ABSTRACT

Vitamin C is a micronutrient with many important physiological roles. It can function as a reducing agent, a free radical scavenger, and an enzyme cofactor. Much research has examined the potential of vitamin C supplements to enhance exercise capacity in trained athletes; however, little is known regarding the effects of vitamin C supplements on the promotion of leisure-time physical activity in the general population. This area deserves attention since 1/3 of Americans have below adequate vitamin C status, and since aversion to exercise, fatigue, and altered mood states are the earliest signs of poor vitamin C status. This study analyzed the effect of supplementing 500 mg twice daily of vitamin C on self-reported leisure-time activity levels and mood states in young men. Twenty-nine healthy, young men, aged 18-35 years, were stratified by age, BMI, smoking status, and plasma vitamin C concentrations and assigned to either a control (CON) or experimental group (VTC) for the 8-week randomized, double-blinded, parallel arm trial. Subjects were instructed to keep track of their leisure-time physical activity by filling out the validated Godin Leisure-Time Exercise Questionnaire weekly for the entire study. In addition, subjects took the self-administered Profile of Mood States (POMS) at baseline, week 4, and week 8 to observe mood states. Plasma vitamin C concentrations were analyzed at the initial screening, week 4, and week 8 of the study. Plasma vitamin C concentrations significantly differed by group at week 4 and week 8. Furthermore, vitamin C supplementation significantly increased self-reported mild, moderate, and strenuous activity levels during the 8-week trial. Overall,
total physical activity scores increased nearly 50% in the VTC group as compared to 18% in the CON group (p=0.001). However, mood states were not significantly impacted by vitamin C supplementation during the trial. This study provides the first experimental evidence that supplementing 500 mg of vitamin C twice daily can be effective in increasing leisure-time physical activity in healthy young men. This study, however, was unable to link improvements in physical activity rates to improved mood states. Since sedentary behaviors have been implicated in the rise of obesity in the U.S., further research should be conducted to substantiate the finding that vitamin C supplementation increases physical activity.
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Chapter 1

Introduction

The National Health and Nutrition Examination Survey (NHANES) statistics for 2003-2004 reported that 66.2% of adults, 20-74 years old, in the United States were classified as either overweight or obese (Nguyen & El-Serag, 2010). These numbers further break down to categorize 33.4% of adults to be overweight and 32.9% to be obese. Due to the fact that obesity has been linked to many chronic conditions such as type 2 diabetes, metabolic syndrome, hyperlipidemia, and cardiovascular disease, a great deal of concern has been expressed for the nation’s future health (Ford, Zhao, & Tsai, 2010; Crawford et al., 2010). Furthermore, it has been found that people are gaining more weight earlier in life. Lee et al. (2010) assessed the trend of obesity across the life cycle and found that a large number of individuals are becoming overweight at a younger age, thus increasing the burden of chronic diseases throughout a person’s lifetime.

Vitamin C is a micronutrient with many important physiological functions. It is a reducing agent, a scavenger of free radicals, and an enzyme cofactor in the biosynthesis of carnitine, histamine, collagen, norepinephrine, and epinephrine (Cahill & El-Sohemy, 2009; Schleicher, Carroll, Ford, & Lacher, 2009). Vitamin C may also have potential protective effects against cancer, cataracts, asthma, and cardiovascular disease (Thompson, 2007). Though vitamin C has a fairly low recommended dietary allowance (RDA) of 75 mg for women and 90 mg for men, the 1994-1996 US Department of Agriculture Continuing Survey of Food Intakes
by Individuals reported that 18% of adults in the United States consumed less than 30 mg of vitamin C per day (Hampl, Taylor, & Johnston, 2004). College-aged students are one sub-category that has low plasma concentrations of vitamin C, and this has been related to low consumption of vitamin C rich foods (Johnston, Solomon, & Corte, 1998). Moreover, males tend to have lower serum concentrations of vitamin C than women between the ages of 12 to 59 years old (Schleicher et al., 2009).

Research suggests that slight but noticeable fatigue and abhorrence to exercise occurs in subjects with mild vitamin C deficiency (Johnston, Swan, & Corte, 1999; Levine et al., 1996). Moreover, symptoms dissipated quickly with vitamin C supplementation. Most research, however, has focused on the potential health benefits of vitamin C supplementation by endurance athletes with conflicting results. Several investigations observed that vitamin C supplementation reduced exercise-induced lipid peroxidation during high intensity exercise, which theoretically would reduce tissue damage and improve muscle recovery (Nakhostin-Roohi, Babaei, Rahmani-Nia, & Bohlooli, 2008; Cesari et al., 2004; Clarkson & Thompson, 2000). Conversely, Gomez-Cabrera et al. (2008) found that vitamin C hindered endurance capacity in exercising subjects by reducing the oxidative stress that triggered gene transcription and adaptive training effects.

Aside from investigations that examine the impact of vitamin C on athletic training, there is little research examining the impact of vitamin C status on sense of well-being and leisure-time physical activity levels. Understanding how
dietary factors influence leisure-time physical activity levels is important since regular activity provides psychological benefits such as a pleasant mood state and lessening of anxiety (Sakuragi & Sugiyama, 2006). Aerobic exercise and weight-training have been observed as an effective alternative treatment for depression in a randomized controlled trial with older adults (Singh et al., 2005). Physically active men and women show less signs of depression and have lower rates of chronic diseases and all-cause mortality (Carlson, Densmore, Fulton, Yore, & Kohl, 2008; Binfarè, Rosa, Lobato, Santos, & Rodriques, 2009).

The primary aim of this randomized parallel arm trial was to examine the effect of supplementing 500 mg vitamin C twice daily in young men, 18-35 years, on self-reported physical activity levels. A secondary aim of this study was to examine the effect of vitamin C supplementation on mood states in young men.

It was hypothesized that vitamin C supplementation (500 mg twice daily for eight weeks) would have no effect on the number of METS recorded by young men. A secondary hypothesis was that vitamin C supplementation (500 mg twice daily for eight weeks) would improve the mood states of young men.

**Definitions**

- BMI: \[ \frac{\text{weight (kg)}}{\text{(height (m))}^2} \]; underweight is <18.5 kg/m², normal is 18.5-24.9 kg/m², overweight is 25.0-29.9 kg/m², obese is >30 kg/m² (American Dietetic Association)

- Regular smoker: greater than or equal to 10 cigarettes per day (Moran, Wechsler, & Rigotti, 2004)
• Social smoker: fewer than 10 cigarettes per day [typically does not smoke daily] and smokes mainly with others in a social scene (Schane, Glantz, & Ling, 2009; Moran et. al, 2004)

• Plasma vitamin C concentrations: >0.4 mg/dL is adequate; 0.2-0.4 mg/dL is low; <0.2 mg/dL is deficient (Jacob & Sotoudeh, 2002)

• METS: Metabolic Equivalent Tasks; <4 is inactive; 4-6 is moderately active; >6 strenuous (Perkins, Owens, Kearney, & Swaine, 2009)

• Physical activity: aerobic activity or small bouts of anaerobic activity; included but not limited to activities such as walking, cycling, hiking, sports, weight-training, domestic chores, and yard work (Tormo et al., 2003)

• Training athlete/purposeful exercise: engaging in purposeful exercise 5 or more times a week (Yfanti et al., 2009)

• Moderate-intensity of physical activity: light sweating or a slight to moderate increase in breathing or heart rate (Carlson et al., 2008)

Delimitations

This study was conducted at the Polytechnic Campus of Arizona State University in Mesa, Arizona. The subjects were non-training, healthy college men aged 18-35 years. The subjects were recruited via flyer distribution throughout the Polytechnic Campus. The study was carried out with 29 subjects which is a relatively small subject pool.
Limitations

During the eight week study, the subjects were given a survey booklet to record their physical activity, cold/flu symptoms, and any use of medication. Subjects were also asked to consume a study capsule twice daily. The subjects were sent weekly reminder e-mails, but complete daily adherence from the subjects cannot be assured. There is a possibility of measurement error due to the fact that self-reported measure of dietary intake and physical activity may not always be an accurate reflection of these factors. In addition, the subjects were to refrain from drinking any sort of juice or fruit-flavored drinks during the course of the study. Subject compliance to this guideline may be a limitation of the study. This study was carried out in the months of January through May.
It is well-known that the absence of fresh fruits and vegetables in the diet will lead to the development of scurvy. In the late 16th century a large number of sailors travelling great lengths across the sea acquired scurvy. James Lind, a Scottish physician, was the first man to demonstrate that scurvy could be treated and prevented by dietary means with the consumption of citrus fruits, such as lemons and oranges (Baron, 2009). James Lind published the famous *Treatise of the Scurvy* in 1753, thus convincing British admiralty to use lemon juice to prevent scurvy among sailors (Baron, 2009).

Vitamin C is a micronutrient that has been studied for decades for its antioxidant and cofactor properties. It is a commonly consumed supplement believed to aid in processes such as shortening durations of colds and preventing lipid peroxidation in the body. This review provides a detailed overview of the properties of vitamin C, investigated relationships between vitamin C and physical activity levels, and observed effects of vitamin C on mood. It will also explore obesity rates in the United States and touch on the importance of physical activity in combating obesity.

**Overview of Vitamin C**

**Biochemistry and Function**

Vitamin C is a water-soluble micronutrient that has many important biological roles within the body. It is a redox system that has two L-isomers. In the reduced state vitamin C is known as ascorbic acid and in the oxidized state it
is known as dehydroascorbic acid (DHA). DHA is exceedingly unstable and does not perform the functions of ascorbic acid; yet, it is readily converted to ascorbic acid in the mitochondrial matrix (Mandl, Szarka, & Bánhegyi, 2009). This is known as intramitochondrial ascorbate recycling. This 'recycled' vitamin C mechanism can occur by multiple enzymatic systems including glutathione-dependent systems or NADPH-dependent systems (Mandl et al., 2009). Vitamin C is a reducing agent, scavenger of free radicals, and an enzyme cofactor for the biosynthesis of carnitine, collagen, norepinephrine, and epinephrine (Cahill & El-Sohemy, 2009; Schleicher, Carroll et al., 2009). The micronutrient may also have potential protective effects against cancer, cataracts, asthma, and cardiovascular disease (Thompson, 2007).

**Vitamin C as an antioxidant**

In its redox interrelationship, vitamin C can function as an antioxidant and an enzyme cofactor. Because it can serve as a reducing agent, vitamin C acts as an antioxidant in free radical-mediated oxidation processes, thus reducing the amount of reactive oxygen species (ROS) in the body (Mandl et al., 2009). ROS can cause oxidative damage to lipids, cellular proteins, and strands of deoxyribonucleic acids (DNA) in the body. Vitamin C is responsible for up to 24% of all total peroxyl radical-trapping antioxidant capability in human plasma (Henning, Zhang, McKee, Swendseid, & Jacob, 1991). Vitamin C status is inversely associated with oxidative stress (Johnston & Cox, 2001) and impacts biomarkers in diabetes, heart disease, neurological disorders, and cancers in case-controlled data (Fogelholm, 2010). Vitamin C also has the capability to bestow
antioxidant protection by rejuvenating the active forms of glutathione and vitamin E (Wintergerst, Maggini, & Horning, 2006).

Vitamin C in combination with vitamin E reduces the oxidation of low-density lipoproteins (LDL) which is a process that is thought to contribute to atherosclerotic plaques on artery walls (Jacob & Sotoudeh, 2002). Acting as a chemoattractant for monocytes, oxidized LDL prevents macrophages from moving from the artery back into plasma, hence creating foam cells which are part of the progression to atherosclerotic disease (Brown & Goodman, 1998). A study using 11 overweight and obese sedentary adults administered continuous intravenous vitamin C (0.05g/kg fat-free mass) and found the micronutrient inhibited an increase in plasma concentrations of oxidized LDL in the postprandial state (Newsom, Paxton, Rynn, & Bell, 2008). The Second National Health and Nutrition Examination Survey (NHANES) reported the relative risk of coronary vascular disease and stroke declined by 26% with serum vitamin C concentrations of 1.1-2.7 mg/dL in contrast to concentrations of 0.1-0.4 mg/dL (Jacob & Sotoudeh, 2002).

**Vitamin C as a cofactor**

Vitamin C is the cofactor that accelerates the hydroxylation reactions in many biosynthetic pathways, including the formation of collagen (Peterkofsky & Udenfriend, 1965). Three enzymes that are necessary for collagen biosynthesis include prolyl 4-hydroxylase, prolyl 3-hydroxylase, and lysyl hydroxylase (Levine, 1986). In these enzymatic reactions, Vitamin C reduces iron to its ferrous state ($Fe^{2+}$) to maintain enzyme activity. Thirty percent of cellular protein
mass is composed of collagen; hence proline hydroxylation is critical in the formation of many proteins containing collagen-like domains (Mandl et al., 2009).

Carnitine is an amino acid that has many important functions in lipid metabolism. Carnitine transports long-chain fatty acids into the mitochondria from the cytosol. Two hydroxylation reactions are needed for the biosynthesis of carnitine. These reactions are catalyzed by α-ketoglutarate-dependent oxygenase which, like the synthesis on collagen, requires Fe\(^{2+}\) and vitamin C as the reducing agent (Rebouche, 1991).

Vitamin C is a vital cofactor needed in multiple processes that bring about maturation of neurons, neuroprotection, and neurotransmission (Kennedy et al., 2010). A hydroxylation reaction that requires vitamin C as a cofactor occurs in the conversion of dopamine to the neurotransmitter norepinephrine. Dopamine β-monooxygenase must be activated by vitamin C in order to catalyze the conversion of dopamine to norepinephrine (Wimalasena & Wimalasena, 1995). Furthermore, in tyrosine metabolism, the formation of homogentisic acid from 4-hydroxyphenylpyruvic acid must be aided by vitamin C (La Du & Zannoni, 1961). Also due to its reducing properties, Vitamin C can enhance the absorption of non-heme iron from the gastrointestinal tract and regulate transport and storage of iron within the cells (Wintergerst et al., 2006). Vitamin C facilitates the intracellular storage of iron by upregulating the translation of ferritin mRNA thus increasing storage of iron and averting cellular iron-induced oxidative damage (Toth & Bridges, 1995).
**Vitamin C and the common cold**

Cellular levels of vitamin C have been linked with immunoresponsiveness. During times of stress, vitamin C may be mobilized as a part of the immune response mechanism to lessen histamine action (Johnston, Retrum, & Srilakshmi, 1992). Histamine is a neurotransmitter that is elicited in times of physiological stress. During this time of immune response, capillary permeability and smooth muscle contraction is increased, thus eliciting immune factors to travel to the site of inflammation (Johnston, Martin, & Cai, 1992). Histamine can have deconstructive effects on circulatory and immunologic homeostasis if levels are too high (Johnston et al., 1992). The imidazole ring of histamine can be broken by vitamin C thus rupturing the compound and inactivating histamine (Nandi, Subramanian, Majumder, & Chatterjee, 1973).

A momentous amount of research has been conducted on the use of vitamin C to decrease incidence and severity of the common cold after it was suggested by Linus Pauling in the 1970s that vitamin C prevents and eases cold symptoms (Hemilä, 2004). Multiple studies have not been able to replicate these results. Vitamin C or a placebo taken at the first sign of cold symptoms has shown no difference in symptom duration period; however, people consuming vitamin C tended to miss less days of work or school during times of a cold as opposed to those taking a placebo which suggests that they felt better (Hemiliä, Chalker, & Douglas, 2007). Research studies using high doses of vitamin C after the onset of symptoms have not shown therapeutic benefit for alleviating cold duration or
symptoms (Douglas, Hemilä, Chalker, & Treacy, 2004). The research in the literature is inconclusive for using vitamin C as a treatment for the common cold

**Absorption, Transport, & Bioavailability**

Vitamin C can be absorbed in the small intestine as ascorbic acid or DHA. The micronutrient requires two transporter proteins for absorption and transport: SVCT1 and SVCT2. Ascorbic acid is absorbed by means of SVCT1 mainly in the ileum and jejunum; DHA is absorbed [at a lesser extent] by facilitated diffusion with high concentrations in the proximal area of the small intestine in the duodenum and jejunum (Malo & Wilson, 2000). Three of the 12 glucose transporter isoforms, GLUT-1, GLUT-3, GLUT-4, have a high specificity to transport DHA down a substrate concentration gradient (Maulén et al., 2003).

Vitamin C transporter gene polymorphisms may play a role in the efficiency of vitamin C absorption. Polymorphisms of the sodium-dependent vitamin C genes *SLC23A1* and *SLC23A2* may impact serum vitamin C levels independently of dietary intake of vitamin C (Cahill & El-Sohemy, 2009). In addition, glutathione S-transferases (GSTs) are a group of enzymes that reduce DHA to ascorbic acid. There are multiple genotypes of GSTs including GSTM1, GSTT1, and GSTP1. Cahill, Fontaine-Bisson, and El-Sohemy (2009) speculated that a polymorphism in a given GST genotype may increase risk of vitamin C deficiency. The Toronto Nutrigenomics and Health Study found that there was an increased risk of vitamin C deficiency in persons with the *GST* null genotype if the recommended intakes of vitamin C were not being met; however, deficiency was avoided regardless of the genotype if a person was consuming adequate
vitamin C daily (Cahill et al., 2009). A deeper understanding of GST polymorphisms could help increase the knowledge base of the impact of circulating vitamin C levels in the body in connection to the incidence of risks of chronic disease states such as diabetes, cardiovascular disease, and cancer (Cahill & El-Sohemy, 2009; Timpson et al., 2010).

Approximately 20% of people in the United States take daily supplements that contain vitamin C (Blanchard et al., 1997). Most vitamin C supplements contain a megadose of the micronutrient as defined by Blanchard et al. (1997) as ≥600 mg of vitamin C, which is almost 10 times greater than the RDA. The bioavailability of vitamin C begins to decrease above 200 mg as a single dose (Levine et al., 1996; Levine, Wang, & Padayatty, 2001). There is only a negligible increase in plasma concentrations of the vitamin C when daily intakes are greater than 200 mg (Blanchard et al., 1997). Levine et al. (1996) observed a decrease in bioavailability from 100% after a solitary dose of 200 mg to 33% in a solitary dose of 1250 mg; thus less than 50% of the vitamin C was absorbed from the 1250 mg dose and almost 100% of this absorbed dose was excreted.

**Vitamin C Status in Individuals**

Scurvy is the most obvious sign of vitamin C deficiency. Symptoms of scurvy include lethargy, bone pain, spongy bleeding gums, limping, edema, and petechiae (Chang, Chen, Wang, Chang, Lin, & Liu, 2007; Popovich, McAlhany, Adewumi, & McKim Barnes, 2009). The appearance of scurvy symptoms will be seen in an individual with a body pool of vitamin C around 300 mg whereas an essentially full body pool is considered to be around 1500 mg (Jacob, 1993). The
disease of scurvy initially led to the development of a recommended daily intake of vitamin C to prevent scurvy in a majority of the healthy population (Blanchard, Tozer, & Rowland, 1997)

**Recommended Intakes**

The Recommended Dietary Allowance (RDA) of vitamin C set by the Institute of Medicine is 90 mg/day for men and 75 mg/day for women over the age of 18 years (Institute of Medicine). The RDA for individuals who smoke has been increased from the normal RDA for men and women by 35 mg (125 mg/day for men and 110 mg/day for women, respectively). Because tobacco smoke contains many toxic chemicals that create oxidative stress in the body, metabolic turnover of vitamin C is 40% higher in smokers as compared to non-smokers (Lykkesfeldt, Christen, Wallock, Chang, Jacob, & Ames, 2000; Schectman, Byrd, & Hoffman, 1991). Pregnant and lactating women are recommended to consume the RDA for their age group. The Tolerable Upper Limit (UL) for vitamin C is 2 g/d for adults; undesirable effects vitamin C overload include osmotic diarrhea and gastrointestinal distress (Institute of Medicine).

**Prevalence of deficiency**

Much research has shown that many individuals are not consuming adequate amounts of vitamin C. The 1994-1996 US Department of Agriculture Continuing Survey of Food Intakes by Individuals reported that 18% of adults in the United States consumed less than 30 mg of vitamin C per day (Hampl et al., 2004).
Schleicher et al. (2009) examined the prevalence of vitamin C deficiency based on data from the 2003-2004 NHANES. The results of NHANES 2003-2004 showed that $7.1 \pm 0.9\% \text{ (±SE)}$ of the total population were deficient (Schleicher et al., 2009). Adult men aged 20-39 years and $\geq 60$ years encountered a higher prevalence of deficiency than of women of the same age. The percentage of males aged 20-39 years that were serum vitamin C deficient was 10.8 as opposed to 7.9% of females of the same age. For adults aged $\geq 60$ years, 7.2% of males were serum vitamin C deficient as opposed to 4.1% of females. NHANES III, which encompassed data from 1988-1994, found similar results. The survey found that 23% of males aged 25-44 years and 20% of women aged 25-44 years were vitamin C deficient (Hampl et al., 2004). Smokers were three times more deficient in vitamin C than counterparts who did not smoke; race-ethnicity groups did not vary and BMI was not related to vitamin C deficiency (Schleicher et al., 2009). College-aged students are a sub-category identified with a high prevalence of vitamin C deficiency (Johnston et al., 1998). This association has been related to a lower consumption of fruit and vegetables among college students. Socioeconomic status has also been correlated with vitamin C deficiency prevalence (Mosdol, Erens, & Brunner, 2008; Schleicher et al., 2009). The Low-Income Diet and Nutrition Survey (LIDNS) which supplies representative statistics of the nutritional standings in low-income individuals within the UK indicated that 46% of men and 35% of women were vitamin C deficient (Mosdol et al., 2008).
Low consumption of fruits and vegetables has been correlated with depleted vitamin C intakes. Taylor, Hampl, and Johnston (2000) found that adults with adequate vitamin C intake had eaten at least five servings of fruits and vegetables (one serving being citrus) daily; whereas those with low intakes consumed less than one serving of citrus a day. The researchers concluded that five to nine servings of fruits and vegetables (one serving being citrus) should be encouraged to maintain adequate vitamin C levels. Orange juice is the most vitamin C-rich food habitually consumed by individuals in the United States (Johnston & Bowling, 2002). However, vitamin C is easily irrevocably oxidized when exposed to oxygen, light, or processed with heat (Johnston & Hale, 2005). Johnston and Hale (2005) found that orange juice from concentrate after it had been reconstituted and refrigerated had a significantly lower vitamin C content at day 8 compared to day 1; antioxidant capacity was also less at day 8 in comparison to day 1. The bioavailability and antioxidant levels in natural and synthetic [supplemental] sources of vitamin C seem to be similar to one another (Johnston, Danncho, & Strong, 2003).

**Biomarkers of Vitamin C**

Common measurements of vitamin C status include blood [serum or plasma] concentrations and dietary intake measures such as 24-hour recalls and food frequency questionnaires (FFQs) (Loria, Whelton, Caufield, Szklo, & Klag, 1998). Loria et al. (1998) have suggested that the two measures should not be used as interchangeable terms because they essentially are measuring different facets of vitamin C; plasma [or serum] concentrations indicates estimated levels
of the body pool, whereas 24-hour recalls and FFQs measures estimated average dietary intake of vitamin C. Both forms of measurement are important in vitamin C research. Blood plasma concentrations show less changeability in vitamin C levels in comparison to erythrocyte vitamin C levels and provide an easier way to determine concentrations than using leukocytes (Jacob, Skala, & Omaye, 1987). A recent meta-analysis using 26 studies found a moderate relationship between plasma vitamin C concentrations and the use of FFQs, alluding to the idea that the two markers are indicators of vitamin C status but may not be quite measuring the same thing (Dehgan, Akhtar-Danesh, McMillan, & Thabane, 2007). These results are in agreement with the findings of Loria et al. The meta-analysis found the combined data for men and women observed only a 12% variance between measurements of vitamin C plasma concentrations and FFQ data and a 21% variance against dietary records (Dehgan et al., 2007). Therefore, it can be concluded that both plasma vitamin C concentrations and dietary intake methods such as 24-hour recalls and FFQs are both important indicators of vitamin C status in individuals.

**Importance of Physical Activity**

Physical activity has favorable effects on lipoprotein subclass distribution, endothelial function, lipoprotein metabolism, enhances inflammatory markers by reducing CRP, serum amyloid A, and tumor necrosis factor-α concentrations, and improves insulin sensitivity (Fogelhom, 2009). Multiple studies have documented beneficial effects of physical activity in respect to rates of mortality. The National Institute of Health’s American Association of Retired Persons Diet
and Health Study composed of 252,925 individuals demonstrated that men and women following the national physical activity guidelines displayed lower risk of death than inactive individuals (Hainer, Toplak, & Stich, 2009). Individuals that participated in at least 30 minutes of moderate physical activity (approximately 30 minutes most days of the week) lowered risk of mortality by 27%, and those engaging in vigorous activity (intense activity for approximately 20 minutes three times per week) reduced risk of mortality by 33%. In addition, a prospective study that recorded Harvard College Alumni’s physical activity levels twice in an 11-15 year time-interval found that men who increased their physical activity levels by 1,250 kcals per week lessened their chances of dying by 43% when compared to men whose physical activity levels stayed stable or decrease physical activity levels (Hainer et al., 2009). The Two Aerobics Center Longitudinal Study (ACLS) using measured cardiorespiratory fitness and body fat percentage found that moderate to high physical activity levels reduced the increased risk of cardiovascular disease, all-cause, and cancer connected to obesity (Lee, Sui, & Blair, 2008). Individuals who were obese yet fit had decreased risk of mortality as opposed to those who were normal weight but unfit in all the ACLS reports. It is speculated that physical activity can improve chronic disease risk in obese individuals; however how it has the potential to eliminate this risk is not clear (Lee et al., 2008).

King, Hopkins, Caudwell, Stubbs, and Blundell suggest changing the focus from a static body weight number to looking at other markers to indicate the health status of individuals (2009). Fifty-eight sedentary men and women that
were overweight or obese (BMI 31.8 kg/m² ± 4.5) participated in a 12-week supervised aerobic physical activity program five times per week found differing results in body weight reduction. Twenty-six of the 58 subjects did not reach their predicted weight loss estimation based off of his or her individual exercise-induced energy expenditure and the mean weight loss was only 0.8 kg. However, the subjects had statistically significant decreases in waist circumference, resting heart rate, systolic and diastolic blood pressure, and an increase in aerobic capacity. Occurrence of increased acute exercise-induced positive mood state was observed in the subjects. Engaging in physical activity is beneficial for improvement of health markers and mood state (King et al., 2009).

**Vitamin C and Exercise Performance**

There is a relationship between vitamin C status in individuals and exercise. Positive and negative outcomes of efficiency in endurance training have been observed from the use of vitamin C supplementation. Few explanations of functional relationships between micronutrients and physical activity have been presented. This is because of the challenge of potential homeostatic changes in the body due to changes of nutrient intake, and study designs have not allowed for enough comprehensive data to be collected for sound conclusions to be made (Lukaski, 2004). The best controlled studies have concluded that vitamin C supplements do not impact endurance or strength-training performance (Clarkson & Thompson, 2000). However, individuals with high intakes of vitamin C, whether it is through diet or supplementation, tend to engage in more physical activity (Lukaski, 2004).
A placebo-controlled, depletion-repletion study assessed the consequent vitamin C plasma concentrations on plasma carnitine concentrations and substrate utilization in nine subjects with low plasma vitamin C concentrations (<28 µmol/L) in a 90-minute treadmill walk at a 50% VO₂ max treadmill walking test (Johnston et al., 1999). In the trial, nine subjects (4 men and 5 women) were vitamin C depleted for three weeks by consuming a placebo capsule and then vitamin C-repleted for two weeks by taking a vitamin C capsule (500 mg) that was identical in appearance to the placebo. Subjects performed a submaximal walking test at the end of week 3 (end of depletion period) and week 5 (end of repletion period). As consistent with previous findings, there was a significant decrease in mean plasma carnitine concentrations when subjects were repleted with the vitamin C supplement possibly indicating impairment of carnitine transport into muscle tissue in a state of vitamin C deficiency (Johnston et al., 1999).

Johnston et al. (2005) detected that individuals (n=15) with low vitamin C plasma concentrations (<34 µmol/L) oxidized a lesser amount of fat than individuals (n=7) with adequate plasma concentrations (≥34 µmol/L) in a 90-minute treadmill walk at a 50% VO₂ max treadmill walking test. The study included a depletion period followed by a repletion period and two submaximal treadmill tests were performed. An improvement of 14% in work efficiency was seen in subjects after the repleted state associated with a 10% raise in VO₂ max. Plasma concentrations of carnitine in the repleted state declined by close to 20%, hence denoting an increase in muscle carnitine (Johnston et al., 2005). The
improvement of VO₂ max could not solely be accredited to a rise in carnitine levels because muscle biopsies were not collected (Johnston et al., 2005).

Too further her investigation of substrate oxidation, Johnston et al. (2005) examined 11 sedentary subjects with low vitamin C concentrations. Subjects participated in a depletion period and did a 60-minute submaximal treadmill test at the end of the period. For the last four weeks of the study, the subjects were randomized and given either a 500 mg capsule of vitamin C or a placebo capsule. Subjects did a 60-minute submaximal treadmill test at the end of week 8. A greater amount of fat energy expenditure during the treadmill test was seen at the end of week 8 in the repleted subjects as opposed to the depleted subjects. Though there was no difference in plasma carnitine concentrations, an inverse relationship was seen between plasma vitamin C and plasma carnitine concentrations. Plasma carnitine concentrations were associated with fat energy expenditure and it was determined that vitamin C plasma concentration status accounted for the 43% disparity in fat oxidation during the subject’s submaximal exercise. Although these studies used small sample sizes, the data present strong evidence that vitamin C influences factors such as fat oxidation, vigor, and exercise performance.

A double-blind, randomized study with 14 healthy, sedentary men, aged 27-36 years, performed a maximal exercise test on a bicycle ergometer with magnetic brakes after eight weeks of training to observe the effects of vitamin C administration on VO₂ max, endurance capacity, and on mitochondrial biogenesis (Gomez-Cabrera et al., 2008). Five men consumed vitamin C (1000 mg daily)
capsules whereas the others consumed a placebo. The study also had an animal component where it used 24 Wistar rats that exercised under two specific protocols during weeks 3 and 6 of the study; 12 rats were orally administered vitamin C (0.24 mg/cm² of body surface area) daily during the study. In both the human and the rat portions of the study, an increase in vitamin C plasma concentrations and a decrease in VO₂ max in the supplemented group were seen. However, the results were not significant in the human study, and due to the fact that only five men were in the supplemented group these results cannot be generalized. Gomez-Cabrera et al., (2008) also found that ROS initiated the expression of antioxidant enzymes and mitochondrial biogenesis in skeletal muscle, but vitamin C inactivated both processes. The conclusion was drawn that vitamin C lessens training efficiency due to the reduction in the expression of major transcription factors that are exercise-induced.

**Effects of Vitamin C and Exercise on Antioxidant Function**

The effects of vitamin C and exercise on antioxidant function in the body have been examined. A randomized crossover trial with a 14 day washout period observed the effect of vitamin C on cortisol, adrenocorticotropic hormone, IL-6, oxidative stress, and neutrophil responses in nine healthy, endurance-trained men (Davison & Gleeson, 2006). Subjects participated in a 2.5 hour exercise test on an ergometer bicycle that equated to a 60% VO₂ max after two weeks of ingesting either 1,000 mg vitamin C daily (two 500 mg capsules, one taken at morning and evening time with meals) or placebo capsules. Supplementing vitamin C daily for two weeks slightly decreased leuckocytosis, cortisol, and neutrophilic responses.
Conversely, no change was seen in adrenocorticotropic hormone or IL-6 concentrations. Davison and Gleeson (2006) reported that a two-week period was not a long enough time-period to provide a shield against neutrophilic function. In addition, the amount of oxidative stress was relatively low because the subjects were exercising at a controlled 60% VO$_2$ max, and it is possible that larger changes may have been seen in neutrophil functions at greater exertion levels (Davison & Gleeson, 2006).

Nakhostin-Roohi et al. (2008) examined the connection between lipid peroxidation induced by exercise and inflammation and the use of vitamin C. Sixteen healthy, untrained male subjects participated in treadmill tests to develop baseline VO$_2$ max data. After a 10-hour fast, subjects reported to a facility where blood samples were taken and a standard breakfast was provided. After breakfast, 500 mg of vitamin C or a placebo capsule was taken. Subjects participated in a 5-minute 50% VO$_2$ max treadmill warm-up and then ran at 75% VO$_2$ max for 30 minutes. There was no observed affect of vitamin C on inflammatory markers including leukocytes, lymphocytes, interleukin-6 (IL-6), or neutrophils. Serum concentrations of creatine kinase (CK) and malondialdehyde (MDA) were measured to estimate muscle damage and lipid peroxidation. Serum CK was elevated after exercise in both the control and placebo groups. Serum MDA was amplified two hours after exercise in the control group whereas the serum MDA only increased only during the pre-exercise time period in the placebo group. Ingestion of vitamin C prior to exercise moderated serum MDA concentrations post-exercise thus preventing lipid peroxidation. Nakhostin-Roohi et al. (2008)
concluded that vitamin C supplementation can help inhibit lipid peroxidation, hence suggesting that the inflammatory and oxidative damage mechanisms may vary and act independently from one another.

A double-blinded placebo-controlled study observed the impact of supplementing 500 mg vitamin C and 400 IU vitamin E daily for 16 weeks on endurance training in 21 healthy, physically active males aged 18-41 years with normal levels of vitamin C and vitamin E at the beginning of the trial (Yfanti et al., 2009). Subjects participated in 12 weeks of strenuous exercise five times per week and a maximal power output assessment \( P_{\text{max}} \) was done at the beginning of each week. The \( P_{\text{max}} \) test was completed with the identical protocol as the VO\(_2\) max test using a bicycle ergometer. Muscle biopsies were taken using the percutaneous needle method at rest, before, and after the training period. Yfanti et al. (2009) did not find changes in maximal oxygen consumption, maximal power output, or lactate threshold workloads, which are commonly used measurements of aerobic activity. No significant differences between the supplemented and placebo group were seen in the mitochondrial enzymes measured from the muscle biopsies such as citrate synthase and \( \beta \)-hydroxyacyl-CoA dehydrogenase. Supplementing vitamin C and vitamin E in individuals with adequate concentrations of the micronutrients had no effect on physical acclimatization to endurance training (Yfanti et al., 2009).

The Invecchiare in Chianti (InCHIANTI) prospective population-based study in Italy quantified the association of plasma antioxidant concentrations and antioxidant dietary intakes with muscular strength and physical performance in
1,156 elderly persons aged 65-102 years (Cesari et al., 2004). Physical performance was assessed by walking speeds, ability to stand from a chair, knee extensions, and capability to uphold balance in gradually more difficult positions. Dietary intakes of micronutrients were collected using the validated European Prospective Investigation into Cancer and Nutrition (EPIC) questionnaire. Higher daily intakes of vitamin C were significantly associated greater knee extension strength and physical performance (Cesari et al., 2004). A significantly positive association linking plasma antioxidant concentrations to muscular strength and physical performance was found. However, the cross-sectional study design did not allow for the assessment of variation in daily nutrient intake on physical performance, and a large portion of the subject population in the study tended to be overweight hence possibly distorting results (Cesari et al., 2004).

Peake (2003) discusses the controversy of multiple studies with differing results of vitamin C plasma concentrations after acute bouts of exercise; some studies have seen a rise in plasma concentrations whereas others have observed a decline. Varying levels of oxidative stress caused by a particular exercise in different studies could account for variability of results of amounts of vitamin C released during physical activity in response to a potential oxidant-antioxidant equilibrium challenge occurring in the body (Peake, 2003).

**Physical Activity Levels Related to Dietary Intake of Vitamin C**

An epidemiological study using a Spanish cohort consisting of 41,447 people (25,813 women and 15,634 males) of the European Prospective Investigation on Cancer (EPIC) evaluated the correlation between different
physical activity levels and dietary intake patterns (Tormo et al., 2003). Dietary intake information for over a year-long period was gathered by validated computer questionnaires. Intake of total energy, food groups, macronutrients, alcohol, vitamins and pro-vitamins including vitamins A, C, E, α- and β-carotenes, lutein, and lycopene was determined. Subjects were given a questionnaire on seasonal physical activities levels of that consisted of sports by the definition of activity that required ≥6 metabolic energy equivalent task (METs) values (football, aerobics, swimming, tennis, gymnastics, etc.) and activities such as walking, cycling, gardening, domestic chores, and do-it-yourself activities than were <6 METs. Tormo et al. (2003) found that people who were physically active consumed more fruits, vegetables, dairy, and fish, and consumed less alcohol, meat, tubercles, and cereals; men and women had similar eating patterns except that women consumed more added fats and sweets. Men and women who were physically active had higher nutrient intake levels. By observing the average gender-weighted percent change in men from comparing a categorized low physical activity level (defined as >30 min – 2 hours/week) to a high physical activity level (defined as >3 hours/week) there was a 10% increase in vitamin C consumption. Subjects in the low physical activity group had a mean daily vitamin C intake of 161 mg/d whereas the high physical activity group had a mean daily vitamin C intake of 165 mg/d (Tormo et al., 2003). The approximation of mean daily intake of vitamin C was estimated by means of a Food Composition Table (FCT) specially compiled for the study from data gathered from subject interviews of habitual dietary intake over a prior year.
period. The results of this study may not show as great of a variance in dietary
intakes as other studies due to potential social desirability on the questionnaires
and that most individuals in the Spanish cohort tended to follow a Mediterranean-
type diet which consists of higher levels of fruits and vegetables (Tormo et al.,
2003).

A cross-sectional assessment of 2,404 Portuguese Caucasian adults in
Porto, Portugal found that higher levels of physical activity were related to higher
intakes of micronutrients (Camões & Lopes, 2007). The study collected an
estimated dietary intake from the previous year using a validated semi-qualitative
food frequency questionnaire and analyzed nutrient intake through Food
Processor Plus (ESHA Research) based on a version that was modified for typical
Portuguese foods. Physical activity levels were measured by calculating METs
based off data submitted from the subjects completing the EPIPorto Physical
Activity Questionnaire. Subjects were classified into two categories: active (≥4
METs) or sedentary (<4 METs). After adjusting for confounders such as age,
education, and BMI, a positive relationship was seen between increased intake of
vitamin C and activity in the male group. This study showed results similar to
multiple other studies. It is important to note that the EPIPorto Physical Activity
Questionnaire was based on the EPIC questionnaire, but it was not validated.
Using only two categories, active and sedentary, may have been a limitation to
this study; however, Camões and Lopes (2007) state that the prevalence of
sedentarism was so high among the subject population that a division between
moderate and vigorous activity would have resulted in very small percentages of the subject populations.

A study utilizing five subjects from the Iowa State Penitentiary found performance changes in men with varying vitamin C body pool sizes (Kinsman & Hood, 1971). Physical performance changes were seen in subjects with lower concentrations of vitamin C body pool size ranging from 190 mg to 63 mg. Physical performance changes were measured by using the Fleishman Physical Battery Tasks including bending movements or twisting of the legs and other psychomotor tasks. The Fleishman Physical Battery Tasks measured physical performance by the following tasks: balancing (eyes opened and eyes closed), leg lifts, broad jumps, extent flexibility (ability to rotate the back and trunk muscles maximally), dynamical flexibility (ability to flex moments rapidly and repeatedly), cable jumps (ability to coordinate simultaneous body movements), push-ups, and hand grips (Kinsman & Hood, 1971). Subjects with a higher vitamin C body pool [mean value: 1,314 mg] had statistically significant higher scores in physical performance activities that involved frequent bending at the knees or twisting of the legs than subjects with a lower vitamin C body pool [mean value: 161 mg]. Clinical symptoms such as musculoskeletal manifestations can develop when vitamin C body pool sizes are less than 300 mg; symptoms include arthalgia in the knees, wrists, and ankles and ultimately bleeding can occur within the joints (Fain, 2005). Arthropathy of the joint in the legs in vitamin C depleted Iowa State Penitentiary subjects was observed in the study (Kinsman & Hood, 1971).
Nutritional status by physical activity levels was analyzed in a cross-sectional study using 287 elementary school students aged 10-12 years in Seoul, Korea (Kim, Kim, Kim, Kim, & Lim, 2010). Anthropometric measurements of BMI-for-age, weight, height were taken. Physical activity levels were assessed using a modified Godin Leisure-Time Exercise Questionnaire (GLTEQ) and dietary intake was recorded using the 24-hour food recall method. Students had a mean BMI of 18.7 kg/m² and 77.6% of the boys and 82.6% of the girls were of normal weight (Kim et al., 2010). The number of students classified as "active", "moderately active", and "sedentary" using the GLTEQ was considerably consistent throughout the three categories in both boys and girls. The physical activity categories were determined by summing the frequency, length of time, and intensity of performed physical activities in a week-long time period and the number of months the students had been participating in physical activities was also taken into account. Analysis of dietary intake revealed that total energy intake was greater in moderately active boys as opposed to sedentary boys ($P=0.0074$). Nutrient density was calculated and intakes of vitamin C, iron, vitamin A, folate, and calcium per 1000 kcal consumed was higher in active boys than in moderately active boys although the difference was not statistically significant for any nutrients except iron. There was no difference in vitamin C, iron, and calcium intakes in girls. The elementary school-aged students in this cross-sectional study, regardless of their physical activity levels, had lower nutrient intakes than the Korean reference values for specified micronutrients (Kim et al., 2010). Even though the students were not consuming recommended
levels of specific micronutrients, the results showed that male students who were
more physically active were consuming greater intakes of vitamin C, calcium, and
iron.

**Metabolic Energy Equivalent Tasks**

A metabolic energy equivalent task (MET) value can be used to compute
energy expenditure utilized during physical activity. The term MET can be
defined as the rate of energy expenditure during activity in comparison to the
resting metabolic rate (Camões & Lopes, 2007). The resting metabolic rate that
can be acquired during sitting in a restful, quiet environment is considered 1 MET
(Ainsworth et al., 2000). Aerobic physical activities are classified as ≥4 METs.
According to recommendations in the literature, a range of 4-6 METs is specified
as moderate physical activity intensity (Perkins et al., 2009).

**Godin Leisure-Time Exercise Questionnaire**

The Godin Leisure-Time Exercise Questionnaire (GLTEQ) is a simple,
reliable and valid questionnaire that is composed of two questions to help analyze
a person’s leisure-time activity level. Leisure-time activity is defined as typical
physical activity with no-specified time constituent (Motl & Snook, 2008). The
reliability and concurrent validity of the GLTEQ were determined using values of
body fat and VO2 max of 306 healthy adult male and female participants based off
of respective Durnin and Womersley skinfold equations and the laboratory
adaptation of the Canadian Home Fitness Test (Godin, 1985). Reliability
coefficients for the functions representing the classifications of body fat and VO2
max were 0.85 and 0.83, respectively (Godin, 1985). The first item on the
GLTEQ measures average frequency of light (minimal effort), moderate (not to point of exhaustion), and strenuous exertion of leisure-time physical activity over a week-long period (Valenti et al., 2008). The average frequencies from the specified week-long period are multiplied by a corresponding MET: 3 [light], 5 [moderate], and 9 [strenuous], respectively, hence resulting in a person’s total activity performance level. The second question observes the incidence of engaging in leisure-time physical activities long enough to begin sweating within a week-long period (Motl & Snook, 2008). The GLTEQ is a useful tool in evaluating leisure-time physical activity and offers the prospect of investigating changes of leisure-time physical activity throughout the course a specified period of time.

Vitamin C—Promoting Mental and Physical Health

Depression has been estimated to be the number one cause of disability globally, and in the United States, by the US National Institute of Mental Health and the World Health Organization (Kemper & Shannon, 2007). Mental and physical health has been linked. Four lifestyle essentials have been promoted as necessary for healthful moods: optimum nutrition, exercise with balance of soothing sleep, healthy environment (including external atmospheric surroundings and supportive psychosocial surroundings), and participation in mind-body therapies (meditation and relaxation techniques) (Kemper & Shannon, 2007).

Effects of Physical Activity and Mood State

Moderate physical activities are known to provide positive health benefits. Positive physical and cognitive health states, such as improved self-confidence,
can be the result of participation in physical activity (Coble, Rhodes, & Wharf Higgins, 2009). In 2001, Nabetani and Tokunaga gave account of heightened benefits of short-term running on instance of congenial mood and lessened anxiety (Sakuragi & Sugiyama, 2006). In a 4-week, randomly-controlled, trial examining subjective symptoms of mood states by assessment of the Profile of Mood States (POMS) questionnaire, an improved mood state was seen in a walking group that was assigned to walk at a speed of 6 km/hr for one hour six times a week (Sakuragi & Sugiyama, 2006).

A randomized controlled 8-week study evaluated the use of three different treatments for clinically depressed adults over 60 years of age. The three treatments included high intensity progressive resistance training (PRT), low intensity PRT, and standard general practitioner care (Singh, Stavrinos, Scarbek, Galambos, Liber, & Fiatarone Singh, 2005). Subjects participated in exercise machine workouts three days per week for eight weeks. Resistance was fixed at 80% maximal load (weight to be lifted one time) for the high PRT group. Subjects were able to do three to eight repetitions per machine and load was increased at each visit. A fixed resistance at 20% maximal load was set for subjects in the low PRT group and load was not increased throughout the study. Subjects assigned to the standard general practitioner care group were to continue with normal care of their depression as controlled by his or her physician but asked not to start any new exercise programs during the study. Depression was measured by the Hamilton Rating Scale of Depression (HRSD), which is a recommended therapist-rated measure for depression (Singh et al., 2005). Using a
repeated measures ANOVA model, the researchers found that subjects in the high PRT group had more than double the relative response (% change) in HSRD scores (initial: 25 ± 8%; final: 52 ± 7%) in the high HRT group which was much larger than the relative response in the low PRT or standard general practitioner care group. The study concluded that high intensity resistance training was a more effective treatment than low intensity resistance training or traditional care by a general practitioner in older adults (Singh et al., 2005).

McKercher, Schmidt, Sanderson, Patton, Dwyer, and Venn (2009) conversely found that a group of men with a higher percentage of depression (n=212, 8.02% depression) in a cross-sectional design study took more steps (7,500-9,999 steps/day) measured on a pedometer than other groups. This may potentially be attributed to more pacing, but not necessarily to more active physical activity. Two groups were categorized in the study to have higher numbers of steps per day with lower percentages of depression (n=151, 5.30% depression, 10,000-12,499 steps/day; n=113, 4.42% depression, 12,500+ steps/day). The study was comprised of 794 men. Due to a low prevalence of depression in men in the study, statistical power was constrained to further investigations among these associations (McKercher et al., 2009).

**Effects of Vitamin C and Mood State**

Vitamin C has been investigated to have anti-depressive effects. While some studies have found vitamin C to aid in depression there has not been enough evidence to make a sound conclusion indicating a direct link. Three patients with idiopathic depression, ranging 7-29 years old, were treated for two weeks with
intravenous vitamin C (50 mg/kg/day) and complete convalescence was observed in all patients throughout the treatment period (Cocchi, Silenzi, Calabri, & Salvi, 1980). Intraperitoneal injection of vitamin C in six adult Swiss mice found anti-depressive effects (Binfaré et al., 2009). The tail suspension test (TST) and forced swimming test (FST) were used in the evaluation. The TST and FST are commonly performed behavioral tests used to estimate the effectiveness of an antidepressant therapy or treatment (Binfaré et al., 2009). Results found that intraperitoneal injected vitamin C generated an anti-depressive effect in the TST, which is dependent of the monoaminergic system which is comprised of neurons in the brain. This system transmits monoamine neurotransmitters such as dopamine, norepinephrine, and serotonin.

A double-blind clinical trial evaluated the anti-depressive effect of vitamin C and vitamin D on mood in 88 acutely hospitalized patients using the POMS questionnaire (Zhang, Robitaillie, Eintracht, & Hoffer, 2010). Of 32 patients that completed the study 62.5% had plasma vitamin C concentrations of <28.4 µmol/L [considered depleted] and 12.5% had <12.5 µmol/L [considered deficient]; 81% of the patient had plasma 25-hydroxyvitamin D concentrations <75 nmol/L [considered deficient]. Patients were randomized into two groups and treated with either 500 mg vitamin C twice daily or 1000 IU vitamin D twice daily for 5-10 days depending on treatment use of vitamin C or vitamin D in the hospital before the beginning of the study. Results found that ingestion of vitamin C for an average of 8.7 days tripled vitamin C plasma concentrations [initial (µmol/L): 26.3 ± 20.1; final (µmol/L): 96.4 ± 35.5] and was related to a 34% decrease in
total mood disturbance score \((P=0.013)\) (Zhang et al., 2010). Vitamin C was found to have an anti-depressive effect in the acutely hospitalized patients. Vitamin D concentrations also increased in patients consuming the micronutrient but no effect was found to be associated with mood.

A prospective study by Evans-Older, Eintracht, and Hoffer investigated the metabolic origin of low vitamin C concentrations in acutely hospitalized patients and evaluated their mood states (2010). Thirty-four participants with low vitamin C plasma concentrations \((16.7 \mu\text{mol/L} \pm 12.7)\) that were comparable in age \((65 \pm 16\) yrs) completed a 7-day treatment which consisted of orally taking 500 mg of vitamin C twice daily. At the beginning and end of the 7-day treatment period the participants completed the POMS-Brief, a validated 30-item questionnaire adapted from the original 65-item questionnaire, and a fasting blood sample. At the end of the treatment period the mean plasma vitamin C concentration of the 34 participants had increased to \(71.0 \mu\text{mol/L} \pm 30.9\) [normal range] and total mood disturbance score was reduced by approximately one-third which was found to be statistically significant \((P=0.008)\) (Evans-Older, Eintracht, & Hoffer, 2010). Likewise, an inverse connection between increase in total mood disturbance score and in degree of rise in vitamin C plasma concentrations \((n=34, r=0.444, P=0.009)\) was observed (Evans-Older, Eintracht, & Hoffer, 2010).

A study with five healthy subjects from the Iowa State Penitentiary using controlled vitamin C deprivation found changes in behavior between different vitamin C body pool sizes (Kinsman & Hood, 1971). Subjects with a high vitamin C body pool size with a mean value of 1,314 mg as opposed to subjects
with a low vitamin C body pool size with a mean value of 161 mg showed statistically significant differences in results in behavioral measures such as depression, hypochondriasis, hysteria, and social introversion (Kinsman & Hood, 1971). The subjects with larger vitamin C body pool sizes showed less signs of depression and were more social.

High doses of vitamin C improved mood in male and females participants [mean age 24.4 years] consuming a 3,000 mg/day sustained-release vitamin C capsule for 14 days (Brody, 2002). Participants with ascertained plasma vitamin C concentrations were divided into two groups, a placebo (n=39) and a VC (n=42), took the Beck Depression Inventory questionnaire at the beginning and end of the trial. At the end of the trial period, a larger decrease in Beck Depression Inventory scores was seen in the VC group [age-adjusted mean point change: VC= -1.56, placebo= -0.16] (Brody, 2002).

**Profile of Mood States—POMS**

The Profile of Mood States (POMS) is a factor-analytically derived measure used to evaluate marked ephemeral mood states in individuals (Baker, Denniston, Zabora, Polland, & Dudley, 2002). The POMS uses a 65-adjective item list that is to be indicated on a five-point Likert scale ranging from “not at all” to “extremely” of how an individual has felt over the past week. It quantifies six mood states: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment (McNair, Lorr, & Droppleman, 1992). A total mood disturbance (TMD) can also be calculated from the POMS by subtracting Vigor-Activity from the sum of the other five sub-
scales. The POMS is a widely used test and has been validated to use in samples of adult and geriatric populations, patients with medical conditions such as arthritis and HIV infections, and among cross-cultural population segments (Nyenhuis, Yamamoto, Luchetta, Terrien, & Parmentier, 1999; Yeun & Shin-Park, 2006).

**The Importance of Adequate Vitamin C Status and Obesity**

**Trends in Obesity**

Obesity in the United States and across the globe has been a common concern in recent decades. Obesity has been linked to chronic conditions such as type 2 diabetes, cardiovascular disease, and metabolic syndrome, and premature mortality (Lee et al., 2010; Ford, Zhao, & Tsai, 2010). NHANES saw statistically significant increases in obesity from 1976-1980 and 1988-1994 in all sex and age categories (Flegal, Carroll, Ogden, & Curtin, 2010). Analyses from 1999-2000 showed statistically significant increases in obesity in all sex and age categories except men aged 40-59 years (Flegal et al., 2010). No significant changes from NHANES data was seen for men or women in 2003-2004 and 2005-2006 hence suggesting a probable stabilization in the rising obesity trend (Nguyen & El-Serag, 2010). Even though the rate of obesity does not seem to be rising as greatly as it has been past decades, a large proportion of Americans are still considered obese. The incidence of obesity in 2007-2008 was 32.3% for adult men and 35.5% for adult women (Flegal et al., 2010). An increase of obesity in children has also been documented. Lee et al. reported that children born in more recent cohorts are becoming more obese in larger numbers at a younger age.
This in turn may lead to earlier development of obesity-related chronic conditions and possibly lower life expectancy or quality of life (Lee et al., 2010).

There are differing beliefs on the potential causes of obesity. A study surveying participants from the 2007 Health Information National Trends Survey (HINTS) found many interesting causal beliefs of obesity (Wang & Coups, 2010). Individuals who were obese tended to believe that obesity was inherited as opposed to those who were not obese; women also tended to feel that obesity was inherited more than men. People with a college-level education tended to have a stronger belief that obesity could be caused by overeating and lack of physical activity than those with a high school education level; some individuals also believed that genetics contributed to obesity in an individual (Wang & Coups, 2010). In addition, demographics played a role in casual beliefs of correlating obesity to physical activity levels. Females, people older in age, a lower education level, being obese, and those believing that obesity was inherited engaged in lower levels of physical activity; however, those who believed that obesity was caused by overeating and not exercising engaged in more physical activity (Wang & Coups, 2010).

Neighborhood socioeconomic status has been analyzed for accessibility to physical activity resources. A study indentifying resource availability, resource accessibility, and neighborhood socioeconomic status found that physical activity levels by individuals in a Midwestern U.S. city was impacted by multiple factors (Estabrooks, Lee, & Gyurcsik, 2003). The study found that approximately 36% of the physical activity resources were pay-for-use and there were more free-for-
use facilities in higher socioeconomic status areas as opposed to low- and medium socioeconomic areas. The researchers concluded that individuals who live in neighborhoods of a lower economic status may have limited capability to control their physical activity due to residing in an inaccessible environment (Estabrooks et al., 2003). Though the researchers did a thorough investigation of resource availability for physical activity, it was only analyzing one Midwestern U.S. city and this study cannot be generalized to all cities in the United States as a whole.

**Influence of Vitamin C on Adiposity**

An inverse relationship between plasma vitamin C concentrations and adiposity has been strongly authenticated in research. Johnston, Beezhold, Mostow, and Swan in a cross-sectional study documented plasma vitamin C is inversely related to BMI, body fat percentage, and waist circumference in both men and women (2007). Increased BMI levels have been linked to elevated incidences of chronic diseases such as type 2 diabetes, hypertension, and dyslipidemia (Crawford et al., 2010). Likewise, an investigation of the relationship between waist circumference and mortality in the Cancer Prevention Study II Nutrition Study composed of 48,500 men and 56,343 women found a two-fold higher mortality risk for both men and women with a larger waist circumference (Jacobs et al., 2010). Waist circumferences were significantly associated with increases in mortality rates in all waist circumference categories >75 cm for women and in all weight circumference categories >90 cm for men (Jacobs et al., 2010). The clinical recommendation cut point for abdominal
obesity in women is ≥88 cm and the clinical recommendation cut point for abdominal obesity in men is ≥102 cm (Jacobs et al., 2010).

In a study using rats, vitamin C supplementation decreased body fat mass without having an effect on energy intake (Campión, Milagro, Fernández, & Martinez, 2008). Wistar eight-week old rats were divided into three groups and provided different diets. One group was fed a “cafeteria” high-fat diet [59.2% lipids, 9.3% protein, 31.5% carbohydrates]. The second was fed a control diet [10.3% lipids, 16.6% protein, 73.1% carbohydrates]. The third group was fed the same proportion of lipids, protein, and carbohydrates as the control diet group but was supplemented with 750 mg/kg body weight vitamin C per day. After 56 days, the rats consuming the control diet with vitamin C had decreased subcutaneous and retroperitoneal fat pads by 41% and 21%, respectively (Campión et al., 2008). Vitamin C supplementation may be an inventive approach in managing immoderate weight gain due to the fact that the course fat deposition may not be only driven by calories consumption (Campión et al., 2008).

**Closure**

Vitamin C has many important physiological functions. The micronutrient is a reducing agent, a free radical scavenger, and an enzyme cofactor for numerous reactions that occur within the body (Schleicher et al., 2009). Protective effects against cancer, cataracts, asthma, and cardiovascular disease may be provided by the powerful antioxidant (Thompson, 2007).
Adult men between the ages of 20-39 years and ≥60 have a higher prevalence of vitamin C deficiency than women of the same age and NHANES III documented that approximately one-fifth of men and women aged 25-44 years were vitamin C deficient (Hampl et al., 2004). Males between the ages of 12 to 59 years are inclined to have lower serum concentrations of vitamin C than women (Schleicher et al., 2009). Furthermore, college-aged students typically have low plasma concentrations of vitamin C due to low intake of foods plentiful in vitamin C. (Johnston, Solomon, & Corte, 1998).

A majority of research has focused on impending health benefits of vitamin C supplementation by endurance athletes. Research in this realm has consistently found disputed results. Vitamin C supplementation has been observed to reduce exercise-induced lipid peroxidation during high intensity exercise, hence notionally reducing tissue damage and improve muscle recovery (Nakhostin-Roohi, Babaei, Rahmani-Nia, & Bohlooli, 2008; Cesari et al., 2004; Clarkson & Thompson, 2000). In opposition, vitamin C has been scrutinized to hinder endurance capacity in exercising subjects by reducing the oxidative stress that triggered gene transcription and adaptive training effects (Gomez-Cabrera et al., 2008).

More research is needed to investigate the relationship between vitamin C status and leisure-time physical activity levels. Regular physical activity has been observed to provide positive psychological benefits such as a amiable mood state and decrease of anxiety (Sakuragi & Sugiyama, 2006). Singh et al. (2005) in a randomized controlled trial with older adults reported that aerobic exercise and
weight-training is an effective alternative treatment for depression. Physically active men and women that show fewer signs of depression also have lower rates of chronic diseases and all-cause mortality (Carlson, Densmore, Fulton, Yore, & Kohl, 2008; Binfarè, Rosa, Lobato, Santos, & Rodriques, 2009). Hence taking part in physical activity is favorable for improving health markers such as lipid levels and insulin sensitivity (King et al., 2009). It is speculated that physical activity can improve chronic disease risk in obese individuals; however the capability of completely eliminating these risks is not clear (Lee et al., 2008). Vitamin C has the potential to provide improved health parameters and increased mood state in young men who have low plasma vitamin C concentrations.
Chapter 3

Methods

Subjects & Study Design

Healthy men, 18-35 years of age, were recruited at the Polytechnic campus of Arizona State University via flyer distribution and college department ListServes. The men were screened by a short phone interview for regular smoking ($\geq 10$ cigarettes per day), BMI $> 34$ kg/m$^2$, use of dietary supplements containing $> 60$ mg/day of vitamin C, and/or prescription medications for chronic conditions. Training athletes or those who engaged in purposeful exercise more than five times a week were also excluded from the study. Eligible volunteers were invited to the initial screening (study visit 1) where they read and signed a written consent. A postprandial blood sample (no food or drink with the exception of water for 5 hours) was collected for vitamin C assessment. To continue in the study, the subject's plasma vitamin C concentration needed to be $< 0.80$ mg/dL indicating below average vitamin C status. A general medical history questionnaire was completed. Height and weight were measured by a stadiometer and calibrated scale. BMI (kg/m$^2$) was calculated by use of a bioelectrical impedance scale (Tanita). Lastly, the Rapid Eating Assessment for Participants-Shortened Version (REAPS) and a 24-hour recall were completed to estimate dietary quality and vitamin C intake. The study was approved by the Institutional Review Board at Arizona State University.

Subjects entered into the 8-week trial were stratified by age, BMI, smoking status, and plasma vitamin C concentrations and randomly assigned to
either a control (CON) or experimental group (VTC). This was a randomized, double-blinded, parallel arm trial; subjects were unaware of the treatment they received. Subjects were informed that they were not to consume any sort of juice or fruit-flavored drinks over the course of the 8-week study. Subjects were given capsules that were identical in appearance and instructed to take two capsules daily; one capsule in the morning and one in the evening. If the subject noted any gastrointestinal distress, they were advised to consume the pill with food. The VTC capsules contained 500 mg vitamin C; the CON capsules, identical in appearance to the vitamin C capsules, were a placebo capsule composed of white flour.

A power analysis was calculated using a probability of 0.05 and a power of 0.8. Utilizing previous research data in a similar population, the sample size was calculated using a standard deviation of 0.2 mg/dL for plasma vitamin C and a 0.2 mg/dL change in plasma vitamin C concentration. A total of 34 subjects were deemed the appropriate sample to enter the two-treatment parallel-design study. An additional power analysis was calculated to verify correct sample size in terms of physical activity. A probability of 0.05 and a power of 0.8 were used. A standard deviation of 2.5 hours of physical activity and a 2.5 hour of physical activity fluctuation were utilized to determine sample size. Thirty-four subjects were deemed the appropriate sample using a verified sample size calculator to enter the two-treatment parallel-design study; thus the power analyses corroborated 34 subjects to be an appropriate sample size. Fifty-four male subjects were interviewed by phone. A total of 43 subjects were invited to
partake in the initial screening and 30 subjects consented to participate in the screening (visit 1). Thirty subjects met all inclusion criteria and were invited to join the study. One subject (CON) was withdrawn from the study due to lack of compliance regarding capsules ingestion twice daily and refraining from fruit juices. Thus 29 subjects completed the study; 15 VTC and 14 CON subjects. Subjects were to return any untaken capsules to the study site at week 4 (study visit 4) and week 8 (study visit 5). Capsule compliance from the subjects was 94%.

At the baseline visit (study visit 2) each subject was given a survey booklet that contained the Godin Leisure-Time Exercise Questionnaire and a FFQ. Subjects were instructed to record their leisure-time physical activity level and dietary intake at the end of each week. During week 1 (study visit 3) subjects brought survey booklets in for review of compliance and accuracy in reporting. The survey booklet was turned in at week 4 (study visit 4) and a new survey booklet used for weeks 5-8 was given to the subjects that was to be filled out and returned at week 8 (study visit 5). At study visits 2, 4, and 5, a fasting blood sample (no food or drink with the exception of water for 8 hours) was collected, and subjects completed the POMS. The survey booklet also contained daily copies of the Wisconsin Upper Respiratory System Survey-21 that was used as a measuring tool for a separate study.

**Blood Analysis**

Plasma vitamin C was determined using the 2,4-dinitrophenylhydrazine method (Omaye, Skala, & Jacob, 1986). A 1 ml aliquot of plasma was mixed
with 1 ml ice-cold 10% trichloroacetic acid. Following centrifuge (3500 x g, 0˚C), the supernatant was frozen (-80˚C) and vitamin C analysis was performed on the sample within one month of storage (Johnston, Martin, & Cai, 1992). Blood histamine levels were also determined for a separate study.

Physical Activity

Leisure-time physical activity levels were assessed using the self-administered Godin-Leisure Time Exercise Questionnaire (GLTEQ). This tool measures the average frequency of light (minimal effort), moderate (not to point of exhaustion), and strenuous exertion of exercise over a week-long period (Valenti et al., 2008). The average frequencies from the week-long period were multiplied by 3 [light], 5 [moderate], and 9 [strenuous] metabolic equivalents, respectively, thus resulting in each subjects’ total physical activity performance level. The second question assesses the occurrence of engaging in normal activities long enough to begin sweating over a week-long period (Motl & Snook, 2008). Body fat and VO₂ max measurements of 306 healthy adult male and female participants based off of respective Durnin and Womersley skinfold equations and the laboratory adaptation of the Canadian Home Fitness Test were used to determine the reliability and concurrent validity of the GLTEQ (Godin, 1985). Reliability coefficients for the functions corresponding to the classifications of body fat and VO₂ max were 0.85 and 0.83, respectively (Godin, 1985).
Mood State

Mood state was assessed using McNair’s Profile of Mood States (POMS) self-administered questionnaire. The POMS is composed of 65 adjectives illustrating feelings and moods a subject has felt over the past week. Each of the 65 adjectives uses a 5-point Likert scale ranging from “not at all” to “extremely”. The POMS reflects six mood states including, Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment (McNair, Lorr, & Droppleman, 1992). Total mood disturbance (TMD) was also calculated by subtracting Vigor-Activity from the sum of the five sub-scales. The TMD measurement is considered reliable due to its intercorrelations with the initial six mood states of the POMS (Baker et al., 2002). Subjects completed the POMS at baseline, week 4, and week 8 (study visits 2, 4, and 5). Based on data from several validity studies and normative samples, reliability coefficients for internal consistency were documented near 0.90 or above within the six mood state sub-categories (McNair, Lorr, & Droppleman, 1992).

Statistical Analysis

Data were reported as the mean ± standard error (SE). Descriptive statistics were used to report subjects' average age, height, weight, BMI, body fat percentage, plasma vitamin C concentration, and dietary intake of vitamin C. Leisure-time physical activity data and POMS scores were transformed to achieve normality. Leisure-time physical activity data were analyzed using the independent t test. POMS data were analyzed utilizing repeated measures.
ANOVA. Statistical analysis was performed using SSPS Statistical Analysis System 19.0. Differences were considered significant at $P \leq 0.05$. 
Chapter 4

Results

Descriptive Characteristics of Subjects

Subjects' age and characteristic data at baseline are shown in Table 1. Male subjects (n=29) were between the ages of 18 and 32 years, with a mean age of 23.2 years. No characteristic data at baseline significantly differed between the two groups except height ($P=0.026$). Subjects had a mean weight of 180.2±3.9 lbs, with a range of 135.2-223.4 lbs. Their mean BMI of 25.0±0.7 kg/m$^2$ was in the overweight category of BMI classification which is 25.0-29.9 kg/m$^2$. Subjects' BMI ranged from 18.6-33.9 kg/m$^2$. Their mean body fat percentage was 18.8±1.2, with a range of 6.6-30.8. Subjects had a mean baseline vitamin C plasma concentration of 0.52±0.03 mg/dL. There was no significant difference between the VTC and CON vitamin C plasma concentrations at baseline ($P=0.649$). The VTC (n=15) had a mean baseline vitamin C plasma concentration of 0.53±0.04 mg/dL and the CON (n=14) had a mean baseline vitamin C plasma concentration of 0.51±0.04 mg/dL. Groups did not significantly vary in dietary intake of vitamin C as reported by a 24-hour recall ($P=0.372$). The VTC had a mean dietary intake of vitamin C of 138±29 mg and the CON had a mean dietary intake of vitamin C of 103±24 mg.
Anthropometrics, dietary quality, and vitamin C plasma concentrations of the subjects are presented in Table 2. The VTC increased their plasma vitamin C concentrations in week 4 and week 8, whereas the CON maintained a relatively consistent vitamin C plasma concentration. Plasma vitamin C concentrations at week 4 and at week 8 were controlled for amount of vitamin C ingested weekly from food. The data between groups were statistically significant at week 4 ($P=0.026$) and week 8 ($P=0.026$). At week 4 the VTC's mean vitamin C plasma concentration increased to 0.73±0.5 mg/dL from 0.53±0.04 mg/dL at baseline. The CON had a week 4 vitamin C mean plasma concentration of 0.56±0.05 mg/dL, which is similar to its mean baseline value of 0.51±0.04 mg/dL. At week 8 the VTC had a mean vitamin C plasma concentration of 0.74±0.5 mg/dL, whereas the CON had a mean vitamin C plasma concentration of 0.57±0.06 mg/dL. In addition to significant differences between groups, trends were observed with an increasing vitamin C plasma concentration at week 4 and week 8 in each group (0.05<$p$<0.10).
The average FFQ from weeks 1, 3, 5, and 7 were analyzed to examine the milligrams of vitamin C that subjects were consuming from food each week. The dietary intake analysis indicated that there was no difference in weekly intake of vitamin C intake from diet between the VTC and CON ($P=0.217$). The examination of the REAPS self-administered assessment completed at baseline and week 8 exhibited a trend ($p>.05$ but $<.1$) in dietary quality; the VTC had a slightly higher dietary quality than the CON. The REAPS implied that the VTC and CON did not significantly differ in their dietary quality ($P=0.082$) throughout the course of the study. The relationship between plasma vitamin C concentration and dietary quality (as measured by REAPS) at baseline was investigated using Spearman’s rho correlation coefficient. There was no significant association between variables, $r=0.035$, $n=29$, $P=0.858$. The relationship between plasma vitamin C concentration and dietary intake vitamin C (milligrams consumed as measured by a 24-hour recall) at baseline was also investigated using Spearman’s rho correlation coefficient. There was no significant association between variables, $r=0.262$, $n=29$, $P=0.170$. 
<table>
<thead>
<tr>
<th>Scale</th>
<th>VTC (n=15)</th>
<th>CON (n=14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma VC&lt;sup&gt;a&lt;/sup&gt; concentration (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.53±0.04</td>
<td>0.51±0.04</td>
<td>0.649&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week 4</td>
<td>0.73±0.05</td>
<td>0.56±0.05</td>
<td>0.026&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week 8</td>
<td>0.74±0.05</td>
<td>0.57±0.06</td>
<td>0.026&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Weight (lbs)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>182.5±5.2</td>
<td>177.7±6.1</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>182.2±5.3</td>
<td>177.1±6.1</td>
<td>0.793&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week 8</td>
<td>181.3±5.4</td>
<td>177.1±6.2</td>
<td>0.637&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>24.5±1.0</td>
<td>25.6±1.1</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>24.9±0.9</td>
<td>25.5±1.1</td>
<td>0.240&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week 8</td>
<td>24.8±0.9</td>
<td>25.5±1.1</td>
<td>0.358&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>FFQ&lt;sup&gt;e&lt;/sup&gt; (mg of VC)</strong></td>
<td>92.73±13.60</td>
<td>101.26±11.88</td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>78.82±11.93</td>
<td>97.72±15.57</td>
<td></td>
</tr>
<tr>
<td>Week 5</td>
<td>100.41±11.02</td>
<td>90.18±18.26</td>
<td></td>
</tr>
<tr>
<td>Week 7</td>
<td>110.83±14.73</td>
<td>99.15±20.83</td>
<td>0.217&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>REAPS&lt;sup&gt;g&lt;/sup&gt; (dietary quality)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>31.13±0.79</td>
<td>30.71±0.92</td>
<td></td>
</tr>
<tr>
<td>Week 8</td>
<td>32.00±0.87</td>
<td>29.79±1.38</td>
<td>0.082</td>
</tr>
</tbody>
</table>

<sup>a</sup>VC=vitamin C  
<sup>b</sup>P value represents independent t-test  
<sup>c</sup>P value represents repeated measures ANOVA, change from baseline to week 4  
<sup>d</sup>P value represents repeated measures ANOVA, change from baseline to week 8  
<sup>e</sup>FFQ=food frequency questionnaire  
<sup>f</sup>P value represents repeated measures ANOVA, change from baseline to week 7  
<sup>g</sup>REAPS=Rapid Eating Assessment for Participants-Shortened Version  
<sup>*</sup>P<0.05
Physical Activity

The subjects’ leisure-time physical activity measured in METS hrs/week at baseline and average change in METS hrs/week during weeks 1-4 and weeks 5-8 using the Godin Leisure-Time Exercise Questionnaire (GLTEQ) are displayed in Table 3. Mild, moderate, and strenuous leisure-time physical activity were multiplied by 3, 5, and 9, respectively, to obtain METS hrs/week, and all activity categories were summed to attain total leisure-time physical activity level. Subjects in the VTC and CON did not vary significantly at baseline in mild or moderate leisure-time physical activity, but significant differences between groups at baseline in strenuous leisure-time physical activity ($P=0.049$) and total leisure-time physical activity ($P=0.024$) were observed. Therefore, in analyzing the average leisure-time physical activity between groups in weeks 1-4 and weeks 5-8, calculated METS hrs/week were adjusted for the related physical activity category at baseline. Independent t-tests were run as opposed to one-way ANOVAs because average activity for mild, moderate, strenuous, and total leisure-time physical activity categories for weeks 1 through 4 and weeks 5 through 8 were calculated for analysis of data. Although there was a significant difference between the VTC and CON in average activity during weeks 5-8 in strenuous leisure-time physical activity and in average activity throughout weeks 1-4 in total leisure-time physical activity, significance was not retained after adjusting for the related physical activity category at baseline. After controlling for baseline total leisure-time physical activity as well as diet quality, a statistically significant difference between the VTC and CON for total leisure-
time physical activity during weeks 5-8 \((P=0.017)\) was discerned. The VTC increased their total leisure-time physical activity by 47%, whereas the CON only increased their total leisure-time physical activity by 15%. Significant differences were seen between groups in the mild leisure-time physical activity category for average activity during weeks 1-4 \((P=0.019)\) and for average activity during weeks 5-8 \((P=0.006)\) after controlling for diet quality. The VTC increased their mild leisure-time physical activity by 54% from baseline to week 8. The CON decreased their mild leisure-time physical activity by 22% throughout the eight weeks. Significant differences were found between groups in the moderate leisure-time physical activity category for average activity during weeks 5-8 \((P=0.043)\). The VTC increased their moderate leisure-time physical activity by 56% from baseline to week 8. The CON increased their moderate leisure-time physical activity by 38% throughout the eight weeks.
Mood State

The subjects' mean POMS scores at baseline, week 4, and week 8 are shown in Table 4. The mean scores of the six mood states including, Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment for the VTC and CON at the three specified time periods are listed. Table 4 also shows the total mood disturbance (TMD) score which was calculated by subtracting Vigor-Activity from the sum of the other five sub-categories for the VTC and CON at baseline, week 4, and week 8. Analyses showed that the POMS scores did not vary between groups at baseline, week 4, or week 8. There was no statistical significance in TMD score between the VTC and CON at baseline, week 4 ($P=0.258$), or week 8 ($P=0.345$). Distribution of POMS scores for each mood state of the VTC and CON are provided in Figure 1. TMD
score distributions for the VTC and CON at baseline, week 4, and weeks 8 are shown in Figure 2. Analysis was also performed to observe time effects over the eight weeks for both groups together. At week 4 there were significant differences between groups in mood state sub-categories Tension-Anxiety ($P=0.037$), Depression-Dejection ($P=0.010$), and Fatigue-Inertia ($P=0.000$).

There was no significant difference in change over time between the VTC and CON from baseline to week 8 in any mood state except Fatigue-Inertia ($P=0.002$). However, there were improvements over time for both groups.

### Table 4. Profile of Mood States—mean scores at baseline, week 4, and week 8

<table>
<thead>
<tr>
<th>Mood States</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Scoring Range</th>
<th>Normative Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension-Anxiety</td>
<td>VTC (n=15)</td>
<td>7.47±1.93</td>
<td>4.07±0.88</td>
<td>6.13±1.52</td>
<td>0-36 12.9</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>8.64±1.43</td>
<td>7.86±1.68</td>
<td>7.14±1.07</td>
<td>0-36 23.5</td>
<td></td>
</tr>
<tr>
<td>Depression-Dejection</td>
<td>VTC (n=15)</td>
<td>5.60±0.83</td>
<td>3.18±1.13</td>
<td>4.60±1.97</td>
<td>0-60 13.1</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>8.50±2.56</td>
<td>7.21±1.01</td>
<td>6.21±1.93</td>
<td>0-36 26.5</td>
<td></td>
</tr>
<tr>
<td>Anger-Hostility</td>
<td>VTC (n=15)</td>
<td>5.80±1.09</td>
<td>3.00±1.13</td>
<td>4.27±1.74</td>
<td>0-48 10.1</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>10.86±3.16</td>
<td>7.98±2.37</td>
<td>5.00±1.12</td>
<td>0-36 26.3</td>
<td></td>
</tr>
<tr>
<td>Vigor-Activity</td>
<td>VTC (n=15)</td>
<td>18.73±1.18</td>
<td>19.80±1.47</td>
<td>19.07±1.54</td>
<td>0-32 15.6</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>17.71±1.79</td>
<td>16.57±1.51</td>
<td>17.64±1.55</td>
<td>0-32 15.9</td>
<td></td>
</tr>
<tr>
<td>Fatigue-Inertia</td>
<td>VTC (n=15)</td>
<td>6.00±1.11</td>
<td>3.40±0.86</td>
<td>3.87±1.05</td>
<td>0-28 10.4</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>10.14±1.70</td>
<td>5.93±1.37</td>
<td>6.07±1.30</td>
<td>0-28 10.0</td>
<td></td>
</tr>
<tr>
<td>Confusion-Bewilderment</td>
<td>VTC (n=15)</td>
<td>5.40±0.76</td>
<td>3.73±0.81</td>
<td>3.60±0.74</td>
<td>0-28 10.2</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>6.00±0.99</td>
<td>6.07±1.35</td>
<td>4.36±0.86</td>
<td>0-20 10.3</td>
<td></td>
</tr>
<tr>
<td>Total Mood Disturbance</td>
<td>VTC (n=15)</td>
<td>11.53±1.29</td>
<td>-2.67±4.30</td>
<td>2.73±6.86</td>
<td>0-200 10.0</td>
</tr>
<tr>
<td>CON (n=14)</td>
<td>26.43±8.89</td>
<td>18.43±9.27</td>
<td>11.14±5.59</td>
<td>0-200 10.0</td>
<td></td>
</tr>
</tbody>
</table>

Reported as mean±SE; mood states did not differ at baseline

*P* value represents independent t-test; transformed log values assessed

*P* value represents repeated measures ANOVA for interaction between groups, change from baseline to week 4 (time effect in brackets); transformed log values assessed

Scoring range provided by Profile of Mood States manual (McNair, Lorr, & Droppleman, 1992)

Profile of Mood States—College Student Normative Sample means (McNair, Lorr, & Droppleman, 1992)
Figure 1. VTC and CON POMS scores at baseline, week 4, and week 8 of each mood state category—Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment. Values are reported as means±SE. Differences between groups did not differ significantly at any point in time ($P>0.050$).
Figure 2. VTC and CON POMS TMD scores at baseline, week 4, and week 8. Values are reported as means±SE. Differences between groups did not differ significantly at any point in time ($P>0.05$).
Chapter 5
Discussion

This study investigated the effects of supplementing 500 mg of vitamin C twice daily in young men, 18-35 years, on self-reported physical activity levels and mood states. Subjects were given a survey booklet every four weeks of the study to log weekly leisure-time physical activity and food frequency intakes. Subjects' mood states were analyzed using the POMS at baseline, week 4, and week 8 of the study.

A total of 29 subjects completed this 8-week randomized, double-blinded, parallel arm trial in its entirety. The VTC (n=15) consumed a 500 mg vitamin C capsule twice daily whereas the CON (n=14) consumed a placebo capsule twice daily that was identical in appearance to the VTC capsule. The FFQ average weekly consumption of vitamin C (in milligrams) from food shows that subjects in the VTC and CON were consuming 96 mg and 102 mg vitamin C per day, respectively, throughout the eight weeks of the study. Therefore, both groups were consuming right around the RDA of vitamin C of 90 mg/day (Institute of Health) on a daily basis. However, there is a possibility that the subjects were not consuming as many milligrams of vitamin C per day as estimated by the FFQ. Errors and incongruities have been seen in estimating nutrient intakes from food composition databases due to assays used in assessing nutrient composition, date of the test foods, and increased import of international agricultural products into the United States (Merchant & Dehghan, 2006). College-aged individuals are recognized as a sub-class that has lower plasma vitamin C concentrations because
of a lessened consumption fruits and vegetables (Johnston et al., 1998). Subjects in the study were not restricted from eating foods containing vitamin C; however subjects were to refrain from drinking juice, fruit-flavored drinks, or supplements besides a multi-vitamin containing vitamin C during the course of the study. In the current study, there was a greater increase in plasma vitamin C concentrations in the VTC than the CON. There was no significant difference between dietary intakes of vitamin C between groups. Thus, it is apparent that the increase in vitamin C plasma concentration was due to the supplementation of vitamin C. Supplementing vitamin C helped to begin to replete subjects in the VTCs’ vitamin C body pools (Jacobs et al., 1987). At week 8 the VTC had increased their mean vitamin C plasma concentration to 0.74±0.5 mg/dL from 0.53±0.04 mg/dL at baseline, whereas the CON at week 8 had a vitamin C plasma concentration of 0.57±0.06 mg/dL from 0.51±0.04 mg/dL at baseline. A plasma vitamin C concentration of 1.0 mg/dL implies a full vitamin C body pool in a healthy, non-smoking man (Kallner, Hartmann, & Hornig, 1979).

The REAPS self-administered assessment revealed a trend (p>.05 but <.1) in dietary quality; the VTC had a slightly higher dietary quality than the CON (P=0.082) throughout the course of the study. In a majority of other studies dietary quality is not controlled for nor is the intake of fruits and vegetables. The REAPS is a validated assessment tool designed to quickly assess the relative intake of fat, fiber, cholesterol, sugar, and selected food groups as well as for estimating servings of fruit, vegetables, milk, and fat typically consumed by an individual (Segal-Isaacson, Wylie-Rosett, & Gans, 2004). The correlation
between the REAPS and well-validated Block 1998 Semi quantitative Food Frequency Questionnaire (Block 1998 FFQ) for vegetable servings and fruit servings are \( r = 0.503 \) and 0.506, respectively (Segal-Issacson et al., 2004). It was originally designed for primary care practitioners to quickly assess dietary and exercise patterns with patients (Segal-Issacson et al., 2004). Lower scores on the REAPS refers to a lower dietary quality. This study controlled for diet quality and showed no change in dietary intake of vitamin C throughout the eight weeks. Therefore, it can be determined that the vitamin C supplement increased the VTC’s plasma vitamin C concentration and that this increase was not due to dietary intake of the nutrient.

A majority of research between vitamin C supplementation and physical activity levels has examined outcomes of efficiency in endurance training. Positive and negative results have been observed. Currently, the best-controlled studies have deduced that vitamin C supplementation does not influence endurance or strength-training performance (Clarkson & Thompson, 2000). Research has shown that people who consume more vitamin C, through diet or supplementation, are inclined to participate in more physical activity (Lukaski, 2004; Tormo et al., 2003). A positive relationship between increased vitamin C intake and physical activity levels has been observed in males (Camões & Lopes, 2007). Similar results were found in a study using elementary-age students in Korea. Boys that were more physically active had higher intakes of vitamin C than those who were sedentary (Kim et al., 2010). Little research has been done
to observe the effects of vitamin C supplementation in means of participating in leisure-time physical activity.

Results of this study show that supplementing 500 mg vitamin C twice daily increased subjects' leisure-time physical activity. The VTC increased average activity per week ($P<0.050$) during weeks 1-4 in mild and during weeks 5-8 in mild, moderate, and total leisure-time physical activity categories. The fact that there were significant differences found in the mild, moderate, and total leisure-time physical activity categories could be because the VTC merely moved around more, hence increasing leisure-time physical activity levels, but they did not necessarily engage in more organized physical activity or extreme physical activity. Activities such as walking, cycling, gardening, domestic activities, and do-it-yourself chores are considered $<$6 METS, thus mild or moderate leisure-time activities, depending on the respective activity (Tormo et al., 2003). This increased movement may possibly be due to an increased concentration of norepinephrine in the vascular smooth muscle. Vitamin C is adept to reduce the oxidation rate of norepinephrine thus increasing the concentration of norepinephrine in the body (Dillon, Root-Bernstein, & Leider, 2004). Norepinephrine is part of the known “fight-or-flight” reflex in the body, and having a greater concentration of the catecholamine in the body may cause one to move around more. Another plausible reason as to why a subject may have moved around more could have been a possible increase of serotonin in the body. Vitamin C is a needed cofactor to convert 5-hydroxytryptophan to serotonin. Serotonin plays a role in the modulation of vasodilation of certain vascular beds.
depending on specific serotonin receptors located in vessel walls of smooth muscle tissue (Kaumann & Levy, 2006).

The GLTEQ is a constructive tool in evaluating leisure-time physical activity and allows an investigator to explore changes and examine a person’s leisure-time physical activity level. The reliability and concurrent validity of the GLTEQ were determined using values of body fat and VO\textsubscript{2} max of 306 healthy adult participants based off of respective Durnin and Womersley skinfold equations and the laboratory adaptation of the Canadian Home Fitness Test (Godin, 1985). Reliability coefficients for the functions representing the classifications of body fat and VO\textsubscript{2} max were 0.85 and 0.83, respectively (Godin, 1985). Because the GLTEQ was to be filled out by the subjects at the end of each week at their leisure, it cannot be ascertained that it was filled out completely accurate every week. It is possible that a subject may have forgotten to document a particular physical activity event he participated in. Also, subjects may not have always properly documented a physical activity he performed. Small bouts of activity, such as walking, for example, throughout one’s day should have been accounted for in the GLTEQ. It is possible that activities with less strenuous work could have been neglected in a subject’s documentation of activity on the GLTEQ. Furthermore, subjects in this study tended to participate in a large amount of strenuous leisure-time physical activity. The median high [strenuous] leisure-time physical activity reported for men in the 1990 decade is just over 80 MET*min/day, or approximately 9 MET*hrs/week (Talbot, Fleg, & Metter, 2003). The subjects in the current study reported much higher strenuous leisure-
time physical activity levels than the aforementioned value. Likewise, the median moderate leisure-time physical activity reported for men in the 1990 decade is approximately 250 MET*min/day, or approximately 29 MET*hrs/week (Talbot et al., 2003). The subjects in the current study were within realm of the reported median range for moderate leisure-time physical activity. There is a possibility that the subjects over-estimated their bouts of strenuous leisure-time physical activity while discounting there mild leisure-time physical activity.

Current research inspecting the role of vitamin C and physical activity is varied and conflicting. There seems to be no true agreement regarding the effects of vitamin C and capacity of physical activity. There has been little research performed on observing effects of vitamin C and leisure-time physical activity levels in individuals. Therefore, there remains a great need for future studies to investigate the role that vitamin C plays on leisure-time physical activity levels in individuals. Many individuals may benefit from studies exploring the use of vitamin C supplementation to promote increased leisure-time physical activity levels.

Anti-depressive effects have been observed with the aid of vitamin C. Studies using hospitalized patients have seen an inverse relationship between vitamin C plasma concentrations and total mood disturbance (Zhang et al., 2010, Evans-Older, Eintracht, & Hoffer, 2010). A larger vitamin C body pool has also been associated with fewer signs of depression and greater social interaction (Kinsman & Hood, 1971). Research has largely favored vitamin C to decrease
total mood disturbance and lessen depression in individuals. This study was unable to replicate those results.

Although an inverse relationship exists between increased vitamin C plasma concentrations and improved mood (Brody, 2002; Cocchi et al., 1980; Evans-Older, Eintracht, & Hoffer, 2010), this study did not find a significant relationship between increased plasma vitamin C concentrations and mood states. In five of the six mood states, including Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and in TMD, the VTC improved the specified mood state [decrease in score] at week 4 but then slightly decreased the specified mood state [increase in score] at week 8. The mood state Confusion-Bewilderment was the only sub-category that observed a gradual decrease, hence improvement of mood state, in the VTC. The CON, however, observed a trend of slight improvement [decrease in score] in mood state categories, Tension-Anxiety, Depression-Dejection, Anger-Hostility, and in TMD, from baseline, week 4 to week 8 (0.05 < p < 0.10). The CON had a considerably small reduction [increase in score] in mood state categories Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment. The VTC observed a much more extreme improvement in sub-categorical mood states than the CON from baseline to week 4.

One possible explanation for the improvement in mood states in a majority of mood state sub-categories for both groups could be that week 4 was the week following Arizona State University’s academic Spring Break. Therefore, when subjects reflected back to indicate their level of the given 65 adjectives listed on
the POMS for the previous week, they may have felt less stressed and more at ease during Spring Break week as opposed to weeks that they were in school. Furthermore, Week 8 for a majority of subjects was the week before academic finals at Arizona State University. The anticipation of academic finals may have possibly increased stress and anxiety in a majority of the subjects. It may be beneficial to repeat this study at a different time of the academic year or in the summer with subjects.

Observing POMS scores at just three points: baseline, week 4, and week 8, throughout the 8-week study may not have been enough times to form a completely accurate depiction of the subjects' mood states. In a future study it may be valuable to have the subjects take the self-administered questionnaire on a weekly basis instead of only three times throughout the study. The use of an additional behavioral measurement besides a self-reported questionnaire may have been a more objective measure of mood state.

The original 65-adjective item POMS used in this study was created in 1971 and contained a few adjectives that are no longer consistently used in the English language. One example of this is the adjective “bushed”. A definition of a word was read from an online dictionary, dictionary.com, if an interpretation of a word was requested by a subject. However, there is a possibility that a subject was unsure about the meaning of an adjective on the POMS but failed to ask for a definition of the adjective. This may have caused a subject to unknowingly misinterpret a word and thus improperly indicate a given mood state adjective using the Likert scale ranging from “not at all” to “extremely”.

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This study analyzed time effects of the POMS mood state sub-categories for the eight weeks of the study. Significant differences were seen at week 4 between groups in mood state sub-categories Tension-Anxiety ($P=0.037$), Depression-Dejection ($P=0.010$), and Fatigue-Inertia ($P=0.000$). However, there was no significant difference in change over time between the VTC and CON from baseline to week 8 in any mood state except Fatigue-Inertia ($P=0.002$). It is not clear why this occurred in the study.

It should be noted that the means for each sub-categorical mood state in this study are well below college student normative samples reported by McNair, Lorr, and Droppleman (1992). The fact that the VTC and CON subjects’ mood state scores were less than the college student normative samples for the POMS could be a reason why no significant differences were seen in the study. The subjects may not have had extreme enough mood disturbances to see a change in mood states with intervention.
Chapter 6
Conclusions & Applications

The results of this study show that supplementing 500 mg of vitamin C twice daily in young men, 18-35 years, increased self-reported average activity per week ($P<0.050$) during weeks 1-4 in mild and during weeks 5-8 in mild, moderate, and total leisure-time physical activity categories. This study offers the first experimental evidence that supplementing 500 mg of vitamin C twice daily can be effective in increasing leisure-time physical activity levels in young, healthy men. Mood states were not significantly impacted by supplementing vitamin C in this study.

Healthy, young men may benefit from the recommendation to increase intake of vitamin C through supplementation in order to augment leisure-time physical activity levels. There is relatively low risk in consuming vitamin C; it is well-tolerated in healthy individuals. The most common undesirable effect of consuming too large of a dose of vitamin C is gastrointestinal distress which can be avoided by taking the supplement with food. Populations that should yield caution or avoid supplementing vitamin C are individuals that are prone to kidney stones, have renal failure, iron-overload pathologies, or glucose-6-phosphate dehydrogenase deficiency (Johnson, 1999). If a healthy individual is considering diet changes to promote improved well-being, vitamin C-rich foods or vitamin C supplementation should be considered. Vitamin C should not be neglected from the diet in order to maintain a healthful lifestyle.
REFERENCES


Genes when Fed a High-Fat Diet. *International Journal of Vitamin & Nutrition Research, 78*(2), 87-95. doi: 10.1024/0300-9831.78.2.87


Adults. *International Journal of Vitamin and Nutrition Research, 69*(1), 41-44.


Intensity Weight Training Versus General Practitioner Care for Clinical Depression in Older Adults. *Journal of Gerontology, 60A*(6), 768-776.


APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL
To: Carol Johnston  
HSC

From: Carol Johnston, Chair  
Biosci IRB

Date: 01/18/2011

Committee Action: Amendment to Approved Protocol

Approval Date: 01/18/2011

Review Type: Expedited F2 F4 F7

IRB Protocol #: 0907004162

Study Title: Plasma vitamin C concentrations, physical activity levels, and mood states in young college men

Expiration Date: 06/02/2011

The amendment to the above-referenced protocol has been APPROVED following Expedited Review by the Institutional Review Board. This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator’s responsibility to obtain review and continued approval of ongoing research before the expiration noted above. Please allow sufficient time for reapproval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination.

This approval by the Biosci IRB does not replace or supersede any departmental or oversight committee review that may be required by institutional policy.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.
APPENDIX B

INFORMED CONSENT
CONSENT FORM
Nutrient Supplementation and Health Parameters

INTRODUCTION
The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

RESEARCHERS
Dr. Carol Johnston, director of the Nutrition program at Arizona State University, as well as graduate students, Gillian Osterclay and Sara Schumacher, have invited your participation in a research study.

STUDY PURPOSE
The purpose of the research is to examine the effect of nutrient supplementation in young college males, aged 18-40, on immune function and psychological status.

DESCRIPTION OF RESEARCH STUDY
If you decide to participate, then as a study participant you will join a study to evaluate the effect of ingestion of a nutrient supplement twice daily for 8 weeks on health markers. You will be instructed to complete a one-page questionnaire daily regarding illness and three one-page questionnaires each week regarding physical activity and diet. If you are interested in joining the study, you will be asked to come to an initial screening where a fasting blood sample (no food or drink for 5 hours with the exception of water) will be drawn, your body weight and height will be measured, and you will complete health history, diet, and mood questionnaires. If you are eligible for the study, you and the other participants will be randomly placed in either the control (placebo) or experimental (nutrient supplement) group. Subjects will be asked to visit the research site on 7 occasions at 0, 1, 2, 4, 7 and 8 weeks. At weeks 0, 4, and 8, a blood sample (requiring overnight fasting for 8 hours) will be drawn, you will be weighed, and you will complete mood and physical activity questionnaires. At each blood sampling approximately 4 tablespoons of blood will be collected. At the study visits, you will need to bring in the questionnaire booklet on diet, illness, and physical activity. We will also ask you to wear a pedometer at your waist for 2 three-day periods at the start and end of the study.

If you say YES, then your participation will last for 8 weeks at the Polytechnic campus of Arizona State University. Approximately 50 of subjects will be participating in this study locally.

RISKS
There may be a slight chance of gastrointestinal distress when the supplement is taken on an empty stomach. This risk is reduced if you ingest the nutrient supplement with a meal. Blood draws may cause light-headedness and fainting, and bruising may occur at the sight of the needle insertion. A nurse or trained phlebotomist will be performing the blood draws.

BENEFITS
Although there may be no direct benefits to you, the possible benefits of your participation in the research is that you will be able to experience what it is like to be a part of a research study that may provide new evidence for health promotion for many college students.

NEW INFORMATION
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY
All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. Your name will not be associated with any data pertaining to the study. In order to maintain confidentiality of your records, Dr. Carol Johnston will assign you a subject number which will be used to identify you throughout the entire course of the study.

ASU IRB
Approved
Sign: [Signature]
Date: [Date]
WITHDRAWAL PRIVILEGE
It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. Your decision will not affect your grades or any relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

COSTS AND PAYMENTS
The researchers want your decision about participating in the study to be absolutely voluntary. Yet they recognize that your participation may pose some costs. In order to help defray your costs you will receive two $10 gift card incentives at the 0 and 4-week visits and a $15 gift card at week 8 for a total of $35.

COMPENSATION FOR ILLNESS AND INJURY
If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT
Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, Principal Investigator and Professor of Nutrition at ASU (480-727-1713), Gillean Osterday, Graduate Student (480-225-4262), or Sara Schumacher, Graduate Student (480-694-5159).

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given (offered) to you.

Your signature below indicates that you consent to participate in the above study.

Subject’s Signature __________ Printed Name __________ Date __________

Preferred contact: phone or email: __________________________

INVESTIGATOR’S STATEMENT
“I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document.”

Signature of Investigator __________ Date __________
APPENDIX C

METHODOLOGY TIMELINE
**Initial Contact**
- Age
- Phone Questionnaire
  - Smoking habits, supplement/prescription medical history, physical activity limitations

**Screening Study Visit 1**
- Informed Consent
- Weight/Height
- Tanita (BMI)
- Postprandial Blood Sample (5-hour fast)
- Medical History Questionnaire
- REAPS-Diet Quality Questionnaire
- 24-hour recall

---

**Randomization**

**Baseline Study Visit 2**
- Fasting Blood Sample (8-hour fast)
- Weight
- POMS
- Survey booklets and capsule distribution (Baseline – Week 4 materials)
- Godin Leisure-Time Exercise Questionnaire

**Week 1 Study Visit 3**
- Survey booklet compliance check

**Week 4 Study Visit 4**
- Fasting Blood Sample (8-hour fast)
- Weight
- POMS
- Survey booklets and capsule distribution (Week 5 – Week 8 materials)

**Week 8 Study Visit 5**
- Fasting Blood Sample (8-hour fast)
- Weight
- POMS
- REAPS-Diet Quality Questionnaire
APPENDIX D

PHONE SCREEN
Hello ___________________.

Thank you for your interest in the Nutrient Supplementation and Health Parameters research study. Do you have time to answer 6 short questions to see if you qualify for the trial?

If no: When would be a better time?
If yes:

1. First of all, how old are you? ___ years

2. Do you currently smoke cigarettes? Yes or No
   a. If yes: How often?
   b. If “a” answered, ask: Approximately how many cigarettes do you smoke [refer to period of time that “a” was answered in]?
   c. If “a” and/or “b” answered, ask: Do you usually smoke with others in a social scene or alone?

3. Do you currently take vitamin and mineral supplements?
   a. If yes: Which supplements do you take?

4. Do you currently take any prescriptions?
   a. If yes: Which prescriptions do you take?

5. Are you currently seeing a doctor for a health condition?
   a. If yes: What condition are you being seen for?

6. Do you have any limitations regarding physical activity and exercise?

→If any of the answers do not meet the requirements for the study, the individual will be excluded.

If meets requirements:

Based on this information, I would like to invite you to be a participant in the Nutrient Supplementation and Health Parameters study. We will be contacting you shortly about the first study visit. Thank you for your time. Have a great day!

If does not meet requirements:

Unfortunately, we will not be able to accept you into the study. We appreciate your interest and time. Have a great day!

Thank you, ____________________________
APPENDIX E

HEALTH HISTORY QUESTIONNAIRE
1. Gender: M    F

2. Age: ________

3. Have you lost or gained more than 5 lbs in the last 12 months?      Yes    No
   If yes, how much lost or gained?       ________   How long ago? _________


5. Ethnicity: (please circle) Native American      African-American      Caucasian      Hispanic      Asian
   Other

6. Do you smoke?  No, never ________
   Yes _______     # Cigarettes per day = ________
   I used to, but I quit ________ months/years (circle) ago

7. Do you take any medications regularly?     Yes    No
   If yes, list type and frequency:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Do you currently take supplements (vitamins, minerals, herbs, etc.)?    Yes    No
   If yes, list type and frequency:

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Dosage</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. Have you ever been hospitalized? ________
   If yes, for what? ____________________________

10. Please ANSWER (YES/NO) if you currently have or if you have ever been diagnosed with any of the following diseases or symptoms:

<table>
<thead>
<tr>
<th>Disease</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary Heart Disease</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Heart Murmur</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Rheumatic Fever</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Irregular Heart Beat</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Varicose Veins</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Stroke</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Diabetes</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Low Blood Sugar</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Bronchial Asthma</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Hay Fever</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Leg or Ankle Swelling</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

| Chest Pain                      | YES | NO |
| Shortness of Breath             | YES | NO |
| Heart Palpitations              | YES | NO |
| Any Heart Problems              | YES | NO |
| Coughing of Blood               | YES | NO |
| Feeling Faint or Dizzy          | YES | NO |
| Lung Disease                    | YES | NO |
| Liver Disease                   | YES | NO |
| Kidney Disease                  | YES | NO |
| Thyroid Disease                 | YES | NO |
| Anemia                           | YES | NO |
| Hormone Imbalances              | YES | NO |

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Please elaborate on any condition listed above.

11. How would you rate your lifestyle?
   Not active ____________  Active ____________  
   Somewhat active __________  Very Active ____________

12. Please circle the total time you spend in each category for an average week.

   **Light activities** such as:
   Slow walking, golf, slow cycling, doubles tennis, easy swimming, gardening
   Hours per week:  0 1 2 3 4 5 6 7 8 9 10+

   **Moderate activities** such as:
   Mod. Walking, mod. cycling, singles tennis, mod. swimming, mod. weight lifting
   Hours per week:  0 1 2 3 4 5 6 7 8 9 10+

   **Vigorous activities** such as:
   Fast walking/jogging, fast cycling, court sports, fast swimming, heavy/intense weight lifting
   Hours per week:  0 1 2 3 4 5 6 7 8 9 10+

13. How much alcohol do you drink? (average drinks per day) __________

14. Do you have any food allergies?   Yes  No  If yes, explain:______________________________

15. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.)   Yes  No
   If yes, explain:____________________________________________________________________
# REAPS (Rapid Eating Assessment for Participants - Shortened Version)

CJ Segal-Isaacs, EDD RD, Judy Wylie-Rosett, EDD RD, Kim Gans, PhD, MPH

<table>
<thead>
<tr>
<th>In an average week, how often do you:</th>
<th>Usually/ Often</th>
<th>Sometimes</th>
<th>Rarely/ Never</th>
<th>Does not apply to me</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Skip breakfast?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>2. Eat 4 or more meals from sit-down or take out restaurants?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>3. Eat less than 2 servings of whole grain products or high fiber starches a day? <strong>Serving</strong> = 1 slice of 100% whole grain bread; 1 cup whole grain cereal like Shredded Wheat, Wheaties, Grape Nuts, high fiber cereals, oatmeal, 3-4 whole grain crackers, 1/2 cup brown rice or whole wheat pasta, boiled or baked potatoes, yuca, yams or plantain.</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>4. Eat less than 2 servings of fruit a day? <strong>Serving</strong> = 1/2 cup or 1 med. fruit or 1/2 cup 100% fruit juice.</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>5. Eat less than 2 servings of vegetables a day? <strong>Serving</strong> = 1/2 cup vegetables, or 1 cup leafy raw vegetables.</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>6. Eat or drink less than 2 servings of milk, yogurt, or cheese a day? <strong>Serving</strong> = 1 cup milk or yogurt; 1/2 - 2 ounces cheese.</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>7. Eat more than 6 ounces (see sizes below) of meat, chicken, turkey or fish per day? <strong>Note</strong>: 3 ounces of meat or chicken is the size of a deck of cards or ONE of the following: 1 regular hamburger, 1 chicken breast or leg (High and drumstick), or 1 pork chop.</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>Rarely eat meat, chicken, turkey or fish O</td>
</tr>
<tr>
<td>8. Use regular processed meats (like bologna, salami, corned beef, hotdogs, sausage or bacon) instead of low fat processed meats (like meat beef, turkey, lean ham; low-fat cold cuts/hotdogs)?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>Rarely eat processed meats O</td>
</tr>
<tr>
<td>9. Eat fried foods such as fried chicken, fried fish, French fries, fried plantains, tostones or fried yucas?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>10. Eat regular potato chips, nacho chips, corn chips, crackers, regular popcorn, nuts instead of pretzels, low-fat chips or low-fat crackers, air-popped popcorn?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>Rarely eat these snack foods O</td>
</tr>
<tr>
<td>11. Add butter, margarine or oil to bread, potatoes, rice or vegetables at the table?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>12. Eat sweets like cake, cookies, pastries, donuts, muffins, chocolate and candies more than 2 times per day.</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>13. Drink 16 ounces or more of non-diet soda, fruit drink/punch or Kool-Aid a day? <strong>Note</strong>: 1 can of soda = 12 ounces</td>
<td>O</td>
<td>O</td>
<td>O</td>
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</table>

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
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</table>

14. You or a member of your family usually shops and cooks rather than eating sit-down or take-out restaurant food? | O | O |
15. Usually feel well enough to shop or cook. | O | O |
16. How willing are you to make changes in your eating habits in order to be healthier?  
   1 Very willing  2  3  4  5 Not at all willing
Please indicate how many times you consumed the following foods THIS WEEK:

- **Citrus fruits and/or juices** (oranges, grapefruit, lemons, etc) □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Apples, pears and/or plums** □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Strawberries and/or other berries** □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Bananas** □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Melons** (cantaloupe, honeydew, watermelon, etc) □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Papaya, mangoes, kiwi** (including juices) □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Cruciferous vegetables** (broccoli, kale, Brussels sprouts, cauliflower, cabbage, asparagus)
  □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Peppers** (sweet green, red, yellow, hot green chili, hot red chili, jalapeno)
  □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Highly fortified breakfast cereals** (total, all bran, 100% bran, honey buckwheat crisp, bran buds, product 19, ovaltine, maypo, instant breakfast, etc)
  □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more

- **Fortified energy/fitness bars/drinks** (power bars, Powerade, etc)
  □ small □ medium □ large
  □ 0 □ 1-3x/wk □ 4-6x/wk □ 7-9x/wk □ 10-12x/wk □ 13-15x/wk □ 15 or more
EXERCISE QUESTIONNAIRE

I. During a past 7-Day period (one week), how many times on average did you do the following kinds of exercise for more than 15 minutes during your free time (fill in the box with the appropriate number).

a) STRENuous EXERCISE  (HEART BEATS RAPIDLY)
(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

Times/week:  

b) MODerate EXERCISE  (NOT EXHAUSTING)
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

Times/week:  

c) MILD EXERCISE  (MINIMAL EFFORT)
(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snowmobiling, easy walking)

Times/week:  

II. During past 7-Day period (one week), in your leisure time, how often did you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

OFTEN  SOMETIMES  NEVER/RARELY

Please think of how you felt over the last week when answering these questions.

The numbers refer to these phrases:

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

<table>
<thead>
<tr>
<th>The numbers refer to these phrases.</th>
<th>Hopeless</th>
<th>Desperate</th>
<th>Slight</th>
<th></th>
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<tbody>
<tr>
<td>Cat 3</td>
<td>1.</td>
<td>4.</td>
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</tr>
<tr>
<td>1. Friendly</td>
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<tr>
<td>2. Tense</td>
<td></td>
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<tr>
<td>3. Angry</td>
<td></td>
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<td>4. Worn out</td>
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<tr>
<td>5. Unhappy</td>
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<td>6. Clueless</td>
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<tr>
<td>7. Lively</td>
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<tr>
<td>8. Confused</td>
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<td>9. Sorry for things done</td>
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<tr>
<td>10. Shaky</td>
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<tr>
<td>11. Listless</td>
<td></td>
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<tr>
<td>12. Pleased</td>
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<tr>
<td>13. Considerate</td>
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<tr>
<td>14. Sad</td>
<td></td>
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<tr>
<td>15. Active</td>
<td></td>
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<tr>
<td>16. On edge</td>
<td></td>
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<tr>
<td>17. Grumpy</td>
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<td>18. Blue</td>
<td></td>
<td></td>
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<td>19. Energetic</td>
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<tr>
<td>20. Reaction</td>
<td></td>
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<td>22. Relaxed</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>23. Unworthy</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>27. Restless</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>28. Unable to concentrate</td>
<td>1.</td>
<td>4.</td>
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<td>29. Fatigued</td>
<td>1.</td>
<td>4.</td>
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<td>30. Expressive</td>
<td>1.</td>
<td>4.</td>
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<td>31. Helped</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>32. Discouraged</td>
<td>1.</td>
<td>4.</td>
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<td></td>
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<tr>
<td>33. Restful</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>34. Nervous</td>
<td>1.</td>
<td>4.</td>
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<td>35. Lonely</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>36. Misunderstood</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>37. Muddled</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>38. Cheerful</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>40. Bitter</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>41. Exhausted</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>42. Ready to fight</td>
<td>1.</td>
<td>4.</td>
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<tr>
<td>43. Good natured</td>
<td>1.</td>
<td>4.</td>
<td></td>
<td></td>
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<tr>
<td>44. Gloomy</td>
<td>1.</td>
<td>4.</td>
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</tbody>
</table>

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.