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Effects of vitamin E supplementation on muscle soreness, plasma creatine kinase, and plasma malondialdehyde following an endurance-ergometer ride

Lewis, Cynthia Lynn, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

EFFECTS OF VITAMIN E SUPPLEMENTATION ON MUSCLE SORENESS, PLASMA CREATINE KINASE, AND PLASMA MALONDIALDEHYDE FOLLOWING AN ENDURANCE-ERGOMETER RIDE

By

Cynthia Lynn Lewis

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Allan H. Goldfarb
(Dissertation Advisor)

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School at The University of North Carolina at Greensboro.

Dissertation Adviser Allan H. Goldfarb

Committee Members Jackie Hudson

Marshall K. Watson

H. Hunt

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To the child within who has struggled long and hard to become free of the dragons of the past. To that childlike wonderment, that universal spark of creativity that asks, "Why?"

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The purpose of this study was to determine the effects of vitamin E supplementation on muscle soreness ratings (MSRs), plasma creatine kinase (CK), and plasma malondialdehyde (MDA) following an endurance-ergometer ride at 70% maximum oxygen consumption (VO_2 max). Eleven trained male cyclists (mean age = 29.0 $\pm$ 2.1 years and mean VO_2 max = 62.3 $ml.kg^{-1}.min^{-1}$) participated in a ten-week, double-blind, cross-over study. They received either placebo or vitamin E (alpha-tocopherol; 800 mg/day) for 4 weeks. At the end of the fourth week, they performed an endurance-ergometer ride at 70% max VO_2 for four hours or to exhaustion, whichever occurred first. Blood samples were taken during the ride from an indwelling catheter placed in an antecubital vein. Post ride 24, 48, and 72 hour blood samples were taken via venipuncture from an antecubital vein. Prior to each blood drawing, MSRs were determined by using a rating scale of 1 (no soreness) to 10 (very, very sore). Dietary intake was monitored for the four days preceding each endurance ride.

After two weeks of no supplementation, cyclists were placed on the opposite treatment for the remaining four weeks. Plasma CK was determined by a modified Hughes procedure and plasma MDA by the thiobarbituric acid method.

Data were analyzed using a Repeated Measures Analysis of Variance. A Pearson product moment correlation was used to determine the time course relationships of MSRs, CK, and MDA and relationship of variables. Paired t-tests were conducted to determine differences in dietary intake. Significance level was set at $p < 0.05$.

The results of the study suggest that Vitamin E supplementation had no effect on attenuating MSRs, plasma CK, or MDA levels compared with the placebo supplementation. No differences were observed in the time course patterns or relationships of variables between the two treatments. It was concluded that vitamin E supplementation of 800 mg/day for 4 weeks did not attenuate MSRs, plasma CK, or plasma MDA in trained male cyclists performing an ergometer ride at 70% max VO_2.

CHAPTER I

INTRODUCTION

Statement of the Problem

Muscle soreness is described as an aching feeling, tenderness, or pain within the muscle. It may occur as a result of strenuous exercise or novel activity. The onset of soreness can develop acutely (during or immediately after exercise) or be delayed up to 24 to 48 hours after exercise. Hough (1902) suggested that acute muscle soreness resulted from different mechanisms than the delayed onset of soreness. He hypothesized that acute soreness had a metabolic origin (biochemical mechanism) and that delayed onset muscle soreness (DOMS) resulted from "some sort of rupture within the muscle" (mechanical mechanism). The research in the last 90 years has generally supported his theories (Armstrong, 1984).

Suggested biochemical mechanisms of acute soreness include metabolic depletion (Mills, Newham, & Edwards, 1982; Rodbard & Pragay, 1968), altered cellular pH (Halonen & Konttinen, 1962), and lipid peroxidation (Balke, Snider, & Bull, 1984; Davies, Quintanilha, Brooks, & Packer, 1982). These biochemical processes can result in calcium ion efflux, impairment of the Na-K-ATPase pump, and/or altered membrane permeability which in turn can disrupt oxidative coupling and lead to cell damage or death (Armstrong, 1984).

In the metabolic depletion theory, researchers theorized that the depletion of substrate could disrupt ATP production. Disruption in ATP production can lead to impairment in the Na-K-ATPase pump and oxidative coupling (Rodbard & Pragay, 1968). When the Na-K-ATPase pump is impaired, sodium and potassium ion concentrations inside and outside the cell are altered leading to altered membrane permeability.

Another possible mechanism of muscle soreness is altered cellular pH (Abraham, 1977). Cellular pH can decrease with high intensity or long duration exercise. Under these exercise conditions, hydrogen ions can accumulate which decreases cellular pH (Fitts & Holloszy, 1976). Decreased cellular pH can lead to impairment in the Na-K-ATPase pump and oxidative coupling.

Lipid peroxidation is yet another possible biochemical mechanism of acute muscle soreness. Lipid peroxidation (lipox) is the addition of a molecule of oxygen to the double-bond structure of lipid membranes. This process produces free radicals, is self replicating, and results in altered membrane structure and function which can lead to cell damage or cell death (Murray, Gradner, Mayer, & Rodwell, 1988). Lipox primarily takes place in the electron transport system within the mitochondria and has been shown to increase with endurance exercise (Balke et al., 1984;

Davies et al., 1982). Lipid peroxidation is often measured by the by-product malondialdehyde (MDA).

Acute muscle soreness is associated with concentric contractions. During concentric contractions the active muscle shortens. While no morphological changes have been observed in muscles biopsied following concentric contractions (Newham, 1988), elevations in concentrations of muscle enzymes in serum and plasma have been measured (Brooke, Carroll, Davie, & Hagberg, 1979; Clarkson, Byrnes, Gillisson, & Harper, 1987; Fowler, Chowdhury, Pearson, Gardner, & Bratton, 1962). One of the muscle enzymes that appears in serum and plasma is creatine kinase (CK). Elevated serum and plasma CK concentrations are a marker of altered membrane permeability (Griffiths, 1965).

Mechanisms of acute muscle soreness are not completely understood. Pain may possibly be mediated by a number of different substances resulting from altered membrane integrity. Suggested pain stimulating substances include bradykinins, serotonin, prostaglandins, potassium and calcium ions, and metabolic by-products (Armstrong, 1984; Smith, 1991).

The mechanisms suggested for DOMS are different from those suggested for acute muscle soreness. These mechanisms include muscle tearing and ischemia. A muscle tearing or mechanical model for DOMS was postulated by Friden,

Sjostrom, and Ekblom (1981). They observed morphological changes, including myofilament disruption and Z disk streaming, in rats that performed downhill treadmill running (eccentric exercise). Similar changes were observed in humans in the eccentric phase (step down) of the step test, but not in the concentric phase (step up) (Newham, McPhail, Mills, & Edwards, 1983).

Another possible mechanism for DOMS is ischemia (Hoppeler, 1986; Sjostrom, Friden, & Ekblom, 1987). In ischemia blood flow to the working muscle is decreased. Blood flow can be diminished by pressure exerted on the blood vessel during static or isometric muscle contractions. During the relaxation phase, blood flow returns to the muscle. Diminished blood flow or occlusion with subsequent reperfusion may result in tissue damage.

Eccentric muscle contractions are associated with DOMS. During eccentric contractions, the active muscle fibers lengthen. Friden et al. (1981) suggested that the lengthened fibers tear as a result of the higher tensions produced within muscle fibers during eccentric contractions compared with concentric contractions.

Following eccentric contractions, marked elevations in serum and plasma CK have been demonstrated (Byrnes, Clarkson, Spencer-White, Hsieh, Fryman, & Maughan, 1985; Clarkson et al., 1987b; Friden, Stakiansas, & Hargens, 1989;

Newham, Jones, & Clarkson, 1987). Elevations in serum and plasma CK may have resulted from disruption of the myofilaments and membrane tearing (Friden et al., 1981). A significant positive correlation in the time course change of CK activity and DOMS following eccentric exercise has been reported (Clarkson, Byrnes, McCormick, Turcotte, & White, 1986).

Pain mediation mechanisms with DOMS, like acute muscle soreness, are still under investigation. One suggested pain mechanism in DOMS is acute inflammation (Smith, McCammon, Smith, Chamness, Israel, & O'Brien, 1989). Macrophages seen in acute inflammation produced prostaglandin E_2 (PGE_2) which sensitize free nerve endings resulting in DOMS.

Pain of muscle soreness has been reported using various self-rating soreness scales. One such scale, devised by Clarkson, rates soreness on a 1 to 10 scale with 1 being no soreness and 10 being very, very sore (Clarkson, Litchfield, Graves, Kirwan, & Byrnes, 1985) (Appendix A). Researchers have reported moderate to marked (3 to 7) muscle soreness ratings following eccentric exercise, but ratings were minimal (less than 2) following concentric exercise (Clarkson et al., 1987b; Clarkson et al., 1986; Newham, Mills, Quigley, & Edwards, 1983). These researchers may not have observed elevated muscle soreness ratings following concentric exercise because the intensity and/or duration

were not sufficient to produce such a response (Tiidus & Innuzzo, 1983).

Lewis, Goldfarb, and Boyer (1991) (Study 1) (Appendix B) theorized that muscle soreness ratings would elevate in concentric exercise, if the activity were of a long duration and high intensity. These researchers measured muscle soreness ratings (MSRs), serum CK, and plasma malondialdehyde (MDA) in cyclists who performed a strenuous 100 mile bicycle road ride with marked elevation gains. Cycling is a primarily a concentric exercise (Faria & Cavanegh, 1978). Yet increased muscle soreness ratings occurred.

In this strenuous concentric activity, MSRs increased immediately after the ride and remained significantly elevated at 24 hours. The peak (5.4) and 24 hour (4.2) MSRs were similar to soreness ratings observed by Clarkson et al. (1985; 1986) and Newham (1983) following eccentric work. Mean peak 24-hour serum CK concentrations (252 IU/L) increased five fold from rest. These concentrations were above normal range (0 to 160 IU/L) and suggested altered membrane permeability and possibly minor muscle damage. Mean peak 48-hour MDA concentrations (2.52 nmol/ml) increased approximately 40% above rest and were above normal range (1 to 2 nmol/ml) (Jenkins, 1988).

The time course pattern of MSRs, serum CK, and plasma MDA were different from each other. MSRs peaked immediately

post ride. Soreness was diminishing, but remained significantly elevated at 24 hours. By 48 hours pain returned to nonsignificant levels. Serum CK peaked at 24 hours. CK decreased at 48 and 72 hours but remained significantly elevated. Plasma MDA peaked at 48 hours and was decreasing by 72 hours but remained above baseline. Lipid peroxidation was a possible mechanism in altered membrane permeability as demonstrated by moderately high MSRs, significant elevations in serum CK levels, and the trend for plasma MDA levels to increase.

Lipid peroxidation and free radical production are attenuated by several defense mechanisms. One primary agent to reduce free radicals and lipox is vitamin E. Vitamin E is an antioxidant incorporated into lipid membranes. It can sequester free radicals and brakes the chain reaction process of lipid peroxidation.

The beneficial effects of vitamin E have been demonstrated in both animal and human research. A number of researchers have demonstrated that lipox increased in animals at rest and after exercise who were fed a vitamin E deficient diet (Davies et al., 1982; Packer, 1984; Quintanilha, 1984; Quintanilha & Packer, 1983). In human studies, vitamin E supplementation decreased lipox as shown by lower MDA concentrations in supplemented subjects compared with unsupplemented controls (Dillard, Litov,

Savin, Dumelin, & Tappel, 1978; Goldfarb, Todd, Boyer, Alessio, & Cutler, 1989).

Vitamin E deficiency and supplementation studies demonstrate an effect of vitamin E on serum CK concentrations. In animal studies, rats fed a vitamin E deficient diet had higher serum CK concentrations than controls fed a diet normal in vitamin E (Amelink, Van Der Wal, Wokke, Van Asbeck, & Bar, 1991). In human studies, males supplemented with vitamin E had lower CK concentrations compared to controls after performing a downhill treadmill run (Cannon, Orencole, Fielding, Meydani M., Meydani S., Fiatarone, & Blumberg, 1990).

In addition to the antioxidant effects of vitamin E, studies suggest that vitamin E may have a modulating effect on pain. Two possible pain stimulating substances in muscle soreness are calcium ions and prostaglandins (PGE_2) (Armstrong, 1984; Smith, 1991). Phoinex, Edward, & Jackson (1989) stated that vitamin E had a stabilizing effect on the sarcolemma and sarcoplasmic reticulum which may prevent excessive calcium ion efflux. Prostaglandin E_2 has also been implicated in sensitizing free nerve endings to pain following exercise (Smith, 1991). Vitamin E supplementation suppressed prostaglandin E_2 production in a study of young and older mice (Meydani, Meydani, Verdon, Shapiro, Blumberg, & Hayes, 1986). Diminishing calcium ion efflux and

suppressing PGE2 may decrease pain or muscle soreness.

In summary, vitamin E deficiency increased lipid peroxidation and serum CK in animals. Vitamin E supplementation decreased lipid peroxidation and serum CK concentrations in human subjects. Also, vitamin E may have a pain modulating effect.

To investigate the effect of vitamin E supplementation on lipid peroxidation and serum CK concentrations, Lewis and Goldfarb (1992) conducted a second 100 mile road-ride study with a marked elevation gain (Study 2) (Appendix C). Cyclists were age matched and randomly assigned to a placebo or vitamin E group. Each group consisted of 9 males and 2 females. The vitamin E group received 800 mg per day. Both groups received supplementation for 2 weeks prior to the road ride.

MSRs again peaked immediately post ride and remained significantly elevated at 24 hours in both groups. Ratings were 5.5 for the vitamin E group and 4.2 for the placebo group. Serum CK again increased significantly from rest concentrations and peaked at 24 hours in both groups. These concentrations represented a 316% increase for the vitamin E group (125.4 IU) and a 268% increase for the placebo group (101.2 IU) over rest concentrations. The time course pattern for MSRs and serum CK was the same as during the first road ride-study (Appendix B).

No significant differences were observed between the placebo and vitamin E group though there was a trend for the vitamin E group to have higher MSRs and higher serum CK concentrations. While both groups were closely matched with respect to mean percent body fat, age, and gender, the placebo group finished in a shorter mean time and trained more miles per week. Even though max VO_2s were similar between the two groups, the placebo group was better trained as demonstrated by their shorter mean finish time and greater number of miles trained per week. In this road study, training, ride intensity, and dietary intake of vitamin E and polyunsaturated fats were not controlled. Another research study to control or monitor these variables was designed and is the topic of this dissertation.

Purpose

The purpose of this study was to determine the effects of vitamin E supplementation (800 mg alpha tocopherol for four weeks) on MSRs, plasma CK, and plasma MDA in trained male cyclists performing an exhaustive endurance cycle ergometer ride under controlled conditions. Cyclists rode at 70% VO_2 max in a double-blind, cross-over design. The time course effect of MSRs, plasma CK, and plasma MDA were examined to determine if there were differences between treatments. The relationships among these variables were also determined.

Hypotheses

The null hypothesis was chosen as the researcher is unaware of the direction of the effect if any for vitamin E supplementation when compared to the placebo supplementation.

The hypotheses were as follows:

1. Perceived muscle soreness ratings will not be different when subjects are given vitamin E supplementation as compared with the placebo supplementation.

2. Peak concentration of plasma CK will not be affected by vitamin E supplementation compared with the placebo supplementation.

3. Peak concentration of plasma MDA will not be affected by vitamin E supplementation compared with the placebo supplementation.

4. The time course response of MSR, CK, and MDA to exercise will not be altered with vitamin E supplementation as compared with the placebo supplementation.

5. The relationship between MSRs, CK, and MDA will not be altered with vitamin E supplementation compared with the placebo supplementation.

Assumptions and Limitations

Subjects were asked to refrain from taking vitamin supplements for two weeks prior to beginning participation in the study. High levels of supplementation from other

sources during the placebo treatment could lead to erroneous findings.

Subjects were healthy males who were experienced cyclists. They ranged in age from 20 to 42 years. Generalizing the findings beyond this group would be limited.

Subjects were asked to maintain their normal routine of training and to refrain from weight lifting one week prior to the endurance-ergometer tests. No subject routinely engaged in weight training. Acute weight training bouts can elevate plasma CK and plasma MDA values.

Subjects completed a four day food record prior to each endurance ride. The records were analyzed to assess total kilocalories, percent carbohydrates, proteins, fats, and dietary vitamin E intake. Additionally, grams of dietary polyunsaturated, monosaturated, and saturated fats were determined. Differences in dietary vitamin E and the amount and kind of fat could alter lipid peroxidation.

CHAPTER II

LITERATURE REVIEW

Muscle Soreness

Theories of Soreness

Muscle soreness which is an aching, tenderness or pain following strenuous or novel exercise can have an acute or delayed onset. Acute and delayed onset muscle soreness (DOMS) may have different mechanisms. Biochemical mechanisms have been proposed for acute muscle soreness. These mechanisms for acute muscle soreness include metabolic depletion, altered cellular pH and lipid peroxidation. Researchers suggested that a metabolic depletion of ATP occurred in strenuous endurance exercise (Rodbard & Pragay, 1968; Oberc & Engel, 1977; Wrogemann & Pena, 1976). This depletion could result in accumulation of cytosolic calcium ions. Calcium ion accumulation can result in alteration in membrane permeability and even cell damage or cell death (Armstrong, 1984, 1986; Cross, Halliwell, Borish, Pryot, Ames, Saul, McCord, & Harman, 1987).

Saltin and Gollnick (1983) argued against a decrease in ATP as being a mechanism of muscle soreness. They suggested that during submaximal exercise, ATP levels in the active muscle appeared to be maintained at near resting values. While ATP depletion may remain controversial as a mechanism

of muscle soreness, alterations in the oxidative coupling mechanism with strenuous exercise may occur and result in calcium ion accumulation and muscle soreness (Armstrong, 1984).

Altered cellular pH as evidenced by changes in serum lactate may be a mechanism in acute muscle soreness. During exhaustive endurance or high intensity exercise lactate can accumulate. Under these conditions there is a buildup in the hydrogen ion concentration which lowers cellular pH. Lower cellular pH can alter the membrane permeability resulting in an efflux of calcium ions and disruption in the Na-K-ATPase pump (Armstrong, 1984).

Lactate levels increase during strenuous exercise, but return to resting levels by 15 to 30 minutes after the completion of exercise (Halone & Konittinen, 1962). Elevation in lactate may fit the time course of acute muscle soreness. Acute muscle soreness is soreness that occurs during or immediately after exercise and returns to non-significant levels after a short period of recovery. Lactate accumulation does not fit the time course of DOMS as lactate levels have returned to within normal limits long before DOMS begins. In addition, lactate may accumulate without the development of muscle soreness.

Lipid peroxidation is a process that is known to be damaging to the cell membrane. Lipid peroxidation is a

process by which the production of free radicals in the electron transport system can attack the double-bond structure of lipids. The double-bond structure found in polyunsaturated and monosaturated fats are most susceptable to lipox (Packer, 1984). In this process, a molecule of oxygen is added to the membrane altering the membrane structure. The integrity of the membrane is altered resulting in increased permeability of the membrane and leaking of enzymes such as CK. Possible mechanisms of acute muscle soreness are alterations is oxidative coupling, lowered cellular pH, and lipid peroxidation.

Because DOMS has a different time of onset, delayed up to 24 or 48 after exercise, mechanical models have been suggested including muscle tearing and ischemia. Several structural changes occur that suggest strenuous, unaccustomed exercise may cause minor muscle tissue damage. Strenuous or novel exercise can result in microscopic disruptions of myofibrils and Z-lines (Friden et al., 1981). Newham et al. (1983) observed that microscopic disruption occurred in the leg performing step down phase of the step test (eccentric exercise), but not in the leg performing the step up phase of the step test (concentric exercise). Subjects experienced DOMS in the leg performing eccentric exercise but not in the leg performing concentric exercise.

Ischemia may also play a role in DOMS (Hoppeler, 1986; Sjostrom et al., 1987). During isometric exercise, static muscle contractions may produce intermuscular pressures that diminish blood flow. Muscle contraction as low as 10% of maximum voluntary contraction (MVC) may diminish blood flow. Muscle contractions of 65% MVC can occlude blood flow (Guyton, 1986). Diminished blood flow or occlusion with subsequent reperfusion may result in tissue damage. Ischemia may particularly be involved in muscle soreness from isometric exercise.

Muscle Soreness Ratings

A muscle soreness rating scale was devised by Clarkson et al., (1985) and has been used in a number of research studies (Buckley-Bleiler, Maughan, Clarkson, Bleiler & Whiting, 1990; Clarkson et al., 1985, 1986, 1987b; Clarkson, Apple, Byrnes, McCormick, & Triffletti, 1987a; Newham et al., 1983b). The scale is a 1 to 10 scale with 1 being no soreness and 10 being very, very sore. The site of muscle soreness is also indicated on the chart (Appendix A).

Muscle Soreness and Type of Muscle Contraction

The type of muscle contraction--eccentric, concentric, or isometric--can affect muscle soreness. During eccentric contractions, the muscle fibers lengthen while the fibers shorten with concentric contractions. The muscle fiber length is unchanged during isometric contractions. Delayed

onset of muscle soreness occurred after eccentric work (Clarkson et al., 1986; Newham et al., 1983b). It has been reported that subjects experienced little if any soreness following the step up portion of the step test and uphill treadmill running (concentric activities). Subjects experienced marked soreness following step down in the step test and downhill treadmill running (eccentric activities) (Newham et al., 1983b; Newham, Jones, & Edwards, 1986).

Clarkson et al. (1986) investigated muscle soreness in subjects performing eccentric, concentric, and isometric biceps curls. The greatest degree of soreness was observed after the eccentric biceps curls (ratings 4 to 5) followed by isometric (rating 2 to 3), and then concentric curls (rating 1 to 1.5).

Jones, Newham, and Clarkson (1987) found a positive correlation with muscle soreness and eccentric upper limb weight lifting. They suggested that connective tissue may be damaged during eccentric contraction. Damage to the noncontractile elements results in increased sensitivity to the muscle receptors.

The mechanism of muscle membrane damage in eccentric contractions may be mechanical. Fewer muscle fibers are recruited in eccentric contractions to exert the same amount of force when compared to concentric contractions. Several temporary morphological changes occur with strenuous

eccentric contractions. These changes include Z disks disturbances (Friden et al., 1981), myofibrillar and sarcolemmal disruptions (Armstrong, Ogilvie & Schwane, 1983; Friden et al., 1981) and A- and I-bands widening (Armstrong et al., 1983; Friden et al., 1981; Newham et al., 1983a).

Friden, Stakianas and Hargens (1986) measured greater intramuscular fluid pressure during eccentric (236 mm Hg) versus concentric (157 mm Hg) contractions of the dorsiflexors. In addition, 24 hours later, the pressure in the anterior compartment of the leg was 10.5 mm Hg in the limb performing the eccentric work, but was only 4.4 mm Hg in the limb doing concentric contraction.

Biochemical changes as well as structural alteration accompany tissue damage following eccentric contractions. These biochemical changes include enhanced protein turn over, increased plasma intracellular proteins, and stimulation of neutrophils.

Enhanced protein breakdown has been measured in both younger and older men following a 45 minute eccentric cycle-ergometer ride at 80% VO_2 max (Fielding, Meredith, O'Reilly, Frontera, Cannon, & Evans, 1991). Disruption of the extracellular matrix as evidenced by increased plasma intracellular proteins and ions has been observed (Stauber, Clarkson, Fritz, & Evans, 1990). Increase intracellular proteins in the extracellular matrix has been suggested as a

mechanism in swelling and pain following eccentric contractions. Evidence of acute inflammatory responses with increased neutrophil and macrophage concentrations has been reported following a 45 minute downhill treadmill run (eccentric exercise) at 45% VO_2 max. No inflammatory response was evidenced after an uphill treadmill run (concentric contractions) at a comparable work load (Smith et al., 1989).

These morophological and biochemical alterations are accompanied by changes in functional characateristics of the muscle. Decreased muscle strength has been demonstrated to last up to 2 weeks following strenuous eccentric exercise (Newham et al., 1987).

Clarkson et al. (1985) reported that muscle soreness ratings (3 to 4) following isometric forearm flexion were related to muscle tension generated during the activity. Subjects who generated higher tensions, experienced greater muscle soreness. During eccentric contraction, fewer muscle fibers do the same absolute work as during concentric contractions. Eccentricly contracting muscle fibers generate greater tension than concentrically contracting muscle fibers. As a result of greater tension an overload is placed on these fibers that is not experienced during concentric activities (Aura & Komi, 1986; Edwards, Mills, & Newham, 1981; Newham, 1988).

Previous researchers reported subjects having little, if any, perceived muscle soreness (rating, < 1.5) following concentric events. However, Lewis et al. (1991) and Lewis and Goldfarb (1992) reported cyclists had increased muscle soreness following strenuous 100 mile road rides. Other studies may not have demonstrated muscle soreness as a result of concentric activity because the duration and/or intensity were not sufficient to elicit muscle soreness.

Time Course Patterns of MSRs

The pattern of muscle soreness varies with the type of muscle contraction. Initial soreness was delayed, with onset occurring 24 or 48 hours post event in eccentric and isometric work (Byrnes & Clarkson, 1986; Clarkson et al., 1986; Newham et al., 1983b, 1986). Soreness peaked as much as 48 to 96 hours later with this type of contraction. Soreness may persist for up to 7 days depending upon the severity of the activity.

Muscle soreness ratings peaked immediately post event following a strenuous endurance-concentric exercise (Lewis et al., 1991; Lewis & Goldfarb, 1992). Soreness was declining at 24 hours and returned to nonsignificant levels by 72 hours.

Muscle Soreness and CK

With equal work time, all three types of muscle contractions exhibited approximately the same increase in CK

concentrations. In a study by Clarkson et al. (1986), subjects performed eccentric, concentric and isometric biceps curls. Serum CK activity increased 36% following eccentric work, 38% following concentric work and 34% following isometric work. Because of the difficulty in equating the same work load during each type of contraction, the researchers chose to keep the work time the same. While CK concentrations were approximately equal with each type of contraction during biceps curls, subjects experienced the greatest soreness with eccentric contractions.

In a total body activity, such as running, subjects experienced greater muscle soreness and higher releases of serum CK following downhill treadmill running (eccentric work) than following uphill running (concentric work) (Newham et al., 1986). After running on a treadmill at a 13 degree incline, subjects had peak serum CK concentrations of less than 200 IU/L. After running downhill at 13 degrees, peak serum CK concentrations were up to 15,000 IU/L. Serum CK peaked in 24 hours after uphill running, but did not peak for 96 hours after downhill running.

Researchers suggested that fewer muscle fibers were activated at a similar workload during eccentric contractions, therefore less muscle mass was involved with downhill running (Aura & Komi, 1986; Edwards et al., 1981; Newham, 1988) Thus, the overload on muscle fibers used

during the exercise appears to effect serum CK release.

One study reported an exception to increased muscle soreness and markedly elevated serum CK following eccentric exercise (Nosaka & Kuramata, 1991). These researchers measured muscle soreness ratings and serum CK following a bout of consecutive drop jumps (mean 114) to exhaustion. Muscle soreness ratings peaked at 24 hours and returned to near baseline values at 72 hours. Serum CK peaked immediately post exercise and returned to near baseline concentrations by 36 hours. Peak serum CK concentrations increased about 1.3 fold from pre-exercise values.

During isometric work, increased tension parallels increased muscle soreness and increased CK concentrations (Clarkson et al., 1985). In the study, eleven untrained male subjects performed two different exercise bouts. Subjects performed 40 maximum voluntary contractions (MVC) of isometric forearm flexion for 10 seconds followed by 20 seconds of rest between contractions (Group A: 40 sets x 10 sec MVC/ 20 sec rest). In the second bout, subjects did the same protocol, but with a 5 second rest between contractions (Group B: 40 sets X 10 sec MVC/5 sec rest). Subjects in group A generated higher tensions than group B. Serum CK concentrations in Group A increased 143%, whereas serum CK concentrations is Group B increased only 52%.

In a later study, Graves, Clarkson, Litchfield, Kirwan, and Norton (1987) used regression analysis to determine the relationship between serum CK release and muscle tension, and serum CK release and amount of muscle mass used during isometric contractions. Subjects did three different isometric exercises: (a) one arm flexion, (b) two arm flexion, and (c) one leg extension. Serum CK concentrations increased 181%, 222%, and 297% respectively. While there was a tendency for increased serum CK concentrations with increased tension and muscle mass, the correlations were not significant.

Increased muscle soreness correlated significantly with increased muscle tension in isometric contractions (Clarkson et al., 1986; Graves et al., 1987; Kirwan, Clarkson, Graves, Litchfield, & Byrnes, 1986) (Table 1). However, increased serum CK was not significantly correlated to increased tension ($r = 0.30$) or muscle mass ($r = 0.28$) during the isometric contractions. During isometric contraction, blood flow can be diminished in contractions that are as low as 10% MVC. Blood flow can be completely occluded at 65 to 70% MVC (Lamb, 1984). The hypoxic state followed by reoxygenation may result in damage to the muscle membrane or connective tissue through lipid peroxidation. Lipid peroxidation is a process of auto-oxidation that is damaging to the cellular membrane. Since serum CK release was not

significantly correlated with tension, soreness may be the result of damage to other tissues beside the membrane.

Lewis et al. (1991) found a 445% increase in CK activity comparing resting and 24 hour concentrations in cyclists following the first road ride (Study 1). Serum CK peaked at 24 hours at 240 IU/L. While serum CK tended to decline after 24 hours, it remained significantly elevated for the 72 hour period. Peak MSRs were reported immediately post event with a mean rating of 5.5. Soreness persisted for 24 hours after the ride, but was declining. By 48 hours, soreness had essentially returned to baseline. The time course of MSRs and CK was not significantly correlated.

Following a second road ride, Lewis and Goldfarb (1992) observed a similar pattern in the rise of serum CK to that seen in subjects participating in the previous study (Lewis et al., 1991). Subjects were divided into two groups, a vitamin E group and a placebo group. The vitamin E group received 800 IU of alpha tocopherol daily. Both groups were supplemented for 2 weeks. While serum CK rose 315% for the vitamin E group and 265% for the placebo group above resting concentrations, serum CK did not elevate out of the normal range. The MSRs peaked immediately post event for both groups with a mean rating of 5.5 for the vitamin E group and 4.2 for the placebo group.

In summary, greatest muscle soreness occurred after eccentric contractions, followed by isometric and then concentric work. Delayed onset of muscle soreness (DOMS) occurred following eccentric and isometric work. The time course of DOMS was correlated to the time course of elevated serum CK concentrations in eccentric contractions. The amount of serum CK appears to be related to muscle overload in eccentric work. In isometric work, muscle soreness appeared to be related to tension; however muscle tension and serum CK activity were not significantly correlated (Clarkson et al., 1985). Acute onset of muscle soreness occurred following strenuous endurance cycling, a concentric event. The time course of muscle soreness and serum CK were not significantly correlated following concentric exercise (Lewis et al., 1991; Lewis & Goldfarb, 1992).

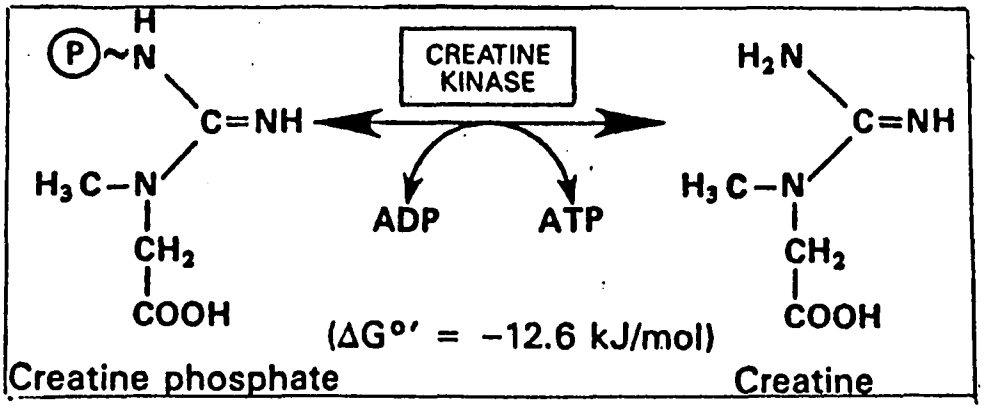
Creatine Kinase

Characteristics

Creatine N-phosphotransferase, subclass EC 2.7.3.2., commonly known as creatine kinase (CK), is a phosphotransferase enzyme, which transports phosphate from one molecule to another (Bennington, 1984; Lang & Wurzburg, 1982). This enzyme catalyzes the conversion of the reversible reaction: ADP + phospho-creatine <----^{CK}----> ATP + creatine (see Figure 1). CK is activated by magnesium, manganese and sulfhydryl agents (Bennington, 1984; Perryman, Knell, &

Roberts, 1984). Under physiological conditions the above reaction is favored in the forward direction from left to right. This pathway is a primary source of ATP resynthesis during intense, short-term exercise and is found primarily in the cytosol of the cell.

Figure 1. CK Phosphotranserase in ATP Synthesis. From Harper's Biochemistry by R. Murray, G. Gardner, P. Mayer and V. Rodwell, 1988, Appleton & Lange, Norwalk, Connecticut p. 97.



CK is a dimer, a compound produced by the combination of two like molecules. The two different subunits of CK are "M" which denotes the muscle subunit and "B" which denotes the brain subunit. (Wevers, Olthuis, Van Niel, Van Wilgenburg, & Soons, 1977; Hood, 1980; Bennington, 1984). Therefore, three isozymes are possible for CK: CK-MM, primarily in skeletal muscle; CK-MB, primarily in the heart and brain; and CK-BB found in the brain (Perryman et al., 1984). The isozymes, CK-MM, CK-MB, and CK-BB, are mostly

tissue specific. Serum is composed of 95% to 97% CK-MM and 3% to 5% CK-MB. CK-BB is not usually detected with most routine techniques (Bennington, 1984).

In addition, CK-MM exists as three isoforms. The isoform CK-MM3 is a precursor of CK MM2 and CK MM1 and is found in muscle tissue and lymph. Upon entering the blood stream CK-MM3 from muscle tissue is converted to CK-MM2 and CK-MM1. CK-MM3 is used as a marker of recent release of CK from tissue (Perryman et al., 1984).

Normal Ranges

Lab Analysis

Normally, serum CK concentration is low and is usually impermeable to cell membranes because of its high molecular weight of 80,000 daltons (Da) (Hortobagyi & Denahan, 1989). Ranges for normal serum concentrations of total serum CK are dependent upon which laboratory analysis techniques are employed to measure the enzyme. Moskowitz and Osband (1984) reported the normal serum CK range to be 0 to 180 IU/L . Garcia (1974) reported the reference values for the Coulter Accuzyme Method to be 5 to 50 IU/L for men.

Gender Differences

Males have slightly higher ranges of serum CK than females. Serum CK levels have been reported to be higher in individuals with greater lean body mass (Garcia, 1974; Novak & Tillery, 1977; Larsen & Rossner, 1983). Norton, Clarkson,

Graves, Litchfield, and Kirwan (1985) noted that when percent body fat and body density were adjusted, males still had higher serum CK resting values than females. Estrogen may play a role in gender differences (Norton et al., 1985).

Racial Differences

Black, Quallich, and Gareleck (1986) observed racial as well as gender differences in serum CK concentrations. African Americans had the highest serum CK concentrations followed by Hispanics and then Caucasians. Males of each race had higher serum CK levels than females. Meltzer (1971) and Olerud, Homer, and Carroll (1976) also measured higher serum CK concentrations in African Americans compared to Caucasians.

	Males	Females
African Americans	146.5 IU/L	66.4 IU/L
Hispanics	84.5 IU/L	41.5 IU/L
Caucasians	60.8 IU/L	37.0 IU/L

Age Differences

Griffiths (1966) reported the median value of serum CK for adult males to be 30.6 IU/L; adult females, 22.3 IU/L; male children, 33.3 IU/L; and female children, 28.4 IU/L. Whitefield and Martin (1986) studied 206 pairs of twins. They suggested that resting serum CK values were genetically determined and that individual variations occurred with physical activity. No circadian variation has been

demonstrated to occur with serum CK.

Serum CK activity at rest has been reported to increase with age (Meltzer, 1971). Gale and Murphy (1979) computed that this increase is approximately 3 IU/L per decade in the normal resting level. Possible age related muscular atrophy and the degenerative process may contribute to higher resting serum CK levels in the sedentary elderly.

Clinical Reasons for Elevated CK

Elevation of serum CK concentrations out of the normal range, indicate a change in membrane permeability as a result of cell membrane damage. With injury, CK passes to the lymph via the interstitial fluid. From the lymph, CK is released into the general blood circulation (Linden, Kupper, Friedel, & Trautschold, 1979). Elevated serum CK concentrations were observed in a number of cardiovascular, muscular, neurologic, and hormonal disorders as well as following strenuous or novel exercise.

Serum CK concentrations above normal ranges were reported following myocardial infarction (Hess, MacDonald, Frederick, Jones, Neeley, & Gross, 1963; Griffiths, 1966; Sobel & Shell, 1972; Galen, 1975), in muscle disorders such as muscular dystrophy (Annesley, Strongwater, & Schnitzer, 1985; Tsug, 1981; Yasmineh, Ibrahim, Abbasnezhand & Awad, 1978), and in neurological disorders (Meltzer, Kupler, Wyatt & Synder, 1970; Brayne, Calloway, Dow & Thompson, 1982).

Hagberg, Michaelson, and Ortelius (1982) found elevated serum CK levels in individuals performing repetitive motion tasks. Serum CK have been elevated in individuals receiving intramuscular injections of antibiotics (Batsakis, Preston, Briere, & Gieson, 1968; Nevins, Saran, Bright, & Lyon, 1969) and in hypothyroid patients (Graig & Smith, 1965).

Alterations in Serum CK With Exercise

Type of Muscle Contraction

Alterations in serum CK concentrations with exercise is a complex, dynamic process. Total serum CK appears to be related to an interplay of a combination of factors. These factors include: (1) type of muscle contraction: eccentric, concentric, or isometric; (2) mode of exercise, impact or non-impact, (3) intensity and duration of exercise, (4) physical conditioning of the subject: trained or untrained; and (6) adaptation to training.

Vejjajiva and Teasdale (1965) were the first to report elevated serum CK following strenuous exercise. Other researchers reported elevated serum CK post exercise (Pearce, 1965; Hansen, Bjerre-Knudsen, Brodthagen, Jordal, & Paulev, 1982; Brown, McClure, & Wang, 1982; Apple & McGue 1983; Galun, 1984). Even moderate exercise can result in an increase in total serum CK in untrained subjects (Nicholson, McLeod, Morgan, Meerkin, Cowan, Bretag, Graham, Hill, Robertson, & Sheffield 1985).

A greater release of CK has been observed following eccentric work than following concentric work (Clarkson et al., 1986; Newham et al., 1983b, 1986; Schwane, Johnson, Vandenakker, & Armstrong, 1983) (see Table 1). Serum CK and DOMS were significantly correlated in subjects who ran downhill on a treadmill (Newham et al., 1983b).

Slight elevations in serum CK were reported after concentric contraction in uphill treadmill running (Newham et al., 1986) and the concentric portion of the step test (Newham et al., 1983b). Spitler, Alexander, Hoffler, Doerr, and Buchanan (1984) reported no significant differences in CK concentrations comparing 12 trained and untrained men and women following a maximal ergometer stress test (concentric contractions). However, Brooke et al. (1979) reported increased serum CK in 12 untrained subjects and 12 patients with myalgia following a 90 minute bicycle ergometer test. Twelve trained subjects in this study had little change in CK. Lewis et al. (1991) found a marked increase in total serum CK immediately following a strenuous 100 mile ride in subjects who had previously trained for the ride. These variable findings may reflect differences in intensity and duration of exercise and training states of the subjects.

During strenuous endurance cycling, the muscle may become glycogen depleted. In the Lewis et al. (1991) study, mean finish time was 7.2 hours with a range of 5.75 to 9.5

hours. Glycogen concentration is a major determinant of muscular endurance (Gollnick, Armstrong, Sembrowich, Shepherd, & Saltin, 1973). Altered actin and myosin coupling and disrupted oxidative phosphorylation may occur as the cellular pH begins to fall in glycogen depletion. At exercise intensities above 70% VO_2 max the rates of glycolysis and subsequent production of hydrogen protons are high (Karlsson, 1971). Following continuous high intensity exercise to exhaustion, muscle pH can drop from a resting value of 7.0 to 6.6 to 6.4 (Sahlin, Alvestrand, Brandt, & Hultman, 1978). Accumulation of hydrogen ions may effect calcium ions binding to troponin thus effecting actin and myosin cross-bridge formation (Dawson, Gadian, & Wilkie, 1980). This process may alter membrane function making the membrane susceptible to lipid peroxidation and subsequent CK release. With endurance exercise, circulating lipid levels increase. A greater potential for lipid peroxidation occurs at the moment when serum lipids increase (Packer, Almada, Rothfuss, & Wilson, 1989).

Variable in serum CK following concentric activity may reflect differences in intensity and durations of exercise. Low intensity and short duration exercise may have minimal effect on membrane permeability. High intensity (> 70% VO_2 max) and long duration (> 90 min.) may have marked effects on serum CK.

Table 1

Muscle Soreness and CK in Ecccentric, Concentric, and Isometric Contractions

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Combined							
Clarkson et al. (1986)	College age	F	28	6 sets 10s work to 10s rest biceps curl, eccentric, concentric isometeric	Unknown	peak CK eccentric 36% concentric 38% isometric 34%	Eccentric > soreness but CK no >, CK not best indicator of degree of injury
Newham et al. (1983b)	28 28	F M	8 5	step test a. step up-concentric b. step down-eccentric	active (untrained for task)	2 patterns: CK a.<400 IU/L 1-2 days peak, b. up to 34,500 IU/L peak 4-5 days	Eccentric > muscle damage than concentric.Sensitive subjects can have a >> rise CK
Newham et al. (1986)	30 30	M F	2 3	a. 13% uphill treadmill (concentric) b. 13% downhill eccentric	healthy (untrained)	a. CK <200 IU/L peak 1 day b. CK to 15,000 IU/L peak 4 days	Eccentric > muscle damage than concentric
Isometric							
Clarkson et al. (1985)	24	M	11	Ison Arm flex a. 40x10 sec MVC 20 sec rest (10/20) b. 40x10 sec MVC 5 sec rest (10/5	untrained	CK 143% (a) CK 52% (b)	CK and soreness with > tension tension > a than b
Graves (1987) et al.	22	M	12	Isometric a. 1 arm flex b. 2 arm flex c. 1 leg exten	untrained	a. CK 181% b. CK 222% c. CK 297	CK↑not = tension or muscle mass r = 0.30 tension r = 0.28 muscle mss
Kirwan (1986)	21	F	13	ison knee extent a. 40, 10sec MVC 20 sec rest (10/20) b. 40, 10/5sec MVC 5 sec rest (10/5)	UN	a. Ck 350 IU/L b. CK 194 IU/L	(a) tension > than (b), CK ↑ with > tension

Table 1 continued

Muscle Soreness and CK in Ecccentric, Concentric, and Isometric Contractions

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Concentric							
Brooke (1979) et al.	23-48 23-48 15-42	M M M	12 12 13	bike ergometer 90 to 120 min 50% VO_2 3 month training	a. trained b. untrained c. patients	a. mean change 31 IU/L, b.= 386 IU/L, c.= 467 IU/L	CK << trained, CK > myalgia patients bike test for screening
Lewis et al. (1991)	35	M	11	Endurance bike ride 100 mi	trained	CK peaked 24 hr at 240 IU/L	Peak soreness post ride, CK & Soreness not sig. correlation r = 0.23
Lewis & Goldfarb (1992)	29	M F	18 4	Endurance bike ride 100 mi 11 vitamin E group 11 placebo group	trained	vitamin E CK= 125 IU/L placebo CK = 103 IU/L	CK peaked 24 hr both groups. CK not significantly different, but trend for lower CK in placebo group
Spitler (1984) et al.	27-55	M F	12 12	Bike Test High fit M High fit F Low fit M Low fit F	trained untrained	Pre Post 160* 183 IU/L 98 117 IU/L 139* 159 IU/L 95 106 IU/L *elevated	No differences CK pre & post max bike. No differences in [CK] trained & untrained
Eccentric							
Jones (1987) et al.	24-43 24-43	M F	3 4	eccentric forearm flex 15 sec MVC contractions for 20 min	active (untrained for task)	CK post exer. ≈ 720 IU/L < elbow angle > soreness	EMG negative soreness not due to spasm. *Suggest damage to connective tissue
Schwane (1983) et al.	20	M	7	treadmill run 0 & 10% downhill grade 9 x 5 min bouts = 45 min total	moderately trained	peak CK 24hr 4x > downhill than level	WBC count no soreness not due to inflammation. Due to structural changesin muscle

Intensity and Duration of the Activity

Researchers have debated if intensity or duration is more important in the release of serum CK (Table 2). Pearce (1965) was among the first to suggest that only exercise of considerable severity can increase serum CK. Mayer and Clarkson (1984) observed that a single bout of exercise for a longer time resulted in a greater rise in serum CK than multiple bouts for a shorter time even when the total amount of work was equal. Duration appeared to play a greater role since work intensity was equated in this study. These data confirmed earlier findings of Nuttal and Jones (1968).

After investigating subjects' serum CK responses to treadmill running and ergometer tests, Fowler, Gardner, Kazerunion, and Louvstad (1968) suggested that serum enzymes, CK and LDH, increased in proportion to intensity of work and duration. They noted that duration appeared to be of greater significance than did intensity following bike ergometer and treadmill tests. After submaximal cycle ergometer trials, Forssell, Nordlander, Nyquist, Orinius, & Styrelius (1975) found that in untrained subjects, serum CK rose little following heavy work of short duration. Ohkuwa, Saiato, and Miyamura (1984) observed a similar response in serum CK in trained subjects following a high intensity short duration 400 meter sprint. Robinson, Williams, Worthington, and Carter (1982) measured serum CK in trained

Olympians. Serum CK was lowest in subjects who trained the shortest time. Average serum CK was 110 IU/L for workouts < two hours and 322 IU/L for workouts > six hours. From these studies, duration appears to play a greater role in CK release than intensity. However, changes in serum CK may be related to greater total amounts of work done during these studies and not to duration alone.

Stansbie, Aston, Dallimore, Williams, and Willis (1983) found serum CK to elevate but only during exercise of severe intensity. They measured serum CK release after a bike ergometer ride, squeezing a ball to exhaustion, and marathon running. Serum CK rose only in marathon running. However, the marathon was also the activity of greatest duration.

Following weight lifting, Tiidus and Innuzzo (1983) reported higher serum CK concentrations in subjects who did the fewest repetitions, but lifted the most weight. They suggested that higher intensity resulted in greater serum CK release than longer duration. Type of exercise may play a role in whether duration or intensity is of greater consequence. In cycle ergometer and treadmill running, duration appears to play a greater role. In weight lifting, intensity appears to play a greater role. Weight lifting at maximum contractions may place greater tension on individual muscle fibers in both the concentric and eccentric phases of lifting than is placed on the muscle in cycling or running.

Table 2

Intensity and Duration

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Forssell (1975) et al.	32-73 ?	M F	15 2	submax bike ergometer test	untrained	73% CK post exercise, but within normal range	Post ex. CK 32 mU/L AMI CK mean 900 mu/L heavy physical work of short duration will not ↑ CK
Fowler (1968) et al.	17-31	M	5	bike ergometer variable intensity & duration	untrained	pre mean CK= 0.76 CPK units post CK= 25.4 units (range UN)	Both intensity & duration important, but duration more important
Kirwan (1986)	21	F	13	isom knee ext a. 40 x 10 sec MVC 20 sec between b. 40 x 10 sec MVC 5 sec between	UN	a. CK 350 IU/L b. CK 194 IU/L	a > tension than b, CK ↑ with > tension (contradicts next study)
Mayer (1984) et al.	college age	M	9	isom knee extension a. 60s hold 60s rest b. 30s h 30s rt (2x) c. 15s h 15s rt (4x) total work equal	UN	a. CK 130% b. CK 74% c. CK 29%	a > CK, ↑ duration tension did not expect findings. CK efflux related to blood pressure?
Pearce (1965)	letter to editor					CK athletes chronic adaptation	Only exercise of considerable severity can ↑ CK activity
Stansbie (1983) et al.	29	UN	UN	a. bike ergometer b. sueeze ball to exhaustion (blood flow occluded)	UN UN	a. no change CK b. no change CK	mild and moderate exercise do not serum CK only severe exercise resulted in an increase
	32	M	69	c. marathoners	recreational to elite	c. CK 5x	
Tiidus (1983) & Innuzzo	20-45	M & F	21	weight lifting 3 groups: 30% 10RM, 545Rep 55% 10RM, 275Rep 80% 10RM, 170Rep	untrained	CK 2x after 80% 10RM, 170	CK ↑ > after high intensity lower repetitions, thus intensity more than duration

Mode of Exercise: Impact or Non-impact

Mode of exercise, whether impact or non-impact (Table 3) is a factor in serum CK release. LaPorte, Linde, Bruce, and Fitzsimmons (1978) found no acute changes in total serum CK in swimmers (non-impact), but found significant increases in runners and weight lifters (impact). Hansen et al. (1982) also reported greater serum CK release in trained and untrained runners (impact); than in trained rowers (non-impact) (Table 3).

Berg and Haralambie (1978) found that for the same duration, serum CK and serum hexose phosphate isomerase (PHI) were higher in impact-type activities such as running and skating and lower in non impact-type activities such as ergometer exercise. These researchers noted that above 65-70% of maximal aerobic capacity, serum CK enzyme activity increased proportional to the exercise time.

In well trained swimmers, no acute rise in total serum CK was observed following a high intensity workout or after a one hour endurance swim (Symanski, McMurray, Silverman, Smith, & Siegel, 1983). Haralambie and Sensen (1980) found a mild increase in total serum CK in swimmers after a 90 minute swim but the increases were within the normal range for CK. Strauss, Lott, Bartels, Fox, and Whitcomb (1986), however, reported elevated CK concentrations in chronic swim trained subjects over the course of the season.

Serum CK release appears to be greater following impact exercise than non-impact exercise. Brown et al. (1982) measured CK concentrations up to 13,280 IU/L in moderately trained runners after a marathon. Noakes, Kotzenberg, McArthur, and Dykman (1983) measured mean total serum CK concentrations of 634 IU/L in trained ultradistance runners. In one high impact study, serum CK was found to change minimally (Nosaka & Kuramata, 1991). These investigators measured a 17% increase in serum CK following consecutive drop jumps. This study was is an exception to the generalization that impact exercise resulted in greater serum CK release than non-impact exercise. In comparison, Brooke et al. (1979) reported elevations of serum CK concentrations of 32 IU/L in trained subjects following a 90 minute ergometer ride, 386 IU/L in untrained subjects and 467 IU/L in patients with myalgia. Haralambis and Senser (1980) measured serum CK concentrations of 124 IU/L in bobsledders compared to 29 IU/L in control subjects, but even the concentrations for bobsledders were within the normal range. When elevation in serum CK concentrations occur in non-impact exercise, the degree of elevation was generally not as great as with impact exercise.

Table 3

Impact and Non-impact Exercise

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Impact Exericse							
Berg (1978) et al.	17-42	M	166 (18 groups)	a. bike ergometer b. ski race c. running	trained	a. mean 4 U/L b. mean 23 U/L c. mean 120 U/L	Impact > CK CK ↑ with duration in skiing & running
Brown (1982) et al.	24	M	1	marathon	moderately trained	Total CK 13,280 IU/L, CK-MB 1.3%	↑ CK post run, no change in CK-MB isozyme
Hansen (1982) et al.	19-44 15-24 22-48	M M M	16 6 9	a. 27 km run b. 12 km run c. 1½ hr rowing	trained runners untrained trained rowers	a. 223 U/L b. 147 U/L c. 221 U/L	CK ↑ = in impact & non-impact. CK ↑ with duration
Noakes (1983) et al.	UN	M	75	88 km race	trained	total CK = 634 U/L. CK-MB (1-19%) X = 4%	Tissue source for ↑ CK-MB not known
Non-impact Exercise							
Brooke (1979)	23-48 23-48 15-42	M M M	12 12 13	bike ergometer 90 to 120 min 50% VO_2 3 month training	a. trained b. untrained c. patients	a. mean change 31 IU/L, b. = 386 IU/L, c. = 467 IU/L	CK << trained, CK > myalgia patients bike test for screening
Haralambie (1976) et al.	25-35 25-35	M M	45 25	bobsled race	trained for controls	post race 124 IU/L. controls 29 IU/L	CK > sled racers due to isometric contractions during race
Haralambis (1980) et al.	22	M	16	90 min 5000 meter swim	trained	CK peak 24 hr 131 IU/L	CK rose, but still in normal range CK much lower than post endurance run
LaPorta et al. (1978)	college age	M	UN	a. 1 hr swim b. 1 hr. run c. weight lift (hard workout) d. controls	3 groups trained 1 group control	a. 588 CK IU/L b. 322 CK IU/L c. 368 CK IU/L d. 115 CK (all 24 hr means)	Statistically only b & c significant. Group a, 2 subjects mean. CK > Impact than non-impact exer.

Table 3 continued

Impact and Non-impact Exercise

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Non-impact Exercise continued							
Strauss (1986) et al.	collage age	M	14	swim training	trained CK	peak 20 U (range 0-12 normal)	Chronic swim training resulted in elevated CK activity
Symanski (1983) et al.	18-36	M	6	tethered swim 1 hr 70% VO_2 max	trained	acute bout no change in CK or CK-MB pre to post test	High intensity swim not sufficient stress to elevate total CK or CK-MB

Training

Several research groups have reported lower peak serum CK concentrations in trained subjects when compared to untrained or less trained subjects (Brooke et al., 1979; Gimenex & Florentz, 1984; Haibach & Hosler, 1985; Nuttal & Jones, 1968; Olerud et al., 1976; Rutledge, 1978) (Table 4). Evans, Meredith, Cannon, Dinarello, and Frontera (1986) and Evans (1987) found that trained individuals had lower peak serum CK values when compared with untrained subjects. Runners and sedentary male subjects pedaled a negative bicycle ergometer at a workload of 250 watts for 45 minutes. Pedaling of the ergometer required eccentric rather than concentric muscle contractions. While absolute workloads were the same, relative intensity was not adjusted for the trained subjects. As a result, the trained subjects were working at a lower relative workload compared to untrained subjects (Table 4).

Peak serum CK concentrations were lower and occurred earlier in trained subjects than in untrained subjects. In trained subjects, serum CK peaked at 24 hours at 207 IU/ml. In untrained subjects, serum CK peaked at 120 hours at 2143 IU/ml (Evans et al., 1986). However, the trained subjects had higher resting serum CK concentrations which were thought to be an adaptive response to endurance activity. Roti, Iori, Guiducci, Emanuele, Robuschi, Bandini, Gnudi &

Roti (1981) also reported higher resting serum CK for trained than untrained subjects, but lower peak serum CK concentrations post exercise in trained subjects.

Ahlborg and Brohult (1967) found that after a high intensity bike ergometer ride that trained subjects had lower peak serum CK concentrations when compared to untrained individuals, but these values were not statistically significant. Therefore, prior training appears to reduce the serum CK response to exercise.

It is unclear why the serum CK response is lower in trained versus untrained subjects. Hunter and Critz (1971) suggested that lower serum CK concentrations in trained subjects may reflect the trained subject's increased ability to generate ATP via oxidative phosphorylation which may help to keep the membrane intact by reducing oxidative stress.

At high intensity and duration, even well trained subjects demonstrated elevated serum CK concentration post event (Jaffe, Garfinkel, Ritter, & Sobel, 1984; Lewis et al., 1991). Likewise, if intensity and duration of the event are not sufficient, differences in serum CK concentrations between trained and untrained subjects were not noted (Misner, Massey, & Williams, 1973; Spitler et al., 1984). A clear demonstration of the training effect in decreasing CK when relative intensities are equal for trained and untrained subjects has not been examined.

Table 4

Trained vs Untrained

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Ahlborg (1967) Brobult	21-23	M	12	Bike ergometer high intensity 90 min	trained (vs untrained prior study)	CK rest 0.4nm post ex. 0.7nm	Resting CK trained, post ex. trained CK
Brooke (1979) et al.	23-48 23-48 15-42	M M M	12 12 13	bike ergometer 90 to 120 min 50% VO_2 3 month training	a. trained b. untrained c. patients	a. mean change 31 IU/L, b. = 386 IU/L, c. = 467 IU/L	CK << trained, CK > myalgia patients bike test for screening
Evans (1986) et al.	UN UN	M M	5 4	Eccentric Bike Ergometer 250 W for 45 min	a. untrained b. trained	a=CK 2143 IU/L peaked on da 5 b=CK 207 IU/L peaked on da 1	Trained CK rest, CK ↑ post exer & peaked sooner than untrained
Gimenez (1984) et al.	UN	M	9	Bike ergometer Square Wave	5 trained M 4 sed. M	CK trained at rest (222%) post ex.(235%)	CK↑ trained chronic adaptation, CK↑ post exercise 12% above baseline
Haibach Hosler (1985)	18-58 UN	M F	20 1	marathon	trained	CK > slowest runner (300 U/L)	> muscle damage in less fit subjects
Hunter Critz (1971)	20-27	M	12	10 wk training bike ergometer step test & phy. work capacity	untrained	CK rest no pre/post train CK post exer. after training	Trained ↑ ATP maintain cell membrane thus enzyme efflux
Jaffe (1984) et al.	UN	M	9	24 hr post professional football game	trained	CK = 717 IU/L CK-MB=17 IU/L % CK-MB/CK <3% (normal <10%)	CK in well trained above normal post exercise. CK-MB in players with > total CK
Lewis (1991) et al.	35	M	11	Endurance bike ride 100 mi	trained	CK peaked 24 hr at 240 IU/L	Peak soreness post ride, CK & Soreness
Misner (1973) et al.	28-42	M	11	15 wk training 5 ran (endurance) 45 min 3da/wk 6 played games 45 min 1½/wk	untrained	CK post ex. in endurance & recreational trained subjects following a max test	CK did not ↑ with endurance or re-creation training (training may not have been intensive enough)

Table 4 continued

Trained vs Untrained

Reference	Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Buttal (1968) Jones	26 31	F M	7 7	Squats 25% MVC 30 reps in 6 min 3 to 5 wk on protocol	untrained untrained	Pre train: max test M CK 2.5x F CK 3x, train test no M or F	CK post training M & F similar postpattern. No circadian variation
Ohkuwa (1984) et al.	19	M	13	400 m sprint	trained	CK activity correlated with sprint velocity (r = - 0.56)	In well trained sprinters plasma CK better indicator training than peak blood lactate
Olerud (1976) et al.	UN	M	337	Marine recruits recruits	untrained basic training	whites-3,814 IU/L blacks-7,794 " others-2,672 "	basic training severe exercise CK & myoglobin
Roti (1981) et al.	UN UN	M M	22 33	pre, 4,8 & 24hr post soccer match	11 trained 11 untrained 33 control	rest trained 137 IU/L untrained 82 IU/L post ex. CK trained	CK > resting trained, but < post exer. Trained near max muscle to plasma efflux
Rutledge (1978)	young ?	M	6	1 hour non-competitive run	untrained	Pre: 99 IU/L post: 181 IU/L	untrained: immeditate CK pre & post run
Spitler (1984) et al.	27-55	M F	12 12	Bike Test High fit M High fit F Low fit M Low fit F	trained untrained	Pre Post 160* 183 IU/L 98 117 IU/L 139* 159 IU/L 95 106 IU/L *elevated	Gender differences apparent. No diffenences in CK trained & untrained

Adaptation to One Bout of Exercise

Serum CK response to stress following even a single bout of a strenuous or novel activity has been reported (Clarkson et al., 1987b; Clarkson & Trembly, 1988; Clarkson & Dedrick, 1988) (Table 5). Subjects who performed a single bout of eccentric forearm flexion exercise had an initial rise in serum CK, but then had a diminished serum CK response to exercise for up to three weeks (Newham et al., 1987). The serum CK response to exercise was attenuated.

Similar findings were observed in subjects who performed exhaustive eccentric knee extension exercises. After the first exercise bout, total serum CK rose significantly. When retested, subjects had little rise in serum CK for up to six weeks post exercise (Triffletti, Litchfield, Clarkson, & Byrnes, 1988). An adaptive response to a single bout of exercise was observed (Table 5).

Total serum CK and CK-MB may be chronically elevated by exercise. Increased serum CK-MB may be an adaptation to endurance training (Apple, Rogers, Sherman, Costill, Hagerman, & Ivy, 1984; Apple, Rogers, Casal, Sherman, & Ivy, 1985). With aerobic conditioning, skeletal muscle enzyme adaption may become more like that of the heart. However, Siegel, Silverman, Evans, and Madar (1983) suggested that there is no firm evidence to indicate whether the skeletal muscle or myocardial tissue or both are releasing the CK-MB.

Table 5

Total CK and CK-MB Adaptation to Exercise

Reference	X Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Total CK Adaptation							
Clarkson (1988) Trembly	22	M	16	Eccentric hamstring 2 bouts 7 days apart	untrained	bout 1 CK 327 IU/L bout 2 CK 167 IU/L	CK lower 2nd bout ↓ than first bout acute training adaptation
Clarkson (1987b) et al.	25	F	8	Eccentric forearm fl. 1. 70 Max 2. 24 Max	untrained	CK 22x 70 Max CK 3x 24 Max CK no change 2nd bout	CK ↑ > work load, but even lower work load had an adaptive response
Clarkson Dedrick (1988) et al.	A. 24 B. 67	F F	10 10	Eccentric forearm fl 24 (115%) Max isometric [bout 1 (bt 1)]	conditioned but untrained for the test	A. bt 1 CK 6 B. bt 1 CK 5 A. bt 2 CK no B. bt 2 CK no	CK ↑ equally A & B in bout 1. B adapted as well as A
Newham (1987) et al.	24-42 24-43	M F	3 5	eccentric forearm flex for 20 min bt 1 bt 2-2wk later bt 3-4wk later	active (untrained for task)	bt 1 CK (1500-11000 IU/L strength to 40%	Faster recovery of strength and force frequencies after bout 2 & 3 than after bout 1
Triffletti (1988) et al.	college age	M	45	MVC isometric knee extension bout 1, bt 2-3wk later bt 3-6wk later bt 4-9wk later	active	bout 1 CK > bout 2 all experiments except at 9 weeks	Adaptation to isom. exercise than can last up to 6 wks
Adaptation of CK-MB							
Apple McGue (1983)	28,29	M	2	Marathon training	trained	CK ↑ parallels increased milage CK-MB 7.2%	Rise in CK & CK-MB adaptation to strenuous exercise
Apple et al. (1984)	a-25 b-?	M M	5 5	marathon controls	trained untrained	CK-MB 7.2% run runners, <1.0% in controls (muscle biopsies)	CK-MB adaptation to endurance training

Table 5 continued

Total CK and CK-MB Adaptation to Exercise

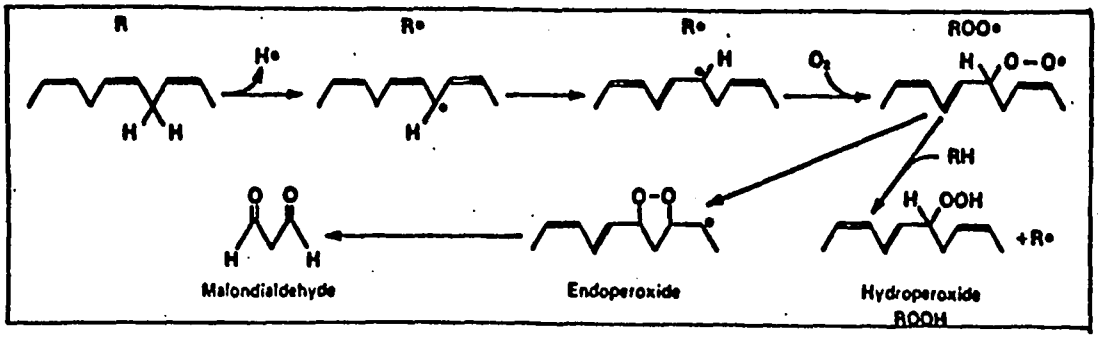
Reference	X Age	Sex	N	Ex. Test	Sub. Status	CK Activity	Conclusion
Adaptation of CK-MB continued							
Siegel (1983) et al.	36	M	25	marathon	trained	CK-MB 8.9% runners, CK-MB 3.3% in controls	Elevated serum & CK-MB from skeletal muscle adaptation

Lipid Peroxidation

Definition

Lipid peroxidation (lipox) is a process of auto-oxidation in which free radicals are formed from the exposure of lipids to oxygen in the presence of a metal ion. The oxidation of a fatty acid is referred to as peroxidation because two molecules of oxygen are added to the lipid molecule at double bond sites. The peroxides formed can lead to further free radical formation. Another result is the release of free radicals which are highly reactive chemical entities with unpaired electrons. Free radicals are implicated in a number of disease processes: cancer, atherosclerosis, essential hypertension, immune deficiency, and the aging process (Cutler, 1984; Zebra, Komorowski, & Faulkner, 1990). Free radicals disrupt methylene double bonds in fatty acids and alter the configuration of the molecule. The oxidation of fatty acids with three or more double bonds can form the by product, malondialdehyde (MDA) (see Figure. 2) (Murray et al., 1988). [Oxidation is the subtraction of an electron, while reduction is the addition of an electron (Murray et al., 1988)]. Lipox can be determined by measurements of its by products: MDA (Tateishi, Yoshimine, & Kuzuka, 1987; Uchiyama & Mihara, 1978;) ethane and pentane (Balke et al., 1984).

Figure 2. Formation of MDA From an Unsaturated Fatty Acid. From Harper's Biochemistry by R. Murray, G. Gardner, P. Mayer and V. Rodwell, 1988, Appleton & Lange, Norwalk, Connecticut, p. 138.



Propagation

Lipox is a chain reaction process providing a continuous supply of free radicals that initiates further peroxidation (Murray et al., 1988) (see Figure 3). The

Figure 3. Propagation of a Free Radical. From Harper's Biochemistry by R. Murray, G. Gardner, P. Mayer and V. Rodwell, 1988, Appleton & Lange, Norwalk, Connecticut, p. 138.

(1) Initiation: Production of R$^{\cdot}$ from a precursor.

$$ROOH + metal^{(n)+} \rightarrow ROO^{\cdot} + metal^{(n-1)+} + H^{+}$$

(2) Propagation:

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$$
$$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}, \text{etc.}$$

(3) Termination:

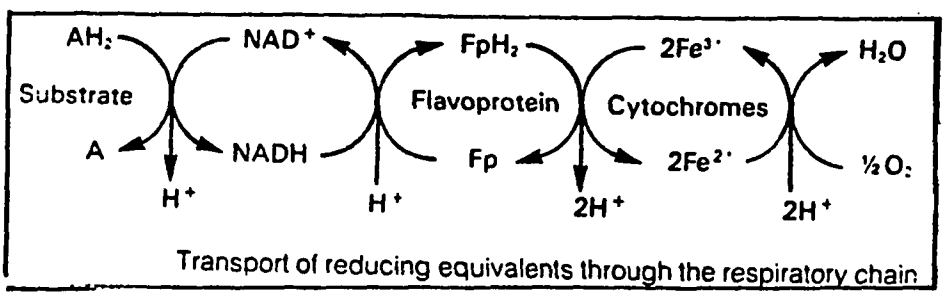
$$ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2$$
$$ROO^{\cdot} + R^{\cdot} \rightarrow ROOR$$
$$R^{\cdot} + R^{\cdot} \rightarrow RR$$

process will continue unless terminated by either an antioxidant, enzyme mechanisms, or if the reactants are bound or completely utilized. A number or sources generate free radicals both extra and intracellularly. Only the intercellular sources will be reviewed because of their relationship to exercise.

Free Radical Sources

Free radicals are produced in the mitochondrial and microsomal electron transport system in the process of reducing oxygen (0_2) to water (H_2O). Ferrous ions in the transport system associated with cytochromes can facilitate free radical formation (Figure 4).

Figure 4. Ferrous Ions in Association With Cytochromes as a Site of Free Radical Formation. From Harper's Biochemistry by R. Murray, G. Gardner, P. Mayer and V. Rodwell, 1988, Appleton & Lange, Norwalk, Connecticut, p 109.

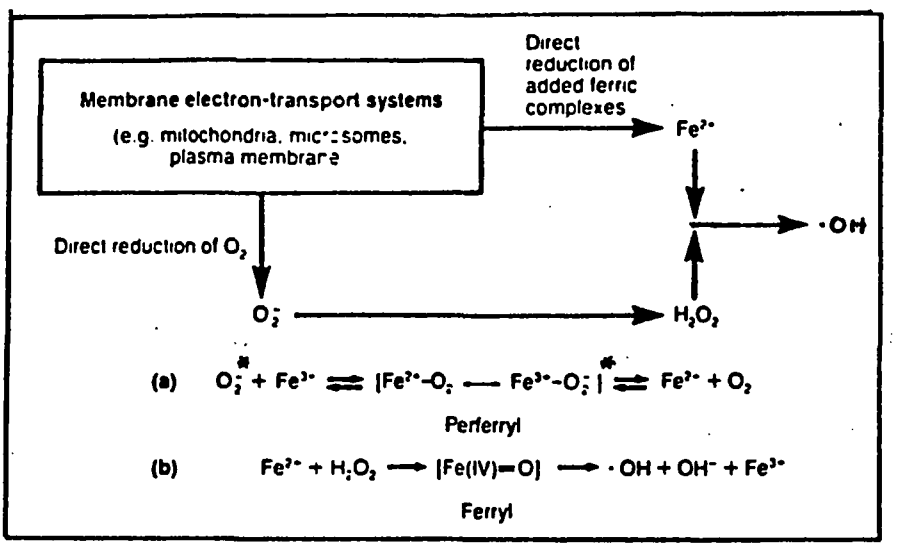


The oxygen molecule has spaces for four electrons in its outer electron shell. While oxygen can accept a total of 4 electrons to form water, it can be reduced in univalent steps (Green & Hill, 1984). The main pathway is the tetravalent reduction of oxygen to water which proceeds in the mitochondria with cytochrome oxidase as the final catalyst. No oxygen intermediates are produced in this pathway (Sjodin, Hellsten, & Apple, 1990). Ernster, Atallah, and Hochstein (1986) suggests that the tetravalent reduction of oxygen accounts for 95 to 98% of the total oxygen consumption. A small fraction of 2 to 5% of the oxygen consumed by cells can be utilized in a univalent pathway in the mitochondria under oxidative stress (Sjodin et al., 1990) In the univalent pathway highly reactive intermediate oxygen radicals can be produced.

The presence of a metal ion, is required for to remove electrons univalantally from oxygen. Heavy metals, such as iron, copper and cobalt, have two valence states with suitable oxidation-reduction potentials that can generally increase the rate of lipid auto-oxidation (Barber & Bernheim, 1967; Dillard, Downey, & Tappel, 1984). The addition of a single electron to the oxygen molecule results in the formation of a superoxide anion (O_2-*) (see Figure 5) (Gutteridge & Halliwell, 1990).

Oxygen, itself, is not a very reactive substance because of its unusual electron spin. Oxygen exists as a diradical, wherein the two unpaired electrons in the outer orbital have parallel spins rather than opposite spins. For oxygen to oxidize a two electron donor, an inversion of spin is necessary for the paired electrons in the orbital to have

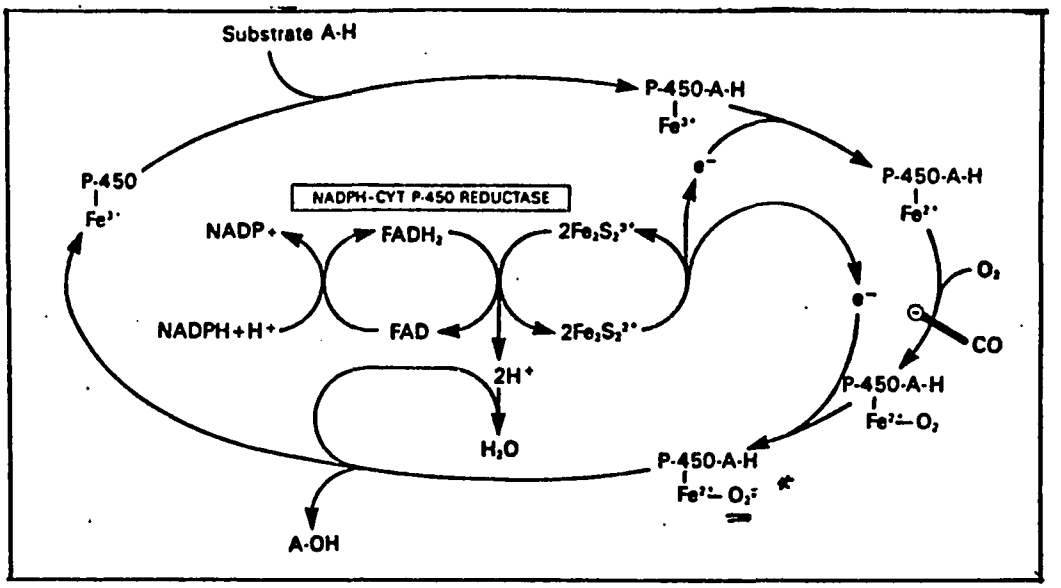
Figure 5. Formation of Hydrogen Peroxide. From J. Gutteridge, and B. Halliwell. 1990, Clinica Chimica Acta, 13, p. 131.



opposite spins (Weiss, 1986). A catalyst is necessary for such a reaction. Enzymes are able to bind oxygen and substrate molecules for sufficient length of time to allow oxidation to occur. The mitochondrial enzyme, cytochrome oxidase, can reduce oxygen directly to water (Weiss, 1986),

but cytochrome P-450 generates the superoxide free radical (Figure 6) (Murray et al., 1988). Superoxide may be protonated to form HO_2*, the perhydroxyl radical. Oxygen can also be divalently reduced to H_2O_2. The addition of an electron to H_2O_2 leads to the formation of the hydroxyl

Figure 6. Free Radicals Formed in the P450 System. From Harper's Biochemistry by R. Murray, G. Gardner, P. Mayer and V. Rodwell, 1988, Appleton & Lange, Norwalk, Connecticut, p 106.



* site of superoxygen anion radical

radical (OH*) and the hydroxyl anion (OH-). The protonated form of the hydroxyl anion (OH-) is water (H_2O). Finally, the addition of the fourth electron to OH* reduces the species to the hydroxyl anion OH-* and water. Superoxide (O_2-*), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH*) have been implicated in the pathophysiology of the

microcirculation (Weiss, 1986). Of these three, the hydroxyl radical (OH*) is the most reactive and damaging chemical species (Halliwell, 1981).

Free radicals can be generated at many cellular sites including mitochondria and its electron transport system, microsomes, peroxisomes, endoplasmic reticulum, nuclear membrane and its electron transport system, and the plasma membranes (Fong, McCay, & Poyer, 1973; Freeman & Crapo, 1982; Freeman 1984). Superoxide radical generation by mitochondria mediated reactions is greatest when respiratory chain carriers located on the inner mitochondrial membrane are highly reduced (Freeman, 1984). Thus during strenuous exercise, superoxide radicals can be generated.

The endogenous factors that influence mitochondrial radical production are those that regulate respiration which include availability of nicotinamide-adenine dinucleotide (NAD), succinate, and adenosine diphosphate (ADP) (Freeman, 1984). Oxygen-related free radicals can disrupt the oxidative processes of glycolysis by depleting reduced nicotinamide-adenine dinucleotide (NADH), reduced glutathione (2G-SH), and adenosine triphosphate (ATP) and give rise to cytosolic calcium (Ca^{+}) ions, all of which can lead to cell damage. (Cross et al., 1987). NADH is an electron carrier, ATP is the energy source of the cell, and 2G-SH is a substrate for the removal of hydrogen peroxide.

Oxidative stress can lead to changes in the metabolism of cellular lipids, altered cellular membranes, altered activity of membrane-bound enzymes such as sarcoplasmic reticulum calcium ATPase, and disturbances in cellular protein turnover and energy production (Murphy & Kehrer, 1989).

Interestingly, the electron transport system that sustains life produces free radicals that can lead to the cell's demise. Consequently, the cell has a number of free radical defense mechanisms (Jenkins, 1988).

Protective Mechanisms

First, the membrane structure shields polyunsaturated fatty acid by two hydrophilic layers. Second, vitamin E which is found within the membrane and beta carotenoids which function at low partial pressures of oxygen (Murray et al., 1988; Krinsky, 1989) are important antioxidants. Other antioxidants include uric acid, cystine, glutathione, vitamin C and glucose (Sevanian, Muakkassah-Kelly, & Montestruque, 1983). Third, heavy metals are bound by transferrin, albumin (Gutteridge, 1986), ceruloplasmin and urate (Davies, Sevanian, Muakkassah-Kelly, & Hochstein, 1986) which makes the metal ions less reactive (Halliwell & Gutteridge, 1984). Fourth, free radicals occurring in the electron transport shuttle are intermediate substances that have a brief existence. Fifth, an array of enzymes exist

that remove the free radicals. These include superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase (Freeman and Crapo, 1982). Finally, the enzyme phospholipase A_2 (Sevanian et al., 1983) has the ability to cleave damaged membrane segments. A summary of the cell's primary free radical defense systems is given in Figure 7 (Jenkins, 1988).

Lipox: Mechanism of Injury

Lipox is not only be a mechanism of membrane damage, but the process occurs whenever tissue has been damaged by any other mechanism. Tissue damaged by any mechanism is more susceptible to lipid peroxidation (see Figure 8) (Halliwell & Gutteridge, 1986; Gutteridge & Halliwell, 1990). Salaris and Babbs (1989) determined that relatively low concentrations of oxygen were sufficient to produce oxidative damage post ischemia.

Increased neutrophil infiltrations have been reported in post-ischemic skeletal muscle following blood flow occlusion and reperfusion in rats. Neutrophils are apart of the acute inflammatory process which also includes stimulation of macrophages and lipid peroxidation in the scavenger mechanism (Smith, Grisham, Neil, Granger, & Korthus, 1989).

Figure 7. Free Radical Defense Mechanisms of Cells. From "Free radical chemistry relationship to exercise" by R. Jenkins, 1988, Sports Medicine, 5, p.162.

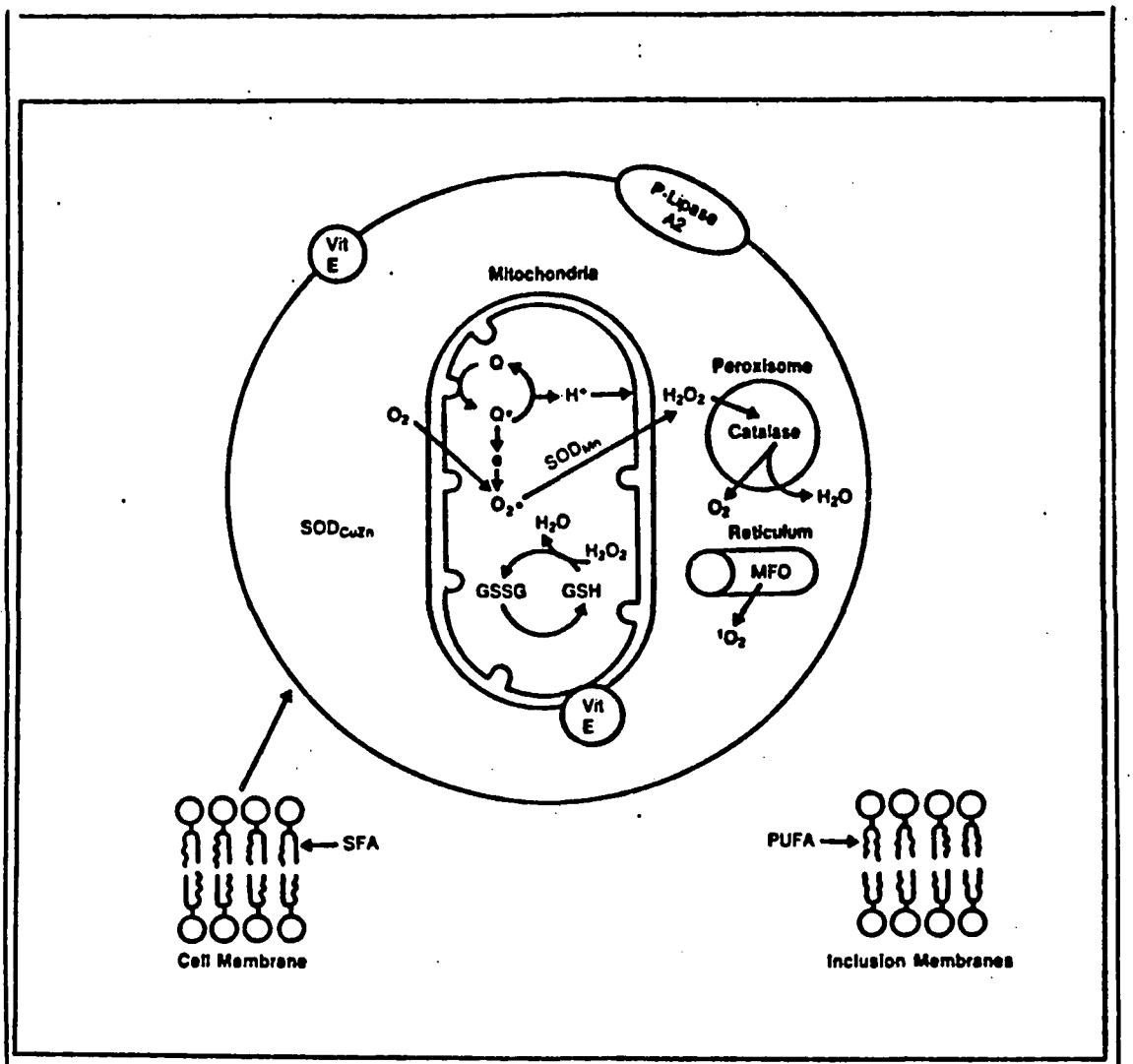
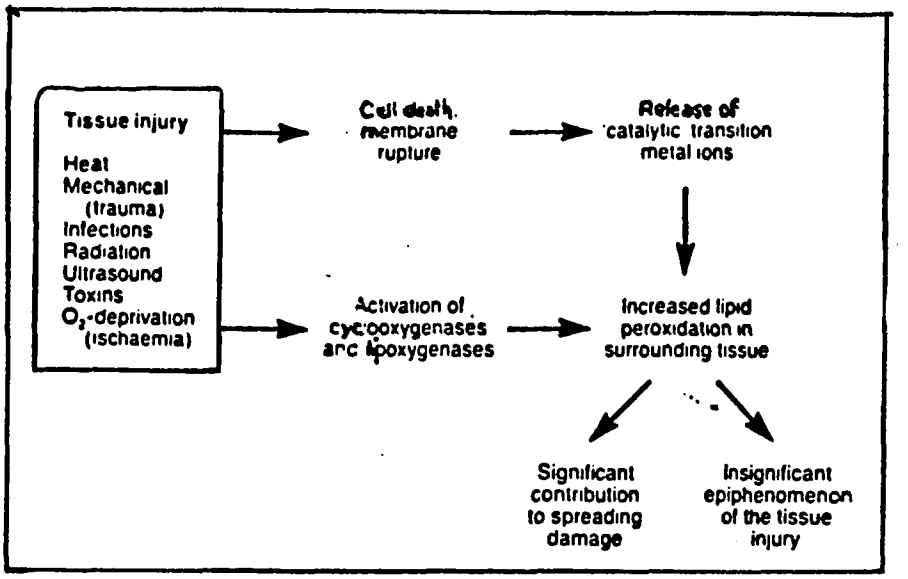


Fig. 3. Summary of cell's free radical defence. P-Lipase A_2 = phospholipase A_2; Q· = quinone radical; SOD_{Mn} = manganese su peroxide dismutase; SOD_{CuZn} = copper zinc superoxide dismutase; MFO = mixed function oxidase or cytochrome.P450; SFA = saturated fatty acid; PUFA = polyunsaturated fatty acid; GSSG = oxidised glutathione; GSH = reduced glutathione.

Figure 8. Damaged Tissue Susceptibility to Lipid Peroxidaton. From J. Gutteridge, and B. Halliwell. 1990, Clinica Chimica Acta, 13, p. 132.



Lipox and Exercise

A number of researchers have demonstrated an increase in lipox as a result of exercise. Male subjects ran downhill (gradient: 12 degrees) at 75% VO_2 max (Maughan, Donnelly, Glesson, Whiting, Walker, & Clough, 1989). Lipox occurred as demonstrated by elevated plasma MDA. Elevated serum muscle enzymes were indicative of muscle membrane damage. Thiobarbituric acid reactive substances (TBARS), which is a procedure used to determined lipid peroxidation, peaked at 6 hours post run as did LDH. Serum CK and aspartate amino-transferase peaked at 24 hours after this eccentric activity. These researchers suggested a relationship between increased lipox and indicators of

muscle membrane damage, but not necessarily a causative effect.

Serum CK and MDA were correlated in another study. Following an 80 kilometer run, serum CK and MDA were significantly correlated, r = 0.84 (Kanter, Lesmes, Kaminsky, La Ham-Saeger, & Nequin, 1988).

Intensity of exercise plays an important role in lipid peroxidation. Alessio and Goldfarb (1988) measured a 77% increase in lipox in muscle tissue following moderate exercise (20 meters/min for 20 min) and a 300% increase in muscle with high intensity (45 meters/min for 1 min) exercise in rats running on a treadmill. Lovlin, Cottle, Pyke, Kavanagh, and Belcastro (1987) measured MDA in male subjects performing an ergometer test at different intensities. Subjects rode at 40% VO_2 max for 5 minutes, rested for 5 minutes and then rode for 5 minutes at 70% VO_2. After a second 5 minute rest, subjects pedaled to voluntary exhaustion. Blood samples were taken prior to the test, in the middle of both rest periods and post test. They reported that MDA plasma levels increased 26% after the exhaustive ride. At 40% VO_2, however, a significant decrease in MDA levels occurred (10.3%) and at 70% levels were still below resting values.

Lewis et al. (1991) found that cyclists performing a strenuous 102 mile bicycle ride demonstrated peak muscle

soreness immediate post event, peak serum CK activity at 24 hours and peak plasma MDA levels at 48 hours post event. Lovlin et al., (1987) measured MDA levels below baseline at intensities between 40 and below 70% VO_2 max. At higher intensities MDA levels rose. In the Lewis et al. (1991) study, because of the long duration of the ride, (mean 7.2 hours) less elite cyclists may have been functioning at intensities of 70% VO_2 max or below. Serum CK and plasma MDA had time course changes that appear to be independent, but intensity was not controlled in this study. Lipid peroxidation may contribute to muscle membrane damage at intensities of 70% VO_2 max and above.

Researchers demonstrated that by-products of lipox respond to a training effect in animal models. Untrained rats had higher lipox indicators than trained rats at the same absolute workload in both red and white muscle (Alessio, Goldfarb, & Cutler, 1988). Their findings supported the results of Salminen and Vihko (1983) who measured lipox in red and white skeletal muscle in mice. Endurance trained mice had lower lipid peroxidation by-products than untrained control mice in both types of muscle tissue.

Kihlstrom (1990) investigated the effects of endurance training against hypoxic and reoxygenation induced injuries. Following blood flow occlusion and reoxygenation, tissue was

more susceptible to oxidative stress. Lipox by-products increased in heart tissue for both endurance trained and untrained control rats following reoxygenation; however, the trained rats had lower MDA levels than untrained controls.

In addition to training, antioxidants have a role in the protective mechanism of the cell membrane against oxidative stress. The mechanism of vitamin E action in lipox, supplementation and deficiency responses to exercise will be presented.

Vitamin E

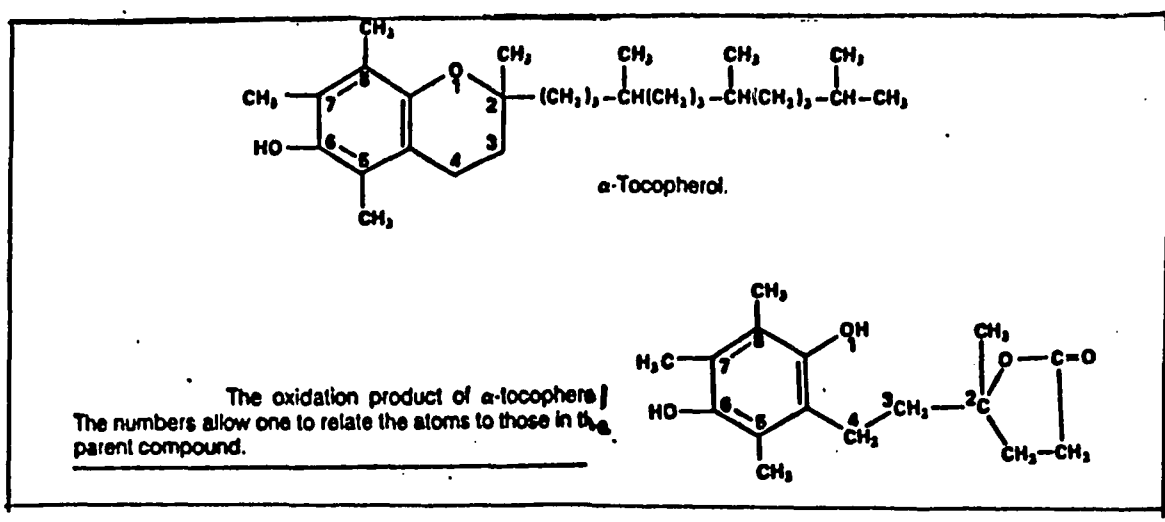
Antioxidant Properties

Vitamin E is the name given to a family of eight tocopherols, alcohols that vary in the structure of their side chain. The most reactive of the tocopherols is d-alpha tocopherol because it has the highest rate constant for reaction (Burton, Cheeseman, Doba, Ingold, & Slater, 1983). Its chemical structure predisposes it to rapid oxidation (Murray et al., 1988) (Figure. 9).

Vitamin E is a lipid soluble vitamin. It exists only in small amounts on the cell membrane in a ratio of one molecule vitamin E to 2000 to 3000 lipid molecules (Bieri, Corash, & Hubbard, 1983), but it is an effective antioxidant (Reed, 1984). Antioxidants fall into two classes: (1) preventive antioxidants which reduce the rate of chain initiation and (2) chain breaking antioxidants which

interfere with chain propagation (Murray et al., 1988). Preventive antioxidants include catalase and other peroxidases that react with ROOH and chelators of metal ions. Chain breaking antioxidants are often phenols or

Figure 9. Vitamin E Molecular Structure.
From Harper's Biochemistry by R. Murray, G. Gardner, P. Mayer and V. Rodwell, 1988, Appleton & Lange, Norwalk, Connecticut, p 139.



aromatic amines. The principal chain-breaking antioxidants are superoxide dismutase (SOD) which acts in the aqueous phase to trap superoxide free radicals, urate, and vitamin E which acts in the lipid phase to trap peroxyl radicals. The chain-breaking antioxidant activity of tocopherols (TocOH) toward peroxyl radicals (ROO*) is illustrated as follows: (Reed, 1984; Murray et al., 1988).

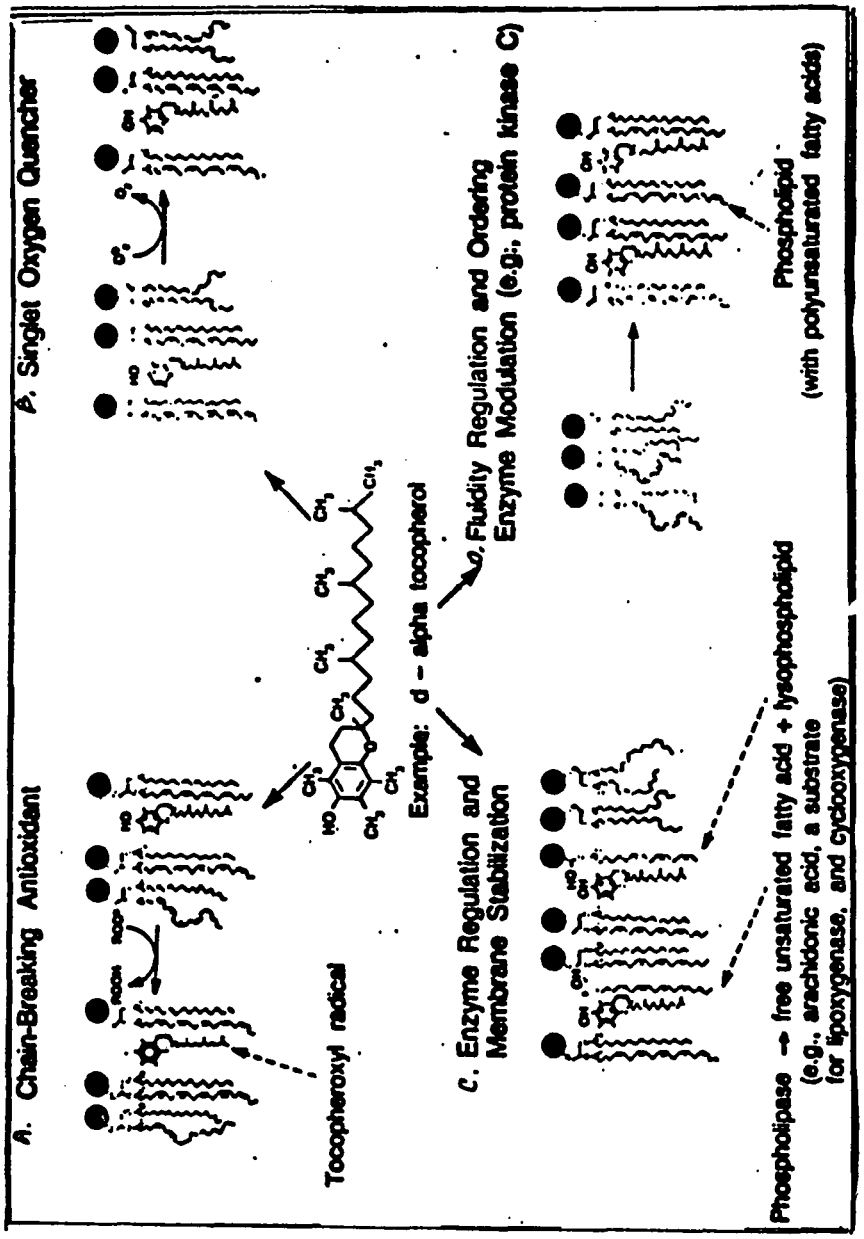
ROO* + TocOH --------> ROOH + TocO* (oxidized tocopherol)

ROO* + TocO* --------> ROOH + nonradical product

Alpha-tocopherol is a more effective hydrogen donor than the other tocopherols since electron-releasing methyl groups, lacking in the other species of Vitamin E, act to stabilize the developing phenoxyl radical and increase the rate of the reaction (Parker, 1989). Fukuzawa and Gebicki, (1983) reported that membrane-bound alpha-tocopherol reacted with $O_2^{\cdot}$, $HO^{\cdot}$, and $HO_2^{\cdot}$ radicals. With the positioning of the phenoxyl group at the surface of the membrane, alpha-tocopherol may intercept radical species prior to their interaction with allylic carbons of polyunsaturated fatty acids, which reside deeper in the membrane (Perly, Smith, Hughes, Burton, & Ingold, 1985).

Vitamin E modifies biological responses of the lipid membrane (Figure 10). Vitamin E acts as a chain-breaking antioxidant, but can also act as a singlet oxygen quencher. It exerts a controlling influence on linoleyl and arachidonyl residues to help stabilize the membrane (Diplock, 1983), and promotes the membrane's fluidity (Packer & Landvik, 1989). Vitamin E sequesters free radicals that could otherwise cause membrane or other structural damage. As a singlet oxygen quencher, vitamin E acts to remove the oxygen radical from biochemical reaction that could result in lipid peroxidation. Vitamin E assists with enzyme regulation and fluidity regulation by maintaining stability of the phospholipid membrane.

Figure 10. Vitamin E as a Biological Response Modifier From "Vitamin E: introduction to biochemistry and health benefits" by L. Packer and S. Landvik, 1989, Annals of New York Academy of Science, p. 2.



The recommended daily allowance (RDA) of Vitamin E is 8 mg. or international units (IU) for females and 10 mg for males. Individuals who consume large amounts of vegetable oil (high percent of unsaturated fatty acids) may have a requirement of 30 IU per day (Reed, 1984). Even elevated requirements can usually be met by the diet. Vitamin E is quite prevalent in foods in both animal and vegetable sources with the richest food source being polyunsaturated oils themselves. Safflower oil and cottonseed oil are particularly rich in alpha tocopherol. Other sources are margarine, whole-grain cereal products and seeds. Amounts of vitamin E in meats, fish, eggs, and dairy products depend on the diet of the animals. Green leafy vegetables are another good source of vitamin E. Vitamin E is stored in the lipid portions of liver, muscles, and adipose tissues in humans.

Vitamin E and Deficiency

Vitamin E deficiency is rare in older children and adults because of its prevalence in the food chain and because of its lipid solubility. Infants, however, are susceptible to vitamin E deficiency. Premature human infants can demonstrate vitamin E deficiency which can be reversed by Vitamin E supplementation (Bieri et al., 1983). Conversely, Packer, and Landvik (1989) noted that it is difficult to deplete adult tissues of vitamin E even after

prolonged dietary E deficiency. They suggested that redistribution of vitamin E between body tissues occurs.

Vitamin E can be regenerated which helps to maintain body stores. The tocopheroxyl radical, itself, is relatively unreactive and therefore does not propagate chain reactions. Vitamin E is regenerated from this radical by tocopheroxyl radical reductase which has been found in liver mitochondrial and microsomal membranes (Packer, Maguire, Mehlhorn, Serbinova, & Kagan, 1989). Additionally, Vitamin C and glutathione are major water-soluble intracellular antioxidants (reductants) in the cytosol that can regenerate reduced vitamin E from the oxidized tocopheroxyl radical or increase scavenging of the free radicals that could otherwise oxidize vitamin E (Packer & Landvik, 1989).

Vitamin E and Lipid Peroxidation

Researchers have examined the role of vitamin E in reducing or preventing lipid peroxidation. In vitro, vitamin E inhibited the hydrogen peroxide iron ion complex, H_2O_2--Fe^{2}+, (Fukuzawa, Kishikawa, Tadokoro, Tokumura, Tsukatani, & Gebicki, 1988; Link, Pinson, Kahane, & Hershko, 1989) from propagating free radical formation. Oxidation of red blood cells was suppressed by vitamin E (Miki, Tamai, Mino, Yamamoto, & Kiki, 1987). When vitamin E fell below a critical level, oxidation of red blood cells occurred.

In 32 healthy elderly subjects $\geq$ 60 years of age, supplementation with vitamin E (800 mg dl-alpha-tocopherol acetate) for 30 days decreased lipid peroxidation products. Cell-mediated immunity was enhanced and prostaglandin E_2 was decreased (Meydani, Barklund, Liu, Meydani, Miller, Cannon, Morrow, Rocklin, & Blumberg, 1990).

In vitamin E deficient animal models, lipid peroxidation increased and endurance decreased. Davies et al. (1982) demonstrated a 40% decrease in endurance time in vitamin E deficient rats compared to control animals. Quintanilha and Packer (1983) observed a progressive and specific increase in the susceptibility of many subcellular membranes to oxidative damage with increasing levels of vitamin E deficiency.

An increase in lipid peroxidation of 44.5% in skeletal muscle and 101.8% in cardiac tissue was measured in vitro, from muscle homogenates of mice who were on a 12 week vitamin E deficient diet (Salminen, Kainulainen, Arstila, & Vihko, 1984). Altered cardiac function and elevated MDA levels were measured in post-weaning rats who were placed on vitamin E deficiency diets (Hubel, Kelly, Griggs, & McLaughlin, 1989). MDA levels were higher in cardiac tissue than liver tissue in rats on a 2 month vitamin E deficient diet (Arkhipenko, Konovalova, Dzhaparidze, Lankin, & Spirichev, 1988). These findings suggested that the

myocardium was more susceptible to oxidative stress than liver in vitamin E deficiency.

Activations of other defensive enzymes were not sufficient to compensate for the loss of vitamin E. Vitamin E does not work in isolation from other enzyme systems. Only when membrane hydroperoxides were continuously reduced by glutathione peroxidase did vitamin E lead to an inhibition of lipid peroxidation (Maiorino, Coassin, Roveri & Ursini, 1989).

Vitamin E Deficiency and Serum CK

Serum CK concentrations following 2 hours of treadmill running were higher in vitamin E deficient rats than in controls (Amelink et al., 1991). By 48 hours, focal necrosis, phagocytosis and cellular infiltrates increased in both male and female rats; but male rats demonstrated a greater response. These responses indicate that altered membrane permeability and tissue damage were greater in vitamin E deficient rats.

Vitamin E Supplementation and Exercise

Vitamin E plays a role in minimizes lipid peroxidation during exercise. Plasma vitamin E was mobilized at the end of exercise in cyclists performing an ergometer test at 45, 60, and 75% VO_2 max as compared with resting concentrations (Camus, Pincemail, Roesgen, Dreezen, Shuse, & Deby, 1990).

Other researchers reported the mobilization of vitamin E with exercise (Quintenilla, 1984; Pincemail, Deby, Camus, Pirnay, Bouchex, Massaux, & Goutier, 1988).

In an attempt to enhance plasma levels of vitamin E, supplementation studies have been done. Following a two week supplementation with 1,200 IU vitamin E (alpha-tocopherol), lipid peroxidation decreased (as measured by pentane) in two subjects who previously demonstrated elevated levels during an ergometer test at 50% VO_2 max (Dillard et al., 1978).

Goldfarb et al. (1989) found a decrease in lipid peroxidation in a double blind cross over study in runners who ran at 80% VO_2 max on a treadmill following 4 weeks of 800 IU/day supplementation with vitamin E (alpha-tocopherol). Indicators of muscle membrane damage decreased in subjects running at 65% VO_2 max on a treadmill following 4 weeks of 800 IU/day supplementation of vitamin E, vitamin C and beta carotene (Viguie, Packer, & Brooks, 1989).

Sumida, Tanaka, Kital, and Nakadomo (1989) investigated the effect of 4 weeks of supplementation of 300 IU vitamin E (d-alpha-tocopherol acetate) daily on lipid peroxidation. They measured a significant increase in MDA levels following a maximum ergometer test to voluntary exhaustion prior to supplementation. After supplementation, MDA levels were significantly decreased compared to the non supplemented

treatment in untrained subjects. However, in very well trained subjects, Viinikka, Vuori, and Ylikorkala (1984), found no change in MDA when subjects were supplemented with 300 IU vitamin E daily for 4 weeks.

Vitamin E supplementation has been reported to decrease serum CK concentrations in supplemented subjects following downhill treadmill running. Subjects supplemented with vitamin E (400 IU/day for 48 days) had lower serum CK concentrations following a 45 minute downhill treadmill run at a -16% grade (Cannon et al., 1990). Following antioxidant supplementation that included 800 IU vitamin E per day, Viguie et al. (1989) reported plasma CK concentrations were lower in supplemented subjects than controls following a -5 degree downhill treadmill run at 65% VO_2 max.

Findings from these studies suggest that vitamin E supplementation may decreased lipid peroxidation in untrained and moderately trained subjects and decreased plasma CK concentrations. The purpose of this study was to determine the effects of vitamin E supplementation (800 IU/day for 4 weeks) on muscle soreness, plasma CK, and plasma MDA in cyclists performing an exhaustive endurance ride at 70% VO_2 max.

CHAPTER III

METHODS

Subjects

Eleven healthy males (20-42 years) participated in this study. Each subject had to meet the criteria that he cycled a minimum of 100 miles per week in training and that he had no musculoskeletal or medical condition that would limit his participation in an endurance-ergometer ride. No individual smoked or had been diagnosed as having diabetes, musculoskeletal disorders, or any other chronic disease that could have influenced his participation in the study. No subject had symptoms of coronary heart disease as determined by the guidelines in Table 1-1 on page 2 of the Guidelines for Exercise Testing and Prescription published by the American College of Sports Medicine (ACSM Guidelines, 1991). The one subject over 40 had physician permission to participate in the study. Subjects received $100.00 for their participation from a grant from the Foundation for Physical Therapy of the American Physical Therapy Association.

Seven subjects were US Cycling Federation Road Racers and were sponsored by local bike shops. Three were Category IV (entry level) racers and four subjects were Category III (intermediate level) racers. Two subjects were triathletes and two were skilled recreational cyclists. The subjects' mean 100 mile ride times were 4.85 hours. On training

rides, their speeds averaged 22 to 25 miles per hour (35 to 42 kilometers per hour) for a 40 mile (67 kilometer) distance.

Subjects were of the same gender to control for gender differences. Males were chosen because there was a larger pool of trained male cyclists than trained female cyclists. Males are higher CK responders than females. Greater CK release or less clearance has been reported in males compared to females. Any differences between the two trials with regard to CK release may have been more apparent in males than in females.

Subjects were of an age range that age differences had minimal effects on plasma CK release (Munjal, McFadden, Matrix, & Coffman, 1983). Ten subjects were white and one was African American. Because the study was a cross-over design each subject served as his own control for population variability of plasma CK and plasma MDA release.

Subjects were recruited through the local bike club, through training rides, and by flyers (Appendix D) displayed at local bicycle shops. Prior to participation, each subject was informed of the procedures, benefits, and risks associated with this study, and of his right to terminate participation at any time. Each subject was informed of procedures both verbally and in written form in accordance with the procedures filed with the Human Subjects Review

Committee. Subjects gave signed consent (Appendix E), completed a medical history form (Appendix F), and a training questionnaire (Appendix G) prior to any testing.

Testing Protocol

Supplementation

Subjects participated in a double-blind, cross-over design. The double-blind model was chosen to eliminate both subject and researcher bias. A third party kept the list of subjects and assigned in a randomized manner the vitamin E and the placebo treatments. The list was presented to the researcher at the completion of the blood analysis. The cross-over model controlled for variability of CK and MDA release among subjects.

Six subjects received placebo supplementation first while five received vitamin E supplementation first. Each was given a four week supply of either 800 mg (2 pills, 400 mg in the morning and at night) of alpha-tocopherol or placebo to be taken daily. The vitamin E and placebo pills were supplied by Hoffman La Roche Laboratories (New Jersey). The pills were of the same oil base and looked that same. They differed only in the vitamin E content. Subjects were asked to discontinue any other supplementation two weeks prior to the test.

Maximal Ergometer Stress Test

At the end of each supplementation period, subjects reported to the Exercise Physiology Lab in the Health and Human Performance building on the UNCG campus and completed a maximum, bicycle-ergometer stress test.

A Monarch (Sweden) bicycle (model #868) ergometer was used in this study. The original bicycle ergometer was modified with the substitution of a racing saddle, drop racing handle bars and toe clips. These modifications approximated the setup for a bike used for road racing. The frame angles of the Monarch were different from a standard road racing bike and could not be altered. Prior to the start of the maximal ergometer tests the seat of the bike was adjusted for height and distance to the subject's comfort level.

The subjects were familiarized with the bike by cycling for five minutes at a resistance of two kiloponds at their self selected cadence which ranged from 90 to 100 cycles per minute. After the warm-up period, the resistance was increased one half kilopond every two minutes until the subject indicated that he could no longer continue, leg fatigue occurred, or until the researcher stopped the test in accordance with ACSM Guidelines (1991). All subjects in both trials continued the test to voluntary exhaustion.

Heart rate was continuously monitored using a three lead system on a Quinton electrocardiograph (ECG) model Q3000 (Seattle, Washington). ECG monitoring complied with ACSM Guidelines (1991). Samples of expired air were collected for one minute for each of the last five minutes of exercise to determine oxygen consumption. Gas collection began when the subject's heart rate reached 150 beats per minute for subjects thirty years old and over and 160 for subjects twenty to twenty nine years old. The oxygen and carbon-dioxide content of each sample was determined using a Ametek (Pittsburg, Pennsylvania) oxygen and carbon dioxide analyzers model #N-22M and #P-61B, respectively. Known concentrations of oxygen and carbon dioxide were used to calibrate both analyzers before each test. Gases were acquired from Air Products. Percent oxygen, percent carbon dioxide and the volume of air were used to calculate VO_2 max. The max VO_2 was used as the basis for determining the 70% sub-maximal workload for the endurance ergometer ride that was performed at the end of each supplementation period.

Four Day Food Record

Subjects were instructed in keeping a food record for each of the four days preceding the endurance rides (Bazzarre & Yuhas, 1983) (Appendix H). They were given written examples of how to record their food intake. The

researcher also verbally instructed subjects in procedures to record breakfast, lunch, dinner and snacks. Their diets were self-selected. They were asked to eat their usual food intake in their customary patterns.

At the time of the second maximal ergometer stress test, subjects were given a copy of their first food record. They were instructed to eat a similar diet for the four days preceding the second endurance ride as they had for the first endurance ride. The food records were used to minimize dietary changes, such as lipid content, or dietary vitamin E that might influence test results. Dietary intake of vitamin E was controlled by having them eat the same diet prior to each endurance ride.

The four day food records were analyzed using a Nutritionist III computer software program by N-Squared Computing Company (Salem, Oregon). Food records were analyzed for total calories, percent carbohydrates, fat, and protein and dietary Vitamin E. Additionally, the amount of fat in polyunsaturated, monosaturated and saturated forms were determined.

Training

Subjects were requested to train in a similar manner before each endurance ride. At the time of the endurance rides, subjects recalled their training distances for the previous three days. Subjects maintained their self-

selected training regimes.

Endurance Ride

Subjects performed an exhaustive endurance ergometer ride at 70% VO_2 max at the end of each supplementation period. The work load of 70% VO_2 max was chosen to optimize both duration for CK response and intensity for MDA response. In aerobic exercise, duration is a greater factor for CK elevations than intensity (Fowler et al., 1968). Intensity and exhaustive exercise are important factors for increases in plasma MDA (Lovlin et al., 1987).

All endurance rides began approximately at 8:00 am. Subjects were in a post absorptive state having not eaten after 10:00 pm the previous evening. Prior to the start of the endurance ride, a venous catheter was inserted into an antecubital vein and a resting blood sample was obtained.

After the resting blood sample was drawn, subjects were given water and carbohydrate replacement in the form of fig newtons and bananas. These foods are commonly consumed by endurance cyclists. Food replacement and water was given throughout the endurance ride (Coggan & Coyle, 1978, 1988, 1989).

Subjects cycled at their predetermined cadence at a resistance of 2 kilopond for the first five minutes. The resistance was increased one-half kilopond every five minutes until the subject's workload was equal to 70% of his

VO_2 max. Revolutions of the fly wheel of the ergometer were counted using an electro-magnetic sensor connected to a computer. If the cyclist dropped below his self-selected cadence during the endurance ride, the computer generated an auditory signal. He then was encouraged to resume his regular cadence. Power output was calculated from total revolutions and resistance.

Expired gases were collected and analyzed every 10 minutes during the first 30 minutes to insure that subjects obtained the desired 70% VO_2 max intensity. Expired gases were collected and analyzed every 30 minutes after steady state was achieved to monitor that the subject continued to cycle at the desired intensity. Subject's heart rate was determined using a Trainer Heart Monitor model #8863. Heart rate was recorded at each gas analysis and was used as an indicator of intensity. Heart rates were monitored throughout the endurance ride. Subjects cycled until they indicated they were no longer able to continue the test or up to four hours, which ever came first.

At the completion of the endurance ride, the subjects received no supplementation for a two-week period. Subjects then received the other treatment for the remaining four weeks of the study. The second half of the study was conducted in an identical fashion to the first half of the study: each subject was given a copy of his first food

record prior to the second endurance ride. Each was asked to eat as closely as possible the diet that he indicated was eaten the four days prior to the first endurance ride.

Blood Sampling

A venous catheter was inserted into an antecubital vein just prior to the beginning of the endurance ride. Seven milliliters of blood were taken prior to the ride and every thirty minutes during the ride. Blood samples were drawn from the catheter using sterile needles and syringes. Samples were placed into collecting tubes on ice containing ethylenediamine-tetraacetic acid (EDTA) to prevent clotting. The catheter was flushed with sterile physiological saline every 5 to 10 minutes. When the catheter not work properly, it was removed. Blood collection was done hourly by venipuncture technique, thus the half hour blood samples were not drawn.

Blood samples were taken 24, 48, and 72 hours after the ride. Samples were drawn from an antecubital vein by sterile vena puncture technique into a 10 ml vacutainer containing 0.10 microliter of 15% EDTA solution and placed on ice. Approximately 0.5 ml was removed from the collecting tubes and the vacutainers for hemoglobin and hematocrit determination prior to centrifugation.

After 0.5 ml of blood was removed for determination of hemoconcentration, the remaining blood samples were

centrifuged at 3000 rpm for 10 minutes at 4^0 C. Plasma was pipetted into collecting tubes and stored at -70^0 C to allow for subsequent determination of CK and MDA. Plasma was used for determination for both CK and MDA to decrease the amount of blood sample taken from the subject.

Muscle Soreness Rating Scale and Exertion

Subjects completed a muscle soreness rating scale (Appendix A) prior to the ride, every thirty minutes during the ride, and at 24, 48, and 72 hours after the ride. Subjects rated their perceived level of soreness on a one to ten scale with one being no soreness and ten being very, very sore. Subjects also indicated the area of soreness on a body chart.

Analysis of Blood Samples

Hemoconcentration

Hematocrit was determined using micropipets in triplicate. Micropipets were spun down at 4000 rpms [will get g force equivalent] for 5 minutes using a hematocrit centrifuge. Hemoglobin was evaluated according to the procedures outlined by Sigma Diagnostics Company (St. Louis, Missouri) using Sigma Kit #525-A. Hemoconcentration during the ride was determined from hematocrit and hemoglobin as outlined by the procedures of Dill and Costill (1974). Plasma CK and plasma MDA concentrations were adjusted for shifts in hemoconcentration.

Total Plasma CK

Total plasma CK was analyzed using Sigma Diagnostic Creatine Phosphokinase Kit #520 which employs a modified Hughes procedure (Hughes, 1962). The kit can be used to analyze either serum or plasma CK. Plasma CK was analyzed in this study so less blood was taken from the subjects.The plasma CK procedure utilized a colorimetric method with spectrophotometric absorption monitored at 520 nm. The amount of color (formed) absorption was proportional to CK activity (Hughes, 1962; Szasz, Gruber, & Bernt, 1976)

ADP + Phosphocreatine ----CK----> ATP + Creatine

Creatine + Naphthol + Diacetyl ----> colored complex

After spectrophotometric determination, absorption of samples were compared to known standard. The CK activity in the samples was determined from a calibration curve. Test and blank tubes were assessed in duplicate. Procedures for plasma CK determination are in Appendix I.

The Sigma Units calculated from the assay were then converted into International Units (IU/L) (Appendix I). Normal serum ranges were 0-20 Sigma Units which was equivalent to 0-165 IU/L.

Plasma MDA

Plasma MDA was determined by the thiobarbituric acid method (TBARS) according to Ohkawa, Ohishi, & Yagi (1979) and Stubbe (1988). Determinations were made with plasma

rather than serum because lysis of red blood cells can elevate MDA concentrations. The plasma MDA procedure utilized a colorimetric method with spectrophotometric absorption monitored at 532 nm. A brief description of the procedures is given below. A full description of the procedures is presented in Appendix J.

0.5 ml plasma + 0.5 ml 10% TCA incubated one hour centrifuged twice to precipitate out proteins and lipoproteins that could interfere with the reaction

0.25 ml supernate + 0.25 ml 0.67% TBA (heated at 95^0 C for 15 minutes) ----> colored complex

Reagents: TBA--0.67% thiobarbituric acid (TBA)
TCA--10% trichloracetic acid (TCA)

The amount of color formed (absorption) was proportional to MDA concentration (Ohkawa et al., 1979; Stubbe, 1988). The plasma samples were compared to known standards. The MDA concentration was determined from a calibration curve. Test and blank tubes were assessed in duplicate. MDA was calculated in nanamoles/ml.

Statistical Analysis

A paired t-test was used to determine if differences in anthropometric measures, VO_2 max, power output of the endurance rides, and dietary intake occurred between the two treatments. Differences in VO_2 and power output would have indicated that the work intensity of the endurance rides were not controlled. Differences in work intensity would

have been problematic.

A paired t-test was use to analyze data from the Nutrition III output to determine differences between the two treatments for total calories, per cent carbohydrates, proteins, fat, grams of dietary vitamin E, and grams of polyunsaturated, monosaturated and saturated fats. Paired t-tests were calculated using a statistical software package, "Statistics with Finesse" (New York, New York).

Differences in MSRs, plasma CK, and plasma MDA concentrations over the time course for the two treatments were determine by an Analysis of Variance (ANOVA) with Repeated Measures. The SAS statistical software package (Cary, NC) was employed to calculate the ANOVA results.

Due to problems in blood sampling, eight half-hour data points were missing. If a half hour data point was missing, the value was estimated by averaging the value before and after to enable the statistical analysis to be completed. For example, if the 90 minute data point was missing, the 60 and 120 values were averaged for this point. ride. The time period with the fewest missing data points was two hours and was used as the end point for this study. The inclusion of these eight estimates allowed the data to be statistically interrupted.

The Greenhouse-Geisser correction factor was used to adjust the degrees of freedom for the F-test (Glass &

Hopkins, 1984). Because a total of eight data points were missing, eight degrees of freedom were subtracted from the total degrees of freedom (Statistical Consulting at UNCG).

A Pearson product-moment correlation was used to determine the relationship of oxygen consumption across the time course of the two endurance rides for each subject. If the oxygen consumptions were significantly correlated, then the oxygen cost for the two treatments were controlled for to eliminate oxygen cost as a factor for the variable measured.

A Pearson correlation was used to determine if the time courses of MSR, plasma CK and MDA were significantly correlated for all subjects. If vitamin E supplementation altered the time courses of variables, the variables would demonstrate different correlations.

The relationship among variables was determined by a Pearson correlation between the two treatments. If vitamin E supplementation influenced the response of variables, then the variables would demonstrate altered correlations with each other during the vitamin E treatment compared to the correlation during placebo treatment.

Statistical significance was set at $p < 0.05$ level. The data are presented in means $\pm$ standard errors of the means.

CHAPTER IV

RESULTS

Subject Characteristics

To control for seasonal training effects, data were collected between early Spring and late Fall. According to subjects training records, they longer training rides in late Spring and Summer because of longer daylight hours.

Subjects verbally reported that they had taken all supplements with one exception, a subject missed taking a supplement for three days. After the blood analysis, it was determined that the supplement not taken was the placebo. Therefore, the supplement not taken had no effect on the results of the study. Throughout the study, subjects were unaware of the supplement they were taking.

Subjects' anthropometric and training characteristics are presented in Table 7. Subjects' height, mass, and percent body fat ranged from 171 to 188 cm, 64 to 84 kg, and 4 to 12%, respectively. Their VO_2 maxs ranged from 52.5 to 69.2 $ml \cdot kg^{-1} \cdot min^{-1}$, with a group mean of 62.3 $ml \cdot kg^{-1} \cdot min^{-1}$. Subjects had trained an average of 9 years (range 6 to 12) and averaged approximately 320 kilometers per week (range 167 to 500) in training. Subjects' anthropometric and training characteristics indicated that the subjects were aerobically trained and fit.

Table 7.

Mean of Subject Anthropometric and Training Characteristics

Age (years)	29.0 ± 2.07
Mass (kg)	73.4 ± 1.3
Height (cm)	179.9 ± 4.6
% Body Fat	7.8 ± 0.9
Training Distances (km/wk)	322.7 ± 37.0
Length of Training (years)	9.1 ± 0.6
Mean VO_2 Max ($ml \cdot kg^{-1} \cdot min^{-1}$)	62.3 ± 1.5

(means and standard errors of the means)

Changes in the aerobic conditioning of the subjects could have effected plasma CK and plasma MDA levels. The mean VO_2 max was 62.4 ± 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ when subjects were supplemented with vitamin E and 62.3 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$ when they were supplemented with the placebo. There were no significant differences for VO_2 maxs between the two treatments.

Plasma CK and plasma MDA levels could also have been effected by significant differences in the ride times and power output of the subjects. The subjects supplemented with vitamin E cycled a mean of 169 ± 12.3 (90 to 240) minutes. When supplemented with the placebo, subjects

cycled a mean of 171.8 ± 11.5 (120 to 240) minutes. The endurance times for the two treatment rides were not significantly different and were unaffected by treatment. Cyclists pedaled at an average power output of 198 ± 6.7 (range 160 to 228) watts when supplemented with vitamin E. When supplemented with the placebo, cyclists pedaled at an average output of 199 ± 6.4 (range 162 to 233) watts. There were no significant differences in the ride times or power outputs between the two treatments.

A Pearson product moment correlation was calculated for each subject to determine the relationship of oxygen consumption across the time course of both treatments (Table 8). The results showed a moderately high correlation ranging from .74 to .99 between the treatments. These results indicate that the treatments were comparable for oxygen consumption (VO_2).

The maximal ergometer stress tests were used to determine each subject's maximum VO_2 from which 70% VO_2 max was calculated. Throughout the rides, the work load was adjusted on the ergometer to maintain the 70% VO_2 max intensity.

No significant differences were obtained in work intensities or power output between the two treatments for each subject. Therefore, total work, oxygen cost, and intensity were controlled in this study between treatments.

Table 8.

Correlations of VO_2 for Both Treatments for the Two Hours Exercise Period

Subject	r
1	0.76*
2	0.74*
3	0.89*
4	0.76*
5	0.92*
6	0.76*
7	0.99*
8	0.83*
9	0.79*
10	0.89*
11	0.83*
$\bar{X}$ =	0.81* ± 0.03

* significant at $p < 0.05$

Four Day Food Records

A summary of the four day food records is presented in Table 9. Analysis of the four day food records indicated no significant differences among the total calories, percent carbohydrates, fat, protein, and dietary vitamin E for the two treatments. Percent and kind of fats, were consistent for both treatments. Differences in these variables could have influenced free radical interactions with lipids

independent of supplementation. Therefore, eliminating differences in kinds and amounts of fat was important in determining the influence of vitamin E.

Table 9.

Food Record Summary: Mean Daily Nutrient Intake for Both Trials

	Vitamin E Trial	Placebo Trial
Total Calories (KCal)	2667 ± 221	2729 ± 181
Carbohydrates (% of total calories)	59.3% ± 2.1	57.8% ± 2.4%
Proteins (% of total calories)	15.7% ± 0.9	15.5% ± 0.9
Fats (% of total calories)	24.0% ± 1.7	26.0% ± 1.6
Polyunsaturated Fat (gm)	14.5% ± 1.6	14.6% ± 1.3
Monosaturated Fat (gm)	30.5% ± 4.4	31.7% ± 3.4
Saturated Fat (gm)	27.7% ± 3.7	30.6% ± 3.0
Dietary Vitamin E (mg)	15.1% ± 2.4	13.3% ± 2.5

Hypothesis 1.

Hypothesis 1: Perceived muscle soreness ratings will not be different when subjects are given vitamin E suppmenetation as compared with the placebo supplementation.

Subjects reported no soreness prior to the beginning of the rides. The MSRs peaked immediately post-ride for both the vitamin E and the placebo treatments. By 24 hours MSRs declined but remained above baseline. By 48 hours MSRs had essentially returned to baseline values. The mean MSRs for each treatment are presented in Table 10 and graphed in Figure 11. Figure 11 shows that the rises in MSRs over time was a similar pattern for both treatments.

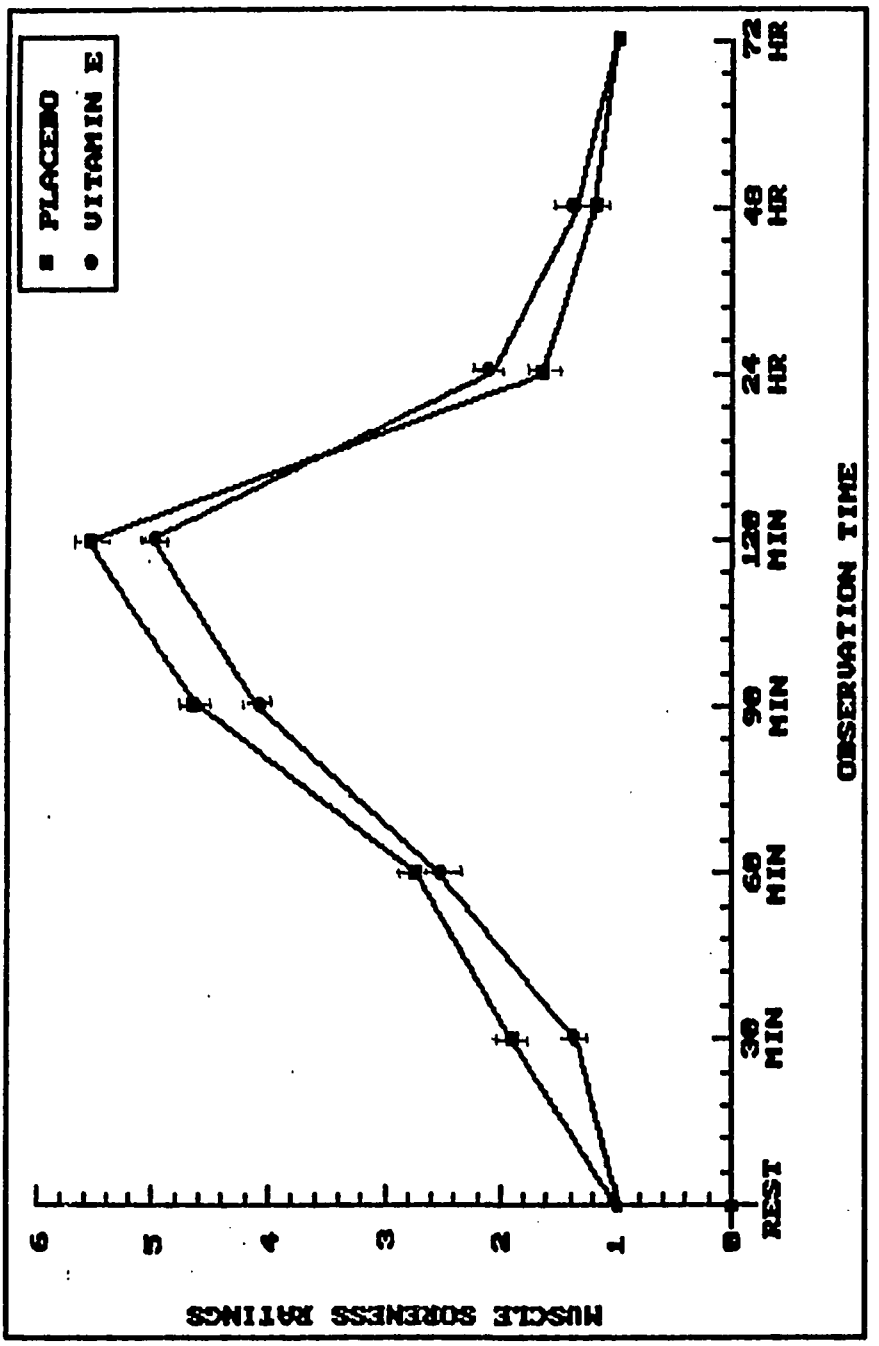
Table 10.

Mean Muscle Soreness Rating Scores of Both Trials

Time	Placebo	Vitamin E
At Rest	1.0 ± 0	1.0 ± 0
30 min.	1.9 ± 0.4	1.4 ± 0.2
60 min	2.7 ± 0.4*	2.6 ± 0.6*
90 min	4.6 ± 0.6*	4.1 ± 0.6*
120 min	5.6 ± 0.6*	5.0 ± 0.5*
24 hr	1.6 ± 0.3	2.1 ± 0.3
48 hr	1.2 ± 0.1	1.4 ± 0.2
72 hr	1.0 ± 0	1.0 ± 0

(means and standard errors of the means)
(* significantly different from rest $p < 0.05$)

Figure 11. Muscle Soreness Ratings (Units) Over the Time Course of the Study



Summary statistics for hypothesis one regarding the effect of vitamin E upon perceived muscle soreness are presented in Tables 11, 12, and 13. Vitamin E supplementation had no significant effect upon MSRs when compared with placebo treatment as demonstrated by the F value (Table 11). The order of supplementation had no effect on MSRs as noted by the F value. Vitamin E had no effect on attenuating muscle soreness ratings. Subjects were sore immediately post ride with both treatments. Statistically no differences in the soreness rating were observed.

Table 11.

Summary Statistics of Effect of Vitamin E on MSRs

Source	DF	SS	MS	F	PR	G-G*
Treatment	1	0.512	0.512	0.180	0.679	...
Treatment*Order Effect	1	8.194	8.194	2.93	0.121	
Error A	9	25.158	2.795			

*G-G Greenhouse-Geisser Correction Factor Probability

Significant time differences for both treatments occurred as demonstrated by the F value for time and

Greenhouse-Geisser correction probability (Table 12). No order effect of supplementation was observed between the two treatments for MRSs with respect to time. The effect of time was significant for both treatments, but not significantly different from each other because there was no treatment effect.

Table 12.

Summary Statistics of MSRs for Time Effect

Source	DF	SS	MS	F	PR	G-G
Time	7	409.730	58.533	42.70	0.000	0.0001
Time Order Effect	7	14.184	2.026	1.48	0.191	0.256
Error B	63	100.534	1.436			

Table 13 presents the combined effect of treatment over time for muscle soreness between the two treatments. The vitamin E treatment did not influence MSRs over the time course when compared with the placebo treatment. No order effect of supplementation was observed in treatment over time.

Table 13.

Summary Statistics of MSR Combined Treatment and Time Effect

Source	DF	SS	MS	F	PR	G-G
TRT*Time	7	5.449	0.778	1.073	0.375	0.389
TRT*TIme Order Effect	7	6.730	0.961	1.380	0.231	0.270
Error C	63	44.008	0.698			

In summary, Table 10 notes that MSRs increased during exercise and peaked immediately post exercise. The MSRs declined and returned to near baseline values at 48 hours. Minimal muscle soreness was noted at 24 hours. Figure 11 graphically presents the data which indicates that the MSRs elevated and declined in a similar manner. Vitamin E supplementation had no significant effect on MSRs compared with placebo. Order of supplementation had no effect on treatment. A significant time effect was observed for MSRs for both treatments. Supplementation order did not effect time. The treatment by time effect was not significant. Therefore, vitamin E had no effect on attenuating the exercise induced MSRs.

Hypothesis 2

Hypothesis 2: Peak concentrations of plasma CK will not be affected by vitamin E supplementation compared with the placebo supplementation.

Plasma CK concentrations were adjusted for shifts in plasma volume as previously outlined in the Method's Section. Plasma CK increased slightly over the exercise period and peaked at 24 hours for both treatments (Table 14). Figure 12 depicts the data in graphic form and indicates a similar pattern for both treatments over time. The mean peak plasma CK concentration for the placebo treatment was 93.0 ± 12.9 IU/L at 24 hours which represents a 78% increase from rest. The peak CK concentration for the vitamin E treatment at 24 hours was 79.0 ± 6.7 IU which was

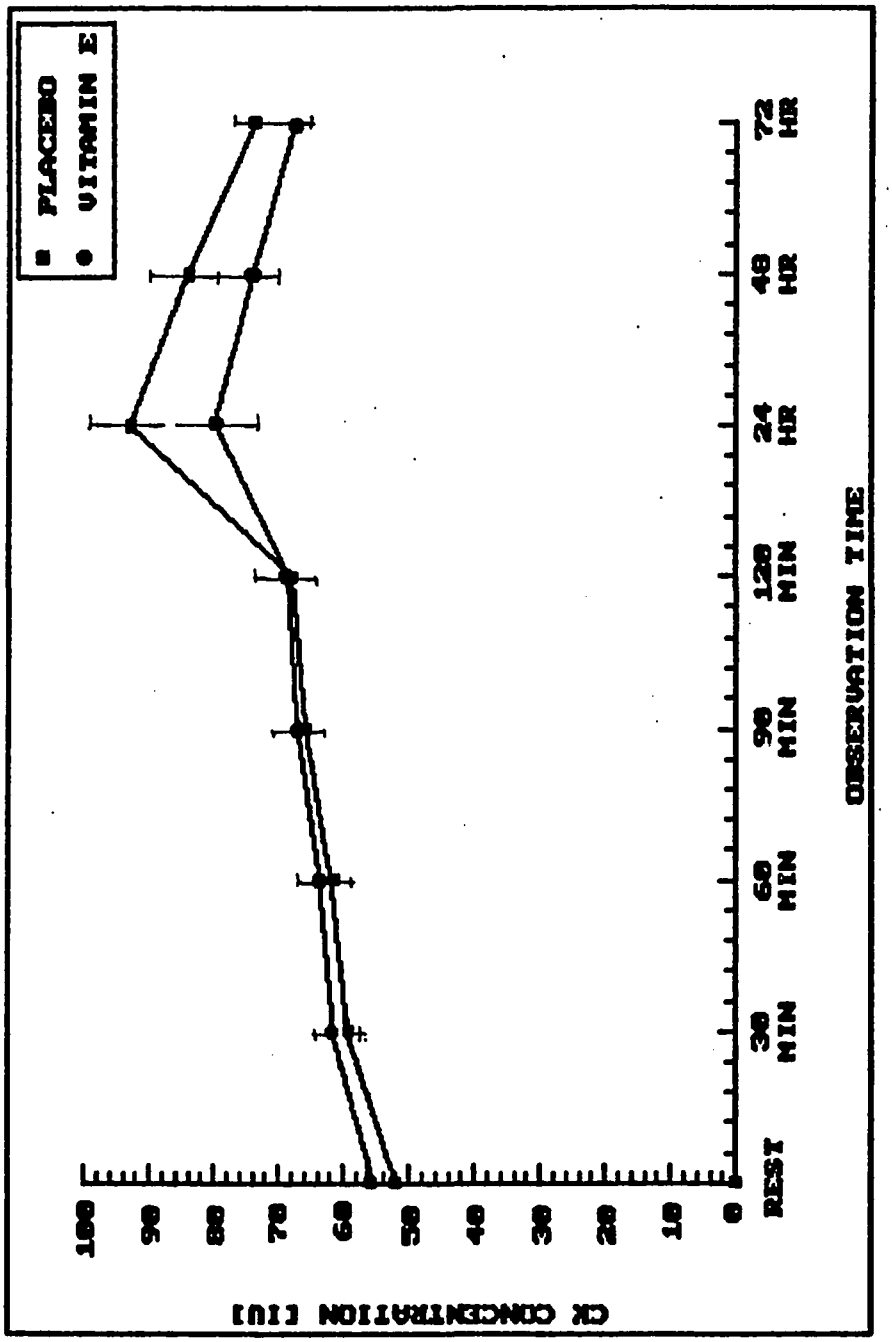
Table 14.

CK Concentration [IU/L] Across the Time Course of Study

Time	Placebo	Vitamin E
At Rest	52.2 ± 5.7	55.9 ± 4.1
30 min.	59.3 ± 5.3	61.8 ± 4.8
60 min	61.4 ± 5.8	63.7 ± 4.9
90 min	65.8 ± 6.4	67.0 ± 5.7
120 min	67.8 ± 7.3	68.9 ± 6.4
24 hr	93.0 ± 12.9*	79.8 ± 6.7*
48 hr	84.1 ± 9.7*	73.9 ± 4.6*
72 hr	73.9 ± 8.5*	67.7 ± 4.5*

(means and standard errors of the means)
(* significantly different from rest $p < 0.05$)

Figure 12. Plasma CK Levels (IU/L) Over the Time Course of the Study.



a 45% increase from rest. At no time were plasma CK concentrations elevated out of the normal range for any subject for either treatment. The mean plasma CK concentration at 72 hours decreased in both treatments, but had not returned to baseline concentrations.

Summary of statistics for plasma CK concentrations between treatments are presented in Tables 15, 16, and 17. No differences were observed in treatment effects between the vitamin E treatment and the placebo treatment for plasma CK concentration as indicated by the F value (Table 15). Order of supplementation had no effect on treatment as noted by the F value.

Table 15.

Summary Statistics of the Effects of Vitamin E on Plasma CK

Source	DF	SS	MS	F	PR	G-G
Treatment	1	15.435	15.435	0.02	0.886	...
Treatment Order Effect	2	3592.945	1796.473	2.48	0.145	...
Error A	8	5784.280	876.885			

Significant differences were found over time for the two treatments (Table 16). This result suggests that the 24 hour peak concentration for both treatments was different from the concentration at rest. Order of supplementation

had no interactive effect with time as indicated by the F value in the time order effect column.

Table 16.

Summary Statistics of CK Time Effects

Source	DF	SS	MS	F	PR	G-G
Time	7	6124.201	874.886	3.760	0.002	0.044
Time Order Effect	14	2819.183	201.370	0.87	0.598	0.478
Error B	56	13026.834	232.622			

No significant differences were observed in the combined effect of treatment over time between the two treatments (Table 17). The significant time effect (Table 16) was in response to exercise for both treatments and not because of supplementation. Though differences were found in plasma CK concentrations over time in both treatments, no main effect for treatment over time was observed.

Table 17.

Summary Statistics of CK Combined Treatment and Time Effects

Source	DF	SS	MS	F	PR	G-G
TRT*Time	7	447.687	63.955	0.90	0.512	0.438
TRT*Time Order Effect	14	662.373	47.312	0.67	0.796	0.646
Error C	70	3971.289	70.916			

In summary, that plasma CK concentrations increased slightly during exercise and peaked at 24 hours independent of treatment. Plasma CK declined, but had not returned to baseline by 72 hours. Vitamin E supplementation had no significant effect on plasma CK compared with placebo. Order of supplementation had no effect on treatment (Table 15). A significant time effect was observed for both treatments and this was unaffected by vitamin E supplementation. Therefore, it appears that vitamin E at 800 IU/day for 4 weeks did not alter the exercise-induced changes in plasma CK concentrations due to cycling.

Hypothesis 3

Hypothesis 3: Peak concentration of plasma MDA will not be affected by vitamin E supplementation compared with the placebo supplementation.

Plasma MDA concentrations were adjusted for shifts in

plasma volume as previously outlined in the Method's Section. Increases in plasma MDA were observed for both treatments over the exercise period (Table 18). Plasma MDA peaked at 48 hours in both treatments. The peak plasma MDA concentration for the placebo treatment was 1.7 $\pm$ 0.1 nmol which represents a 30% increase from rest. The peak MDA concentration for the vitamin E treatment was 1.8 $\pm$ 0.1 nmol which was a 28% increase from rest. At no time did plasma MDA concentrations elevate out of the normal range for any subject for either treatment. Mean plasma MDA concentrations decreased by 72 hours in both treatments, but had not returned to baseline by this time. Figure 13 presents the graphic form of the data and indicates a similar pattern for both treatments over time. The only exception to a similar response was a slight insignificant decrease in plasma MDA concentration at 30 minutes of exercise for the placebo treatment.

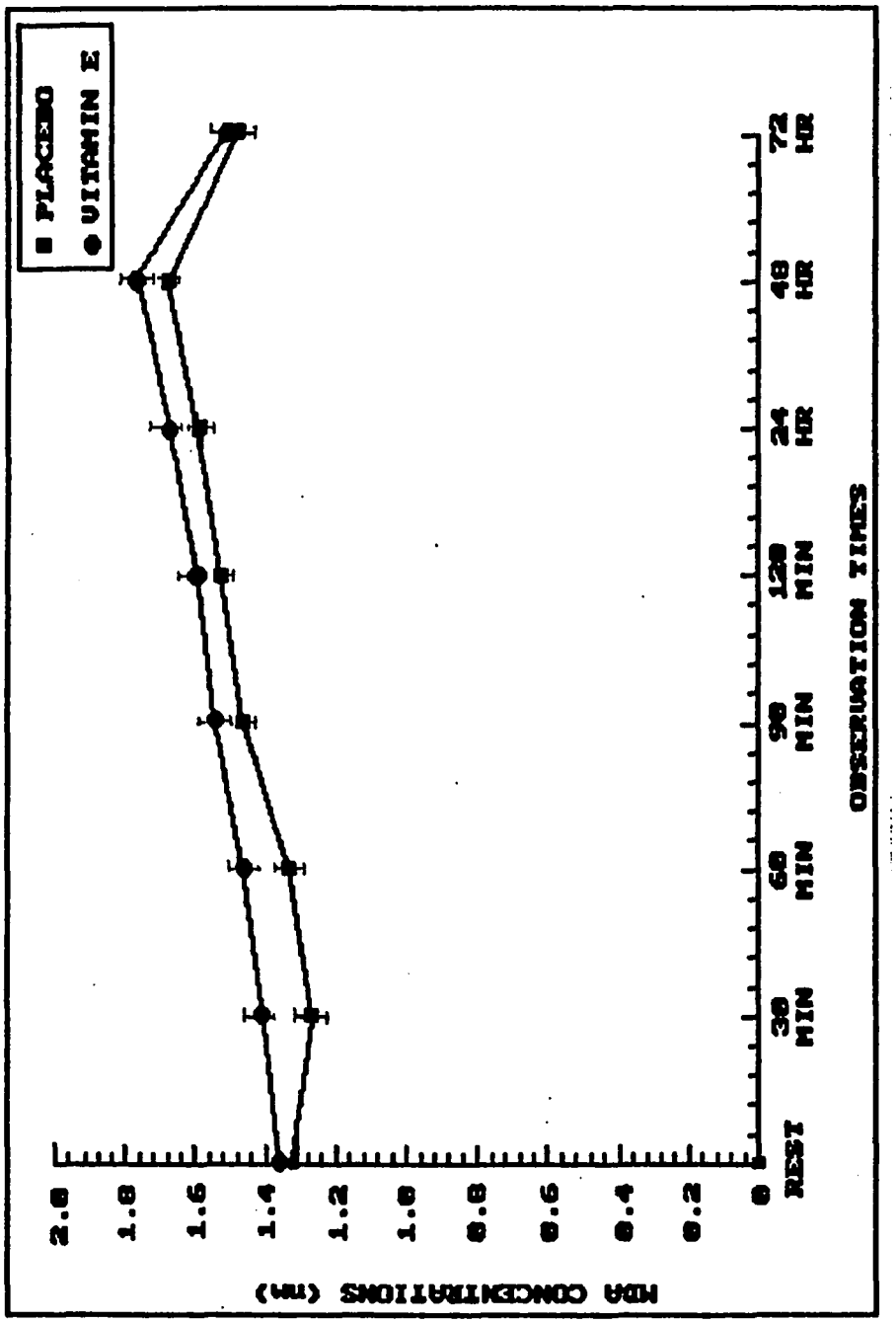
Table 18.

MDA Concentrations (nm) Across the Time Course

Time	Placebo	Vitamin E
At Rest	1.32 ± 0.1	1.36 ± 0.1
30 min.	1.27 ± 0.1	1.41 ± 0.1
60 min	1.33 ± 0.1	1.46 ± 0.1
90 min	1.46 ± 0.1	1.54 ± 0.1*
120 min	1.52 ± 0.1*	1.59 ± 0.1*
24 hr	1.59 ± 0.1*	1.67 ± 0.1*
48 hr	1.68 ± 0.1*	1.77 ± 0.1*
72 hr	1.48 ± 0.1*	1.51 ± 0.1*

(means and standard error of the means)
(* significantly different from rest $p < 0.05$)

Figure 13. Plasma MDA Levels (nmol) Over the Time Course.



Summaries of statistics for plasma MDA are presented in Tables 19, 20, and 21. The vitamin E treatment was not statistically different from the placebo treatment for MDA concentrations as noted by the F value in the treatment row (Table 19). Order of supplementation had no significant effect upon treatment as noted by the low F value in the treatment order effect row.

Table 19.

Summary Statistics of Effects of Vitamin E on Plasma MDA

Source	DF	SS	MS	F	PR	G-G
Treatment	1	0.793	0.793	2.50	0.153	...
Treatment Order Effect	2	0.547	0.273	0.86	0.458	
Error A	8	2.549	0.317			

Significant differences were found over time for both treatments as demonstrated by the large F value in the time row in Table 20. This statistic suggests that the peak rise in MDA concentrations at 48 hours were different from the resting concentrations.

Table 20.

Summary Statistics of MDA Time Effects

Source	DF	SS	MS	F	PR	G-G
Time	7	1.637	0.234	61.64	0.000	0.000
Time Order Effect	14	0.402	0.029	7.56	0.224	0.113
Error B	70	0.945	0.014			

The combined effects of treatment over time were not significantly different for the two treatments (Table 21). No differences in treatment effect over time were observed as noted by the low F value in the treatment*time effect.

Table 21.

Summary Statistics of MDA Combined Treatment and Time Effect

Source	DF	SS	MS	F	PR	G-G
TRT*Time	7	0.021	0.003	0.21	0.982	0.771
TRT*Time Order Effect	14	0.104	0.007	0.53	0.904	0.683
Error C	70	0.783	0.014			

In summary, mean plasma MDA concentration increased moderately during exercise and peaked at 48 hours after exercise for both treatments. The concentrations of MDA elevated and declined in a similar matter for both treatments indicating that vitamin E supplementation had no significant effect on plasma MDA compared with placebo (Table 19). Therefore, supplementation with vitamin E (800 IU/day for 4 weeks) had no effect on attenuating the exercise-induced increases in plasma MDA concentrations.

Hypothesis 4

Hypothesis 4: The time-course response of MSRs, CK, and MDA to exercise will <u>not</u> be altered with vitamin E supplementation as compared with the placebo supplementation.

A Pearson product moment correlation coefficient was calculated to determine the response of the variables between treatments. Separate correlations for each variable for each subject is presented in Table 22.

Table 22.

Time Course Correlations of Variables Comparing Treatments

Subject	MSR	CK	MDA
1	0.99*	0.63*	0.81*
2	0.95*	0.96*	0.76*
3	0.76*	0.89*	0.75*
4	0.83*	0.52	0.91*
5	0.93*	0.59	0.86*
6	0.74*	0.87*	0.49
7	0.80*	0.66*	0.57
8	0.90*	0.79*	0.81*
9	0.53	0.75*	0.77*
10	0.65*	0.89*	0.93*
11	0.62*	0.56	0.90*
$\bar{X}$ =	0.79* (± 0.05)	0.74* (± 0.05)	0.78* (± 0.04)

* significance at $p < 0.05$

The mean correlations for MSR, plasma CK and plasma MDA were significant between the two treatments. The correlations were consistent and reproducible between treatments. There was considerable variability within subjects as the reproducibility of the variables. Since the variables were highly correlated between treatment, this

finding suggests that vitamin E supplementation had little effect on these variables.

Hypothesis 5

Hypothesis 5: Relationships between MSRs, CK, and MDA will not be altered with vitamin E supplementation compared with placebo supplementation.

A Pearson product-moment correlation was calculated to determine if the variables were correlated with each other for either the vitamin E or placebo treatments. The correlations indicated that these variables were not related to each other. If variables had shown significant changes in either trial, then this finding would suggest an effect of supplementation on the relationship of these variables. The data suggest that vitamin E had no effect on the relationship of these variables as indicated in Tables 23 through 28.

Plasma CK and plasma MDA concentration increased over the two hour endurance ride except that plasma MDA dipped insignificantly at 30 minutes of exercise during the placebo trial. Plasma CK and plasma MDA continued to increase at 24 hours. Plasma CK was decreasing while plasma MDA continued to increase at 48 hours. Both variables decreased by 72 hours. At no time period were plasma CK and plasma MDA significantly correlated for the placebo treatment.

Table 23.

Placebo Trial: Plasma CK Correlation With MDA Over the Time Course

CK-REST	30	60	90	120	24	48	72
MDA REST .13							
MDA 30 mim	-.14						
MDA 60 min		.23					
MDA 90 min			.11				
MDA 120 min				.29			
MDA 24 hr					.20		
MDA 48 hr						-.25	
MDA 72 hr							.23

The correlation of plasma CK and plasma MDA with vitamin E are presented in Table 24. Plasma CK and plasma MDA increased from rest to 24 hours. Plasma CK decreased from 24 to 48 hours while plasma MDA continued to increase.

Table 24.

Vitamin E Trial: Plasma CK Correlation With MDA Over the Time Course

CK-REST	30	60	90	120	24	48	72
MDA REST .07							
MDA 30 mim	.13						
MDA 60 min		.10					
MDA 90 min			.09				
MDA 120 min				.21			
MDA 24 hr					.12		
MDA 48 hr						-.52	
MDA 72 hr							.39

The response of plasma CK and MDA was to decrease at 72 hours. No significant correlations were obtained at any point. The data in Table 23 and Table 24 suggest that vitamin E supplementation did not alter the relationship between plasma CK and plasma MDA compared with placebo.

In Tables 25 through 28 the correlations of variables at rest and 72 hours were zero in part because MSRs were 1 for all subjects at these two times. This resulted in no variance in the MSRs, and as such, correlations were zero.

The correlation of plasma CK and MSRs with placebo are presented in Table 25. Plasma CK and MSRs increased during exercise. Plasma CK increased while MSRs decreased at 24 hours. Plasma CK and MSRs decreased at 48 and 72 hours. No significant correlations were obtained at any point.

Table 25.

Placebo Trial: Plasma CK Correlation With MSR Over the Time Course

CK-REST	30	60	90	120	24	48	72
MSR REST 0.0							
MSR 30 min	.04						
MSR 60 min		.02					
MSR 90 min			.05				
MSR 120 min				.22			
MSR 24 hr					-.16		
MSR 48 hr						.02	
MSR 72 hr							0.0

Plasma CK concentration and MSR ratings during exercise for the vitamin E treatment are presented in Table 26. Both variables increased during exercise. Plasma CK continued to increase at 24 hours while MSR decreased. Both variables were decreasing by 48 hours and continued to decrease by 72 hours. No significant correlations were obtained at any point for the vitamin E treatment. The data in Table 25 and Table 26 suggest that vitamin E supplementation did not alter the relationship between plasma CK and MSRs compared with placebo.

Table 26

Vitamin E Trial: Plasma CK Correlation With MSR Over the Time Course

CK-REST	30	60	90	120	24	48	72
MSR REST 0.0							
MSR 30 min	.11						
MSR 60 min		.29					
MSR 90 min			.38				
MSR 120 min				.27			
MSR 24 hr					-.39		
MSR 48 hr						.52	
MSR 72 hr							0.0

The correlation of plasma MDA and MSRs with placebo are presented in Table 27. Plasma MDA and MSRs increased during exercise except for a slight decrease in plasma MDA at 30 minutes. Plasma MDA increased while MSRs decreased by 24 and 48 hours. Plasma MDA and MSRs decreases by 72 hours.

No significant correlations were obtained at any point.

Table 27.

Placebo Trial: Plasma MDA Correlation With MSR Over the Time Course

MDA-REST	30	60	90	120	24	48	72
MSR REST 0.0							
MSR 30 min	-.11						
MSR 60 min		.01					
MSR 90 min			.35				
MSR 120 min				.15			
MSR 24 hr					-.11		
MSR 48 hr						-.16	
MSR 72 hr							0.0

The correlation of plasma MDA and MSRs with vitamin E are presented in Table 28. Plasma MDA and MSRs increased during exercise. Plasma MDA increased while MSRs decreased by 24 and 48 hours. Plasma MDA and MSRs decreases by 72 hours. No significant correlations were obtained.

Table 28.

Vitamin E Trial: MDA Correlation With MSR Over Time Course

MDA-REST	30	60	90	120	24	48	72
MSR REST 0.0							
MSR 30 min	.52						
MSR 60 min		.34					
MSR 90 min			.10				
MSR 120 min				.42			
MSR 24 hr					-.26		
MSR 48 hr						-.52	
MSR 72 hr							0.0

No significant correlations were obtained for plasma MDA and MRSs for placebo supplementation (Table 27) or for vitamin E supplementation (Table 28). These findings suggest vitamin E supplementation did not alter the plasma MDA and MSR relationship.

No significant correlations were observed for any variable. Vitamin E had no effect on the relationships of plasma CK, plasma MDA and MSRs compared with the placebo.

Summary of the Results

Hypothesis 1 was accepted. No differences in MSRs were obtained between the two trials. Hypothesis 2 was accepted because no significant differences were obtained comparing the two treatments for plasma CK. No significant differences were found between the two treatments for plasma MDA at any time period. Therefore, hypothesis 3 was accepted. The response of the variables across treatment was not altered as demonstrated by the high correlations within subjects. Therefore, hypothesis 4 was accepted. Hypothesis 5 was accepted as there were no significant change in the relationship comparing the vitamin E or placebo treatments.

These data suggest that, vitamin E supplementation (800 mg/day for four weeks) has no effect on attenuating MSRs, plasma CK, plasma MDA, the time course patterns of variables, or the relationship of these variables.

CHAPTER V

DISCUSSION

Vitamin E supplementation had no significant effect on exercise induced changes in MSRs, plasma CK, and plasma MDA concentrations in aerobically trained cyclists. The endurance ergometer ride resulted in elevated MSRs and increased plasma CK and plasma MDA independent of treatment and order. Peak values for plasma CK and MDA increased significantly, however, the values remained within the normal range. Vitamin E supplementation had no significant effect on the time course patterns or the relationship of the MSR, CK, and MDA variables. These findings suggest that the responses of the variables may not be attributable directly to oxidative stress. The discussion will deal with the comparison of the results of exercise studies using vitamin E supplementation in humans with the results of the present investigation. The interruption of the results will lead to possible mechanisms to explain the exercise-induced response of the variables.

Vitamin E Supplementation Studies

The effect of vitamin E supplementation on lipid peroxidation in cyclists has been investigated in two other studies. Sumida et al. (1989) measured a statistically significant decrease in serum MDA in vitamin E supplemented

subjects performing a short duration, high intensity ride. The maximal cycle ergometer test was used as their control situation. Subjects were then supplemented with vitamin E (300 mg/day for 4 weeks) and performed a second maximum ergometer test. Plasma MDA increased from 4.4 to 4.5 nmol during the control ride. Plasma MDA decreased from 3.8 to 3.5 nmol after vitamin E supplementation. Changes in plasma MDA concentrations were small. In this pre-test, post-test study design, there was no control for the effect of training or the order of supplementation. Therefore, it is unclear in this study if the observed decrease in serum MDA was a result of vitamin E supplementation or a result of the previous maximal cycle ergometer test.

The effects of vitamin E supplementation on MSRs, serum CK, and plasma MDA in moderately trained cyclists was investigated by Lewis and Goldfarb (1992). Subjects performed a 100 mile road ride that had marked elevation gains. They were randomly assigned to a vitamin E group (800 mg/day for 2 weeks) or a placebo group. Elevations in MSRs, serum CK, and plasma MDA occurred in both treatments. No significant differences were obtained in the vitamin E supplemented group compared with the placebo group. It was concluded that the two week vitamin E supplementation period may have been insufficient to attenuate the exercise-induced response for these variables. Additionally, this study did

not control for the intensity of the ride, variability of serum CK and plasma MDA within the population, and differences in subjects' aerobic capacity.

Other researchers have reported the beneficial effect of vitamin E supplementation in runners. Vitamin E supplementation (800 mg/day for 4 weeks) decreased lipid hydroperoxide, a marker of lipid peroxidation, following a 30 minute treadmill run with a zero degree grade at 80% VO_2 max (Goldfarb et al., 1989). There was no change in exercise-induced MDA as a result of the vitamin E supplementation.

In contrast, an antioxidant supplement decreased plasma CK following a -5 degree downhill treadmill run at 65% VO_2 max. Vitamin E, 800 mg/day, was part of the antioxidant supplement which also included 1000 mg vitamin C/day and 10 mg beta-carotene/day (Viguie et al., 1989). This supplement regime also decreased oxidative stress as indicated by the amount of reduced glutathione.

The beneficial effects of vitamin E supplementation on lowering CK concentrations in males (22-29) performing a downhill treadmill run was reported by Cannon et al. (1990). Subjects received 400 IU of vitamin E daily for 48 days. They ran at 75% VO_2 max for three 15 minute periods on a downhill (-16% grade) treadmill.

The effect of vitamin E supplementation has not been extensively studied. The literature with vitamin E supplementation and cycling appears to suggest minimal if any changes in MDA. Vitamin E supplementation appears to have some beneficial effect in running. The two different exercise modes, cycling and running, may in part influence the observed responses.

Responses of Variables to Cycling and Running

Cycling is primarily a concentric exercise whereas running is both an eccentric and concentric exercise. The differences in to these exercise modes maybe reflected in part by the relationship of CK and MDA. Kanter et al. (1988) reported that the exercise-induced responses of serum CK and plasma MDA were significantly correlated ($r = 0.84$) following an eighty kilometer run. Serum CK and plasma MDA were measured only after the run and correlations over time were not investigated.

In the present study, plasma CK and plasma MDA relationships over time was investigated. Plasma CK and plasma MDA were not significantly correlated at any point during the endurance cycling ride. The low correlation of CK and MDA (range 0.07 to -0.52) following concentric exercise (cycling) was different from the response to a combined eccentric/ concentric exercise (running). It is clear that the mode of exercise and the type of contraction

influenced the response of these variables.

Design Factors of the Study

The design of this study tried to control or factor in components not controlled for in previous investigations. A primarily concentric exercise, cycling, was chosen to minimize the potential muscle fiber tearing that can accompany an eccentric/concentric exercise such as running (Friden et al., 1981; Newham et al., 1983). The possible muscle tearing, from eccentric contractions during running, may have influenced the response of serum CK and markers of lipid peroxidation in the three running studies (Goldfarb et al., 1989; Viguie et al., 1989; Cannon et al., 1990).

Conditioned subjects were tested to minimize the training effects of the endurance rides. To eliminate a possible order effect of supplementation, subjects were randomly assigned to the supplement they received first. The cross-over design controlled for variability of CK and MDA responses and aerobic conditioning within the sample study.

This study was the first that attempted to maximize both duration and intensity in cycling and investigate the effect of vitamin E supplementation of MSRs, plasma CK and plasma MDA. Duration of the activity appears to be a greater factor than intensity for serum or plasma CK response in endurance exercise (Fowler et al., 1968;

Robinson et al., 1982; Van Der Meulen, Kuipers, & Drukker, 1991). Intensity appears to have a greater effect than duration on plasma MDA concentration (Lovlin et al., 1987).

Dietary intake of vitamin E, and amounts and kinds of fat were monitored in this study. Dietary intakes were not reported in the other investigations (Goldfarb et al., 1989; Lewis et al., 1992; Sumida et al., 1989; Viguie et al., 1989). Differences in vitamin E intake, and kinds and amounts of fats could possibly alter lipid peroxidation independent of supplementation. The double-bond structure of polyunsaturated and monosaturated fats are susceptible to lipid peroxidation (Parker, 1989).

In the present study, blood samples were taken during the exercise period and for three days after. Blood samples taken during the exercise enabled the investigator to determine the changes in plasma CK and MDA and their relationship while exercising. It also allowed for changes in MSRs to be compared with biochemical measures during the exercise as opposed to only after the exercise was completed.

The exercise induced responses of the MSRs, plasma CK, and plasma MDA will be discussed with regard to the endurance ergometer rides. These responses will be compared with responses from other studies.

Exercise-Induced Response of Variables

Muscle Soreness Ratings

Peak muscle soreness ratings post ride were comparable to the peak MSRs reported in the road-ride studies by Lewis et al. (1991), and Lewis and Goldfarb (1992) (Appendix B and C). However, in the present study, the 24-hour MSRs were lower compared to the two road-ride studies. Factors influencing the lower 24 hour MSRs in the endurance ergometer rides may have been duration of the exercise, aerobic conditioning of the subjects, and ride position.

The mean finish times in the endurance-ergometer rides were significantly shorter that the mean finish road-ride times. These shorter ride times in the present study may have resulted in less subject fatigue and therefore lower 24-hour MSRs.

Subjects in the endurance ergometer rides had higher VO_2 maxs, trained more miles/week, and had trained for more years than the subjects in the second road-ride study (Lewis & Goldfarb, 1992). Subjects in the endurance ergometer rides may have been better trained than subjects in the road rides. Studies suggest that muscle soreness decreases with training (Clarkson et al., 1985, 1986, 1987). The fact that subjects in this study were aerobically trained may partially account for the lower 24-hour MSRs.

Subjects in the endurance-ergometer rides and the two road rides reported different primary sites of soreness. In the endurance ergometer rides, subjects reported that their muscle soreness was primarily in the lower limb muscles. Following the road rides, subjects reported they were as equally sore in the neck, lower back extensors, and the lower limb muscles. Differences in ride positions may have influenced the sites of muscle soreness.

Cyclists maintained a more upright posture in the endurance-ergometer rides than in the road rides. The horizontal posture on the drop handlebars during the road rides may have required more co-contractions of the neck extensors and static holding of the low back extensors in a lengthened position. The fact that the upright posture assumed in the endurance-ergometer ride did not stress the neck and low back extensors, may have accounted for the differences in the sites of muscle soreness. The lower 24-hour MSRs may have reflected less musculo-skeletal stress in the subjects performing the endurance-ergometer rides. Additionally, subjects cycled on the level during the endurance rides whereas the road rides had significant elevations.

Plasma CK Response

Mean plasma CK concentrations were lower in the present study compared with the two road-ride studies. Peak 24-

hour plasma CK increased over 400% from resting in the first road ride (Lewis et al., 1991) and about 300% for the combined groups in the second road ride (Lewis & Goldfarb, 1992). Plasma CK increased only 60% from rest following the endurance ergometer rides independent of treatment. Duration and aerobic conditioning of the subjects may have attenuated the plasma CK changes with exercise in the present investigation.

As previously noted, the mean endurance ergometer-ride times were half as long as the mean road-ride times. A lower response in plasma CK with a shorter duration exercise agrees with findings by several other researches (Forssell et al., 1975; Fowler et al., 1968; Robinson et al., 1982; Van Der Meulen, Kuipers, & Drukker, 1991).

Aerobic conditioning of the subjects may have been a contributing factor diminishing effecting the peak plasma CK concentrations observed in the present study. Investigators have reported lower plasma CK concentrations post exercise in trained subjects when compared with untrained subjects (Ahlborg et al., 1967; Evans et al., 1986; Gimenex et al., 1984; Hunter & Critz, 1971; Nuttal et al., 1968; Roti et al., 1981). Brooke et al. (1979) reported greater mean peak serum CK response in untrained subjects compared to trained subjects following a 2 hour bicycle ergometer ride at 50% VO_2 max. Subjects in the second road study (Lewis &

Goldfarb, 1992) were moderately trained and demonstrated elevated CK responses. The fact that subjects in the present study were well trained probably contributed to the lower peak plasma CK concentrations compared to previous investigation. The data, in the present study, confirms previous reports that suggest that lower CK responses occur in well conditioned subjects.

Plasma MDA Response

Mean peak plasma MDA concentrations after exercise were lower in the present study compared with the first road ride study. Peak 48-hour plasma MDA increased about 40% from resting in the first road ride (Lewis et al., 1991). Plasma MDA increased only 28% in the present investigation. Ride intensity may have been a factor in the lower peak MDA concentrations observed in the present study.

Plasma MDA concentrations have been shown to increase with strenuous exercise. In the present study, cyclists may have been working harder at a higher intensity at the end ride of the because of the marked gains in elevation. The increase in intensity at the end of the road rides may have contributed to higher plasma MDA concentrations. In the present study, exercise intensity was maintained at 70% VO_2 max. Perhaps the intensity was not sufficient to increase plasma MDA concentrations out of the normal range.

Time Course Patterns of Muscle Soreness

The time course pattern of MSRs in the present study showed MSRs peaking immediately post exercise, declining by 24 hours, and returning towards baseline ratings at 48 hours. This pattern was similar to the pattern observed in the two road-ride studies (Lewis et al., 1991; Lewis & Goldfarb, 1992). The present study and the two road-ride studies are the only studies to investigations found to examine the time course patterns of MSRs to endurance concentric exercise (cycling).

The time course pattern of muscle soreness following an endurance concentric exercise differs from the pattern observed following eccentric exercise. The time course pattern for muscle soreness in eccentric exercise is for soreness to be delayed for 24 to 48 hours, peak 48 to 96 hours, and then return towards baseline (Byrnes & Clarkson, 1986; Clarkson et al., 1986; Newham et al., 1983). The differences observed in concentric and eccentric contractions may reflect the possible muscle tearing and inflammatory events that can occur following eccentric work (Friden et al., 1981; Newham et al., 1983a; Smith et al.,1991). It is not know what mechanisms are responsible for the muscle soreness in concentric exercise. Future investigation should examine possible biochemical mechanisms for muscle soreness in concentric exercise.

Time Course Pattern in Plasma CK

The time course pattern for plasma CK demonstrated slight increases during exercise, peaking at 24 hours, and declining, but not return to baseline concentration by 72 hours. This CK pattern was similar to the CK pattern observed in the previous two road-ride studies (Lewis et al., 1991; Lewis & Goldfarb, 1992). These findings concur with Brooke et al. (1979) who reported a similar pattern in CK response in well trained subjects following a 90 minute cycle ergometer ride at 50% VO_2 max.

The time course pattern in plasma CK following a concentric endurance exercise is different from the pattern observed in endurance eccentric exercise. Eccentric exercise demonstrated a delayed response and peaks 48 to 72 hours after the exercise (Byrnes & Clarkson, 1986; Clarkson et al., 1986; Newham et al., 1983). The mechanisms of the different responses of plasma CK to concentric and eccentric work is not known. Possibly the acute inflammatory repair process following eccentric exercise may result in further tissue damage and subsequent elevations in CK concentrations 48 and 72 hours after the exercise.

Time Course Pattern in Plasma MDA

The time course pattern for plasma MDA in the present study was to increase slightly during exercise and peak at 48 hours. Plasma MDA was declining by 72 hours, but had not

returned to baseline concentrations. These results concur with the results from the first road-ride study (Lewis et al., 1991). No other studies have investigated plasma MDA time course to an endurance concentric exercise. Previous studies have reported plasma MDA concentrations only before and after exercises of different intensities and durations (Cannon et al., 1990; Goldfarb et al., 1989; Viguie et al., 1989).

The rise in plasma MDA during exercise was a attributable to oxidative stress and an indication of lipid peroxidation. The peak 48-hour concentration was likely a response to lipid peroxidation and may have taken time to be released into the circulation as lipid peroxidation of membranes may occur quickly but cleavage may take time.

Lipid peroxidation and MDA may not only be generated by hydrogen peroxide and free radical production with oxidative stress during exercise, but can also arise from removal of necrotic tissue post injury (Halliwell & Gutteridge, 1986; Gutteridge & Halliwell, 1990). Tissue damaged by any mechanism is more susceptible to lipid peroxidation. Since vitamin E did not alter the amount nor pattern of MDA, this suggests that the amount of vitamin E was not enough to prevent oxidative injury.

Relationship of Variables

The MSRs, plasma CK, and plasma MDA were not significantly correlated in the present study. This lack of significant correlation was also observed in results for MSRs and plasma CK from the first road-ride study (Lewis et al., 1991).

Positive correlations between some variables have been observed with eccentric exercise. Clarkson et al. (1986) observed a significant positive correlation in MSRs and serum CK following bouts of weight lifting. Kanter et al. (1987) reported a significant positive significant correlation between serum CK and plasma MDA following an eight kilometer run. The correlations were only determined from post exercise serum CK and plasm MDA concentrations. The relationship of serum CK and plasma MDA across a time course of 4 to 7 days which would include peak concentrations of both serum CK and plasma MDA has not been examined.

Other Possible Mechanisms to Explain Findings

Lipid peroxidation does not appear to be a primary mechanism for elevated MSRs, plasma CK, and plasma MDA because vitamin E had no significant effect on the peak rise, time course, and relationship of the variables. Other mechanisms need consideration to explain changes in variables. One mechanism for consideration is lactate

accumulation (Abraham, 1977; Armstrong, 1984).

A rise in plasma lactate reflects an increase in hydrogen concentration and therefore a lower cellular pH. Armstrong (1984) and Cross et al. (1987) stated that lowered cellular pH was associated with disruption of cellular ATP production, alteration in the Na^{+}-K^{+}-ATPase pump, and a rise in cytosolic calcium ions which can result in altered in membrane permeability. This mechanism may have contributed to the moderate increase in MSRs, plasma CK, and plasma MDA following the endurance-ergometer rides.

Researchers have reported a rise in lactate with exhaustive exercise (Fitts and Holloszy, 1976; Jacobs, 1981; Karlsson, Nordesjo, Jorfeldt, & Saltin, 1972). Lactate peaks at the end of strenuous exercise and returns to normal shortly after secession of the exercise. Possibly a paralleled increase in lactate and MSRs occurred during the endurance ergometer rides. Peak MSRs could have occurred at the time of peak lactate accumulation. The elevated plasma CK was possibly a reflection of the alteration in cell membrane permeability resulting from lowered cellular pH. Elevation of plasma MDA at 48 hours was possibly a reflection of minor muscle damage and altered appearance and clearance of lipid peroxidation. Further study is suggested to compare the relationships of lactate accumulation to MSRs, plasma CK, and plasma MDA.

Altered membrane permeability as a result of substrate depletion or disruption in oxidative coupling with exhaustive endurance exercise is a another possible mechanism. When oxidative coupling is disrupted, the Na^{+}-K^{+}-ATPase pump is impaired (Oberc & Engel, 1977; Wrogemann & Pena, 1976). Impairment in the Na^{+}-K^{+}-ATPase pump can result in accumulation of cytosolic calcium ions (Armstrong, 1984). Elevations in cytosolic calcium ions can lead to alteration in membrane permeability and even cell damage or death (Armstrong, 1984, 1986; Cross et al., 1987).

The mechanism effecting the observed changes in variables may be combined effect of lactate accumulation and disruption of oxidative coupling from substrate depletion or from other pain substances such as prostaglandins, bradykinins or immune response.

Summary

In summary, Vitamin E supplementation (800 mg/day for 4 weeks) in a double-blind cross-over study no significant effect on MSRs, peak plasma CK, and plasma MDA compared to the placebo supplementation in aerobically conditioned subjects performing an exhaustive endurance cycle ergometer ride at 70% VO_2 max had . Vitamin E supplementation had no effect on the time course response or the relationship between variables when compared with the placebo supplementation.

Other possible mechanisms were discussed that could have effected the observed increase in MSRs, plasma CK, and plasma MDA. The increase in muscle soreness over the time course of the treatment may have paralleled lactate accumulation. The muscle soreness and plasma CK increases may be the result of altered membrane permeability because of lowered cellular pH. The moderate elevation of plasma MDA at 48 hours may have reflected alternations in clearance and release.

CHAPTER VI

RECOMMENDATIONS

Vitamin E supplementation (800 IU/day for 4 weeks) had no effect on the exercise-induced elevation of MSRs, plasma CK, and plasma MDA in trained subjects following endurance cycling. The results of the study lead to the following recommendations for investigation: (1) lactate mechanism, (2) other markers of lipox, (3) other agents, (4) effects of vitamin E supplementation in untrained subjects, (5) effects of vitamin E supplementation in older subjects, and (6) comparison of eccentric versus concentric exercise.

(1). Further investigation of the relationships of lactate accumulation to MSRs, plasma CK, and plasma MDA is warranted in concentric exercise.

(2). Other markers of lipid peroxidation may be more sensitive indicators of oxidative stress during exercise. The TBARS method for MDA is not a specific measurement of lipid hydroperoxides. Lipid hydroperoxides as an indicator of lipid peroxidation with concentric exercise needs further investigation. Enzymes responding to lipid peroxidation warrant investigation. Further study of the effect of vitamin E supplementation on the responses of superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione peroxidase in concentric exercise is suggested.

(3). Other agents such as prostaglandins, bradykinins, and immune function factors all have been implicated as possible inflammatory agents and need further investigation in concentric exercise.

(4). The aerobic conditioning of the subjects may have minimized their response to lipid peroxidation in oxidative stress. Untrained subjects may have demonstrated more effect of exercise stress by responding with greater changes in physiological measures. Untrained subjects may benefit from vitamin E supplementation whereas well trained individuals may not require vitamin E supplementation.

(5). The age of the subjects may have minimized their response to oxidative stress. Older subjects may benefit more from vitamin E supplementation than younger subjects (Meydani et al., 1990).

(6). Vitamin E may be more beneficial in eccentric exercise versus concentric exercise. Vitamin E could possibly be more effective in eccentric exercise as it may protect during times of reperfusion as shown reperfusion injury studies (Salaris & Babbs, 1989).

A more complete and accurate understanding of the mechanisms of both acute and delayed muscle soreness could aid in the development of exercise protocols and preventative measures. Further investigations of the effects of vitamin E on these mechanisms is warranted.

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Appendix A

(Muscle Soreness Scale)

Perceived Delayed Onset of Muscle Soreness Rating Scale

1	2	3	4	5	6	7	8	9	10
Normal			Uncomfortable				Sore		Very Sore

In row x, please indicate the general degree of soreness of the entire muscle when using or moving it (without pressing it).
In row y, please rate the degree of soreness of different parts of your muscle when you palpate (press lightly) the areas indicated on the diagram.

Hands & Arms (palm up)
(general) x _____

(specific) y (a) _____
(b) _____
(c) _____
(d) _____

Hands and Arms (palm down)
(general) x _____

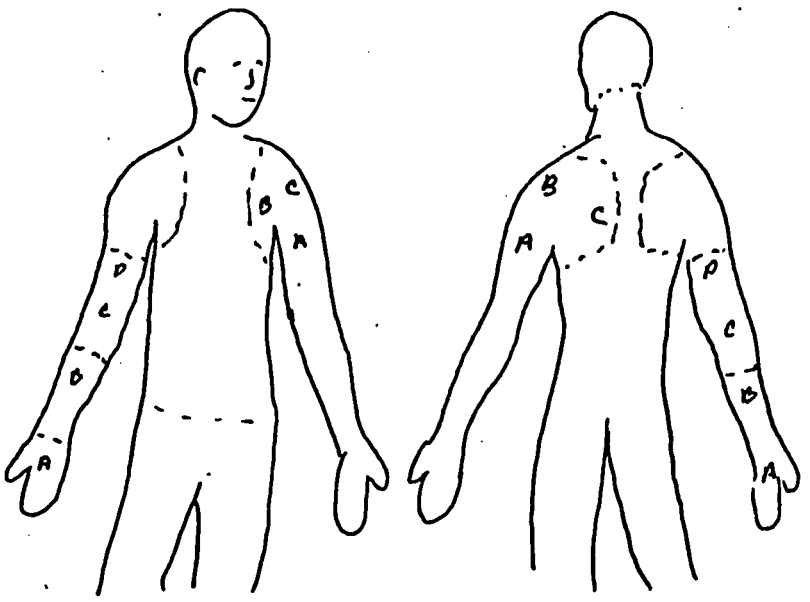
(specific) y (a) _____
(b) _____
(c) _____
(d) _____

Shoulders (front)

(shoulders general) x _____
(shoulders specific) y (a) _____
(b) _____
(c) _____

Shoulder (back)

(shoulders general) x _____
(shoulders specific) y (a)_____
(b_____
(c)_____



Rate the degree or soreness in the: Neck, upper back, mid back, and low back.

	X	Y
general	_____	(a) _____
		(b) _____
		(c) _____
		(d) _____

Legs & Thighs (front)

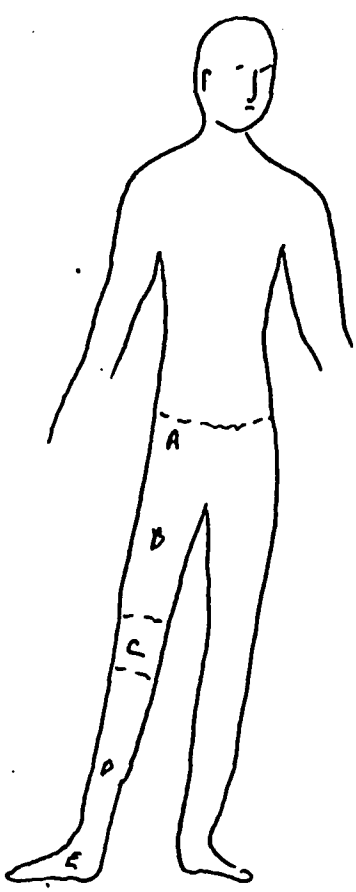
(general) x _____

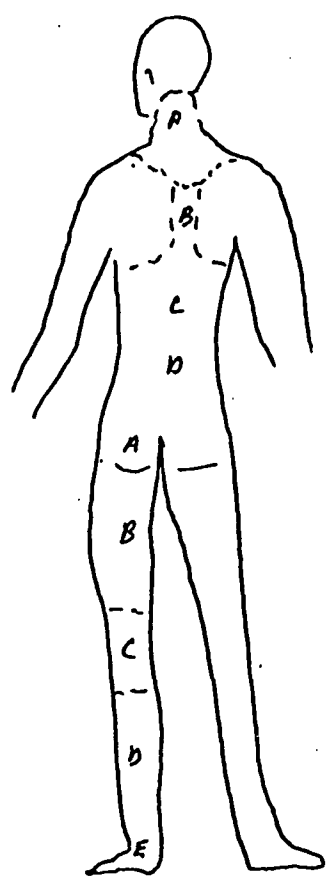
(specific) y (a) _____
(b) _____
(c) _____
(d) _____
(e) _____

Legs & Hips (back)

(general) x _____

(specific) y (a) _____
(b) _____
(c) _____
(d) _____
(e) _____





Appendix B

Lewis, Goldfarb, and Boyer, 1991

(Study 1)

SERUM CREATINE KINASE, PLASMA MALONDIALDEHYDE, AND DELAYED ONSET OF MUSCLE SORENESS IN BICYCLE RIDERS COMPLETING A STRENUOUS 100 MILE RIDE. C.L. Lewis, A.H. Goldfarb, & B. Boyer, Exercise Science Dept. UNC Greensboro, Greensboro, NC 27403

This study examined the time course relationship of serum creatine kinase (CK), plasma malondialdehyde (MDA) and perceived delayed onset of muscle soreness (DOMS) ratings following a strenuous concentric event to determine if oxidative stress is related to muscle soreness in concentric contractions. Eleven males, 35.4 yrs (± 3.98), who trained 130 mi/wk (±10.9) completed the 1990 Assault on Mt. Mitchell, a 102 mile bicycle ride with a gain of 6000 ft. elevation. Mean finish time was 7.2 hrs (± 0.44). Blood was collected prior, immediately post, 24, 48, and 72 hours post event. Serum CK and plasma MDA were analyzed spectrophotometricly. After blood collection, DOMS was rated on a scale of 1 (no soreness) to 10 (very, very sore). Repeated measures ANOVA ($p \leq .05$) determined significance and a Pearson Correlation determined relationships between variables.

		pre	post	24hr	48hr	72hr
CK IU:	$\bar{x}$ =	53.6	167.2*	239.2*	202.1*	169.8*
	se =	(±6.0)	(±30.3)	(±50.9)	(±49.8)	(±50.8)
MDA (nmoles/ml)						
	$\bar{x}$ =	1.81	1.93	2.34	2.52	1.74
	se =	(±0.23)	(±0.36)	(±0.39)	(±0.40)	(±0.28)
DOMS	$\bar{x}$ =	1.0	5.4*	4.2*	1.7	1.1
	se =	(±0)	(±0.6)	(±0.5)	(±0.3)	(±0.1)

* statistical significance

CK and DOMS, and CK and MDA correlations were not significant. These data suggest that strenuous concentric cycling, which produces elevated serum CK and increased DOMS, is unrelated to oxidative stress as indicated by plasma MDA concentrations.

Appendix C

Lewis and Goldfarb, 1992

(Study 2)

EFFECT OF VITAMIN E ON SERUM CREATINE KINASE, AND MUSCLE SORENESS RATINGS IN BICYCLE RIDERS COMPLETING A STRENUOUS 100 MILE RIDE. C.L. Lewis, A.H. Goldfarb, Exercise Science Dept. UNC Greensboro, Greensboro, NC 27403

This study examined the effect of vitamin E on the time course relationship of serum creatine kinase (CK), and perceived muscle soreness ratings (PMSR) in twenty four age and sex matched subjects participating in the 1990 Bridge to Bridge Ride. Subjects were divided into two groups receiving two week supplementation of placebo (P) or 800 IU of vitamin E daily prior to the ride. Mean age of both groups was 29 (± 2.3) years. Mean training milage, VO_2, and finish time were 142 (± 10) mi/wk, 53.2 (± 3.3) ml/kg·min, and 7.3 hr (± 0.4) for the vitamin E group; and 182 mi/wk (± 25), 53.3 (± 2.3). ml/kg·min and 6.5 (± 0.5) hours for the placebo group Mean Blood was collected prior, immediately post, 24, 48, 72 and 96 hours post event. Serum CK was analyzed spectrophotometricly. After blood collection, PMSR was rated on a scale of 1 (no soreness) to 10 (very, very sore). Repeated measures ANOVA ($p \leq .05$) determined significance.

Vitamin E	pre	post	24hr	48hr	72hr	96hr
CK IU	39.6	89.8*	125.4*	85.1*	72.6*	58.3
se	(±2.4)	(±14.5)	(± 23.2)	(±13.2)	(±11.5)	(±12.5)
PMSR	1.0	5.5*	4.2*	2.3	1.4	1.2
se	(±0.0)	(±0.6)	(±0.6)	(±0.3)	(±0.2)	(±0.1)
Placebo	pre	post	24hr	48hr	72hr	96hr
CK IU	37.8	68.1*	101.2*	67.7*	47.8	36.8
se	(±4.8)	(±7.8)	(±18.1)	(±10.0)	(±7.0)	(±7.1)
PMSR	1.0	4.2*	3.3*	2.2	1.6	1.1
se	(±0.0)	(±0.7)	(±0.6)	(±0.3)	(±0.2)	(±0.1)

* statistical significance

Immediate post, 24, and 48 hr determinations were significantly different from pre ride levels for serum CK and PMSR for both the vitamin E and the placebo groups. However no significant differences were found between the two groups. The greater training milage, and shorter finish time of the placebo group may reflect greater cycling efficiency even though there was no significant difference in VO_2 max between the two groups. This efficiency may negate any effect of vitamin E to minimize membrane damage in moderately young trained populations.

Appendix D

Recruitment Flyer

TRIAD BIKERS

I am Cynthia Lewis, a doctoral student in Exercise Physiology at UNC Greensboro, a physical therapist, and recreational cyclist.

YOU ARE WANTED FOR AN EXERCISE PHYSIOLOGY STUDY.

The purpose of the study is to determine if vitamin E can reduce the minor muscle damaging effects of strenuous bike riding. This study will benefit researchers and individuals concerned with training protocol and injury recovery.

To be a subject, you have to be a male

18 to 45 who rides 100 miles a week during the training season.

You will be asked to:

1. Sign a consent form which explains the purpose of the study.
2. Complete questionnaires regarding your training program, medical history, and diet.
3. Take vitamin E or a placebo for two 4 weeks periods.
4. Come to the Exercise Physiology Lab at UNCG and complete two max bike tests and two submaximal bike rides.
5. Have a small amount of blood drawn from a vein in your arm before, during, immediately after, and once daily for 3 days after each submaximal ride. Blood will be drawn by a trained person using sterile technique.
6. Indicate any muscle soreness on a rating scale each time blood is drawn.

IF grant funding is approved, subjects will be given $100.00. Please call--

Cynthia Lewis
at 852-7697

Appendix E

(Consent Forms)

CONSENT FORM FOR HUMAN SUBJECTS
THE UNIVERSITY OF NORTH CAROLINA AT GREENSBORO
DEPARTMENT OF EXERCISE AND SPORT SCIENCE

Subject's Name: _____

Date of consent: _____

Project Title: "Effects of Vitamin E on Muscle Soreness, Creatine Kinase, and Malondialdehyde Following a Strenuous Endurance Bicycle Ergometer Ride"

Description and Explanation of Procedures:

I understand that the purpose of this study is to investigate the effects of vitamin E on changes in plasma creatine kinase, lipid peroxidation and muscle soreness response to an exhaustive submaximal ergometer ride. I am presently on no prescription medications or taking any over the counter medication on a regular basis. If during the course of this study, I am placed on any medication by my physician, or voluntarily take any medication, I will inform the researcher. I have no known musculoskeletal injury that will interfere with my participation in the study.

I understand that during the study, I will do my regular activities including bicycle training. I agree not to do weight lifting one week prior to the submaximal ride.

Prior to the start of the study, I will complete a consent form, medical history, training questionnaire, and four day food record. I will perform two maximal ergometer stress tests and two submaximal tests at 75% VO2 max.

I will take a supplement, either 800IU of vitamin E or a placebo for four weeks prior to the submaximal ride. Neither I nor the principal researcher will know which supplement I am taking.

One week prior to the submaximal test, I will do a maximum bicycle ergometer stress test. The resistance on the bicycle is gradually increased until I reach my maximum oxygen consumption at the prescribed pace. If I choose, I may stop the test at any time. I will breath into collecting tubes for analyses of my expired oxygen. I know that the American College of Sports Medicine guidelines for exercise stress testing will be followed. During my visit for the stress test, I will have my per cent body fat determined using skin fold calipers.

I understand that a venous catheter will be inserted. Ten mls of blood will be drawn prior to the ride, and at 30, 60, 90, 120, 150, 180, 210, 240 minutes during the ride and 20 minutes post ride. I will return to the Exercise Physiology Lab at UNCG 24, 48, and 72 after the ride for

blood collection after each of the two submaximal rides. The amount of blood drawn before the ride will have negligible effect on my performance. I will indicate a muscle soreness rating score prior to each blood collection.

Risks and Discomforts:

The possibility exists of certain physiological changes occurring during the maximal bike stress test. These include abnormal blood pressure or heart beats, fainting, and in very rare instance a risk of heart attack (less than 3 in 10,000). My risks are minimized since I am a healthy conditioned individual. I know that risks will be minimized by the researchers adherence to the American College of Sports Medicine guidelines. Researchers conducting the test are trained exercise physiologists. Researchers will closely monitor me. If I would appear to be having an adverse reaction, the test will be immediately stopped. The muscle soreness that I may experience after the submaximal tests is temporary and usually subsides in 2 to 3 days post event. I understand that I may experience some slight discomfort or bruising with the blood drawing, but these factors will be reduced by using direct pressure until all bleeding stops. I know that a slight risk of infection is associated with blood drawing as is associated with any skin prick. However, the risk will be minimal since only sterile techniques will be used; and a person trained in catheterization and venipuncture technique will be collecting the blood samples.

Potential Benefits:

Benefits to the individual directly are knowledge of his maximal oxygen capacity, percent body fat and dietary intake. Knowing this data can assist the subject in him in his training protocol. Data from this study may advance knowledge in the field of exercise physiology and specifically in the area of strenuous concentric work on lipid peroxidation and how vitamin E may prevent this reaction.

Compensation/Treatment for Injury:

No compensation will be made for treatment for injury. Risks from the bicycle ergometer stress test will be minimized by adherence to the American College of Sports Medicine guidelines.
Risks from blood collection have been reduced by using sterile technique and trained personnel. I, the subject, am

responsible for medical treatment expenses if any should arise in this study.

Consent:

I have been satisfactorily informed about the procedures described above and the possible risks and benefits of the project, and I agree to participate in this project. Any questions that I have about the procedures have been answered. I understand that this project and this consent form have been approved by the University Institutional Review Board which ensures that research projects involving human subjects follow federal regulations. If I have any questions about this, I will call the Office of Research Services at (919) 334-5878.

I understand that I am free to withdraw my consent to participate in the project at any time without penalty or prejudice. In addition, I will not be identified by name as a participant in this project.

Any new information that might develop during the project will be provided to me if that information might affect my willingness to participate in the project.

_____ _____

Signature of Subject Witness to Signature

_____

Address

_____

Phone

_____

Date

Appendix F

Medical History Quesitonnaire

Medical History Questionnaire

Directions: Please check the blank yes or no or fill in the blank when a longer answer is requested.

Name: _____

Height: _____ Weight: _____

Birth date: _____

	(yes)	(no)
1. Do you currently have any functional limitations of your upper limbs,	_____	_____
of your neck and back	_____	_____
or of your lower limbs	_____	_____
that would prevent your participation in this ride?		

2. If yes explain:_____

	(yes)	(no)
3. Have you had an injury of the upper limb, neck and back, or of the lower limbs in the past that required medical treatment?	_____	_____

4. If yes, explain each treatment:

_____

_____

_____

	(yes)	(no)
5. Are you recovered from this past injury?	_____	_____

6. If no explain:_____

_____

7. Do you have any of the following:	(yes)	(no
a. high blood pressure	_____	_____
b. frequent chest pain	_____	_____
c. frequent dizziness	_____	_____
d. heart trouble of any kind	_____	_____
e. diabetes	_____	_____
f. high cholesterol	_____	_____

8. Do you smoke?	_____	_____
9. Are you taking medication for a musculoskeletal disorder or cardiovascular disease?	_____	_____

10. If yes explain:_____

_____

Appendix G

(Racing/Riding Questionnaires)

RACING/RIDING QUESTIONNAIRE

Directions: Complete each of the following questions.

1. Do you consider yourself a:
   a. racing cyclist
   b. biathlete
   c. triathlete
   d. recreational cyclist _____

2. Total number of years you have been a recreational bicyclist? _____

3. Total number of years have you ridden as a racing cyclist? _____

4. If you are a racing cyclist, what is your ranking? (category 1, 2, 3, or 4
   _____

5. Do you have a professional sponsor?
   _____

6. a. Do you ride for a professional racing team?
   _____

   b. for a club team?
   _____

7. a. How many months of the year do you train?
   _____

   b. During training, how many miles do you train a week on the average?
   _____

   c. How many days a week do you train?
   _____

   d. How long do you train each day?
   _____

8. a. What is your fastest 10 mile time trial?
   _____

   b. your fastest 40 k time trial?
   _____

c. your fastest 50 mile ride time?

_____

d. your fastest 100 mile ride time?

_____

9. a. Do you weight train as a part of your training protocol? yes _____ no _____

b. Did you weight train the immediate week preceding the Assault? yes _____ no _____

10. a. Do you cross train with running or jogging? yes _____ no _____

b. If yes, how many miles did you train?

_____

c. Do you cross train with high impact aerobics? yes _____ no_____

d. Do you cross train with swimming? yes _____ no_____

11. What type of bike are you riding?

_____

12. What gearing are you presently using in training?

_____

13. List the exercise activities you did the last 3 days prior to the submaximal ride.

a. 3 days prior

_____

b. 2 days prior

_____

c. 1 day prior

_____

Appendix H

(Four Day Food Records)

Subject's Name

_____

FOUR DAY FOOD RECORD

Please list what you eat during the day for breakfast, lunch, dinner and for snacks. List what you eat, the amount you eat and how it is prepared.

Example: Breakfast

1. two egg scrambled in one teaspoon margarine
2. one piece whole wheat toast (Kerns Country Grain) with one teaspoon margarine
3. one cup decaf coffee with one tablespoon coffee rich
4. two slices bacon (name brand)

(when possible list brand names)

Lunch

1. one 4 oz. grilled hamburger steak with 2 tablespoons catsup
2. tossed salad (1 cup lettuce, 1/2 medium tomato, 1/4 cup mushrooms, 4 green olives) with 4 soda crackers (no salt tops) 2 table spoon regular blue cheese dressing
3. medium sized baked potato with one tablespoon sour cream
4. one slice Texas toast with 1 pat of butter.
5. two cups regular coffee with 1 tablespoon half and half
6. one two inch wedge apple pie (no crust top)
   1/2 cup regular ice cream

If a food package lists the number of calories in the food product please indicate the number of calories and amount.

For example a 1.3 oz package of corn chips is 250 calories.

The more specific and detailed you can be about what you eat, the more information I can give you regarding you food intake.

Subject's Name _____

Date

Breakfast:

Lunch

Dinner

Snacks

Subject's Name _____

Date

Breakfast:

Lunch

Dinner

Snacks

Subject's Name _____

Date

Breakfast:

Lunch

Dinner

Snacks

Subject's Name _____

Date

Breakfast:

Lunch

Dinner

Snacks

Appendix I

(Procedures for CK)

Plasma CK Procedures

1. 50 ul phosphocreatine was added to duplicate blanks (tubes without sample) and 40 ul added to the test sample tubes. 10 ul of each plasma sample were added to duplicate tubes. Then 10 ul of double distilled water was added to each blank and test sample.

2. Blank and test samples were placed in a 37 ^{0}C water bath for 5 minutes.

3. After removal from the water bath, 20 ul of ADP glutathione were added to each tube. Blanks and samples were then incubated in a 37 ^{0}C water bath for 30 minutes. The CK in the sample phosphorylated ADP to APT plus creatine.

4. After removal from the water bath, p-hydroxymercuribenzoate was added to each blank and sample to stop the reaction. Coloring reagents naphthol (0.1 ml) and diacetyl solution (0.1 ml) were added to each tube. Then .3 ml of double distilled water were added to each tube to bring the solution to a volume of 1 ml.

5. Tubes were again incubated in a water bath at 37 ^{0}C. Incubation time was 20 minutes. Blanks and samples were read using a spectrophotometer at 520 nm.

Appendix J

Procedures for MDA

Plasma MDA Procedures

1. 0.5 ml duplicate samples were placed into 10 ml tubes containing 1/2 ml 10% chilled trichloracetic acid (TCA) and then mixed. Chilled TCA precipitated out proteins and lipoproteins that could interfere with the reaction.

2. The tubes were placed into a 37 ^{0}C water bath for one hour.

3. After one hour the samples were mixed and centrifuged twice at 3000 rpm for 10 minutes at 4 ^{0}C. After the first centrifugation the supernate was poured into another tube leaving most of the residue in the initial tube. After the second centrifugation, a 0.25 ml sample of the supernate was carefully pipetted into another tube so as not to get any of the residue into the sample. Samples were then covered with parafim and refrigerated up to 24 hours or immediately processed.

4. The 0.25 ml sample of the supernate was combined with 0.25 ml of 0.67% thiobarbituric acid (TBA) and mixed.

5. The samples were heated at 95 ^{0}C for 15 minutes in a hot water bath.

6. Samples taken out of the hot water bath were immediately cooled in an ice bath and read at 532 nm on a Guilford spectrophotometer.

Reagents: TBA--0.67% thiobarbituric acid (TBA)

TCA--10% trichloracetic acid (TCA)