Effects of Antioxidant Vitamin Supplementation on Resistance Exercise Induced Lipid Peroxidation in Trained and Untrained Participants

by:

Peter Viitala ©

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Abstract

High intensity aerobic-type exercise produces oxygen free radicals that can cause damage to lipid membranes (lipid peroxidation) that may lead to many problems such as the inactivation of cell membrane enzymes, the progression of degenerative diseases (cardiovascular disease and cancer) and lessening of the effectiveness of the immune system. Little research has examined lipid peroxidation associated with high intensity resistance exercise, nor the protective effects of antioxidant supplementation. Additionally training state, which could conceivably affect one's ability to counter free radical damage, has not been adequately studied. Fourteen untrained participants and 13 trained participants took part in a double blind, placebo controlled crossover experimental design study. They received 885 mg tocopherol acetate/day for a 2 week period, received the placebo during another 2 week period and at the end of each period performed the Resistance Exercise Test (RET). A 2 group (trained vs untrained) x 2 treatment (vitamin E vs placebo) x 3 time (pre exercise vs immediate post exercise vs 6 hours post exercise) factorial ANOVA with repeated measures on time was performed on Malondialdehyde (MDA) values. There were no significant group or treatment effects found between the 4 groups assessed. There was only one significant difference found and that was in the main effect for time ($F [2,49] = 22.41$, $p = 0.00$). This study concluded that the RET caused a significant increase in MDA in all 4 groups at 6 hours post exercise. There was no evidence that vitamin E supplementation was effective in reducing oxidative damage in comparison to the placebo group. As well, there was no difference between the trained and untrained groups with respect to their impact on lipid peroxidation measures.

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CHAPTER 1

INTRODUCTION

Moderate to high intensity aerobic-type exercise produces an increase of oxygen free radicals that can cause damage to lipid membranes, proteins, deoxyribonucleic acid (DNA) and other cellular components (Dragon et al., 1991; Kanter et al., 1993; Lovlin et al., 1987). The damage to lipid membranes, otherwise known as lipid peroxidation, is defined as the oxidative deterioration of polyunsaturated fats in cellular lipid membranes caused by oxygen free radicals. It might be expected that there may be similar increases in free radical formation and lipid peroxidation caused by resistance exercise and aerobic-type exercise, but perhaps produced by different mechanisms.

In resistance exercise, the ischemia-reperfusion injury is a widely held hypothesis (McCord, 1985). Intense muscle contractions can result in a temporary decrease in blood flow and oxygen availability and subsequent ischemia. The following reperfusion period (muscle relaxation) produces an abundant reintroduction of O_2 and results in the formation of the O_2^- radical. Mechanical stress is another hypothesis used to explain an increase in free radicals. In particular, eccentric exercise causes high levels of force that has been shown to initiate muscle tissue damage. This initiates the inflammation process that eventually produces oxygen free radicals (Saxton et al., 1992). Research is required to investigate whether high intensity resistance exercise produces a significant increase in lipid peroxidation.

The area of antioxidant supplementation and exercise and subsequent effects on free radical production and lipid peroxidation has attracted much attention in recent years. Some studies involving vitamin E antioxidant supplementation and aerobic exercise have shown that vitamin E was effective in reducing exercise induced lipid peroxidation (Sumida et al., 1989; Rokitzki et al., 1994). Nevertheless, there has been mixed results. Little research has been done with high intensity resistance exercise while exploring the potential beneficial effects of antioxidant supplementation on attenuating possible free radical formation and lipid peroxidation.

Most of the research exploring the effects of training and its impact on antioxidant enzymes has been with aerobic-type exercise. Aerobic training has been shown to enhance internal antioxidant enzymes and make subjects less susceptible to free radical damage (Allesio and Goldfarb, 1998; Robertson et al., 1991). These studies have suggested that beginner exercisers might derive a greater benefit from nutrition supplements than the trained exerciser, due in part because of their less developed antioxidant enzyme system. Research is thus required to determine the role that resistance training plays on the internal

antioxidant system. Subsequently, does resistance training over an extended period of time increase the body's internal antioxidants and will the trained state interact with vitamin E supplementation in reducing free radical production ?

Vitamin E supplementation has produced increases in plasma vitamin E (α tocopherol) levels in the human body (Princen et al., 1995; Baker et al., 1996). The most frequently used analysis of vitamin E involves the measure of plasma vitamin E levels. A few studies have shown that plasma vitamin E levels will increase 2x the normal concentration within 2 weeks with 800 to 1200 mg of α tocopherol supplementation (Machlin and Gabriel, 1982; Dimetrov et al., 1991). It is important to measure and observe plasma vitamin E changes as a result of supplementation. This eliminates some potential confounding variables (seasonal variations) or problems with the treatment (substandard vitamin E) itself. It also enables the researcher to be confident in making statements with regards to a successful treatment (increase in plasma vitamin E) and it's effect on the dependent variable (MDA).

Purpose

The primary objectives of this investigation are to determine: (1) whether a high intensity, whole body Resistance Exercise Test (RET) will cause lipid peroxidation measures to significantly increase, (2) the effects of a 2 week period of antioxidant vitamin supplementation (Vitamin E) on the levels of lipid peroxidation measures caused by the RET, and (3) the impact that the training state (untrained vs trained) will have on possible lipid peroxidation measures caused by the RET. In addition, the interaction of training state and antioxidant supplementation will be investigated.

Secondary purposes are to confirm whether a 2 week treatment period with vitamin E supplementation will significantly increase blood plasma vitamin E levels and whether a 4 week washout period will return blood plasma vitamin E levels to baseline measures.

These purposes will be achieved by implementing a double blind, placebo-controlled crossover experimental design.

The researchers hypothesize that the RET will significantly increase lipid peroxidation. Secondly, both the vitamin E supplementation and the trained resistance participant will aid in significantly reducing the lipid peroxidation measures. The plasma vitamin E levels will be changed as mentioned above.

Significance of the Study

The potential benefits of antioxidant vitamin supplementation that quench oxygen free radicals may be enormous. It is hypothesized that damage to lipids caused by the oxygen

free radicals can result in many problems such as the inactivation of cell membrane enzymes, increased permeability of ions across membranes (decreased muscle cell functioning) and increased platelet aggregation in blood vessels (Esterbauer et al., 1989; Galeotti et al., 1990). Other problems may include the progression of degenerative diseases (cardiovascular disease and cancer) and lessening of the effectiveness of the immune system (Seyama, 1993; Halliwell and Chirico, 1993). Antioxidant supplementation may provide protection against negative health consequences caused by resistance exercise. If this is true, there will be health care savings within the medical field.

If the vitamin E supplementation proves to be successful in decreasing resistance exercise induced lipid peroxidation, another benefit will also develop. The marketing of vitamin E will increase considerably. If there were a 'no effect' result with the vitamin E supplementation, individuals will then save money that would have been wasted on supplementation. They will look for other ways to combat the potential damage caused by exercise induced lipid peroxidation.

If the trained participant realizes a decrease in exercise induced lipid peroxidation in the study, trained participants will gain the effects of long term training: the protection of the human body from increased levels of exercise induced oxygen free radicals.

Definitions

Antioxidants: are substances that help reduce the severity of oxidation damage by forming a lesser active radical or quenching the reaction. Vitamin E, vitamin C and beta carotene are the most well known nutritional antioxidants (Goldfarb, 1993). The most important chain breaking antioxidant in human lipids is vitamin E (α tocopherol) (Burton and Ingold, 1989).

Heavy Resistance 10/1 Protocol: 10 represents the 10 RM load and 1 for the 1 minute rest periods between exercises (Kraemer et al., 1992).

Malondialdehyde (MDA): During the Thiobarbituric Acid (TBA) biochemical assay, TBA is added to the blood plasma and reacts with the MDA (an aldehyde) found in the lipid hydroperoxides. MDA can then be measured and serves as a measure of lipid damage (Chirico, 1994).

Oxygen Free Radicals: are oxygen species that carry one or more unpaired electrons that are highly reactive (Sjodin et al., 1990). They include such species as Superoxide (O_2^-) or Hydroxyl ($OH\cdot$) which attack molecules by removing hydrogen.

Repetition Maximum (RM): One Repetition Maximum (1 RM) is the maximum weight that a participant can lift for only one repetition in a specific exercise (Wathen, 1994). A

Ten Repetition Maximum (10 RM) occurs when a participant can lift a particular weight 10 (not 11) repetitions on a specific exercise (Wathen, 1994).

Resistance Exercise Test (RET): a high intensity, whole body, modified circuit training session that provides significant overload to the working muscles. The RET that will be used was initially used by McBride et al. (1997). It produced significant increases in oxygen free radicals in recreationally trained (minimum of 1 year) resistance athletes and resulted in increases in lipid peroxidation. The intensity of the exercise protocol used is a primary factor in creating a physiological environment for increased free radical production.

Limitations

1) A methodology concern has been identified within this field of study. The reference values for Malondialdehyde (MDA) utilizing the modified TBA test with Butylated Hydroxytoluene (BHT) have not been reported often and therefore require further investigation.
2) The analysis of the dietary intakes will be dependent upon the accuracy of the information recorded by each participant, on whether the three days analysed will represent the true intakes, on the accuracy and consistency of the investigators interpretations and the accuracy of the nutrition software program Foodworks (2000).
3) The analysis of the measures within the daily log will be dependent upon the accuracy and consistency of the information recorded by each participant.
4) Compliance in ingesting the prescribed supplement will be checked by pill counts, but ultimately compliance is dependent on the honesty of the subject.
5) The analysis of the intensity of the RET will be partly restricted to the accuracy of determining the 10 repetition maximum for each participant.
6) The analysis of the intensity of the RET will also be partly restricted to each participant doing each exercise correctly and with full effort.
7) The analysis of the intensity of the RET will also be partly restricted to the subjective ratings provided by the participants with the Borg CR-10 scale (Noble et al., 1983).

Delimitations

1) Participants will include 27 apparently healthy males and females who range in age from 19 to 30 years of age and reside in Thunder Bay, Ontario, Canada.
2) Resistance participants will include individuals who either matched the trained or untrained grouping criteria.
3) Participants will include individuals with normal plasma α tocopherol concentrations between 12 to 37 umol/L (Vitamin E Research and Information Service [VERIS], 2000).

4) Screening will include the absence of malabsorption syndromes, medical problems, drugs or supplements (within 1 month of the study and during) and smokers.
5) Blood assays will include malondialdehyde, vitamin E, haemoglobin and hematocrit.
6) Dietary analysis will be conducted during the two treatment periods only.
7) Self-reported variables in the training log will include number of workouts/week, length of exercise session, level of muscle soreness, minor injuries and illnesses, stressful events, gastrointestinal difficulties.
8) Only 885 mg/day α tocopherol acetate will be administered as supplementation.
9) Only 2 week treatment periods will be utilized with a 4 week washout period between.
10) The RET will be a high intensity resistance test consisting of a modified circuit involving 8 different resistance exercises.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The purpose of this chapter is to: a) review the production of oxygen free radicals and subsequent lipid peroxidation, b) present an overview of the techniques used to assess lipid peroxidation, c) outline the functions of vitamin E and explain the movement of vitamin E within the human body, d) discuss various forms of exercise in relation to how they may possibly affect oxygen free radicals and lipid peroxidation, and lastly e) consider the training state of the individual while determining if exercise and supplementation has an impact on lipid peroxidation measures.

All of these concepts lead up to the review by which vitamin E antioxidant supplementation may affect possible exercise induced oxygen free radicals and lipid peroxidation.

OXYGEN FREE RADICALS AND LIPID PEROXIDATION

Oxygen Free Radicals

Oxygen is essential for human life but in some forms can be very damaging to the body. Thousands of oxygen free radicals are produced in each resting cell every day. It has been estimated that for every 25 oxygen molecules reduced by normal respiration, one oxygen free radical is produced (McCord, 1979). Living organisms have developed a sophisticated antioxidant system to cope with the damaging products of oxygen reduction (Ji, 1992). These systems include chemical compounds such as vitamins E and C, beta carotene, and glutathione (G), as well as a series of enzymes (superoxide dismutase [SOD], catalase [CAT] and glutathione peroxidase [GPX]) specializing in reducing free radicals to more stable species. The existence of other free radicals that are not oxygen centered such as the trichloromethyl radical also exist in the human body and they can lead to cell damage as well.

Oxygen free radical generation occurs by means of an increased electron flux through the cytochrome chain in the mitochondria electron transport system and subsequent interaction of the electrons with molecular oxygen (Kanter, 1995). Molecular oxygen (O_2) has two unpaired electrons and therefore the oxygen molecule can accept four electrons at most and forms water. However, the addition of one, two or three electrons to O_2 will produce superoxide radical (O_2^-), the radical form of hydrogen peroxide (H_2O_2) or the hydroxyl radical (·OH) respectively (Kanter, 1995). Also, the initial formation of O_2^- can also produce the addition of H_2O_2 (in radical form) and ·OH radicals through the following reactions (Clarkson, 1995; Gutteridge, 1994):

$$O_2^- + O_2^- + 2H+ = H_2O_2 + O_2$$

With the presence of transitional metal ions such as ferrous iron (Fe^{2+}) or cuprous copper (Cu^+) present, the formation of the most highly reactive oxygen free radical ·OH is enhanced. This radical is the primary source of the destructive mechanism of oxygen free radicals (Cheeseman and Slater, 1993).

$$H_2O_2 + Fe^{2+} = \cdot OH + OH^- + Fe^{3+}$$

The amount of oxygen free radicals produced appears to be related to exercise intensity. During heavy prolonged exertion, the energy for increased metabolic demand is produced by oxidative metabolism and this increases the risk for oxidative stress (Allessio, 1993). The rate of O_2 consumption may increase 10 to 15 fold and the rate of O_2 flux into working muscles may increase 100 fold (Sen, 1995). When O_2 is used in metabolism, most ends up combined with hydrogen to form water. However, aerobic metabolism also produces

energy, waste products such as carbon dioxide and a small, but steady stream of oxygen free radicals. The generation of oxygen free radicals is increased during exercise; four to five percent of O_2 will form O_2^- (Dekkers et al., 1996).

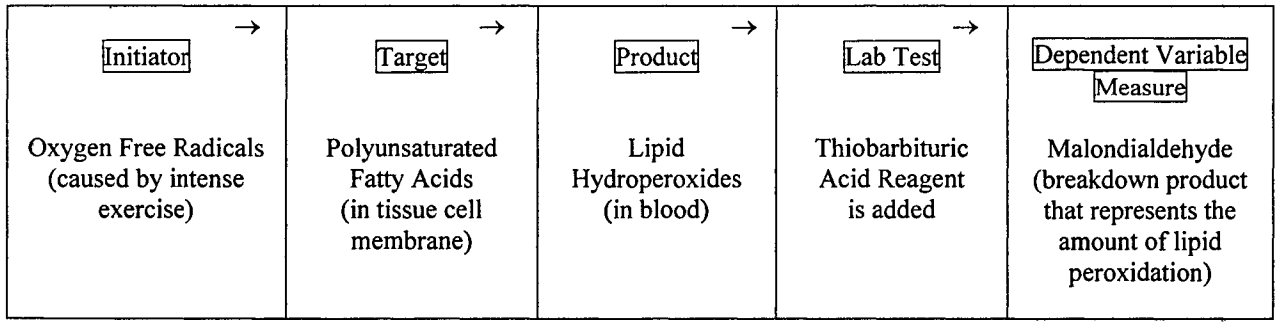
Oxygen free radicals damage the body cells by attacking lipids, proteins and DNA. In the particular case of lipids, the ·OH radical, O_2^- radical and the radical form of H_2O_2 attack polyunsaturated fatty acids found in tissue cellular membranes. This causes a hydrogen atom to be removed at the carbon chain and a lipid radical is formed. This is the start of lipid peroxidation

Lipid Peroxidation

The following explanation outlines the process of lipid peroxidation from the production of oxygen free radicals to the formation of the Thiobarbituric $Acid_2$ – Malondialdehyde ($[TBA]_2$ – MDA) product (Halliwell and Chirico, 1993). Polyunsaturated fatty acids (PUFAs), as mentioned above, found in tissue cellular membranes are attacked by the · OH, O_2^- and H_2O_2 free radicals causing a carbon-centred lipid radical to be produced. This lipid radical further reacts with similar lipid radicals or other fatty acid side chains. The result is free radical chain reactions. Lipid hydroperoxides are then formed within the cell membrane and leak into the blood. The decomposition of lipid hydroperoxides results in the formation of aldehydes of various chain lengths. Malondialdehyde (MDA), a three carbon chain aldehyde is one of the primary aldehydes formed. During the Thiobarbituric Acid (TBA) assay, TBA is added to blood plasma and combines with MDA, which then serves as the measure of free radical damage to lipids (Chirico, 1994).

Figure 1. The Process and the Measurement of Lipid Peroxidation

The following diagram outlines the stages that occur in lipid peroxidation starting with oxygen free radicals attacking the target (PUFA's) to the production of the measurable end product, MDA.



Assessment Methods of Lipid Peroxidation

Many assays are available to measure lipid peroxidation, but no single assay is an accurate measure of the whole process (Halliwell and Chirico, 1993). The extent of lipid peroxidation can be determined by measuring the following: losses of unsaturated fatty acids at the Target stage (see above Figure 1. Lipid Peroxidation), the amounts of primary peroxidation products at the Product stage and the amounts of secondary products at the Dependent Variable stage. The biomarkers used to detect lipid peroxidation include thiobarbituric acid reactive substances (TBARS), conjugated dienes, pentane, ethane, F_2-isoprostanes and electron spin resonance (ESR) products (Institute of Medicine, 2000). They are measured in plasma, tissue, urine or breath. The terms TBARS, TBA, MDA and $[TBA]_2$- MDA have been used interchangeably.

Halliwell and Chirico (1993) have summarized the available methods of measuring lipid peroxidation in biological systems. They state that the increased concentration of end products of lipid peroxidation (MDA) is the evidence most commonly used and frequently quoted for the involvement of free radicals in human disease (Halliwell and Chirico, 1993). Some flaws have also been identified with some of the techniques. For example, Halliwell and Gutteridge (1993) state that although hydrocarbon gases (pentane and ethane) constitute a noninvasive means of measuring lipid peroxidation, the gases are considered to be minor end products of lipid peroxidation.

It is possible to detect carbon and oxygen centered radicals by electron spin resonance (ESR) combined with the use of spin traps. Kanter (1995) states that this is the only method that can directly detect the presence of free radicals, but the use of ESR in vivo is in its infancy. The use of spin traps or ESR take advantage of the physical properties of radicals as they spin and measures the strength of the signals emitted. The greater the resonance signal peak, the greater the free radical production (Alessio, 1993). Nevertheless, Yaki (1994) states that "the radical species disappear very rapidly and although radical species can be measured by use of trapping agents under special conditions such as in vitro experiments, this cannot be applied to the clinical specimens, since the radical reaction occurring in the specimens has already been terminated by the time of analysis (p. 3)". Recent papers have also identified a series of F_2-isoprostane like compounds that may arise as a result of lipid peroxidation. Specificity is achieved by the use of antibody techniques directed against low density lipoproteins that have undergone peroxidation, and now these can also be applied to plasma samples. Halliwell and Chirico (1993) state that further work is required to evaluate the possibility that these markers may be useful.

A few problems associated with the original TBA test involve non specificity and amplification of the MDA biomarker. Now, more and more researchers are separating the various peroxidation products before measuring them. This is done by using High Performance Liquid Chromatography (HPLC). It improves the specificity of the TBA test. The TBA test was further modified by Chirico and Halliwell (1993) and Chirico

(1994) (Appendix C). During the heating stage of the test, the amplification of peroxidation is prevented by adding the chain breaking antioxidant Butylated Hydroxytoluene (BHT) to the sample before the TBA reagents. Chirico and Halliwell (1993) further add that the modified test avoids the problems of the original test and should be the method of choice for investigation for lipid peroxidation in human material.

VITAMIN E

Background and Dietary Intakes

Vitamin E is an essential, fat soluble vitamin; it is used generally as a term for a group of tocopherols and tocotrienols. Alpha tocopherol is the most biologically active form (Bjorneboe et al., 1990). Of the eight naturally occurring forms of vitamin E only the α tocopherol form (as opposed to δ, β and γ tocopherols and tocotrienols) of vitamin E is maintained in human plasma (Institute of Medicine, 2000). Other naturally occurring forms of vitamin E do not contribute toward meeting the vitamin E requirement because although absorbed, they are not converted to α tocopherol by humans and are poorly recognized by α tocopherol transfer protein (α TTP) in the liver.

The richest dietary source of vitamin E includes vegetable oils, nuts, whole grains, green plants, cereal grains and wheat germ. The highest categories include fats and oils used as spreads which contributed 20.2 percent of the total vitamin E; vegetables, 15.1 percent; meat, poultry and fish, 12.6 percent (Murphy et al., 1990). The recommended dietary allowance (RDA) for adults in Canada and the United States is 15 mg/day (22.5 International Units [IU]) of α tocopherol (Institute of Medicine, 2000). The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 %) apparently healthy individuals in a particular life stage or gender group (Institute of Medicine, 2000). The RDA is intended to be used as a goal for daily intake.

Vitamin E deficiency is seen rarely in humans (Vitamin E Research and Information Science [VERIS], 1993; Institute of Medicine, 2000). Upon review of 2 major surveys and 2 studies, the Institute of Medicine (2000) stated that the mean intakes of healthy adults in the United States and Canada are likely to be above the RDA of 15 mg/day of α tocopherol. Therefore, current dietary patterns appear to provide sufficient vitamin E to prevent deficiency syndromes (Institute of Medicine, 2000). Deficiency syndromes are usually as a result of lipid fat malabsorbtion syndromes or lipoprotein deficiencies.

Absorption and Distribution

In humans, orally administered deuterated α tocopherol is first secreted from the intestine in chylomicrons into the intestinal lymph and transported to the liver. It is then transferred out from the liver in very low density lipoproteins (VLDL), is metabolized and appears in blood associated with low and high density lipoproteins (LDL and HDL) (Kayden and Traber, 1993). Low density lipoprotein (LDL) is the lipoprotein fraction that contains most alpha tocopherol in humans.

The precise rate of absorption in humans is not known, but the efficiency of vitamin E absorption varies considerably (Institute of Medicine, 2000). The fractional absorption of vitamin E in humans has been estimated to be from 35 to 80 %, based on fecal recovery and an oral dose of radioactive α tocopherol (Kelleher and Losowsky, 1970). The amount of vitamin E absorption in the human body depends largely on three factors – fat absorption, bile acids and the α TTP. Most dietary vitamin E is found in foods that contain fat (Institute of Medicine, 2000). Therefore, the absorption of vitamin E depends on an individual's ability to digest and absorb fat.

Vitamin E absorption also requires the presence of bile acids for micelle formation (Kayden and Traber, 1993). Bile acids are produced in the gall bladder and function to aid in digestion of dietary fat. The level of vitamin E in the plasma is also limited by the ability of α TTP that is located in the liver. It incorporates vitamin E into VLDP (Tiidus and Houston, 1995). The α TTP discriminates between tocopherols and is responsible for the preferential plasma enrichment with α tocopherol (Institute of Medicine, 2000).

Human tissues are dependent upon vitamin E from blood plasma (Traber, 1999). Vitamin E rapidly transfers between lipoproteins and also between lipoproteins and tissue membranes. Vitamin E concentrations in body tissues vary considerably; the highest levels are in lipid rich cell fractions. The liver, adrenal glands, skeletal muscle and adipose tissue have the capacity to accumulate the largest amounts of α tocopherol (Bjorneboe and Drevon, 1989; VERIS, 1993). Plasma concentrations of α tocopherol largely represent changes in tissue α tocopherol concentrations (Burton et al, 1998).

Physiological Role and Other Functions

Unlike most nutrients, a specific role for vitamin E in a required metabolic function has not been found (Institute of Medicine, 2000). The major function of vitamin E is to work as a chain-breaking antioxidant in a fat soluble environment that prevents the propagation of free radical reactions (Burton and Ingold, 1986; Packer, 1984). It is probably the most focused upon and important non-enzymatic antioxidant substance in the body. The vitamin is a lipid radical scavenger and especially protects polyunsaturated fatty acids (PUFA) within membrane phospholipids and in plasma lipoproteins (Burton et al., 1983).

Lipid radicals react with vitamin E 1,000 times more rapidly than they do with PUFA (Packer, 1994). Vitamin E provides an easily donated hydrogen to the lipid reaction and an antioxidant radical is created (Halliwell and Chirico, 1993). Then, the new antioxidant radical combines with other antioxidant radicals and disappears.

Other vitamins such as vitamin C have antioxidant capabilities. As well, vitamins function in different environments. Vitamin C acts as an antioxidant as it quenches a variety of reactive oxygen species and reactive nitrogen species in aqueous environments. Evidence for in vivo antioxidant functions of ascorbate acid include the scavenging of reactive oxidants in activated leukocytes, lung, and gastric mucosa and diminished lipid peroxidation as measured by urinary excretion (Institute of Medicine, 2000). Beta carotene, associated with vitamin A is also a scavenger of single oxygen (Bucci, 1995).

Vitamin E may have significant impacts with regards to the prevention of chronic diseases such as cardiovascular disease and cancer and the improvement of the immune function. Vitamin E inhibits LDL oxidation as seen in culture, which is a causative agent in the development of cardiovascular disease (Steinberg et al., 1989). It could also affect atherosclerosis at a number of steps; vitamin E inhibits platelet adhesion, aggregation and platelet release reactions in vitro (Freedman et al., 1996). The Institute of Medicine (2000) states that there has been some support at the clinical level for the effect of vitamin E on cardiovascular disease, but additional large, randomized, intervention studies are required.

Cancer is believed to develop as the result of an accumulation of mutations that are unrepaired (Institute of Medicine, 2000). Deoxyribonucleic Acid (DNA) is constantly undergoing damage due to interaction with free radicals, and therefore one mechanism by which vitamin E might inhibit cancer formation is by quenching these free radicals. Overall, the epidemiological evidence for an effect of vitamin E on cancer is weaker than that for vitamin E and cardiovascular disease. At this point, studies provide only limited evidence for a protective association and only for some cancer sites (Institute of Medicine, 2000). Halliwell (1999) states that Vitamin E has not been shown to directly protect DNA or proteins against oxidative damage.

It has been established that several aspects of immune function decline with increasing age (Bendich, 1994). In addition, supplementation with vitamin E is able to reverse some of these deficits in some individuals. Meydani et al. (1997) studied 88 healthy subjects of at least 65 years of age. After supplementation with 200 or 800 mg/day of vitamin E for 235 days, they had higher antibody responses to hepatitis B vaccine. Pallast et al. (1999) found that the number of T helper cell dependent immune functions increased with increasing doses of vitamin E in elderly persons aged 65 – 80 yr. The positive results from limited research are strong enough to support continued research (Institute of Medicine, 2000).

Supplement Intakes and Washout

A range of doses of vitamin E supplementation has been used to increase levels of plasma α tocopherol within the human body. The effectiveness of vitamin E supplementation has been most frequently determined by measuring the plasma α tocopherol levels. Bieri et al. (1983) have found that in general, a 10 fold increase over and above dietary intake of vitamin E intake is required to double baseline plasma α tocopherol concentrations. Baseline measures in normal populations of alpha tocopherol levels range from 12 to 37 umol/L (Vitamin & Research and Information Service [VERIS], 2000]. Princen et al. (1995) produced concentrations of α tocopherol in plasma that were 2.6 times higher than baseline values after 2 weeks of 540 mg/day of α tocopherol acetate.

In other studies, oral supplementation with large amounts of vitamin E (as much as 100 times the daily requirement of 15 mg), plasma α tocopherol concentrations increased 2 – 4 fold (Dimitrov et al., 1991; Farrell and Bieri, 1975). Machlin and Gabriel (1982) suggest that it is advisable to treat subjects with high levels (400 to 1600 mg/day) of vitamin E for at least 1 – 3 wk to attain maximal plasma levels. Baker et al. (1996) showed that the feeding of 800 mg/day of vitamin E significantly increased plasma vitamin E after only 2 days. This increase indicates that supplement vitamin E is absorbed into blood plasma quickly.

Vitamin E occurs in a natural free alcohol form, but acetate and succinate derivatives are often used in natural and synthetic vitamin E supplements. Acetate or succinate is added to the chromanol ring of the free alcohol form of vitamin E and this prevents its oxidation and extends the shelf life. The esters of alpha tocopherol are considerably more stable and therefore offer greater latitude in product formulation. Vitamin E acetate, which is an oil, can be used in liquid vitamin drops and soft gelatin capsules because of its great stability (Institute of Medicine, 2000). Vitamin E acetate and succinate esters are readily hydrolyzed in the digestive tract to the biologically active free tocopherol and are absorbed as efficiently as α tocopherol (Chessemen et al., 1995; VERIS, 2000). Prior to absorption, pancreatic enzymes are believed to participate in removal of the acetate and then the free tocopherol is absorbed (VERIS, 1993).

An effective washout period is necessary to produce usable results when using a Two Period Crossover Research design (Armitage and Hills, 1992). The main focus of research with vitamin E has been on attempts to increase plasma α tocopherol levels using various treatment periods and doses of supplementation. Therefore only a few studies have been done that have indirectly shown the washout effects of vitamin E after supplementation. Discontinuation of the vitamin E supplement has been associated with a decline in plasma α tocopherol that returned to pretreatment conditions in just over 2 weeks (Baker et al., 1980; Dimetrov et al., 1996). It took 15 days for vitamin E plasma levels to decrease to baseline levels after 21 days of 800 mg/day of synthetic dl-α tocopherol acetate (Baker et al., 1980). Dimetrov et al. (1996) also found that after

supplementation with 807 mg/day of α tocopherol acetate for 4 weeks, plasma tocopherol levels returned to baseline within 16 days after discontinuation.

Safety

The Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals (Institute of Medicine, 2000). In a review of the literature, Bendich and Machlin (1988) indicated that relatively few side effects have been reported in double blind studies of vitamin E even at high doses as high as 3,200 mg/day. They further stated that in six double blind placebo controlled studies involving oral intake of 600 – 3200 mg/day vitamin E for 3 weeks to 6 months conducted since 1974, very few adverse side effects were noted and no specific side effect was consistently noted in all the studies. In another review of vitamin E toxicity studies, it was concluded that humans show few side effects following supplemental doses below 2,100 mg/day of tocopherol (Kappus and Diplock, 1992). The Institute of Medicine (2000) has set the UL for adults at 1,000 mg/day of any form of α tocopherol supplementation based on the adverse effect of increased tendency to hemorrhage. The Vitamin E Research Institute Service (2000) also states that vitamin E is safe and very well tolerated over a wide range of daily doses over long periods of time. As well, it should be noted that excess absorbed tocopherols are readily excreted in bile, due to the limiting capacity of the α TTP (Kayden and Traber, 1993).

EXERCISE AND LIPID PEROXIDATION

Aerobic Exercise and Lipid Peroxidation

A number of studies have shown that aerobic type exercise of sufficient intensity and duration produces oxygen free radicals that cause significant damage to lipid membranes (Lovlin et al., 1987; Dragon et al., 1991; Kanter et al., 1993). As mentioned earlier, lipid peroxidation is defined as the oxidative deterioration of polyunsaturated fats in cellular lipid membranes caused by oxygen free radicals. Kanter et al. (1993) utilized 20 young males who performed 30 minutes of treadmill running at 60 % of VO_2 max followed by 5 minutes at 90 % VO_2 max. Significantly elevated markers of lipid peroxidation (MDA in blood serum and breath pentane samples) were produced. Lovlin et al. (1987) measured plasma levels of MDA of 8 healthy men during an intermittent graded cycling test to exhaustion. There was a significant increase in MDA when samples were taken immediately after the exercise session (resting value of 2.26 mmol/l to a final value of 2.88 mmol/l). The authors concluded that strenuous exhaustive exercise induced free radical generation.

A few studies have produced contradictory findings with regards to the effects of exercise on lipid peroxidation. Possible reasons for such findings could include the nonspecificity of the measuring device used to measure lipid peroxidation (Wong et al., 1987), the timing of the lipid peroxidation measure (Maughan et al., 1989), the absence of analysis of plasma volume changes due to exercise (Surmen-Gur et al., 1999), the intensity and mode of exercise investigated (Kanter and Eddy, 1992) and the training state of the athlete (Maxwell, 1993).

For example, Maxwell et al. (1993) had 24 healthy students perform 1 hr of box stepping at 60 % of VO_2 max. There was no significant increase in blood plasma MDA and subsequent lipid peroxidation. It was suggested by the author that the mode of exercise or intensity of exercise were important variables that contributed to the absence of a significant increase in free radical production.

Resistance Exercise and Lipid Peroxidation

It might be expected that there would be similar increases in free radical production and lipid peroxidation with resistance exercise as is evidenced with aerobic exercise. Only a small number of studies have investigated though. Some of the these studies (Sahlin et al., 1992; Saxton et al., 1994; Ortenblad et al., 1997) have demonstrated no evidence of free radical production during resistance exercise. Sahlin et al. (1992) had 7 healthy men perform repetitive static exercise (10 seconds on and 10 seconds off with two legged intermittent knee extensions) for 80 minutes or to fatigue. There was no increase in lipid peroxidation measures. Similar results were produced by Saxton et al. (1994) who monitored plasma and skeletal muscle markers of free radical damage in 14 males subjected to alternate bouts of concentric and eccentric muscle actions. Lipid peroxidation as shown by plasma markers (TBARS) and skeletal muscle markers (MDA) did not change immediately after exercise, prompting the authors to conclude that oxygen free radicals were not involved in exercise induce lipid peroxidation and muscle damage. Ortenblad et al., (1997) had 8 trained, elite male volleyball players and 8 nontrained males perform a series of 6 bouts of maximal continuous jumping for 30 seconds with 2 minute rest periods between each bout. Plasma and muscle MDA after the jump test were not significantly different from rest.

There may be a few reasons why resistance studies produce conflicting results with regards to the production of free radicals and lipid peroxidation. Maughan et al. (1988) and McBride et al. (1997) showed that MDA concentrations had peak values observed at 6 hours post exercise. Perhaps some investigators have not taken this into consideration. Alessio et al. (1999) had subjects perform maximum voluntary contractions of a handgrip dynamometer (isometric exercise) until exhaustion and measured 2 biomarkers of oxidative stress. They compared the scores to those obtained on a treadmill test to exhaustion. Levels of MDA were not affected by either high intensity isometric exercise

or the aerobic exercise, while levels of lipid hydroperoxides increased 36 % above rest during isometric exercise and 24 % during aerobic exercise. The study produced conflicting results; the authors concluded that there was evidence of oxidative stress after both exhaustive aerobic and isometric exercise. Perhaps, elevated MDA values did not appear because the time course affects of MDA were not considered.

Another reason for inconsistent results could be attributed to exercise intensity. There are a variety of resistance exercise sessions that could be used as potential inducers of oxygen free radicals and lipid peroxidation. McBride et al. (1997) believed that the intensity of the exercise protocol, plus the number of muscle groups exercised is vital in causing measurable changes in MDA. McBride et al. (1997) indicated that two studies (Sahlin et al., 1992; Saxton et al., 1994) used resistance exercise involving low intensities, involved only a limited number of muscle groups and as well produced lower lactate levels. McBride et al. (1997) felt that this is why there was no increase seen in lipid peroxidation measures in the two studies.

McBride et al. (1997) felt that studies involving high intensity resistance exercise including several of the body's major muscle groups (similar to a typical resistance training workout) were required. Therefore within their study, McBride et al. (1997) used a heavy resistance, whole body protocol (8 different exercises) during the resistance exercise test (RET). It consisted of a protocol similar to the 10/1 protocol used by Kraemer et al. (1992). There was a significant increase in plasma MDA concentration from pre exercise to post exercise test in both the placebo and supplement group. They concluded that the heavy resistance protocol used (3 sets of 8 exercises at 10 RM) in combination with the selected rest periods (2, 1.5 and 1 minute) created a physiological stress that was conducive to an increase in free radical formation.

It is also important to consider that potential injuries and early fatigue could result from a RET with a high intensity level. Resistance participants can have large differences in resistance training experience and strength. Subsequently, a RET will need to meet the needs of the selected participants and groups involved. Wathen (1994) recommends that beginner participants in a resistance training program use moderate volumes of exercise in the early stages of training; workouts should include one to three sets of 6 to 12 repetitions. In the McBride et al. (1997) study, each participant performed 3 sets of each exercise at his 10 repetition maximum (RM). This format met the guidelines outlined by Wathen (1994) in relation to working with beginner participants.

It has been proposed that factors other than the main principle (electrons interacting with molecular oxygen in the mitochondria transfer chain) can influence the production of free radicals (Alessio et al., 1999). Intense muscle contractions associated with resistance exercise could result in a temporary decrease in blood flow and oxygen availability and subsequent ischemia-reperfusion at the site of the active muscle. The ischemia-reperfusion injury is a widely held hypothesis. Ischemia and the following reperfusion period have been shown to constitute a powerful stimulus of free radical generation

through the xanthine oxidase reaction (McCord, 1985; Clarkson and Tremblay; 1988; Friedl et al., 1990). McCord (1985) adds that this occurs as a result of catabolism of adenosine triphosphate to hypoxanthine and the conversion of xanthine dehydrogenase to the enzyme xanthine oxidase during the ischemic period. The abundant reintroduction of O_2 during the reperfusion period (muscle relaxation) results in the formation of the O_2^- radical.

Another mechanism for resistance exercise induced free radicals may also include mechanical stress. In particular, eccentric exercise, where the muscle generates high levels of force while lengthening have been shown to initiate muscle tissue damage and the inflammation process (Saxton et al., 1992). Inflammation also occurs with unaccustomed exercise. There is a strong possibility that there is a relationship between muscle damage and free radical activity following exercise.

Activation of circulating neutrophils and monocytes in blood and macrophages in tissue (phagocytosis) occurs as part of the inflammatory response and the body's natural immune response to injury. This process is also called the respiratory burst, which is accompanied by increased activity of the enzyme nicotinamide adenine dinucleotide (NADH) that results in an increased production of both the O_2^- and H_2O_2 radicals (Weiss, 1986; McCully and Faulkner, 1985).

From the mechanisms mentioned above, it would be logical to contrive that resistance training may result in a significant rise in the production of oxygen free radicals in the active muscle site. Therefore, it is possible that a resistance protocol involving a stimulation of great muscle mass at a high intensity may result in lipid peroxidation.

A question still unresolved with regards to the effects of resistance exercise on free radicals is: are free radicals products of muscle tissue injury or do free radicals actually promote exercise induced free tissue injury ? One study by Zerba et al., (1990) found that the loss of force production immediately after eccentric exercise in older mice was attenuated by treatment with the free radical scavenger superoxide dismutase suggesting oxidative damage could contribute to the muscle tissue injury.

Gender Differences

Much research has been done in the area of antioxidant supplementation, exercise, the production of oxygen free radicals and lipid peroxidation. Eighty percent of past human research has utilized male participants only. Some studies (Dillard et al., 1978; Maxwell et al., 1993; Gopal et al., 1999) have used female and male human participants, but only the Gopal et al. 1999 study attempted to make observations and conclusions based on gender differences. They suggest that females that exercised in the luteal and follicular phases of their menstrual cycle have elevated estrogen or estradiol levels. Estrogen has been show to have antioxidant properties. Nevertheless, the study concluded that there

were no differences between females and males for post exercise plasma MDA, lipid hydroperoxides and blood glutathione. In a study done by Tiidus and Houston, 1993, the researchers found that female rats are less susceptible than male rats to exercise induced lipid membrane damage, due to higher levels of estradiol, which resulted in membrane stability.

Kanter (1998) states that little is known about the effects of the monthly menstruation cycle and blood loss (coupled with exercise) on antioxidant requirements. No concrete conclusions have been made with regards to whether male and female participants produce the same amount of free radicals and lipid peroxidation due to an exercise bout. As well, the effects of antioxidant supplementation and the trained state on lipid peroxidation have not been explored with regards to gender differences.

VITAMIN E SUPPLEMENTATION

Vitamin E Supplementation, Aerobic Exercise and Lipid Peroxidation

There is a lot of evidence that suggests that antioxidant supplementation (particularly vitamins E and C) has favourable effects on markers of lipid peroxidation following exercise (Kanter, 1998). Still, the overall results are somewhat unclear, as considerable contradictory data does exist (see Table 1). Table 1 was prepared in order to examine past studies that demonstrated the effects of vitamin E supplementation on exercise induced (aerobic and resistance) lipid peroxidation in vitamin E sufficient human participants. A comprehensive search was performed and 10 vitamin E studies were selected and analyzed. Some of the reasons for such conflicting findings could be due in part to the difference in intensities and types of exercise used, the absence of analysis of plasma volume changes due to exercise (Surmen-Gur et al., 1999) and the training state of the athlete (Robertson et al., 1991; Mena et al., 1991).

Vitamin E has been identified as the major lipid soluble, free radical scavenging antioxidant in biological membranes. Its function, as mentioned earlier is to protect polyunsaturated fatty acids against oxidative damage by scavenging lipid free radicals. A few studies (see Table 1) involving vitamin E antioxidant supplementation and aerobic exercise have produced positive results in attenuating lipid peroxidation (Sumida et al., 1988; Meydani et al., 1993). For example, a group of 21 moderately trained or sedentary college students exercised to exhaustion on a cycle ergometer; there was a significant rise in the concentration of MDA in plasma (Sumida et al., 1989). Repetition of the exercise test after 4 weeks of supplementation with 300 mg/day vitamin E showed that the production of MDA was significantly lower than at baseline without vitamin E. Sumida et al. (1989) concluded that lipid peroxidation resulting from acute heavy aerobic exercise could be inhibited by vitamin E supplementation.

Table 1. Vitamin E Supplementation Studies

A. Studies showing no effect

Reference	Participants	Supplement Dose/Duration	Exercise Test (mode & intensity)	Research Design	Other Controls	Dep. Variables & Supp. Measure	Dep. Values (range & units)	Results	Comments
Maxwell et al., 1993	24 untrained students 16 m, 8 f 17.9 – 21.8 yrs.	3 separate groups. 400 mg/day dl tocopherol, 400 mg/day vit C, 3 weeks	1 hr of box stepping (eccentric exercise) 60 % VO_2 max	random placebo gr. included single blind	• overnight fast before exercise. • no vit. allowed during study. • non smokers only	plasma MDA (HPLC) (at 1 hr.,2 hr, 1 day) plasma vit. E plasma volume change	.89-2.33 umol/L 27.2-32.7 umol/L	• no significant change in MDA in all three groups • vit. E ↑, but not significant	• intensity of exercise ? • MDA & HPLC √ • vit E increase – was it high enough ?
Kanter et al., 1993	20 males 5 well trained 5 mod-trained 10 untrained 20-29 yrs.	combined supplement 592 mg/day dl tocopherol , 1000 mg/day ascorbic, 30 mg/day beta carotene 6 weeks	treadmill running 60 % VO max – 30 min., plus 90 % VO max – 5 min. 2 exercise tests separated by the 6 weeks	random single blind placebo gr. (trigl. capsule)	• no exercise 1 day before exercise test. • abnormal serum vit. & C excluded from study. • non smokers only.	serum MDA (HPLC) (immediate) expired pentane serum vit. E	4-6.5 nmol/ml 10-30 pmol/kg/min 82-1.92 mg/ml	• ↑ in MDA in supp. gr. after exer. test	• 3 vitamins ?
Rokitzki et al., 1994	30 trained male cyclists 23.0 ± 2.8 yrs.	330 mg/day dl tocopherol acetate 20 weeks	cycle ergometer 100 % VO_2 max (30 to 40 min) 2 exercise tests separated by 5 months	random double blind placebo (soy bean oil)	• no exercise 1 day before exercise test • 12 fast before test • smokers allowed	plasma MDA (BHT added) (immediate) plasma vit. E plasma volume (hematocrit) change	4.9-7.0 umol/L 24.9-40.4 umol/L	• ↑ MDA in supp. gr. • vit E sign. ↑	• study stated it was a pos. result – but ↑ MDA was due to 5 mon. supp. (pre exer. test to pre exer. test) • no change in plasma vol. is unusual ?
Harris and McMurry 1995	14 active males 18-35 yrs.	595 mg/day d tocopherol acetate Third group - control group had no placebo or supp. (n=4) 7 weeks	20 min. bench stepping exer. (20 lbs. of wt. around the waist) 2 exer. tests separated by 48 days	all part. had placebo before 1st test, than supp. before 2nd test single blind placebo (corn starch)	• no exercise allowed 1 day before exercise test. • same diet 1 week before tests • smokers allowed	plasma MDA immediate, 6, 24,48 hrs.	2.60-3.14 umol/L	• no sign. change in all 3 groups	• intensity of exercise ? • no analysis of plasma vitamin E levels or plasma volume change.

A. Studies showing no effect (continued)

Reference	Participants	Supplement Dose/Duration	Exercise Test (mode & intensity)	Research Design	Other Controls	Dep. Variables & Supp.Measure	Dep. Values (range & units)	Results	Comments
McBride et al., 1997	12 moderately trained resistance male part. 18-30 yrs.	992 mg/day d α tocopherol succinate 2 weeks	a high intensity resistance exercise test (7 exercises and 3 circuits)	random single blind placebo (cellulose capsule) matched participants	• no exercise allowed 2 days before and after tests • 3 day dietary analysis during study. • no controls for smokers mentioned	• plasma MDA (immediate, 6 6, 24 hrs.) • plasma volume change	1.8-5.3 umol/L less than 10% for all post exer. time pts.	• no sign diff. between supp. & placebo groups for all MDA post exercise time pts.	• small N (6) used, but researchers state adequate power • no plasma vitamin E status monitoring • confusing results - repeated measures ANOVA not displayed
Kaikkonen et al., 1998	37 trained male marathon runners 40 ± 7.0 yrs.	combined supplement 13.5 mg/day d tocopherol acetate, 90 mg/day coenzyme Q 10 3 weeks	marathon run	random double blind placebo (contents not mentioned)	• no vit. in beverages during run • also measured baseline vit. C co-enzyme Q glutatione peroxidase • non smokers	lipid peroxidation (immediate) as measured by lipo protein damage to VLDL & LDL. plasma vit E plasma volume measured	11.3-13.9 % 28.8-33.5 umol/L	• both supp. & placebo gr. had ↑ in prop. of of LDL- of LDL. • ↑ vit E 16 %	• plasma vol. measured √ • placebo unknown ?
Surmen-Gur et al., 1999	36 untrained male students 18-24 yrs. 17 smokers 19 non smokers	400 mg/day dl tocopherol 4 different groups 4 weeks	A) cycle ergometer 100 % VO_2 max (7-10 min.) B) 3 min. rest, than C) cybex isokinetic dynamometer 3 x 20 repetitions leg extension./flexion (maximal effort)	random control gr. (used no placebo)	• no exercise day before the exer. test • light breakfast allowed • participants with unusual diet excluded	plasma MDA (at 6 min.) serum vit. E plasma volume measured	4.3-5.1 nmol/ml 1.4-2.2 mg/dl	• no increase in MDA in all 4 grs. • serum vit E sig. ↑ • plasma volume decreased 7.3-11 %	• plasma MDA not measured at 6 hrs post exer. ? • placebo controls missing. • did not meet single blind standards
Viitala et al., 2003	Untrained gr. - (7 males & 7 females) Trained gr. - (8 males & 5 females) 19-30 yrs.	885 mg/day d α tocopherol acetate 2 weeks	a high intensity resistance exercise test (8 exercises, 3 circuits) 2 tests separated by 6 weeks	random double blind placebo (soy oil capsule) crossover study (4 week washout period)	• no exercise allowed the day before the exer. test & 6 hrs. post. • 1 dietary analysis completed during each treatment period. • non smokers only	plasma MDA (BHT added) (immed., 6 hrs.) plasma vit. E plasma volume measured	21-2.2 umol/L 17-77 umol/L 2% decrease in plasma volume	• a sign. ↑ was found in MDA in all 4 grs. (6 hrs post exercise), therefore no diff. between placebo & supp. grs. and untrained and trained grs. • only supp. grs. had sign. ↑ in plasma vitamin E levels.	• plasma vit. E measured • plasma MDA with BHT √, but no HPLC ?

B. Studies showing positive effects

Reference	Participants	Supplement Dose/Duration	Exercise Test (mode & intensity)	Research Design	Other Controls	Dep. Variables & Supp. Measure	Dep. Values (range & units)	Results	Comments
Sumida et al., 1989	21 untrained male students 20.3 ± 0.3 yrs.	300 mg/day d tocopherol acetate 4 weeks	cycle ergometer 100 % VO_2 max 2 tests separated by 4 weeks	single blind no placebo controls	• overnight fast • no controls for smokers mentioned	serum MDA (immediate, 1 hr, 3 hrs.) serum vit. E plasma volume change	3.8-4.5 nmol/ml 9.6-22.3 ug/ml	• ↓ in MDA in supp. gr. after exer. test. • vit. E sign. ↑ • blood volume hematocrit ↑ 7-12 %	• no control group.
Meydani et al., 1993	21 males untrained 20-29 yrs. (n=9) 55-74 yrs. (n=12)	728 mgday dl tocopherol 7 weeks	eccentric running on incline treadmill 75 % of max HR 3 x 15 min intervals; rest 5 min. between	random double blind placebo gr. (soy bean oil)	• meat free 3 days before urine collected • no exercise vitamin supp., antinflamatory drugs during study • smokers allowed	urinary MDA (1,2,5,12, days) conjugated deines plasma vit E plasma volume change	3.18-5.12 n/mol/24 hrs. .2-.8 Δ OD 6-17 ug/ml	• no increase in MDA in supp. grs. after exer. test. • vit E ↑ • plasm vol. ↓	• very small N • dep. variable (Urinary MDA) - 12 days – a very long delay. • conjugated deines - a poor dependent variable.

Legend

vit. E, vitamin E; exer. test, exercise test; m, male; f, female; yrs., years; mg, milligrams; %, percent; VO_2 max, maximum volume oxygen uptake; min., minutes; ml, millilitres; N, number; gr(s)., group(s); dep., dependent; supp, supplement; trigl., triglicerides; HPLC, high performance liquid chromotography; d, natural; dl, synthetic; sign, significant; vit. C; vitamin C; BHT, butylated hydroxytoleune; pos. result, positive result; neg. result, negative result; VLDL, very low density lipoprotein lipid, LDL, low density lipoprotein lipid; umol/L, micromol per litre; nmol/ml, nanomoles per millilitre; nmol/3min, nanomoles per 3 min.; ug/ml, micrograms per millilitre; pmol/kg/min, picomoles per kilogram per min; Δ OD, change in oxidative damage, MDA, Malondialdeyhde. hrs., hours; pts., points, ↑, increase; ↓, decrease; mon., month.

There have been quite a number of studies involving vitamin E supplementation and lipid peroxidation that surprising has shown 'no effect' of the treatment (see Table 1). One study that does not support the efficacy of vitamin E antioxidant supplementation is the research of Surmen-Gur et al. (1998). The authors studied the effect of 400 mg/day of vitamin E supplementation on post exercise MDA levels in smokers and non smokers. It was stated in their introduction that "cigarette smoke, as a pollutant, has been demonstrated to contain oxidant or free radicals that can directly and indirectly initiate and propagate the process of lipid peroxidation" (pp. 472).

All 36 participants performed a maximal test on a cycle ergometer followed by exercising to exhaustion using an isokinetic leg extension/flexion machine. All four groups (non smoker, supplement group; non smoker, placebo group; smoker, supplement group; smoker, placebo group) had no significant changes in adjusted pre to post exercise plasma MDA concentrations. They would have seen significant increases in MDA if not for the adjustment due to plasma volume changes. Adjustments were needed in the post MDA measures because a significant water shift from the blood plasma compartment to muscle tissue occurred as a result of the exercise performed. The authors used the adjusted MDA measures and they concluded that there was no effect of vitamin E supplementation or smoking on plasma lipid peroxidation. The authors also make an addition point "previous reports about exercise induced oxidative damage and positive supplementation findings should be reevaluated if plasma volume shifts were not taken into consideration (pp. 477)." They obviously feel that previous studies were not thorough enough in their methodologies and produced false results.

There have been many other studies showing the positive effects of other vitamins such as vitamin C, plus beta carotene, selenium and antioxidant mixtures (plus vitamin E) on lipid peroxidation induced by exercise. Upon review of many studies, Dekkers et al. (1996) concluded that supplementation with antioxidant vitamins has favourable effects on the process of lipid peroxidation. Kanter (1998) also adds that although the physiological implications of these effects remain to be elucidated, the prudent use of an antioxidant supplement can provide insurance against elevated demands of physical activity.

Vitamin E Supplementation, Resistance Exercise and Lipid Peroxidation

Little research has been done investigating the effects of vitamin E supplementation on resistance exercise induced lipid peroxidation. Only one study by McBride et al. (1997) (see Table 1) examined this question and it was done with 12 recreationally weight trained males. Upon further review of Table 1, it is clear that only a small amount of research involving vitamin E supplementation and exercise induced lipid peroxidation has been done with female participants (20%). Other studies mentioned previously involving resistance type exercise did not include vitamin E antioxidant supplementation.

McBride et al. (1997) found that supplementation with 992 mg/day of vitamin E in the form of α tocopherol succinate for a period of 2 weeks resulted in no significant differences between the placebo and supplement groups for MDA measures. The authors used the TBA test and MDA as the quantitative marker for lipid peroxidation. This may be perceived as a problem in methodology as they did not use HPLC and the reagent Butylated Hydroxytoluene (BHT). This may have added to a lack of specificity in measuring MDA and inflated MDA measures respectively.

Other factors that could have impacted on the results of the McBride et al. (1997) study include the type of research design and subjects used. Perhaps, using a stronger design such as the two period crossover design would eliminate inter-subject variability between groups when reviewing the effects of the treatment. Their study was limited to only one group, recreationally trained males. They did not investigate the potential effects of training on the natural antioxidant enzyme levels in the human body. Further research is required to support the conclusion made by McBride et al. (1997).

THE TRAINING STATE AND ANTIOXIDANT ENZYMES

An elaborate antioxidant system has evolved to protect living organisms from oxidative damage (Chance et al., 1979). Some examples of the body's natural antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) and nonenzymatic antioxidants such as glutathione (G) and vitamins. As oxygen free radical generation in the cell is highly localized, the distribution of these enzymes is such that they destroy the radical species produced in the same cell. For example, almost all O_2^- formed in the mitochondria respiratory chain is converted to H_2O_2 by the mitochondria enzyme SOD.

Habitual physical training has been shown to enhance the natural antioxidant enzymes (Robertson et al., 1991; Mena et al., 1991). Subudhi (1999) states " while the bad news is that training may produce free radicals, the good news is that this same training over a long duration may cause a positive response and actually build up an adaptive defence to free radicals" (p. 16). Kanter (1998) agrees with this as he feels that there is a relative consistent finding of an increase in antioxidant activity in various tissues of trained participants that is highly suggestive of a protective adaptation to the habitual stress of exercise. He also notes that free-radical species are continuously produced in the human body and that some have further beneficial effects, notably as a part of the body's natural immune system. Robertson et al. (1991) performed blood tests on 20 distance runners and 6 sedentary individuals. All running subjects were required to have been running regularly for at least 2 years. They found that the activities of the GPX and CAT within erythrocytes were significantly correlated with the weekly training distance and also total G was higher in the training group. Nevertheless, the groups had similar plasma concentrations of TBARS. They also noticed that there was a significant negative correlation between VO_2 max and plasma TBARS concentration. They concluded that

there was an increase in blood antioxidant defence mechanisms associated with endurance training, but despite this there is still a degree of muscle damage in these individuals. Mena et al. (1991) also found that resting activities of SOD, CAT and GSH in erythrocytes of professional cyclists characterized by a high aerobic capacity were significantly higher than the inexperienced ones.

Antioxidant enzymes have been measured in blood plasma, the myocardium, muscle tissue and attached to erythrocytes in blood. It is believed that the antioxidant defence system is in dynamic equilibrium, so that a decrease in one of the factors will to a certain extent be compensated through increased capacity of other antioxidants (Ortenblad et al., 1997). This implies that evaluating the capacity of the antioxidant defence system can be difficult and requires determination of the whole antioxidant status at the time. In a study done by Ortenblad et al. (1997) with elite volleyball players and non-trained individuals, there were mixed results with regards to antioxidant enzyme concentrations. Resting muscle antioxidant activities for SOD and GPX were significantly higher in jump-trained compared with non-trained individuals. The blood antioxidant enzyme activities, plus the muscle CAT were not different between the 2 groups. Ji (1993) states that training studies with either animals or human subjects have not been able to consistently demonstrate that antioxidant enzymes can be increased by chronic exercise. He further points out that muscle SOD and CAT activities have been shown to increase with training by some researchers (Quintanilha, 1984; Higuchi et al., 1985), whereas others reported no change (Alessio and Goldfarb, 1988; Laughlin et al., 1990).

While reviewing research studies, Maxwell (1993) noted that a study by Viinkka et al., (1984) found that there was no increase in plasma peroxides following exhaustive exercise in top athletes undergoing bicycle ergometry. He also noted that runners completing an 80.5 kilometre ultra marathon had increased plasma lipid peroxidation (Kanter et al., 1988), while Duthie et al., (1990) found that TBARS did not rise in plasma following a half marathon. Maxwell (1993) concluded that vigorous exercise in athletes can certainly result in increases in oxidative damage in some circumstances. Maxwell's review shows the inconsistent results found in studies in this area of research.

According to Jenkins (1988) and Ji (1993), antioxidant enzyme adaptation is one of the fundamental changes in skeletal muscle in response to exercise training and free radicals. In a review article presented by Dekkers et al. (1996), the authors suggest that in general endurance training should increase oxidative stress, which should induce some positive adaptations in some of the antioxidant enzymes. In contrast, one investigator has stated that "100 to 200 IU of vitamin E should be recommended to all athletes; it would be bordering on improper coaching not to point out the benefits of supplementation to the athlete" (Simon-Schnass, 1993, p. 144). Other experts also believe that there is sufficient information to suggest that athletes supplement their diets with antioxidants in excess of the RDA. On the other hand, many sports nutritionists recommend that adequate amounts of antioxidants be obtained in the diet and that athletes should make intelligent food choices.

Conflicting opinions have developed with regards to supplementation and trained participants. The results are confusing and do not provide a definitive answer at this time.

SUMMARY

Only one study supports the increased production of oxygen free radicals and lipid peroxidation as a result of resistance exercise, while a number of studies do not. The one study by McBride et al. (1997) used a higher intensity of exercise and a larger amount of muscle mass (whole body) during the exercise bout. There appears to be valid theories supporting the concept that resistance exercise could lead to an increase in the production of oxygen free radicals in active muscle sites. Further research is required to prove that resistance exercise produces significant increases in free radicals and lipid peroxidation.

Only one resistance exercise study (McBride et al., 1997) has been done utilizing antioxidant supplementation with vitamin E. The results showed that vitamin E supplementation was ineffective in reducing resistance exercise induced lipid peroxidation. A few studies have provided support of vitamin E supplementation in the reduction of lipid peroxidation measures caused by aerobic exercise. Yet, the results remain unclear as there is also a body of non-supporting research. It is quite possible that vitamin E could work as it has with the aerobic exercise studies by quenching possible oxygen free radicals caused by resistance exercise.

Opposing views have developed with regards to whether trained participants have increased protection against oxygen free radicals. Researchers have explored antioxidant vitamins and enzymes that are found in blood plasma, in local tissue and within erythrocytes. Research performed in varying locations has caused conflicting results. Further research is required.

CHAPTER 3

METHODOLOGY

Participants - Screening Criteria

Each participant has filled out an Activity/Medical History questionnaire (Appendix A) prior to the study. In order to be enrolled in the study, the participant had to meet the following criteria:

1) Had no clinical history of malabsorption syndromes such as cystic fibrosis, celiac disease and biliary atresia that would interfere with the normal absorption of dietary fat and Vitamin E (Bieri et al., 1983).
2) Had not taken dietary supplements or drugs within one month of the study.
3) Had no medical problems (i.e. – musculoskeletal, inflammatory, cardiovascular) that would prevent full and safe participation in a resistance exercise protocol.
4) Was a non-smoker.
5) Had the expectation and willingness to maintain their regular physical activity patterns (see below for specific group requirements) and diet throughout the study and to perform two - 3 day dietary records during the 2 treatment periods.
6) Was between the ages of 19 to 30 years of age.
7) Matched either the trained or untrained grouping criteria.

After meeting the above screening criteria, the participants were placed in the appropriate group.

Resistance Groups

Group A - 14 Untrained Resistance Participants

To be placed in this group, the participant had to:

1) Be an individual who has had very little resistance training experience (i.e. less than 6 months)
2) Be currently doing resistance training 0 – 1 x/week (just starting out)

Group B – 13 Trained Resistance Participants

To be placed in this group, the participant had to:

1) Have been doing resistance training for a minimum of 3 years to a maximum of 10 years (can have a few periods off training – i.e. 1-2 weeks, only 1 – 3 month break within a year period.
2) Be doing weight training regularly - a minimum of 3x/week to a maximum of 5x/week (on average)
3) Not be a competitive power lifter or body builder (does not enter competitions).

A power test utilizing previous data on MDA measures from Wong et al., (1987) indicated that a sample group size of 12 should be sufficient to reach a power of 0.80 in detecting an effect size of .03 u/mol/L.

Schedule of Events

The study proceeded as follows:

1. Recruitment of participants was done through radio, television and newspaper advertisements and with postings at Lakehead University and at local fitness facilities. As mentioned above, screening of participants involved participants filling out Activity/Medical History questionnaires. Approved participants, having met the screening criteria, resistance group requirements and medical requirements then completed the Participant Consent Form (Appendix D – approved by Lakehead University Ethics Review Committee) and were included as participants in the study. Recruitment continued until 20 participants were obtained in each group to allow for attrition during the study.

2. An orientation session for both groups took place which included safety in the weight room and a review of the RET. This was more extensive for the beginners. Individual sessions were conducted to secure descriptive measures (age, height, weight, % body fat, years of weight training experience, vitamin E (α tocopherol) level in blood plasma) and the establishment of a 10 RM for all 8 exercises in the RET (includes standard body positions - grip width & joint angles and speed of lift). All participants were completely familiarized with the exercises and the RET protocol.

3. Treatment Period #1 In order to maintain consistency of testing times between groups during the day and to have small enough groups for the RET (circuit format), the Untrained and Trained Groups was split in half. Untrained resistance participants (Group A) were randomly assigned so that there was 7 participants in Group A-1 and 7 participants in group A-2. Trained resistance participants (Group B) were also be randomly assigned leaving 7 and 6 participants in Groups B-1 and B-2 respectively.

Participants in Group B-1 and A-1 arrived on the 1st Saturday at 8:30 and 9 am respectively at the Lakehead University Fieldhouse for initial blood testing by the phlebotomist. A blood sample was taken and blood plasma vitamin E (α tocopherol) levels were measured using HPLC apparatus (Ingold et al., (1987).

Participants then received the supplement or placebo (double blind) for the 2 week treatment period, plus a daily log (Appendix B) into which they recorded relevant exercise, lifestyle and health information. The participants also performed a 3 day diet analysis (Foodworks, 2000) that enabled monitoring of unusual dietary vitamin E, vitamin C and vitamin A (beta carotene) intakes. The following Saturday, participants in Group B-2 and A-2 arrived at 8:30 and 9 am respectively for initial blood testing. They were given similar instructions.

4. Resistance Exercise Test #1 (RET) took place at the completion of the 2 week Treatment Period. Participants in Group A-1 and B-1 reported to the Lakehead University Fieldhouse on Saturday at 8 and 10:30 am respectively, whereas participants in Groups A-2 and B-2 reported 1 week later at the 8 and 10:30 am times respectively. Participants refrained from exercise 24 hours and taking alcohol 12 hours preceding the RET and had a light breakfast (specified foods with little to no vitamin E content) before the RET. The RET was performed by each participant. A blood sample was taken before the RET to determine blood plasma vitamin E levels after Treatment Period #1. Additional blood samples were taken and analyzed for Malondialdehyde (MDA) levels. They were taken before the RET, immediately after the RET and 6 hr post RET. The method of Chirico (1994) was used to measure lipid peroxidation and the levels of MDA using the modified TBA test with BHT added. No exercise was permitted until after the 2nd post RET blood test.

   Rate of Perceived Exertion (RPE) was taken at the mid point and at the end of RET. The Borg CR-10 scale (Noble et al., 1983) was used to evaluate the level of intensity (RPE) of each participant within each group.

   Hematocrit (Hct) and Haemoglobin (Hb) was also measured (pre and post RET) and plasma volume shifts were determined using the equation suggested by Van Beaumont et al., (1981).

5. The Washout Period started immediately after RET #1. No supplement or placebo was provided to the participants for a 4 week period and they were instructed to maintain normal exercise and diet. This was monitored by the daily log (Appendix B). Dimitrov et al. (1996) showed that after 4 weeks of 807 mg/day of α tocopherol acetate supplementation, plasma vitamin levels returned back to baseline after just 16 days.

6. Treatment Period # 2 followed the washout period. Groups A-1 and B-1 arrived at 8:30 and 9 am respectively for blood testing. The following Saturday, participants in

Group A-2 and B-2 arrived at 8:30 and 9 am respectively for blood testing. All four groups were given the same instructions as in Treatment Period # 1. However, each participant received the opposite treatment (supplement or placebo) during this 2 week Treatment Period in comparison to Treatment Period #1.

7. Resistance Exercise Test #2 (RET) took place at the completion of the Treatment Period #2. Participants in Group B-1 and A-1 reported to the Lakehead University Fieldhouse on Saturday at 8 and 10:30 am respectively. They followed the same instructions as outlined in RET #1. One week later, Groups B-2 and A-2 arrived at 8 and 10:30 respectively. They followed the same format as previously mentioned.

Exclusion Criteria

Exclusion criteria for the participants during the 2 treatment periods and the washout period included:

1) Failure to ingest at least 75 % of the prescribed supplement (checked by returned capsule count)
2) Failure to maintain normal exercise routine ($\pm$ 25 % exercise sessions)
3) Failure to be tested within $\pm$ 2 days of scheduled RET date (due to illness, injury or travel)
4) Being injured or ill (enough to prevent exercising in their normal pattern) for more than one week during the treatment periods.
5) Participants with baseline plasma Vitamin E (α tocopherol) levels below the normal level of 12 umol/L (Vitamin E Research and Information Science [VERIS], 1993).
6) Inability to do the exercises in the RET as prescribed.

Treatment Period

During each 2 week period, all participants were asked to take three gel capsules per day. The capsule was either the placebo (containing soy oil) or the vitamin E dosage of 295 mg α tocopherol acetate. The treatment dosage of vitamin E (885 mg/day α tocopherol acetate) was below the Tolerable Upper Intake Level of 1,000 mg/day established by the Institute of Medicine (2000) and posed no negative side effects.

Resistance Exercise Test

1) The Resistance Exercise Test (RET) was performed in a modified, circuit type fashion that includes 8 different resistance exercises. The participants

performed the RET within their designated group (A-1, B-1, A-2, B-2) at the Lakehead University Fieldhouse.

The RET Circuit

1) bench press	universal gym	5) arm curls	free weights
2) incline leg press	incline machine	6) leg extensions	leg extension machine
3) side lat raises	free weights	7) lat pull downs	universal gym
4) leg curls	leg curl machine	8) triceps press	universal gym

2) The heavy resistance protocol used during the RET consisted of a protocol similar to the 10/1 protocol used by Kraemer et al. (1992). Each participant was lifting weights at their 10 repetition maximum (RM). This ensured that each participant was exercising at a relative intensity fairly equal to other participants with different strength levels. They were lifting a similar volume in relation to their own strength.
3) There was one warm-up circuit where each subject performed 10 repetitions of the 8 exercises at 50% of the participants previously determined 1 RM.
4) The 1st circuit of 8 exercises was performed at 10 RM. Each participant did 10 repetitions of each exercise; they performed each exercise to muscle exhaustion at approximately the 10^{th} repetition. Ninety seconds of rest was allowed between each exercise in all three circuits. Two minutes was allowed between circuits.
5) The 2^{nd} circuit of 8 exercises was performed at the 10 RM, but with a weight approximately 8 % lower than the 1^{st} set 10 RM (McBride et al., 1997).
6) The 3^{rd} circuit consisted of 8 exercises with the weight set at approximately 20 % lower than the 1^{st} set 10 RM (McBride et al., 1997).

Blood Processing

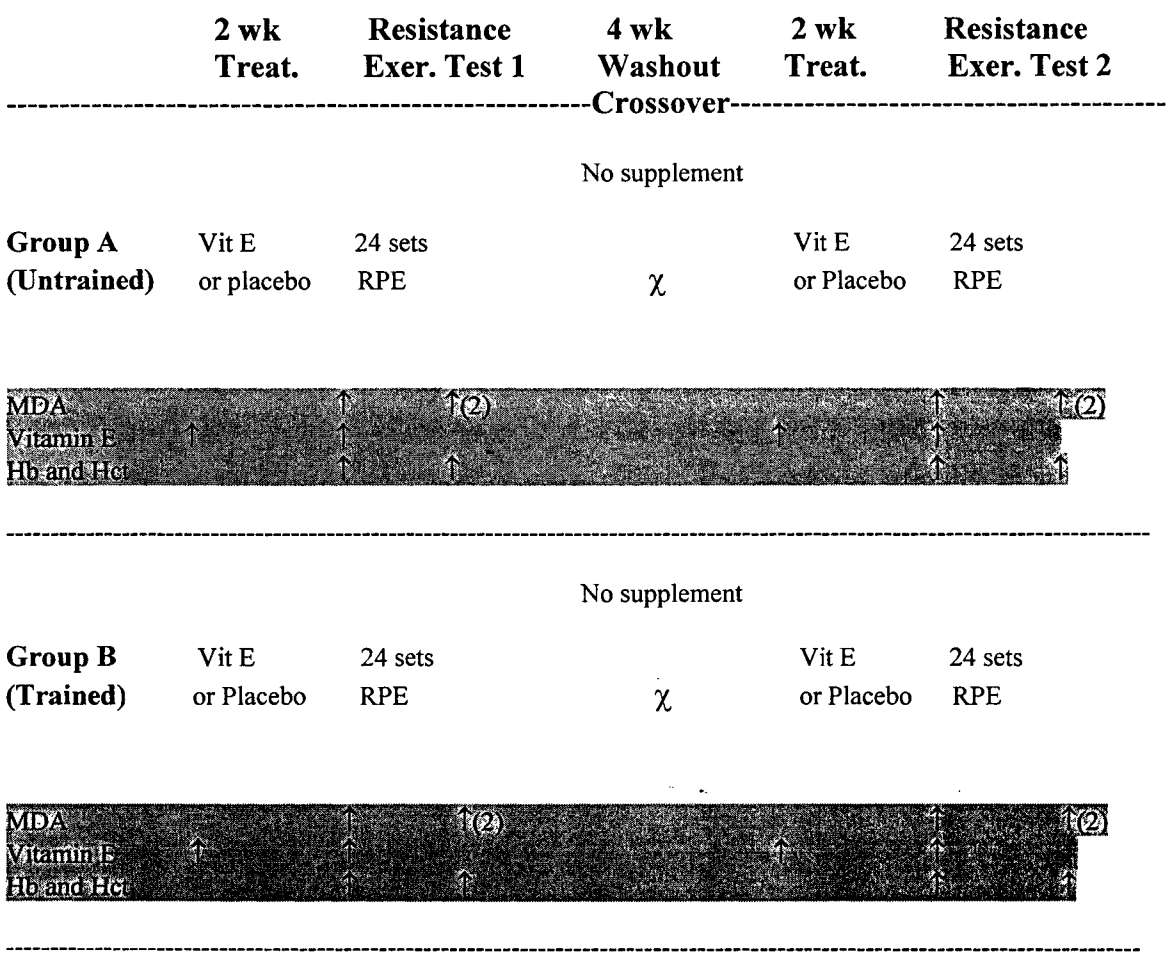
Eight blood samples was be taken from each participant during the study by a certified phlebotomist. The amount of blood was small (20 ml) and it was obtained from an antecubital vein. At certain times during the study (see Figure 1. Experimental Design - The 2 Period Crossover Trial), a smaller amount of blood was needed only for the analysis of plasma α tocopherol levels. At other times during the study (the two pre RET blood sample times), the venous blood samples was divided into three portions:

(1) 10.0 ml of blood into 10 ml green top test tubes containing heparin as an anticoagulant.
(2) 2.5 ml of blood into 5.0 pink top test tubes containing EDTA as an anticoagulant.
(3) 0.50 ml of blood into 1.0 ml polypropylene eppendorf micro test tubes.

The first portion was centrifuged immediately after collection; the plasma was separated and stored at – 70 °C until analysed to determine MDA levels (Chirico, 1994). The second portion was directly transported to the Thunder Bay Regional Hospital Haematology laboratory in

order to determine haemoglobin and hematocrit concentration levels (Van Beaumont et al., 1981). The third portion was immediately stored on dry ice, then transported to the Reference Testing Centre, St. Joseph's Health Centre in London in order to have the plasma α tocopherol levels analysed (Ingold et al., 1987).

Figure 2. Experimental Design - The Two Period Crossover Trial



Abbreviations and symbols: Vit E = vitamin E; RPE = rate of perceived exertion; MDA = malondialdehyde; Hb = haemoglobin; Hct = Hematocrit; 2 week treat. = 2 weeks of treatment; χ = crossover of treatment; ↑ = time of measurement.

Statistical Analysis

The dependent variables in this study were adjusted MDA and blood plasma vitamin E (α tocopherol). The independent variables included group (trained vs untrained), treatment (supplement vs placebo) and time (pre, immediate post and 6 hours post exercise).

Gender differences in lipid peroxidation was determined by analyzing MDA change scores (pre to immediate post exercise; pre to 6 hours post exercise) through the use of two- 2 group x 2 treatment x 2 gender factorial ANOVAs.

An independent t-test was performed between two groups (plasma/supplement; supplement/ placebo) on the plasma vitamin E change scores (pre to post treatment) in order to determine if a carryover effect was present (i.e., if the washout period was ineffective) (Armitage and Hills, 1982). The washout was found to be effective; data was then pooled to test for differences between groups, treatments and time on MDA measures.

The control variables that were used to ensure that the four groups (untrained placebo; untrained supplement; trained placebo; trained supplement) were similar during the study included dietary vitamin E intake, dietary vitamin C intake, dietary vitamin A intake, the number of resistance training sessions, the number of aerobic training sessions, RPE middle and RPE end. A 2 group x 2 treatment factorial ANOVA was performed separately on each of the control variable change scores (treatment period 1 to treatment period 2).

To determine whether the 2 week treatment periods of vitamin E were effective in significantly increasing plasma vitamin E levels, a 2 group x 2 treatment factorial ANOVA was performed on blood plasma vitamin E change scores (pre to post treatment). An effective supplementation period was critical before the investigation can proceed to show whether vitamin E influences lipid peroxidation levels. Also, the nutritional dietary intakes and daily logs were examined so that any discrepancies in routine can be taken into account.

Vitamin E supplementation and placebo effects were determined through the use of a 2 group (trained vs untrained) x 2 treatment (vitamin E vs placebo) x 3 time (pre exercise vs immediate post exercise vs 6 hours post exercise) factorial ANOVA on MDA values. The effects of the 2 levels of training on MDA measures were also determined with the factorial ANOVA.

The Pairwise Comparison Post Hoc test was be used to determine interaction effects. Statistical significance will be $P \leq 0.05$.

CHAPTER 4

RESULTS

Group Descriptive Measures and Gender Differences

The group descriptive measures (untrained vs trained) and weights lifted (10 repetition maximum RM) for each exercise during the resistance exercise test are listed in Table 2.

Table 2.
Group Descriptive Measures and Resistance Exercise Test Weights

PARAMETER	UNTRAINED (Means ± SD) n = 14	TRAINED n = 13	T-TEST
Age (yrs)	23.3 ± 3.8	24.2 ± 3.7	t_{25} = - .60, p = .55
Gender	M = 7, F = 7	M = 8, F = 5	t-test = n/a
Height (cm)	170.2 ± 8.5	173.4 ± 9.9	t_{25} = - .90, p = .36
Weight (kg)	72.5 ± 15.3	73.5 ± 15.1	t_{25} = - .17, p = .87
Predicted Percent Body Fat (%)	22.1 ± 5.9	16.2 ± 4.9	t_{25} = 2.82, p = .01
Vitamin E Baseline (umol/L)	19.6 ± 4.1	19.2 ± 5.5	t_{25} = .22, p = .82
Past Weight Train Experience (yrs)	0.1 ± 0.1	5.2 ± 2.6	t_{25} = - 7.55, p = .00

EXERCISE (10 RM)	UNTRAINED (Means ± SD)	TRAINED	T-TEST
Leg Press (lbs)	146.6 ± 31.5	438.5 ± 187.8	t_{25} = - 5.83, p = .00
Bicep Curls (lbs)	37.9 ± 10.9	60.0 ± 20.0	t_{25} = - 3.61, p = .00
Leg Curl (lbs)	49.1 ± 13.7	74.2 ± 27.7	t_{25} = - 3.02, p = .01
Bench Press (lbs)	75.5 ± 22.9	138.1 ± 58.3	t_{25} = - 3.72, p = .00
Leg Extension (lbs)	88.0 ± 28.4	116.0 ± 35.7	t_{25} = - 2.28, p = .03
Side Lat Raise (lbs)	9.8 ± 2.8	18.9 ± 7.9	t_{25} = - 4.07, p = .00
Lat Pull Down lbs)	83.0 ± 17.9	113.2 ± 31.8	t_{25} = - 3.07, p = .01
Triceps Press (lbs)	62.1 ± 12.1	101.3 ± 40.0	t_{25} = - 4.05, p = .00

All 27 participants were found to be in the normal range of plasma vitamin E levels (12 to 37 umol/L) before the study commenced (Vitamin E Research and Information Service [VERIS], 2000). There were no significant differences between the untrained and trained groups with regards to the general descriptive measures taken except within the body fat percentage category and past resistance training experience category. The percentage of body fat was significantly higher in the untrained group (22.1 % ± 5.9) in comparison to the trained group (16.2 % ± 4.9) and as established by the enrolment criteria, the untrained group had greatly reduced experience in the weight room (.1 year ± .1) in comparison to the trained group (5.2 years ± 2.6). There were significant differences found between the untrained and trained groups in weights lifted in all weight training exercises. The differences found between the untrained and trained groups in weights lifted was obviously due to the substantial differences in past resistance training experience and the greater proportion of males in the trained group.

There were no significant gender differences in lipid peroxidation caused by the resistance exercise test. A 2 group (trained vs untrained) by 2 treatment (supplement vs placebo) by 2 gender (male vs female) factorial ANOVA on MDA change scores (pre exercise to immediate post exercise) found no significant difference (significance level at .05). Another 2 group (trained versus untrained) by 2 treatment (supplement vs placebo) by 2 gender (male vs female) factorial ANOVA on MDA change scores (pre exercise to 6 hours post exercise) also found no significant difference. The gender factorial ANOVA results are listed in Table 3.

Table 3.

Gender Factorial ANOVA (on pre to immediate post exercise MDA change scores)			
Treatment Main Effect	**Group Main Effect**	**Gender Main Effect**	**Interaction (Group x Treatment x Gender Effect)**
F (7,46) = .03	F (7,46) = .51	F (7,46) = .65	F (7,46) = .01
p = .87	p = .48	p = .43	P = .93

Gender Factorial ANOVA (on pre to 6 hours post exercise MDA change scores)			
Treatment Main Effect	**Group Main Effect**	**Gender Main Effect**	**Interaction (Group x Treatment x Gender Effect)**
F (7,46) = .50	F (7,46) = .00	F (7,46) = .34	F (7,46) = .01
p = .48	p = .99	p = .56	P = .92

There were no significant gender differences, subsequently the 7 females and 7 males were pooled in the untrained group (n = 14) and 5 females and 8 males were pooled in the trained group (n = 13).

Control Variables

The control variables that were used to ensure that the four groups (untrained placebo; untrained supplement; trained placebo; trained supplement) were similar during the study included dietary vitamin E intake, dietary vitamin C intake, number of resistance training sessions, number of aerobic training sessions, and the rate of perceived exertion at the middle of the exercise test (RPE mid) and at the end of the exercise test (RPE end). The Participant Daily Logs and 2 - 3 Day Diet Analyses provided much of the data as seen in Table 4.

Table 4. Daily Logs and Diet Analyses

VARIABLE	UNTRAINED GROUP (n = 14) (Means ± SD)	TRAINED GROUP (n = 13)	T-TEST
Vitamin E Intake (mg/day)	12.36 ± 10.63	8.88 ± 3.53	$t_{25} = 1.09$, $p = .29$
Vitamin C Intake (mg/day)	189.82 ± 163.00	192.00 ± 139.40	$t_{25} = -0.01$, $p = .99$
# of Resistance Sessions (week)	1.46 ± 0.41	4.00 ± 0.82	$t_{25} = -10.29$, $p = .00$
# of Aerobic Sessions (week)	1.93 ± 1.89	2.19 ± 1.80	$t_{25} = -0.371$, $p = .71$

The only difference between the two groups was found in the number of resistance training sessions. The trained group had a significantly higher number of resistance training sessions in comparison to the untrained group as established by the exercise criteria for each group.

A 2 group (trained vs untrained) x 2 treatment (placebo vs supplement) factorial ANOVA was performed on all 6 variable change scores (treatment period 1 to treatment period 2) separately and the results are presented in Table 5.

Table 5. Control Variables

Control Variable	Overall F Statistic	Treatment Main Effect	Group Main Effect	Interaction Effect
RPE mid	F (3,23) = .28, p = .84	F (3,23) = .60, p = .45	F (3,23) = .01, p = .92	F (3,23) = .25, p = .62
RPE end	F (3,23) = .24, p = .87	F (3,23) = .02, p = .88	F (3,23) = .01, p = .95	F (3,23) = .67, p = .42
Resistance Training Sessions	F (3,23) = .14, p = .93	F (3,23) = .36, p = .56	F (3,23) = .03, p = .87	F (3,23) = .03, p = .87
Aerobic Training Sessions	F (3,23) = .38, p = .76	F (3,23) = .04, p = .95	F (3,23) = .34, p = .57	F (3,23) = .93, p = .34
Vitamin C Intake	F (3,23) = .12, p = .95	F (3,23) = .12, p = .73	F (3,23) = .23, p = .63	F (3,23) = .02, p = .89
Vitamin E Intake	F (3,23) = 1.2, p = .33	F (3,23) = .85, p = .37	F (3,23) = 2.6, p = .12	F (3,23) = .13, p = .72

All four groups had similar RPE mid and RPE end change score measures as indicated by the non significant status under the Overall F Statistic column. The two factorial ANOVA tests indicate that the four groups had exercised at an intensity level that did not change from one period to the next. There was no significant difference between the untrained group and the trained group (t_{25} = -.04, p = .96) for RPE mid and as well there was also no significant difference between the untrained group and the trained group (t_{25} = .70, p = .48) for RPE end. This indicates that the two groups (trained vs untrained) were exercising at similar relative intensities.

All four groups had similar change score values (treatment period 1 to treatment period 2) for the number of resistance workouts (F (3,23) = .14, p = .93) and aerobic workouts (F (3,23) = .38, p = .76). Therefore, there was no change in their workout patterns during the length of the study.

The dietary vitamin C intake results showed no statistical differences (F (3,23) = .12, p = .95) between the four groups. Therefore, there were similar changes in the four groups. There were no significant differences in the dietary vitamin E intake change scores between the four groups (F (3,23) = 1.2, p = .33). Overall, we see that there were no significant differences within the 4 groups in the 6 control variables selected. These 6 potential confounding variables were controlled to ensure that they did not compromise the results of the study.

Test for Carry Over Effect

The 27 participants had a two week period of vitamin E supplementation (885 mg tocopherol/day - SU) and a two week period of placebo (soy bean oil capsule - PL) separated by the washout. In studies with crossover designs, it is necessary to test for a carryover effect, i.e., to see if the supplemental vitamin E (or placebo) was transferred from the first treatment period to the second treatment period (Armitage and Hills, 1982). A number of authors (Grizzle, 1965; Armitage and Hills, 1982; Woods, Williams and Tavel, 1989) recommend that an independent t-test on change scores (treatment period 1 to treatment period 2) between the two treatment groups be performed in order to determine whether there was a carryover effect. This is also referred to as the 'treatment by period' interaction.

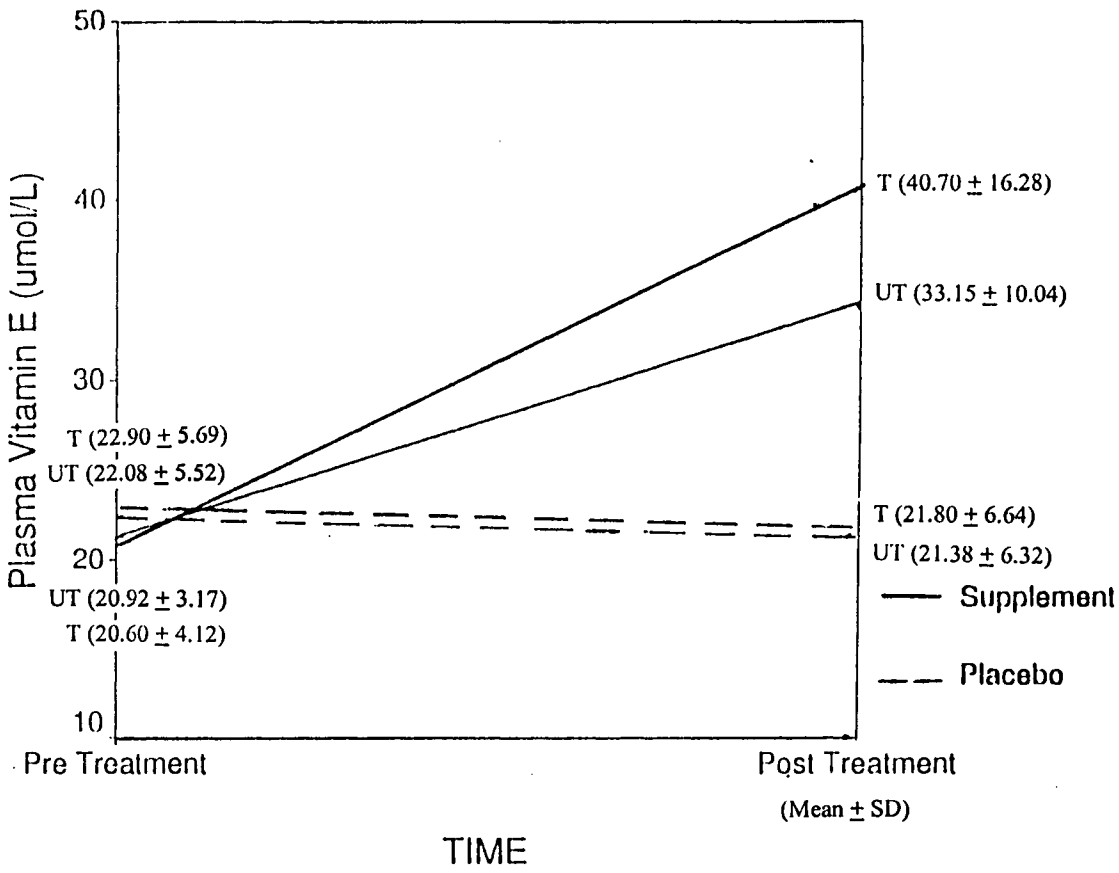
An independent t-test was performed between the two groups (PL/SU; SU/PL) on plasma vitamin E change scores (pre to post treatment) ($t_{22} = 0.49$, $p = .62$). The test did not provide evidence of a 'treatment by period' interaction. This test showed that there was no significant difference between the two groups and therefore the data from the two treatment periods was pooled.

Dependent Variables

The two dependent variables that were assessed included plasma vitamin E and MDA. Plasma vitamin E was evaluated to determine the effectiveness of vitamin E supplementation. Malondialdehyde was assessed to determine if it significantly increased as a result of the RET. Secondly, MDA values were also analyzed to see whether post exercise MDA measures were influenced by the vitamin E supplementation or the state of training of the participant.

A 2 treatment (supplement vs placebo) x 2 group (untrained vs trained) factorial ANOVA was performed on plasma vitamin E change scores (pre to post treatment) ($F(3,42) = 16.94$, $p = .00$). There was a significant difference found in the main effect for treatment ($F(3,42) = 46.50$, $p = .00$, as the two supplemented groups had large increases (almost double) in their plasma vitamin E levels, while the two placebo groups had very small plasma vitamin E changes. There was no significant difference found for the main effect for group ($F(3,42) = 3.19$, $p = .08$), plus no significant difference found for the treatment by group interaction ($F(3,42) = 3.74$, $p = .06$). Figure 3 shows the changes in plasma vitamin E as a result of the vitamin E and placebo treatments.

Figure 3. Vitamin E and Placebo Supplementation



Hematocrit and hemoglobin blood tests were performed on pre RET and immediate post RET blood samples in order to determine the plasma volume change as a result of exercise. It was determined that there was a 2 % decrease in plasma volume. Immediate post exercise MDA values were adjusted before the following MDA statistical tests were done.

A 2 group (trained vs untrained) x 2 treatment (vitamin E vs placebo) x 3 time (pre exercise vs immediate post exercise vs 6 hours post exercise) factorial ANOVA with repeated measures on time was performed on MDA values. The results are listed in Table 6. There were no significant differences found between the four groups (untrained/placebo; untrained/supplement; trained/placebo; trained/supplement) assessed. There was only one significant difference found and that was in the main effect for time (Wilks' $\Lambda = .52$, $F(2,49) = 22.41$, $p = .00$).

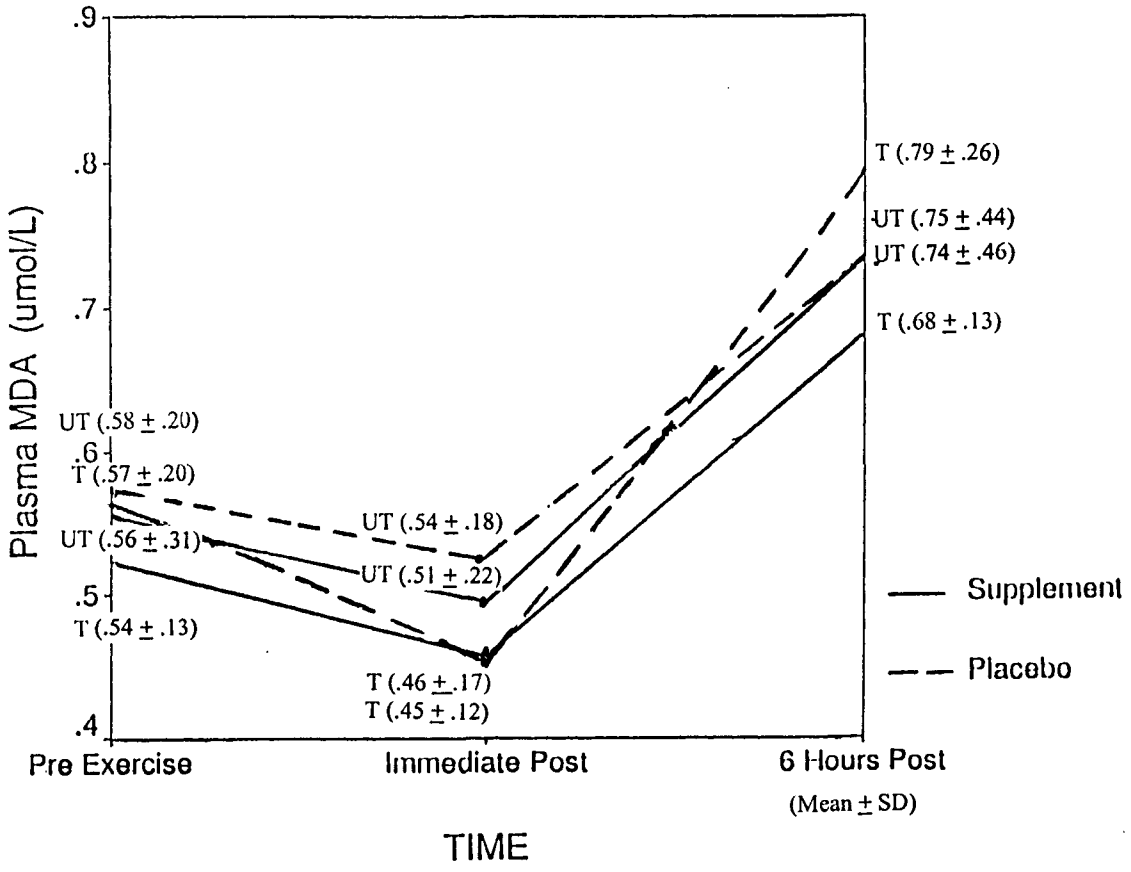
Table 6. Group x Treatment x Time Factorial ANOVA

Treatment Main Effect	**Group Main Effect**	**Time Main Effect**	**Interaction (Group x Treatment x Time) Effect**
Wilks' $\Lambda = .92$	Wilks' $\Lambda = .88$	Wilks' $\Lambda = .52$	Wilks' $\Lambda = .98$
$F(2,49) = .37$	$F(2,49) = .34$	$F(2,49) = 22.41$	$F(2,49) = .63$
$p = .55$	$p = .56$	$p = .00$	$p = .54$

Using the Pairwise Comparison Post Hoc test for the main effect for time, it was found that the 6 hour post exercise MDA values were significantly higher in comparison to the pre exercise MDA levels ($t_{53} = -4.15$, $p = .00$). Therefore, the RET did cause a significant increase in MDA and lipid peroxidation. Immediate post exercise MDA values were significantly lower than pre exercise MDA levels ($t_{53} = 2.52$, $p = .02$) and the 6 hour post exercise MDA values were significantly higher than the immediate post exercise MDA values ($t_{53} = -6.56$, $p = .00$).

In summary, there was no evidence that vitamin E supplementation was effective in reducing oxidative damage in comparison to the placebo group. As well, there was no difference between the trained and untrained groups with respect to their impact on lipid peroxidation. Figure 4 displays the MDA measures from pre to immediate post exercise and 6 hours post exercise for the four groups.

Figure 4 – Vitamin E Supplementation and The State of Training



CHAPTER 5

DISCUSSION

Two preliminary results followed an expected pattern – a high intensity resistance exercise bout resulted in significant increases in lipid peroxidation and vitamin E supplementation was able to significantly increase the levels of plasma vitamin E in participants. The end result of the study showed that the two weeks of vitamin E

supplementation (885mg/day α tocopherol acetate) did not attenuate resistance exercise induced lipid peroxidation in both untrained or trained resistance participants. If the assumption is correct that acute measures of increased levels of lipid peroxidation contribute to long term negative health consequences, then the implications of this research would indicate that vitamin E antioxidant supplementation does not combat the negative health consequences. Also, the trained participant had no added protection against free radical damage.

One of the purposes of this study was to explore the effects of a high intensity resistance exercise test on lipid peroxidation levels. Clarkson (1995) states that in the Maughan et al. (1989) study, peak changes in MDA occurred at 6 hours post exercise, while other studies have only examined immediate post exercise MDA values. The current study found a significant decrease in MDA immediate post exercise in both groups (supplemented and placebo); nevertheless, the key measurement to utilize is the measure occurring at the peak time of MDA at 6 hours post exercise. The significant increase in MDA (at 6 hours post exercise) agrees with the majority of research in the field of aerobic exercise and as well the McBride et al. (1997) high intensity resistance exercise study. In the current study, the supplementation treatment was successful. It was found that plasma vitamin E levels significantly increased with the supplementation of 885 mg/day of vitamin E for 14 days. Nevertheless, vitamin E supplementation was not able to decrease the lipid peroxidation caused by intense resistance exercise.

Other studies (Surmen Gur et al., 1999; Maxwell et al., 1993; Kanter et al., 1993) have also shown that vitamin E supplementation was not effective in reducing lipid peroxidation, although these studies used aerobic exercise as the mode of exercise. The reason for the 'no effect' of vitamin E supplementation in the current resistance exercise study is not clear. This study controlled for many outside confounding variables that might have affected the results. Plus, the plasma vitamin E levels were significantly increased in the supplemented group due to the treatment. Subsequently, one might speculate that the increased levels of vitamin E in the plasma were not transferred rapidly enough to the specific muscle tissue sites to assist in destroying the large influx of free radicals. Therefore, the creation of higher levels of vitamin E in human plasma may be an ineffective method to combat exercise induced free radicals. A second explanation might be that unknown human biological substances or factors impact or impede the antioxidant process. Further investigation is necessary to provide a definite answer to this problem.

This was the first study to explore the training state (untrained vs trained) in resistance participants to determine if the level of training had an impact on the amount of free radical damage. The two groups were significantly different in past resistance training experience and yet there was no difference found between the two groups in the production of lipid peroxidation after the resistance exercise bout. Past resistance training did not assist in reducing lipid peroxidation measures after an acute exercise bout.

Upon examination of the RET, it was realized that the trained group had lifted much heavier weights, but the elevated post exercise MDA values were the same as the untrained group MDA values. This is likely due to physiological adaptations within the trained group (due to years of resistance training) that enabled them to keep MDA production at the same level as the untrained group. Measurement of a number of the antioxidant substances would assist in determining whether there had been biological differences between the two groups as a result of the large differences in training experience.

Lastly, two limitations will be discussed that may have hidden the effectiveness of vitamin E in this study. It may be possible that vitamin E still works as one part of a bigger system and that the effectiveness of this vitamin supplement was masked by the ineffectiveness of another substance. Frei et al. (1991) found that vitamin C formed the first line of antioxidant defense in plasma under different oxidative stress and was the only endogenous antioxidant capable of completely protecting lipids against oxidative damage. Niki (1991) adds that vitamin E (tocopherols) act as the primary defense against lipophilic radicals, whereas ascorbic acid acts as the primary defense against hydrophilic radicals. In other words, various types of free radicals can develop and move in different environments such as muscle cell lipid membranes (lipid environment) or human plasma (aqueous), but a specific antioxidant will be only effective with a specific radical.

In an examination of the post exercise MDA measures, it was noted that there was a large variability in response. For example, vitamin E supplementation assisted 5 of 13 trained, supplemented participants as they had no increase in lipid peroxidation, while other participants in this group had significant increases (+ 1.4 umol/L MDA). A complex system of tissue and plasma damage is confronted by an array of antioxidant defenses. These include many antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase, as well as vitamins such as vitamin C, vitamin E and beta carotene. Prior to the 2 weeks of vitamin E treatment, the 5 participants mentioned above could possibly have had the complete package of antioxidants necessary, except for vitamin E. Perhaps the other participants had a deficiency in one or two of the antioxidant defenses (other than vitamin E) and as a result, increases in MDA developed. It would be critically important to measure many antioxidants in order to find deficiencies in participants that might interfere with an evaluation of the effectiveness of vitamin E.

A second limitation involves a methodology concern that has been identified within this field of study. There have been a variety of methodologies used to measure lipid peroxidation. It has been suggested that some of the equipment is insensitive (Alessio, 1993; Halliwell and Chirico, 1993; Kanter, 1998). For example, upon review of 3 studies involving vitamin E antioxidant supplementation, considerable differences were found in the lipid peroxidation (MDA) measures. Three slightly different methodologies were used to measure the blood plasma MDA variable. Both the McBride et al. (1997) and the Surmen-Gur et al. (1999) study used different variations of the standard MDA

procedure to determine their results and produced considerably higher values (1.8 – 5.2 umol/L; 4.3 – 5.1 umol/L respectively) in comparison to the current study (.2 – 2.2 umol/L) which included the addition of Butylated Hydroxytoleune (BHT). Therefore, the inflated values may have potentially created more room for error.

Almost all studies that have explored exercise induced oxidative stress have used indirect markers of lipid peroxidation and have not measured direct free radical generation. Perhaps with more sophisticated measuring apparatus, more precise measures can take place. Then, a definite answer to the antioxidant supplementation question may become known.

In summary, a high intensity resistance exercise bout resulted in significant increases in lipid peroxidation. Both the vitamin E supplementation and the increased levels of resistance training did not provide protection against exercise induced free radical damage.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

The results showed a high intensity RET resulted in significant increases in lipid peroxidation; the researchers initial hypothesis should be accepted. The major hypothesis of this study proposed that vitamin E supplementation would be effective in reducing lipid peroxidation. The results of this study concluded that two weeks of vitamin E supplementation (885mg/day α tocopherol acetate) did not attenuate resistance exercise induced lipid peroxidation and subsequently the hypothesis is rejected. Lastly, the investigators predicated that the trained group would show lower levels of lipid peroxidation. Upon review of lipid peroxidation results, trained individuals cannot expect lower levels of exercise induced oxidative damage compared to untrained participants.

This study was unique in that it explored the trained state (untrained vs trained resistance participants) and assessed whether there were differences in free radical damage between the two groups. Also, it utilized a more developed biochemical procedure (MDA plus BHT) to measure the dependent variable and used both females and males in an antioxidant supplementation study, whereas 80 % of studies in this field have used only males.

Recommendations for further research include the following:

1) Many of the endogenous antioxidant enzymes and vitamins should be monitored during research. This would provide a detailed picture of the changes that occur as a result of chronic training or an acute exercise bout. Most studies have failed to observe the complete picture.
2) New biochemical procedures to measure the dependent variable need to be explored. This may involve a search for new techniques that may provide a more direct measure of lipid peroxidation damage or free radical production. Kanter (1998) states that more research is required before any particular marker can be considered the method of choice in studies measuring lipid peroxidation in biological samples.
3) Females and males should be utilized as participants. Not enough research has been done with females as participants. For example, lower amounts of exercise induced lipid peroxidation may be produced in the female body due to increased antioxidant properties as a result of elevated estrogen levels.
4) Further research is needed in the field of resistance exercise and antioxidant supplementation in order to find a way to reduce exercise induced free radical damage.
5) Further investigations are required in the area of the trained versus untrained participant. There seems to be some support for long term training producing positive adaptations in the antioxidant composition in the human body.

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APPENDICES

Appendix A – Activity/Medical History Questionnaire

Name:_____ Date:_____
Address_____ Age:_____
Phone:_____ Birthdate:yr_____mon___day___

Please fill out the following questions as completely and accurately as possible.

1. Record the number of resistance exercise sessions you currently do in an average week (over the last 6 months).
   -
   -
   -
2. Record the number of hours that you spend in an average resistance session. Do not include stretching or lightly warming up or cooling down. If your resistance exercise sessions vary, record the suspected amount of hours that you would exercise during this study (spring of 2001).
   -
   -
   -
3. How many years or months have you been doing resistance exercise training ? Have you taken any breaks and for how long ?
   -
   -
4. List each sport, plus other types of aerobic exercise that you have been involved in over the last six months and how many hours a week do you participate in each.
   -
   -
   -
5. Name all vitamin and/or minerals or any other supplements that you are presently taking or have been in the past three months (Include brand names, names of vitamins/minerals, quantities).
   -
   -
6. Do you currently smoke ? (cigarettes, cigars, pipe) Yes____ No____

7. Please check all that apply.

Do you have or have you had ?	Yes	When	Treatment	No
A. Diabetes	____	_____	_____	____
B. High Blood Pressure	____	_____	_____	____
C. Epilepsy	____	_____	_____	____
D. Anemia	____	_____	_____	____
E. Malabsorption syndromes (cystic fibrosis, celiac disease)	____	_____	_____	____
F. Heart Problems	____	_____	_____	____
G. Coronary Artery Disease	____	_____	_____	____
H. High Cholesterol	____	_____	_____	____
I. Chest Pain	____	_____	_____	____
J. Gout	____	_____	_____	____
K. Arthritis	____	_____	_____	____
L. Fainting/Dizziness	____	_____	_____	____
M. Inflammatory Problems (swelling)	____	_____	_____	____
N. Bone or Joint Problems (back, hip, knee, shoulder, etc.)	____	_____	_____	____
O. Alcoholism	____	_____	_____	____
P. Nutritional Problems	____	_____	_____	____

If any, please describe _____

_____

8. Answer the following questions:

Have you ever been hospitalized as a patient ?	Yes____ No____
Do you now have allergies or asthma ?	Yes____ No____
Do you have any injury or illness at present ?	Yes____ No____
Are you taking any medication at present ?	Yes____ No____
Recent head injury or loss of consciousness	Yes____ No____

If yes, please specify_____

9. What do you consider your occupation ? Inactive ____Active ____
   Type of occupation_____

10. Do you experience unusual discomfort, shortness of breath or pain with moderate to heavy exercise ? Yes____ No____

I certify the information above is correct to the best of my knowledge.

Participant signature_____ Date_____

Appendix B – Daily Log

Group (A or B):_____ Session (1st, 2nd, or Washout)_____
Name:_____ Start/Finish dates of session_____

- Please fill out this daily log as completely and accurately as possible.
- For questions with many choices (i.e., #1, 4, 5), shade in the more appropriate box.
- Fill out **all questions every evening**, just before retiring to bed.

1. Minor illnesses (i.e., cold, flu,)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Shade in																												

Comments regarding above illness (type, symptoms experienced, medication)

2. Exercise Session (length – minutes [exclude stretching]; type – resistance or aerobic)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Length (min)																												
Type																												

3. Level of Muscle Soreness (1 = very much, 2 = much, 3 = normal, 4 = little, 5 = very little or none)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Level																												

4. Minor Injuries (i.e., shin splints, pulled muscle)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Shade In																												

Comments regarding above (i.e., type, how occurred, seriousness ?)

5. Gastrointestinal difficulties

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Shade In																												

Comments regarding above (i.e. type - stomach pains, diarrhea)

6. Major stressful events

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Type																												

Comments regarding above (i.e., exams, job, family)

Additional Comments (i.e., major changes in sleep, dietary routine, appetite)

Appendix C – The Modified Thiobarbituric Acid Test with Butylated Hydroxytoleune

Procedure

1. To the plasma sample (0.5 ml) or a 1,1,3,3-tetraethoxypropane (TEP) standard (prepared in a 400 ml/liter solution of ethanol), add 50 µl of 0.2% (w/v) Butylated Hydroxytoluene (BHT) in ethanol.

2. Divide the mixture into two tubes (for duplicate determinations) and add 1.5 ml of 0.44 *M* H_3PO_4 to each.

3. Wait at least 10 minutes at room temperature before adding 0.5 ml Thiobarbituric Acid (TBA) solution (w/v) in distilled water heated gently to 60° to dissolve TBA).

4. Heat the mixture at 90° for 45 min, and then cool on ice.

5. Add the new plasma (includes $[TBA]_2$ MDA) to the spectrophotometer and read the absorbance value at the fluorescence of 532 Nm.

Appendix D – Participant Consent Form

Study Title: "The Effects of Antioxidant Supplementation on Resistance Induced Lipid Peroxidation in Two Levels of Resistance Participants".

Investigators: Peter Viitala, MSc candidate, Ian Newhouse, Ph.D., Norm Lavoie, Ph.D., Christine Gottardo, Ph.D.

I, _____(please print name) hereby volunteer to participate in this research study entitled, **"The Effects of Antioxidant Supplementation on Resistance Induced Lipid Peroxidation in Two Levels of Resistance Participants"** under the direction of Ian Newhouse, Ph.D., School of Kinesiology, Lakehead University. The purpose of the study is to determine if oxygen free radical production will increase after a high intensity resistance exercise bout and if antioxidant vitamin supplementation (vitamin E) prior to the exercise bout has a significant effect on lipid peroxidation measures. Lastly, the researchers hope to determine if the training state, plus antioxidant supplementation has an affect on the production of oxygen free radicals and lipid peroxidation.

Free radical production has been related to the progression of many diseases such as cardiovascular disease and cancer. Antioxidant vitamin supplementation may be essential for beginner participants or resistance athletes to decrease their risk of developing life threatening diseases and curtail short term exercise induced muscle damage.

Any risks involved in this study have been explained to me. The treatment dosage of vitamin E (885 mg/day α tocopherol acetate) is below the Tolerable Upper Intake Level of 1,000 mg/day established by the Institute of Medicine and should pose no negative side effects. A certified phlebotomist will conduct blood sampling. The amount of blood will be small (20 ml) and there will be little discomfort with the procedure. There may be slight bruising and/or tenderness at the point of puncture.

The resistance exercise test (RET) will include eight exercises that will be performed in a circuit type fashion. The exercises include the bench press, incline leg press, lateral raises, leg curls, arm curls, leg extensions, lat pull downs and abdominal curls. The RET will use the 10 repetition maximums (RM) protocol. It is called 10 RM as I can only do a maximum of 10 repetitions with a specific weight for the muscle group exercised. Machine exercises and free weights will be used.

Risks for the RET are minimal, but can involve a pull or strain to a muscle which can cause temporary soreness or discomfort. Before the RET, I will be warmed up properly, taught how to perform each exercise and be properly positioned. The risk will be further reduced or eliminated by close supervision by experienced personnel during the RET so that proper form and no 'jerking' movements occur during the exercises. Other risks could include

nausea, fainting, muscle soreness and post exercise fatigue. I will be free to stop the exercise session at any time if I feel I cannot continue. There will be close supervision during the workout and monitoring during the post exercise recovery period.

Lactate measures using the Sport Lactate Analyzer and a Rating of Perceived Exertion (RPE) using the Borg CR-10 wall scale will be used to evaluate the level of intensity of the RET within each group. Lactate measures will be taken at the mid point and at the end of the RET. The penlit II blood sampler and microlance make a small pin prick on the index finger that produces a minute amount of blood. This is picked up by a capillary tube and placed into the Lactate Analyzer for analysis. There may be a little discomfort with this procedure.

The only benefits to me from participating in this study will be the information on my exercise performance abilities during the RET and blood responses. In addition, I will be making a valid contribution to science and medicine by participating in the study. Upon completion of the study, I will be briefed on my individual results and will receive a summary of the study.

The data derived from individual participants will remain confidential and publication of the results will not reveal participant identity, as the participants will be referenced by number.

I also understand that I may withdraw from the study at any time, even after signing this form, and this will in no way affect the regular care that I receive.

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

I have explained the nature of the study to the participant and believe that he/she has understood it.

_____ _____

Signature of Researcher Date

Nutrition Assessment

How to fill out the Nutritional Diary (attached sheet)

It is important to record all that you eat or drink during the day. One helpful tip is to keep your recording diary taped to the cupboard that you open the most or keep it taped to the fridge. Make sure to record 2 weekdays and 1 weekend day.

When filling out the diary, it is very important that the kind and amount of food recorded be accurate. You should even include details like brand names, whether the product is light, smooth or crunchy. Here are some examples that will demonstrate how to make a clear presentation of what you eat.

If you eat a banana and cereal, you would record the following details:

- 1 ½ cups of 2% milk
- 1 tsp sugar
- 1 large banana
- 2 cups of regular Kellogg's Corn Flakes

If you eat spaghetti and meatballs, record the following details:

- 2 cups of spaghetti noodles
- ¼ cup green peppers
- ¼ cup onions
- ¼ cup carrots
- tsp garlic powder
- 3 slices of garlic (1/2 tsp of garlic and butter mix) on white bread
- 2 cups of milk

Nutrition Diary

Name of Participant:_____ Day of the week_____			
Meal	**Description of Food or Drink**	**Brand Name**	**Amount**
Breakfast			
Lunch			
Dinner			
Snack(s)			