

Micronutrient Needs for the Prevention of Oxidative Damage
in Collegiate Male Cross Country Runners


by

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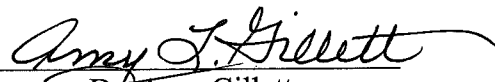
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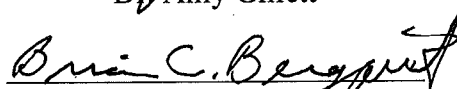
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ABSTRACT

The purpose of this study was to investigate the micronutrient needs of male cross country runners for the prevention of oxidative damage. Subjects for this study consisted of 11 male collegiate cross country runners at a Division III University. A nutrition assessment consisting of a 3-day food record and measurements of height, weight, body fat percent, and VO₂ max using the V max Encore instrument was conducted on each athlete before the track season and at peak performance during the track season. Each athlete was also asked several questions in regards to training increases, and the use of medications including supplements.

The results of this study are based on differences in nutrition assessment measurements and the consumption of micronutrients at pre-season and at peak performance in comparison to the RDA's. The pre-season intake of vitamin A exceeded and vitamin E and C intakes were significantly lower than the RDA's. Similarly vitamin A was significantly higher and vitamin E was significantly lower at peak performance. However, the RDA for vitamin C was met at peak

performance. Pre-season mineral intakes, except for iron, were not significantly different than the RDA's. At pre-season and peak performance iron significantly exceeded the RDA. In conclusion, male cross country runners exhibited adequate consumption of micronutrients, with the exception of vitamin E, to meet the RDA's. Additional research would be needed to determine if meeting the RDA's would ensure that an adequate antioxidant defense system could be maintained.

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Chapter I: Introduction

Endurance athletes consume large amounts of oxygen, which increases the production of reactive oxygen species (ROS) and leads to oxidative stress (Williams, Strobel, Lexis, & Coombes, 2006). The major source of ROS is thought to be the mitochondria of active muscle, but free radicals are also produced by red blood cells or during inflammatory response. When the antioxidant system is not adapted to excessive production of ROS, oxidative stress is initiated (Margaritis, Palazzetti, Rousseau, Richard, & Favier, 2003). The high production of ROS during exhaustive exercise could be responsible for membrane damage, several physiological and biochemical changes, and also for muscular damage leading to a consequent drop in muscular functionality, enzyme release to plasma, histological changes, and muscular soreness (Aguilo, Tauler, Sureda, Cases, Tur, & Pons, 2007). Reactive oxygen species act as a signal to induce adaptive responses, including the antioxidant defense system for homeostasis and the prevention of oxidative damage (Pattwell & Jackson, 2004). To maintain the antioxidant defense system and to prevent oxidative damage appropriate nutrition is essential. In particular, correct nutrition is critically important for improvement of athletic performance, conditioning, recovery from fatigue after exercise, avoidance of injury, and prevention of oxidative damage (Aoi, Naito, & Yoshikawa, 2006). Having a balanced diet is also important for the maintenance of an athletes' antioxidant defense system. The micronutrients important to maintain the enzymatic and non-enzymatic antioxidants include: vitamin A, vitamin C, vitamin E, selenium, copper, zinc, iron, and manganese.

Statement of the Problem

The purpose of this study was to investigate a NCAA Division III University men's cross country team's micronutrient needs to prevent and minimize oxidative damage. During the track

season in the spring of 2008, a nutrition assessment was conducted on each athlete at pre-season and at peak performance. The nutrition assessment consisted of a 3-day dietary food log and measurements of height, weight, RMR, body fat percent, and VO₂ max. Each athlete was also asked several questions relating to factors affecting oxidative damage.

Research Questions

1. Are collegiate male cross country runners at increased risk for oxidative damage based on micronutrient needs to minimize and prevent oxidative damage?
2. Do male cross country athletes consume the recommended amounts of daily Vitamin A (carotenoids and retinol), Vitamin C, and Vitamin E before training?
3. Do male cross country athletes consume the recommended amounts of daily Vitamin A (carotenoids and retinol), Vitamin C, and Vitamin E at peak performance?
4. Do male cross country athletes consume the recommended amounts of daily selenium, copper, iron, zinc, and manganese before training?
5. Do male cross country athletes consume the recommended amounts of daily selenium, copper, iron, zinc, and manganese at peak performance?
6. Do male cross country athletes consume recommended levels of micronutrients for antioxidant defense as training levels increase?

Definition of Terms

For conveying the operational definition used by the researcher the subsequent terms are defined as follows:

Antioxidants. The important vitamins and minerals that are used by enzymes and non-enzymes for the defense against oxidative damage.

Antioxidant defense systems. Enzymes and non-enzymes used by the body to defend against oxidative stress.

Oxidative damage. Actual damage inflicted by the reactive oxygen species in the body at the cellular level.

Oxidative stress. Disturbance in the equilibrium of the prooxidant/antioxidant system where the prooxidants outbalance the antioxidants.

Reactive oxygen species (ROS). Any atom that contains one or more orbital electrons with unpaired spin states. These oxygen species are also referred to as free radicals.

VO₂ max. The maximum volume of oxygen consumed in a given time period.

Assumptions and Limitations

In reviewing this research it is important to consider the underlying assumptions and limitations. First, it was assumed that the 3-day dietary food log was an accurate account of the nutrient intakes by the athletes' and that the athletes were not influenced by perceptions of what they should eat or inaccurate by estimated portion sizes consumed. Second, it was assumed that the nutrient consumption by the athletes' was reflective of the nutrient amounts in the body. It was also assumed that the athletes VO₂ max was reflective of their actual cardiovascular capacity for oxygen transportation and that a higher VO₂ max was reflective of higher concentrations of free radicals in the body. Limitations to the study included the accuracy of the 3-day dietary food logs used to evaluate nutrient intakes because of day to day and seasonal changes in participants' diets. Also, due to the fact that the sample was recruited from one University, this study may not apply to other male cross country runners in other locations. Finally, there are variables that this study did not cover, such as intensity of training or unknown factors that could alter the results and conclusions.

Chapter II: Literature Review

This chapter examines endurance runners and the development of oxidative damage through aerobic metabolism and training. This chapter further explores the antioxidant defense system, enzymatic and non-enzymatic, with a focus on vitamins and minerals important for the maintenance of the antioxidant defense system and running performance. The micronutrients examined in depth in this chapter include: vitamin A, vitamin C, vitamin E, selenium, copper, iron, zinc, and manganese.

The three aerobic pathways supporting energy production are pyruvate metabolism from glycolysis, beta oxidation of fatty acids, and oxidation of amino acids (Wildman & Miller, 2004). Endurance running is an aerobic sport where the body relies on oxygen to continue the production of energy to maintain the physical demands of repeated and rhythmic contractions placed on the lower body (Scott, Littlefield, Chason, Bunker, & Asselin, 2006). Numerous studies have demonstrated that long and strenuous aerobic exercise perturbs the physiological balance between oxidative products and the antioxidant system increasing the likelihood of exercise-induced oxidative stress (Machefer et al., 2007).

Pyruvate Metabolism

Pyruvate sits at the crossroads of several metabolic pathways. Pyruvate, depending on the cell type and metabolic state, can be used for fuel in mitochondria via conversion to acetyl coenzyme A (acetyl CoA), converted to lactate for exportation from a cell as in red blood cells or skeletal muscle, used to make glucose in the liver, or to make the amino acid alanine in skeletal muscle (Wildman & Miller, 2004). Acetyl CoA, derived from pyruvic acid, enters the citric acid cycle in the mitochondria of the cell where oxygen molecules are utilized in the cycle reactions. Water enters the citric acid cycle and leaves the citric acid cycle as a waste product (Sherwood,

2004). In red blood cells the only possible fate of pyruvate is conversion to lactic acid by lactate dehydrogenase. In muscle cells the degree of lactic acid formation depends on the availability of oxygen and mitochondrial content (Wildman & Miller, 2004).

Amino Acid Oxidation

Three-quarters of the free amino acid pool in the body is found within skeletal muscle tissue. Because skeletal muscle is the predominant tissue in the body and thus has a large free amino acid pool, skeletal muscle is fundamentally involved in the coordinated exchange and metabolism of amino acids. The breakdown of certain amino acids in skeletal muscle during exercise might serve to maintain levels of Krebs cycle intermediates for energy production within those cells (Wildman & Miller, 2004). Amino acids fall into three categories: glucogenic, ketogenic, or glucogenic and ketogenic. Glucogenic amino acids are those that give rise to a net production of pyruvate or citric acid cycle intermediates, which are precursors to glucose via gluconeogenesis. Lysine and leucine are the only amino acids that are solely ketogenic which can not bring about net glucose production. A small group of amino acids comprised of isoleucine, phenylalanine, threonine, tryptophan, and tyrosine give rise to both glucose and fatty acid precursors and are characterized as glucogenic and ketogenic (King, 2008b). Beyond the categories of amino acids the last fate of amino acids occurs during starvation or prolonged exercise. When glycogen stores are depleted, in muscle during exertion, catabolism of muscle proteins to amino acids contributes the major source of carbon for maintenance of blood glucose levels (King, 2008a). During physiological stress, skeletal muscle releases amino acids into circulation. Glutamine and alanine account for 50-80% of the amino acids released during prolonged exercise or stress (Wildman & Miller, 2004).

Beta Oxidation of Fatty Acids

Fatty acids are used for fuel by a number of cell types. In order for the cells to utilize fatty acids they must have mitochondria and an adequate oxygen supply. Fatty acids are primarily oxidized in a metabolic pathway referred to as beta-oxidation. Each round of beta-oxidation produces one mole of NADH, one mole of FADH₂, and one mole of acetyl CoA. The acetyl CoA, the end product of each round of beta-oxidation, may then enter the Krebs cycle, and the reduced electron is transferred to the electron transport chain. The acetyl-CoA is oxidized to CO₂ with the concomitant generation of three moles of NADH, one mole of FADH₂, and one mole of ATP (King, 2008c). The key enzymes involved in beta-oxidation are acyl-CoA dehydrogenase, 3-hydroxyacylacyl-CoA dehydrogenase, and thiolase (Wildman & Miller, 2004).

During exercise more and more fatty acids will be used as fuel, especially at lower to moderate intensities and in Type I and IIa muscle fibers. Type I muscle fibers, slow twitch muscle, have more mitochondria and myoglobin and are associated with more capillaries than fast twitch muscle fibers (Type II) (Slomianka, 2006). Type IIa, fast-oxidative-glycolytic, are called intermediate fibers since they have increased mitochondria and demonstrate some aerobic and fatigue resistant characteristics like Type I (Slomianka, 2006). Increased mitochondria in Type I and Type IIa muscle increases the use of fatty acids in beta-oxidation. Therefore, more fatty acids can enter mitochondria in muscle cells for the production of energy to sustain the activity level of the athlete (Wildman & Miller, 2004).

VO₂ Maximum

Oxygen's presence largely determines one's capacity for ATP production and the ability for sustaining high-intensity, endurance exercise (McArdle, Katch, & Katch, 2005). Increased exercise work rates require larger volumes of oxygen (VO₂) in order to aerobically produce ATP

in muscle fibers. VO_2 increases linearly with increases in work rate to the maximal level (VO_2 max). VO_2 max values are influenced by a combination of factors such as heredity, fitness level, sex, physical size, and age (Wildman & Miller, 2004). This maximum work rate is the result of a complex interaction between heart and skeletal muscle factors that combine to establish the measured maximum rate of oxygen use by the muscles at that peak work rate (VO_2 max). The measured peak rate of oxygen consumption is the result of the peak work rate achieved (Noakes, 2003). VO_2 max varies in individuals and even between athletes in the same sport. The general VO_2 max range for cross country runners is 60 to 85 ml/ kg/ min (Wilmore & Costill, 2005). The extent by which VO_2 max can change with training depends on the starting point of the athletes' training. The fitter the individual the less potential there is for an increase in VO_2 max. Once the upper limit is reached by the athlete further improvements in performance are still seen with training. This means that the athlete is able to perform at a higher percentage of their VO_2 max for longer periods of time (Costill, 1986). Over time greater volumes of training or strenuous exercise may have detrimental effects, like decreased performance, due to increased oxygen consumption (Baechle & Earle, 2000).

Vmax Encore. The Vmax Encore instrument (VIASYS Respiratory Care Inc., Yorba Linda, CA) is used to measure the VO_2 maximum. To understand more about the Vmax Encore a phone interview was conducted on March 18, 2009 with Mr. David Hensley, a respiratory therapist from Viasys. He stated the measurement of VO_2 maximum is broken down into two systems, flow and gases (oxygen, carbon dioxide, carbon monoxide, methane, acetylene). Mr. Hensley stated the main parts of the Vmax Encore include: the mass flow sensor, quadraplatinum wired anemometer, oxygen analyzer, and an infrared analyzer. The flow of gases is measured with a mass flow sensor. This sensor is used to find out the mass of air as it changes in density

through expansion/contraction with temperature and pressure as the air mass enters the machine. The mass flow sensor also measures the minutes of ventilation during the test. The quadraplatinum wired anemometer measures the velocity of the air (D. Hensley, personal interview, March 18, 2009). The wire temperature is maintained at 700 degrees by electrical currents. The wire's electrical resistance increases as the wire's temperature increases, which limits electrical current flowing through the circuit. As air flows past the wire, the wire cools, which in turn allows more current to flow through the circuit. As more current flows, the wire's temperature increases until the temperature reaches equilibrium again (D. Hensley, personal interview, March 18, 2009). The amount of current required to maintain the wire's temperature is directly proportional to the mass of air flowing past the wire. For the Vmax Encore, the test rate is 125 volts per second to maintain the temperature of the wire. The gases are measured by two sensors, the oxygen analyzer and infrared analyzer (D. Hensley, personal interview, March 18, 2009). The oxygen analyzer is only sensitive to oxygen. The gas runs through the oxygen analyzer and an average of inspiration/expiration is measured. The infrared analyzer measures the carbon based gases (carbon dioxide, carbon monoxide, methane, acetylene). Mr. David Hensley stated that the carbon based gases are measured as an average of inspiration/expiration and any further detail is proprietary information.

In this maximal test the participant ventilates through a hose attached to the metabolic cart to determine the volume of oxygen used (oxygen inspired minus oxygen expired = arterial-venous difference). The heart rate and blood pressure of the heart is also measured to determine the cardiac output (Q). This indicates how much oxygenated blood is being delivered per minute to working muscle. To get the volume of oxygen this equation is used: $VO_2 = (\text{heart rate}) (\text{stroke volume})(\text{arterial-venous difference})$ (Wildman & Miller, 2004). VO_2 max is calculated when the

VO₂ and heart rate finally plateau with elevated work rates. At this point the delivery of oxygen could not meet the demands of the working muscle (Wildman & Miller, 2004).

Cellular respiration is the use of oxygen and the production of carbon dioxide in the production of energy. The respiratory exchange ratio (RER) is generated by the volume of expired gas (carbon dioxide) divided by the volume of inspired gas (oxygen). This means the amount of oxygen needed to oxidize an energy molecule is proportional to the amount of carbon dioxide produced (Wildman & Miller, 2004). The RER helps depict what kind of energy source is being utilized. When the RER is closer to 1.0, more carbohydrate is being used for energy. If the RER is closer to 0.7 more fatty acids are used for energy. It takes more oxygen to oxidize a fatty acid for energy production (Wildman & Miller, 2004). The respiratory exchange quotient (RQ) is the measurement for changing oxygen and carbon dioxide levels in a specific cell or isolated tissue of interest, and is sometimes interchanged with RER (Wildman & Miller, 2004).

Oxidative Stress

During intense exercise skeletal muscle is exposed to levels of mechanical and metabolic insult that would seriously injure or kill most other cells. No other tissue of the body undergoes such drastic incremental changes in oxygen (O₂) metabolism during physical activity. The O₂ flux through the mitochondria can increase 100 times, when going from rest to maximum exercise in highly trained oxidative muscle fibers (Clanton, Zuo, & Klawitter, 1999). This increase in oxygen consumption is associated with a rise in the production of reactive oxygen species (ROS) (Leeuwenburgh & Heinecke, 2001). Only 2% to 5% of oxygen, in the mitochondria, is not completely reduced to water and forms reactive oxygen species (Sjodin, Westing, & Apple, 1990). During high intensity exercise, ROS generation can be generated by an influx of neutrophils and macrophages into the muscle and an activation of cytokines

secondary to muscle damage. Further exercise stimulates a redistribution of blood flow which provokes hypoxia and re-oxygenation in some tissues. This may increase the production of superoxide by xanthine oxidase in muscle. When skeletal muscle is subjected to excessive contractile activity, prostanoids and their free radical intermediates are also released. There may also be disruptions to the homeostasis of calcium or damage to iron-containing proteins within the muscle that may activate reactive oxygen species production (Sacheck & Blumberg, 2001). ROS, such as hydrogen peroxide and hypochlorous acid, are termed free radicals or oxygen intermediates. These molecules are capable of independent existence and contain an unpaired electron in the outer orbital, making them extremely unstable (Williams, Strobel, Lexis, & Coombes, 2006). The excessive production of reactive oxygen species (ROS) during exhaustive exercise induces oxidative stress (Vina et al., 2000). ROS, related to oxidative stress, are an underlying aetiology in exercise-induced disturbance in muscle homeostasis which are associated with muscle fatigue and may also contribute to the late phase of exercise-induced injury (Jackson, 1998). To counteract ROS the body utilizes ROS as a signal to induce adaptive responses, including the maintenance of oxidative homeostasis and the prevention of oxidative damage (Pattwell & Jackson, 2004). However, when the antioxidant defense system is not maintained or exercise produces an over abundance of ROS an imbalance occurs in the oxidant/antioxidant systems leading into oxidative stress (Urso & Clarkson, 2003).

Antioxidant Defense System

Reactive oxygen species occur in tissues and can damage DNA, proteins, carbohydrates, and lipids. These potentially deleterious reactions are controlled by a system of antioxidants which eliminate pro-oxidants and scavenge free radicals (Mascio, Murphy, & Sies, 1991). Antioxidants are chemical compounds that can bind to free radicals and thus prevent them from

damaging healthy cells (Janicki-Deverts, 2000). There are two major classes of antioxidants that work together in cells to reduce the harmful effects of radicals: 1) enzymatic and 2) non-enzymatic antioxidants (Powers, 2002). Several antioxidant enzymes are produced by the body, with the three major classes being catalase, the glutathione (GSH) peroxidases, and the superoxide dismutases (SODs). Non-enzymatic antioxidants include the innate compound glutathione as well as antioxidant vitamins obtained through the diet, such as alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), and beta-carotene (vitamin A) (Janicki-Deverts, 2000). Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environments to detoxify ROS; these antioxidants work as a complex team to remove various types of ROS. To maximize intracellular protection, these scavengers are located throughout the cell and provide protection against ROS toxicity using different approaches (Powers & Lennon, 1999).

Enzymatic Antioxidants

Superoxide dismutase (SOD) dismutates superoxide radicals to form hydrogen peroxide (H_2O_2) and oxygen (O_2). Two isozymes of SOD exist in mammalian skeletal muscle. The Cu-ZnSOD isoform is located primarily in the cytosol, whereas the MnSOD isoform is found in the mitochondria (Ji, 1995). GSH peroxidase (GPX) is an enzyme responsible for reducing hydrogen peroxide (H_2O_2) or organic hydroperoxides to water and alcohol respectively. This enzyme uses GSH as the electron donor and requires selenium as a cofactor. GPX is located in both the cytosol and the mitochondria (Halliwell & Gutteridge, 1989). Catalase (CAT), an antioxidant enzyme, catalyses the breakdown of H_2O_2 to form water and O_2 . To maintain catalytic activity, CAT requires Fe^{3+} as a cofactor. Although CAT is widely distributed in the

cell, high concentrations are found in both peroxisomes and mitochondria (Halliwell & Gutteridge, 1989).

Non-Enzymatic Antioxidants

Vitamin E is an important antioxidant in cell membranes and other lipid components of the cell. Alpha-tocopherol is the most investigated vitamin E isomer and contains the most potent antioxidant activity in biological systems (Packer, 1991). Vitamin E is particularly important because of its capacity to convert superoxide, hydroxyl, and lipid peroxy radicals to less reactive forms. It can also break down lipid peroxidation chain reactions which occur during ROS-mediated damage to cell membranes (Janero, 1991). Vitamin C antioxidant roles are numerous. This antioxidant can directly scavenge superoxide, hydroxyl, and lipid hydroperoxide radicals. Furthermore, this antioxidant plays a key role in recycling vitamin E (Packer, Slater, & Willson, 1979). Vitamin A, beta-carotene, is a lipid soluble antioxidant located in cellular membranes. The structural arrangement, long chains of conjugated double bonds, permits the scavenging of several ROS forms (Yu, 1994).

Vitamin A

Vitamin A plays a major role in many physiological functions. The major functions include: vision, a hormone affecting gene expression, cell growth in functional maintenance and mechanisms, glycoprotein synthesis, and repairing damaged tissue from free radicals (Helwig, 2007). The current Recommended Dietary Allowance for vitamin A is 3000 IU for males and 2,333 IU for females (Drake, 2007c). Some common foods containing high amounts of Vitamin A include: cod liver oil, sweet potato, canned pumpkin, raw carrot, milk, butter, cantaloupe, spinach, kale, collards, and butternut squash (Drake, 2007c). Provitamin A carotenoids represent a group of compounds that are precursors of vitamin A. Structurally, carotenoids have an

expanded carbon chain containing conjugated double bonds usually, but not always, with an unsubstituted beta-ionone ring at one or both ends of the chain. There are actually more than 650 natural carotenoids; many of these carotenoids such as lutein, zeaxanthin, and lycopene, may have more potent antioxidant effects, although more research is necessary in regards to their antioxidant properties. Of all the carotenoids, beta-carotene is the most researched and studied (Gropper, Smith, & Groff, 2005). The precursor beta-carotene typically has the antioxidant properties to expunge the singlet oxygen species (Groff, Gropper, & Hunt, 1995). Limited studies have been performed addressing the possible role of beta carotene in the prevention of muscle damage. Unfortunately the studies used vitamin A as part of an antioxidant cocktail mixture (Helwig, 2007). In one study by Kanter, Nolte, and Holloszy (1993), supplementation of the antioxidant vitamins C, E, and A, simultaneously decreased the absolute levels of lipid peroxide markers produced during exercise. Kanter et al. stated, "It is hard to say if the beneficial effect is due to an individual vitamin or a cumulative effect of the vitamin mixture" (1993, p. 968). In another study by Aguilo et al. (2007), 15 male endurance athletes were assigned to either a placebo group or an antioxidant supplement group. The antioxidant cocktail supplement consisted of 500 mg vitamin E, 30 mg beta-carotene, and the last 15 days with 1 g of vitamin C. The participants consumed the supplement or placebo for 90 days while continuing to train and compete. This study showed the high antioxidant intake in the supplemented group increased the antioxidant concentrations and helped to neutralize the higher production of ROS induced by exhaustive exercise. Within three months of training and supplementation, maximal blood lactate concentrations were lower in the supplement group versus the placebo group (Aguilo et al., 2007). Even with the positive results, again, the benefits of the supplementation could not be placed with one particular vitamin.

Vitamin C

Vitamin C, also known as ascorbic acid, has long been popular and more people can name good food sources of this vitamin than any other. This vitamin also has a long history as a supplement for athletes (Wildman & Miller, 2004). The Recommended Dietary Allowances for vitamin C are 75 mg for females and 90 mg for males (Frei, 2008). Some common foods containing high amounts of Vitamin C include: orange juice, grapefruit juice, orange, grapefruit, strawberries, tomato, sweet red pepper, broccoli, and potato (Frei, 2008). Vitamin C works with key enzymes in the production of collagen, which provides structure to connective tissue. This makes vitamin C extremely important to athletes to minimize sprains, strains, and fractures. It also participates in the formation of catecholamines (norepinephrine and epinephrine), carnitine, maybe testosterone, and as an antioxidant. All of these functions make vitamin C desirable to athletes for improving performance (Wildman & Miller, 2004). Nieman, Butler, Pollett, Dietrich, and Lutz (1989), reported that the average daily intake of vitamin C for marathoners was significantly higher than the recommended dietary allowances. The male marathoners consumed an average of 147 mg and female marathoners consumed 115 mg of vitamin C daily. Vitamin C is an essential water soluble vitamin, and it is considered the most important antioxidant in the watery fluids inside and outside the body cells (Bendich, Machlin, Scandurra, Burton, & Wayner, 1986). The main antioxidant properties include the regeneration of reduced vitamin E, stabilization of hydroxyl radicals, and quenching singlet oxygen (Frei, England, & Ames, 1989). As an antioxidant, ascorbic acid may react in blood or intracellularly with a variety of reactive oxygen species and give the radicals an electron in the form of hydrogen. Thus, ascorbic acid acts as a reducing agent to reverse the oxidation reaction and regenerate vitamin E (Gropper, Smith, & Groff, 2005). In the study by Frei, England, and Ames (1989),

ascorbate trapped virtually all peroxy radicals before escaping into the plasma lipids. When ascorbate was completely consumed the peroxy radicals initiated lipid peroxidation. Adding more ascorbate to the plasma, after initiation, brought the lipid peroxidation to a complete standstill. Another study by Kramarenko, Hummel, Martin, and Buettner (2006) looked at the reaction of singlet oxygen and ascorbate. This study used Photofrin, a photosensitizer used in the treatment of cancer. The exposure to light produces singlet oxygen that initiates oxidations leading to cell death. When ascorbate was added to the solution with Photofrin and exposed to light, ascorbate quenched the singlet oxygen. The very fast reaction of ascorbate with singlet oxygen and its high concentration in the water space of cells suggests that ascorbate could be an important sink for singlet oxygen *in vivo* (Kramarenko, Hummel, Martin, & Buettner, 2006).

Vitamin E

Vitamin E, also known as alpha-tocopherol, is well known as an antioxidant that helps protect cell membranes, particularly unsaturated fatty acid components of phospholipids, against oxidation by free radicals. This includes not only the plasma membrane but also organelle membranes. Vitamin E is a popular nutritional supplement, and more than 50% of athletes use a multivitamin and mineral supplement. Athletes who restrict their dietary intake or those who consume a very low fat diet may consume less vitamin E than recommended (Wildman & Miller, 2004). The Recommended Dietary Allowance for vitamin E is 15 mg (22.5 IU) for both males and females (Traber, 2008). Some common foods containing high amounts of Vitamin E include: olive oil, soybean oil, corn oil, canola oil, safflower oil, sunflower oil, almonds, hazelnuts, peanuts, spinach, carrots, and avocados (Traber, 2008). The main antioxidant functions of vitamin E include the quenching of singlet oxygen, stabilization of superoxide anions, and stabilization of hydroxyl radicals (Kanter, 1998). The structure of vitamin E,

specifically its phenolic ring, enables hydrogen ions to be donated to free radicals. The hydrogen ions from alpha-tocopherol effectively and quickly react with and terminate a variety of free radicals before free radicals can destroy cell membranes and other cell components (Gropper, Smith, & Groff, 2005). A study by Zoppi and colleagues looked at the supplemental effects of ascorbic acid and alpha-tocopherol in elite soccer players. The study demonstrated that vitamins C and E may reduce lipid peroxidation and muscle damage during high intensity efforts in soccer players (Zoppi et al., 2006). Since this study used a cocktail mixture of antioxidants the benefits of the supplementation can not be placed with one particular vitamin. Another study by Fischer and colleagues demonstrated the effects of antioxidant supplementation on the production of interleukin-6, a pro-inflammatory and anti-inflammatory cytokine (Fischer et al., 2004). Interleukin-6 is produced from muscle in response to muscle contraction or exercise (Sherwood, 2004). The principal finding of the study demonstrated the supplementation of an antioxidant cocktail, vitamin C and E, inhibited the release of interleukin-6 from contracting skeletal muscle. The antioxidants thus caused a significant decrease of interleukin-6 in circulation and blunted the increase of interleukin-1ra and cortisol, which are also markers of stress in the body (Fischer et al., 2004). Both of these studies, like others, support the benefits of vitamin E in quenching radicals and singlet oxygen to decrease oxidative stress in the body.

Selenium

Selenium is an essential trace element required for a number of selenium dependent enzymes called selenoproteins. Of these selenoproteins, glutathione peroxidases are involved in antioxidant processes (Whanger, 2007). One of the most clearly established functions of selenium is as an essential cofactor for the enzyme glutathione peroxidase (Gropper, Smith, & Groff, 2005). Five selenium containing glutathione peroxidases (GPx) have been identified:

cellular or classical GPx, plasma or extracellular GPx, phospholipid hydroperoxide GPx, gastrointestinal GPx, and olfactory GPx (Whanger, 2007). These enzymes are established throughout the body to increase the efficiency of the antioxidant defense system. Glutathione peroxidases are the first line of defense against free radicals and are also the key in coordinating water and lipid soluble antioxidant defense systems (Balakrishnan & Anuradha, 1998). Reduced glutathione is used by the peroxidases to stop the peroxidation of cells by free radicals such as hydrogen peroxide, and lipid peroxide (Groff, Gropper, & Hunt, 1995). In order for glutathione to have effective antioxidant properties adequate levels of selenium are required. Without selenium GPx relinquishes the ability to degrade hydrogen peroxide and other free radicals (Powers & Ji, 1999). The Recommended Dietary Allowance for selenium is 55 mcg/day for males and females (Whanger, 2007). Some common foods containing high amounts of selenium include: Brazil nuts, shrimp, crab meat, salmon, halibut, enriched noodles, brown rice, light chicken meat, pork, beef, and whole wheat bread. In a study by Laughlin et al. (1990), male rats, exercised, trained, and sedentary, were used to test whether exercise training increases skeletal muscle antioxidant enzymes. This study demonstrated that GPx increases with exercise and is related to skeletal muscle oxidative capacity (Laughlin et al., 1990). Another study supporting this theory showed an increase in GPx in the gastrocnemius muscle in rats with an increase in exercise duration (Powers et al., 1994). Both of these studies showed increases of GPx with increased exercise and oxidative capacity. However, these studies may not be generalizable to the human population. In one study that used human subjects, previously sedentary, in a 40 week training session to run a half marathon, glutathione increased as the training increased. Glutathione levels returned back to normal 5 days after the training was over (Evelo, Palmen, Artur, & Janssen, 1992). All of these studies showed the quick increase and reaction of GPx to

oxidation of muscle contraction due to exercise duration. Without selenium GPx antioxidant functions decrease, and the other antioxidant enzymes would have to adapt to minimize oxidative damage (Ji, Stratman, & Lardy, 1988).

Zinc and Copper

Zinc is the most ubiquitous mineral in the body and is involved in nearly every human operation. Skeletal muscle and bone contain roughly 60% and 30% of all the zinc (Wildman & Miller, 2004). The Recommended Dietary Allowance for zinc in adults is 11 mg/d for males and 8 mg/d for females (Ho, 2008). Some common foods that contain high amounts of zinc include: oysters, Dungeness crab, beef, pork, dark chicken meat, dark turkey meat, baked beans, fruit yogurt, cashews, almonds, and garbanzo beans (Ho, 2008). Zinc plays important roles in growth, development, immune response, neurological function, and reproduction. On the cellular level, zinc plays an important role in the structure of proteins like copper-zinc superoxide dismutase (Ho, 2008). Zinc functions through the catalysis of various enzymes, the maintenance of the structural integrity of proteins, and the regulation of gene expression (Panel on Micronutrients, 2000).

Copper is an essential trace element for humans and animals due to its ability to easily accept and donate electrons. Copper (Cu) shifts between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) forms but is mainly found in the body as the cupric form (Drake, 2007b). The Recommended Dietary Allowance (RDA) for copper in adult males and females is 900 mcg/d. Some common foods that contain copper include: beef liver, oysters, clams, crab meat, cashews, sunflower seeds, hazelnuts, almonds, lentils, and raw mushrooms (Drake, 2007b). Copper has multiple roles in the body including energy production, connective tissue formation, neurotransmitter synthesis, antioxidant functions, gene expression, and other many roles. One of copper's many

roles is as an antioxidant in superoxide dismutase 1 (Drake, 2007b). Copper functions to catalyze the activity of many copper metalloenzymes that act as oxidases to achieve the reduction of molecular oxygen (Panel on Micronutrients, 2000).

Both copper and zinc function in the enzyme superoxide dismutase 1 (SOD1). The SOD1 enzyme is typically found in the cytoplasm to convert superoxide into hydrogen peroxide and molecular oxygen (Juarez et al., 2008). The copper/zinc superoxide dismutase (Cu/Zn SOD) uses two copper atoms to convert the superoxide anion ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2). Zinc atoms play a structural role in the formation of the enzyme. SOD1 plays an important role in providing a defense against oxidative damage from superoxide radicals that, if uncontrolled, lead to other damaging reactive oxygen species (Panel on Micronutrients, 2000). In a study by Koury et al. (2004), the comparison of zinc and copper biochemical indices of antioxidant status and their relationship in elite athletes was performed on triathletes, long distance runners, short distance runners, and swimmers. The body composition and 24 hour recall was evaluated on all participants. Venous blood samples were drawn 16-20 hours after competition to assess erythrocyte zinc, superoxide dismutase, and metallothionein. In all of the athletes, zinc and copper intakes were not different and did not affect the biochemical indices. This study showed an increase of the biochemical indices for increased antioxidant protection in long distance/aerobic modalities in comparison to the short distance/anaerobic modalities. In addition, the study demonstrated correlations between erythrocyte zinc, superoxide dismutase, and metallothionein consistent with the importance of an adequate zinc status in the response to increased antioxidant mechanisms with increased exercise (Koury et al., 2004).

Manganese

The essential nutrient manganese is involved in the formation of bone and in amino acid, cholesterol, and carbohydrate metabolism. The adequate intakes for manganese in adult males and females are 2.3 and 1.8 mg/d, respectively. Some of the common foods that have high amounts of manganese include: raw pineapple, pineapple juice, pecans, almonds, instant oatmeal, raisin bran, brown rice, and green tea (Drake, 2007a). The metalloenzymes related to manganese include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase (Panel on Micronutrients, 2000). Manganese superoxide dismutase is the principal antioxidant in the mitochondria. The mitochondria are especially vulnerable to oxidative stress since they consume over 90% of the oxygen utilized by cells. Manganese superoxide dismutase catalyzes superoxide radicals to hydrogen peroxide, which is reduced to water by other enzymes (Drake, 2007a). In a study by Davison, Hughes, and Bell (2005), manganese was supplemented in a vitamin and mineral cocktail to see if there were any effects on DNA damage after exercise. Fourteen healthy male subjects were randomly placed into a placebo group or treatment group. The treatment group received an antioxidant cocktail comprised of 400 mg alpha-lipoic acid, 200 mg co-enzyme Q10, 12 mg manganese, 600 mg ascorbic acid, 800 mg N-acetyl cysteine, 400 micrograms selenium, and 400 IU alpha-tocopherol. The main findings of this study demonstrated exhaustive exercise induces DNA damage, while antioxidant supplementation did not protect against damage. The results of this study may not be accurate due to inappropriate amounts of antioxidant supplementation as other cocktail supplementation studies have had success (Davison, Hughes, & Bell, 2005).

Iron

Iron is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component in proteins and enzymes (Higdon, 2006). Over 65% of body iron is found in hemoglobin, up to about 10% is found as myoglobin, 1 to 5% is found as part of enzymes, and the remaining iron is found in blood or stored (Gropper, Smith, & Groff, 2005). The Recommended Dietary Allowance (RDA) for iron in adult males and females below the age of 51 is 8 and 18 mg/d, respectively. Some of the common foods high in iron include: beef, dark chicken meat, oysters, shrimp, light tuna, black-strap molasses, raisin bran, raisins, prune juice, dried prunes, kidney beans, lentils, and tofu (Higdon, 2006). Iron serves in the body as part of several proteins and as a cofactor for multiple enzymes. Iron is part of these main protein and enzyme functional groups: hemoglobin, myoglobin, cytochromes, enzymes involved in electron transport, monooxygenases, dioxygenases, peroxidases, oxidoreductases, and other iron-containing proteins. The catalase enzyme, part of the peroxidases, which requires iron for activity converts hydrogen peroxide to water and molecular oxygen. Catalase helps prevent cellular damage from hydrogen peroxide and is thus considered an important antioxidant (Gropper, Smith, & Groff, 2005). In a study by Peeling et al. (2007), an investigation was completed on sixteen iron depleted female athletes to examine the effects of intramuscular iron injections on aerobic performance. These women completed VO₂ max tests and were assigned to a supplement or placebo group. To check iron status blood draws were taken prior to the injections, 20 days after the injections, and 28 days after the injections. All of the participants had to keep dietary logs for 4 days to assess iron and vitamin C intakes. This study demonstrated that intramuscular iron injections significantly increased serum ferritin levels from baseline data. The supplement group was also noted to be working at a greater intensity level in comparison to

the placebo group maintaining at the same work intensity. No significant effects of iron supplementation could be identified in the VO_2 max test or between groups. It was noted that effects on workload might not be seen in iron deficient women because these measures act to prevent anemia (Peeling et al., 2007). In another study by Aguilo et al (2004), a antioxidant cocktail (vitamin E, C, and beta-carotene) were supplemented to eighteen amateur male athletes to see the effects on basal iron status in a three month training/competition period. The participants were randomly placed in a placebo or supplement group. Hematological parameters, dietary intake, physical activity intensity, antioxidant status, and basal iron status were determined before and after intervention trials. This study demonstrated that prolonged intense exercise decreased antioxidant defenses in the placebo group but not in the antioxidant-supplement group. The placebo group also showed a high oxidative stress index, and decreases in serum iron and iron saturation index (Aguilo et al., 2004). Endurance physical activity increases the development of oxidative stress in athletes and may induce sports anemia with low iron plasma levels and storage. The acute phase immune response, induced by long duration/intense exercise, includes the redistribution of extracellular iron to intracellular compartments to combat reactive oxygen species. In the presence of reactive oxygen intermediates, iron catalyzes the generation of hydroxyl radicals (Aguilo et al., 2004).

Three Day Dietary Food Log

To assess the above consumption of vitamins and minerals a three day dietary food log was used for the present study. The dietary recall and food log techniques attempt to uncover all foods and beverages consumed during a specified time frame. Dietary recalls can suffer from recall bias or patient short term memory. Food logs risk biasing patient intake because the

patient has to record it (Miller, 2005). Evidence suggests that all self reporting dietary measures may share certain person-specific biases or errors (i.e. portion sizes) (Ke et al., 2005).

Chapter III: Methodology

The purpose of this study was to investigate a Division III men's cross country team's micronutrient needs for the prevention of oxidative damage. This chapter includes a description of how the subjects were selected, a description of the sample, and a description of the instruments used for data collection. The method for collecting data and how the data was analyzed is also discussed. The last part of this chapter addresses the limitations in the methodology.

Subject Selection and Description

Subjects for this study were selected from a National Collegiate Athletic Association (NCAA) Division III University men's cross country team. Two team meetings were held in the fall of 2007 to explain the study and ask for the athletes consent to participate in the study. All of the athletes on the roster of the men's cross country team were asked to participate. For the athletes who were unable to attend either meeting, individual sessions were set up between the researcher and athlete to go over the study and to ask the athlete to participate. Participation in this study was voluntary. Athletes who chose to participate in the study signed consent forms (refer to Appendix A) and VO₂ Max waivers (refer to Appendix B). Fifteen of the cross country runners on the official men's cross country roster consented to participate and ranged in age from 18 to 22.

Instrumentation

The researcher developed a nutrition assessment form (refer to Appendix C) that included the areas being addressed in this study. The nutrition assessment was directed towards identifying general information for the identification of micronutrient needs. The general information included on the form required the following information: date, gender, age,

kilocalories, body fat percentage, height, weight, resting metabolic rate (RMR), medications including supplements, body mass index (BMI), VO_2 max, training difference between pre and post sessions, and present injuries or illness.

A detailed three day dietary food log (refer to Appendix D) was used by the researcher to assess each subject's diet for average caloric intake, and average micronutrient (retinol/ carotenoids, alpha-tocopherol, ascorbic acid, selenium, copper, iron, zinc, and manganese) intakes. The three day food log required the following information to be filled in: subject's name, date, and days of the week the food log was completed. Each food log contained directions for proper use of the dietary logs, and instructions for accuracy when logging food or fluids consumed.

Height was measured using a Tanita body composition analyzer (model TBF-215) to the nearest 0.5 inch. Weight was measured using a Tanita body composition analyzer (model TBF-215) to the nearest 0.1 pound. The Tanita body composition analyzer (model TBF-215) also calculated body mass index, resting metabolic rate, and body fat percent. VO_2 maximum was measured with the Vmax Encore instrument (VIASYS Respiratory Care Inc., Yorba Linda, CA).

Data Collection Procedures

A nutrition assessment aimed at identifying micronutrient needs of male cross country athletes was performed on each subject before the 2008 track season and at peak performance in the spring of 2008, spanning a 3 month time period. Each subject recorded a three day dietary food log, for each nutrition session, consisting of two weekdays and one weekend day, prior to each nutrition assessment appointment.

For each nutrition assessment appointment, for pre-season and peak performance, a standard procedure was used. The first part of the nutrition assessment was a personal interview

in which several questions were asked and the responses were recorded on the nutrition assessment form. The subject was asked his name and age. Each subject was also asked if he used any medications including supplements, and if so, what specific supplements were used. The subject was then asked to describe his current training, if any during pre-season and how much he increased during the season. Each subject increased his training mileage over the test period by 20 miles by peak performance. Finally the subject was asked to describe any illness or injuries at the present time.

The subject was then asked for his three day dietary food log. The researcher and the subject went over the log together to clarify any questions about the type and amount of food consumed during the three day period. The researcher noted that the subject's name was recorded on the three day dietary food log to be able to match it to the nutrition assessment form in case clarification was needed throughout the duration of the study. Once the baseline data was collected, the researcher began the physical part of the nutrition assessment.

Each subject's height and weight was measured with the Tanita body composition analyzer (model TBF-215). Before the subject was allowed to step on the scale, the scale was sterilized with an alcohol pad. The scale was also sterilized with an alcohol pad after the subject stepped off. One pound of clothing was entered in as a standard weight for each subject since each subject was wearing minimal running attire consisting of shorts and a T-shirt.

The next measurement was the VO_2 maximum which was measured with the Vmax Encore instrument (VIASYS Respiratory Care Inc., Yorba Linda, CA). Before the subjects were tested the researcher took time to explain the process of the test with each athlete. The subject was then required to put on a heart rate monitor and get fitted for the head gear. To be consistent, before each test, the researcher recalibrated the VO_2 Max machine to make sure the

instrument was running properly. At least one half hour before each test, the researcher turned on the machine for a warm-up. Once the machine was calibrated the researcher hooked up the subject to the VO₂ max machine. To keep the test consistent for all athletes the researcher took two minutes of baseline data, five minutes at a warm-up (with the treadmill set at 4 % grade and 7 miles per hour), five minutes at increasing incline (1 % grade increase per minute), then increasing speed till exhaustion (1 mile per hour increase per minute), and then a walking recovery until the subjects heart rate returned to 120 to 130 beats per minute. All of the data collected was coded and kept in a locked area where only the researcher and the researcher's advisor had access. After data analysis, the data was destroyed.

Data Analysis

The Food Processor SQL Edition version 9.9 computer software program was used to analyze the 3-day dietary food logs. The Statistical Program for Social Sciences (SPSS) version 15.0 computer software program was used to analyze the data for micronutrient needs. Descriptive statistics including mean and standard deviation were conducted on the interval and ratio data. Paired t-tests were conducted to compare data from pre-season and peak performance to address any differences in the population for weight, BMI, resting metabolic rate, body fat percent, VO₂ maximum, and intakes of kilocalories, vitamins (A, E, and C), and minerals (iron, manganese, selenium, copper, and zinc). One sample t-tests were conducted to identify if the population consumed enough of vitamins A, E, and C and minerals iron, manganese, selenium, copper, and zinc in comparison to the Recommended Dietary Allowances (RDA) for these vitamins and minerals in pre-season and at peak performance.

Limitations

A major limitation to this study was the small sample size (N= 11). Another limitation was that only one male cross country team was asked to participate in this study. A third limitation was that each athlete filled out their own three day dietary food log, and subjects may have under or over estimated foods consumed. The food logs were assumed to be accurate and a good predictor of the athlete's actual consumption and current micronutrient status. The training increase over the testing time period was assumed to be strenuous enough to induce oxidative stress/damage. There also may be limitations with the equipment used in the nutrition assessment. The Tanita body composition analyzer (model TBF-215) used was assumed to be calibrated and accurately measuring height, weight, BMI, RMR, and body fat%. The VO₂ maximum machine may not accurately represent the true VO₂ max for each subject depending on proper calibration, and/or health of the subject. This study also assumed that measurement of higher VO₂ maximums in the athletes' reflected higher free radical production in the body.

Chapter IV: Results

Introduction

The primary purpose of this study was to investigate a NCAA Division III University men's cross country team's micronutrient needs to prevent and minimize oxidative damage. In the spring of 2008, a nutrition assessment was conducted on each athlete at pre-season and at peak performance. This chapter discusses the outcomes of this study specifically looking at participants' age, height, body weight, BMI, RMR, VO₂ maximum, kilocalories, body fat percent, and intake of vitamins (A, E, and C) and minerals (iron, manganese, selenium, zinc, and copper) at pre-season and peak performance. The intakes of pre-season and peak performance vitamins (A, E, and C) and minerals (iron, manganese, selenium, zinc, and copper) were also compared to the RDAs' for these vitamins and minerals.

Age and Height

Initially 15 cross country runners were assessed at the pre-season assessment. During the season, 3 runners were injured and one runner decided not to participate. There were a total of 11 runners to complete both assessments at pre-season and at peak performance. Of the 11 cross country runners who participated, three were 18 years of age, two were 19 years of age, four were 20 years of age, and the two oldest were 21 and 22 years old, respectively. Of the cross country participants, a majority of the runners were 18, 19, or 20 years old (Figure 1). The height of the male cross country athletes ranged from 69 inches to 74 inches. The frequency of height in inches of the cross country runners is as listed in Figure 2. Five were 69 inches, two were 70 inches and 72 inches, and the two tallest were 73 and 74 inches, respectively.

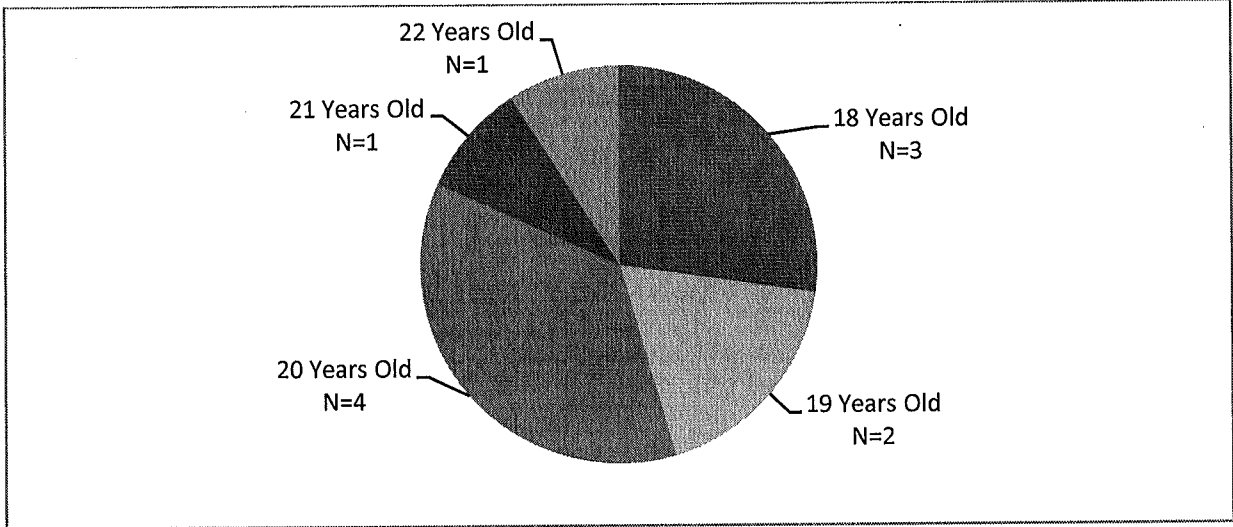


Figure 1. Frequency of age for the male cross country runners who participated in the study (N=11)

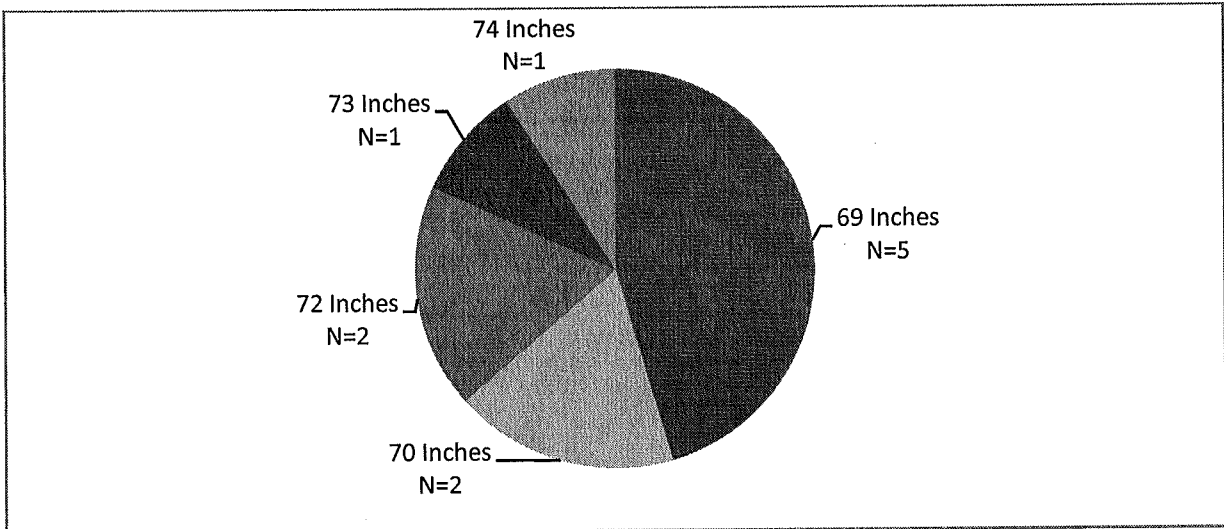


Figure 2. Frequency of height for the male cross country runners who participated in the study (N=11)

Weight, BMI, RMR, VO₂ Maximum, Kilocalories, Body Fat Percent

Pre-season and peak performance body weight, body mass index, resting metabolic rate, VO₂ maximum, kilocalories, and body fat percent of the male cross country athletes were compared and were analyzed with a paired t-test. The mean pre-season weight was 69.09 kg and the mean peak performance weight was 68.93 kg (Table 1). The mean difference between pre-season and peak performance weight was a decrease of 0.16 kg, which was not a significant

difference (Table 2). Similarly, the decrease in mean differences of pre-season and peak performance BMI (0.04 kg/m^2), RMR (3.09), $\text{VO}_{2 \text{ max}}$ (1.4 ml/kg/min), and kilocalories consumed (221.16 kcals) were not significant. Pre-season means were 21.4 kg/m^2 for BMI, 1782.73 for RMR, 71.8 ml/kg/min for $\text{VO}_{2 \text{ max}}$, and 3385.01 kcals for kilocalories consumed, whereas peak performance mean values for BMI (21.4 kg/m^2), RMR (1779.64), $\text{VO}_{2 \text{ max}}$ (70.4 ml/kg/min), and kilocalories consumed (3163.85 kcals) decreased, but these values were not significantly different from the pre-season mean intakes. The decrease in mean difference (0.8%) in body fat percent tended to be significant ($p=0.054$) between pre-season (6.6%) and peak performance (5.8%).

Table 1

Comparison of the Means for Pre-season and Peak Performance Body Weights, Body Mass Index (BMI), Resting Metabolic Rate (RMR), VO₂ Maximum (VO₂ max), Kilocalories (Kcals), and Body Fat Percent of Participants from a NCAA Division III University Men's Cross Country Team

	Mean (N=11)	Standard Deviation
Pre-season Weight (kg)	69.09	7.4725
Peak Performance Weight (kg)	68.93	6.4641
Pre-season BMI (kg/m ²)	21.4	2.301
Peak Performance BMI (kg/m ²)	21.4	2.052
Pre-season RMR	1782.73	108.6650
Peak Performance RMR	1779.64	95.2200
Pre-season VO ₂ max (ml/kg/min)	71.8	4.992
Peak Performance VO ₂ max (ml/kg/min)	70.4	8.045
Pre-season Kcals Consumed	3385.01	981.4974
Peak Performance Kcals Consumed	3163.85	1005.0532
Pre-season Body Fat %	6.6	3.083
Peak Performance Body Fat %	5.8	3.043

Note. kg = kilograms; kg/m² = kilograms per meter squared; ml/kg/min = milliliters per kilogram per minute.

Table 2

Mean Differences of Pre-season and Peak Performance Body Weights, Body Mass Index (BMI), Resting Metabolic Rate (RMR), VO₂ Maximum (VO₂ max), Kilocalories (Kcals), and Body Fat Percent of Participants from a NCAA Division III University Men's Cross Country Team

	Mean Differences (N=11)	SD	t-value	Significance
Weight (kg)	0.16	2.53	0.206	0.841
BMI (kg/m ²)	0.04	0.82	0.147	0.886
RMR	3.09	34.408	0.298	0.772
VO ₂ max (ml/kg/min)	1.4	5.218	0.873	0.403
Kcals Consumed	221.16	1070.1479	0.685	0.509
Body Fat %	0.8	1.19	2.179	0.054

Note. SD = Standard Deviation; kg = kilograms; kg/m² = kilograms per meter squared; ml/kg/min = milliliters per kilogram per minute.

Pre-season and Peak Performance Vitamins

The intakes of three vitamins (A, E, and C) were analyzed with a paired t-test at pre-season and peak performance. The mean pre-season vitamin A was 7596.91 IU/d and the mean peak performance vitamin A was 6182.24 IU/d (Table 3). The mean difference between pre-season and peak performance of vitamin A was an increase of 1414.67 IU/d, which was not significantly different (Table 4). The mean difference (0.86 mg/d) revealed that vitamin E was lower at pre-season (6.17 mg/d) compared to peak performance (7.03 mg/d), however, this was not a significant difference. Although the intakes of vitamin A and E were not significantly different, the intake of vitamin C was significantly lower ($p=0.029$), at pre-season (60.22 mg/d) versus peak performance (114.59 mg/d). The mean difference of vitamin C at these two time periods was 54.37 mg/d.

Table 3

Comparison of the Means for Pre-season and Peak Performance Vitamin A, Vitamin E, and Vitamin C Intakes of Participants from a NCAA Division III University Men's Cross Country Team

	Mean (N=11)	Standard Deviation
Pre-season Vitamin A (IU/d)	7596.91	4686.5054
Peak Performance Vitamin A (IU/d)	6182.24	4141.0824
Pre-season Vitamin E (mg/d)	6.17	4.0462
Peak Performance Vitamin E (mg/d)	7.03	7.0722
Pre-season Vitamin C (mg/d)	60.22	34.6163
Peak Performance Vitamin C (mg/d)	114.59	89.4951

Note. IU/d = International Units per day; mg/d = milligrams per day.

Table 4

Mean Differences of Pre-Season and Peak Performance Vitamin A, Vitamin E, and Vitamin C Intakes of Participants from a NCAA Division III University Men's Cross Country Team

	Mean Differences (N=11)	SD	t-value	Significance
Vitamin A (IU/d)	1414.67	4474.5690	1.049	0.319
Vitamin E (mg/d)	-0.86	4.95	-0.576	0.578
Vitamin C (mg/d)	-54.37	70.7321	-2.549	0.029

Note. SD = Standard Deviation; IU/d = International Units per day; mg/d = milligrams per day.

Pre-season and Peak Performance Minerals

Iron, manganese, selenium, zinc, and copper intakes were analyzed by a paired t-test at pre-season and peak performance. The peak performance (25.07 mg/d) iron intake was higher than pre-season (22.70 mg/d) (Table 5). This mean difference of 2.37 mg/d for iron intake was not significantly different (Table 6). Manganese intakes at pre-season and peak performance were 2.44 and 2.45 mg/d, respectively, representing a non-significant mean difference of 0.01. There was a 24.12 mcg/d mean decrease in selenium at peak performance compared to pre-

season, with intakes of 59.31 and 83.43 mcg/d, respectively. This decrease from the pre-season value tended towards significance ($p=0.076$). Peak performance zinc was 12.92 mg/d, whereas pre-season zinc was 12.16 mg/d. This slight mean decrease in zinc intake (0.77 mg/d) was not significant. The slight increase in peak performance copper of 0.63 mcg/d tended to be significant ($p=0.059$). The mean intakes for copper were 1.92 and 1.29 mcg/d for peak performance and pre-season, respectively.

Table 5

Comparison of the Means for Pre-season and Peak Performance Iron, Manganese, Selenium, Zinc, and Copper Intakes of Participants from a NCAA Division III University Men's Cross Country Team

	Mean (N=11)	Standard Deviation
Pre-season Iron (mg/d)	22.70	8.4365
Peak Performance Iron (mg/d)	25.07	11.5570
Pre-season Manganese (mg/d)	2.44	1.6787
Peak Performance Manganese (mg/d)	2.45	1.2004
Pre-season Selenium (mcg/d)	83.43	43.6784
Peak Performance Selenium (mcg/d)	59.31	28.3954
Pre-season Zinc (mg/d)	12.16	6.0969
Peak Performance Zinc (mg/d)	12.92	7.1235
Pre-season Copper (mcg/d)	1.29	1.1877
Peak Performance Copper (mcg/d)	1.92	1.6889

Note. mg/d = milligrams per day; mcg/d = micrograms per day.

Table 6

Mean Differences of Pre-season and Peak Performance Iron, Manganese, Selenium, Zinc, and Copper Intakes of Participants from a NCAA Division III University Men's Cross Country Team

	Mean Differences (N=11)	SD	t-value	Significance
Iron (mg/d)	-2.37	11.8650	-0.662	0.523
Manganese (mg/d)	-0.01	1.8001	-0.025	0.980
Selenium (mcg/d)	24.12	40.4485	1.978	0.076
Zinc (mg/d)	-0.77	7.4662	-0.340	0.741
Copper (mcg/d)	-0.63	0.9788	-2.132	0.059

Note. mg/d = milligrams per day; mcg/d = micrograms per day; SD = standard deviation.

After the pre-season and peak performance analyses comparing the intakes of the vitamins and minerals were completed, t-tests were utilized to compare the intakes of these same vitamins and minerals to the Recommended Dietary Allowances (RDA) for pre-season and peak performance separately. Pre-season analyses appear in Tables 7, 8, 9, and 10. Note peak performance analyses follow in Tables 11-14.

Pre-season Vitamins and RDAs

In the comparison of pre-season intakes of vitamins A, E, and C to the RDA, vitamin A intake (7596.91 IU/d) (Table 7) significantly ($p=0.009$) exceeded the RDA of 3000 IU/d by 4596.91 IU/d (Table 8). In contrast, pre-season intake of vitamin E at 6.17 mg/d was significantly lower ($p=0.001$) than the RDA of 15 mg/d, which represented a mean difference of 8.82 mg/d. Similarly, the mean difference (29.77 mg/d) of pre-season vitamin C was significantly lower ($p=0.017$) than the RDA (90 mg/d). The mean intake was 60.22 mg/d.

Table 7

Comparison of the Means for Pre-season to the Recommended Dietary Allowances (RDA) for Vitamins A, E, and C Intakes of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean (N=11)	Standard Deviation
Vitamin A (IU/d)	3000	7596.91	4686.5054
Vitamin E (mg/d)	15	6.17	4.0462
Vitamin C (mg/d)	90	60.22	34.6163

Note. IU/d = International Units per day; mg/d = milligrams per day.

Table 8

Mean Differences of Pre-season to the Recommended Dietary Allowances (RDA) for Vitamins A, E, and C Intakes of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean Difference (N=11)	t -Value	Significance
Vitamin A (IU/d)	3000	4596.91	3.253	0.009
Vitamin E (mg/d)	15	-8.83	-7.235	0.001
Vitamin C (mg/d)	90	-29.78	-2.853	0.017

Note. IU/d = International Units per day; mg/d = milligrams per day.

Pre-season Minerals and RDAs

A t-test was utilized to compare the pre-season intakes of iron, manganese, selenium, zinc, and copper to the RDA. The mean iron intake (22.70 mg/d) significantly ($p=0.001$) exceeded the RDA (8 mg/d) by 14.70 mg/d (Tables 9 and 10). The mean difference (0.14 mg/d) between the pre-season manganese intake (2.44 mg/d) and the RDA (2.3 mg/d) was not significant. Selenium intake (83.43 mg/d) at pre-season tended to be significantly higher ($p=0.056$) than the RDA (55 mg/d). This represented a mean difference of 28.43 mg/d. Zinc and copper intakes (12.16 mg/d and 1.29 mcg/d, respectively) at pre-season were not significantly different than the RDAs (11 mg/d and 0.9 mcg/d, respectively).

Table 9

Comparison of the Means for Pre-season to the Recommended Dietary Allowances (RDA) for Minerals Iron, Manganese, Selenium, Zinc, and Copper of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean (N=11)	Standard Deviation
Iron (mg/d)	8	22.70	8.4365
Manganese (mg/d)	2.3	2.44	1.6787
Selenium (mcg/d)	55	83.43	43.6784
Zinc (mg/d)	11	12.16	6.0969
Copper (mcg/d)	0.9	1.29	1.1877

Note. mg/d = milligrams per day; mcg/d = micrograms per day.

Table 10

Mean Differences of Pre-season to the Recommended Dietary Allowances (RDA) for Minerals Iron, Manganese, Selenium, Zinc, and Copper of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean Difference (N=11)	t-Value	Significance
Iron (mg/d)	8	14.70	5.779	0.001
Manganese (mg/d)	2.3	0.14	0.277	0.788
Selenium (mcg/d)	55	28.43	2.158	0.056
Zinc (mg/d)	11	1.16	0.630	0.543
Copper (mcg/d)	0.9	0.40	1.092	0.301

Note. mg/d = milligrams per day; mcg/d = micrograms per day.

Similar to the analysis of the pre-season data, peak performance vitamins and minerals were also analyzed with t-tests to compare the intakes of these same vitamins and minerals to the Recommended Dietary Allowances (RDA). Tables 11 and 12 compare vitamins A, E, and C at peak performance to the RDA. Tables 13 and 14 compare minerals iron, manganese, selenium, zinc, and copper at peak performance to the RDA.

Peak Performance Vitamins and RDAs

In the comparison of the peak performance vitamins to the RDA, peak performance vitamin A (6182.24 IU/d) intakes were significantly ($p=0.029$) higher than the RDA (3000 IU/d), with a mean difference of 3182.24 IU/d (Tables 11 and 12). In contrast, peak performance vitamin E had a mean difference of 7.97 mg/d and was significantly ($p=0.004$) lower than the RDA (15 mg/d). The mean intake of vitamin E was 7.03 mg/d. Vitamin C was not significantly different than the RDA (90 mg/d), with a mean intake of 114.59 mg/d and a mean difference of 24.59 mg/d.

Table 11

Comparison of the Means for Peak Performance to the Recommended Dietary Allowances (RDA) for Vitamins A, E, and C of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean (N=11)	Standard Deviation
Vitamin A (IU/d)	3000	6182.24	4141.0824
Vitamin E (mg/d)	15	7.03	7.0722
Vitamin C (mg/d)	90	114.59	89.4951

Note. IU/d = International Units per day; mg/d = milligrams per day.

Table 12

Mean Differences of Peak Performance to the Recommended Dietary Allowances (RDA) for Vitamins A, E, and C of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean Difference (N=11)	t-Value	Significance
Vitamin A (IU/d)	3000	3182.24	2.549	0.029
Vitamin E (mg/d)	15	-7.97	-3.736	0.004
Vitamin C (mg/d)	90	24.59	0.911	0.384

Note. IU/d = International Units per day; mg/d = milligrams per day.

Peak Performance Minerals and RDAs

At peak performance, the intakes of the minerals iron, manganese, selenium, zinc, and copper were compared with the RDA utilizing a t-test. The mean (25.07 mg/d) intake of iron was significantly (0.001) higher than the RDA (8 mg/d), with a mean difference of 17.07 mg/d. The intakes of manganese, selenium, and zinc (2.45 mg/d, 59.31 mcg/d, and 12.92 mg/d, respectively) were not significantly different than the RDA's (2.3 mg/d, 55 mcg/d, and 11 mg/d, respectively). Manganese, selenium, and zinc had mean differences of 0.15 mg/d, 4.30 mcg/d, and 1.92 mg/d, respectively. In contrast, copper (1.92 mcg/d) intake tended to be significantly ($p=0.073$) higher than the RDA (0.9 mcg/d), with a mean difference of 1.02 mcg/d.

Table 13

Comparison of the Means for Peak Performance to the Recommended Dietary Allowances (RDA) for Minerals Iron, Manganese, Selenium, Zinc, and Copper of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean (N=11)	Standard Deviation
Iron (mg/d)	8	25.07	11.5570
Manganese (mg/d)	2.3	2.45	1.2004
Selenium (mcg/d)	55	59.31	28.3954
Zinc (mg/d)	11	12.92	7.1235
Copper (mcg/d)	0.9	1.92	1.6888

Note. mg/d = milligrams per day; mcg/d = micrograms per day.

Table 14

Mean Differences of Peak Performance to the Recommended Dietary Allowances (RDA) for Minerals Iron, Manganese, Selenium, Zinc, and Copper of Participants from a NCAA Division III University Men's Cross Country Team

	RDA	Mean Difference (N=11)	t-Value	Significance
Iron (mg/d)	8	17.07	4.898	0.001
Manganese (mg/d)	2.3	0.15	0.424	0.680
Selenium (mcg/d)	55	4.30	0.503	0.626
Zinc (mg/d)	11	1.92	0.896	0.391
Copper (mcg/d)	0.9	1.02	2.003	0.073

Note. mg/d = milligrams per day; mcg/d = micrograms per day.

Summary

Of the team's 15 athletes, from a NCAA Division III University men's cross country team, 11 athletes participated in the investigation of micronutrient needs for the prevention of oxidative damage. The athletes ranged in age from 18 to 22 years, and in heights of 69 to 74 inches. The participants' weight, BMI, RMR, and $VO_{2\max}$ were not significantly different between pre-season and peak performance. In contrast, the runners' body fat percent tended to be significant ($p=0.054$) between pre-season and peak performance. The intakes of vitamins A, E, and C, and minerals iron, manganese, selenium, zinc, and copper were compared by paired t-tests at pre-season and peak performance. Of the vitamins studied, vitamin C was significantly lower at pre-season in comparison to peak performance, with a significance level of $p=0.029$. Minerals iron, manganese, and zinc were not significantly different between pre-season and peak performance, whereas the decrease in selenium and increase copper tended to be significant ($p=0.076$ and $p=0.059$, respectively).

After analyzing the differences between pre-season and peak performance vitamins and minerals, the intakes of these same vitamins and minerals were compared to the RDA's for pre-season and peak performance separately. The vitamins A, E, and C for pre-season were significantly different than the RDA's. Vitamin A was significantly higher with a significance level of $p=0.009$. Vitamins E and C were significantly lower ($p=0.001$, and $p=0.017$, respectively) than the RDA's. Pre-season minerals manganese, zinc, and copper were not significantly different than the RDA's, although selenium tended to be significantly higher ($p=0.056$), and iron exceeded the RDA with a significance of $p=0.001$.

Vitamin A was significantly higher and vitamin E was significantly lower at peak performance than the RDA's. The significance levels were $p=0.029$ and $p=0.004$ for vitamins A and E, respectively. Vitamin C was not significantly different than the RDA. The intakes of minerals manganese, selenium, and zinc, in comparison to the RDA's at peak performance were not significantly different. In contrast, the iron intake exceeded the RDA, with a significance level of $p=0.001$ and copper tended to be significant ($p=0.073$), with a difference higher than the RDA at peak performance. All of these results will be discussed further in Chapter 5.

Chapter V: Discussion

Introduction

This study investigated a NCAA Division III University men's cross country team's micronutrient needs to prevent and minimize oxidative damage. The nutrition assessment consisted of a 3-day dietary food log and measurements of height, weight, RMR, body fat percent, and VO_2 max at pre-season and peak performance. Each athlete was also asked several questions relating to factors affecting oxidative damage. This chapter states the limitations to the study, draws conclusions from the results, and makes recommendations for future studies.

Limitations

A major limitation to this study was the small sample size ($N=11$); which means the statistics should be examined with caution. Another limitation of this study was that only one university male cross country team was recruited to participate and may not be applicable to other male cross country teams in other areas. A third limitation was that each participant filled out their own 3-day dietary food log and participants may have underestimated or overestimated foods consumed. The food logs were assumed to be a good predictor of the athlete's actual consumption and current micronutrient status. The training increase over the testing time period was also assumed to be strenuous enough to induce oxidative stress/damage. There may also be limitations in the equipment utilized in assessing VO_2 max, weight, height, BMI, RMR, and body fat percentage. The Tanita body composition analyzer (model TBF-215) was assumed to be calibrated for measuring height, weight, BMI, RMR, and body fat percentage accurately. The VO_2 max machine may not accurately represent the true VO_2 max for each participant depending on proper calibration and/or the health of the participant. This study assumed that the higher the VO_2 maximum the higher the free radicals produced in the body.

Discussion

There were 11 athletes who completed the study. They ranged in age from 18 to 22 and in height from 69 to 74 inches. The pre-season weights, BMI, RMR, and VO₂ max values did not change significantly with increased mileage over the training period. This means that the athletes were already at a physical state comparable to peak performance at the beginning of the season. Costill (1986) stated the fitter the individual, the less potential there is for an increase in VO₂ max, however, the fitter athlete is able to perform at a higher percentage of their VO₂ max for longer periods of time with increased training. The decrease in body fat tended to be significant ($p=0.054$) from pre-season to peak performance. In a study by Coetzer et al. (1995), the male marathon runners had on average 4 to 7% body fat, which is half the amount found in an average male. The athletes in the present study had mean body fat percentages of 6.6 % at pre-season and the mean body fat percentage at peak performance was 5.8 %.

Intakes of vitamins A and E at pre-season were not significantly different than peak performance intakes. Vitamin C intakes at peak performance were significantly ($p=0.029$) higher versus pre-season intakes. Also intakes of pre-season iron, manganese, and zinc were not significantly different at peak performance. Copper tended to be significantly higher ($p=0.059$) at peak performance and the decrease in selenium tended to be significant ($p=0.076$) at peak performance. Minimal variance between pre-season and peak performance intakes, besides vitamin C and copper, indicated only slight differences in foods consumed in the diet. The differences in food selections at peak performance provided higher amounts of vitamin C and copper. In the study by Nieman, Butler, Pollett, Dietrich, and Lutz (1989), male marathoners consumed an average of 147 mg of vitamin C. The athletes in the present study consumed a

mean of 60.22 mg per day of vitamin C at pre-season and 114.59 mg per day by peak performance.

The intakes of these same vitamins and minerals were compared to the RDA's for pre-season and peak performance. The vitamins A, E, and C for pre-season were significantly different than the RDA's. Vitamin A was significantly ($p=0.009$) higher and vitamins E and C were significantly lower ($p=0.001$ and $p=0.017$, respectively) than the RDA's. At peak performance, vitamin A ($p=0.029$) was significantly higher and vitamin E ($p=0.004$) was significantly lower, than the RDA's. The consumption of vitamin A remained high through out the study and was higher than the RDA, whereas vitamin E intakes at both times remained lower than the RDA. It can be assumed that the athletes did not consume enough foods with vitamin E or did not consume foods that were a significant source of vitamin E. These athletes often restrict fat, and the oils that may be restricted are the best source of vitamin E. Vitamin C was not significantly different than the RDA at peak performance. In a study by Nieman, Butler, Pollett, Dietrich, and Lutz (1989), it was reported that the average daily vitamin C intake (147 mg) of marathoners was significantly higher than the recommended dietary allowances (90 mg). In this study, the athletes' intakes of vitamin C was considerably lower at pre-season (60.22 mg) and then exceeded the RDA at peak performance (114.59 mg). Since vitamin C is considered the most important antioxidant in the watery portions of cells (Bendich et al., 1986), exceeding the RDA may protect the cells from free radical damage as well as regenerate vitamin E, which could be at sub-optimal levels because intakes were significantly below the RDA.

Pre-season minerals manganese, zinc, and copper were not significantly different than the RDA's; however, selenium tended to be significantly higher ($p=0.056$), and iron ($p=0.001$) exceeded the RDA. The intakes of manganese, selenium, and zinc in comparison to the RDA's,

at peak performance, were not significantly different. In contrast, the iron ($p=0.001$) intake exceeded the RDA and copper tended to be significant ($p=0.073$) with a difference higher than the RDA at peak performance. In a study by Niekamp and Baer (1995), 13 out of 14 male cross country runners consumed a variety of foods to maintain adequate intakes of micronutrients vitamin A, vitamin C, thiamin, riboflavin, niacin, vitamin B₆, folate, iron, magnesium, zinc, and calcium. Overall, the athletes in this study consumed adequate amounts of the micronutrients at peak performance to meet dietary recommendations of vitamins A, and C, minerals iron, manganese, selenium, copper, and zinc. The only micronutrient not meeting the RDA was vitamin E.

Conclusion

Based on this study, Division III male cross country runners exhibited adequate consumption of micronutrients throughout the study to maintain the antioxidant defense system based on the RDA. This also means the athletes consumed a wide variety of foods to meet these needs. The athletes may benefit from nutrition education on antioxidants (especially vitamin C and E) and the best foods to consume for supplying these nutrients. The athletes may want to consider taking a low dose of vitamin E supplement, since their intake is only half of the RDA. The supplementation may decrease lipid peroxidation and muscle damage during high intensity efforts (Zoppi et al., 2006). There needs to be more research conducted on oxidative damage in endurance athletes to recommend vitamin and mineral supplements.

Recommendations

There have been very few studies conducted regarding nutritional intakes of endurance athletes, therefore this study offers a glimpse of current nutritional intakes of these athletes. A recommendation for future studies is the use of more than one university Division III cross

country team for a larger representative sample. Another recommendation would be to utilize the Dual Energy X-ray Absorptiometry (DEXA) machine to assess body fat percent and lean body mass. This machine has been recently acquired by the human performance laboratory. To verify that free radicals are being produced in the athletes', plasma could be drawn and the T-bars assay used at pre-season and peak performance. Another recommended test involving plasma is the total radical-antioxidant parameter (TRAP), which would assess the levels of antioxidants at pre-season and peak performance. Another option would be to take plasma to show levels of vitamin A, C, and E, and minerals iron (serum ferritin) and zinc in the plasma. Copper/zinc and manganese could be tested functionally in superoxide dismutase. Finally, the training demands and techniques (including increasing mileage, hill runs, climate differences, plyometrics, etc.) should be explored as these training techniques may increase oxidative stress in different ways.

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Appendix A: Consent to Participate Form

This research has been approved by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46.

Consent to Participate in UW-Stout Approved Research

Title: VO₂ Maximum and Micronutrient Needs of 18-26 Year Old Male Collegiate Cross Country Athletes

Description:

The goal of this study is to explore a National Collegiate Athletic Association Division III university men's cross country team for micronutrient consumption to prevent oxidative damage of their lean mass during a typical season. The nutrition assessment will consist of a 3-day food record, age; measurements of height, weight, body-fat %, VO₂ maximum, and questions in regards to the recorded daily diet. These measurements will be taken twice during the season: before training begins and at peak performance.

Risks and Benefits:

Participation in this study carries some risk. For example, you will be asked to provide information of a confidential nature regarding your age, weight, alcohol intake, and dietary intake. Another risk involved is extreme fatigue in being tested for a VO₂ maximum. Benefits to this study would include making you more aware of your current nutritional status, which may assist you in making dietary and lifestyle changes to help improve both your performance and health. Benefits to this study also include the possibility of identifying possible oxidative damage and micronutrient needs of male cross-country runners at a Division III university.

Time Commitment:

For this study, you will be asked to record everything that you eat for three consecutive days, including two-week days and one weekend day. Once you have completed the 3-day food record you will be asked to set up an individual appointment with the researcher to complete the rest of the nutrition assessment. The nutrition assessment will be done on campus in Room 427 of the Home Economics Building. The actual nutrition assessment will take approximately 60 minutes. To thank you for your time, the researcher will gladly discuss the results of your nutrition assessment with you.

Confidentiality:

To ensure your data is kept confidential individual appointments for the nutrition assessment will be set up in which only you and the researcher will be in the lab. All the data collected during the nutrition assessment will be kept in a locked area in which only the researcher and researcher's advisor will have access. At the completion of this research all data that identifies individual participants will be shredded.

Right to Withdraw:

Your participation in this study is entirely voluntary. You may choose not to participate without any adverse consequences to you. Should you choose to participate and later wish to withdraw from the study, you may discontinue your participation at this time without incurring adverse consequences.

IRB Approval:

This study has been reviewed and approved by The University of Wisconsin-Stout's Institutional Review Board (IRB). The IRB has determined that this study meets the ethical obligations required by federal law and University policies. If you have questions or concerns regarding this study please contact the Investigator or Advisor. If you have any questions, concerns, or reports regarding your rights as a research subject, please contact the IRB Administrator.

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Statement of Consent:

By signing this consent form you agree to participate in this nutrition assessment of male collegiate cross-country runners.

Signature _____ Date _____

Appendix B: VO₂ max Waiver

Human Performance Laboratory
VO₂ Max Exercise Testing

I have been informed that the purpose of this test is to determine my maximum exercise capacity, and that I will be required either to walk/run on a treadmill at different combinations of speed and grade (elevation), or that I will be required to pedal an exercise cycle at progressively harder workloads. In all cases, the test will continue until I become fatigued and decide to stop. I have been informed that I may be required to wear an apparatus that allows my exhaled air to be analyzed. The apparatus consists of a mouthpiece and breathing valve similar to a scuba diving mouthpiece, with a nose clip to prevent me from breathing through my nose.

I have been informed that my time commitment is about 45-60 minutes, and that I should wear clothes that allow for free movement during vigorous exercise. I have been informed that the test will begin with an exercise warm-up period of a few minutes, and that subsequently the exercise workload will be progressively increased until I become fatigued. Both leg fatigue and breathlessness are common sensations of the fatigue that I may experience, which could possibly result in injuries due to fall.

I have been informed that throughout the test my heart rate may be monitored either by wearing a chest strap that allows use of a heart rate monitor, or by electrodes that measure electrical activity in my heart (electrocardiogram). Appropriately trained personnel will monitor my exercise. I have been informed that I will need to communicate with the lab personnel during the test, either by hand signals or by the use of a perceived exertion chart.

I have been informed that the risk involved in this testing for healthy persons is thought to be zero. For persons with known or suspected heart disease, 1/10,000 will die and 6/10,000 will have a life-threatening emergency. I have been informed that one of the benefits of this testing is the determination of my exercise capacity. This information may be useful to develop an individualized exercise program.

In the unlikely event that any injury or illness occurs as a result of this research the University of Wisconsin-Stout and affiliates do not automatically provide reimbursement for medical care or other compensation. I have been informed that payment for treatment of any injury or illness must be provided by me or my third-party, such as my health insurer or Medicare. If any injury or illness occurs in the course of research, or for more information, I will notify the investigator in charge. I have been informed that I am not waiving any rights that I may have for injury resulting from negligence of any person or the institution.

 Participant Signature

Date

Appendix C: Nutrition Assessment Form

Name: _____

.....**Cut Here**.....

Nutrition Assessment Form

Code Number:		Date:
Gender:		Age:
Kilocalories (Tanita Scale):		
Body Fat % (Tanita Scale):		
Medications: <input type="checkbox"/> Supplements: <input type="checkbox"/> Prescription:		
3-Day Food Record: <input type="checkbox"/> Completed: Yes No <input type="checkbox"/> Subject's Name on 3-Day Food Record: Yes No (To be replaced by code) <input type="checkbox"/> Record Verified with Subject: Yes No		
Height – Tanita Scale (to the nearest 0.5 inch):		BMI (Tanita Scale):
Weight – Tanita Scale (to the nearest 0.1 pound):		Classification:
VO ₂ Maximum Results (See Attached Charts):		
Other Comments:		

