SERUM CREATINE KINASE ACTIVITY MODELED FOLLOWING ECCENTRIC AND CONCENTRIC EXERCISE

by

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B.Sc. (P.E.)(Hons.), Montana State University, 1987

THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the School

of

Kinesiology

Simon Fraser University

July 1992

ISBN 0-315-83634-2

APPROVAL

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Degree: Master of Science (Kinesiology)

Title of Thesis: Serum Creatine Kinase Activity Modeled Following Eccentric and Concentric Exercise

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ABSTRACT

An elevated serum enzyme activity (ESEA) has been widely reported to result from eccentric and concentric muscle contraction as a consequence of injury to the muscle fibre. In order to model the time course of this process the present study measured creatine kinase serially together with an objective measure of strength (a one repetition maximum (1 RM) of quadriceps contraction) throughout 10 days following a maximal exercise stimulus in a group of young men who were separated into an eccentric and a concentric exercise group. Each group performed 70 maximum isokinetic contractions of the right quadriceps muscle. These respective stimulus/response sequences were repeated on 2 further occasions 10 days apart in each group. Time-to-peak ESEA following all of the exercise bouts was not significantly different ($p > 0.05$) between the two experimental groups (group means 34.6 ± 14.1 and 15.7 ± 3.8 hours for eccentric and concentric exercise, respectively). Three of 5 subjects, who performed eccentric exercise, exhibited a slightly delayed peak in ESEA. This only occurred after the first training session. Subsequent bouts of 70 eccentric contractions in these three subjects elicited a time to peak ESEA similar to all other subjects. Peak ESEA decreased significantly ($p \leq 0.05$) in exercise bouts subsequent to the first, in both groups. The 1 RM was decreased after the first exercise session but there was no decrease in the pre-contraction stimulus value after the second and third exercise bouts. A two-component model has been developed to account for the observed time course of ESEA. In this model the

first component represents creatine kinase (CK) leakage to the blood from the muscle. The second component depicts degradation of CK from the blood space. This model fitted the ESEA data very well. The model parameters are discussed in terms of physiological and ultrastructural correlates in the literature describing events which produce the so-called damage to muscle resulting from exceptionally heavy physical activity.

ACKNOWLEDGEMENTS

First, I would like to thank the members of my masters committee, Dr. Eric Banister and Dr. Murray Allen for the time and attention they devoted to this study. I am grateful for the expert assistance provided by Michael L. Walsh, Dr. Yoshiyuki Fukuba, Francois Bellavance and Brian Pothier and most grateful to Dr. Eric Banister for his patience, support and enthusiasm throughout my work, which provided me with inspiration.

I would also like to thank all of my subjects who participated in my study for all of their time and sacrifices. Without their dedication this study would not have been possible. To Craig Asmundson, Vic Stobbs, and the secretaries in the office who were always available for help and encouragement, making my educational experience a memorable one; I express my appreciation.

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INTRODUCTION

There are several effects of exercise on muscle which include muscle soreness, hypertrophy, possibly injury, and an accompanying elevated serum enzyme activity (ESEA). Thus, after an exercise bout of sufficient intensity or duration a persistent but reversible ultra-structural change is found in skeletal muscle (i.e., Z-band streaming) (Friden and Ekblom, 1983; Warhol *et al.*, 1985) associated with an elevated serum enzyme activity (ESEA) (Tiidus and Ianuzzo, 1983). The amount of damage to active muscle fibers, has been defined by Armstrong (1990) as microinjury because the initial lesions are usually subcellular and occur in a relatively small proportion of muscle fibers. Several enzymes are typically involved in the ESEA response including aspartate transaminase, aldolase, lactate dehydrogenase, and creatine kinase (CK) (Noakes, 1987). In addition, proteins such as myoglobin (Munjal *et al.*, 1983; Roxin *et al.*, 1984) and myosin heavy chain fragments (Mair *et al.*, 1992) are elevated in the serum following strenuous exercise.

Time Course of ESEA

The literature regarding post-exercise time course of ESEA is quite varied and appears dependent on the type of muscle activity previously undertaken. A faster time to peak ESEA follows a concentric (muscle shortening) (Newham *et al.*, 1986a) in comparison with an eccentric (a forced muscle lengthening during stimulation) contraction (Clarkson and Tremblay, 1988). Downhill

running involving predominantly eccentric contraction, however results in a non-delayed peak in ESEA resulting in a similar time course of ESEA to that following uphill running (Schwane *et al.*, 1989), or exercise involving isolated concentric contractions (Newham *et al.*, 1986a). A peak ESEA following cycling (Berg and Haralambie, 1978) or swimming (Critz and Cunningham, 1972) is substantially less in comparison with marathon running (Dressendorfer and Wade, 1983; Noakes and Carter, 1982). Although the time course of ESEA varies widely and appears to depend on the type of activity previously undertaken, the mechanism inducing ESEA (to be discussed later) is thought to be similar (Russell *et al.*, 1992).

ESEA Following Acute Training

There is some evidence that the ESEA response to an acute exercise stimulus is modified and alleviated somewhat during subsequent stimuli as adaptation to training takes place (Clarkson and Tremblay, 1988). Others have observed an effect of a prior submaximal effort reducing post-exercise ESEA in subsequent heavy exercise 2 to 6 weeks after the initial activity (Byrnes *et al.*, 1985; Graves *et al.*, 1987; Triffletti *et al.*, 1989). However, Schwane *et al.* (1989) failed to observe a significant decrease in ESEA 24 hours (h) after a 45 minute downhill run on a 10 % slope in two groups of subjects whether or not they trained for 1 or 2 weeks by either uphill or downhill running, respectively, prior to the test. Serial measurement of a defined criterion performance has been infrequently made throughout training in such training studies,

hence any impairment in such an index related to the pattern of ESEA due to training is also not well defined.

ESEA and Performance Following Acute Training

An association between ESEA and performance ability is evident in acute strength training bouts. Tiidus and Ianuzzo (1983) reported that 48 h after performing an exercise bout consisting of concentric exercise, 75 % of the subjects experiencing a large ESEA were unable to lift 90 % of their one repetition maximum (1 RM). In contrast, 80 % of the subjects exhibiting a low ESEA were able to lift 90 % of their 1 RM. Clarkson and Tremblay (1988) however, reported that the poorest daily criterion performance preceded peak ESEA by at least 4 days after performing 70 maximum eccentric forearm contractions. The difference in subsequent peak physical effort with respect to these two similar acute exercise periods may possibly be attributed to the peak post-exercise ESEA occurring 3-6 days after the eccentric compared with 8-24 h after the concentric stimulus (Newham *et al.,* 1986a). Clarkson and Tremblay (1988) further observed no ESEA despite a reduction in performance after 70 maximum eccentric contractions when the task was preceded by a relatively few (i.e., 24) similar contractions 2 weeks earlier. The apparent contradiction of the latter experiments to the former described above may be because an immediate ESEA response is not an inevitable consequence of exercise, after exercise apparently of such severity to induce skeletal muscle damage (Newham *et al.*, 1986b)

ESEA Following Chronic Training

In an endurance training study (Bansiter *et al.*, 1992) in which the amount (dose) of the exercise undertaken each day was quantitatively measured, the time course of ESEA was directly related to the dose of exercise and inversely related to the pattern of criterion performance (running a standard distance time trial). When training was heavy, ESEA was high and physical performance on a standard test declined. ESEA returned to a base line level during peaking (detraining) as performance increased. In addition, in the later stages of heavy training, just prior to peaking, ESEA decreased from a peak towards a lower but still elevated level compared with the base line measure. The criterion physical performance itself also began to recover towards its original base line value during the final stages immediately before peaking. Dressendorfer and Wade (1983) have also reported an adaptation in ESEA during a 20 day period of heavy racing averaging 17.3 miles per day.

Quantification of Muscle Damage

In an entirely different context, ESEA has been used by several investigators to estimate the degree of cardiac tissue damage resulting from myocardial infarction in the hours following infarction (Norris *et al.*, 1975; Roberts *et al.*, 1975; Shell *et al.*, 1971; Shibata *et al.*, 1985; Sobel *et al.*, 1972). This estimation

involved calculating the total amount of enzyme appearing in the circulatory system by integrating the ESEA curve and accounting for the amount of enzyme degraded in the blood by determining the time constant for the latter portion of the ESEA decay curve when it was assumed that enzyme influx into the circulatory system was complete. The total amount of muscle damage then could be calculated by knowing the proportion of enzyme released from the damaged site which then enters the vascular space and the difference in enzyme concentration between damaged and normal cardiac tissue. The rationale of this technique has been used to quantify the extent of skeletal muscle injury resulting from severe exercise (Apple and Rhodes, 1988). In none of these cases, has the model been extended to define the complete, developing time course of ESEA.

Skeletal Muscle Damage

Skeletal muscle damage to subcellular components is evident after muscle activity, particularly when the exercise is relatively intense, of long duration, or includes eccentric contraction. Structural damage involves both the cytoskeleton and the lipid bilayer membrane structures. Myofilament damage is a rapid process in which increased intracellular calcium concentration ($[Ca^{2+}]_i$) appears to play a role (Duncan, 1987). Increased $[Ca^{2+}]_i$ may be implicated in induction of the cytosolic thiol proteases calpain I and II producing direct protein breakdown in muscle (Furono and Goldgerg, 1986). Calpain receptors have also been reported to be

located directly on the sarcoplasmic reticulum (SR) in proximity to the Ca^{2+} flux initiating muscle contraction. However when these proteases are inhibited muscle proteolysis still occurs (Furono and Goldberg, 1986). The second type of muscle damage is a slower process which effects the plasma membrane permitting the efflux of cellular enzymes (i.e., CK) into the extracellular fluid (Duncan and Rudge, 1988).

The muscle damage resulting from exercise has been rationalized with a logical progression of steps caused by one or more of several possible initiating events (Armstrong, 1990). Firstly, the overall high specific tension mechanically disrupting sarcolemma, SR, and myofilaments may itself induce an inflammatory response. Secondly, metabolic factors such as a high local temperature in the muscle have been related to muscle protein degradation (Baracos *et al.*, 1984). Thirdly, Ca^{2+} aggregation in mitochondria and a consequent reduction in respiration could also lead to an attenuated ATP level, which might impair Ca^{2+} uptake from the cytosol via an ATP-dependent calcium pump in the sarcolemma, mitochondria, and SR. Fourthly, general local damage may impede local microvascular circulation (Suval *et al.*, 1987) and perhaps lymphatic flow the former inducing an ischemia followed by a reperfusion which could result in free radical production and membrane lipid peroxidation (Davies *et al.*, 1982). Lastly, exercise induced phospholipase A_2 (PLA_2) activity may be permitted to come into contact physically with phospholipid substrates in the cell membrane (Duncan, 1988; Duncan and Rudge, 1988) and lyse structural components of the sarcolemma. PLA_2 is an enzyme found

in the membrane of a cell and when activated with the appropriate stimulus (Ca^{2+}, hormone, neurotransmitter, drug, or toxic agent), cleaves the membrane phospholipids to produce arachidonic acid (AA). It appears the production of AA from PLA_2 is a rate limiting step in a cascade of events leading to the inflammatory response. There are two major branches of arachidonic acid metabolism, one catalyzes the enzyme cyclooxygenase leading to the formation of prostaglandins, prostacyclin and thromboxanes none of which appears to be involved in producing ESEA (Duncan and Rudge, 1988). The other pathway, catalyzed by the enzyme lipoxygenase, leading to the formation of fatty acids known as leukotrienes (Chang *et al.*, 1987). These fatty acids, which have a detergent effect in addition to the digestive effect of lysophospholipids caused from lipid peroxidation of its membrane, can also disrupt the integrity of the cell membrane and permit enzyme efflux from the cytoplasm (Duncan and Rudge, 1988).

The hypothetical initiating events listed above could all lead to, or result from, an elevated $[Ca^{2+}]_i$. In addition, CK efflux from incubated mouse soleus muscle, induced by 2,4-dinitrophenol (DNP), has been shown to be dependent on extracellular Ca^{2+} (Duncan and Jackson, 1987). Furthermore, when PLA_2 is stimulated by 10^{-6} molar Ca^{2+} influx and inhibited by chlorpromazine, cellular efflux of CK is prevented (Jackson *et al.*, 1984). Therefore, if $[Ca^{2+}]_i$ homeostasis is altered to approximately 10^{-6} molar as a result of exercise, Ca^{2+} is likely to be an important factor leading to skeletal muscle damage. As discussed previously, $[Ca^{2+}]_i$ also stimulates

PLA_2 which produces the several effects detrimental to the cell discussed previously.

As yet there are no data available on the time course of PLA_2 activity in the muscle or blood following exercise. However, the time course of PLA_2 in blood during acute adult respiratory distress syndrome appears similar to ESEA but the time course of onset occurs earlier (Koeniger *et al.*, 1989). Following exhaustive exercise maximum $[Ca^{2+}]_i$ uptake capacity in isolated SR is lower (Byrd *et al.*, 1989) and $[Ca^{2+}]_i$ accumulates in an injured muscle immediately following downhill walking in rats (Duan *et al.*, 1990). These findings are consistent with implicating Ca^{2+}-activated PLA_2 activity an important possible initiator of injury to the muscle cell.

Regeneration of Damaged Skeletal Muscle

After the initial autogenic proteolytic and lipolytic response disrupting cellular structures an evolving inflammatory response probably dominates the process of developing injury. The latter phase includes the invasion of the damage site by mononuclear cells (macrophages) from the blood which occur 3-4 h following the exercise when foci of muscle damage is already widespread (Fisher *et al.*, 1990).

Several studies involving acute blunt trauma to animal muscle have provided insight into post-trauma ultrastructural events occurring in skeletal muscle (Fisher *et al.*, 1990; Hurme and Klimo 1992; Russell *et al.*, 1992). In these studies various severe muscle stimuli were used to induce injury including eccentric exercise,

stretch, chronic stimulation, tension overload and cold injury. These reports show that hemorrhage, inflammation, non-necrotic degradation, regeneration involving myoblast formation and satellite cell activation by growth factors encompass a 30 day period. Immediate (3-24 h) effects of the clinically-produced trauma revealed gross tearing and disruption of normal cells and small blood vessels. A large number of mononuclear cells in the intracellular connective tissue and within the damaged muscle were observed. By 24-48 h there were a large number of sarcolemmal nuclei observed some of which were likely of satellite cell origin (Fisher *et al.*, 1990) which have both a myogenic and regeneration capability in skeletal muscle (Carlson and Faulkner, 1983). During the next several days regeneration of the sarcomere occurred and by day 14, 21 and 30 post-trauma the muscle cell appeared to have healed as no abnormalities were observed. The foregoing studies which used a variety of ultrastructural, immunochemical, cellular and molecular techniques now confirm and extend the gross features of intrinsic degradation, cell-mediated breakdown, and regeneration described earlier by others (Armstrong 1990; Carlson and Faulkner, 1983; Carlson, 1972).

The purpose of the present study was to investigate the time course of ESEA in order to develop a simple systems model of the fine structural and biochemical degenerative/regenerative process described above. The present research will attempt to provide insight into the initiating events inducing the rise and subsequent decline in serum enzyme activity following heavy exercise in young male subjects.

HYPOTHESES

The working hypotheses of this study are that:

1. A mathematical model of the time course of ESEA following a single substantial bout (dose) of exercise (70 eccentric or concentric contractions) of the right quadriceps muscle group may be deduced which will account for the ESEA actually observed.

2. Parameter definition of the model determined by iterative modeling of the theoretical time course of ESEA against experimentally determined serial measures of ESEA following exercise of the quadriceps muscle, will depend on the type of contraction made and the immediate history of previous exposure of the muscle to a similar stimulus.

METHODS

Training Groups and Regimen

Ten volunteer male subjects were allocated randomly to a concentric or eccentric training group (n=5). Subject data is shown in Table 1. This study was approved by the Simon Fraser University Ethics Committee. Before commencing the first of three exercise sessions separated by ten day intervals (Figure 1), each subject was medically examined and approved to take part in the study and each signed an informed consent letter. In order to control for physical activity the subjects were asked not to engage in exercise 7 days prior to and during the study. This was confirmed by questioning the subject daily, and if additional exercise was undertaken the subject was removed from the study. Furthermore, subjects were chosen who had not been involved in activities involving concentric or eccentric resistive leg extension exercises within the 3 months prior to commencing the study .

Table 1. Showing the age (yr), weight (kg) and height (cm) of each male subject.

Subject	1	2	3	4	5	6	7	8	9	10
Age	36	25	25	27	30	28	29	30	26	26
Height	185	168	183	160	193	178	180	178	170	178
Weight	85	66	76	67	84	86	80	66	50	68

Exercise involved 70 maximum leg extension exercises (concentric contraction) or resistance to a torque, forcing leg flexion from an extended position (eccentric contraction) against a maximum resistive force exerted by the subject on an isokinetic (Biodex™) apparatus for measuring strength. Raw data (torque and work) were sampled at 100 Hz throughout both flexion and extension movement in either a concentric or eccentric exercise mode at a speed of 30° per second. The Biodex™ also recorded isometric torque. The means of torque and work respectively were able to be calculated for each separate contraction of a repetitive sequence of contractions by an individual.

The sequence of exercise sessions and data collection throughout the experimental period is shown in Fig. 1. During the exercise period each muscle contraction (70 in total) was performed at a speed of 30 degrees per sec (3 seconds per contraction) with an 11 second rest interval between each contraction. The range of motion of the concentric exercise was from the flexed (90°) to the fully extended (0°) leg position. The subject exerted maximal force for each contraction. The speed was controlled by the Biodex™. The Biodex™ mechanism returned the leg passively to a starting position between repetitions to avoid any antagonist muscle group activity to the designated contraction. Similarly, the group of subjects undergoing the eccentric exercise stimulus exerted a maximum resistive effort against a superior load forcing the leg from extension (0°) to 90° flexion, after which the leg was returned passively to the extended position. The subject was seated and

secured at the waist and shoulder to immobilize the upper body while he was able to maintain free movement of the exercising leg.

A physiological/mathematical model of the dose/response effect of training was formulated (see later) in order to describe, quantitatively, the empirical pattern of ESEA development and recovery associated with each type of successive high intensity exercise stimulus.

Blood Sampling and Analyses

A physician was on call during the time when blood was taken and only a qualified individual drew the venous blood sample from the subject.

A blood sample was drawn 2 days prior to commencing the first exercise bout to establish basal serum enzyme activity. If this basal CK activity was high due to previous physical activity the initial exercise bout would be postponed until a basal level was obtained. A blood sample was also drawn immediately before and after each of the 3 exercise sessions and at 5 h intervals for the first 40 h, excluding the hours in the late evening and early morning, and every 15 h thereafter until ESEA approximated the basal level. Each blood sample taken was allowed to clot and then centrifuged to separate serum which was extracted and refrigerated for no longer than 12 h before analysis. Total serum CK activity, $\mu moles \cdot min^{-1} \cdot L^{-1}$ ($iU \cdot L^{-1}$), was measured on a Roche Cobas Bio analyzer at 30°C using a BMC (Boehringer-Mannheim CmdH, Mannheim, West Germany) reagent as outlined by Szaz *et al.* (1976). The coefficient of variation in

repeated daily analyses of standard samples of this enzyme ranged between 3-5 % during the period of the study.

Criterion Performance

A 1 RM, criterion performance was determined previous to each standard exercise bout and daily throughout the following 10 day recovery period until a subject could attain the developed pre-exercise torque. The 1 RM consisted of the subject performing two-3 second maximum isometric contractions in an extended leg position, in which the right knee was bent at a 45° angle. The highest torque of two trials determined the criterion performance for the given day.

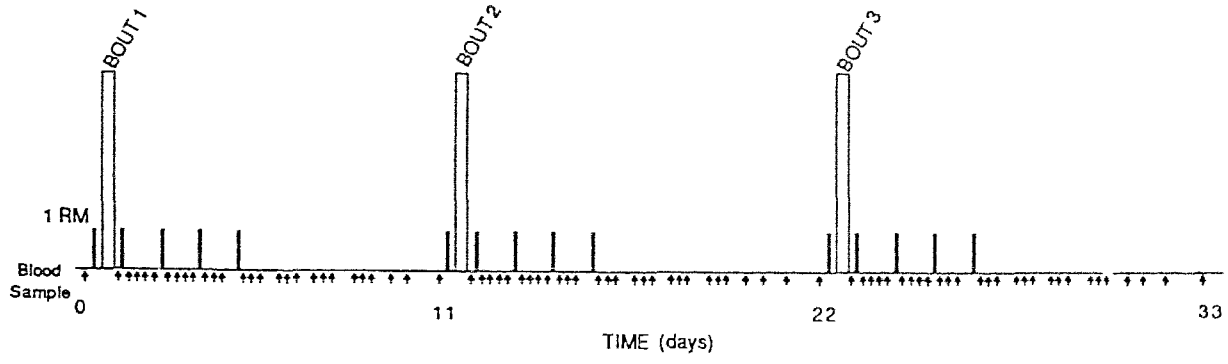


Figure 1. Showing experimental design of the study and the type and sequence of measurements made on a subject following a single dose (set of 70 eccentric or concentric) of exercise.

Theory

Modeling Exercise Induced Serum Enzyme Activity

A theoretical two-component model of enzyme flux from the muscle cell to the vascular space was developed to explain the empirical pattern of ESEA found in each 10 day period following an exercise stimulus. In the hypothesized model determining the time course of ESEA, it was assumed that enzyme from the cytoplasmic and mitochondrial compartments of the cell is lost principally to the to the extracellular space. This enzyme efflux may be attributed to cell membrane damage potentiated by any one of several initial events described by Armstrong (1990) including fibre disruption, calcium-induced lipolysis (Duncan, 1987; Jackson *et al.*, 1984), free radical mediated membrane lipid peroxidation (Davies *et al.*, 1982) or other induced cellular inflammatory responses. Enzyme lost from the cell was considered transferred directly or indirectly (via interstitial, lymphatic system) to the vascular compartment in which the time course of enzyme cellular efflux was reflected by ESEA. Although it has been shown that an increase in plasma CK activity after ligation of a dog hind limb could be prevented by collection of thoracic duct lymph (Lindena *et al.*, 1979), it is currently undetermined how CK enters the blood after local disruption due to intense exercise. Due to the relatively greater increased vascularity of the affected area after exercise compared with the above study (Lindena *et al.*, 1979), it is possible that a

proportion of CK is lost directly from the cytosol to the blood after exercise.

Two-Component Model System: ESEA

Figure 2 shows a hypothesized two-compartment model of developing membrane damage and concomitant healing precipitating an intramuscular enzyme efflux to the vascular space together with constant natural degradation of enzyme activity from the vascular space. In this figure, flow of enzyme activity from the muscle contributing to the already existing quantity of enzyme activity in the vascular space (X) is f(t). Degradation of enzyme activity from the vascular space is proportional to the product of overall enzyme activity per litre and the flow loss in $L \cdot min^{-1}$ (F) from the vascular space.

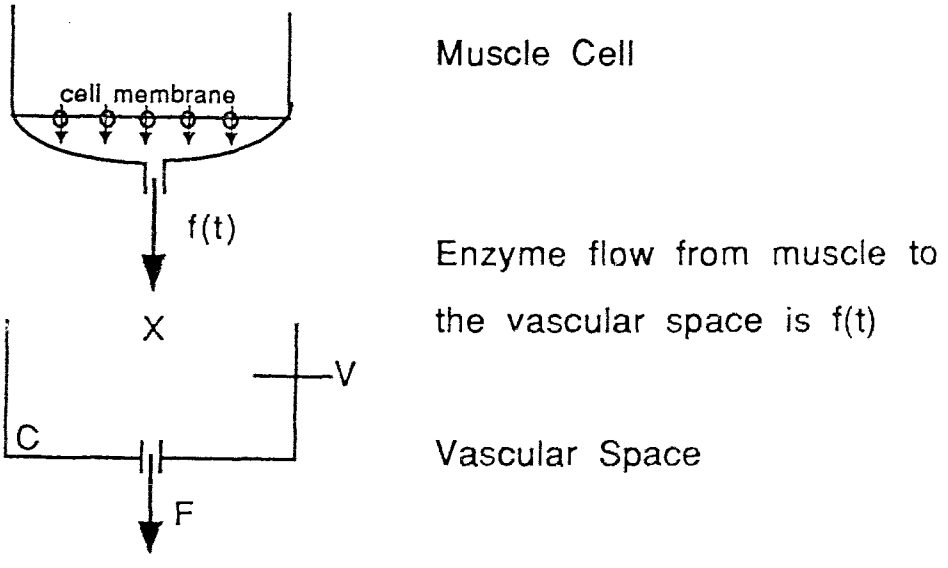


Figure 2. Showing the two-component model of leakage of enzyme from the muscle f(t) and degradation of CK from the vascular space (F). The open circles diagrammatically represent the developing membrane damage.

where:

$f(t)$ = enzyme leakage into the blood ($iU \cdot min^{-1}$)

F = flow loss from the vascular space ($L \cdot min^{-1}$)

X = enzyme activity above base line (iU) in the vascular space

V = Volume of vascular space (litre)

C = Enzyme activity (per litre) in plasma above base line ($iU \cdot L^{-1}$)

and:

$F \cdot X/V$ = degradation of enzyme activity.

Thus, the rate of change of enzyme activity per volume of vascular space (X) above base line in the vascular space is given

by: $$d/dt X = f(t) - F \cdot X/V \qquad (1)$$

and: $$f(t) = d/dt X + F/V \cdot X.$$

Integrating during the period of enzyme loss from muscle, from 0 to time T [0→T]:

$$\int_0^T f(t)\,dt = X(T) + F/V \int_0^T X(t)\,dt. \qquad (2)$$

Thus, total enzyme loss from the muscle is the total enzyme in the vascular compartment remaining above base line at time T plus the

area under the ESEA curve corrected for the ongoing loss due to catabolism (F·C'

and: $$\int_0^T f(t)dt = V \cdot C(T) + F \int_0^T C(t)dt. \quad (3)$$

Thus, the shape of the ESEA time curve reflects the kinetics of the release of enzyme from the muscle cell.

In this model, therefore, estimation of the integral $\int_0^T f(t)dt$, representing the quantity of exercise-induced enzyme loss from muscle in a period $0 \rightarrow T$, may be made from an analysis of the time course of enzyme measured serially in blood.

Mathematically the two phase (rise and decay) time course of ESEA in Fig. 2 may be represented by the two-component expcnential:

$$y_{(t)} = C_{(t)} = A\,(e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \quad (4)$$

where:

$A = a \cdot k_1/k_2 - k_1$

and

a = potential enzyme loss from muscle due to the exercise stimulus ($iU \cdot L^{-1}$)

k_1 = rate constant for enzyme leakage (min^{-1} $x10^{-4}$)

k_2 = rate constant for CK degradation (min^{-1} $x10^{-4}$)

$C_{(t)}$ = enzyme activity ($iU \cdot L^{-1}$) in blood above base line

and $A < 0$, $k_2 > k_1 > 0$.

The constant A represents the combined flow-volume characteristics of the vascular compartment and the degradation of enzyme from this area. $C_{(t)}$ represents the rise and decay of enzyme activity per unit volume in the vascular compartment due to an exercise-induced developing porosity and recovery of the cell membrane at any given time above base line activity per unit volume. The leakage of CK from the muscle f(t), as shown diagrammatically in Fig. 2 through open circles along the membrane, is followed by their plugging (by red blood cells, macrophage and etc.) or by pore closure following adaptation or recovery from the exercise stress. These events affecting enzyme leakage into the vascular space, are represented by the rate constant k_1 of equation 4. The continuous removal or degradation of CK in blood per unit volume is controlled by F in Fig. 2 and is represented by rate constant k_2 in equation 4.

The Modeling Process

Parameter definition of the constants, A, k_1 and k_2, of equation 4 involved a systematic process. ESEA data were first displayed on a computer screen. The theoretical data points of the time course of ESEA were generated from the model theoretical equation using initial estimated values for A, k_1, and k_2. A first iteration of the predicted ESEA pattern was superimposed on real data throughout the time course of each ESEA (CK) curve. An accurate parameter definition was then made from a reiterative

process changing each parameter through a sequential, computer-generated process to achieve a least-squares best fit of the predicted to real time course of ESEA values. At this point a change in any one of the least-squares model values by a single decimal place resulted in a greater total mean squared error.

Statistics

The significance of the difference between the time and magnitude of the peak ESEA, the model parameters (A, k_1 and k_2) and the average torque in the concentric and eccentric training groups for bouts 1-3, respectively was established by using a two-way analysis of variance with repeated measurements on one factor. A post hoc comparison between exercise bouts was performed using the Bonferroni method. A least-squares best fit line was calculated for the relationship between the emperical and theoretical modeled ESEA (CK) data in the concentric and eccentric groups for bouts 1-3, respectively. The mean and standard deviation for the time to peak ESEA (CK) value for each group were also calculated.

RESULTS

ESEA Response to Exercise

Figure 3 illustrates the time course of ESEA for subjects 1-5 exposed to a concentric stimulus and subjects 6-10 who were exposed to an eccentric stimulus. There was no significant difference between the concentric and eccentric groups with respect to time to peak ESEA and peak amplitude of ESEA. Therefore, to increase statistical power, the two groups were combined to determine if there was a significant difference between the 3 training bouts with respect to the time to peak ESEA and the peak amplitude ESEA value.

The time to peak ESEA following the initial exercise bout was not significantly different ($p > 0.05$) between the two exercise groups (group mean value 34.6 ± 14.1 and 15.7 ± 3.8 h for eccentric and concentric exercise, respectively). Subjects 8, 9, and 10 who performed eccentric exercise exhibited a slightly delayed peak in ESEA, which only occurred after the first exercise session. For bouts 2 and 3 of 70 contractions in these three subjects, the time to peak ESEA (CK) was similar to that of all other subjects in the two groups.

The peak amplitude of the time course of ESEA decreased significantly ($p \leq 0.05$) compared with the initial ESEA peak in both groups following subsequent exercise bouts after the first. There was no significant difference in peak ESEA between the second and third exercise stimulus.

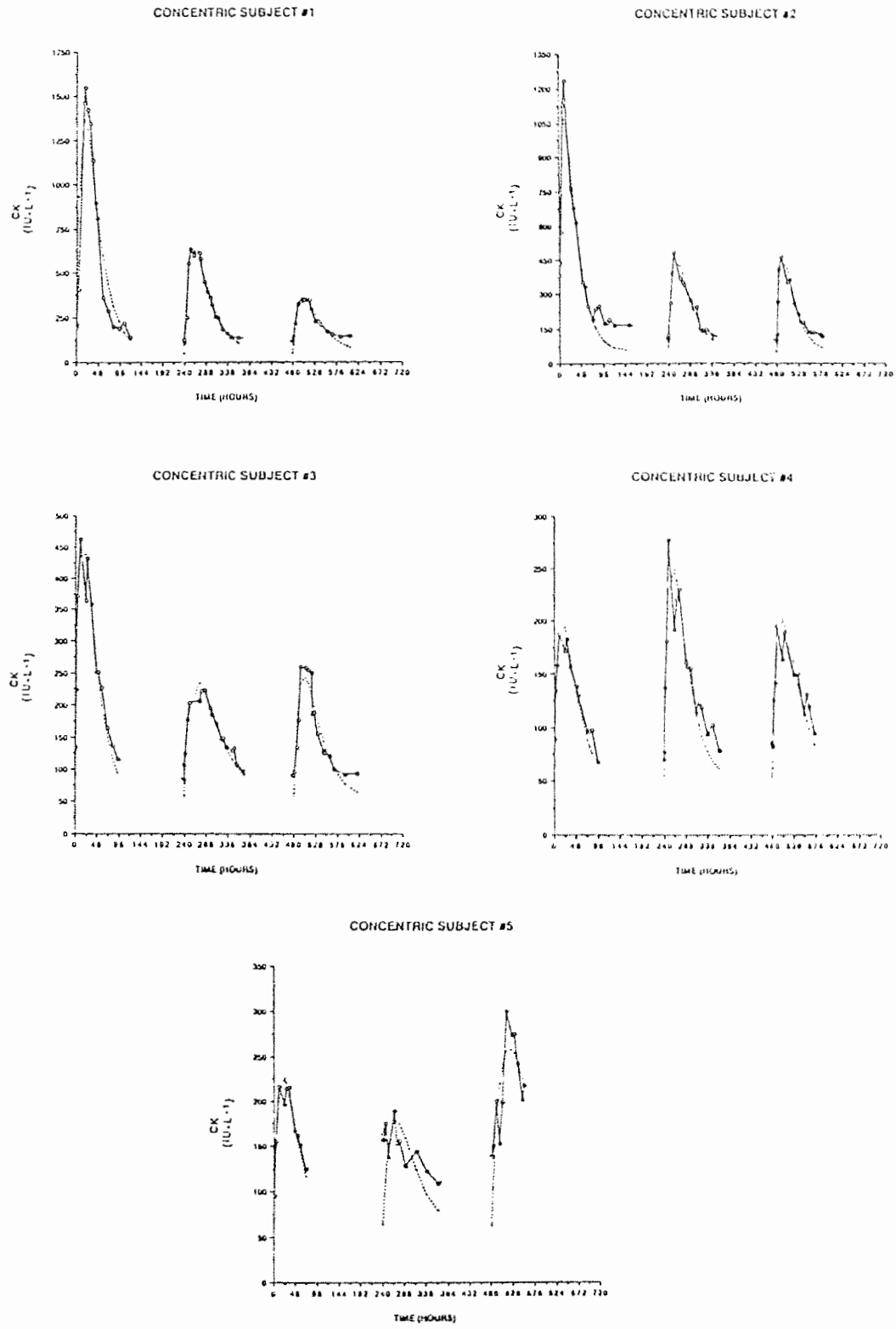


Figure 3. Showing the real (o—o) and modeled (o--o) ESEA time course in male subjects following completion of concentric (1-5) or eccentric (6-10) 70 muscle contractions set, respectively on 3 occasions 10 days apart.

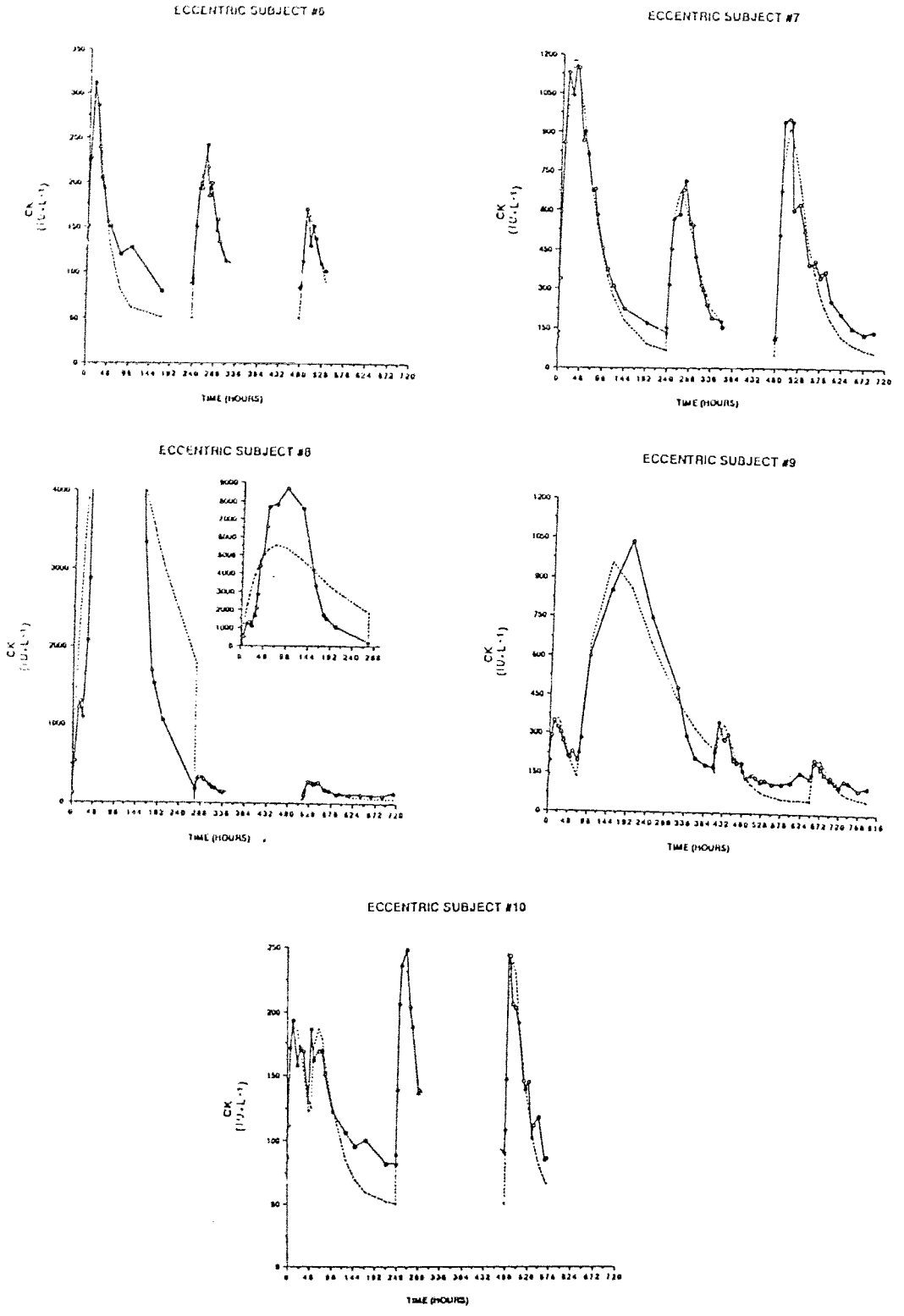


Figure 3 continued.

Modeling Creatine Kinase Activity

Figure 3 shows the result of modeling serial CK activity to achieve a least squares best fit of the time course of predicted measures of ESEA (CK) to those actually observed. Parameter definition A ($iU \cdot L^{-1}$), k_1, and k_2 (min^{-1} $x10^{-4}$) for the two-component exponential iterative modeling is shown in Table 2. The R^2 value, which is the fraction of the explained variance in y resulting from the prediction of y from the modeled values of x in the equation $y = mx + c$ and the mean square error (MSE) for subjects 1-10, are also shown in Table 2.

Criterion Performance

A one repetition maximum (RM) isometric contraction was serially measured daily following each 70 contraction bout of exercise until the pre-contraction level was reestablished. This measure, termed a criterion performance is shown in Table 3. The 1 RM decreased following the first exercise session and recovered to the pre-70 contraction value in 2-3 days for most subjects. However, subjects 2 (concentric group) and 8, and 9 (eccentric group) required 4-5 days before they were capable of achieving their pre-training torque. For the second and third training session the 1 RM equalled or surpassed the pre-training 1 RM value by 1 or 2 days following the exercise stimulus.

Average Torque

Figure 4 shows the results of the average peak torque value of the 70 maximum muscle contractions for each training bout for the concentric and eccentric training groups. There was no significant difference in the average peak torque value between the two training groups or within the training bouts.

Model Parameters

Figures 5 and 6 show the mean values of the model parameters A, and k_1 and k_2, respectively. There was no significant difference in any of the model parameters between the concentric and eccentric groups or between any of the training bouts.

Table 2. Parameter definition A, ($iU \cdot L^{-1}$) k_1, and k_2 (min^{-1} $x10^{-4}$) for the two-component $C_{(t)} = A\,(e^{-k1 \cdot t} - e^{-k2 \cdot t})$ exponential defining the time course of ESEA. The fraction of variance (R^2) and the mean squared error (MSE) of empirical ESEA of CK data points predicted from the theoretical model of ESEA are also shown. Subjects 1-5 and 6-10 trained concentrically and eccentrically, respectively.

	Bout	A	k_1	k_2	R^2	MSE
Subject 1:	1	3965.6	14.0	5.5	0.896	34213.5
	2	1751.5	12.1	5.5	0.958	1738.7
	3	932.2	11.2	4.4	0.952	952.8
Subject 2:	1	3061.7	18.0	7.1	0.872	20340.0
	2	1176.6	14.0	5.5	0.942	1158.9
	3	1242.6	17.7	6.9	0.957	1450.5
Subject 3:	1	1236.0	14.9	5.8	0.934	1412.6
	2	558.1	8.5	3.3	0.941	392.7
	3	589.0	11.7	4.5	0.864	650.5
Subject 4	1	589.3	15.6	6.1	0.949	320.0
	2	797.6	16.4	5.8	0.874	696.5
	3	605.4	12.7	5.0	0.850	568.4
Subject 5	1	533.9	12.1	4.7	0.923	511.9
	2	386.5	9.0	3.5	0.048	3311.7
	3	627.8	11.7	4.6	0.649	2609.7
Subject 6	1	732.0	17.3	6.8	0.901	1776.7
	2	497.3	11.7	4.6	0.897	364.4
	3	332.2	15.9	6.2	0.850	331.5
Subject 7	1	3400.9	9.5	3.7	0.966	5930.2
	2	1867.6	18.9	7.4	0.948	2045.9
	3	2603.7	10.6	4.2	0.909	9393.7
Subject 8	1	16416.5	3.4	1.4	0.691	3291252.0
	2	919.6	18.9	7.4	0.836	3451.3
	3	698.3	13.3	5.2	0.908	1374.8
Subject 9	1	940.4	14.4	5.6	0.909	2352.7
	1 *	2723.5	3.3	1.3		
	2	867.7	11.7	4.6	0.775	3759.9
	3	503.7	14.0	5.5	0.679	1925.8
Subject 10	1	433.0	15.5	6.1	0.828	413.7
	1 *	416.1	12.9	5.0		1584.9
	2	574.8	14.9	16.4	0.950	258.1
	3	565.6	16.4	6.4	0.877	671.1

* due to a biphasic curve

Table 3. 1 RM criterion performance (foot-pounds) for the concentric (subjects 1-5) and eccentric (subjects 6-10) training groups, respectively.

	Bout	Pre	Post	Day 1	Day 2	Day 3	Day 4	Day 5
Subject 1	1	264	272	294	284	303		
	2	286	280	292				
	3	292	274	300				
Subject 2	1	208	177	-	-	189	202	
	2	210	191	211				
	3	221	193	230				
Subject 3	1	192	215	216				
	2	220	221	216				
	3	229	251					
Subject 4	1	158	139	166				
	2	170	179	191				
	3	205	195	207				
Subject 5	1	186	188					
	2	199	172	195				
	3	214	190	211				
Subject 6	1	-	191	216	210			
	2	219	230	234	241			
	3	235	208	208	226	226		
Subject 7	1	131	126	153				
	2	155	156	156				
	3	177	161					
Subject 8	1	215	195	177	201	197	185	216
	2	215	183	219	189			
	3	201	182	183	197	205		
Subject 9	1	129	83	54	58	102	123	147
	2	142	124	153				
	3	160	131	165				
Subject 10	1	145	163	161				
	2	177	164	172				
	3	198	175	209				

- indicates data not obtained

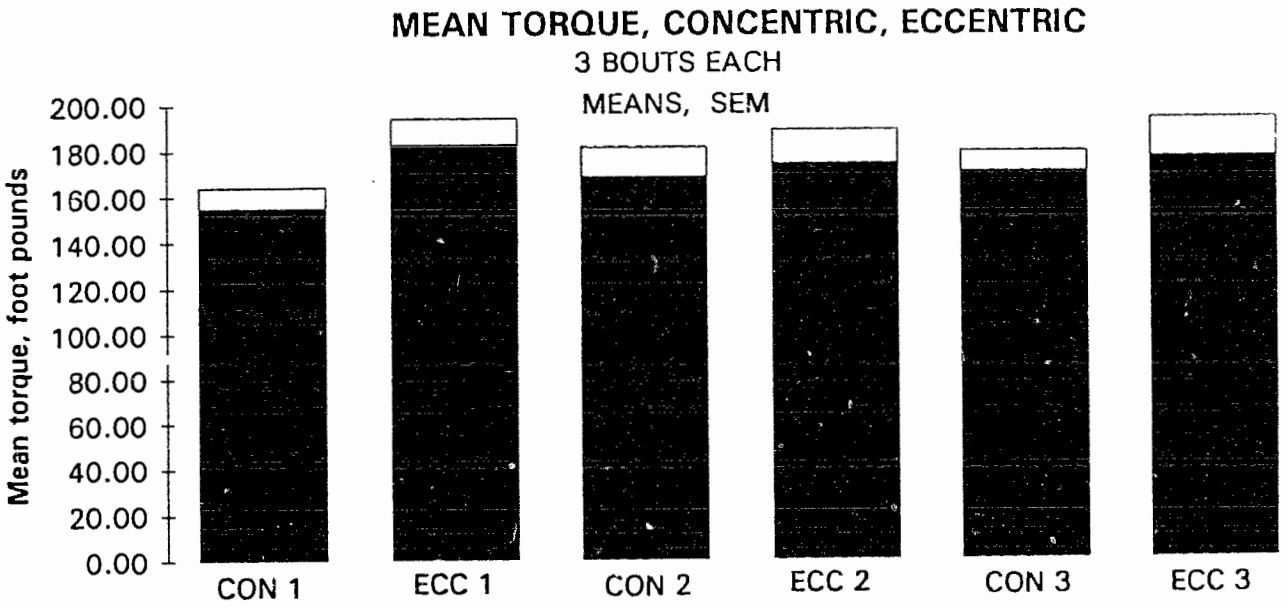


Figure 4. Showing the average peak torque value and S.E.M. for the 70 maximum contractions for the 3 training bouts for the concentric and eccentric training groups.

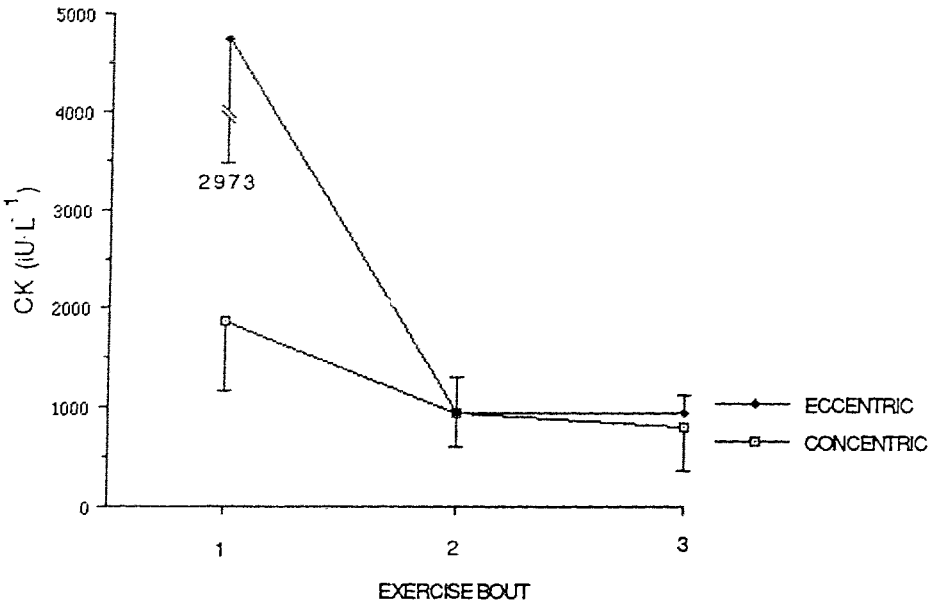


Figure 5. Showing the average value and S.E.M. of model parameter A for the 3 training bouts with both training groups combined.

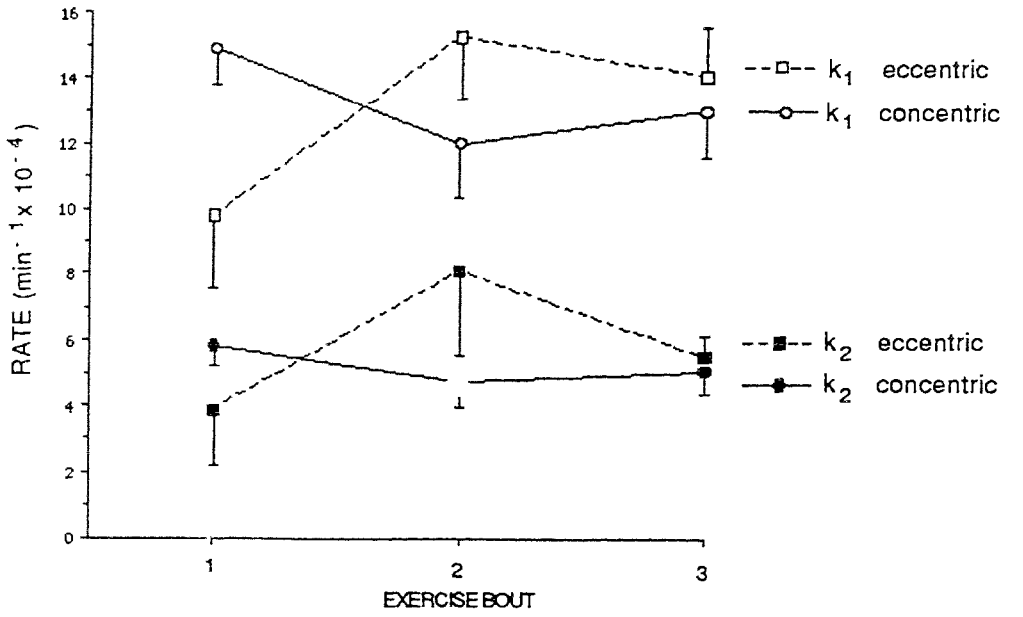


Figure 6. Showing the average value and S.E.M. of model parameters k_1 and k_2 for the concentric and eccentric training groups for the 3 training bouts.

DISCUSSION

Modeling the Exercise Response

Data from subjects 1-10 (Fig. 3) were fitted well with a two-component model system, therefore confirming hypothesis one.

Two subjects (8 and 9) who performed eccentric training found the first exercise stimulus extremely stressful and showed an extended ESEA time course before its return to base line. The model for these two subjects produced a poor fit throughout the middle period of the time course. A possible physiological explanation will be discussed.

When good coincidence (i.e., high R^2) is achieved by iterative modeling of predicted against real values of CK in the serum, the predictive equation 4 becomes a valid representation of the real time course of the hypothesized components and model constants A, k_1 and k_2 may be determined.

There was no significant difference in the rate constants k_1 and k_2 between the 2 training groups or the 3 exercise bouts which does not confirm hypothesis two. However, in retrospect, physiologically this makes sense since the rate constants, k_1 and k_2, represent the rate of leakage and degradation of enzyme, respectively which are both processes and therefore should not change. However, the magnitude of the ESEA response should change with subsequent exercise bouts. The experimental peak amplitude of ESEA significantly decreased with subsequent exercise bouts.

However, the model constant A which represents the magnitude of the ESEA response, was not significant different with subsequent exercise bouts, which does not confirm hypothesis two. The reason the former but not the later was significantly different is that the model constant A is composed of the rate constants k_1 and k_2 which could compound the amount of error which occurs.

The model shows that with subsequent training the magnitude of the response decreases (perhaps due to the decrease in the number of focal damage sites) and that each focal damage site involves the same process of leakage (k_1) and degradation (k_2) of enzyme.

In the present study the degradation rate constant of ESEA (CK) from the vascular space was found to range from k_2 = 0.0003 → 0.0007 min^{-1} (Table 1) which is of the same order of magnitude, expressed in minutes, as variously found for this parameter, (usually defined as k_D), in post myocardial infarct patients (k_D = 0.0008 → 0.002 min; Roberts *et al.*, 1975; 0.0002 → 0.0008, Shibta *et al.*, 1985; 0.0006 → 0.00109, Norris *et al.*, 1975 and Sobel *et al.*, 1972) and in runners after a marathon (k_D = 0.0012, Apple *et al.*, 1984)

Conceptually, in the model, even as membrane porosity develops (mirrored by rising blood enzyme activity) it slows during the hours after the contraction stimulus ceases as holes are plugged or repaired. Concomitantly, enzyme leaked to the vascular space is continuously degraded or otherwise effluxed from the blood. The former process is represented by f(t) and the latter by F, in Fig. 1. Figure 7, representing equation 4 below, shows this clearly. The rising front of the serum CK activity time curve above baseline

shown by the middle curve in Fig. 7 is driven by enzyme leakage to the vascular space controlled by the fast rate constant k_1 of equation 4 shown in the bottom curve of Fig. 7. The degradation rate of enzyme activity from the blood is dominated by the rate constant k_2 shown in the top curve Fig. 7 representing, in the later stages, pure degradation or efflux of enzyme from the vascular space (see coincidence of top and middle curves of Fig. 7 as time becomes large) as exercise-induced leakage into the vascular compartment diminishes.

Where equation 4 is:

$$y_{(t)} = C_{(t)} = A\,(e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \qquad (4)$$

where

$$A = a \cdot k_1 / k_2 - k_1$$

and

a = potential amount of enzyme loss from muscle due to actions set *in train* by the high intensity exercise exercise stimulus.

k_1 = rate constant for enzyme leakage

k_2 = rate constant for CK degradation

$C_{(t)}$ = enzyme activity ($iU \cdot L^{-1}$) in blood above base line.

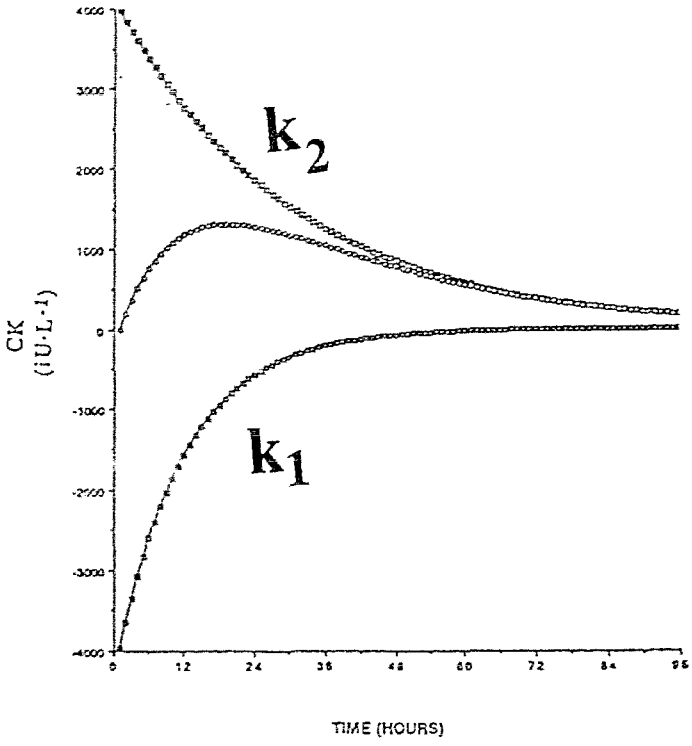


Figure 7. Showing how the process of enzyme leak to, and degradation from, the vascular space may be represented by first order differential equations whose difference at any point in time describes the time course of ESEA (middle curve). The fast rate constant (k_1) controlling leak of enzyme from the muscle to the vascular space is shown in the lower half of the graph and the slow rate constant (k_2) controls degradation of ESEA from the vascular space and is shown in the top half of the graph.

Flow Pattern of CK

The early time course of ESEA responding to a single large dose of exercise must reflect the kinetics of cell membrane damage following enzyme release from the muscle cell. The model described in this paper, shown in Fig. 2, assumes that an exercise-induced, enhanced CK release from muscle occurs as a potential impulse and is delivered principally to the vascular space (possibly via the lymph

system) from which it is continually degraded and plasma CK returns exponentially to base line when the effect caused by the disruptive exercise stimulus declines. Figure 7 illustrates the influx, (k_1), of CK into the vascular space and the continual degradation (k_2) of CK from the vascular space. The empirical time course of CK elevation for all subjects in Fig. 3 shows that most had an immediate onset (<4 h) of ESEA (CK) after each exercise session indicating that cellular loss of CK to the vascular compartment is immediate and not all of CK leaking from the damaged muscle is transferred to blood via slow lymph flow as indicated by Lindena *et. al.* (1979).

The present model characterizes the time course of developing muscle cell membrane instability and recovery induced by exercise from the expression of ESEA in the blood (Fig. 7). A legitimate regulator of these processes therefore must possess an even faster time course than ESEA (CK) resulting from a disruptive exercise stimulus.

Delayed or Biphasic ESEA Time Course

A biphasic or delayed shape of the ESEA time course in which a delayed high ESEA peak has been observed developing many hours or even days after the precipitating exercise stimulus (Armstrong, 1990; Clarkson and Tremblay, 1988; Newham *et al.*, 1986a). This observation may be due to the restricted access that phagocytic activity has to more severely damaged muscle loci until repair of the surrounding lymphatic or microvascular circulation takes place. Suval *et al.* (1987) demonstrated areas of no flow in the

microcirculation after induced ischemia followed by reperfusion in rat skeletal muscle. Normally phagocytic action by monocyte invasion of damaged tissue occurs 3-4 h following exercise when their digestive action probably begins to dominate the disruptive process resulting in an ESEA. Exercise involving unaccustomed high muscle tension (i.e., eccentric contractions) may result in enough local tissue disruption or local hemorrhage to restrict or stop local blood flow. Thus the delayed response in ESEA observed in subjects 8, 9, and 10 (Fig. 3) which is similar to previous observations (Armstrong, 1990; Clarkson and Tremblay, 1988) may be due to just such an effect. Until local blood flow is slowly re-established an ESEA effect from the damage may not be apparent. It is possible that the revascularization of focal damage sites would also occur at various times depending on the different extent of the focal damage at each site.

Several other subjects (subjects 3, 4, 5, 6, and 7 of Fig. 3) exhibited a series of inflections during the time course of ESEA. These inflections may be caused by a similar mechanism as described above. However, another explanation is also possible. If CK is transported to the vascular space predominantly by the lymphatic system (Lindena *et al.*, 1979), an increase in lymph flow (containing a basal level of enzyme) would cause a small increase in ESEA. In fact, following massage therapy ESEA is observed (Arkko *et al.*, 1983) and possibly is due to increased flow of lymph fluid into the vascular space as a result of the manipulation. In the present study, it is possible that the maximum muscle contraction produced during the criterion performance (1 RM) increased lymph flow (in

this case high in leaked enzyme) to the vascular system. The end result would be an injection of enzyme-rich lymph fluid to the vascular system causing an inflection in the pattern of ESEA. The irregular inflections in the pattern of ESEA observed in the above subjects of Fig. 3 were commonly observed in the blood samples immediately following a criterion performance.

Delayed/Biphasic Response of ESEA

Several investigators (Armstrong, 1990; Clarkson and Tremblay, 1988; Newham *et al.*, 1986a) have documented both the delayed response in ESEA following an eccentric stimulus together with a significant decrease in peak ESEA in subsequent similar exercise session. The present study observed a training effect in eccentric trained subjects 8, 9, and 10 (Fig. 3) unlike any previously documented data. These subjects exhibited a delayed/biphasic response after their first training session. However, in any subsequent exposure to an exercise session these subjects presented a monophasic peak ESEA (CK) similar to all other subjects, with the peak ESEA occurring between 12-20 h post-exercise. A criticism of the earlier studies (Clarkson and Tremblay, 1988; Newham *et al.*, 1986a) is that a blood sample was taken too infrequently (i.e., once per day), and for too short a time period (up to 5 days), following the initial intensive, exercise stimulus for these isolated data points to define the pattern of ESEA time course accurately. It is possible, therefore, that the time delay or the appearance of the biphasic

response of ESEA observed in the present experiment may have been missed in prior investigations.

ESEA Response to Exercise

Notably, a repeated dose of the same contraction torque (Fig. 4) 10 days apart produced a decreased physiological response in the muscle. Exercise sessions 2 and 3, respectively, produced a significantly ($p \leq 0.05$) lower peak ESEA value than session 1. There was a trend in the group mean peak ESEA value to decrease during successive exercise trials, however the difference was not significant. These findings are consistent with other studies (Brynes *et al.*, 1985; Clarkson and Tremblay, 1988; Graves *et al.*, 1987; Triffletti *et al.*, 1989) involving two training sessions spaced 10 days to 6 months apart in which an ameliorating effect of the exercise session on the physiological/biochemical response to a succeeding exercise bout occurred. The adaptive process leading to a decreased level of ESEA following subsequent isolated training sessions observed in the present study and the other previous studies is also visible during chronic endurance training (Banister *et al.*, 1992; Dressendorfer and Wade, 1983). This adaptive process accounts for the lower level of peak ESEA in trained compared with untrained individuals after strenuous activity (Noakes and Carter, 1982).

Criterion Performance

Clarkson and Tremblay (1988) showed performance, as determined by 1 RM isometric contraction, was impaired in subjects following 70 maximum eccentric forearm contractions. The present study also determined performance using a 1 RM isometric contraction, however performance was only impaired following the initial exercise bout in eccentric trained subjects 8 and 9. Despite obtaining high levels of ESEA, all other subjects regained their pre-exercise torque values immediately or within the next day following the 70 maximum contractions. The apparent contraction in the impaired performance between the previously described observations is that these exercise bouts, which result in muscle damage severe enough to induce ESEA, may be so focal in nature (Armstrong, 1990) that the overall function of the muscle is not impaired.

Hypothetical Rationalization of the Exercise-Induced Degradation of Muscle

The seemingly irrational exercise-induced degradation of muscle described in this thesis may be in fact a rationale response inducing adaptation of muscle to a new level of activity after an initial induced protein catabolism. Mader (1988) has described the induction of a transcription-translation activation feed-back circuit from so-called protein specific fragment (PSF) formation. These PSFs result from a wide variety of proteins escaping from the

damaged area after intense exercise such as myoglobin (Roxin *et al.*, 1984; Munjal *et al.*, 1983), CK (Noakes, 1987), or myosin heavy chain fragments (Mair *et al.*, 1992). Generally developed ideas on the regulation of both nuclear and mitochondrial gene expression by contractile activity of muscle (Booth and Thomason, 1991) also argue that degradation precedes growth and the organism does not perceive the need for the latter until the former occurs. Fisher *et. al.* (1990) observed a marked catabolic response where total protein content decreased (27 %) within 48 h in rat skeletal following acute blunt trauma. In the same study, muscle protein accumulation commenced after day 3. However, complete repletion of the lost protein did not occur until day 21 post-injury. Thus, it appears that enhanced physical performance is only attained by initially stimulating an exaggerated catabolism, sparked by exercise induction of several types of digestive enzymes.

Therefore, the exercise-induced degradative action upon muscle may be an adaptive response to exercise, hence skeletal muscle damage may be an inappropriate term.

Limitations of the Model

The two-component model used to account for the rise and subsequent decline ESEA has limitations. First, the model assumes that there is loss of muscle enzyme directly to the vascular space or to the interstital space from where lymphatic collection subsequently transports enzyme to the thoracic duct. An emphasis on the former process has been rationalized from considering the

relatively slow lymphatic flow (Hermens *et al.*, 1982) compared with the vascular flow rate and the demonstrated increased vascular permeability observed during exercise (Suval *et al.*, 1987). Hence, the model does not distinguish between the relative contributions made by these two compartments respectively to ESEA.

Lastly, the model does not yet distinguish precisely between the postulated processes of membrane disruption and repair since both are represented here by the single rate constant k_1.

REFERENCES

Apple F.S., Rogers M.A., Sherman W.M., Costill D.L., Hagerman F.C., Ivy J.L. Profile of creatine kinase isozymes in skeletal muscles of marathon runners. Clin. Chem. 30(3): 413-416, 1984.

Apple F.S., Rhodes M. Enzymatic estimation of skeletal muscle damage by analysis of changes in serum creatine kinase. J. Appl. Physiol. 65(6): 2598-2600, 1988.

Arkko P.J., Pakarinen A.J., Koshinen K. Effects of whole body massage on serum protein, electrolyte and hormone concentrations, enzyme activities, and hematological parameters. Int. J. Sports Med. 4: 265-267, 1983.

Armstrong R.B. Initial events in exercise-induced muscular injury. Med. Sci. Sports Exerc. 22: 429-435, 1990.

Banister E.W., Morton R.H., Fitz-Clarke J. Serum enzyme activity during and after frequent strenuous exercise modeled from training. J. Physiol. Anththrop. (Japan) In Press, 1992.

Baracos V.E., Wilson E.J. and Goldberg A.L. Effects of temperature on protein turnover in isolated rat skeletal muscle. Am. J. Physiol. 246:C125-C130, 1984.

Berg A. and Haralambie G. Changes in serum creatine kinase and hexose phosphate isomerase activity with exercise duration. Eur. J. Appl. Physiol. 39: 191-201, 1978.

Booth F.W.and Thomason D.B. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. Physiol. Rev. 71: 541-585, 1991.

Brynes W.C., Clarkson P.M., White J.S., Hsieh S.S., Frykman P.N., Maughan R.J. Delayed onset soreness following repeated bouts of downhill running. J. Appl. Physiol. 59: 710-715, 1985.

Byrd S.K., Bode A.K., Klug G.A. Effects of exercise of varying duration on sarcoplasmic reticulum function. J. Appl. Physiol. 66: 1383-1389, 1989a.

Carlson B.M. Regeneration of minced muscles. Basel: S. Karger, 1972.

Carlson B.M., Faulkner J.A. The regeneration of skeletal muscle fibers following injury: a review. Med. Sci. Sports Exerc. 15: 187-198, 1983.

Chang J., Musser J.H., McGregor H. Phospholipase A_2: function and pharmacological regulation. Biochem. Pharmacol. 30: 2429-2436, 1987.

Clarkson P.M., Tremblay I. Exercise-induced muscle damage, repair, and adaptation in humans. J. Appl. Physiol. 65: 1-6, 1988.

Critz J.B., Cunningham D.A. Plasma enzyme levels in man after different physical activities. J. Sports Med. and Physical Fit. 12: 143-149, 1972.

Davies K.J.A., Quintanilha A.T., Brooks G.A., Packert. Free radical and tissue damage produced by exercise. Biochem. and Biophys Res. Commun. 107: 1198-1205, 1982.

Dressendorfer R.H., Wade C.E. The muscular overuse syndrome in long-distance runners. Phys. and Sports Med. 11: 116-127, 1983.

Duan C., Delp M.D., Hayes D.A., Delp P.D., Armstrong R.B. Skeletal muscle Ca^{2+} overload and injury from eccentric exercise. J. Appl. Physiol. 68:1241-1251,1990.

Duncan C.J. Role of calcium in triggering rapid ultrastructural damage in muscle: a study with chemically skinned fibres. J. Cell Sci. 87: 581-594, 1987.

Duncan C.J., Jackson M.J. Different mechanisms mediate structural changes and intracellular enzyme efflux following damage to skeletal muscle. J. Cell Sci. 87: 183-188, 1987.

Duncan C.J. The role of phospholipase A2 in calcium-induced damage in cardiac and skeletal muscle. Cell Tissue Res. 253: 457-462, 1988.

Duncan C.J., Rudge M.F. Are lysosomal enzymes involved in rapid damage in vertebrate muscle cells?: A study of the separate

pathways leading to cellular damage. Cell Tissue Res. 253: 447-455, 1988.

Fisher B.D., Baracos V.E., Shnitka T.K., Mendryk S.W., Reid D.C. Ultrastructural events following acute muscle trauma. Med. Sci. Sports Exerc. 22: 185-193, 1990.

Friden J., Fkblom B. Myofibrillar damage following intense eccentric exercise in man. Int. J. Sports Med. 4: 170-176, 1983.

Furono K., Goldberg A.L. The activation of protein degradation in muscle by Ca^{2+} or muscle injury does not involve a lysosomal mechanism. Biochem. J. 237: 859-864, 1986.

Graves J.E., Clarkson P.M., Litchfield P., Kirwan J.P., Norton J.P. Serum creatine kinase activity following repeated bouts of isometric exercise with different muscle groups. Eur. J. Appl. Physiol. 56: 657-661, 1987.

Hermens W., Willems G.M., Visses M.P. Quantification of circulating proteins: theory and application based on analysis of protein levels. Martinus Nishoff Publishers, 1982.

Hurme T., Kalimo H. Activation of myogenic precursor cells after muscle injury. Med. Sci. Sports Exerc. 24: 197-205, 1992.

Jackson M.J., Jones D.A., Edwards R.H.T. Experimental skeletal muscle damage: the nature of the calcium-activated degenerative processes. Eur. J. Clin. Inves. 14: 369-374, 1984.

Koeniger R., Hoffmann G.E., Schmid, Th. O. Serum activities of phosphoplipase A in acute posttraumatic pulmonary insufficiency. Klin Wochenschr. 67:212-216,1989.

Lindena J., Kupper W., Friedel R., and Trautschold I. Lymphatic transport of cellular enzymes from muscle into the intravacular compartment. Enzyme. 24:120-131, 1979.

Mader A. A transcription-translation activation feedback circuit as a function of protein degradation, with the quality of protein mass adaptation related to the average functional load. J. Theor. Biol. 134: 135-157, 1988.

Mair J., Koller A., Artner-Dworzak E., Haid C., Wicke K., Judmaier W., Puschendorf B. Effects of exercise on plasma heavy chain fragments and MRI of skeletal muscle. J. Appl. Physiol. 72: 656-663, 1992.

Munjal D.D., McFadden J.A., Matix P.A., Coffman K.D., Cattaneo S.M. Changes in serum myoglobin, total creatine kinase, lactate dehydrogenase and creatine kinase MB levels in runners. Clin. Biochem. 16: 195-199, 1983.

Newham D.J., Jones D.A., Edwards R.H.T. Plasma creatine kinase changes after eccentric and concentric contractions. Muscle and Nerve 9: 59-63, 1986a.

Newham D.J., Jones D.A., Tolfree S.E.J., Edwards R.H.T. Skeletal muscle damage: a study of isotope uptake, enzyme efflux and pain after stepping. Eur. J. Appl. Physiol. 55: 106-112, 1986b.

Noakes T.D. Effects of Exercise on Serum Enzyme Activities in Humans. Sports Med. 4:245-267, 1987.

Noakes T.D., Carter J.W. The response of plasma biochemical parameters to a 56-km race in novice and experienced ultramarathon runners. Eur. J. Appl. Physiol. 49: 179-186, 1982.

Norris R.M., Whitlock R.M.L., Barratt-Boyes C., Small C.W. Clinical measurements of myocardial infarct size: modification of a method for the estimation of total creatine kinase phospholipase release after myocardial infarction. Circ. 51: 614-619, 1975.

Roberts R., Henry P.D., Sobel B.E. An improved basis for enzymatic estimation of infarct size. Circ. 52: 743-754, 1975.

Roxin L., Venge P., Friman F. Variations in serum myoglobin after a 2-minute isokinetic exercise test and the effects of training. Eur. J. Appl. Physiol. 53: 47-55, 1984.

Russel B., Dix D.J., Haller D.L., Jacobs-El J. Repair of injured skeletal muscle: a molecular approach. Med. Sci. Sports Exerc. 24: 189-196, 1992.

Schwane J.A., Williams J.S., Sloan J.H. Effects of training on delayed muscle soreness and serum creatine kinase activity after running. Med. Sci. Sport 19: 584-590, 1989.

Shell W.E., Kjekshus J.K., Sobel B.E. Quantitative assessment of the extent of myocardial infarction in the conscious dog by means of analysis of serial changes in serum creatine phosphokinase activity. J. Clin. Chem. 50: 2614-2625, 1971.

Shibata T., Hashimoto H., Ito T., Ogawa K., Satake T., Sassa H. Late estimation of myocardial infarct size by total creatine kinase nomogram. Am. Heart J. 109: 1238-1252, 1985.

Sobel B.E., Bresnahan G.F., Shell W.E., Yoder R.D. Estimation of infarct size in man and its relation to prognosis. Circ. 46: 640, 1972.

Suval W.D., Duran W.N., Boric M.P., Hobson R.W., Berendsen P.B., Ritter A.B. Microvascular transport and endothelial cell alterations preceding skeletal muscle damage in ischemia and reperfusion injury. Am. J. Surgery. 154: 211-218, 1987.

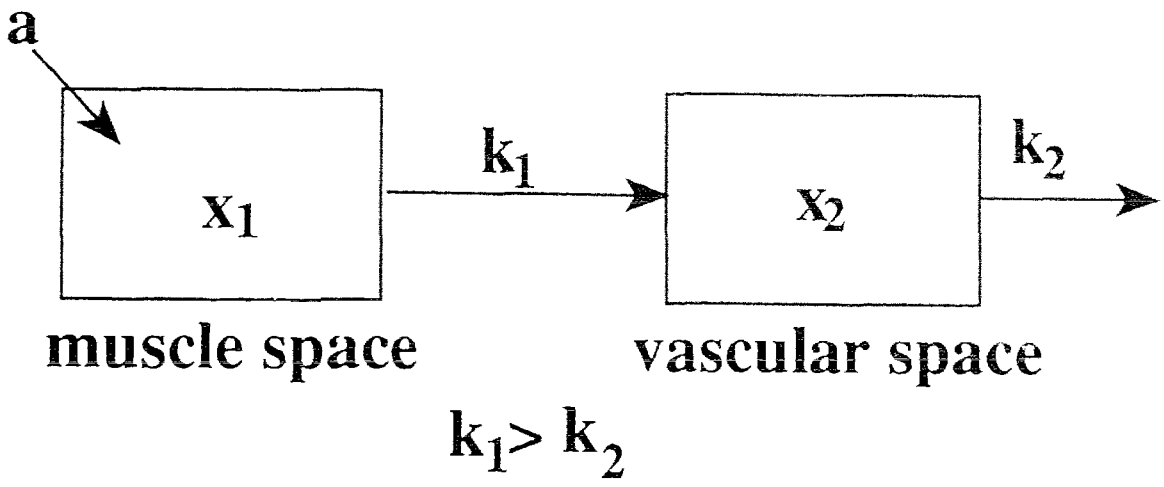
Szaz G., Gruber W., Bernt E. Creatine kinase in serum: 1. determination of optimal reaction conditions. Clin. Chem. 22: 650-656, 1976.

Tiidus P.M., Ianuzzo C.D. Effects of intensity and duration of muscular exercise on delayed soreness and serum enzyme activities. Med. Sci. Sport. 15: 461-465, 1983.

Trifflerri P., Litchfield P.E., Clarkson P.M., and Brynes W.C. Creatine kinase and muscle soreness after repeated isometric exercise. Med. Sci. Sport. 3: 242-248, 1989.

Warhol M.J., Siegel A.J., Evans W.J., Silverman L.M. Skeletal muscle injury and repair in marathon runners after competition. Am. J. Pathol. 118: 331-339, 1985.

APPENDIX 1 Development of the ESEA Model



where a = the potential quantity of enzyme (activity) lost to the vascular space by the physiological/biochemical event set *in train* by the disruptive exercise stimulus.

x_1 and x_2 are the quantity of enzyme in the cell and vascular space, respectively.

k_1 and k_2 are rate constants of enzyme loss (quantity) from the cell space and vascular space, respectively.

t = time domain
s = s domain

Thus the rate of enzyme loss from the cell space is:

$$dx_1/dt = -k_1 \cdot x_1 \qquad (1)$$

and the rate of degradation of enzyme from the vascular space is:

$$dx_2/dt = k_1 \cdot x_1 - k_2 \cdot x_2 \qquad (2)$$

From the Laplace Transformation $\mathcal{L}\, x_{(t)}$:

$$s\, x_1(s) - x_{1(0)} = -k_1 \cdot x_1(s) \qquad (1)$$

$$s\, x_2(s) - x_{2(0)} = -k_1 \cdot x_1(s) - k_2 \cdot x_2(s) \qquad (2)$$

$$(s + k_1)\, x_1(s) = x_{1(0)} = a \qquad (1)$$

$$(s + k_2)\, x_2(s) = k_1 \cdot x_{1(0)} + x_{1(0)} \qquad (2)$$

$$(s + k_2)\, x_2(s) = k_1 \cdot x_{1(0)} + 0 \qquad (2)$$

therefore, from (1)

$$x_1(s) = a/(s + k_1) \qquad (1)$$

Substituting from (1) for x_1 in (2):

$$(s + k_1)\, x_1 = ak_1/(s + k_1) \qquad (2)$$

Solving for $x_2(s)$:

$$x_2(s) = ak_1/(s + k_1)\,(s + k_2)$$

and:

$$x_2(s) = ak_1\, [1/(s + k_1) - 1/(s + k_2)]$$

$$x_2(s) = ak_1 \left[(s + k_2 - s - k_1)/(s + k_1)(s + k_2) \right]$$

$$x_2(s) = ak_1 \left[(k_2 - k_1)/(s + k_1)(s + k_2) \right]$$

$$x_2(s) = ak_1 /(k_2 - k_1) \left[1/(s + k_1) - 1/(s + k_2) \right]$$

and $\mathcal{L}^{-1} x_2(s)$:

$$x_{2(t)} = ak_1/(k_2-k_1) \left[e^{-k_1 t} - e^{-k_2 t}\right]$$

$$x_{2(t)} = ak_1/(k_1-k_2) \left[e^{-k_1 t} - e^{-k_2 t}\right]$$

$$x_{2(t)} = A \left[e^{-k_1 t} - e^{-k_2 t}\right]$$

APPENDIX 2 Actual and Predicted Data

Subject #1 Concentric

	Serum Creatine Kinase Activity (iU/L)					
Time (h)	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	196	50	111	50	115	50
1.00	217	241				
1.25			128	141	143	95
5.25					215	206
5.50			251	374		
6.30	404	936				
9.75			551	515		
10.00					316	290
13.00					329	323
13.50			636	586		
16.30	1464	1361				
17.50			626	624	335	350
18.30	1551	1369				
21.50			594	633	347	360
24.50	1423	1315				
26.00					348	358
27.75			623	612		
30.00	1342	1210				
32.50					345	342
34.00			613	567		
36.50	1137	1060				
37.50			577	537	347	323
41.75					297	305
42.00	901	931				
44.50			451	474		
46.00	810	842				
50.00					231	268
51.00			393	416		
56.50					229	241
57.00			363	367		
58.80	361	596				
61.25			321	335		
62.50					211	217
69.00			255	283		
70.30	287	433				
75.00			250	249		
77.50					170	167
82.00	202	314				
84.75			187	203		
87.00					158	142
94.30	192	228				
95.75			161	163		
102.75			143	143		
105.75					141	107
107.00	218	168				
119.50			138	108		
120.00	142	127				
125.75					146	84

Subject # 2 Concentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	381	50	104	50	103	50
0.45					124	85
0.75			125	93		
1.00	437	237				
3.75					268	279
5.25			262	283		
5.75					407	354
6.00	569	824				
9.25			390	377		
9.75	1129	1009				
11.50					461	454
12.75			480	421		
13.50	1232	1067				
23.75					352	413
25.25			367	421		
26.25	767	879				
29.25					361	362
31.00			352	387		
32.50	679	733				
36.75			338	347		
37.50	615	623				
38.50					261	280
47.75					213	213
48.75			273	266		
50.25	350	402				
52.50					182	185
55.00			248	230		
56.25	330	326				
58.75					177	155
59.00			246	210		
62.25	250	266				
71.00			150	161		
71.50					136	113
74.25	191	181				
76.25			141	143		
76.50					131	101
79.50	237	155				
82.00			148	128		
83.50					135	88
87.00	248	127				
95.50					123	73
96.75			122	98		
99.50	174	95				
99.75					118	70
105.25	179	86				
109.75	192	79				
122.00	165	68				
153.25	167	55				

Subject # 3 Concentric

	Serum Creatine Kinase Activity (iU/L)					
Time (h)	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	136	50	84	50	91	50
0.50			107	58		
0.75					97	58
1.25	134	128				
3.25	224	229	124	100		
6.25					134	165
6.50	370	343				
8.75			176	161		
10.25					176	206
11.50	462	434				
12.25			203	188		
15.00					260	233
24.00	363	439				
26.75					259	243
27.25	432	418				
32.25					254	233
34.00			206	235		
35.25	357	357				
38.75			220	230		
39.25					250	215
41.25					186	209
45.00					187	198
46.00			222	220		
47.50	252	267				
51.00	252	244				
52.50					155	177
58.00			194	197		
59.00	226	200				
60.75			184	191		
65.50					128	144
68.25					125	138
70.25			170	172		
71.50	163	149				
79.00					121	118
82.00			146	150		
83.00	137	117				
86.50			148	143		
89.25					100	102
94.25			134	131		
95.50	116	94				
106.30			128	115		
110.30			132	110		
114.00					92	77
116.80			108	103		
131.00			96	90		
139.00					94	64

Subject # 4 Concentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	88	50	70	50	86	50
0.75			77	70	83	63
1.00	89	71				
2.75			137	117		
3.00					126	98
3.25	134	109				
6.50					142	139
7.00			181	187		
7.50	158	159				
10.25					195	169
11.00			277	225		
11.50	188	184				
23.00			192	249		
24.00	172	194			164	203
28.25			220	238	189	200
28.75	183	185				
34.50			230	219		
35.75	157	167				
47.25			161	175		
48.00					149	161
48.75	138	133				
51.00			157	162		
52.75	130	123				
58.25					150	139
58.50			155	141		
71.50			114	111	112	114
73.00	96	88				
76.25			122	102		
76.50					131	106
77.50	96	82				
82.25					119	98.2
82.75			118	92		
84.00	97	76				
94.75					95	84
95.25			94	78		
96.75	68	67				
106.8			102	69		
121.00			79	62		

Subject # 5 Concentric

	Serum Creatine Kinase Activity (iU/L)					
Time (h)	*Actual* Bout 1	*Predicted* Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	101.00	50.00	157.00	50.00	138.00	50.00
0.075					138.00	61.20
1.00	96.00	72.30	164.00	62.20		
4.00					149.00	104.70
5.75	155.00	151.4				
6.25			175.00	112.90		
10.50					200.00	170.20
10.75	216.00	198.70				
12.75		138	151.30			
18.50					153.00	220.40
24.00					199.00	240.70
24.25	197.00	226.20				
25.50			189.00	177.90		
28.50	214.00	220.20				
30.25			153.00	178.40		
31.25					300.00	255.00
35.00	215.00	205.70				
36.25			156.00	174.90		
43.50					274.00	257.40
47.75	167.00	171.10				
48.75					275.00	253.10
49.75			129.00	158.50		
53.50	162.00	156.20				
56.00					242.00	244.20
58.25	151.00	144.60				
67.75					202.00	225.30
71.75	125.00	116.90				
72.25					217.00	217.30
73.75			144.00	123.90		
97.75			123.00	96.80		
121.25			109.00	79.30		

Subject #6 Eccentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	134	50	89	50	84	50
0.75			94	65		
1.00	151	93			86	68
5.00					113	119
6.00	227	231				
6.25			151	148		
11.50					171	155
12.30	312	290				
12.50			193	196		
14.25					164	160
15.50			199	207		
18.25			194	213		
18.30	287	288				
20.25					131	158
24.60	239	263				
25.25					152	150
26.50			242	212		
29.50			217	208		
30.30	205	232				
31.25					138	137
33.75			185	200		
36.30	194	201				
38.75			200	188		
44.00					110	109
47.80	155	150				
51.00			146	158		
53.80	151	130				
54.00			159	151		
55.50					102	90
57.50			135	143		
72.50			113	114		
77.80	120	81				
101.80	128	62				
172.30	80	51				

Subject #7 Eccentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	118	50	134	50	111	50
1.00	137	163	155	118	120	146
3.50	339	409				
4.75			318	324		
6.50					508	542
8.25	857	753			677	629
8.50			455	468		
12.00					942	766
12.50	1130	955	565	569		
24.00					952	916
24.50	1042	1176				
24.75			584	671		
26.00						
27.75						
29.50	1149	1177			941	900
30.25			677	659		
36.00					605	849
36.50	1151	1131	713	625		
48.25					625	713
48.75	864	985	556	531		
53.50	903	919	542	498		
60.25					524	575
60.50	814	823				
61.25			423	434		
72.25					394	455
72.50	673	670				
75.00			316	349		
77.00	679	618				
77.50			298	321		
83.00					393	367
84.00	584	544			409	360
87.00			243	276		
96.25	457	433			345	282
100.00					359	262
102.75			190	220		
107.00					368	229
108.50	375	345				
119.50			178	109		
120.00					259	181
121.50	312	273	156	148		
144.25					208	122
145.50	223	182				
168.50					154	89
193.75					132	71
197.30	170	92				
216.25					140	62
239.80	134	66				

Subject #8 Eccentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	111	50	178	50	104	50
0.75			185	95		
1.00	126	252			110	82
3.50					162	147
6.00	539	1181				
6.50			312	299		
9.75			337	342	281	244
13.00	1215	2270				
15.25					265	276
16.50	1296	2731				
20.50			299	330		
21.50					251	281
22.50	1093	3410				
27.50	1659	3880				
27.75					261	267
31.20	2081	4177				
33.10	2870	4314				
33.25			232	239		
33.75					263	246
38.00			201	208		
38.40	4390	4647				
44.00			189	174		
44.90	5010	4964				
46.00					182	199
50.00					169	184
51.20	6530	5191				
54.20	7655	5274				
57.00			143	122		
57.50					158	159
62.00			142	108		
71.50	7800	5506				
73.50					115	119
78.50					121	109
94.50	8690	5367				
101.50					112	79
126.00					113	64
128.75	7530	4707				
148.50					102	57
152.25	4163	4148				
159.60	3332	3972				
172.50					105	53
177.60	1694	3550				
183.80	1528	3410				
197.00					132	52
204.00	1062	2976				

Subject #9 Eccentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Boutl	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	141	50	175	50	136	50
0.75					137	68
1.00	149	97	189	85		
2.50	198	156				
2.75					155	110
3.50			233	159		
6.00			259	216		
6.75					194	168
7.00	292	279				
10.25	349	327				
10.50			348	284	210	198
22.25	321	356				
23.75			285	338		
24.25					174	211
26.25			282	334		
26.75					187	206
27.00	306	337				
34.25	271	297				
34.50			300	310	155	184
46.25	213	230				
47.75			214	254		
48.25					133	144
50.50			204	242		
50.75					144	138
51.75	209	203				
58.25	231	176				
59.25			195	208		
61.75					125	113
70.50	197	135				
71.75			196	166		
72.75					108	95
74.50			164	158		
75.50	228	205				
80.25	285	332				
82.50			139	138		
84.00					131	81
95.75	599	641				
96.00			144	112		
96.25					123	71
98.50			146	108		
106.25			139	97		
119.25			122	83		
121.00					95	59
121.75			129	81		
130.00			126	75		
143.25	850	955				
143.75					105	54
145.75			115	66		
169.75			115	58		
192.25			122	55		
193.00	1035	858				
215.00			156	52		
240.25			136	51		
247.50	747	652				
313.75	481	434				
338.25	294	372				
361.25	210	321				
385.50	183	276				
407.25	175	248				

Subject #10 Eccentric

Time (h)	Serum Creatine Kinase Activity (iU/L)					
	Actual Bout 1	Predicted Bout1	Actual Bout 2	Predicted Bout 2	Actual Bout 3	Predicted Bout 3
0.00	88	50	82	50	91	50
0.75	86	68	89	72		
1.00					108	82
3.25	112	115				
4.00					147	153
4.50			139	156		
7.00					244	198
7.25	171	162				
8.50			206	208		
10.75					243	228
12.00			235	231		
12.50	193	189				
15.50					206	238
21.25					203	229
23.50	158	185				
24.00			248	231		
29.00	171	171				
30.00					191	199
30.25			204	211		
35.30	168	153				
36.25			188	189		
40.25					146	159
45.25					140	142
47.30	129	122				
49.00			137	147		
51.50					145	124
52.30	186	125				
52.50			139	137		
58.50	162	171				
59.25					103	106
63.50					112	98
71.00	168	186				
75.00					118	81
76.80	169	178				
84.30	151	162				
88.00					85	69
92.00					86	66
101.00	122	125				
129.00	106	84				
149.00	95	69				
172.00	100	60				
217.00	82	52				