 47%, and a mean intra-individual coefficient of variation of 19%, in fifteen subjects.

The finding that the coefficients of variation, in this study, were much smaller than those found by Nicholson et al. (1985a), is most likely due to more specific pre-test instructions and the use of a pre-test screening procedure. The importance of not engaging in any strenuous, new, or excessively prolonged physical activity was stressed to each of the subjects frequently throughout the study period. This request may have been reinforced to a greater extent, and more specifically, than when apparently given by Nicholson et al. (1985a). In their study, the subjects were apparently asked only "to avoid all forms of recreational exercise on the three days prior to each venesection".

Furthermore, unlike the procedure used by Nicholson et al. (1985a), a pre-test screening procedure was used in this study. Thus, if total plasma CK activity were found to be above criterion levels, an extra day of rest was given, in order that the exercise treatment days began with near-basal activity levels. This was particularly important for this study, since it was expected that the exercise treatment intensities given would evoke only relatively small changes in total plasma CK activity.

In comparing the effects of different intensities upon the total plasma CK activity, at given times of sampling, it was found that the 50% MVC treatment produced significant elevations ($p<0.05$) above the 10%

MVC and 30% MVC treatments, but only at the 12, 14, and 24 hour sampling time (see Tables 4.8, 4.9, 4.10). No significant differences were found between the 10% MVC and 30% MVC treatments, at any of the times of sampling.

A close examination of the ANOVA summary in Table 4.7 will reveal that significant differences in the total plasma CK activity due to the simple main effects of the exercise intensity at given times of sampling occur also at the 8 and 10 hour sampling time. However, Tukey post-hoc analyses did not reveal any significant differences at these times of sampling at the $p<0.05$ level of significance. This is explained by the relatively greater conservativeness found in the Tukey post-hoc procedure towards type I errors, than found in other post-hoc procedures (Keppel, 1973). This post-hoc approach was specifically selected for this study in the interest of reducing the experimentwise error rate (ie. the probability of making one or more type I errors in a set of multiple comparisons). Unfortunately, the result of this conservative approach is a decreased sensitivity for detecting real differences when they exist (Keppel, 1973). Significant differences in total plasma CK activity among the times for the 30% MVC treatment (see Table 4.7) were not found in the post-hoc analyses for the same reason.

As expected, significant differences were not seen until the later sampling times, due to a delay in the elevated total plasma CK activity, which has been well-documented in numerous studies. However, it was surprising to note that the elevation in activity, due to the 50% MVC intensity, was still significantly higher than the measurements at the 10% MVC and 30% MVC levels, at the 24 hour sampling time. This is particu-

larly interesting in light of the fact that the 50% MVC treatment was only given for a total of 3.5 minutes.

It was earlier suspected that significant differences in activity levels might have been found, at some time, between the 10% MVC and 30% MVC treatments. However this was not shown. This hypothesis was partially based upon the findings of Gollnick et al. (1974a), who examined the glycogen depletion patterns between FT and ST muscle fibres, following sustained isometric contractions. They revealed through the histochemical analyses of biopsy samples, that at intensities below 20% MVC, there is a major use of ST fibres, and above this critical tension, there is a selective use of FT fibres.

It was postulated in this study that some cell injury might occur as muscular tension rises above this threshold. At levels below 20% MVC, where the demands of ATP are aptly met by the aerobic ST fibres, and where the number of motor units recruited are relatively small, it would seem unlikely that the integrity of the muscle cell would be affected.

The differences expected may not have been seen due to two possibilities. Firstly, the actual FT recruitment threshold in the subjects of this study may have been higher than suggested by Gollnick et al. (1974a). In this case, relatively few FT motor units would have been recruited, thus reducing the size of the pool of fibres which might have been affected. Consequently, proportionately less myofibrillar damage would likely have occurred. Secondly, a number of fibres may have been actually injured at the 30% MVC intensity. However, this may have occurred in too small a number to be seen as an effect in the measurement of changes in total plasma CK activity.

Additionally, after an inspection of the graph of changes in mean total plasma CK activities in Figure 4.1, it would seem that there might have been significant trends following the 10% and 30% MVC treatments had a greater number of subjects been used. Slightly higher total plasma CK activity levels, following exercise at these two lower intensities, were measured between 8 and 12 hours post-exercise. In consideration of this, further studies which involve such low intensities of exercise might reserve judgement upon the speculation that no significant changes will occur following 10% and 30% MVC treatments.

An analysis of the changes in total plasma CK activity over time, following the 50% MVC intensity treatment, also yielded some interesting results. An examination of Table 4.11 will reveal that total plasma CK activity rose significantly above the pre-exercise levels, as early as four hours post-exercise. Beyond the 4 hour sample, another significant increase occurred at the 12 hour sample time and remained so for the subsequent 14 hour sample. Again, the effectiveness of the 50% MVC treatment is illustrated, as the total plasma CK activity levels are still well above the pre-exercise levels, after 24 hours post-exercise.

The peak level of 119.8 U/l in total plasma CK activity at the 50% MVC level represents a modest increase when compared to elevations seen post-exercise in other studies. For example, Schwane et al. (1983) found mean elevations of greater than 450 U/l, after bouts of downhill running. Also, Newham et al. (1983) found elevations as large as 34,500 U/l, five days following a prolonged stepping exercise. However, such large increases in total plasma CK activity were not expected in this study, since low intensity exercise, in brief intervals, was used, and

thus, extensive injury to the muscles was not expected to occur. Also, the muscle mass used in the exercises in this study were relatively smaller than would be expected in a stepping or running exercise.

Finally, these results concur with the previous findings of Tiidus and Ianuzzo (1983), in that the peak level of total plasma CK activity will occur between 8 and 24 hours post-exercise. It is, however, unfortunate that blood samples could not be practically taken, in this study, between the 14th and 24th hour post-exercise. In an examination of Figure 4.1 it can be seen that the actual peak total CK activity, for the group, may have occurred in the range between 14 and 24 hours post-exercise. This possibility is reinforced by the fact that peak activity was measured at either the 14th or the 24th hour post-exercise in six of the nine subjects in this study (see Appendix C).

Chapter V

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The purpose of this study was to examine the effect of controlled isometric exercise, at three selected intensities, upon the post-exercise pattern of total plasma CK activity.

The following was determined from the results of this study:

1. Significant increases above pre-exercise levels in the total plasma CK activity appeared following only the treatment intensity of 50% MVC.

2. No significant changes in total plasma CK activity were found following or between the treatment intensities of 10% MVC and 30% MVC.

3. The levels of total plasma CK activity rose significantly above pre-exercise levels as early as 4 hours following the 50% MVC trials. A further significant increase, beyond the level of activity at 4 hours post-exercise, occurred at the 12 hour point, and remained so for the subsequent 14 hour sample.

4. A significant interaction exists between the treatment intensity and the time of sampling. Differences between the 50% MVC treatment and the 30% or 10% MVC treatment were not evident until 12 hours post-exercise.

In addition, it was demonstrated that a small amount of exercise, such as that used in the present study, would evoke significant changes in the measured total plasma CK activity, lasting for as long as 24 hours post-exercise. Also, the outlined experimental pre-test conditions and screening procedure produced exceptionally good estimates of what appear to be basal levels of total plasma CK activity, in each individual.

5.2 Recommendations

Based on the findings of this study, the following recommendations have been made, for consideration in similar research endeavors:

1. The daily physical activity levels of the subject must be monitored and controlled, since very small amounts of exercise have been shown to alter total plasma CK activity significantly.

2. Pre-test screening procedures and conditions, as outlined, should be used to establish basal levels of total plasma CK activity, since a large amount of inter-individual variation may be found within the test group.

3. If peak activity levels are of interest, several serial sampling times should be used. Usually this will be between 8 hours and 24 hours post-exercise. However, the extent of sampling will depend upon the type of treatment and some characteristics of the subject group.

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Appendix A

INFORMED CONSENT FORM

For Participation as a Subject
in a
M.Sc. (KIN) Thesis Project
for
Richard Chin
Department of Kinanthropology
University of Ottawa

Project Title

The Effects of Selected Intensities of Repeated Isometric Knee Extension Exercises Upon Subsequent Total Plasma Creatine Kinase Activities.

Purpose

The purpose of this study is to examine the effects of three different intensities of an isometric knee extension exercise upon the activity levels of an enzyme (ie. Creatine Kinase) in the blood, afterwards throughout the day.

Description of the Study

As a subject for this study, you will be requested to not engage in any strenuous, new, or excessively prolonged physical activities for the duration of the study. The complete schedule consists of fourteen days, described as follows:

DAY 1: SCREENING DAY

Under the condition that you have not engaged in any strenuous, new, or prolonged activities in the two days prior to this day, a small fingertip blood sample will be taken with a lancet and collected into small capillary tubes. The analysis of this sample will determine if another day of rest is needed.

DAY 2: STRENGTH TEST DAY

In a secure seated position on a table, you will be required to perform leg extensions against a padded board, situated just above your ankles. Through brief maximum efforts, your maximum knee extension strength will be determined through a series of twenty trials.

DAY 3: REST DAY

No testing will take place on this day. However, you will be reminded that you should not engage in any strenuous, new, or excessively prolonged physical activities during this period.

DAY 4: SCREENING DAY

No testing will take place on this day. However, as on DAY 1, one blood sample will be taken and analyzed to determine if another day of rest is needed.

DAY 5: EXERCISE DAY

Prior to the start of exercise, another fingertip blood sample will be taken.

Briefly afterwards, seated with the same apparatus as on DAY 2 you will be asked to perform a series of ten second contractions, between which you will be given a twenty second rest. The amount of force generated by the legs will be seen on an oscilloscope monitor, during the contractions. This will enable you to maintain the force at a preselected amount, by keeping the oscilloscope light beam at a given level on the monitor.

At the completion of the exercise, the time will be noted, and another blood sample will be taken. Thereafter, blood samples will be similarly taken at 2, 4, 6, 8, 10, 12 and 14 hours post-exercise.

DAY 6: 24 HOUR POST-EXERCISE BLOOD SAMPLE AND SECOND STRENGTH TEST

On this day, one 24 hr post-exercise blood sample will be taken and, as on DAY 2, a determination of maximum strength will again be made.

DAY 7: REST DAY

No testing will take place on this day, as in DAY 3.

DAY 8: SCREENING DAY

As in DAY 4, no testing will take place. However, one blood sample will be taken and analyzed to determine if another day of rest is needed.

DAY 9: SECOND EXERCISE DAY

Testing and blood sampling will be the same as on DAY 5, except the exercise will be at another pre-selected intensity.

DAY 10: 24 HOUR POST-EXERCISE BLOOD SAMPLE AND THIRD STRENGTH TEST

The procedures on this day are the same as on DAY 6.

DAY 11: REST DAY

No testing will take place on this day, as on DAYS 3 and 7.

DAY 12: SCREENING DAY

As on DAYS 4 and 8, no testing will take place on this day. However, a blood sample will be taken and analyzed to determine if another day of rest is needed.

DAY 13: THIRD EXERCISE DAY AND BLOOD SAMPLING

Testing and blood sampling will be the same as on DAYS 5 and 9, except the exercise will be done at the third of the three pre-selected intensities.

DAY 14: ONE 24 HOUR POST-EXERCISE BLOOD SAMPLE

One final 24 hr post-exercise blood sample will be taken.

Risks to Subjects

In agreeing to be a subject for this project, it must be realized that there are possibilities of some health risks and some discomfort.

In the course of taking several fingertip blood samples, there will be some pain during the lancing. There is a risk of infection if the wounds are not kept clean. However, some swelling and tenderness of the fingertips is more likely to occur, and should become reduced in one or two days. The fingertips may take several days to completely heal, whereupon scars or marks can no longer be seen.

During the exercise periods, subjects will experience muscular fatigue in the thighs (ie. quadriceps) and possibly in the shins (ie. anterior tibial muscles). However, this fatigue is very transient. Muscular soreness may develop later and may persist for one or two days afterwards. Furthermore, some bruising may develop on the shins.

Final Notes and Signed Consent

All of the data and information collected will be treated with confidentiality. Also, the subject has the right to any further explanation of any of the procedures or risks involved.

The subject reserves the right to withdraw consent and discontinue participation in this project at any time. As a student of the university, the subject is ensured that his academic standing will not, in any way, be affected by whether he participates in this project or not.

I, ____________________________, have read the description of this project and understand the possible risks involved therein. Thus, having had the opportunity to ask and receive answers for my subsequent queries, I hereby give my consent to be a participant.

signed:______________________________

date:________________________________

experimenter:________________________

witness:_____________________________

Appendix B

Results of the Individual Maximal Voluntary Contraction (MVC) Tests and the Subsequent Treatment Intensities.

SUBJECT	TEST DAY	MEASURED MVC (NEWTONS)	TREATMENT INTENSITY (% MVC)	ABSOLUTE TREATMENT INTENSITY (NEWTONS)
PS	1	1736	50	868
	2	1601	10	160
	3	1822	30	547
KM	1	1875	10	187
	2	2091	50	1045
	3	2006	30	602
JW	1	2111	30	633
	2	2302	10	230
	3	2333	50	1167
AG	1	825	10	82
	2	964	30	289
	3	1038	50	519
MD	1	1619	30	486
	2	1690	10	169
	3	1558	50	779
BH	1	1432	10	143
	2	1529	50	765
	3	1506	30	452
PM	1	1546	50	773
	2	1774	30	532
	3	1681	10	168
PT	1	1726	50	863
	2	2189	30	657
	3	2018	10	202
TM	1	1466	30	440
	2	1595	50	798
	3	1550	10	155

Appendix C

Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: JW

TIME OF SAMPLE	TOTAL PLASMA CK ACTIVITY MEASUREMENT 10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	78.7 U/l	64.9 U/l	76.7 U/l
POST-EX	74.9	69.9	81.5
2 HR	75.9	75.3	80.3
4 HR	77.4	87.3	101.4
6 HR	83.1	75.7	85.0
8 HR	92.6	85.8	86.8
10 HR	95.6	86.8	92.2
12 HR	82.1	93.0	108.8
14 HR	79.4	74.7	81.9
24 HR	85.2	71.5	81.0

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject:PS

TOTAL PLASMA CK ACTIVITY MEASUREMENT

TIME OF SAMPLE	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	82.2 U/l	73.4 U/l	92.6 U/l
POST-EX	85.8	86.5	89.6
2 HR	89.8	80.7	100.4
4 HR	94.8	90.4	106.7
6 HR	100.6	89.7	118.4
8 HR	97.7	99.4	122.6
10 HR	115.1	101.1	110.3
12 HR	97.0	101.3	121.3
14 HR	94.2	111.0	105.2
24 HR	75.8	88.1	100.1

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: KM

TIME OF SAMPLE	TOTAL PLASMA CK ACTIVITY MEASUREMENT		
	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	67.2 U/l	66.4 U/l	74.6 U/l
POST-EX	76.4	69.1	96.4
2 HR	79.2	66.5	105.5
4 HR	57.7	63.1	114.5
6 HR	85.0	76.8	124.7
8 HR	69.3	78.4	144.1
10 HR	63.5	76.1	137.8
12 HR	63.8	83.1	147.3
14 HR	74.4	96.9	230.0
24 HR	73.4	88.6	126.3

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: PT

TOTAL PLASMA CK ACTIVITY MEASUREMENT

TIME OF SAMPLE	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	91.6 U/l	90.0 U/l	89.2 U/l
POST-EX	87.8	94.2	97.6
2 HR	93.3	102.0	105.2
4 HR	92.3	105.9	108.7
6 HR	94.1	106.7	110.6
8 HR	102.5	105.1	126.6
10 HR	100.2	112.7	134.4
12 HR	95.9	111.5	139.1
14 HR	94.0	104.2	140.2
24 HR	82.2	123.7	143.4

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: PM

TOTAL PLASMA CK ACTIVITY MEASUREMENT

TIME OF SAMPLE	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	88.8 U/1	82.3 U/1	90.5 U/1
POST-EX	89.6	75.4	88.8
2 HR	93.1	84.4	98.9
4 HR	98.6	82.0	109.0
6 HR	100.7	89.7	122.9
8 HR	99.8	95.8	137.3
10 HR	106.6	86.8	152.4
12 HR	89.9	96.4	163.7
14 HR	97.5	80.6	163.2
24 HR	89.0	74.2	213.9

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: BH

TIME OF SAMPLE	TOTAL PLASMA CK ACTIVITY MEASUREMENT		
	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	31.4 U/l	38.4 U/l	52.4 U/l
POST-EX	38.1	39.9	53.1
2 HR	37.5	38.3	53.8
4 HR	39.8	40.5	60.5
6 HR	43.1	41.2	73.9
8 HR	49.7	40.1	82.1
10 HR	40.0	37.8	81.7
12 HR	46.2	40.2	83.2
14 HR	41.8	37.8	83.6
24 HR	38.0	33.1	77.0

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: TM

TOTAL PLASMA CK ACTIVITY MEASUREMENT

TIME OF SAMPLE	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	69.4 U/l	72.7 U/l	60.4 U/l
POST-EX	65.1	67.5	61.3
2 HR	69.5	67.3	71.7
4 HR	64.3	63.9	78.8
6 HR	68.9	62.7	91.8
8 HR	70.8	71.5	88.7
10 HR	62.9	65.4	95.5
12 HR	71.9	62.4	95.8
14 HR	70.2	61.5	87.7
24 HR	67.2	52.8	71.5

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: MD

TOTAL PLASMA CK ACTIVITY MEASUREMENT

TIME OF SAMPLE	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	82.7 U/l	86.6 U/l	76.3 U/l
POST-EX	88.9	84.9	84.0
2 HR	81.3	87.2	89.9
4 HR	92.6	91.9	95.6
6 HR	82.8	100.9	99.2
8 HR	85.7	104.2	101.5
10 HR	86.0	104.4	102.9
12 HR	87.8	101.8	105.8
14 HR	88.4	95.2	106.8
24 HR	80.8	99.0	93.5

Appendix C (continued): Individual changes measured in total plasma CK activity (U/l at 37 deg. C.) in response to the effects of exercise intensity and the time of sampling.

Subject: AG

TOTAL PLASMA CK ACTIVITY MEASUREMENT

TIME OF SAMPLE	10% MVC TREATMENT	30% MVC TREATMENT	50% MVC TREATMENT
PRE-EX	83.4 U/l	82.4 U/l	62.9 U/l
POST-EX	76.5	86.1	67.9
2 HR	78.0	86.5	71.2
4 HR	87.1	95.8	73.3
6 HR	90.6	103.6	75.9
8 HR	99.9	101.4	73.9
10 HR	95.8	108.0	76.7
12 HR	105.7	106.0	73.6
14 HR	96.3	105.8	80.0
24 HR	79.7	~~97.9~~	71.1