

**THE EFFECT OF BETA-CAROTENE, VITAMIN E, AND VITAMIN C
SUPPLEMENTATION UPON WORK CAPACITY DURING A PROTOCOL
OF ARM CURL EXERCISE USING THE BIODEX DYANAMOMETER**

A Dissertation

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Abstract

The effect of acute and chronic supplementation upon work capacity in trained and untrained subjects was examined using 10 normal healthy subjects (ages 19-23). The subjects were tested using a double-blind crossover design. During the two supplemental stages subjects ingested either the placebo or antioxidant mixture capsules (beta-carotene, vitamin C, vitamin E). There was a 28 day washout period between the two supplemental stages. Subjects were tested during each stage for an acute effect (one day of supplementation) and chronic effect (twenty-eight days of supplementation). Testing consisted of seven sets of arm curl exercises with a 60-second rest period between each set. Work, during the last three sets of each phase, was recorded and compared for each subject. Hypotheses tested was whether an acute (one day) and chronic (28 days) supplementation protocol would have an effect upon work capacity in untrained as compared to trained subjects. No significant differences were seen after acute or chronic supplementation in either trained or untrained subjects. However, power for the study was too low to make a determination without a high risk of Type II error. Significant subject dropout and work measure variability contributed to the low power.

Chapter 1 Introduction / Review of Literature

The recent interest in antioxidants and free radicals has prompted an increase in the supplementation of these substances by many in the general public. Athletes believe that supplementing their diet with antioxidants helps to maintain optimum fitness. To this end vitamin manufacturers market antioxidants to athletes, professional and amateur alike, as an ergogenic aid counteracting the affect of oxidative stress during exercise (Urso and Clarkson, 2003; Applegate, 1999; Juhn, 2003; American Dietetic Association [ADA], 2000). It is well accepted that exercise causes an increase in the production of free radicals and other reactive oxygen species (ROS) (Powers and Hamilton, 1999). Among these ROS are O₂⁻ (superoxide radical), H₂O₂ (hydrogen peroxide), and OH (hydroxy free radicals). A proliferation of these free radicals can cause a decrease in the function of affected cells and can result in a decreased ability of muscles to maintain work. Some may believe that the ingestion of antioxidants will help to stave off this proliferation of free radicals during exercise and thus provide a beneficial effect.

Vitamin E, vitamin C, and beta-carotene are three antioxidants that have garnered the most attention from those who wish to supplement their diets with exogenous nutrients. Vitamin E works to protect cell membranes by breaking the chain reactions that attack the lipid portions of those membranes. Vitamin C works in conjunction with vitamin E and is located in the cytosol of cells. It helps to regenerate vitamin E that has been used to fight the lipid peroxidation occurring within cell membranes. Beta-carotene is a form of vitamin A called pro-vitamin A. It can be converted into vitamin A as needed by the body, and much of it is located between the lipid bilayers of cell membranes. In this position, it also acts as an antioxidant protecting cell membranes.

During strenuous exercise, there is an increase in oxygen uptake by working muscles. As

work intensifies, oxygen uptake increases. This increase in oxygen uptake results in an increase in ROS that may result in the fatiguing of working muscles (Powers and Hamilton, 1999). A study by Reid and Shoji et al (1992) tested the muscle fibers of rat diaphragm in vitro to determine if, while working, these fibers produced an above normal increase in superoxide radicals (O_2^-). Fibers were bathed in a Krebs-Ringer solution containing cytochrome C and stimulated artificially (550 mA, 13 V) for one hour. Afterwards, measurements of O_2^- were taken at an absorbance at 550 nm using a spectrophotometer (model 260, Gilford). Repetitive tetanic contractions resulted in an increase in absorbance of 400% relative to relaxed controls, indicating an increase in O_2^- release in active fibers. Addition of superoxide dismutase to the medium resulted in an inhibition of this increase in absorbance. This confirmed that O_2^- was responsible for the increase in absorbance. Davies, Quintanilha, Brooks, and Packer (1982) exercised rats to exhaustion on a treadmill to observe the magnitude of increase of free radical concentration. The male Long Evans rats were exercised at a sub-maximal work intensity level, and immediately sacrificed. Using the electron pragmatic resonance (EPR) spectroscopy method, researchers found that there was a two- to three-fold increase in free radical concentration in muscle and liver, immediately following exercise.

The human body has adequate antioxidant defenses under normal physiological conditions. These defenses include enzymatic and nonenzymatic antioxidants within the interior and exterior of cells. The non-enzymatic include antioxidant vitamins, uric acid, glutathione, alpha lipoic acid, bilirubin, and ubiquinones (Powers and Hamilton, 1999). Enzymatic antioxidants are located mainly within the mitochondria and cytosol, and include superoxide dismutase, glutathione peroxidase, and catalase. The functions of each of these antioxidant molecules are illustrated in tables 1.1 and 1.2. It also appears that the body can make adaptations

in enzymatic antioxidants depending upon the oxidative capacity of muscle tissue. Jenkins, Friedland, and Howald (1984) have shown in the past that, superoxide dismutase and catalase show a linear relationship with respect to VO_2 max. In a study in which 12 healthy subjects had their VO_2 max as well as, superoxide dismutase, and catalase activity measured (in the vastus lateralis), it was discovered that those subjects with a high aerobic capacity (defined as VO_2 max over 60 ml/kg/min) had significantly higher levels of both antioxidants, than the subjects with lower VO_2 max. This suggests that the human body will adapt to a change or difference in oxygen consumption and the possibility of a higher than normal influx of ROS in to muscle tissue.

In another study by Robertson, Maughan, Duthie, and Morrice (1991), blood antioxidants were measured in six untrained and 12 trained subjects (runners). Twelve runners were assigned into two sub-groups - a high mileage group (80 - 147 km/week) and low mileage group (16 - 43 km/week). Results showed that plasma creatine kinase activity was significantly correlated with weekly training distance, indicating that there was muscle damage. The erythrocyte activity of glutathione peroxidase and catalase was significantly and positively correlated with the runners weekly mileage. This suggests that endurance training may increase blood antioxidant defenses.

During times when ROS is above normal, such as during prolonged exercise, or when antioxidant defense is hampered by nutritional deficiencies, the increase in free radicals may cause cell damage (Ji, 1994), decrease endurance capacity by as much as 40% (Davies, Quintanilha, Brooks, and Packer 1982), or attenuate force production by as much as 20% (Reid and Haack et al, 1992). Because of the recent emphasis on antioxidants in the diet, and their connection with optimum health, many individuals have increased their intake of these molecules through daily supplementation of multivitamins. Chief among the ingredients of most

multivitamins are the antioxidant vitamins E, C, and beta-carotene.

Function and Role of Beta Carotene

Beta-carotene is a pre-cursor vitamin A referred to as pro-vitamin A. That is, the human body can convert beta-carotene to vitamin A as needed. It is essentially composed of two molecules of vitamin A joined together (Guthrie and Picciano, 1995). It also belongs to a class

Table 1.1 Functions of Enzymatic Antioxidant Molecules

Enzymatic Antioxidants	Functions
Superoxide dismutase	Catalyzes the conversion of two superoxide radicals (O ₂ ⁻) into hydrogen peroxide and molecular oxygen
Glutathione Peroxidase	Eliminates hydrogen peroxide and organic hydroperoxides
Catalase	Eliminates hydrogen peroxide

Table 1.2. Functions of Nonenzymatic Antioxidant Molecules

Non Enzymatic Antioxidants	Functions
Vitamin E	Protects cell membranes from oxidation
Vitamin C	Radical scavenger and regenerates vitamin E
Uric Acid	Scavenges hydroxyl radicals
Glutathione	Serves multiple roles in the cellular antioxidant defense
Alpha Lipoic Acid	Effective as an antioxidant and in the recycling of vitamin C
Carotenoids (including Beta Carotene)	Lipid soluble antioxidants located primarily between lipid bilayers of cells
Bilirubin	May serve as an extra cellular antioxidant
Ubiquinones	Efficient antioxidants in its reduced form

The data in these tables was taken from Powers and Hamilton (1999)

compounds known as carotenoids, which are pigments synthesized within a number of plants.

Carotenoids are found in abundance in fruits and vegetables and dairy fats.

The term vitamin A is used to refer to the chemical name of retinol. In the small intestine, beta-carotene is converted to retinal, which is subsequently converted to retinol. This conversion and subsequent absorption takes place in the mucosal cell (Groff et al, 1995) and is dependent upon the vitamin A status of the individual and vitamin A content of any given meal (Blomstrand and Werner, 1967). Carotenoids and retinols released from consumed foods are packaged into micelles and subsequently cross the brush border of the small intestine to enter the mucosal cells. Within the mucosal cells, retinol is converted to retinyl esters and incorporated into the chylomicrons for transport via the lymphatic system, to the liver. Beta-carotene can be converted to retinal, which is converted to retinol and subsequently converted to retinyl esters to follow the same fate as absorbed retinol. However, some beta-carotene is not converted to retinal and is incorporated into the chylomicrons and distributed throughout the lipids of body tissues, mainly adipose tissue and the adrenal glands (Guthrie and Picciano, 1995).

Beta-carotene is located in between the lipid bilayers of tissue cells. It lies parallel with the membrane surface deep within the hydrophobic core of the two layers (van de Ven et al, 1984). It is a lipid soluble antioxidant that aids in the reduction of lipid peroxidation within cells (Powers and Lennon, 1999). Beta-carotene has the ability to quench some free radical reactions within membrane systems. This process entails the inactivation of electronically excited molecules such as singlet oxygen (O_2), generated from lipid peroxidation of cell membranes. In singlet oxygen, the peripheral electron is excited to an orbital above that which it usually occupies. Singlet oxygen can damage cells unless removed by antioxidants. Beta-carotene can also react with peroxy radicals that are involved in the oxidation of lipids, (Mascio, Murphy, and Sies, 1991) thus contributing to the defense of tissue cells. This ability to quench free radicals and other ROS can be attributed to the conjugated double bonds within the chemical structure of

carotenoids. Beta-carotene reacts with singlet oxygen by transferring excitation energy from the oxygen so that it may return to its ground state, without any chemical change to the beta-carotene.

Recommended Dietary Allowances and Toxicity

The Food and Nutrition Board of the National Academy of Sciences sets the recommended dietary allowances (RDA) for selected vitamins and minerals. The RDA for vitamin A is currently 2300 (women) - 3000 (men) IU. On most commercial labels, vitamin A is measured in international units (IU). One IU is equal to 0.3 ug retinol and 3.6 ug beta-carotene, or 0.3 retinol activity equivalents (RAE). Currently there is no RDA for beta-carotene or any of the other vitamin A precursors. It is not uncommon to see levels as high as 25,000 IU of vitamin A in the form of beta-carotene, in commercial vitamin supplements or mixtures. Intakes as high as 180 mg (Groff et al, 1995) appear to pose no harmful side effects. This is due in part by the body's selective absorption of beta-carotene as discussed earlier. As consumption of beta-carotene increases, the percentage of absorption decreases. A condition known as carotenoderma may occur. This causes a yellowing of the skin, especially on the soles of the feet and palms of the hands. However, this condition is harmless, and disappears following removal of the carotenoids from the diet.

Beta-Carotene Supplementation and Exercise

Most research concerning antioxidants with exercise, have investigated how these compounds may improve human performance. However, to date there is very little research examining the effects of beta-carotene on muscular fatigue or endurance.

One study has shown differences, following supplementation with beta-carotene. LeBlanc (1998) conducted a study in which trained runners ran a 5K race at maximum effort.

The subjects were assigned to two groups. One group received a placebo, and the other 15 mg of beta-carotene per day for a period of one month. At the end of this period, 5K race time was examined again. Afterward, subjects underwent a washout period of 2 months. Following this washout period the groups were crossed over, and the group that had received the placebo received the beta-carotene and vice versa, for one month. Following beta-carotene supplementation, five kilometer race times showed an improvement from 19.91 minutes (while taking the placebo), to 19.36 minutes. This improvement was found to be statistically significant with a p value of 0.004. There was no clear cause for the increase in exercise performance, and the study concludes that there was not a clear understanding of the mechanism for this improvement. However, what is seen in this particular experiment may be a result of an increased ability to resist fatigue rather than an improvement in muscular performance per se. In endurance races a decrease in race time may be the result of a decreased rate of fatigue, rather than an increase in speed or strength.

Function and Role of Vitamin E

There are eight compounds produced by plants that could be classified as vitamin E. These compounds fall into two classes: 1) the tocopherols; 2) and the tocotrienols. The most biologically active are the four tocopherols or tocopherols. They are alpha-, beta-, gamma-, and delta-tocopherol. The most biologically active of those four is alpha tocopherol (Farrel and Roberts, 1994). Vitamin E content in food or tissue is measured in terms of tocopherol equivalents (TEs). One TE corresponds to 1 mg of alpha-tocopherol, and is equal to one IU of alpha tocopherol.

Vitamin E is distributed in a wide range of foods. Vegetable oils, green leafy plants, and the bran and germ of some plants represent the majority of dietary vitamin E sources. Vegetable oils have the highest content of vitamin E per gram. However, the average serving of these in the

diet is relatively small. The next highest food sources are fortified cereals followed by vegetables (Guthrie and Picciano, 1995).

Absorption of vitamin E occurs in the jejunum via passive diffusion (Erdman et al, 1988; Sokol, 1988). The vitamin is carried via micelles into the enterocytes. Similar to other fat soluble vitamins, absorption of vitamin E improves when lipids are present in the diet (Bender, 1992). Similar to beta-carotene, as vitamin E intake increases absorption decreases. Absorption rate can vary between 20 to 50% (Groff et al, 1995) but, in special conditions may rise as high as 80%. The supplemental use of vitamin E in doses as high as 200 mg, may result in a drop in absorption to 10% (Farrel and Roberts, 1994).

Once vitamin E enters the enterocyte, it is incorporated into chylomicrons and transported via the lymph. While in the lymph, vitamin E equilibrates with plasma lipoproteins, (high density lipoproteins [HDLs], low density lipoproteins [LDLs]) and with erythrocytes (red blood cells). In the erythrocytes, vitamin E is incorporated into the cell membrane.

Vitamin E (tocopherol) is distributed throughout the body primarily by the LDLs (Groff et al, 1995). Uptake of vitamin E by tissue cells happens mainly in two ways: 1) uptake of LDLs by LDL receptors (Traber and Kayden, 1984; Sokol, 1988); 2) or lipoprotein lipase hydrolysis of the chylomicrons and very low density lipoproteins (VLDLs) (Groff et al, 1995). Once inside the cell it binds to tocopherol binding proteins for transport to cell, mitochondrial, and microsomal membranes (Farrel and Roberts, 1994). Vitamin E is distributed throughout the body tissues. Most is stored in the adipose tissue with smaller amounts in the liver, lungs, heart, muscles, adrenal glands and the brain. The amount of vitamin E in the adipose tissue increases as intake increases. However, levels in the other tissues stays constant or increase at a very slow rate. When intake is low, withdrawal from the liver and plasma is fairly rapid but, occurs slowly from

the adipose tissue (Diplock, 1984). Depletion of vitamin E from the muscle occurs at an intermediate rate.

The main function of vitamin E in the human body is as an antioxidant. It is the body's first line of defense against cellular membrane lipid peroxidation. In tissue cells, vitamin E is embedded within the lipid bilayers. There, it can assert its ability to stabilize cell membranes and break the chain reactions caused by free radicals and other ROS (Powers and Hamilton, 1999). The reduction of molecular oxygen in tissue cells can generate a superoxide radical (O_2^-) and later hydrogen peroxide (H_2O_2). These can lead to the formation of lipid peroxy radicals (LOO). Lipid peroxy radicals can remove a hydrogen atom from polyunsaturated fatty acids (PUFA). If these PUFA are located within the membrane of tissue cells, it can cause a chain reaction throughout the cell membrane. These chain reactions must be broken to keep minimal the amount of damage to cell membranes. Vitamin E can break these chain reactions by reacting with lipid peroxy radicals, thus preventing their abstraction of hydrogen in PUFA (Groff et al, 1995). This is done by providing hydrogen for the reduction of the lipid peroxy radical. Furthermore, clinical tests suggest that it (vitamin E) may actually reduce the susceptibility of low-density lipoproteins to oxidation by free radicals (Jialal and Grundy, 1992). Once vitamin E has broken this reaction it is oxidized to create an alpha tocopheroxyl radical and must be regenerated. This regeneration is carried out by ascorbate (vitamin C) via a series of interactions between NADPH, and reduced glutathione (Packer et al, 1979; Groff et al, 1995; Wefers and Sies, 1988). This relationship is further illustrated in a study by Brown, Morrice, and Duthie (1997). They studied the results of a vitamin E supplementation protocol upon oxidative stress in smokers and non-smokers. Smokers because of their habit, are in a perpetual state of oxidative stress. Smoking brings into the body a higher than normal amount of ROS with which the

antioxidant defenses must deal. In this study, fifty smokers had habitually lower plasma ascorbate concentrations ($p < 0.02$) and higher susceptibility to hydrogen peroxide (H_2O_2) stimulated erythrocyte peroxidation ($p < 0.001$) than did nonsmokers. They found that after 20 weeks of supplementation with either 70, 140, 560, 1050 mg alpha tocopherol/day, there was a significant decrease in erythrocyte susceptibility to oxidation ($p < 0.001$) in the group of smokers. Moreover, there was a decline in plasma ascorbate concentration ($p < 0.02$) in the nonsmokers after prolonged alpha tocopherol supplementation. This may be an illustration of the relationship between vitamin E and vitamin C discussed previously.

Recommended Dietary Allowances and Toxicity

The daily requirement for vitamin E increases as the intake of PUFA increases. On the other hand, most foods high in PUFA are also high in vitamin E (Lehman et al, 1986). The RDA for women and men is, 8 mg TE/day and 10 mg TE/day respectively. The normal intake of vitamin E in American diets ranges from 4 to 22 mg TE/day in adults, who are not supplementing their diet with exogenous vitamin E (Di Mascio et al, 1991). Depending upon the intake of PUFA in the diet the actual need for vitamin E can range from 5 to 20 mg TE/day (Guthrie and Picciano, 1995). The RDA for vitamin E is high enough to maintain a blood level of around 0.5 mg/dl, which is considered safe. Deficiency in humans is extremely rare (Groff et al, 1995). Usually individuals exhibiting deficiency in vitamin E are low birth weight infants, individuals with malabsorption syndromes, and those suffering from abetalipoproteinemia. Abetalipoproteinemia is a genetic disease in which apolipoprotein B, necessary for the formation of chylomicrons, LDLs, and VLDLs, is lacking (Sokol, 1988). In addition, any disorder that affects the absorption of fat will have an affect on the absorption of vitamin E. In the rare instance of vitamin E deficiency, anemia can occur because of the hemolysis of red blood cells,

as well as retinopathy, muscle weakness, and neurologic problems.

It appears that large doses of vitamin E may be consumed without harmful side effects. Intakes between 400 and 800 mg TE/day have been taken for months or even years without any harm (Council on Scientific Affairs, 1987). High dosages of vitamin E may interfere with the functions of other fat soluble vitamins (vitamin K, beta-carotene) but, this is only seen in dosages exceeding 1 g/day (Groff et al, 1995)

Vitamin E Supplementation and Exercise

A relationship between vitamin E and athletic training was first posed in the 1950s (Cureton 1954). Cureton suggested that ingestion of wheat germ oil would enhance the rate of improvement seen in physical performance following aerobic training in human subjects. At that time, it was thought that vitamin E was the active agent within wheat germ oil. Since that time, most research investigating vitamin E and exercise performance has involved animals and not humans. In 1990 Novelli, Bracciotti, and Falsini (1990) saw a 50% increase in endurance time in rats during exercise, following treatment with vitamin E. Swiss mice were injected intraperitoneally with a solution of alpha tocopherol in olive oil (100mg / kg body weight), a solution of three spin trappers (N-tert-butyl-alpha-phenyl nitron [PBN], 5-5-dimethyl-1-pirrolyn-N-oxide [DMPO], and alpha-4-pyridyl-1-oxide-N-tert-butyl-nitron [POBN]) in saline solution, or saline solution alone. Spin trappers are compounds that react with free radicals and make them less reactive. Rats were exercised to exhaustion, four at a time, before and after treatment by attaching 1 gram weights to their tails and allowing them to swim in a bath until exhausted (exhaustion was defined as when a rat's head submersed three times). Average time to exhaustion for the alpha tocopherol treated mice went from 276 seconds to 415 seconds. Time to exhaustion for the spin trapper treated mice also increased significantly: 1) PBN treated mice

increased from 298 to 393 seconds; 2) POBN increased to 493 seconds; 3) DMPO increased to 555 seconds. The researchers believe the results of their study indicate that the trapping and / or inactivation of free radicals may increase endurance during exercise.

In an interesting study by Sacheck, Decker, and Clarkson (2000) the connection between vitamin E intake and moderate intensity exercise was examined. They recruited 24 female rowers from a local college rowing team. After completing a 3-day food diary within one week of the exercise test vitamin E intake was assessed. The rowers were assigned to two groups of 12 subjects. One group was a low fat intake group and the other was a high fat intake group. The low-fat group was classified as those ingesting less than 40 grams of fat/day, and the high-fat subjects ingested more than 60 grams of fat/day. None of the subjects were supplementing with any nutritional substances, taking medications, using oral contraceptives, or consuming alcohol during the study time period. Following diary assessment it was discovered that the low-fat group was consuming 2.9 mg vitamin E/day and the high fat group 9.8 mg vitamin E/day. The low fat group was consuming less than the normal intake range for vitamin E in American diets (Di Mascio et al, 1991). However, they were also ingesting a very low fat diet which consequently is low in PUFA. The ratio of vitamin E to PUFA (0.56 mg/g) was well above the recommended ratio of 0.4 mg/g (National Research Council, 1989) for the low fat group. The ratio for the high fat group was 0.69 mg/g. Subjects were instructed to run on a treadmill for 5 min at a 0% grade, thereafter they ran at a speed that elicited a rise to 75% of their age related maximum heart rate ($220 - \text{age}$), at a -10% grade (downhill) for 45 minutes. Venous blood was collected immediately prior to exercise, immediately post exercise, and at 6, 24, and 48 hours after exercise. Serum vitamin E levels were not significantly different between the two groups pre- or post-exercise, nor were there any significant differences seen between low-fat and high-

fat groups in regards to creatine kinase, malondialdehyde (an indicator of lipid peroxidation), or conjugated dienes (formed during the peroxidation of PUFA) pre- or post-exercise. There were significant increases in plasma creatine kinase at 6, 24, and 48 hours post exercise in both groups, when compared to pre-exercise values, but as previously expressed there were no significant differences between the two groups (no significant interaction). They concluded that, although the low fat group did not consume as much vitamin E as the high fat group, and they were below the RDA for vitamin E, the intake of vitamin E was sufficient to help protect against the oxidative damage brought about by the moderate-intensity exercise. Furthermore, since their serum vitamin E levels were normal throughout the study, perhaps the body may be able to compensate for low vitamin E intake in the low-fat subjects. It also may be possible that the ratio of vitamin E to PUFA plays an important part as well. Although the intake of vitamin E was low, the ratio was within the normal values. It is well established that the need for vitamin E increases as PUFA intake rises. However, if these low fat individuals were consuming a lower than normal amount of PUFA, then the amount of vitamin E they were ingesting may have been sufficient. Another question that remains to be answered. Does the training status of these rowers have more of an influence upon their ability to stave off the effects of oxidation during moderate-intensity exercise than their vitamin E status? Being endurance athletes one would expect there to be a larger than normal production of enzymatic antioxidants because of training (Roberson et al, 1991). It remains to be seen how athletes or untrained individuals would respond to a bout of high-intensity exercise, in respect to vitamin E status.

Although some animal studies may indicate that supplementation with vitamin E may decrease exercise induced oxidative stress, in humans most research to date has not indicated any improvement in endurance. Sumida, Tanaka, Kitao, and Nakadomo (1988) supplemented

twenty-one healthy male college volunteers with 300 mg vitamin E for four weeks. The subjects performed a pre- supplementation and post-supplementation protocol of exercising to exhaustion using an incremental exercise test upon a Monark cycle-ergometer. The exercise test involved subjects pedaling a Monark bicycle-ergometer at an initial work rate of 4 minutes at zero watts. Thereafter, the work rate was increased by 15 watts every minute until a pedaling rate of 60 rpm could no longer be maintained. Following data collection, the investigators found no significant difference in $VO_{2\text{ peak}}$ (Control - 48.9 ml/kg/min; Vit E - 49.1 ml/kg/min) or time to exhaustion (Control - 19.0 min; Vit E - 19.1 min). However, this was not a double-blind placebo controlled trial. Lawrence et al (1975) supplemented groups of trained swimmers with 900 IU of alpha tocopherol or placebo in a double-blind protocol for 6 months, but also found no difference between the supplemented and placebo groups. Subjects were given 21 tablets of 300 IU of alpha tocopherol or a placebo, every week. They were asked to perform an endurance swimming test consisting of 10 laps of 100 yards with a 10 second rest in between. After one month of supplementation, the two groups decreased their swimming time by an average of 3%. After six months, those in the alpha tocopherol group had decreased their time by 6.8%, and the placebo group had increased theirs by 6.2%. These differences however, were not found to be statistically significant. This suggests the possibility that a crossover design, in which subjects are their own control, might be helpful in detecting differences that may have been overlooked. Though there is much research still to be done, it remains possible that vitamin E may need to be taken in conjunction with other antioxidants (Balakrishnan and Anuradha, 1998; Powers and Hamilton, 1999; Schroder et al, 2000; LeBlanc 1998), to have an effect upon muscular endurance and / or fatigue, and not just in the reduction of free radicals. It may be possible that these combinations will have an effect upon the ability to sustain a given amount of work over time

Researchers have used many different protocols and subjects to investigate the effect that vitamin E would have upon muscular fatigue. Of the studies previously cited none used the double blind-placebo controlled design that is most preferred. One injected each of three groups with a compound (either spin trappers, alpha tocopherol, or saline solution) to examine their effects (Novelli et al, 1990) however, this was not a double blind procedure. Nor was the study by Sumida et al (1988), in which they investigated the differences in VO₂ and exhaustion, a double blind study. Lawrence et al (1975) did use the double blind design in their investigation. However, they chose not to employ a crossover within their study. There have also been a number of different dosages and methods for ingesting the supplement. Some researchers injected subjects with alpha-tocopherol and some had subjects ingest it in tablet form. Thus, there is no standard dosage that is used to determine the effect of vitamin E upon work or fatigue. Interestingly, Sacheck et al (2000) chose to monitor vitamin E intake levels rather than have subjects supplement with exogenous vitamin E. However, no differences were seen in those ingesting a high fat or low fat diet. With that in mind, training status may result in a lack of any effect of the two diets in that particular investigation. This may also have been the reason no significant effect was seen with Lawrence et al (1975) as well.

Function and Role of Vitamin C

Vitamin C is probably the most supplemented and researched vitamin known to man. The chemical name for vitamin C is ascorbic acid or ascorbate. At one point in history, it was known as antiscorbutic because of its ability to prevent scurvy (Guthrie and Picciano, 1995). Human beings are one of the few mammals that cannot synthesize ascorbic acid. So therefore, they must obtain their supplies of this vitamin through food sources.

The best sources of ascorbic acid are found in foods of plant origin. Foods such as

oranges, cantaloupe, broccoli, green peppers, grapefruit, lemons, and strawberries are among the highest in vitamin C content. When any of these foods are cooked or processed using heat, they can reduce the amount of vitamin C content. Most plants containing vitamin C are best consumed raw to avoid any loss in the content of the vitamin.

Ascorbic acid has many functions within the human body. It is a water soluble vitamin vital in carnitine synthesis (needed for the transport of fatty acids into the mitochondria), collagen formation, neurotransmitter synthesis, and most importantly, as an antioxidant. It also plays a part in the process to convert cholesterol to bile acids and aids iron absorption (Guthrie and Picciano, 1995).

After ingestion, absorption of vitamin C occurs in the jejunum (Groff et al, 1995). Absorption takes place primarily via active transport into the intestinal cells. Before being absorbed, it is possible that some ascorbate will be oxidized to form dehydroascorbate because of the presence of Fe^{+++} present in the intestine (Englard and Seifter, 1986; Guthrie and Picciano, 1995). Dehydroascorbate can be absorbed by passive diffusion into the cells. Once inside the cell dehydroascorbate is reduced back to ascorbic acid by dehydroascorbate reductase. Ascorbate moves across the basolateral membrane of the intestinal cells by sodium-independent carrier-mediated transport (Groff et al, 1995). After moving through the cells, ascorbate moves into the blood stream and is carried by the plasma throughout the body. It moves with ease into body cells. Some tissues have a greater concentration than others. The highest concentration is found in the adrenal and pituitary gland (50 times the concentration in serum). More intermediate levels are found in the liver, pancreas, lungs, and kidneys (5 to 30 times the concentration in serum). Lower levels are found in the muscles, and red blood cells. When amounts up to 100 mg are consumed, 80-90% of all vitamin C is absorbed (Guthrie and Picciano, 1995). However, as the

amount of vitamin C ingested increases, absorption decreases. As little as 16% is absorbed when amounts around 12 grams are consumed.

The amount of vitamin C within the entire body can be between 1.2 to 2.0 grams when 100 mg/day of the vitamin are ingested. This is the level deemed necessary to protect the body from scurvy for 90 days (Guthrie and Picciano, 1995). If large amounts of vitamin C are consumed the body will only absorb and retain enough to maintain a plasma concentration of 1.2 to 1.5 mg/100 ml. Anything beyond that needed to maintain these levels is excreted.

As an antioxidant, ascorbic acid may react with OH (hydroxyl radical), O_2^- (superoxide radical), H_2O_2 (hydrogen peroxide), and HO_2 (hydroperoxyl radical). Once ascorbate reacts with these ROS, semidehydroascorbate radical (also called ascorbyl) and water, are formed. Two ascorbyls react to form ascorbate and dehydroascorbate (Groff et al, 1995). Dehydroascorbate can react with glutathione to form ascorbate. As a reductant, vitamin C can reduce cupric (Cu^{2+}) to cuprous (Cu^+) ions, and ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions (which aids in their absorption in the small intestine). These two products (Cu^+ , Fe^{2+}) may react with free radicals and other ROS, and may cause damage to cells via the generation of more ROS (Groff et al, 1995). Ascorbate can react in aqueous environments within the body, including blood, extracellular fluid, and cell interiors, before any oxidative damage may occur to lipids (Frei, 1991).

Recommended Dietary Allowance and Toxicity

The RDA for vitamin C is currently set at 60 mg/day for adult men and women. This is the amount deemed necessary to ensure that body tissues are saturated with the vitamin (a body pool of 1500 mg). Some nutritionists believe that the RDA should not be greater than the amount needed to prevent scurvy (10 to 12 mg/day) (Guthrie and Picciano, 1995). Conversely, there is some evidence that saturation of the tissues of young men with ascorbic acid may require an

intake of 138 mg/day (Jacob et al, 1987).

Vitamin C is relatively non toxic (Alhadeff et al, 1984). However, toxicity is more likely when large doses (in excess of 1 gram) are ingested several times a day. This is because absorption of vitamin C in the small intestine is saturable and dose dependent (Groff et al, 1995). This results in a more pronounced absorption of vitamin C throughout the day as opposed to taking one large dose. However, any vitamin C in excess of a body pool between 1200 and 2000 mg is filtered by the kidneys and excreted in the urine. Finally, unabsorbed vitamin C in the intestine can cause diarrhea because of its osmotic effect (Alhadeff et al, 1984), which draws water into the intestinal tract.

Vitamin C Supplementation and Exercise

For years, athletes have been supplementing with vitamin C with the belief that it will aid or at least help to maintain athletic performance. However, much of the research aimed at investigating this long held belief has been inconclusive. In one particular animal study, Lang, Gohil, and Packer (1986) supplemented guinea pigs with up to 4 grams/ kg of body weight of vitamin C for two weeks and observed an increase in endurance capacity. At the same time, they did not see a concomitant increase in vitamin C tissue levels, but saw a decrease in muscle vitamin E levels. In addition, ubiquinone levels stayed constant. They suggested that this might show a tissue specific interaction between C, E, and ubiquinone. Human studies reveal conflicting results. As early as 1966, Spioch, Kobza, and Mazur (1966) injected volunteers with 500 mg of vitamin C before a 5-minute step test to evaluate the effectiveness of supplementation on human performance. They found a reduction in oxygen consumption (by 12%), oxygen debt (by 40%), and pulse rate (by 11%). Unfortunately, this study was uncontrolled. Consequently there was no way to know if the improvements were attributed to the supplementation or training

effect. In 1985 a vitamin C supplemented group of 16 healthy female Indian physical education college students, saw improvement in physical efficiency (by 5.0 on the Harvard Step Test), and a 9-minute walk / run (236.77 meters) (Samanta and Biswas, 1985). However, only the improvement in 9-minute walk in the supplemented group was significant at $p < 0.05$. Students were randomly assigned to an experimental group and a control group. Each subject in the experimental group was given a 500mg vitamin C tablet every day for four weeks.

Unfortunately, they did not compare results against a sedentary group to see differences between physically active individuals and the latter. In a study using 286 American Air Force officers, half of whom supplemented with 1 gram of vitamin C daily for 12 weeks, a small improvement was seen in endurance performance (12-minute run) between supplemented and unsupplemented subjects (Gey, Cooper, Bottenberg, 1970). However, this improvement was not statistically significant (less than 1%). Keren and Epstein (1980) saw no improvement in maximum oxygen uptake during a cycle-ergometer test, in untrained subjects after they ingested 1 gram of vitamin C daily. The untrained subjects were assigned to a supplemented group or a placebo group. Each group was given either 1000mg per day of vitamin C or a placebo for 21 days in a double-blind manner. Before and after supplementation VO_2 max and anaerobic capacity were measured. After 21 days of aerobic training (running and hiking), in conjunction with the supplementation protocol, no differences were seen ($p < 0.05$) in the aerobic or anaerobic capacities of the two groups.

In 1990, a group attempted to see if a vitamin C restricted diet would have an impact upon physical performance in a group of healthy volunteers (van der Beek et al, 1990). Twelve healthy subjects were prescribed diets providing low vitamin C intake (20% of the Dutch RDA) for seven weeks. In a double blind manner, six of the subjects were assigned to an experimental

group and the other six were assigned to the control group. Both groups also received twice the RDA for all other vitamins during the study to ensure no deficiency other than vitamin C. There was a 3-week introductory period preceding the 7-week experimental period. The first 2 weeks of the introductory period subjects consumed their regular diet and vitamin C equal to the Dutch RDA (DRDA) (50 mg/day). At the last week of the introductory period subjects began consuming the experimental diet. Once the 7-week experimental period began, the control group consumed the prescribed vitamin C deficient diet with twice the DRDA of all vitamins other than vitamin K, for the entire supplementation period. The experimental group continued on the diet but at weeks 1-3 they also supplemented with twice the DRDA for all vitamins other than C and K. At weeks 4-7 they ingested an additional 15 mg vitamin C per day (for a total of 25 mg/day). Following this 7-week vitamin C deficient regimen the experimental group received a dose of vitamin C per day that was twice as high as the DRDA. Blood was drawn at the before the experiment and at weeks 3, 7, and 10. Measures of VO_2 max and onset of blood lactate (OBLA) were measured at the times that blood was drawn. VO_2 max was determined via an incremental cycle-ergometer test. Subjects pedaled at 50 watts at a rate of 60-80 rpm. After 2 minutes work-rate was increased 50 watts and subsequently 25 watts every 2 minutes thereafter to exhaustion. Blood was drawn during the last 30 seconds of each step to determine blood lactate. Following data analysis it was found that there were no significant differences between the experimental group and the control group for aerobic power or OBLA. There were significant differences between the experimental group and the control group with respect to serum vitamin C levels ($p < 0.01$). There was a slight increase in heart rate at each of the three exercise tests in the experimental group, but this increase was not significant. However, the authors hypothesized that this increase may have been related to the lower than normal serum vitamin C level and the

effect upon carnitine synthesis and / or catecholamine receptor sensitivity. But no evidence of this has been confirmed. Being that subjects were never totally restricted from ingesting vitamin C during the course of the study, it may be difficult to ascertain its true effect upon aerobic power and OBLA. In this study subjects were supplemented with the DRDA for vitamin C prior to the initial restriction period and then for the first three weeks were restricted to 20% of the DRDA and then the next four weeks to 50% of the DRDA. Perhaps, for that short period, the body was able to maintain a level of serum vitamin C high enough to maintain aerobic capacity, by releasing some vitamin C from other tissues throughout the body (i.e. adrenal gland, pituitary gland, liver, etc.).

As with vitamin E, many diverse protocols have been used to test the effectiveness of vitamin C on work capacity. Some, like Lang et al (1986), have used animals and, some have used human subjects. There have been varying results between species among the studies cited, and there does not appear to be a clear-cut answer as to how effective vitamin C may be. Some have used trained volunteers (Samanta and Biswas, 1985; Gey et al, 1970;), while others have used untrained subjects (Spioch et al, 1966; Keren and Epstein, 1980; van der Beek et al, 1990). There has also been a wide variety of experimental designs. Some used no controls, while others used a control group. At least two used a double-blind method (Keren and Epstein, 1980; van der Beek, 1990) while most did not. In human subjects, the dosages range between 500mg and 1000mg per day. Most studies examined the chronic effect of vitamin C supplementation upon muscular work and / or aerobic capacity, while only one examined the effect of an acute dosage of the vitamin. In addition, one study examined the effect of chronic versus acute dosing upon human subjects. Allesio et al (1997) supplemented subjects with 1000 milligrams of vitamin C or a placebo, per day for 1 day and 2 weeks. At 1 day and at 2 weeks subjects performed 30 minutes

of submaximal exercise. Before and immediately following exercise plasma thiobarbituric acid and reacting substances (TBARS) and, oxygen radical absorbance capacity (ORAC) were measured. TBARS are biomarkers of oxidative damage (LeBlanc, 1998) used to indirectly measure the extent of lipid peroxidation in biological systems. They (Alessio et al, 1997) found that following the exercise TBARS were 12.6 and 33% above resting values in the supplemented group, compared to 46% higher in the placebo group. No changes were seen in ORAC. When comparing oxidative stress (represented by the ratio of TBARS to ORAC), the placebo group noticed a 32% increase compared to 5.8 and 25.8% in the vitamin C group at 1 day and 2 weeks, respectively. This led to the conclusion that oxidative stress was higher in those who ingested the placebo, than those who ingested vitamin C.

None of the aforementioned investigations were conducted in a double-blind crossover design. This design would be the most thorough method of detecting any differences between or within an experimental group and, it would be the method of choice for a subsequent study on this subject.

Antioxidant Combinations

There have been a few groups who have attempted to study the effects of some antioxidants in combination and how they may effect muscular work or endurance. Rokitzki et al (1994) set out to examine if supplementation with antioxidants could influence lipid peroxidation and muscle enzymes. The subjects were 22 trained long distance runners. Half of the subjects ingested an antioxidant capsule of 400 IU alpha tocopherol and 200 mg vitamin C per day, while the others received a placebo for a total of 4.5 weeks. At the end of the supplementation period the subjects participated in a marathon race. Serum measures of retinol, ascorbic acid, beta-carotene, alpha tocopherol, malondialdehyde, uric acid, glutathione peroxidase, and catalase

were taken at 4.5 weeks before, immediately before and after, and 24 hours after the marathon. Serum concentrations of alpha tocopherol and ascorbic acid increased significantly after the 4.5 week supplementation period in the supplemented group. There were no significant changes in glutathione peroxidase or catalase. There was a significant increase in creatine kinase concentration immediately after and 24 hours after the marathon, in both the supplement group and control group. However, the creatine kinase increase in the supplemented group was significantly lower than in the control group. As creatine kinase is an indicator of muscle damage it was concluded that the supplementation with the alpha tocopherol / vitamin C combination helped to reduce the amount of blood creatine kinase increase during exercise. However, one drawback is that there was no way of measuring the intensity at which the subjects ran the race. It may be possible that they were running at a much lower pace than usual, which would not necessarily be comparative to results after running as fast as they could cover the distance. Similar results were found by another group recently (Bryant, Ryder, Martino, Kim, and Craig, 2003). They used seven trained cyclists in which each ingested two capsules per day containing these four treatments: placebo (placebo plus placebo); vitamin E (400 IU per kg plus placebo); vitamin C (1 gram plus placebo); and vitamin C and E (1 gram of C and 200 IU per kg vitamin E). The treatment order was placebo, vitamin C, vitamin C and E, vitamin E. Each subject ingested the treatment in the same order. Each supplementation phase lasted for 3 weeks for a total of 12 weeks. At the end of each phase subjects performed a steady state ride on a cycle ergometer for one hour followed by a 30-minute performance ride. The steady state ride was at 70% of VO_2 max at a pedal rate of 90 rpm. Immediately afterwards, the performance ride was at the same workload, but subjects could establish their own pedal rate. Blood samples were collected immediately before and after the exercise trials and analyzed for malondialdehyde and

lactic acid. Results showed that malondialdehyde levels pre-exercise (2.94 umol and 4.81 umol) as well as post exercise (4.32 umol and 7.89 umol), were lower during the vitamin E phase than during the placebo phase. Lactic acid levels were also lower in the vitamin E supplementation period than the other three periods, although not statistically significant. They also noted that total work levels decreased after each supplementation phase. Although interesting it would appear that because these subjects were training during the study, their training may be having more of an effect upon these measures than the actual supplementation regimen. Additionally, each supplement was only taken for 3 weeks which may not be enough time to evaluate each individually especially considering that the third phase was a combination between C and E. A larger study utilizing a crossover design, untrained subjects, and perhaps a washout period would give a clearer picture to the effect of either of these antioxidants. However, the authors point out that because of time restraints they were unable to perform a washout in this particular study.

Another group set out to investigate the influence of a vitamin C and E combination upon muscular damage using trained athletes similar to an aforementioned study. Dawson et al, (2002) had 15 male distance runners divided into two groups. One group supplemented with 500 mg of vitamin C and 500 IU of vitamin E, or 1000 mg vitamin C and 1000 IU vitamin E, for 4 weeks. The other group received a placebo. Following this first supplementation period subjects ran a 21 km race as fast as possible. Afterwards, there was a 4-week washout period followed by a crossover in the supplementation regimen for each subject. Following another 4-week supplementation period another 21 km race was run. Venous blood was taken before, immediately after, and 24 hours after each run. Samples were analyzed for creatine kinase, myoglobin, malondialdehyde, and both vitamins. There was also a subgroup that had muscle biopsies taken 24 hours before and 24 hours after each run. Both vitamin dosages produced very

similar results so, vitamin groups were combined into one group. There were significant within group differences ($p < 0.05$) seen in serum creatine kinase, and myoglobin levels, but not malondialdehyde, after the run. However there were no between group differences for those same measures. In the biopsy samples there were changes seen consistent with endurance training but, again no significant differences between the placebo and supplemented conditions. They concluded that this dosage of vitamin C and E did not reduce biochemical or muscle structural indices for damage in these runners. It appears that it may be possible that these runners were so highly trained that they may already have more than enough enzymatic protection through adaptation, against the affects of oxidative stress in working muscles. A 21 km run is an extremely long event and there is no indication of the workload that these individuals were using in the study. The authors assumed that the athletes were running as fast as possible, but there was no way to measure this. In a long event as this it is quite possible that these runners never reached a point at which their already enhanced enzymatic antioxidant systems was over stressed. If the authors had required the subjects to run on a treadmill, true workload may have been quantified and compared rather than relying on the subjects honest effort.

Pro-oxidant Activity of Beta Carotene, Vitamin E, and Vitamin C

Beta-carotene, vitamin E, and vitamin C are antioxidants. However, it has been suggested that they may also serve as pro-oxidants in certain situations. They may promote oxidation via their action upon certain molecules, such as cupric (Cu^{2+}) and ferric (Fe^{3+}) ions, or depending upon which nutrient, by the failure to regenerate after serving in their role as an antioxidant.

There have been some reports of pro-oxidant effects of beta-carotene in vitro when high doses have been administered. However, the in vitro pro-oxidant effect of beta-carotene was

dose dependent only in an environment of 100% oxygen pressure (Palozza et al, 1995). Palozza et al, (1995) used rat liver microsomal membranes exposed to 2,2'-azobis (2-amidinopropane) (AAPH), a radical initiator, to illustrate this point. They showed that at a PO₂ of 150 mm Hg beta-carotene acted as an antioxidant, inhibiting AAPH induced malondialdehyde (MDA) formation. However, at 760 mm Hg PO₂ beta-carotene lost its antioxidant properties resulting in an increase in lipid peroxidation. Their group used four different concentrations of beta-carotene to illustrate the point that different dosages would elicit a slightly different effect. At a PO₂ of 150 mm Hg, there was a decrease in MDA at each of four successively higher concentrations of beta-carotene. On the other hand, at a PO₂ of 760 an opposite effect was seen. At the same four successive concentrations of beta-carotene an increase in MDA is seen. Later they showed that the effect was prevented by a concomitant addition of alpha tocopherol, illustrating the cooperative interaction of these two antioxidants. Another study showed that beta-carotene acted as an antioxidant and inhibited AAPH induced lipid peroxidation in normal and in tumor cells at 150 mm Hg PO₂ (Palozza, P. et al, 1997). However, when PO₂ was increased to 760 mm Hg beta-carotene exhibited a pro-oxidant effect, in addition to losing its antioxidant effectiveness. It was noted that the pro-oxidant activity was more pronounced in the tumor cells than in the normal cells. More study is needed to investigate these effects in vivo. It is possible that beta-carotene is incorporated into biological membranes in vitro differently than in vivo (Liebler, D.C. et al, 1997). PO₂ in living tissues is much lower than those in the aforementioned studies. Arterial blood PO₂ is 100 mm Hg and in most tissues it is 40 mm Hg or less (Vander et al, 1990). Therefore, it is likely that any pro-oxidant properties displayed by beta-carotene in vivo would not be caused by a high partial pressure of oxygen.

The pro-oxidant effect of beta-carotene may be caused by the auto-oxidation of beta-

carotene at higher PO₂ values in vitro. It may be possible that at high oxygen PO₂, a chain-carrying beta-carotene peroxy radical is formed (Burton, 1989; Burton and Ingold, 1984; Kennedy and Liebler, 1991). This may be the reason why antioxidant effectiveness is reduced in high PO₂ environments (Kennedy and Liebler, 1992). Carotenoids may interact with free radicals in three ways: 1) electron transfer; 2) hydrogen abstraction; 3) and radical species addition (Young and Gordon, 2001). The products of these reactions may react with oxygen to produce peroxy radicals. In the end, it may not be a pro-oxidant effect that is seen, but a reduced antioxidant effect (Palozza et al, 1995). An environment of 100% oxygen is not physiologically tangible so, it is not likely that beta-carotene would display a pro-oxidant effect in vivo (Burton and Ingold, 1984). However, as there are varying PO₂ throughout body tissues, all less than 40 mm Hg, it is possible that beta-carotene may be more or less effective as an antioxidant depending upon the tissue.

Few reports show any pro-oxidant effects of vitamin E. Most, if not all, have been in vitro studies, and most have been in abnormal physiological environments. As discussed previously, alpha tocopherol is a major antioxidant present in LDLs (Sokol, 1988). It is assumed that increasing the amount of alpha tocopherol in LDLs would make them less susceptible to oxidation. However, under mild oxidative conditions it has been shown that increasing tocopherol within the LDL may increase LDL susceptibility to oxidation (Bowry et al, 1992). The reason for this increase in oxidizability is the action of the alpha tocopheroxyl radical. This radical, sustained within the LDL for a sufficient amount of time, will begin to oxidize the PUFAs within LDL in vitro (Bowry and Stocker, 1993). Since alpha tocopherol scavenges free radicals and other ROS, the more alpha tocopherol present, the increased amount of radicals that can or will be scavenged (Witting et al, 1999). During high fluxes of ROS, vitamin E prevents

lipid oxidation. However, when there is a low level of ROS, lipid peroxidation may be caused by vitamin E, specifically alpha tocopheroxyl radical. Normally, alpha tocopheroxyl radical can also participate in the reduction of lipid peroxy radical into non radical products (Witting et al, 1999). When lipid peroxy radical levels are not high, alpha tocopheroxyl radical will begin to oxidize PUFAs if there are insufficient co-antioxidants, such as vitamin C, to regenerate alpha tocopherol (Bowry et al, 1995). In an experiment by Kontush et al (1996) they showed that, in vitro, this pro-oxidant effect is apparent under certain circumstances. In human plasma and LDLs alpha-tocopherol developed antioxidant and pro-oxidant activity depending upon oxidative conditions and presence of vitamin C. In what was termed a “strong oxidative condition” (plasma diluted 150 fold or two fold, with 50 micromoles Cu^{2+} and 25 millimoles of AAPH, respectively) alpha-tocopherol displayed antioxidant behavior in both plasma and LDLs regardless of the amount of vitamin C in the sample. Under “mild oxidative condition” (plasma diluted 150 fold with 0.5 micromoles and 333 micromoles of AAPH respectively) alpha-tocopherol was shown to have a pro-oxidant effect. However, it was noted that this was only seen when the samples were “virtually free” of vitamin C or the amount was much lower than physiological levels (Kontush et al, 1996). Once vitamin C was added to these samples antioxidant activity of alpha-tocopherol was restored. In the final analysis it was pointed out that alpha-tocopherol should always function as an antioxidant, as long as the concentration of other co-antioxidants, such as vitamin C is high enough to regenerate alpha-tocopheroxyl radical back to alpha tocopherol. Considering vitamin C’s high concentration in human plasma it is highly probable, in vivo, vitamin E should act as an antioxidant regardless of the oxidative conditions (Kontush et al, 1996). We know that, in vivo, vitamin E indeed usually acts as an antioxidant (Groff et al, 1995; Janero, 1991). It appears that only in situations where co-antioxidants, such as

vitamin C, are exhausted or, there is an abnormal condition like thalassemia (Olivieri et al, 1994) or Wilson's disease (Ogihara et al, 1995) is there a chance of pro-oxidant activity by vitamin E in vivo.

There are very few studies showing any pro-oxidant effects of vitamin C. Generally, the pro-oxidant effect of vitamin C is not seen via its direct action upon other molecules, rather, it is with its action upon Cu^{2+} and / or Fe^{3+} (Herbert, 1996). As previously stated, after ascorbic acid reduction the products, Cu^+ and Fe^{2+} , can react with hydrogen peroxide or oxygen to form hydroxy radicals or superoxide radicals (Groff et al, 1995). Afterwards these ROS can damage membranes by attacking lipids and proteins embedded in the membrane itself. Most reports of ascorbic acid and a possible pro-oxidant effect were seen in studies investigating tumor growth and cancer (Alcain et al, 1990). In certain populations in which there is the potential for the accumulation of iron, such as hemochromatosis or thalassemia, ascorbic acid supplementation may cause an accumulation of toxic levels of iron (Schwartz, 1996). In addition, high levels of iron provide a large supply of substrate for hydroxyl ion formation, which ultimately can lead to lipid peroxidation (Wagner et al, 1993).

Muscular Fatigue

Muscular fatigue has been defined as “the failure of a muscle fiber to maintain tension as a result of previous contractile activity” (Vander et al, 1990). High muscular forces can be maintained for a shorter period than low muscular forces (Lieber 1992), and a direct result of muscular fatigue is a decline in the rate of force production. It has been shown that substrate depletion can be a major factor in the fatigue of contracting muscle, depending upon the intensity of the exercise being performed. In low force, endurance type activities, fatigue may depend on the amount of glycogen available. In high force, shorter time interval activities, fatigue may be

dependent upon the muscles ability to replenish ATP via ATP-PC system, anaerobic glycolysis, and / or oxidative phosphorylation. If muscle ATP demands are not adequately met, then force production attenuates over time (Lieber 1992). Also, fatigue in shorter time, high force activity is caused in part by the increase in intracellular acidity brought about by lactic acid buildup (Vander et al, 1990).

In addition to substrate availability, there are numerous other theories for the mechanisms involved in fatigue. Among those are disturbances in electrolyte balance and / or changes in skeletal muscle oxygen-reduction status (Powers and Lennon 1999). In the study by Novelli et al (1990), spin trappers were used to scavenge free radicals in exercising rats. Spin trappers are compounds reacting with free radicals, and may even be classified as a free radical scavenger. The rats were exercised to exhaustion, and compared to rats that had undergone the same protocol, but without receiving the spin trappers. Those that had received the spin trappers had a longer time to exhaustion (on average 185.7 seconds longer). Reid, Haack, et al (1992) determined that: 1) muscular contraction increases intracellular oxidant levels; and 2) reactive oxygen intermediates promote low frequency (fibers stimulated at 30 Hz as opposed to 200 Hz, for five minutes) fatigue. Their study involved rat diaphragm bundles mounted in chambers containing Krebs-Ringer solution loaded with 2',7'-dichlorofluorescein (a fluorochrome that emits at 520 nm when oxidized). Bundles were artificially stimulated to contract repetitively at 30 Hz. Increase in ROS were shown during stimulation via the fluorochrome during the period of repetitive contractions.

Measuring fatigue is a very complex notion, as it can be influenced by both physical and psychological factors (Astrand and Rodahl, 1986). It is not uncommon to perceive a certain work output as being strenuous one day as opposed to the previous day. An example of the

psychological influence of fatigue is in track and field. Many times we have seen an athlete finish a race in victory, and take a victory lap, all while his competitors are sprawled on the track. All of the athletes are fatigued but, for some reason the winner always seems to have the energy to celebrate. This is most certainly due to some psychological factor. Factors that may possibly play a part in muscular fatigue include central nervous system fatigue, peripheral nerve fatigue, and neuromuscular junction fatigue. The control sequence for activation of voluntary muscle as posed by Astrand (1986) occurs in this order; Mood - Brain - Spinal Cord - Peripheral Nerve - Neuromuscular Junction - Muscle Fiber Membrane - Sarcoplasmic Reticulum - Ca^{2+} Ions - Actin and Myosin Interaction. Central nervous system fatigue is sometimes referred to as psychological fatigue (Vander et al, 1990). This is illustrated by a failure of the cerebral cortex to send signals to the motor neurons, which can lead to the failure of muscle fibers to contract, even though the muscles are not fatigued. In the case of an athlete his performance may depend on his “will to win” (or mood / motivation) to overcome this type of fatigue. However, Merton (1954) showed that this may not necessarily be the case. In his classic study he had subjects performing a number of repeated contractions and as time wore on he would superimpose a large electrical stimulation over the voluntary contraction. He hypothesized that if CNS fatigue had any influence upon muscular fatigue then the artificial stimulation would increase force produced during a voluntary contraction. No force increase was observed in this study so he concluded that CNS fatigue was not a factor in muscle fatigue. Bigland-Ritchie, Kakulka, Lippold, and Woods (1982) showed that although force may decrease over time within a muscle, they demonstrated that there is no change in muscle mass action potential and would suggest that the neuromuscular junction is not the weak link in muscular fatigue. Gandevia, Herbert, and Leeper (1998) also showed that although there may be development of muscular fatigue as muscular work is

occurring, the capacity to maintain maximal levels of activation can be maintained.

Measuring fatigue in a non-invasive fashion has become a lot easier today with the help of computerized dynamometers. Researchers have used a number of different protocols to measure fatigue, however most have been based upon the premise that as a muscle(s) fatigues, force attenuates. The most common measurements used are peak torque, work, and power. For example, Svantesson et al (1998) studied fatigue during repeated eccentric and concentric contractions of the plantar flexors. Subjects were tested to see if there was a difference in fatigue development during concentric actions versus concentric-eccentric actions. A protocol for measurement of the concentric was performed and compared against a similar protocol for measurement of the eccentric, using plantar flexion as the action tested on the isokinetic dynamometer. The test was performed to exhaustion, and the total amount of work was recorded at the end of each test. They found that work declined similarly over time in both tests and, that when evaluating fatigue during muscular performance, the concentric-eccentric test should be used.

In a study by Pincivero, Gear, Moyna, and Robertson (1999), the effects of rest interval on quadriceps torque were tested. The study used two groups of subjects who were each assigned a different protocol to perform on a Biodex System II Isokinetic Dynamometer. All the subjects performed four sets of twenty maximal isokinetic knee extensions at an angular velocity of 180 degrees per second. Group one was given a 40 second rest between each set and, group two was given a 160 second rest between sets. The results showed that there was a significantly greater decline in peak torque ($p < 0.05$), total work ($p < 0.05$), and average power ($p < 0.05$) with group one when compared to group two.

In a unique study by Ellenbecker and Roetert (1999), they attempted to show if there was

a difference in the fatigue rate between shoulder external and internal rotation of the dominant and non dominant arms, in elite junior tennis players. The protocol consisted of the subjects undergoing bilateral isokinetic testing with 90 degrees of glenohumeral joint abduction. They did twenty maximal effort concentric contractions of the external and internal rotation, at a speed of 300 degrees per second. The fatigue was measured by calculating a fatigue ratio. The fatigue ratio was calculated by dividing the work in the last 10 repetitions by the work in the first 10 repetitions. They found significant difference between the fatigue ratios for the external versus the internal rotations but, no difference between the dominant and non dominant arms. Therefore, it would seem that work is a viable measurement of fatigue rate of muscles while investigating the effect of supplementation on fatigue.

Summary

As mentioned previously, athletes and fit individuals are constantly under a barrage of advertising from vitamin and supplement manufacturers promoting the use of vitamin supplements. Many young athletes spend upwards of hundreds of dollars on these supplements every month. However, it has been shown that most athletes consume more than enough of the RDA for most nutrients (ADA, 2000) in their normal diets - which contains more calories on average than the average person. If this is the case, and the manufacturers know this, then the constant advice to ingest vitamin supplements is not based upon science but, rather the ignorance of the target audience.

As it has been shown, there are any number of protocols and dosages that have been employed in investigating the effectiveness of the antioxidants in question, both alone and in combination. However, there is no consistency among the many methods and combinations employed. It is accepted that vitamin E is the main chain breaking antioxidant with respect to the

protection of the membranes of tissue cells. Although one study has shown an improvement in time to exhaustion while supplementing with vitamin E in rats (Novelli et al, 1990), it has not shown to be an effective substance to help reduce fatigue by itself (Sacheck et al, 2000; Sumida et al, 1989; Lawrence et al, 1975). However, there are some diverse methods used in some studies to test the effectiveness of vitamin E. One consistent characteristic in some of the investigations is the use of endurance trained subjects (Sacheck et al, 2000; Lawrence et al, 1975). As mentioned previously, there is an accompanying increase in the enzymatic antioxidants in endurance trained subjects (Miller, 1992). It may be possible that their training status may supercede any effect of supplementing in these individuals in during submaximal exercise. Sumida et al (1989) found no difference with vitamin E supplementation in cycle time to exhaustion or VO₂ max while using untrained healthy subjects for the investigation. However, the study was neither double blind and involved no placebo group, consequently there is no guarantee that the results are not biased.

As stated earlier, vitamin C is probably the most studied vitamin among the three antioxidants mentioned. There have been all manner of protocols followed to investigate the antioxidant properties of vitamin C and its influence upon muscular work. In the study by Lang et al (1986) we see an improvement in endurance capacity in guinea pigs who have been supplemented with 4 g/kg of vitamin C. In human subjects, nonetheless, there are mixed results. Samanta and Biswas (1985) supplemented 16 healthy adult females with 500 mg vitamin C or placebo for 4 weeks. They saw improvement in distance covered (1813.83 meters pre-test, 2026.75 meters post-test) during a nine minute walk / run in the vitamin C group after the 4 weeks when compared to before supplementation ($p < 0.05$). The placebo group saw no improvement. Gey et al (1970) supplemented 286 male air force officers with 1000 mg vitamin

C per day for 12 weeks. Before and after the 12 weeks subjects were tested on a 12 minute walk / run test. Afterwards there was a small but insignificant increase in distance covered during the 12 minute test. But, neither of these investigators chose to compare their groups against sedentary subjects to test if there was any training effect. Keren and Epstein (1980) used a double blind placebo controlled protocol, but did not see an improvement in aerobic or anaerobic capacity (on the cycle ergometer) in either control or placebo group, after three weeks of running and hiking training. Ideally the test of aerobic or anaerobic capacity should be in the same activity in which participants were training. In an interesting twist van der Beek et al (1990) used vitamin C restricted diets to show no negative effects upon aerobic power and onset of blood lactate. However, this might have been more informative if they had compared their subjects to trained individuals to see if the same effect is seen. Again, we see a variety of different methods to test the effectiveness of vitamin C but, no clear cut determination of its influence upon muscular work. But, it is clear that more investigation should be done using trained and untrained subjects as opposed to one or the other.

Beta-carotene has not been widely studied in relation to its influence upon muscular work. LeBlanc investigated it in connection to its influence upon 5k race times. They supplemented with 15 mg beta-carotene or placebo per day for one month. Following a washout period the groups were crossed over, and it was determined that there was a significant improvement in 5k race times. Since beta-carotene is located between the lipid bilayers of cell membranes, it may be more possible that beta-carotene should be taken in combination with vitamin C and E. A few studies have looked at combination of vitamin C and E together, but without beta-carotene. Dawson et al (2002) used 15 experienced runners and divided them into two groups (vitamin or placebo). The vitamin supplement was a combination of 500 mg vitamin

C and 500 IU vitamin E, or 1000 mg vitamin C and 1000 IU vitamin E. The runners supplemented for 4 weeks then completed a half marathon run. Afterwards a four week washout period followed before the subjects were crossed over and received the other capsule for four weeks. After that second period another half marathon was run. Following each run blood was collected and analyzed for creatine kinase and malondialdehyde. There were significant increases seen in creatine kinase ($p < 0.05$) but not malondialdehyde between the vitamin and placebo groups. Rokitzki et al (1994) used runners in their study in a similar fashion. Subjects were assigned to either a placebo or vitamin (400 IU vitamin E, 200 mg vitamin C) for 4.5 weeks. Following supplementation they ran a marathon race. Comparing immediately after the race to 24 hours post race, there were significant increases in creatine kinase in the supplemented and placebo group. However, the increase in creatine kinase in supplemented subjects was significantly lower than in the placebo subjects ($p < 0.01$). There were no differences seen in malondialdehyde levels between the two groups. However, there was no way to know if subjects were giving an all out effort during the run, as there was no way to quantify the actual work performed. In the study by Bryant et al (2003) mentioned earlier, the same case can be made. Although subjects supplemented with vitamin C and E, considering the supplementation protocol there was no way to determine if lower malondialdehyde levels were a product of training or the antioxidant combination.

There is no conclusive evidence supporting the ingestion of antioxidant supplements. Much of the advocacy of supplementation with these nutrients is based upon theoretical ideas. In table 1.3 we see that there are a number of studies looking at each of the aforementioned antioxidants. There is no conclusive evidence from these studies that either of the antioxidants can affect muscular work. There are mixed results for each antioxidant and, most did not use two

groups of volunteers. They either used trained or untrained subjects but usually not both. In addition, only one of these studies investigated subjects' deficiency status for each nutrient, prior to the supplementation protocol. LeBlanc (1998) investigated subject status for beta-carotene and discovered that on average, they were on the lower end of the typical beta-carotene intake of American adults (Henderson et al, 1989).

Finally, none of the studies cited have used the mixture of beta-carotene, vitamin C, and vitamin E when investigating antioxidant supplementation upon muscular work. Although there has been no conclusive evidence to support the supplementation of these nutrients, perhaps the combination of these three may have a significant effect considering their interrelationship within the cell membrane.

Main Points

1. No one has compared the effect of beta-carotene, vitamin E, and vitamin C in combination upon muscular fatigue.
2. No one has compared the effect of beta-carotene, vitamin E, and vitamin C in combination after acute and chronic supplementation, while using a double-blind crossover experimental design.
3. Few studies compared trained and untrained subjects
4. The literature is limited concerning the effect of beta carotene upon fatigue and more research is warranted
5. As Vitamin C helps to regenerate vitamin E within the cell, via its interaction with the alpha tocopheroxyl radical, it may be possible that these vitamins may work in conjunction to affect muscle fatigue during intermittent work

Project Rationale

Muscular work causes an increase in oxygen consumption and ultimately an increase in free radicals and other ROS. This increase in ROS can attenuate muscle work capacity and may cause the cessation of work production altogether if their removal is not prompted in a timely fashion. Many popular sports involve short bursts of activity followed by a rest period or period of reduced activity (basketball, track and field, football, tennis), whether during training or in competition. Since these activities involve these short time periods of activity it may be of importance to stave off the effects of fatigue, during intermittent time periods of work, than the overall endurance capacity. With this being the case, any positive effect that antioxidant supplementation may have upon the improvement of work capacity during these times would most likely be welcome. Conversely, if the case were that supplementation with these antioxidants has a negative effect, surely most athletes would not be encouraged to supplement them in their diets.

Preliminary Studies

In a pilot study conducted in our laboratory we instructed the subjects to perform a prescribed intermittent work protocol of five sets of 20 arm curl repetitions, with 60 seconds rest. The rest period was long enough to allow the aerobic energy system to contribute towards energy production (Astrand, 1986, pp. 304-307) and to ensure that subjects could give an “all out” effort. Only the last two sets were used to evaluate fatigue. However, supplementation with a beta-carotene, Vitamin E, and Vitamin C combination resulted in mixed findings. A two-way factorial (mixed model) ANOVA design with repeated measures, was used for analysis. There were six untrained and ten trained subjects which resulted in unbalanced groups. However, as with most statistical software (Freund, 1997, pp. 486), the SAS software accounted for this

difference in the analysis by using the Proc Mixed procedure. Three conditions were compared: 1) Pre-supplementation; 2) Acute supplementation (one day of supplementation); 3) and 25 days of supplementation. At $p < 0.10$, there were significant main effects for subjects' status (trained or untrained) ($p = 0.10$), the interaction of status and supplemental state ($p = 0.05$), and the interaction of supplemental state and the day (pre, acute, 25 days) ($p = 0.09$). In figure 1.2 we see slightly lower work, in acute as compared to chronic supplementation in trained subjects. In untrained subjects we see (figure 1.3) an increase from pre to acute, and an increase from acute to chronic supplementation with regards to work. Although the results seem to be mixed, it may be that chronic supplementation with the antioxidant mixture may have a positive impact upon work in the untrained subjects. Unfortunately, subjects dietary status for the antioxidants is not known. It may be possible that the untrained were below the safe intake levels of one or more of the antioxidants, and that possibly this may affect muscular fatigue during intermittent work. With this information it may be more conclusive to conduct a study with a larger number of subjects using a crossover design, in which subjects are able to supplement on both placebo and the antioxidants. In addition, having knowledge of the subjects average dietary intake for the antioxidants would give a more clear picture of their body status for the supplements.

Problem Statement

The purpose of this study is to investigate the effect supplementation with an antioxidant mixture of beta-carotene, vitamin E, and vitamin C, will have upon work capacity in trained and untrained subjects.

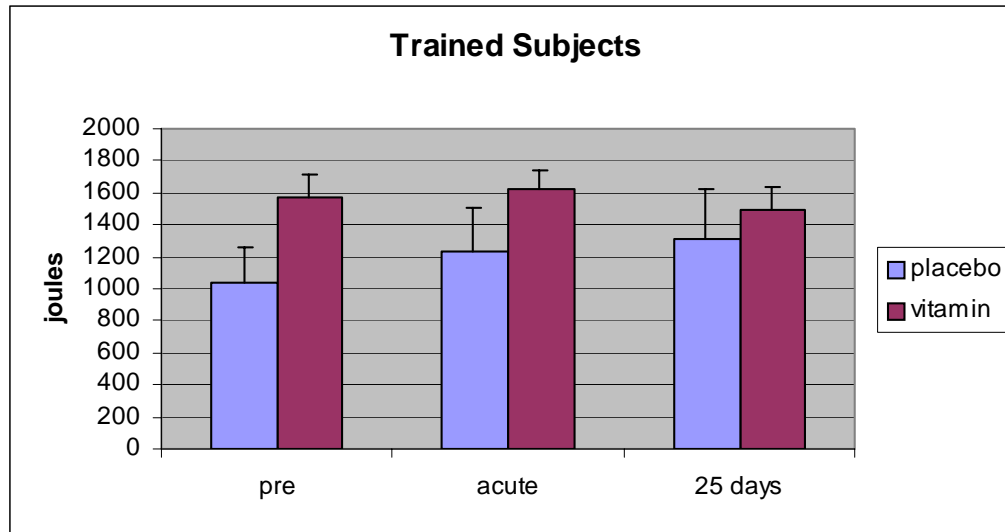


Figure 1.2 Work measures after supplementation with either placebo or antioxidant mixture of beta-carotene, vitamin C, and vitamin E in trained subjects.

Objectives

1. To examine whether supplementation with an antioxidant vitamin mixture will affect fatigue in exercising muscles, in normal healthy untrained subjects
2. To examine whether supplementation with an antioxidant vitamin mixture will affect fatigue in exercising muscles, in normal healthy arm trained subjects
3. To examine whether there is a difference in the effect of supplementing antioxidant vitamins upon normal healthy untrained subjects, when compared to arm-trained subjects

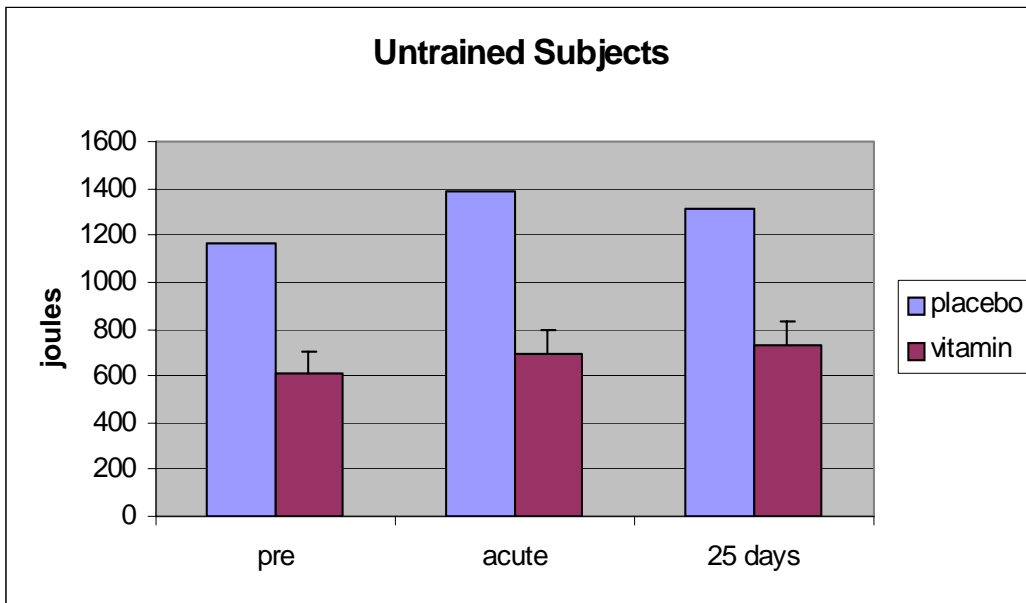


Figure 1.3 Work measures after supplementation with either placebo or antioxidant mixture of beta-carotene, vitamin C, and vitamin E in untrained subjects.(Note: n=1 for placebo)

Hypothesis

It is expected that there would be a group by condition interaction ($p \leq 0.10$) such that acute (one day) and chronic supplementation (28 days) with the antioxidant mixture (beta-carotene, vitamin C, vitamin E) would result in a decreased rate of fatigue in the untrained as compared to the placebo, but not in the trained. Additionally, while empirical, it is also expected that a greater quantity of work will be observed in the untrained following chronic supplementation when compared to acute supplementation, but that this would not be evident in the trained subjects.

Limitations / Delimitations

Because of the proximity of the subject pool at the university, the age of our subjects will be age 18 to 30. Therefore, the results may not be applicable to the ages outside this population. A food diary will be used to assess group antioxidant status, and accuracy of those diaries will be

dependent upon subject compliance. Only serum vitamin C will be measured from blood samples. Due to the multifaceted aspects of fatigue (i.e. neuromuscular, CNS, muscular blood flow, calcium availability, etc.), altering one aspect may not cause a sufficient detectable change in work measures. Finally, the exercise protocol will involve performing arm curls on the dynamometer, thus the results may not be applicable when discussing other exercises or other muscle groups.

Table 1.3 Previous Studies Using Antioxidants

Author	Exercise Mode	Nutrient	Result	Placebo Controlled	Subjects
LeBlanc 1998	outdoor running	beta-carotene	improved 5K race time	yes	trained runners
Novelli 1990	swimming	vitamin E	increase exhaustion time	yes	Mice
Sumida 1988	cycle ergometer	vitamin E	no difference	no	healthy male volunteers
Lawrence 1975	swimming	vitamin E	no difference	yes	trained swimmers
Spioch 1966	step test	vitamin C	decrease in O ₂ consumption, debt, and pulse rate	no	healthy male volunteers
Samanta 1985	9-minute walk / run	vitamin C	improved walk distance	yes	physically active students
Gey 1970	12 minute run	vitamin C	improved run distance (less than 1%), not statistically sig.	no	air force officers
Keren 1980	cycle ergometer	vitamin C	no improvement in VO ₂ max	yes	untrained subjects
Allesio 1997	cycle ergometer	vitamin C	higher oxidative stress in placebo	yes	healthy subjects
Sacheck 2000	downhill running	vitamin E	low vit E vs high vit E: No change in Oxidative stress	no	trained rowers
van der Beek 1990	cycle ergometer	vitamin C	low vit C vs suppl vit C: no change in VO ₂ max or OBLA	yes	healthy subjects
Bryant 2003	cycle ergometer	vitamin C and E	lower lactic acid (not sig.): higher malondialdehyde in vit C compared to placebo	yes	trained cyclists
Dawson 2002	half marathon run	vitamin C and E	no difference between groups in CK, myoglobin, malondialdehyde	yes	trained runners
Lang 1986	run to exhaustion	vitamin C	improvement in endurance capacity	yes	guinea pigs

Chapter 2 Methods

Subjects

Thirty-one healthy subjects, both male and female, were recruited from student populations at a local university, and at a local health club. Of those volunteers only ten completed the entire protocol (see the next chapter for details). None of the participants were currently taking medication, nor were they currently supplementing with antioxidant vitamins. Informed consent was obtained from each subject.

The subjects were assigned to one of two groups: a trained group (Tr); and an untrained group (Untr). Untrained were defined as individuals who did not currently participate in any arm resistance training activities. Trained subjects were those who were currently participating in an arm resistance training program at least one day per week. In addition, trained subjects must have been training for a minimum of one year. Each subject compiled a 3-day food diary to assess antioxidant vitamin status, and underwent an initial arm curl exercise protocol on the dynamometer, as a practice run, before supplementation. This allowed each participant to become accustomed with the requirements of the subsequent exercise tests.

Supplementation Protocol

Before the supplementation protocol began, each subject compiled a 3-day food diary to assess antioxidant vitamin status. The diary length was selected based upon previous research that showed that beyond this time period, the quality of record keeping declines (Gersovitz et al, 1978; Lee and Nieman, 2003;). Subjects' diaries were assessed for RDA levels of the selected vitamins using the software Food Processor for Windows (version 7.30) by ESHA Research. This allowed estimation of the extent to which the RDA for the selected antioxidants was being met, or exceeded, in the regular diet.

Subjects ingested either one antioxidant capsule consisting of 25,000 international units (IU) of vitamin A in the form of beta-carotene, 400 IU of vitamin E, and 500 milligrams (mg) of vitamin C, or one placebo capsule. The provided capsule was ingested at the same time of day for 28 days following the initial pre-supplementation fatigue test. The antioxidant capsules were produced by Vitamin World, Inc. according to the standards of the United States Pharmacopeia / National Formulary requirements. After supplementation, subjects underwent a 28 day washout period to allow antioxidant levels to return to normal. At this point, the capsules were crossed over, and each individual ingested the other tablet for 28 days. All subjects were given a 28 day supply of capsules at the beginning of each supplementation period, and compliance was ascertained by the number of capsules remaining at the end of each specific supplementation period.

Exercise Fatigue Testing Protocol

The exercise fatigue testing procedure consists of an initial practice test using a Biodex dynamometer, and subsequent tests at Day-1 and Day-28 of each supplementation period. The subjects were unaware that the first session (the preceding the supplementation period) was not being recorded. For each of the subsequent tests, each subject was tested within a 12-hour range of the original test. For example, if the original test was at noon, then the tests at Day-1 and Day-28 were between 6:00 a.m. and 6:00 p.m. on those days. The exercise protocol consisted of seven sets of arm curl exercises, using the non-dominant arm, with a 1-minute rest between each set. One set consisted of 20 repetitions. The speed of movement was set at 60 degrees per second for flexion, and a maximum of 400 degrees per second for extension (this speed allowed subjects to relax on the eccentric phase). The subjects were instructed to give all out effort for each set. Subsequently, total work (force multiplied by distance) for each set was measured by

the dynamometer, and used as the measure of fatigue. Only the total work from the last three sets was used in determining fatigue.

Blood Analysis.

Serum vitamin C levels were assessed during the washout period and one day before the end of the second supplementation period. Vitamin C was assessed using the method developed by Dr. Richard Tulley (Tulley, 1992) in which serum samples are analyzed using the Beckman CX5 clinical chemistry analyzer. Each subject had blood samples drawn at the end of the washout period and one day before the end of the second supplementation period. Blood draw and analysis were performed at the Pennington Biomedical Research Center, Baton Rouge, Louisiana.

Data

Total work for each set was collected for each subject, for each supplementation phase. Thus, each subject performed seven sets of the exercise on five separate occasions. Only the last three sets of the last four sessions, however, were used in the fatigue calculations. To examine the effect of supplementation upon fatigue, the total work of the last three sets was summed, and used for statistical comparison and analysis using the SAS statistical software.

Power Analysis

In order to determine sample size a power analysis was carried out by the SAS software. In consultation with Mr. Xiaobing Fang (M.S., Experimental Statistics, Louisiana State University) it was decided that, because more than one main effect was to be considered, power for the study would be determined by the method described by Kuel (2000, pp. 63):

The power of a test of hypothesis is the probability of rejecting a false null hypothesis. The statistics $F_o = MST/MSE$ is used to test the null hypothesis $H_o: \tau_i = 0$. The power of the test is $1 - \beta = P(F > F_{\alpha, v_1, v_2} | H_o \text{ false})$, where v_1 and v_2 are the numerator and denominator degrees of freedom, respectively. When H_o is false, F_o has the non-central F

distribution and non-centrality parameter. If the null hypothesis is true, then the non-centrality parameter is zero and F_0 has the central F distribution.

Based upon this method a SAS program was designed to estimate sample size with the target for power the interaction of supplement and time at 0.70 (Appendix B)(the program gives a number of sample size, alpha level, and power combinations). Based upon this calculation it was determined that for a power = 0.8, and an alpha level = 0.10, there would be 28 subjects required for the later study. The final study will be a crossover design as opposed to the pilot study. This may allow for less variation than the original study.

Statistics

Following data collection all results will be analyzed and compared for significance. A mixed model two-way repeated measures ANOVA with an alpha level of $p < 0.10$, was used to compare within and between group differences in the work measures. The repeated measures will be acute placebo, chronic placebo, acute vitamin, and chronic vitamin. For the serum vitamin C measures a paired t-test will be used to compare placebo and supplementation phase vitamin C levels.

Table 2.1 Timeline For the Study

Days 1-7	Subjects record diet for three days. At the beginning of the recording period, subjects will have the initial assessment on the dynamometer.
Day 14 and 15	Subjects begin the supplementation period. On the second day, they will again be assessed on the dynamometer.
Day 42	Subjects continue supplementation, and on the twenty-eighth day are assessed on the dynamometer
Day 44 - 70	Subjects undergo washout period
Day 71 and 72	Subjects begin the second supplementation period. On the second day, they will again be assessed on the dynamometer.
Day 100	On the last day of supplementation, subjects will again be assessed on the dynamometer

Chapter 3 Results

Subjects

The study originally began with 31 subjects. Out of that group 20 completed the first supplementation phase, but did not participate in the crossover. Ten subjects completed the entire study and participated in both phases. Subjects were selected from kinesiology courses at the university. The mean age of all subjects was 20.9 years with a maximum of 23 years and minimum of 19 years (Table 3.1). All completed a statement of informed consent (Appendix D).

Each subject completed a twenty-eight day supplementation period followed by a one month washout period then, followed by another twenty-eight day supplementation period. They were instructed to pick up their packet of twenty-eight capsules one week prior to the beginning of each supplementation period. There were two packets - one containing the placebo, and the other the antioxidant mixture. Because the study was double blind, neither the investigator nor the subjects had knowledge of the contents of the packet that each received at the specified intervals.

Table 3.1 Subjects Descriptive Statistics

N	Mean	Std Dev	Std Err	Max	Min
10	20.90	1.101	0.348	23.00	19.00

Food Diary Measures

Subjects completed a 3-day food diary to assess current intake for the antioxidants being ingested. The information therein was analyzed using the software, *Food Processor for Windows* (version 7.30) by ESHA Research, as described earlier. The results for each subject are listed in table 3.2. The means for each antioxidant are listed for each subject for the three day period. Descriptive statistics were calculated and appear in table 3.3.

Table 3.2 Antioxidant Vitamin Measures From Food Diary Data

Subjects	Status	Beta-car. (mcg)	Vitamin C (mg)	Vitamin E (mg)
1	Trained	811.12	98.42	2.68
2	Untrained	498.62	87.89	5.30
3	Trained	3122.46	1017.19	14.56
4	Untrained	1388.48	63.19	4.21
5	Untrained	330.18	99.37	3.98
6	Trained	9.38	31.12	4.96
7	Trained	574.32	107.25	8.50
8	Untrained	223.93	102.29	1.58
9	Trained	7494.26	31.42	2.72
10	Trained	3461.40	118.31	18.29

Table 3.3 Descriptive Statistics for Antioxidant Intakes

Nutrient	N	Mean	Std Dev	Std Err
beta-car (mcg)	10	1791.415	2336.351	738.819
vitamin C (mg)	10	175.645	297.277	94.007
vitamin E (mg)	10	6.678	5.539	1.752

Serum Vitamin C Levels

Subjects were asked to volunteer to give blood samples during the 27th day of each supplementation phase. Blood samples were analyzed for vitamin C content at the clinical chemistry laboratory at the Pennington Biomedical Research Center in Baton Rouge, Louisiana. A 5.0 milliliter sample was collected from each subject at each blood draw. Samples were

analyzed using a Beckman Synchron CX5 Chemistry Analyzer. The method is the same described by Tulley (1992) and currently used at Pennington.

Eight of the ten subjects volunteered to have their blood analyzed. Table 3.4 lists the results of the blood sample analysis for each subject. As indicated, blood was drawn one day before the end of each supplementation protocol. Each subject had one sample taken while on the placebo and one taken while on the antioxidant mixture.

Table 3.4 Vitamin C Sample Results

Subject #	Placebo	Vitamin C
1	13.20	16.30
2	10.40	11.40
3	6.10	23.60
4	10.50	17.40
5	8.20	9.30
6	9.20	9.40
7	4.70	6.20
8	2.30	12.10

Following vitamin C data collection, a statistical analysis was performed to obtain descriptive statistics and to observe any differences between the two supplementation periods. A paired t test was chosen to describe any differences. Table 3.5 lists the descriptive statistics for the vitamin c samples.

Table 3.5 Vitamin C Samples Descriptive Statistics

	N	Mean	Std Dev	Std Err
placebo	8	8.075	3.535	1.250
vitamin	8	13.213	5.587	1.975

Results of the paired t test are presented in table 3.6, and show significant differences

($p=0.046$) between samples taken while subjects were on the placebo compared to those taken while subjects were on the antioxidant mixture. Results of the paired t test show that serum vitamin C levels increased following the antioxidant supplementation when compared to the placebo samples.

Table 3.6 Vitamin C Samples Paired T-Test

	Mean	Std Dev	Std Err	Mean Diff	T Value	Prob	DF
placebo	8.075	3.535	1.250				
Vitamin	13.213	5.587	1.975	-5.138	-2.418	0.046	7

Arm Curl Exercise Measures

Following the arm curl exercise, work achieved for each set was recorded in joules for each subject. Table 3.7 shows the results for each subject at each phase of supplementation. The protocol was performed on a Biodex dynamometer in the kinesiology lab at Louisiana State University. Figure 3.1 indicates the acute vitamin condition was 4% lower when compared to the chronic vitamin condition, in the trained subjects. The untrained subjects were 3% lower from the chronic placebo compared to the acute vitamin condition, and a 7% from the acute vitamin compared to the chronic vitamin condition (Figure 3.2).

A two-way repeated measures ANOVA was performed to detect any differences. The ANOVA results are listed in table 3.8. There was no significant difference for the main effect of supstatus (acute placebo, chronic placebo, acute vitamin, chronic vitamin) ($p=0.90$). Additionally, there was no significant effect for the interaction of training and supstatus ($p=0.95$). There was, however, significant differences for the main effect of training (train vs. untrain) ($p=0.07$). Power for all tests are low and indicate a high possibility of making a Type II error (Table 3.9). Power was determined by the SAS software (Appendix C) using the method described by Kuel (2000, pp. 63).

Table 3.7 Work Measurements for Each subject

Subject	Acute Plac	Chronic Plac	Acute Vit	Chronic Vit	training
1	1006.00	1768.50	1877.80	2262.70	trained
2	773.40	616.70	1096.10	789.30	trained
3	2489.00	2780.10	1854.60	1577.20	trained
4	1819.40	2400.40	3007.80	2787.30	trained
5	1372.20	1046.90	1347.10	1199.40	trained
6	752.10	1271.40	1359.40	1489.60	trained
7	1042.50	1089.60	1267.20	1037.60	untrained
8	2101.70	2425.50	1834.20	1800.50	untrained
9	966.60	844.30	1078.80	1159.20	untrained
10	504.60	616.30	670.60	528.40	untrained

To summarize, it appears that the supplementation protocol did not have a significant impact upon work output although there is a significant main effect of training ($p=0.07$). Clearly, no statistically significant change in work output was seen under either condition, trained or untrained. Additionally, power is too low to make a decision without risk of Type II error, regarding the research hypothesis.

Table 3.8 Two Way Repeated Measures ANOVA for Work Results

Source	DF	SS	MS	F	Pr
train	1	1765764.150	1765764.150	3.62	0.07
suppstatus	3	274571.469	91523.823	0.19	0.90
train X suppstatus	3	180431.321	60143.774	0.12	0.95
error	32	15604409.30	487637.79		
total	39	17922768.64			

Table 3.9 Power Measurements at Alpha = 0.10

Effect	Power
train	0.476
supp	<0.10
trainX suppstatus	<0.10

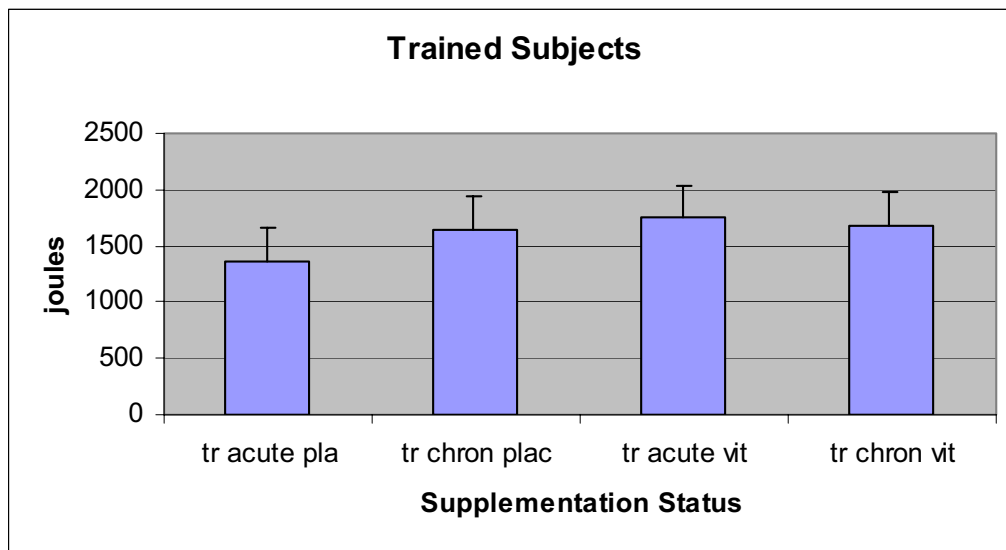
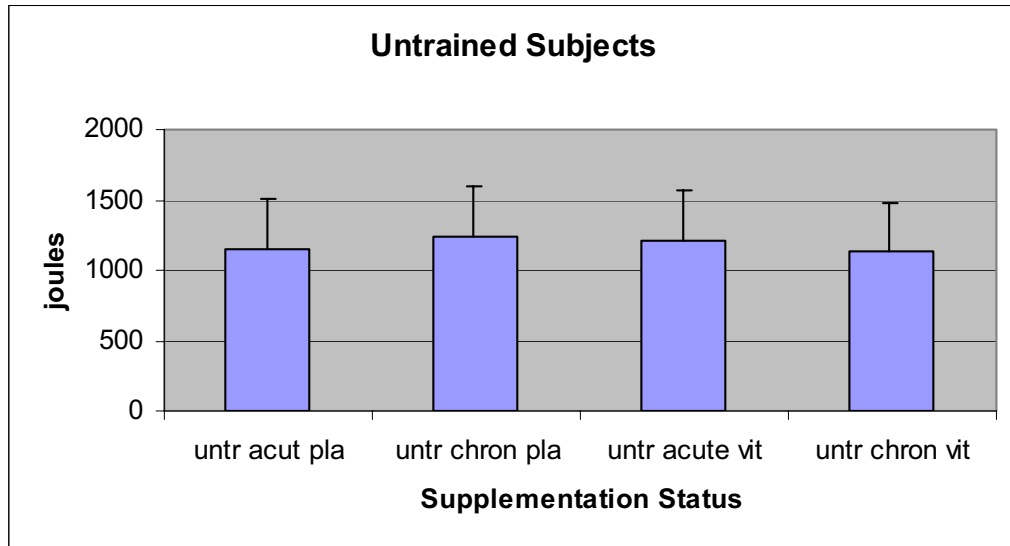


Figure 3.2 Work Output for Untrained Subjects



Chapter 4 Discussion

Antioxidants are continuously marketed to athletes and fitness enthusiasts as being beneficial to muscular performance (ADA, 2000). This is because there is a concomitant increase in oxygen consumption during muscular work which results in an accompanying increase in free radicals and other ROS. Nevertheless, the human body has developed a system of enzymatic and non-enzymatic antioxidants that help to protect it during “steady state” (Powers and Hamilton, 1999; Powers and Lennon, 1999) . However, during times of increased work these systems may be overwhelmed and impair the ability of a muscle to sustain a given level of work production.

Studies have investigated the possibilities that supplementing exogenous antioxidants beyond the RDA for those nutrients that may have a beneficial effect against the deleterious consequence of an increase in ROS. These antioxidants, beta-carotene, vitamin E, and vitamin C have been studied individually in exercising subjects with mixed results.

This investigation supplemented subjects with a combination of these three antioxidant nutrients in an attempt to answer questions regarding their affect upon muscular work. Very few studies have investigated these substances together, and none have used a protocol similar to the one used in this study. Based upon previous literature and the pilot study mentioned earlier, this more thorough investigation was carried out. To this end it was decided that an intermittent work protocol using trained and untrained subjects, who were not deficient in any of the tested antioxidants was the ideal design.

Food Diary Measures

Vitamins C and E, and beta-carotene intakes were estimated using 3-day food diaries. Each subject was given a diary to completely record everything that was eaten during a 3-day period. As invasive measures of these two of the nutrients were not investigated, it was

determined that recording daily intake of these nutrients would provide the best estimate of tissue levels for this particular group. Assessment of the subjects' status in regards to the antioxidants studied, was necessary to ensure that there were none of them starting from a point of deficiency, prior to the start of the experiment.

Currently there is no RDA for beta-carotene in humans. However, the RDA for vitamin A is 0.8 - 1.0 retinol equivalents (RE) (Groff et al, 1995). Beta-carotene is a precursor to vitamin A, and is often called pro-vitamin A. The body cleaves stored beta-carotene as needed to maintain serum vitamin A levels within the body. There appears to be no serious side effects when ingesting beta-carotene in excess of the RDA for vitamin A because it is not converted to vitamin A until the body needs it.

Following diet analysis for each subject, the software displayed information for each vitamin and mineral with an RDA and also other nutrients, such as beta-carotene, that do not currently have an RDA. The *Food Processor for Windows* software reported total vitamin A / provitamin A in the diet. It also provided beta-carotene content as a separate measure from total vitamin A content. Our subjects mean intake for beta-carotene during the 3 days of listing food intake was 1791.415 mcg (Table 3.3). Six micrograms of beta-carotene is equal to one RE. Again, this measure was just a part of the total vitamin A intake, but it does illustrate the extent to which the subjects were meeting the RDA for vitamin A. On average the subjects ingested 298.57 RE per day for the three day period. This far exceeds the RDA for vitamin A and ensures, barring any malabsorptive disorders (pancreatic, liver, or gall bladder diseases), that the subject pool was not vitamin A deficient prior to the supplementation regimen (Beta-carotene ingestion for each subject is listed in table 3.2).

The RDA for vitamin E is 8 mg and 10 mg tocopherol equivalents (TE) per day, for

women and men respectively. The normal intake for vitamin E in American diets is 4 mg TE to 22 mg TE/day for individuals not supplementing their diet with exogenous vitamin E (Di Mascio et al, 1991). Again, vitamin E requirements increase as the intake of PUFA increases or decreases. Because of this dependency upon PUFA content, determining a precise requirement for vitamin E has been difficult. However, because most foods high in PUFA are also high in vitamin E, there is not much fear of deficiency. In fact, vitamin E deficiency in humans is very rare. As in the case with beta-carotene, deficiency in adults is only seen in those suffering from malabsorption diseases, especially those that affect the absorption of fats.

The diet analysis determined that the mean intake for vitamin E for each subject for the three day period was 6.678 mg TE/day (Table 3.3). This is just below the RDA for vitamin E but is well within the normal intake for those consuming a typical American diet (Di Mascio et al, 1991). This also falls between the range stated by Guthrie and Picciano (1995), between 5 and 20 mg TE/day, that would be considered safe depending upon PUFA intake. Considering the rarity of vitamin E deficiency, and the fact that our subjects intake of vitamin E falls between the range for normal intake, it is highly unlikely that these subjects were vitamin E deficient before beginning the supplementation regimen.

The RDA for vitamin C is 60 mg/day for adult men and women. This amount was chosen based upon the amount required to maintain a body pool of 1500 mg (the amount of vitamin C that can be held within the body without excretion via the kidneys). The subjects' food diaries showed that the mean intake for each subject per day was 175.645 mg of vitamin C per day (Table 3.3). This number exceeds the amount in the RDA, but is far below the amount indicated in vitamin C toxicity (Alhadeff et al, 1984). Again, it is highly unlikely that subjects were vitamin C deficient before beginning the supplementation protocol.

Serum Vitamin C Levels

Blood samples were collected from each subject on the 27th day of supplementation, and subsequently analyzed for serum vitamin C levels. Serum vitamin C analysis was carried out at the Pennington Biomedical Research Center clinical chemistry laboratory, as previously noted. Each subject was tested while supplementing on both placebo, and vitamin C. While on the placebo, mean serum vitamin C levels (Table 3.5) were 8.075 mg/L, which is well within normal values (5 - 15 mg/L) for adequate vitamin C status (Tietz, 1997). Following the 28 day vitamin C supplementation period (which followed a twenty-eight day washout period) mean serum vitamin C levels increased to 13.213 mg/L. The subsequent paired t-test for differences showed a significant difference between placebo and vitamin C periods ($p=0.046$)(Table 3.6).

Clearly the supplementation of exogenous vitamin C was well tolerated, and had an affect upon subjects' serum vitamin C. The increase in serum vitamin C tends to support the absorbability of the chosen supplement which, as was indicated, fully complied with United States Pharmacopeia / National Formulary requirements. It also supports that supplementing with vitamin C increases plasma vitamin C levels (Polidori et al, 2004).

The increase in vitamin C also gives insight into the status of another antioxidant used in this study. As long as the levels of serum vitamin C are at or above normal levels we can expect vitamin E to act as an antioxidant rather than a pro-oxidant. Only when vitamin C levels are below normal can we expect a reduction in vitamin E levels because of ascorbate's crucial role in regenerating the alpha-tocopheroxyl radical (Kontush et al, 1996; Brown et al, 1997; Chan, 1993). As we have seen vitamin C levels increase significantly, we feel confident in the fact that vitamin E and beta-carotene was well tolerated, and increased the total body pools of both in each subject.

Power and Significance

During this study there were a number of subjects who dropped out at various points throughout the protocol. The study began with thirty-one subjects and ended with ten who completed the entire protocol. Since sample size is one of the factors affecting power (Norton and Strube, 2001; Thomas and Nelson, 2001;) any loss in subjects will have a profound effect upon power. Power allows for a level of confidence that the decision to be made from statistical results is the correct decision, or more simply put, it is the probability of not making a Type II error (failing to reject the null hypothesis when it is false) (appendix E). The power analysis for this study revealed extremely low power for the main effects as well as their interaction. Power for supplementation status, training, and their interaction was <0.10 , 0.35 , and <0.10 respectively. Therefore, due to the the loss of power in the study, we cannot have confidence that we would not make a Type II error if we were to accept the null hypotheses. The low power almost guarantees making a Type II error.

Variability is another factor that may affect power. Each of the main effects, and the interaction have large variances in this study. For instance, if the distribution of scores were represented by a curve, the narrower the curve is the smaller the variability about the mean; conversely, the wider the curve the greater the variability about the mean (Norton and Strube, 2001). If there were a pair of distribution of scores on the scale, the wider the range of scores the more overlap there would be between the two curves. If one of those curves represents the assumption that the null hypothesis is true and the other represents the distribution based upon the research hypothesis being true, the more overlap there is between the scores the less chance there is of detecting a real difference. As the overlap becomes larger beta increases and thus, $1 - \beta$, or power, decreases. As the main effects and interaction variances are large in this study

(appendix C) the variability, as well as the number of subjects, has also had an effect upon the power of this study. In addition, the variability makes the reproducibility of this study very difficult under the same conditions. Consequently, it is with these facts in mind that we will report the results and any inferences that may be taken from them.

Acute Supplementation Work Values

Subjects were supplemented with a placebo and the antioxidant vitamin mixture (beta-carotene, vitamin E, and vitamin C) to see if an acute dose would cause an affect upon the work capacity during the arm curl protocol. As was noted, there is scant research investigating the affect of an acute (one day) ingestion of either of the three antioxidant nutrients upon muscular fatigue during the arm curl protocol prescribed in this study. In addition, no studies using the intermittent arm curl protocol were found throughout the literature.

Neither untrained nor trained participants showed a significant difference in work values from the acute placebo to chronic placebo phase (untrained $p=0.86$; trained $p=0.49$). Nor were there any significant differences between the chronic placebo and acute vitamin phase (untrained $p=0.95$; trained $p=0.79$). The chronic placebo was 3% lower when compared to acute vitamin phase, and the acute vitamin was 7% lower when compared to chronic vitamin phase in work output. However, with the significance level and power of the analysis we cannot make a conclusion about this and have little confidence in the decrease seen in these work values. Again, the unanticipated dropout rate of the subjects have resulted in the low power of the test, and make it highly likely to commit a Type II error (appendix E).

Chronic Supplementation Work Values in Untrained

Chronic supplementation work values in untrained subjects were compared following the supplementation period. As in the case of the acute supplementation, scant research exists that

compares the influence of this antioxidant mixture upon muscular work capacity during an intermittent work protocol. In the untrained subjects no significant difference in work was observed from the chronic placebo supplementation to the acute vitamin supplementation ($p=0.95$), nor from the acute vitamin to chronic vitamin supplementation ($p=0.87$). Once again we cannot have any confidence in a determination to make because of the low power of the statistical analysis. So, although the p values would indicate that we should fail to reject the null, the power does not allow for there to be any confidence in that decision.

Chronic Supplementation Work Values in Trained

In the trained subjects, no significant difference in work was observed from the acute vitamin to chronic vitamin supplementation ($p=0.86$). In a trend seen similar to that in the acute work values, acute vitamin was 4% lower compared to the chronic vitamin phase in the trained group. Again, normally at the current p value, we would fail to reject the null. However, the low power indicates the high probability for Type II error. So, we would have no confidence in making that determination. Although there may be a trend seen, this would make any percent changes in work statistically meaningless.

Main Effects and Interactions

When comparing the main effects for trained vs the untrained state there is a significant difference observed ($p=0.07$) which, was not unexpected. We would expect the trained subjects to have higher work values than untrained subjects simply because of their constant training state throughout the investigation.

For the interaction of the supplement status (acute placebo, chronic placebo, acute vitamin, chronic vitamin) and training (trained, untrained), $p=0.95$ (table 3.8). It was hypothesized that there may be a significant interaction between the two however, p value

indicates no significance. Additionally tables 3.1 and 3.2 graphically show no apparent indication or trend towards an interaction between the two states (trained or untrained) under any of the four supplemental phases.

Antioxidant Status

Much of the research in this area has been observing the influence of single antioxidants upon work capacity rather than the antioxidant mixture (beta-carotene, vitamin E, vitamin C) employed in the present study. However, also of note, was the lack of data concerning the antioxidant nutrient status of subjects before participation in said studies. Some lacked the information altogether (Spioch et al, 1966; Gey et al, 1970; Keren and Epstein, 1980; Allesio et al, 1997; Novelli et al, 1990; Sumida et al, 1988; Lawrence et al, 1975), and one reported that subjects were deficient in the selected antioxidant (LeBlanc, 1998) before supplementation began. In this study, there was an effort to establish that each subject was not deficient in any of the supplemented antioxidants (Table 3.2) before beginning the supplementation period. Descriptive statistics for each nutrient for each subject were analyzed and showed deficiency to be unlikely in the subject pool. This was performed to ensure that any differences observed were not caused by a subject's being deficient in any of the antioxidant nutrients. The ADA and American College of Sports Medicine report that most athletes do not require supplementation of vitamins and minerals above the current RDAs (ADA, 2000). This is because most athletes are consuming a larger amount of food than normal healthy individuals. This high intake usually ensures that they are not deficient in most nutrients. It is more than likely that our trained subjects would fit this profile. The exception is made in the case of those athletes who "restrict energy intake, eliminate one of the four food groups from their diet, or consume high carbohydrate, low micronutrient-dense diets".

Limitations and Other Concerns

There are a number of pitfalls that may have hampered the ability of the current study to detect differences and obtain sufficient power. First would be the unexpected drop out rate of the participating subjects. There is no apparent reason for the high attrition rate that is seen in this study. In previous pilot work the unusually high attrition rate was not observed and there was no indication that it would transpire here. Subjects used in the current study were university students. Based upon some of the motivational techniques recommended in Appendix F, I arranged, with their professors, for the subjects to receive extra points toward their final grade in the class that I drew them from (they were drawn from a pool of four classes at the university). As it turned out, many of the students dropped the class they were in and thus, dropped out of the study. Some students forgot about their appointment date in the lab and were dropped. Clearly, this has had the largest impact upon the study.

Still those that participated were instructed to give an all out effort on each set of arm curls. If they did not give the effort required this may also have an effect upon the results. If subjects are just “going through the motions” it would make it difficult to see any changes in work values over the four trials. This may be a result of lack of motivation or boredom. Perhaps having meetings at different times throughout the study could have served as a time to renew motivation that may have waned since the start of the study (Appendix F).

The subjects were instructed to take the capsules everyday with a meal. However, there was no way of knowing if they actually followed this advice. It is conceivable that some students missed days and attempted to “double up” by taking their missed dosage and their scheduled dosage at the same time. If this were done on more than one occasion by more than one subject this may have an effect upon the work values as well because of the antioxidant levels that are

affected.

The subjects in the study were found to be at the normal intake for the antioxidants after food diaries were measured. Upon observation it may have been beneficial to look at subjects who were restricted in their diet. Those athletes / individuals who are more likely to have a restrictive diet (i.e. middle distance runners or swimmers) may have shown more of an effect under the intermittent work protocol that was used here. Those who are more prone to restrict the intake of PUFA or fruits and vegetables may have exhibited a lower than normal intake of vitamin E and / or vitamin C. We used college students who were either engaging in strength training regularly or who did no strength training at all. However, none were preparing for a competition as an athlete would, and so they did not exhibit the kind of restrictive diet that the aforementioned athletes may. Finally, and perhaps most importantly, we trusted that the subjects completed their food diaries accurately and truthfully. If they did not this may also have had an effect upon the outcome of the study. The fact that analyzing serum vitamin E and / or beta-carotene levels was financially beyond the scope of this study, was a limitation that we were well aware of. It may be that these measures would have told a different story concerning subjects status for the selected antioxidants.

The exercise protocol chosen may not elicit a large enough oxidative stress to see the results that were anticipated. Arm curls involve mainly the biceps brachii. Perhaps had the protocol involved knee extensions or leg presses this would have caused a high enough oxygen demand to observe an affect of the antioxidants. In the future this should be considered when examining these supplements in a similar manner.

As has been discussed, fatigue is a very complex concept in regard to the human body. There are many factors that can contribute to fatigue including physiological and psychological

(Astrand, 1986) components. Among those previously mentioned are substrate depletion (depending upon the intensity of the exercise), muscle oxidation-reduction state, psychological state / mood, CNS fatigue, and local blood flow.

This study set out to investigate the effect supplementation with the beta-carotene, vitamin C, and vitamin E would have upon work capacity during an intermittent work protocol of seven sets of arm curls with one minute rest between each set. As oxidative stress has shown to play a part in muscular fatigue (Reid, Hack et al, 1992; Novelli et al, 1990) the theory was that supplementing with these antioxidants would help to affect the amount of work being produced through their neutralizing some ROS. Nonetheless, it may be possible that indeed they did help neutralize some of the ROS, but perhaps that the effect is so small as to not make a noticeable difference. To measure this effect, determining the work using the dynamometer may not be the best method. Perhaps another invasive measure unknown to this investigator is available to determine this effect.

As this study involved the arm curl protocol and the measurement of work by the dynamometer, there was no way to address the possibility of there being a psychological factor contributing to fatigue. The protocol was not set out to evaluate psychological factors contributing to fatigue. It may be possible that psychological factors have played a part in this study. As mentioned earlier mood, plays a large part in fatigue. If subjects were not in a good or “energetic” mood, or had feelings of depression this may have had an effect upon the results of their tests on the dynamometer. Unfortunately, this cannot be accounted for under the conditions set forth.

Neural fatigue was not evaluated during this study. Although it seems unlikely that CNS fatigue was a factor, according to the results by Merton (1954), there was not a way to evaluate

this on the dynamometer in the laboratory. However, this type of fatigue would more than likely be measured with an EMG. This is available in the university laboratory and should be considered for the future when a study of this undertaking is being considered. In addition, blood flow was not evaluated under the current study conditions. Although, this measure would probably be determined with some kind of blood flow occlusion device (similar to a blood pressure cuff). However, this seems unlikely as this kind of device may have created more of an oxidative stress than without.

It is unfortunate that this study had such a high attrition rate and thus, low power. In the future there are some suggestions to enhance the chances of having a larger power measurement. Perhaps an increase in the number of subjects in the study, or longer supplementation period would help to guard against the high attrition rate, or allow a stronger effect of the supplement. Also, an increase in the number of subjects from a more diverse population or specialized population (i.e. diet restricted athletes, the elderly, all women, etc.) will shed more light upon the possibilities. Subjects were primarily college age students from a university population. If subjects were taken from a wider age range and a larger pool we may see significant differences. For this study we used the population that was most accessible. It may be that this trend would not prove to be significant with these changes. However, if it were found to be significant this would give some validity to the notion that supplementation with exogenous antioxidants is not only of no benefit but, possibly detrimental to muscular performance during exercise.

Chapter 5 Summary and Conclusions

The purpose of this study was to investigate the possible effect that supplementation with a mixture of beta-carotene, vitamin C, and vitamin E may have upon fatigue, during a protocol of intermittent work. Exercise has been shown to increase the proliferation of free radicals and ROS within working muscles due to an increase in oxygen consumption (Powers and Hamilton, 1999). The antioxidants beta-carotene, vitamin C, and vitamin E have been shown to neutralize some ROS within tissue cells. Manufacturers market these antioxidants as being necessary to the general public, but also to athletes and fit individuals specifically (ADA, 2000). Although research has shown that athletes usually ingest more than the RDA for most nutrients through their diet alone, the supplementation of antioxidants is still promoted by these manufacturers.

It has been demonstrated that the proliferation of ROS during muscular work can contribute towards fatigue in working muscles (Reid and Haack, 1992). To this end, investigators have attempted to test if supplementation with non-enzymatic antioxidants would have an effect upon muscular work. Results have been inconclusive, and have not looked at how combining the three selected antioxidants (beta-carotene, vitamin C, vitamin E) may influence muscular work. Therefore, this study was designed to investigate the following hypothesis:

There would be a group by condition interaction ($p \leq 0.10$) such that acute (one day) and chronic supplementation (28 days) with the antioxidant mixture (beta-carotene, vitamin C, vitamin E) would result in a decreased rate of fatigue in the untrained as compared to the placebo, but not in the trained. Additionally, while empirical, it is also expected that a greater quantity of work will be observed in the untrained following chronic supplementation when compared to acute supplementation, but that this would not be evident in the trained subjects.

Results indicated that there was no effect of the antioxidant supplementation protocol upon muscular work during the intermittent work regimen. There was a significant effect of training as was expected ($P=0.07$), but not with the interaction of training and supplementation

($P=0.95$). Unfortunately, because of the low power of this study, we cannot place any confidence in a determination made about the hypothesis.

In the future, investigators may want to look at how a longer period of supplementation influences work capacity. Most athletes would not take these supplements for just a 28 day period as in this study. Many would take it over the course of an entire competitive season. In addition, what would be the dose response? Is the RDA of each nutrient enough or too much? Does more than that cause unwanted effects? This information would benefit this group specifically performance-wise, as well as financially. Also, a study with a much larger pool of subjects may increase power so, that determinations can be made with confidence. Afterwards if indeed a significant effect is found, this would be of utmost importance to not only athletes but fit individuals supplementing their diet as well.

Finally, it may be of interest to investigate if the supplementation of these antioxidants is causing a proliferation of ROS themselves during moderate to high intensity exercise, when taken in excess of the RDA. We know that vitamin E and vitamin C must be regenerated after they neutralize any ROS. If they are not regenerated for some reason, might they be contributing towards muscular fatigue? This would be an interesting finding if indeed this were the case.

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Appendix A: Food Diary Questionnaire

**Food Diary / Record Packet for Participants in the
Antioxidant / Fatigue Study**

These are the forms needed to report food intake for three days as required of subjects participating in the aforementioned study. Please follow strictly follow directions in order for your data to be usable.

- List all foods and beverages consumed at and between meals for the three days.
- Don't forget such items as gravy, jams, sauces, salad dressing, nuts, candy, margarine / butter, milk on cereal, fruit, or social beverages
- Record foods immediately after eating (don't rely on your memory)
- List separately the foods that make up one dietary item (i.e. for a sandwich list bun, type and amount of meat, butter, mustard, mayonnaise, etc.)
- Express amounts of meat in ounces or grams, fruits and vegetables in cups or number of that particular item (i.e., one orange, one tomato, etc.)
- Bread may be recorded as number of slices
- Beverages should be recorded in ounces or cups
- Include at least one day during the weekend, among the three days recorded

Example:

Meal	Time	Food	Description	Amount
Breakfast	8 am	corn flakes		1.5 cups
		2% milk		2 cups
Snack	10 am	Banana	raw	one
Lunch	1 pm	pasta	cooked	1.5 cups
		marinara sauce	cooked	1 cup
		iced tea		20 ounces
		chicken breast	cooked	8 ounces

Appendix B: Pilot Study Data and Power Analysis for Sample Size

Pre = Pre-supplementation

acute = One day of supplementation

25 days = Twenty fifth day of supplementation

Table B.1 Data Chart for collected results

State	Subject	supp	time	work
untr	1	plac	pre	1163.8
untr	1	plac	acute	1385.8
untr	1	plac	25days	1314.7
untr	2	vit	pre	328.9
untr	2	vit	acute	326.1
untr	2	vit	25days	396.7
untr	3	vit	pre	735.4
untr	3	vit	acute	884.3
untr	3	vit	25days	1034.3
untr	4	vit	pre	732.2
untr	4	vit	acute	675.2
untr	4	vit	25days	807.7
untr	5	vit	pre	806.5
untr	5	vit	acute	789.4
untr	5	vit	25days	576.1
untr	6	vit	pre	448.1
untr	6	vit	acute	796.9
untr	6	vit	25days	719.6
tr	7	plac	pre	516.2
tr	7	plac	acute	622.4
tr	7	plac	25days	690.2
tr	8	plac	pre	1513.3
tr	8	plac	acute	1801.5
tr	8	plac	25days	2080.4
tr	9	plac	pre	1290.4
tr	9	plac	acute	1545.6
tr	9	plac	25days	1501.2
tr	10	plac	pre	826.8
tr	10	plac	acute	980.4
tr	10	plac	25days	993.5
tr	11	vit	pre	1378.6
tr	11	vit	acute	1493.9
tr	11	vit	25days	1212.9

tr	12	vit	pre	1155.5
tr	12	vit	acute	1154.8
tr	12	vit	25days	1021.1
tr	13	vit	pre	1418.2
tr	13	vit	acute	1486.9
tr	13	vit	25days	1316.3
tr	14	vit	pre	1945
tr	14	vit	acute	1939.1

Figure B.1 ANOVA Results

Type 3 Tests of Fixed Effects

Pr > F	Effect	Num DF	Den DF	F Value
0.1045	status	1	12	3.09
0.5974	Supple	1	12	0.29
0.0517	status*Supple	1	12	4.67
0.0128	Day	2	24	5.25
0.9057	status*Day	2	24	0.10
0.0931	Supple*Day	2	24	2.63
0.1468	status*Supple*Day	2	24	2.08

Least Squares Means

Effect	DF	t Value	status Pr > t	Supple	Day	Estimate	Standard Error
status			tr			1379.67	114.75
12	12.02	<.0001					
status			untr			982.63	194.74
12	5.05	0.0003					
status*Supple			tr	plac		1196.82	177.78
6.73	<.0001						12
status*Supple			tr	vit		1562.52	145.15
10.76	<.0001						12
status*Supple			untr	plac		1288.10	355.55
3.62	0.0035						12
status*Supple			untr	vit		677.16	159.01
4.26	0.0011						12
Day					25days	1212.42	116.02
24	10.45	<.0001					
Day					acute	1233.88	116.02
24	10.64	<.0001					
Day					pre	1097.15	116.02
24	9.46	<.0001					
Supple*Day				plac	25days	1315.51	204.03
24	6.45	<.0001					
Supple*Day				plac	acute	1311.64	204.03
24	6.43	<.0001					
Supple*Day				plac	pre	1100.24	
204.03	24	5.39	<.0001				
Supple*Day				vit	25days	1109.32	110.50
24	10.04	<.0001					
Supple*Day				vit	acute	1156.13	
110.50	24	10.46	<.0001				
Supple*Day				vit	pre	1094.06	
110.50	24	9.90	<.0001				

Differences of Least Squares Means

Standard Effect	status	Supple	Day	_status	_Supple	_Day
Estimate	Error	DF	t Value			
status	tr			untr		
397.04	226.04	12	1.76			
status*Supple	tr	plac		tr	vit	-365.69
229.51	12	-1.59				

The Mixed Procedure

Differences of Least Squares Means

Standard Effect	status	Supple	Day	_status	_Supple	_Day
Estimate	Error	DF	t Value			
status*Supple	tr	plac		untr	plac	-
91.2750	397.52	12	-0.23			
status*Supple	tr	plac		untr	vit	
519.66	238.51	12	2.18			
status*Supple	tr	vit		untr	plac	
274.42	384.04	12	0.71			
status*Supple	tr	vit		untr	vit	
885.36	215.30	12	4.11			
status*Supple	untr	plac		untr	vit	
610.94	389.49	12	1.57			
Day			25days			acute -
21.4667	45.3770	24	-0.47			
Day			25days			pre
115.27	45.3770	24	2.54			
Day			acute			pre
136.74	45.3770	24	3.01			
Supple*Day		plac	25days		plac	acute

3.8750	79.8014	24	0.05				
Supple*Day			plac	25days	plac	pre	
215.28	79.8014	24	2.70				
Supple*Day			plac	25days	vit	25days	
206.19	232.03	24	0.89				
Supple*Day			plac	25days	vit	acute	
159.38	232.03	24	0.69				
Supple*Day			plac	25days	vit	pre	
221.45	232.03	24	0.95				
Supple*Day			plac	acute	plac	pre	
211.40	79.8014	24	2.65				
Supple*Day			plac	acute	vit	25days	
202.31	232.03	24	0.87				
Supple*Day			plac	acute	vit	acute	
155.51	232.03	24	0.67				
Supple*Day			plac	acute	vit	pre	
217.58	232.03	24	0.94				
Supple*Day			plac	pre	vit	25days	-
9.0858	232.03	24	-0.04				
Supple*Day			plac	pre	vit	acute	-
55.8942	232.03	24	-0.24				
Supple*Day			plac	pre	vit	pre	
6.1775	232.03	24	0.03				
Supple*Day			vit	25days	vit	acute	-
46.8083	43.2206	24	-1.08				
Supple*Day			vit	25days	vit	pre	
15.2633	43.2206	24	0.35				
Supple*Day			vit	acute	vit	pre	
62.0717	43.2206	24	1.44				

Differences of Least Squares Means

_Day	Effect	status	Supple	Day	_status	_Supple
	Pr > t					
0.1045	status	tr			untr	
0.1371	status*Supple	tr	plac		tr	vit
0.8223	status*Supple	tr	plac		untr	plac
0.0500	status*Supple	tr	plac		untr	vit
0.4886	status*Supple	tr	vit		untr	plac
0.0014	status*Supple	tr	vit		untr	vit
0.1427	status*Supple	untr	plac		untr	vit
acute	Day			25days		
		0.6404				
pre	Day			25days		
		0.0180				
pre	Day			acute		
		0.0060				
acute	Supple*Day		plac	25days		plac
		0.9617				
	Supple*Day		plac	25days		plac

pre	0.0126				
	Supple*Day	plac	25days	vit	
25days	0.3830				
	Supple*Day	plac	25days	vit	
acute	0.4987				
	Supple*Day	plac	25days	vit	
pre	0.3494				
	Supple*Day	plac	acute	plac	
pre	0.0140				
	Supple*Day	plac	acute	vit	
25days	0.3919				
	Supple*Day	plac	acute	vit	
acute	0.5091				
	Supple*Day	plac	acute	vit	
pre	0.3577				
	Supple*Day	plac	pre	vit	
25days	0.9691				
	Supple*Day	plac	pre	vit	
acute	0.8117				
	Supple*Day	plac	pre	vit	
pre	0.9790				
	Supple*Day	vit	25days	vit	
acute	0.2896				
	Supple*Day	vit	25days	vit	
pre	0.7271				
	Supple*Day	vit	acute	vit	
pre	0.1639				

Table B.2 Power Analysis for Sample Size

Subjects Per Group (2 groups)	Alpha	Power
12	0.10	0.74
14	0.10	0.80

Figure B.2 SAS Code for Power Analysis

```
Data SizeFix;
*Base specification;
a=2;dfS=1;
b=3;dfT=2;
ab=6;dfST=2;
*Specifications for power loop;
*Change aS, aT, aST level to 0.1 for type I=0.1. Change n;
aS = .05; cS = .75; cSsq = cS**2; powS = 0; ends = .70; r=2;
aT = .05; cT = .75; cTsq = cT**2; powT = 0; endT = .70; n=12;
aST=.05; cST=.75; cSTsq=cST**2; powST=0; endST=.70; w=1;
*Power loop;
Do until (powS >= ends and powT >= endT and powST >= endST);
  nObs = r*a*n*b*w; *Experiment size and error degrees of freedom;
  dfE1 = dfS*(r-1) + r*a*(n-1);
  dfE2 = dfT*(r-1) + dfT*dfE1 + r*a*n*b*(w-1);
  nS = r*n*b*w; *nTildas for noncentrality parameters;
  nT = r*a*n*w;
  nST = r*n*w;
  parmS = nS * cSsq /2; *Noncentrality parameters;
  parmT = nT * cTsq /2;
  parmST = nST *cSTsq /2;
  fS = Finv(1-aS , dfS, dfE1, 0); *Critical F values;
  fT = Finv(1-aT , dfT, dfE2, 0);
  fST =Finv(1-aST, dfST, dfE2, 0);
  powS = 1 - ProbF(fS, dfS, dfE1, parmS); *Power;
  powT = 1 - ProbF(fT, dfT, dfE2, parmT);
  powST = 1 - ProbF(fST, dfST, dfE2, parmST);
  output;
  r=r+1; *nTilda ratches;
  *n=n+1;
  *w=w+1;
End;
Proc Print; Var r n w dfE1 dfE2 powS powT powST nObs;
run; quit;
```

Appendix C: ANOVA Statistics Dissertation Study

Table B.1 ANOVA table

Source	DF	SS	MS	F	Pr
train	1	1765764.150	1765764.150	3.62	0.07
supp	3	274571.469	91523.823	0.19	0.90
train X supp	3	180431.321	60143.774	0.12	0.95
error	32	15604409.30	487637.79		
total	39	17922768.64			

Table B.2 Bonferroni Pairwise Tests

<u>supp</u>	<u>train</u>	<u>supp</u>	<u>train</u>	<u>t-value</u>	<u>Pr</u>
acute pla	trained	acute pla	untrained	0.48	0.6369
acute pla	trained	acute vit	trained	0.96	0.3425
acute pla	trained	acute vit	untrained	0.35	0.7316
acute pla	trained	chronic	trained	0.69	0.4945
acute pla	trained	chronic	untrained	0.28	0.7837
acute pla	trained	chronic	trained	0.78	0.4395
acute pla	trained	chronic	untrained	0.53	0.6023
acute pla	untrained	acute vit	trained	1.34	0.1902
acute pla	untrained	acute vit	untrained	0.12	0.9059
acute pla	untrained	chronic	trained	1.09	0.2818
acute pla	untrained	chronic	untrained	0.18	0.8564
acute pla	untrained	chronic	trained	1.18	0.248
acute pla	untrained	chronic	untrained	0.05	0.9641
acute vit	trained	acute vit	untrained	1.21	0.236
acute vit	trained	chronic	trained	0.27	0.7871
acute vit	trained	chronic	untrained	1.14	0.2633
acute vit	trained	chronic	trained	0.18	0.8577
acute vit	trained	chronic	untrained	1.39	0.1747
acute vit	untrained	chronic	trained	0.96	0.3422
acute vit	untrained	chronic	untrained	0.06	0.95
acute vit	untrained	chronic	trained	1.05	0.3033
acute vit	untrained	chronic	untrained	0.16	0.8703
chronic	trained	chronic	untrained	0.89	0.3775
chronic	trained	chronic	trained	0.09	0.9276

chronic	trained	chronic	untrained	1.14	0.2609
chronic	untrained	chronic	trained	0.98	0.336
chronic	untrained	chronic	untrained	0.23	0.8212
chronic	trained	chronic	untrained	1.23	0.229

Figure B.1 SAS Code for Power Analysis

```

/*Dissertation power analysis*/
data Power;
FSUPPSTATUS=Finv(0.90, 3, 32,0); PSUPPSTATUS=1-
ProbF(FSUPPSTATUS,3,32,-2.43);
FTRAINING=Finv(0.90, 1, 32, 0); PTRAINING=1-
ProbF(FTRAINING,1,32,2.62);
FSUbyTRAIN=Finv(0.90, 3, 32,0); PSUbyTRAIN=1-
ProbF(FSUbyTRAIN,3,32,-2.64);
proc print NoObs;
run;
quit;

```

Appendix D: Consent Form

1. Study Title:

The effect of an antioxidant supplementation protocol upon muscular fatigue in trained and untrained subjects during repetitive arm curl exercise

2. Performance Sites:

Exercise testing will be done in room B13 of the Gym Aromory

3. Contacts:

Dr. Arnold Nelson
112 Long Field House, LSU
Baton Rouge, LA 70803
phone (work) (225) 578-2036 (7 am - 4 pm)
Home (225) 766-4621 (5 pm - 10 pm)
e-mail: anelso@lsu.edu

Ivan K Guillory
1476 Lake Calais Ct, Apt D
Baton Rouge, LA 70808
phone (225) 757-9527
e-mail: iguillo@attglobal.net

4. Purpose of the Study:

The purpose of this study is to determine whether or not supplementing with vitamins A, C, and E will attenuate the fatigue seen performing a prescribed arm curl exercise protocol.

5. Subjects:

A. Inclusion Criteria

Forty healthy subjects are needed. Both males and females are invited to participate. Participation is voluntary. Two types of subjects are needed, trained and untrained group. Untrained is defined as individuals who do not currently participate in strength building exercise. Trained subjects are those currently undergoing a strength building program with a frequency of no less than three days per week. All potential participants must complete the Physical Activity Readiness Questionnaire (PAR-Q) before inclusion into the study. Only those individuals who answer “no” to all questions will be used as research participants.

B. Exclusion Criteria

Any person on any type of medication, or currently supplementing with any antioxidant vitamins. Also anyone who knows that they are hypersensitive to vitamins A, C, or E.

C. Maximum number of subjects: (including controls)

40

6. Study Procedures:

Supplementation Protocol

Before the supplementation protocol can begin each subject will compile a seven day food diary to assess antioxidant vitamin status. Each diary will be assessed for RDA levels of the selected vitamins, and to what extent that those levels have been met or exceeded. Subjects will ingest an antioxidant tablet containing 25,000 international units of beta carotene, 400 international units of vitamin E, and 500 milligrams of vitamin C. or a placebo (sugar pill). Subjects will supplement on an antioxidant vitamin tablet containing the aforementioned substances, for a total of fifteen days. After a thirty day washout period, they will be ingesting a placebo for fifteen days. Beta carotene is an antioxidant that is a precursor to vitamin A, often called pro-vitamin A. Vitamin E is used in the body to protect cell membranes from oxidation. Vitamin C, among its other functions, acts as an antioxidant and helps to regenerate vitamin E. The dosages that we are using are considered safe and are even within the guidelines established by the United States Olympic Sports Medicine Committee (Table 1)

Table 1.

Nutrient	Supplement Range
Beta Carotene	5,000 – 33,000 IU
Vitamin C	250 – 1,000 mg
Vitamin E	100 – 400 IU

Invasive Procedures (OPTIONAL)

To have a better understanding of the residual amount in the blood of the above vitamins, blood will be taken at two different time during the study. The first blood sample will be taken at the end of the washout period and before the second supplementation period. The second blood sample will be taken at the end of the study. All blood draws will be performed at the cmedical clinic of the Pennington Biomedical Research Center. A total of 5 ml of blood will be taken by a certified phlebotomist at each of the two times mentioned above. The blood samples will be analyzed for vitamin concentration by the clinical chemistry specialists at the Pennington Biomedical Research Center. PLEASE NOTE THAT THESE BLOOD DRAWS ARE OPTIONAL. ANYONE WHO DOES NOT DESIRE TO HAVE THEIR BLOOD SAMPLED WILL NOT BE ASKED TO DO SO NOR BE ASKED TO TERMINATE THEIR PARTICIPATION IN THE STUDY.

Testing Protocol

The testing procedure will consist of an initial assessment using a Biodex dynamometer., and subsequent tests at day one and day fifteen of each supplementation period. The exercise protocol will consist of six sets of arm curl exercises, using the left arm, with a one minute rest between each set. Twenty repetitions will constitute one full set. The speed of movement will be set at 60 degrees per second for flexion and, 400 degrees per second for extension (this speed will allow subjects to relax on the eccentric phase). The subjects will be instructed to give all out effort for each set. Subsequently total work (force multiplied by distance) for each set will be measured by the dynamometer in order to give a measure of the rate of fatigue.

Time line for Experiment

Days 1-7	Subjects record diet for seven days. At the end of the recording period, subjects will have the initial assessment on the dynamometer.
Day 14 and 15	Subjects begin the supplementation period. On the second day, they will again be assessed on the dynamometer.
Day 28	Subjects continue supplementation, and on the fifteenth day are assessed on the dynamometer
Day 29 - 58	Subjects undergo washout period
Day 58	Volunteers will report to the Pennington Clinic for a blood draw.

Day 59 and 60	Subjects begin the second supplementation period. On the second day, they will again be assessed on the dynamometer.
Day 73	On the last day of supplementation, subjects will again be assessed on the dynamometer
Day 74	Volunteers will report to the Pennington Clinic for a blood draw.

7. Benefits:

Subjects may benefit from the study from learning their status for these selected antioxidants after completing the food diary. They may also discover a beneficial effect upon muscular fatigue individually, after supplementing with the antioxidants. Also, general knowledge about the practice of supplementing with vitamins and their affect upon muscle fatigue will be learned and added to the current body of literature on the subject.

8. Risks/Discomforts:

There are small inherent risks involved with taking place in this study. It's possible that the subject could strain a muscle, experience light headedness, or nausea as they will be required to give maximal effort. There is no report risk for the supplementing these vitamins at the proposed dosage. At this time, however, the researcher will read to you the manufacturer's warning label

Additional risks may be encountered by those individuals who volunteer for the two blood samples. A person having a blood sample runs the risk of pain at the site of the blood draw, bruising at the same site, and the development of blood clots (hematoma) at the blood draw site. The chances of either bruising and developing blood clots are greatly reduced if the person follows the instructions of the phlebotomist.

9. Measures taken to reduce risk

All subjects will do a warm-up routine before exercising, this will help reduce the risk in the trained subjects. Warm-up will reduce exercise risk in untrained individuals very little. The only way to them to reduce the risks of the exercise is not to perform the exercise.

To reduce the risks associated with a blood drawn, only trained certified personnel in the employment of the Pennington Center will be allowed to take the blood samples.

10. Right to Refuse:

Il volunteers may terminate their participation at anytime and without warning. The individual just needs to stop the activity and leave the weight room. No attempt will be made by the research personnel to stop the volunteer. In fact, the research personal will do all in their power to help the volunteer leave at any time.

11. Privacy:

The results of this study will be tabulated in an anonymous manner. This means that the researchers will be unable to give you your results, as they will not be able to identify yours from the rest.

12. Financial Information:

This project will not effect the participants finances in either a positive or a negative manner.

13. Withdrawal:

Withdrawal form the study can occur at anytime without any fear of punitive action.

14. Removal:

If the researcher has trouble scheduling the volunteer to come to the test, or if it is discovered

that the subject is not taken the pills in the prescribed manner, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at anytime before or during the study.

Part 5: Signatures:

1. 'The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Chairman, LSU Institutional Review Board, (225)578_8692. I agree to participate in the study described above and acknowledge the researchers' obligation to provide me with a copy of this consent form if signed by me.'

Subject Signature _____ Date _____

2. 'The study subject has indicated to me that he/she is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.'

Signature of Reader _____ Date _____

Appendix E: Power and Statistical Significance

When conducting statistical tests to describe the characteristics of a group of data there are certain distinct measures that come in to play. Those measures include, probability (alpha and beta), effect size, and power.

Probability is defined as the chance that a certain event will occur. An example used frequently is in the case of flipping a coin. One would expect that if a coin is tossed 50 times it would come up heads 25 times and tails 25 times or 50% (0.50). This may or may not occur, however if this test were repeated a number of times you would expect the frequency of scores to be closely huddled around 50% (Thomas and Nelson, 2001). In statistics probability statements are used to describe confidence in the findings of the research. In research, probability is used to protect against making two types of error - Type I and Type II. Type I error occurs when the null hypothesis is rejected although it is true. Type II error is to fail to reject the null hypothesis when it is false.

The probability statistic used to guard against a Type I error is called alpha. The statement used to describe the confidence placed in statistical findings is called a probability statement (Thomas and Nelson, 2001; Freund and Wilson, 1997). A typical probability statement may appear as $p < 0.01$, meaning the difference detected would be expected less than 1% of the time as a result of chance. Within the science community the alpha level is almost always set at either 0.01 or 0.05. As mentioned previously this is done to protect against a Type I error. For example, in an experiment in which a supplement is given, Type I error protects against detecting a difference when the supplementation really had no effect. The higher the alpha level the more opportunity that is given to show any difference but, conversely the chance of Type I error increases. There is no official reasoning as to why 0.05 and 0.01 have been accepted by the

scientific community as the “gold standard” for alpha level. The decision comes down to which type of error is more acceptable to the researcher, Type I or Type II. In a study in which a drug is being tested, but has never shown to be harmful, it might be acceptable to set alpha at 0.10 to give that drug every opportunity to have an effect. In a paper by Franks and Huck (1987) it is posed that there are indeed instances when a higher significance level may be warranted. Sample size can be determined via a formula that takes into account, among other things, alpha level. However, in many instances in exercise and physical activity research, there are times when there is only a limited sample available. In addition, there are factors that are beyond control such as costs of the experiment, subject dropouts and / or availability, and incomplete data (Franks and Huck, 1987). In this case setting alpha at 0.10 or another value may be worth the added risk of Type I error when weighted against the consequence of committing a Type II error. Finally, in the example of the drug testing, in an exploratory study it may actually be more costly to experience a Type II error, that is, the drug actually has an effect, but the researcher accepts the null hypothesis of no effect.

Beta is the test statistic used to specify the probability of making a Type II error. Again, the Type II error is a failure to reject the null hypothesis when it is false. If sample size stays the same beta will grow smaller as alpha is increased (Freund and Wilson, 1997). Conversely, power is the probability of rejecting the null hypothesis when it is false (Thomas and Nelson, 2001). The subject of power will be covered later in this paper.

Effect size (ES), or meaningfulness of differences, has been defined as “the magnitude of the real effect to be detected” (Thomas, Lochbaum, Landers, and He, 1997). Simply put it is the meaningfulness of difference between two means (Thomas and Nelson, 2001). It is calculated as:

$$(M_e - M_c)/S_c$$

This calculation is the mean of the experimental group minus the mean of the control group with the difference divided by the standard deviation of the control group (Thomas, Lochbaum, Landers, and He, 1997). If there is no control group, a pooled standard deviation between the two group means should be used (Thomas and Nelson, 2001; Hedges and Olkin, 1985). Guidelines for behavioral research were suggested by Cohen (1969) and within those guidelines he suggested an ES of 0.2 as small; 0.5 as moderate; and 0.8 as large differences. Thomas, Salazar, and Landers (1991) posed the idea that all authors of research should report ES in their analysis. One reason for this is the chance that small differences may be declared significant because of a combination of a large sample and small variance, or a large variance and small sample size. In a paper by Cohen (1990) it is argued that the main purpose of research should be to measure effect size rather than p values. The logic behind this is that a single study based upon a decision at the $p < 0.05$ level is not likely to have an impact upon theory, but that reporting effect size allows there to be a standard of comparison between past, present, and future research. Clearly this would help to guide research on the selected topic for the future. As will be discussed later, ES, alpha level, and sample size are instrumental in helping to determine power for a given study. If the sample size is low, as is the case many times in physical education research, a higher than normal alpha level, or large ES, or both may have to be adopted to maintain a certain desired level of power (Thomas and Nelson, 2001)

Statistical power has been described as “the probability of not making a Type II error” (Norton and Strube, 2001). In other words, it is the probability of making a correct determination. Consequently, if beta is representative of the probability of making a Type II error, then the probability of not making a Type II error is mathematically represented as $1 - \beta$. Therefore, $1 - \beta$ may be labeled as the mathematical expression of power. The

greater the power, the higher the chance of detecting a real difference in the analysis. The four main components that affect power are alpha level, group means, sample size and variability among subjects (Norton and Strube, 2001). Alpha level affects power indirectly, through its influence upon Type I and Type II error. When alpha is increased, the chance of making a Type I error is increased, while at the same time the chance of making a Type II error is decreased because beta is decreased. A subsequent decrease in beta results in an increase in power, because of the mathematical relationship of beta and power ($\text{power} = 1 - \text{beta}$). Group means have an influence on power through their differences between the group means for the null hypothesis and experimental group means. Logic tells us that the larger the difference between group means, the easier the detection of any differences. In other words, power increases when the difference between the two means becomes larger. Subject variability may also have an effect on power. In a graphic comparison between two distribution of scores, one distribution curve may have the same mean of another distribution curve. However, one curve may be wider than the other because of greater variability among the scores within its distribution. If these two curves overlap and one represents the distribution of the null hypothesis and the other the research hypothesis, the greater the overlap in the scores between the two distributions, the larger the beta and therefore a subsequent decrease in power (Norton, and Strube, 2001). Finally, the sample size may also influence power. Basically, as sample size is increased power is increased. Increasing sample size reduces the variability of the sample group means (Thomas and Nelson, 2001; Norton and Strube, 2001).

Via the use of a power analysis it is possible to determine the number of subjects or observations necessary when planning a study. Using ES, alpha level and power it is possible to calculate a value for sample size. As stated previously, ES may be determined by a previous pilot

study, or it may be determined from the means of a number of related studies (Thomas et al, 1997). It is suggested that the ratio of alpha level to beta level should be 1 to 4 (Thomas and Nelson, 2001). For example, if an alpha of 0.05 was selected then beta should be set at 0.20 and, likewise when alpha is set at 0.10 beta should be set at 0.40 (power = 0.60). Again, the researcher must determine what type of error is most important to avoid within the planned study. Upon obtaining the ES, alpha level and effect size, the sample size is determined via effect size curves (Thomas and Nelson, 2001; Thomas et al, 1997), using simple formulas (Cohen and Cohen, 1983), or relying upon statistical software such as SAS or SPSS. As noted earlier, sample size has a large influence upon power. For example, for an ES = 0.5 and an alpha of 0.05, a sample size of 10 results in a power = 0.20 and a sample size of 20 would result in power = 0.30 (Thomas and Nelson, 2001).

Power analysis may also be used after the completion of a study. After the study is completed, a power analysis can determine if the level of power is adequate to justify any kind of conclusion (Norton and Strube, 2001). For instance, after the power analysis is finished if the power level is determined to be lower than the desired level, then a decision must be made by the researcher to either not accept the result due to inadequate power or risk the increased chance of a Type II error. This kind of problem may occur if there are a significant amount of subjects that drop out or discontinue participation in the study. Again, power analysis is usually determined using statistical software such as SPSS or SAS.

It is well known that statistical significance is representative of a test of the difference between experimental groups (Thomas et al, 1997). Either a difference between group means is significant at the specified alpha level or it is not significant. However, there is a distinction between the significance of the differences and the meaningfulness of the differences. By

definition meaningfulness is “the importance or practical significance of an effect or relationship” (Thomas and Nelson, 2001). It is plausible then, for a study to have outcomes that are not statistically significant but, because of the effect size(s), may have a meaningful outcome when compared to effect sizes within the existing literature (Thomas et al, 1997; Norton and Strube, 2001; Thomas and Nelson, 2001). Effect size makes it possible to compare results across many different studies, that address a similar question, because of the way it standardizes the effect magnitude to a value that can be compared, although scaling properties of the measurements of each study are different (Norton and Strube, 2001). So, regardless of the statistical significance at a given alpha level for a given study, studies still can be compared and may still contribute to the existing knowledge in regards to the particular question being addressed.

As an example of determining sample size, we could consider the improvement of world class sprinters attempting to improve their time in the 400-meter run. If we wanted to see if a certain supplement, in this case creatine phosphate, improved 400-meter sprint times we would first conduct a pilot study in which one group (experimental group) of sprinters ingested the creatine supplement for a given amount of time, and the other group (control group) ingested a placebo. There are 10 athletes in each group. Each sprinter would be timed in the 400 meters before the supplementation began, and at the end of the supplementation period. Afterwards, their improvement in hundredths of seconds would be recorded. In world class sprinters we would not expect a large improvement. Improvements in that event are sometimes measured in tenths or hundredths of seconds. However, that small improvement may well be the difference between an Olympic gold medal and fourth place. So, following a pilot study we calculated the mean of the difference in pre- and post-supplementation times for the five athletes in the

experimental group and the control group. The experimental group received five grams of creatine per day for thirty days and the control group received a placebo for the same amount of time. Afterwards, their improvements were recorded. The mean improvement for the experimental group was 0.1 seconds and the control group was 0.05 seconds. The standard deviation of the control group is equal to 0.03. So, with this information we can calculate a sample size needed for a larger study. We use the same formula mentioned earlier to first find the effect size:

$$(M_e - M_c)/S_c$$

$$(0.10 - 0.05)/0.03 = 1.6$$

With an ES = 1.6, we will assume a desired power as 0.8 and an alpha level of 0.05. Using Sigma Stat statistical software package to calculate a sample size for a one way ANOVA, we would find that we need two groups of seven subjects to detect a meaningful difference between the two groups, supplemented and placebo.

Now that we have the minimum sample size we can collect the data from the main study. Since we need a minimum of 7 subjects per group we recruit twenty subjects. Then we set up a similar protocol to the pilot study. Ten subjects will ingest the creatine for thirty days and the other ten will ingest the placebo. Four hundred meter sprint times are tested before and immediately after supplementation. In the experimental group the mean of the differences between pre and post supplementation is equal to 0.33, and for the placebo group 0.06. Following a one way ANOVA it is found that there is a significant difference between the experimental group and the placebo group such that $p < 0.001$. Following the test, we determine the power to be equal to 1.0 taking into account the difference of the means (0.27) and a $p = .001$. So, following this test we would conclude that supplementation with creatine is beneficial to

world class 400-meter sprinters in reducing their 400-meter sprint times. However, if the case had been that the $p=0.07$ and the power were $.75$ then the researcher would have to make the decision as to which error is most acceptable. With an alpha level at 0.07 the chances of rejecting the null hypothesis when it is true is 7 times out of 100. Conversely, the power is 0.75 which is not 0.8 , but very close. In a study such as the aforementioned, the researcher is using a supplement (creatine) that has not been shown to be harmful with only 30 days of supplementation. So, the decision may be made to reject the null (of no benefit) to give the supplement every opportunity to work. This may not be a bad decision with the power being 0.75 . A power at that level would indicate that there is a 25% chance of making a Type II error. But, again, as the null is that there is no benefit from taking creatine, making a Type II error would not be harmful to the participants and may be worth the risk to the investigator. This would have to be the options weighed before a decision is made whether to accept or reject the null hypothesis.

Appendix F: Obtaining and Motivating Subjects

When conducting a study that entails subjects having to take a drug / supplement and then to perform an exercise protocol at specified intervals throughout a calendar year, there are inherently some issues that rise up that affect subject compliance and motivation.

One concern that has been apparent is the subjects initial apprehension towards the supplement. Since, most lay people are not educated about nutrition or experimental research some questions arise as to the harmful or possible side effects of the supplement. At the beginning of any study involving the supplementation of a nutritional aid, the researcher should explain in detail the nature of the action that the supplement has within the body. This should be done through a group question and answer period and in a detailed written description of the supplement and its action / side effects.

Obtaining subjects for physical activity research can be difficult if there is no incentive for subjects to participate. For example, Cohen, Chelland, Ball, and LeMura (2002) used monetary reward to subjects participating in a physical activity study involving riding a cycle ergometer. They found that the monetary rewards motivated the subjects to ride for a longer time on the cycles than when no money was awarded. For many researchers the awarding of a monetary sum to all subjects is just not financially feasible. However, if they were to make it into a contest or lottery situation in which the subject with the best effort, or best attendance, won a reward, this would serve to be a motivating factor because it would appeal to the subjects competitiveness. Another example of a reward system would involve soliciting college students to participate, whereupon the researcher negotiates rewards for their participation via the college / university or individual professors, preferably in courses related to physical activity.

It may be difficult to keep subjects motivated if a study is long or involved. There are

also factors such as boredom, activity preferences, and even group cohesion that may affect subjects lack of compliance during a study. Perhaps having periodical meetings of the subjects would help to motivate individuals. These meetings could serve as question and answer, as well as motivational periods. When the subjects see that they are a part of a larger group, this may help to motivate them to continue in the study (Spink and Carron, 1992). Encouragement and accountability to the group may serve as a motivating factor for individuals who feel isolated and are not sure that their participation makes any kind of difference. If subjects do not enjoy the mode of exercise being used in a study, then the likelihood of them staying motivated and participating in the study is diminished. When individuals are enjoying an activity they get positive feedback from that participation (ACSM, 1993, pp. 447). However, if they do not then they experience the opposite feelings (Stutts, 2001).

If time permits researchers may be able to use advance advertisement to recruit volunteers. Yancy et al (2001) used what is referred to as passive and active recruitment. Passive recruitment involved placing ads in the local paper or radio station (public service announcements), and also social networking. They also posted flyers at community sites and organizations such as churches, grocery stores, and neighborhood centers. This strategy could easily be adopted to a university setting by utilizing the school newspaper, radio station, student health center, recreational center, or personal friendships / relationships. They also used a method called active recruitment in which potential subjects were contacted directly. The methods used included mass mailings, presentations at selected churches, meetings of community organizations, and community health fairs. Again, in the case of recruiting from a university population these methods may be adapted to that particular community. Investigators could send out detailed letters to professors and leaders of student organizations to define what

the study is investigating, in hopes that these leaders will motivate their students or members to participate in the study. Likewise the investigators could make presentations at the meetings of said student organizations to introduce the students to the type of work being performed and how the students can help, and possibly even benefit from participation. During these presentations they can appeal to the audience's desire to help others, if the study has some medical or health ramifications. As most universities now have student health fairs during the school year, these are prime locations to introduce the work that the group is doing, and to request volunteers for the upcoming study. To ensure the target population are being admitted to the study, researchers could also use a screening questionnaire. In the case of a study needing subjects from a certain age range, and / or fitness level, a questionnaire could be tailored to include domains such as smoking status, disease status, age range, etc. (Yancy et al, 2001). These methods have been used by many researchers to transcend the obstacles to recruiting subjects for physical activity research.

Appendix G: Journal Article

Introduction

The recent interest in antioxidants and free radicals has prompted an increase in the supplementation of these substances by many in the general public. Athletes believe that supplementing their diet with antioxidants helps to maintain their optimum fitness level. To this end vitamin manufacturers market antioxidants to athletes, professional and amateur alike, as an ergogenic aid counteracting the effect of oxidative stress during exercise (Urso and Clarkson, 2003; Applegate, 1999; Juhn, 2003; American Dietetic Association [ADA], 2000). It is well accepted that exercise causes an increase in the production of free radicals and other reactive oxygen species (ROS) (Powers and Hamilton, 1999). Among these ROS are O_2^- (superoxide radical), H_2O_2 (hydrogen peroxide), and OH (hydroxy free radicals). A proliferation of these free radicals can cause a decrease in the function of affected cells and can result in a decreased ability of muscles to maintain work. Some may believe that the ingestion of antioxidants will help to stave off this proliferation of free radicals during exercise and thus provide a beneficial effect.

The human body has adequate antioxidant defenses under normal physiological conditions. These defenses include enzymatic and nonenzymatic antioxidants within the interior and exterior of cells. The non-enzymatic include antioxidant vitamins, uric acid, glutathione, alpha lipoic acid, bilirubin, and ubiquinones (Powers and Hamilton, 1999). Enzymatic antioxidants are located mainly within the mitochondria and cytosol, and include superoxide dismutase, glutathione peroxidase, and catalase

Vitamin E, vitamin C, and beta-carotene are three antioxidants that have garnered the most attention from those who wish to supplement their diets with exogenous nutrients. Vitamin E works to protect cell membranes by breaking the chain reactions that attack the lipid portions

of those membranes. Vitamin C is an antioxidant that works in conjunction with vitamin E and is located in the cytosol of cells. It helps to regenerate vitamin E that has been used to fight the lipid peroxidation occurring within cell membranes. Beta Carotene is a form of vitamin A called pro-vitamin A. It can be converted into vitamin A as needed by the body, and much of it is located between the lipid bilayers of cell membranes. In this position, it also acts as an antioxidant protecting cell membranes.

During strenuous exercise, there is an increase in oxygen uptake by working muscles. As work intensifies, oxygen uptake increases. This increase in oxygen uptake results in an increase in ROS that may result in the fatiguing of working muscles (Powers and Hamilton, 1999; Davies, Quintanilha, Brooks, and Packer, 1982). There is evidence that suggests supplementing the diet with antioxidant nutrients may help to stave off the effects of an increase in ROS (Novelli, Bracciotti, and Falsini, 1990; LeBlanc, 1998; Lang, Gohil, and Packer, 1986; Samanta and Biswas, 1985; Brown et al, 1997; Allesio et al, 1997). However, most studies have investigated the effect of these antioxidants upon continuous endurance exercise, and have not used an intermittent work protocol, nor the three antioxidants in combination, being utilized here.

In this study we have investigated the effect that an acute and a chronic supplementation regimen, with an antioxidant mixture of beta-carotene, vitamin C, and vitamin E, has upon work capacity during an intermittent work protocol in trained and untrained subjects. Additionally, we will observe if there is any significant interaction between the trained and untrained subjects work values from the acute placebo phase through the chronic vitamin phase.

Methods

Subjects. Thirty-six male and female volunteers were recruited, aged 19-34 years, who,

after completing an informed consent form, were deemed healthy and on no medications. Subjects were classified as either trained (n=24) or untrained (n=12). Trained were those who engage in strength training on a regular basis (at least one day per week) for a period of at least one year. Untrained were those who do not participate in any strength training. All subjects were required to be nonsmokers and to not be taking any vitamin supplements at the time of the study.

Dietary Assessment. A subgroup of ten subjects completed a 3-day food diary prior to supplementation to assess current intake for the antioxidants being ingested. The information therein was analyzed using the software, *Food Processor for Windows* (version 7.30) by ESHA Research.

Experimental Design. Subjects ingested either one antioxidant capsule containing (25,000 IU of beta-carotene, 400 IU of vitamin E, and 500 mg of vitamin C), or a disguised placebo, per day for a period of 25 days. The capsules were administered in a double blind fashion so as to remove bias from the investigation. The antioxidant capsules are produced by Vitamin World, Inc. and, are manufactured to the standards of the United States Pharmacopeia / National Formulary requirements.

The testing procedure consisted of an initial practice session using the Biodex dynamometer (one week prior to supplementation), and subsequent tests at day one and day twenty-eight of each supplementation period. Each subject was tested within a 12 hour range of the time of the original test, for each of the subsequent tests. For example, if the original test was at noon, then the tests at one day and twenty-eight days would be between six a.m. and six p.m. on those days. The exercise protocol consisted of five sets of arm curl exercises, using the non-dominant arm, with a 1 minute rest between each set. Twenty repetitions constituted one full set. The speed of movement was set at 60 degrees per second for flexion and, a maximum of 400

degrees per second for extension (this speed allowed subjects to relax on the eccentric phase). The subjects were instructed to give all out effort for each set. Subsequently, total work (force multiplied by distance) for each set was measured by the dynamometer in order to give a measure of the rate of fatigue. The sum of the total work of the last two sets was used in determining fatigue.

Statistical Analysis. Following data collection all results will be analyzed and compared for significance. A two-way (treatment X day) ANOVA with an alpha level of $p < 0.10$, was used to compare within and between group differences.

Results

Dietary Assessment. Subjects completed the 3-day food diary to assess current intake for the supplemented antioxidants. The information therein was analyzed using the software, *Food Processor for Windows* (version 7.30) by ESHA Research, as described earlier. The results for each subject are listed in table 1. The means for each antioxidant are listed for each subject for the three day period. This allowed estimation of the extent to which the RDA for the selected antioxidants are being met or exceeded, in the regular diet. Mean group values for beta-carotene, vitamin C, and vitamin E respectively, were equal to 1791.415 mcg (St err = 738.819), 175.645 mg (St err = 94.007), and 6.678 mg (St err = 1.752).

Table 1 Antioxidant Vitamin Measures From Food Diary Data

Subjects	Beta-car. (mcg)	Vitamin C (mg)	Vitamin E (mg)
1	811.12	98.42	2.68
2	498.62	87.89	5.30
3	3122.46	1017.19	14.56
4	1388.48	63.19	4.21

5	330.18	99.37	3.98
6	9.38	31.12	4.96
7	574.32	107.25	8.50
8	223.93	102.29	1.58
9	7494.26	31.42	2.72
10	3461.40	118.31	18.29

Work Measures. Following the intermittent work protocol, work measures for each subject at each supplementation period were recorded (Table 2). The protocol was conducted on the Biodex dynamometer in the kinesiology lab at Louisiana State University. Mean values for each phase are reported in Table 3.

Table 2. Work Measures for Each Subject

status	acute plac	chronic plac	acute vit	chronic vit
untrained			326.1	396.7
untrained			884.3	1034.3
untrained			675.2	807.7
untrained			789.4	676.1
untrained			460.9	599.9
untrained			1194.7	1116.1
untrained			806.1	1219.6
untrained			796.9	719.6
trained	622.4	690.2		
trained	699.2	1039.6		
trained	1213.1	1628.4		
trained	1801.5	2080.4		
trained	1545.6	1501.2		
trained	980.4	993.5		
trained	1322.5	1413.3		
trained	1234.1	1028.9		
trained			1493.9	1212.9
trained			1154.8	1021.1
trained			1486.9	1316.3

trained			1939.1	1688.1
trained			1753.4	1740.1
trained			1879.2	1972.1
trained			754.7	583.6
trained			1168.4	1022.2
trained			1288.4	1072.7
trained			915.8	679.4
trained			923.5	969.4
trained			1186.1	1321.7
trained			688.4	731.2
trained			1490.1	1414.9
trained			1484.9	2228.6
trained			1217.4	1058.4
untrained	673.8	754.1		
untrained	1385.8	1314.7		
untrained	678	530.7		
untrained	209.6	411.5		

Table 3 Work Mean Values

Status	Supp. Phase	Mean Work
trained	acute placebo	1178.29
trained	chronic placebo	1321.14
trained	acute vitamin	1142.12
trained	chronic vitamin	1072.59
untrained	acute placebo	823.60
untrained	chronic placebo	827.70
untrained	acute vitamin	1048.93
untrained	chronic vitamin	1195.53

A two way ANOVA was used to detect any differences in the data. Although upon viewing the data graphically (Figures 1 and 2) there appears to be an influence upon the untrained subjects supplementing with the antioxidant, the data show otherwise. For the main effects supplementation status, and training, there were p values of 0.88 and 0.09 respectively. For the

interaction of supplementation status and training, p value was equal to 0.25. There was a small effect size of 0.26 for the effect of training. Power for supplement phase, training, and their interaction was <0.10, 0.29, and <0.10 respectively.

Discussion

Dietary Assessment. Vitamins C and E, and beta-carotene intakes were estimated using 3-day food diaries. Each subject was given a diary to completely record everything that was eaten during a three day period. As invasive measures of these two of the nutrients were not investigated, it was determined that recording daily intake of these nutrients would provide the best estimate of tissue levels for this particular group. Assessment of the subjects' status in regards to the antioxidants studied, was necessary to ensure that there were none of them starting from a point of deficiency, prior to the start of the experiment.

Currently there is no RDA for beta-carotene in humans. However, the RDA for vitamin A is 0.8 - 1.0 retinol equivalents (RE) (Groff et al, 1995). Beta-carotene is a precursor to vitamin A, and is often called pro-vitamin A.. The body cleaves stored beta carotene as needed to maintain serum vitamin A levels within the body.

There appears to be no serious side effects when ingesting beta-carotene in excess of the RDA for vitamin A because it is not converted to vitamin A until the body needs it.

Following diet analysis for each subject, the software displayed information for each vitamin and mineral with an RDA and also other nutrients, such as beta-carotene, that do not currently have an RDA. The *Food Processor for Windows* software reported total vitamin A / provitamin A in the diet. It also gave beta-carotene content as a separate measure from total

Figure 1 Trained Subjects Work Graph

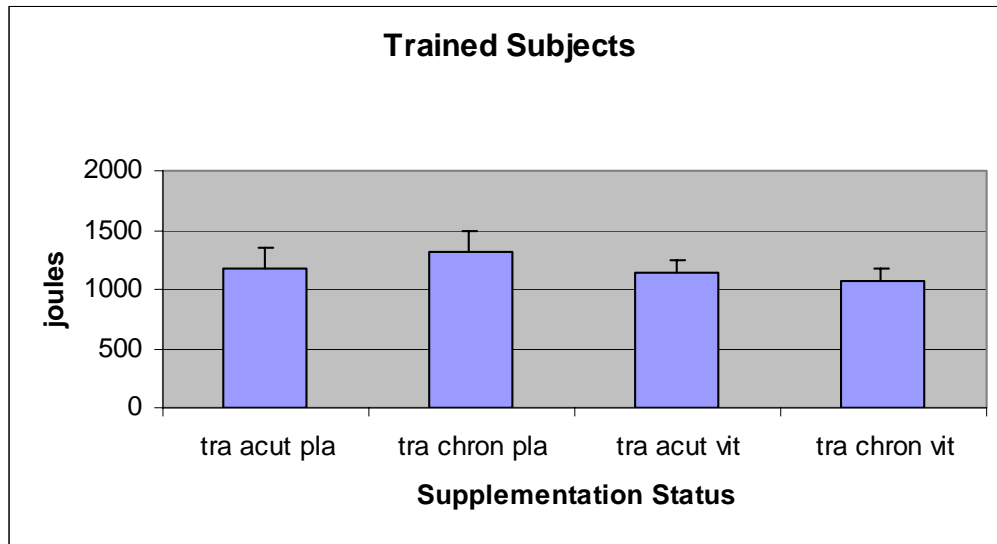
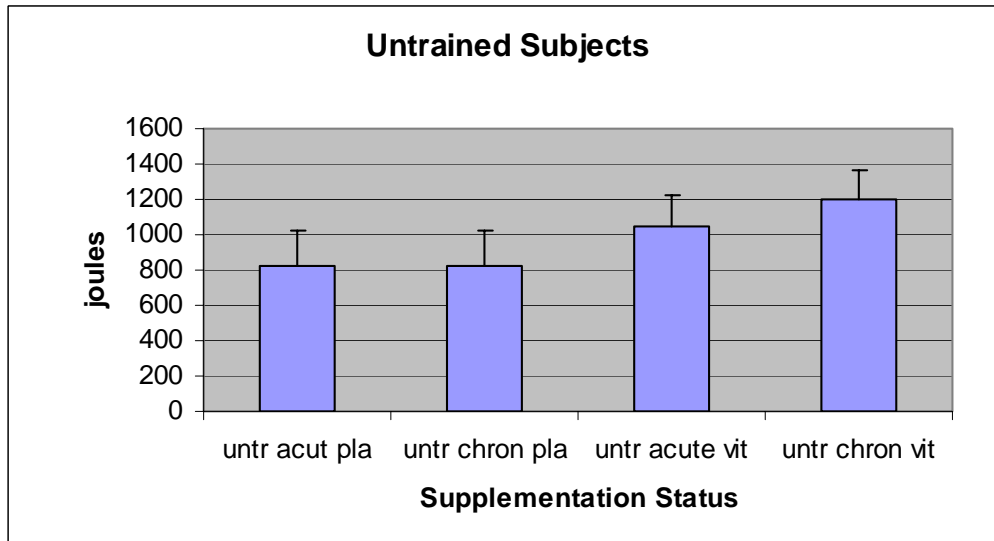


Figure 2. Untrained Subjects Work Graph



vitamin A content. Our subjects mean intake for beta-carotene during the three days of listing food intake was 1791.415 mcg (Std = 2336.35). Six micrograms of beta-carotene is equal to one RE. Again, this measure was just a part of the total vitamin A intake, but it does illustrate the extent to which the subjects were meeting the RDA for vitamin A. On average the subjects ingested 298.57 RE per day for the three day period. This far exceeds the RDA for vitamin A and ensures, barring any malabsorptive disorders (pancreatic, liver, or gall bladder diseases), that the subject pool was not vitamin A deficient prior to the supplementation regimen (Beta-carotene ingestion for each subject is listed in table 1).

The RDA for vitamin E is 8 mg and 10 mg tocopherol equivalents (TE) per day, for women and men respectively. The normal intake for vitamin E in American diets is 4 mg TE to 22 mg TE/day for individuals not supplementing their diet with exogenous vitamin E (Di Mascio et al, 1991). Again, vitamin E requirements increase as the intake of PUFA increases or decreases. Because of this dependency upon PUFA content, determining a precise requirement for vitamin E has been difficult. However, because most foods high in PUFA are also high in vitamin E, there is not much fear of deficiency. In fact, vitamin E deficiency in humans is very rare. As in the case with beta-carotene, deficiency in adults is only seen in those suffering from malabsorption diseases, especially those that affect the absorption of fats.

The diet analysis determined that the mean intake for vitamin E for each subject for the three day period was 6.678 mg TE/day (Std dev=5.54). This is just below the RDA for vitamin E but is well within the normal intake for those consuming a typical American diet (Di Mascio et al, 1991). This also falls between the range stated by Guthrie and Picciano (1995), between 5 and 20 mg TE/day, that would be considered safe depending upon PUFA intake. Considering the rarity of vitamin E deficiency, and the fact that our subjects intake of vitamin E falls between the

range for normal intake, it is highly unlikely that these subjects were vitamin E deficient before beginning the supplementation regimen.

The RDA for vitamin C is 60 mg/day for adult men and women. This amount was chosen based upon the amount required to maintain a body pool of 1500 mg (the amount of vitamin C that can be held within the body without excretion via the kidneys). The subjects' food diaries showed that the mean intake for each subject per day was 175.645 mg of vitamin C per day (Std dev=297.28). This number exceeds the amount in the RDA, but is far below the amount indicated in vitamin C toxicity (Alhadeff et al, 1984). Again, it is highly unlikely that subjects were vitamin C deficient before beginning the supplementation protocol.

Work Measures. Subjects were supplemented with a placebo or the antioxidant vitamin mixture (beta-carotene, vitamin E, and vitamin C) to see if a chronic dose would cause an affect upon the work capacity during the arm curl protocol, from acute to chronic supplementation phase. As was noted, there is scant research investigating the affect of an acute (one day) versus chronic (25 days) ingestion of either of the three antioxidant nutrients upon muscular fatigue during the arm curl protocol prescribed in this study. In addition, no studies using the intermittent arm curl protocol were found throughout the literature. Although, there were differences seen graphically as described earlier, there were no significant differences seen in the interaction of supplement status and training state ($p=0.25$). There was a significant effect for training state ($p=0.09$) which was not unexpected, since the trained subjects were continuing in their strength training program during the time of supplementation and work testing.

With the significance level determined from statistical analysis, we observed that the interaction did not reach significance ($p=0.25$). However, we cannot make this determination with any confidence because of the power being <0.10 . The power is the probability of making a

correct decision (rejecting the null hypothesis when it is false). So, under these conditions it is not possible to make a decision and be confident in it.

Although there is not a significant interaction, there does appear to be a trend towards an interaction graphically (figures 1 and 2). We know that the analysis for this investigation resulted in a low power. However, power can be increased by increasing the sample size. It may be possible that with an increase in subjects, the interaction between supplemental status and training may become significant. In the graph we notice a fairly even mean scores from acute placebo to chronic vitamin states in the trained individuals. These subjects may have such an adaptation to increased ROS via their training activities, that they may not notice any influence of supplemented antioxidants upon work. Conversely the untrained subjects appear to benefit from the vitamin supplementation graphically (figure 2), from the acute supplementation compared to the chronic supplementation. Finally, it is believed that for the future a larger sample size should be utilized and may possibly show a significant interaction between the trained and untrained subjects.

Power, in this instance, appears to have been heavily influenced by the variability of the results. The variance for the supplement status, training phase, and their interaction is 44524.25 (Std dev.=211.01), 614972.03 (Std dev.=784.20), and 283840.37 (Std dev.=532.77). These are large values and show that if the scores were represented by a curve on a scale, the curve would be extremely wide. The narrower the curve the more chance there is of detecting a difference between the curve representing the null hypothesis and the curve representing the research hypothesis.

Additionally, the concept fatigue is multifaceted and encompasses physiological as well as psychological factors (Astrand, 1986). It may be possible that the influence of the antioxidant

supplement may not be enough to make a noticeable impact upon work measures.

In summary, there were no determinations that could be made from the work measure values because of the low power. However, we were able to evaluate the dietary status of each subject for the prescribed antioxidants via the food diary. For the future, researchers may want to consider a longer supplementation period or larger sample size to be able to detect significant differences between the trained and untrained groups using the same protocol. Additionally, a study design entailing a crossover may allow for less variability since each subject would have the opportunity to fall under both treatments.

Note: References listed in reference list; None of the subjects are the same in this study (i.e. there are no repeats because this data is the combined data of pilot and dissertation work)

Vita

Ivan Kenyon Guillory was born on February 27, 1968, in Los Angeles, California. At the age of two, his parents moved the family to Compton, California, where he spent most of his childhood. He attended Brethren High School where, he participated in basketball, cross country and track and field. During his senior year he earned all-league honors in cross country and all-league and all-district honors in track and field. He graduated from Brethren in 1986 and subsequently attended a local college, Cerritos Community College. While at Cerritos he earned conference championships and all-state honors in the 800 meter run at the California Community College State Track and Field Championships. His experience participating in athletics sparked an interest in exercise science and in 1989 he transferred to Morehouse College in Atlanta, Georgia, having received an athletic scholarship in track and field. While at Morehouse he won the Southern Intercollegiate Championship in the 800 meter run during both of his years of competition. In 1993 he received his bachelor of arts degree in health and physical education from Morehouse. From 1993-1994 he worked for a local health club in Atlanta. He enrolled at Louisiana State University in 1994, with an interest in exercise and nutrition. In 1996 he received the master of science degree in clinical exercise physiology. Afterward, he decided to continue his education at Louisiana State in the doctoral program. In regards to this research he has presented abstracts at the American College of Sports Medicine conference and the National Black Graduate Students Association conference. He has plans to continue in academia following completion of the degree requirements.