

Vitamin C Supplementation and Physical Activity Levels in Young Men

by

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ABSTRACT

Among its many roles in the body, ascorbic acid functions as a cofactor in carnitine and catecholamine synthesis, metabolites involved in fat oxidation and mood regulation, respectively. Given that fat oxidation and mood affect one's feelings of vigor, I hypothesized that those with lower levels of plasma ascorbic acid would be less likely to exercise at high levels than individuals with adequate or high levels of vitamin C. To test this, I conducted a double-blind, placebo-controlled intervention. A group of healthy, non-smoking males between the ages of 18 and 40 were put on a vitamin C-restricted diet for two weeks and then randomized to a control group that received placebo capsules for six weeks or an intervention group that received 500 mg of vitamin C daily for six weeks. The men were restricted from eating foods high in vitamin C, instructed to wear a pedometer daily and to record their step counts, and to take a pill daily (either the placebo or vitamin C supplement). Unexpectedly, the subjects receiving the intervention had lower step counts than the control group; the control group, rather than the vitamin C group, significantly ($p=0.017$) increased their steps at week 8 compared to week 2. However, I also estimated daily Metabolic Equivalent Tasks (METs), and subjects receiving the placebo had lower MET outputs than subjects receiving vitamin C at the end of the trial, in spite of having higher step counts. This means the intensity of their activity was higher, based on METs expenditure. Additionally, depression scores (POMS-D) as measured by the Profile of Mood States (POMS) questionnaire were significantly higher ($p=0.041$) among subjects receiving the placebo at the end of the study. These

latter results are consistent with my expectations that subjects with higher levels of plasma vitamin C would have improved mood and higher energy output than subjects with low levels of vitamin C.

DEDICATION

To my parents, Harold and Ruth Netland, for their unending support of my educational pursuits, and to my boyfriend, Sean Lynch, for his constant encouragement and affirmation along this journey. I love you all and would never have made it to this point without your generosity, words of encouragement, and advice. Thank you!

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Chapter 1

Introduction

“The first indication of the approach of this disease is. . . a listlessness to action or an aversion to any sort of exercise. . . much fatigue and. . . breathlessness or panting” (James Lind, English physician, 1716-1794). The disease thus described is scurvy, or inadequate serum vitamin C status. Current research affirms Lind’s observation that the first sign of vitamin C inadequacy is fatigue. The implications of this observation are far-reaching, and current research is beginning to study how this link between inadequate vitamin C stores and fatigue may affect physical activity levels.

Ingesting or supplementing vitamin C has long been touted to promote optimal health and to prevent or treat the common cold. While there is evidence to support vitamin C’s role in the immune system {{33 Ferrer,M.D. 2009; }}, recent research is beginning to explore other promising physiological effects of vitamin C. While most of vitamin C’s functions are due to its antioxidant nature, an intriguing area of research linking vitamin C with weight management and physical activity is based upon the co-factor roles of vitamin C in the biosynthesis of carnitine and/or catecholamine, metabolites which can impact mood and fatigue.

Theoretically, if someone is fatigued from having inadequate stores of vitamin C, that person would be less likely to exercise and to exercise at higher intensities. Some research has been conducted examining vitamin C’s effect on

work efficiency. However, the implications of vitamin C supplementation for physical activity and weight management have yet to be fully elucidated.

Therefore, there is a need in the scientific literature to study whether serum vitamin C status directly affects levels of physical activity since this is very relevant to the current obesity epidemic.

While few people believe that vitamin C deficiency is a common problem today in America, researchers have established that about 14% of men and 10% of women are deficient in vitamin C stores (Hampl, Taylor, & Johnston, 2004). This is a serious public health concern given the wide variety of physiological benefits associated with vitamin C. It has been reported that vitamin C plays a role in cancer prevention, optimal immune system function (Carrillo, Murphy, & Cheung, 2008b; Johnston, 1989; Johnston, 1990; Johnston, 2005; Zojaji et al., 2009), cardiovascular health ((Bohm, Settergren, & Pernow, 2007; Buttros, Bergamaschi, Ribeiro, Fracalossi, & Campos, 2009; Chambers, McGregor, Jean-Marie, Obeid, & Kooner, 1999; Gey, Stahelin, & Eichholzer, 1993; Johnston, Dancho, & Strong, 2003; Montano, Fernandez, & McNamara, 1998; Pleiner, Mittermayer, Schaller, MacAllister, & Wolzt, 2002; Smith, Visioli, & Hagen, 2002), insulin regulation (Cunningham, Ellis, McVeigh, Levine, & Calles-Escandon, 1991; Johnston & Yen, 1994), mood (Binfare, Rosa, Lobato, Santos, & Rodrigues, 2009; Brody, 2002; Dakhale, Khanzode, Khanzode, & Saoji, 2005; Eren, Naziroglu, & Demirdas, 2007; Jaser, Holl, Jefferson, & Grey, 2009), and weight control (Canoy et al., 2005; Galinier et al., 2006; Johnston, Corte, & Swan,

2006b; Johnston, Beezhold, Mostow, & Swan, 2007; Watters, Satia, & Kupper, 2008).

Studies have examined the implications of vitamin C's role in metabolism, and its relationship with physical activity. Vitamin C has been shown to attenuate oxidative stress and a rise in cortisol concentrations following exercise (Carrillo, Murphy, & Cheung, 2008a; Davison, Gleeson, & Phillips, 2007; Ferrer, Tauler, Sureda, Tur, & Pons, 2009), and it appears to enhance vasodilation in some cases during exercise (Eskurza, Monahan, Robinson, & Seals, 2004; Kirby et al., 2009). A correlational observation notes that people with high serum concentrations of vitamin C tend to be more active than those with lower concentrations (Camoés & Lopes, 2008). However, this was not an intervention trial, so it is not possible to isolate the role that vitamin C may play in influencing one's activity. To our knowledge, at the present time there are no intervention studies analyzing the effect of vitamin C supplementation in vitamin C-deficient individuals on objectively measured levels of physical activity. Such a study is important because it would have implications for increasing levels of physical activity, which is important to manage chronic diseases associated with inactivity. Since this study will be conducted in males between the ages of 18 and 40, results will be most relevant for this demographic, which is also the demographic at the highest risk of being deficient in vitamin C (Hampl et al., 2004).

The purpose of this study was to determine the impact of serum concentrations of vitamin C on spontaneous physical activity. I determined the

effect of supplementation with 500 mg of vitamin C daily on increasing spontaneous physical activity in persons with inadequate plasma vitamin C, as recorded by steps using a pedometer. The present study was a double-blind, placebo-controlled, parallel-arm trial. Research was conducted in the Nutrition and Exercise Wellness programs located at Arizona State University Polytechnic in Mesa, Arizona. Our sample population consisted of 30 nonsmoking, fairly sedentary men (not presently engaging in cardiovascular exercise more than 3 times per week) between the ages of 18 and 40. I hypothesized that 6 weeks of daily 500 mg vitamin C supplementation in men with below adequate serum vitamin C would increase their level of physical activity as determined by steps recorded on a pedometer compared to the placebo control group.

Definition of Terms

Below adequate serum concentrations of vitamin C: Fasting serum concentrations of vitamin C <0.50 mg/dL, which is equivalent to 28 $\mu\text{mol/L}$

METs: Metabolic Equivalent Tasks; calculated from my physical activity questionnaire. Each hour of light activity was multiplied by 3, moderate activity by 5, and vigorous activity by 9. The results were summed for a total MET value.

Physical Activity: Movement accumulated throughout the day that is measured objectively through the pedometer and subjectively through the physical activity questionnaire

Step Counts: The total number of steps shown on the pedometer at the end of each day

Delimitations

Since the subjects will be exclusively non-smoking, fairly sedentary males between the ages of 18 and 40, results of this study may not be generalizable to other age groups, females, smokers, or very active individuals.

Limitations

Pedometers can only measure steps taken. They do not record vigor (that is, they do not differentiate between running and walking), nor do they record physical activity such as fidgeting. An accelerometer would be able to do this, but this would have been more costly and still not be able to account for activity such as swimming or cycling. Step counts may become inflated, however, by activities such as vigorously twirling the pedometer, a habit in which many subjects reported or were observed engaging. It is possible that physical activity levels may change in these ways and not be recorded by the pedometer. Subjects will be asked to restrict their intake of fruits and vegetables that are high in vitamin C, foods fortified with vitamin C, and supplements containing vitamin C, as indicated on a chart with which they will be provided; however, I could not guarantee that they would adhere to this request.

Chapter 2

Review Of The Literature

Vitamin C

Chemical structure

Vitamin C is a comprehensive term referring to substances that exhibit anti-scorbutic properties. Specifically, it refers to ascorbic acid (AA), the reduced and physiologically functional form, and dehydroascorbic acid (DHA), the oxidized form that is readily reduced to AA. Naturally occurring vitamin C appears as an asymmetric six-carbon ring in the enantiomeric D-form. Ascorbic acid derives its acidic and reducing character from its two enolic hydrogen atoms and their electrons. The oxidation product of AA is an ascorbyl radical, which converts to AA and DHA or is irreversibly catabolized to 2,3-diketogluonic acid (Institute of Medicine of the National Academies. Food and Nutrition Board.,). While most animals are able to synthesize ascorbic acid, humans lack the rate-limiting enzyme L-gulonolactone oxidase necessary to convert L-gulonolactone to 2-keto-L-gulonolactone, which spontaneously becomes L-ascorbate ((Ramirez et al., 2006), (Frikke-Schmidt & Lykkesfeldt, 2009)²⁴). Humans, therefore, must obtain sufficient vitamin C from a dietary source.

Absorption

AA enters endothelial cells by the sodium-dependent vitamin C transporters (SVCT) 1 and 2 which are specific for AA (Wilson, 2009). This is a saturable process, so that as intake of vitamin C increases, absorption decreases.

Absorption occurs along the entire length of the small intestine; however, AA is mainly taken up in the distal ileum whereas DHA is taken up mainly in the jejunum. Vitamin C follows a distal to proximal uptake, which is opposite that of glucose and may be beneficial for both of their absorptions since they compete for uptake (Malo & Wilson, 2000). While SDVCT1 is the primary route of ascorbate absorption, passive diffusion contributes slightly, particularly with high intakes. Facilitative glucose transporters (GLUTs) take up DHA, at which point it is reduced to ascorbate (Wilson, 2009). Thus, DHA competes with glucose for uptake since they utilize the same transporters.

Transport

Vitamin C is primarily transported in the serum as free, unbound AA. Serum is not saturated with AA until intakes reach one gram per day (Johnston & Cox, 2001). Tissue saturation is achieved at lower intakes, ~200 mg/day (Levine et al., 1996). These levels are well above the Dietary Recommended Intake (DRI) of 75 mg daily for women or 90 mg daily for men (DRI).

Excretion

The kidneys are the key regulators of ascorbic acid homeostatic control. Concentrations are regulated by urinary output. With intakes less than 100 mg per day, there is little urinary output of AA or its metabolites. Intakes near 100 mg per day are associated with a 25% excretion rate, primarily as AA metabolites oxalate and urate (Levine et al., 1996). When intakes exceed 500 mg per day,

most absorbed AA reappears in the urine as AA and, to a lesser degree, AA metabolites.

Toxicity

An Upper Limit (UL) intake of 2 g per day was established for vitamin C, using gastrointestinal distress as the marker of adverse effects. With intakes above two grams per day, individuals are more likely to develop osmotic diarrhea and nausea. Other possible adverse effects at high concentrations include increased kidney stone formation in susceptible individuals due to increased urinary oxalic and uric acid, a suggested pro-oxidant effect by reducing free iron and copper (and hence increasing their pro-oxidant effects), interfering with urinary glucose and fecal blood tests, and in rare occasions leading to hemolytic anemia in persons with the inborn error of metabolism glucose-6-dehydrogenase deficiency. However, a review by Hathcock et al. found that there was no significant evidence supporting these claims of adverse effects (Hathcock et al., 2005).

Deficiency (Scurvy and subclinical deficiency)

There are varying degrees of vitamin C deficiency. Adequate vitamin C status means having vitamin C plasma concentrations at least 23 umol/L. Low concentrations of vitamin plasma vitamin C are defined as 11.4-23 umol/L, and values less than 11.4 umol/L are considered deficient (Jacob & Sotoudeh, 2002). Severe vitamin C deficiency is known as scurvy, and is characterized by fatigue, inflammation of the joints, hair and tooth loss, and easily bruising or bleeding

(Linus Pauling Institute,). Since these symptoms are non-specific to scurvy and resemble those of other more common diseases, scurvy is often initially misdiagnosed. Although death occurs from severe scurvy, if the patient is supplemented with vitamin C scorbutic symptoms are usually reversed (Leger, 2008; Vitale et al., 2009). While both DHA and AA have been documented to improve symptoms of scurvy, ascorbic acid exerts about twice the antiscorbutic activity of DHA (Otsuka, Kurata, & Arakawa, 1986).

Prevalence of deficiency and depletion in the United States today

While many consider scurvy a disease of the past, there are still clinical cases of scurvy in the United States today. Furthermore, there is a fairly large percentage of Americans with subclinical scurvy and hypovitaminosis C, a suboptimal vitamin C state. Hampl et al. analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) to determine the prevalence of vitamin C deficiency and depletion. They found that 14% of males and 10% of females were vitamin C deficient, defined as serum values of less than 11 $\mu\text{mol/L}$. This is surprising since average vitamin C intakes met the RDA for age groups. Certain demographics tended to be more prone to deficiency, such as smokers and those who do not use nutritional supplements. Ethnicity also affected deficiency prevalence; non-Hispanic black males experienced more deficiency than white males, who themselves were more prone to deficiency than Mexican American males (Hampl et al., 2004).

One of the demographics most prone to vitamin C deficiency or depletion is smokers. Lykkesfeldt et al. studied the effect of smoking alone on vitamin C status by controlling dietary and supplemental intake between male smokers and nonsmokers. They found that ascorbic acid is the only antioxidant that is depleted by smoking; however, the smokers were able to replete their vitamin C stores via supplementation. Furthermore, plasma concentrations of vitamin C increased more among smokers than nonsmokers during the supplemental period (Lykkesfeldt et al., 2000).

It is also important to ensure that the elderly are consuming adequate vitamin C-rich foods since Siomek et al. have documented an age-related decline in plasma vitamin C concentrations in a study of 255 subjects representing a variety of ages (Siomek et al., 2007).

Sources

Best sources of vitamin C are citrus fruits, bell peppers, strawberries, and broccoli (Linus Pauling Institute,). Vitamin C is very labile and degrades quickly with exposure to light, air, or heat. Therefore, vitamin C content listed on food labels often does not accurately reflect actual content of the food at the time of consumption. Johnston and Bowling found that vitamin C content of readily available orange juice decreased approximately 2% per day from the date of purchase, and that ready-to-drink orange juice was significantly more oxidized than frozen, reconstituted orange juice (Johnston & Bowling, 2002). Ilic et al. studied vitamin C concentrations in plain and raspberry-flavored yogurt over a

period of six weeks. While the concentration of the vitamin decreased in both types of yogurt, the raspberry yogurt preserved the vitamin C better than the plain yogurt, presumably due to the added reductants from the raspberries (Ilic & Ashoor, 1988).

Recommended intakes

Currently, U.S. dietary recommended intakes of vitamin C for adults are 75 mg daily for women and 90 mg daily for men. These figures are based upon a review by Gey completed in 1998 that suggested plasma vitamin C concentrations of 50 umol/L provide the optimal physiological benefits pertaining to cancer and cardiovascular health (Institute of Medicine of the National Academies. Food and Nutrition Board.,).

A national health promotion, 5-A-Day sponsored by the National Cancer Institute (NCI), encourages Americans to consume at least 5 fruits and vegetables per day. However, a study by Taylor et al. analyzed data from the Continuing Survey of Food Intakes by Individuals (CSFII), using data from 1994-1996, and these results showed that 18% of the population had low vitamin C intakes (defined as less than 30 mg per day), 24% had marginal intakes of 30-60 mg per day, and only 58% had adequate intakes of 60 mg or more daily (Taylor, Hampl, & Johnston, 2000). Simply consuming five servings of fruit and vegetables daily does not ensure that Americans would be consuming adequate vitamin C. In fact, based upon the reported choices of fruits and vegetables, Americans would need to consume seven servings of the type of produce they are selecting to consume

adequate vitamin C. An important caveat that ought to be added to the 5-A-Day message would be to include at least one serving of citrus fruit to increase intake of vitamin C (Taylor et al., 2000). Furthermore, since the publication of this article, revised guidelines for the Recommended Dietary Allowances have been released, raising the minimum level of adequate intake to 75 mg daily for women and 90 mg daily for men; this requirement is increased further for smokers (Institute of Medicine of the National Academies. Food and Nutrition Board.,). Therefore, it is likely that even fewer people consume adequate vitamin C than these statistics suggest.

Hampl et al. also analyzed data from the 1994-1996 CSFII to see what types of fruits and vegetables were commonly consumed. Although Americans reported consuming some fruits and vegetables (average intake was 3.6 +/- 2.3 servings of vegetables and 1.6 +/- 2.0 servings of fruit daily), intake of produce high in vitamin C was remarkably low (average intake of dark green vegetable consumption was 2.0 servings daily and average intake of citrus, berries, or melon was almost 0.8 servings daily)(Hampl et al., 2004) This supports the recommendation that if Americans are to continue eating the same types of produce that they currently are, intake will have to increase to provide adequate vitamin C.

In the study by Hampl et al. which utilized the third National Health and Nutrition Examination Survey, conducted from 1988 to 1994, 13-23% of respondents were vitamin C depleted, defined as serum vitamin C values of

between 11 and 28 $\mu\text{mol/L}$. This was in spite of normal mean dietary intake and mean serum concentrations (Hampl et al., 2004). These data indicate that higher dietary reference intakes may be needed in order to ensure adequate vitamin C status in the population.

Many studies have examined potential beneficial effects of vitamin C supplementation, but these studies use amounts varying from doses of just a couple of hundred milligrams up to 8 grams daily. It can be difficult, therefore, to know how much vitamin C is actually needed to optimize health, and if there is a level of intake above which no further benefits are noted. Johnston and Cox addressed this question in a study designed to determine what intake of vitamin C provides maximal antioxidant protection and saturates plasma. They compared the effect of supplementing with 500, 1000, and 2000 mg of vitamin C daily on total lipid peroxidation and Heinz bodies, the designated markers of oxidative stress. Their results demonstrate that antioxidant protection did not differ significantly between the 1000 and 2000 dosages, leading them to conclude that optimal supplementation is achieved by consuming between 500 and 1000 mg of vitamin C daily (Johnston & Cox, 2001).

One point to consider when comparing the findings of different studies is that it is important to compare status markers of the same kind. That is, a person may be classified in different status categories depending upon whether dietary intake or serum concentrations are studied. Various factors such as smoking and

medication use may also affect serum vitamin C in addition to intake (Loria, Whelton, Caulfield, Szklo, & Klag, 1998).

How to improve intake of vitamin C

Given the many reported beneficial effects of vitamin C, it is imperative that health practitioners develop methods effective at increasing consumption of vitamin C-rich foods among those who do not currently consume adequate amounts of vitamin C. Various studies have been conducted to evaluate effective ways of changing people's behavior. Hanlon et al found that conducting a full health check on worksite employees significantly improved their self-reported intake of fruits and vegetables (Hanlon et al., 1995). Watters observed that people who report using herbal supplements tend to have concentrations of vitamin C 30% higher than nonusers (Watters et al., 2008).

One consistently popular beverage is orange juice. Sanchez et al. studied the effect on plasma vitamin C of drinking two cups of 250 mL of orange juice per day (total 500 mL; 250 mg vitamin C). Both smokers and nonsmokers experienced significantly higher concentrations of plasma vitamin C and lower concentrations of 8-*epi*-PGF2a (a vasoconstrictor associated with pulmonary oxygen toxicity) and uric acid, and this effect was greater among smokers (Sanchez-Moreno et al., 2003).

Physiological roles

Antioxidant

Vitamin C has many varied physiological functions related to its roles as a reductant and a cofactor in numerous reactions. Perhaps most notably, vitamin C acts as an antioxidant, halting free radical and lipid peroxidation perpetuation (Canoy et al., 2005). Importantly, ascorbate is one of the first compounds in the body to be oxidized. In an in vitro study by Vatsery et al., compounds were oxidized in the following order: ascorbate>a-tocopherol>sulphydryls>cholesterol (Vatassery, 1995). Krukoski et al measured the effect of vitamin C and several other compounds on oxidative damage induced by the oxidant *tert*-butyl hydroperoxide (BHP) and found that vitamin C was able to partially inhibit the action of BHP (Krukoski, Comar, Claro, Leonart, & do Nascimento, 2009).

Chambers et al. studied whether vitamin C supplementation could relieve the negative effects of hyperhomocysteinemia on vascular function. Results indicate that elevated homocysteine concentrations are associated with short-term impaired vascular endothelial function, but that treatment with vitamin C (one gram per day for one week) prevented this effect (Chambers et al., 1999).

Neri et al. examined the effect of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction compared to baseline values. The supplement consisted of 600 g of *N*-acetylcysteine, 300 g of vitamin E, and 250 g of vitamin C per day for 15 days prior to testing. After antioxidant supplementation for two weeks, postprandial values for von Willebrand factor (vWF), vascular cell adhesion molecule-1 (VCAM-1), and concentrations of oxidants were all significantly decreased compared to baseline values (Neri et al.,

2005). This demonstrates that even necessary activities such as eating initiate oxidative damage that is preventable by antioxidants.

Not only does vitamin C work as an antioxidant, but it also interacts with other antioxidants, such as glutathione. Reduced glutathione (GSH) serves as an antioxidant, protecting the cytosol and mitochondria from the effects of hydroperoxides which are formed during metabolism. GSH is important for maintaining vitamin C concentrations in vivo since it recycles vitamin C by reducing DHA to ascorbic acid, the physiologically active form (HUGHES, 1964; Martensson & Meister, 1991). NADH appears to recycle DHA back to ascorbic acid as well, but to a much lesser degree (May, Qu, Whitesell, & Cobb, 1996). Conversely, GSH concentrations appear to be maintained by ascorbic acid, although GSH concentrations may not fall until ascorbic acid concentrations are quite low. Henning et al. found that following 60 days of low ascorbic acid intake, subjects had lower total glutathione concentrations and a reduced glutathione: oxidized glutathione ratio in plasma. Simultaneously, NAD and NADP concentrations in red blood cells were elevated (Henning, Zhang, McKee, Swendseid, & Jacob, 1991). Johnston et al. studied the effect of various doses of supplemental ascorbic acid on red blood cell glutathione. Although the ascorbic acid increased red blood cell glutathione at both dosages (500 mg and 2,000 mg daily), it did not increase linearly. The 2,000 mg ascorbic acid treatment did not produce significantly different glutathione concentrations from those resulting from the 500 mg level (Johnston, Meyer, & Srilakshmi, 1993).

Vitamin C also serves to regenerate other antioxidants, such as vitamin E (Jore, Kaouadji, & Ferradini, 1990). Thus, its antioxidant functions extend beyond its cofactor properties. As indicated by Vatassery, vitamin C is oxidized before vitamin E, another way that it spares this antioxidant for further use (Vatassery, 1995).

Cancer

Parenteral administration can bypass normal physiological control of ascorbic acid concentrations, permitting plasma ascorbate concentrations to exceed 20 $\mu\text{mol/L}$. Chen et al. administered 4 g ascorbate per kg body weight parenterally in mice with cancer tumors that were 5-7 mm in diameter. This treatment by itself significantly decreased tumor growth and weight in three types of tumors. Furthermore, metastases were not present in the mice receiving ascorbic acid (Chen, Jones, Stone, Ching, & Chamley, 2009). Du et al. performed a similar experiment in mice, intravenously administering 4g ascorbate per kg body weight in pancreatic cancer cells. Results also showed that ascorbate inhibited tumor growth and prolonged survival rates (Du et al., 2010).

Padadatty et al. analyzed three human case studies where subjects with cancerous tumors received large dosages (15-30 grams of vitamin C twice per week) intravenously instead of conventional treatment. Two of the three subjects survived and the cancer went into remission. The cases suggest that intravenous, but not oral, consumption of very large doses of vitamin C can raise plasma concentrations of vitamin C up to 14,000 $\mu\text{mol/L}$ (whereas consuming five

servings of fruits and vegetables daily results in plasma vitamin C concentrations of about 70-80 umol/L and oral consumption of the highest tolerated level of vitamin C, 18 g per day, results in plasma concentrations of 200 umol/L). Plasma concentrations of vitamin C in the range elevated by intravenous administration can become toxic to certain cancer cells, possibly due to the formation of hydrogen peroxide in extracellular fluid (Padayatty et al., 2006). In a commentary Levine strongly advocated pursuing further research with pharmacologic intravenous administration of vitamin C as a treatment for cancer cells based upon the available evidence (Levine, Espey, & Chen, 2009)

Carnitine

Carnitine is a molecule required for transport of long-chain fatty acids into the mitochondria where they are oxidized to acetyl coenzyme A (CoA). While humans can endogenously synthesize almost all of the carnitine necessary for functioning, vitamin C is a required cofactor for two hydroxylation steps in this process. Therefore, inadequate concentrations of vitamin C can hinder synthesis of carnitine and therefore fatty acid metabolism. Johnston and Corte examined the relationship between muscle and plasma carnitine concentrations and supplementation with ascorbic acid in guinea pigs. They found that while muscle total acid-soluble carnitine concentrations tended to correspond positively with plasma vitamin C, plasma free carnitine showed an inverse relationship to liver vitamin C and to muscle total acid-soluble carnitine. The study protocol involved a depletion phase followed by supplemental repletion. During the repletion phase

plasma free carnitine concentrations fell 30%, and muscle total acid-soluble carnitine rose 30%. These data indicate that an indicator of inadequate vitamin C status may be elevated plasma free carnitine (Johnston & Corte, 1999).

In a human study Johnston, Corte, and Swan administered a submaximal exercise test to subjects with marginal or adequate vitamin C concentrations to compare rates of fat oxidation. Subsequently, some of the subjects with below adequate vitamin C status participated in a depletion-repletion study with submaximal exercise testing. Results showed that subjects with marginal vitamin C status oxidized 25% less fat while exercising compared to those with adequate vitamin C, and that fat oxidation during exercise was inversely related to self-reported fatigue while exercising. Once repleted, subjects previously with marginal vitamin C status increased their fat energy consumption fourfold compared with controls. Authors speculate that inadequate vitamin C status may affect adiposity in two ways: by exercise intolerance due to fatigue and by lipid accumulation (Johnston, Corte, & Swan, 2006b).

Sepsis

Galley et al. showed in a study that patients with sepsis have remarkably diminished total vitamin C concentrations compared with healthy subjects. This could be due to vitamin C being used in the redox cycling of iron or to scavenging free radicals. This is problematic since there is inadequate vitamin C available to act as an antioxidant and prevent tissue damage (Galley, Davies, & Webster, 1996).

Sepsis can impair capillary blood flow, and the effect of ascorbate injection to reverse this occurrence was studied in mice. Tyml et al. noted that ascorbate was able to restore capillary blood flow as soon as ten minutes post injection and that this effect continued for at least 12 hours (Tyml, Li, & Wilson, 2008).

Immune protection

Cortisol is a hormone that is released from the adrenal glands during times of physiological or psychological stress. While it is beneficial in the fight-or-flight response, extended periods of time with high concentrations of cortisol may be immunosuppressive. Although exercise is beneficial to overall health, it temporarily raises cortisol concentrations in participants. Carrillo et al. found that vitamin C supplementation at levels of 1500 mg of vitamin C per day significantly attenuated concentrations of post-exercise cortisol in a linear fashion in male research participants (Carrillo, Murphy, & Cheung, 2008a). Biological importance of this mechanism may be to preserve immune function following intense bouts of exercise.

Another observed immunoprotective effect of vitamin C is that it has been shown to increase body temperature when ingested at high supplemental levels, as shown by Johnston et al. Increased body temperature is part of the immune response to foreign pathogens. This effect has been documented both in guinea pigs receiving ascorbic acid at levels 5-25 times the recommended daily intake

level for guinea pigs (Johnston, 1989) and in humans taking 1 gram of L-ascorbic acid (Johnston, 1990).

Johnston's study of vitamin C and its hyperthermic effect on humans yielded another interesting observation pertaining to the immune response to vitamin C. Subjects taking 1 gram of L-ascorbic acid had significantly lower serum iron concentrations at 24 hours post-dose. This is characteristic of acute phase immune responses (Johnston, 1990).

While histamine initially operates as a stimulant of the immune response, later it acts as an immunosuppressant, perhaps to contain the inflammation (Brostoff, Pack, & Lydyard, 1980). Johnston et al. studied the effect of vitamin C supplementation on histamine and neutrophils chemotaxis. Their results showed lowered concentrations of blood histamine and increased neutrophil chemotaxis, which were inversely related (Johnston, Martin, & Cai, 1992).

Another study by Johnston pertaining to antihistamine effects of vitamin C found that while taking 500 mg of vitamin C per day did not alter blood histamine concentrations significantly; taking 2,000 mg of vitamin C daily reduced blood histamine in vivo by about 40%. Authors concluded that the antihistamine properties of vitamin C appear to be due to plasma concentrations of reduced ascorbate, not total vitamin C concentrations (Johnston et al., 1992).

Infection by the microbe *Helicobacter pylori* is common medical problem today, often treated with the drugs clarithromycin, amoxicillin or metronidazole and a proton pump inhibitor. Zojaji et al., however, studied the efficacy of

administering 500 mg of vitamin C per day with amoxicillin, metronidazole, bismuth, and omeprazole compared to the same treatment without vitamin C supplementation. Results showed an increase from 49% to 78% in eradication rates using the vitamin C supplement (Zojaji et al., 2009).

Cofactor

Vitamin C is essential for the synthesis of collagen, carnitine, norepinephrine, tyrosine, complement component C1q, and corticosteroids. More specifically, vitamin C is required for modifying polypeptide precursors to collagen after translation has occurred (Johnston, 2005). Not only does vitamin C serve as an antioxidant itself, but it also serves to regenerate other beneficial compounds such as vitamin E, glutathione, and flavonoids. It limits low-density lipoprotein (LDL) cholesterol oxidation, platelet aggregation, and DNA and membrane oxidative damage too. Another key role of vitamin C is that it increases production of nitric oxide.

Pulmonary function

Asthma is becoming an increasingly common problem and has been shown to be exacerbated by exposure to ozone. In an effort to mimic the effect of ozone on lungs and vitamin C's potential regulatory response, Trenga et al. exposed study participants to an ozone-induced bronchial challenge. Subjects were either given two placebos or two antioxidant supplements containing 400 IU of vitamin E and 500 mg of vitamin C each for one week prior to exposure to the sulfur dioxide. Results indicate that participants taking the antioxidants

responded less severely to the sulfur dioxide test than those receiving the placebo (Trenga, Koenig, & Williams, 2001).

Feng et al. induced lung damage in rats by injecting them with lipopolysaccharides (LPS) and subsequently injected them with vitamin C one hour later to study the effect of vitamin C on the lung damage. Beneficial effects of vitamin C on the induced lung damage include reversing hypotension, reducing the amount of exhaled nitric oxide and plasma nitrates, preventing injury, and lessening free radical release by white blood cells (Feng et al., 2004).

Banerjee et al. studied smoke-induced lung damage in mice and the effect of vitamin C supplementation on these effects. Mice who received 15 mg of vitamin C daily did not experience the deleterious effects of smoke exposure like the control mice. Specifically, protein damage, inflammation, apoptosis, and lung injury were prevented (Banerjee et al., 2008)

Another significant pulmonary problem is obstructive airways disease. Sargeant et al. compared occurrence of obstructive airways disease (OAD) among smokers with varying concentrations of plasma vitamin C. Subjects with higher concentrations of vitamin C in their blood had a significantly reduced risk of developing OAD (Sargeant, Jaeckel, & Wareham, 2000)

Mooney et al. compared risk of developing lung cancer among smokers who took an antioxidant supplement (500 mg vitamin C and 400 IU vitamin E) with those receiving a placebo. Specifically, they looked at concentrations of benzo(a)pyrene [B(a)P]-DNA adducts, compounds associated with increased risk

of developing lung cancer in current smokers. While there was no significant difference among male subjects receiving the treatment compared to the placebo, women receiving the antioxidant supplement had significantly lower concentrations of [B(a)P]-DNA adducts, thus suggesting that vitamin C and E may help reduce the risk of lung cancer, especially among certain demographics (Mooney et al., 2005)

Weight control

Observational studies have noted an inverse relationship between serum vitamin C concentrations and adiposity. Canoy et al noted that plasma ascorbic acid was inversely related to waist-to-hip ratios for men and women, independent of body mass index (BMI) (Canoy et al., 2005). Johnston et al reported that plasma ascorbic acid was inversely related to BMI, body fat percentage, and waist circumference in men and women; it was also found to be indirectly related to plasma insulin and to be positively correlated with plasma adiponectin in women (Johnston et al., 2007). Watters also found that waist circumference correlates inversely with plasma concentrations of vitamin C (Watters et al., 2008).

One possible explanation for this could be that vitamin C is a cofactor in the synthesis of carnitine, which is essential for fatty acid oxidation. If vitamin C is insufficient, it may hinder synthesis of carnitine, which would lead to decreased fatty acid metabolism. Johnston et al. found that individuals with marginal vitamin C status (<34 $\mu\text{mol/L}$) oxidized 25% less fat than individuals with vitamin C concentrations >34 $\mu\text{mol/L}$ during a submaximal treadmill test. Fat

oxidation was inversely correlated with fatigue ($r = -0.611$, $p = 0.009$). This led them to conclude that vitamin C deficiency may affect weight regulation in two ways: through increased fatigue leading to a higher likelihood to stop physical activity sooner and also an impaired fat utilization for fuel (Johnston, Corte, & Swan, 2006a).

An interesting biochemical finding pertaining to vitamin C and adiposity is that fat accumulation in overweight rats is associated with vitamin C being in a reduced redox state and lower lipid peroxidation than those concentrations observed in lean rats. This appears to contribute to the accumulation of triglycerides. Redox state refers to the percentage of oxidized vitamin C, meaning that lower redox states have lower ratios of the oxidized forms of vitamin C. Since the purpose of this study was to investigate biochemical differences in adipose tissue of obese compared to lean rats and was not an intervention trial, authors did not suggest ways to alter these biochemical variations observed. It is worth noting, however, that adipose tissue biochemical markers do vary between obese and lean rats, and further studies ought to investigate why these markers vary in this pattern, as well as the effect of antioxidant supplementation on these markers (Galinier et al., 2006).

Cardiovascular health

An interesting finding that must be kept in mind when interpreting *in vitro* cardiovascular studies is that standard culture media used for studying human aortic endothelial cells do not contain vitamin C. Smith et al. looked at how this

bears on findings of other cardiovascular studies. They found that cells in standard culture used in cardiovascular studies were virtually scorbutic. Upon addition of vitamin C to the media, total glutathione (GSH), glutathione/glutathione disulfide (GSSG), and NAD(P)H/NAD(P)⁺ ratios increased over 24 hours. Endothelial nitric oxide synthase (eNOS) activity increased by 600% following the addition of vitamin C. Authors propose adding some vitamin C to cultured cells to best simulate *in vivo* conditions (Smith et al., 2002).

Buttrose et al. studied the effect of vitamin C supplementation in rats that were subsequently subjected to induced myocardial infarctions. They found that the group fed a diet high in vitamin C experienced less myocardial damage and improved autonomic balancing of the heart following isoproterenol (ISO)-induced myocardial infarction than the control group (Buttros et al., 2009).

Vasodilation appears to be very responsive to vitamin C supplementation, but the timing of supplementation varies vessel response. Bohm et al. studied the effects of vitamin C supplementation on vessel response to endothelin-1 (ET-1) injection. ET-1 administration decreased endothelium-dependent and independent vasodilation and increased venous interleukin-6 (IL-6) concentrations. Subjects did not experience any vascular changes from the vitamin C administration when it was given after the IL-6 infusion. However, pre-treatment with vitamin C blocked the decrease in both endothelium-dependent and independent vasodilation and the increase in IL-6 concentrations (Bohm et

al., 2007). Authors conclude that since vitamin C operates as an antioxidant, the impaired vascular functioning resulting from ET-1 is due to oxidative stress.

High concentrations of homocysteine are known to impair vascular function and lead to vascular disease. Chambers et al. studied the effect of pretreatment with vitamin C in subjects taking oral methionine- the precursor to homocysteine in metabolism. While the vitamin C did not significantly affect the homocysteine concentrations after oral methionine, the typical decrease in flow-mediated dilation was prevented by taking vitamin C (Chambers et al., 1999). Authors conclude on the basis of these observations that the vascular damage induced by high homocysteine is due to oxidative damage, since vitamin C is known to operate as an antioxidant.

Pleiner et al. examined vitamin C's effect on vasodilation and forearm blood flow following injection of a low-dose of *Escherichia coli* endotoxin (LPS). While the LPS initially decreased forearm blood flow by 30%, the addition of intra-arterial vitamin C completely reversed these effects. There was no effect on blood flow among control subjects (Pleiner et al., 2002).

Given vitamin C's documented role in regulating blood flow, it is not surprising that low concentrations of plasma vitamin C are related to a significantly increased risk of ischemic heart disease and stroke (Gey et al., 1993). Gey et al. analyzed data from the 12-year Basel Prospective Study and found that low concentrations of vitamin C increased the risk of these diseases independent of concentrations of other vitamins such as vitamins A or E.

Vitamin C appears to be a key modulator in rates of cholesterol and other lipid synthesis. Guinea pigs fed suboptimal vitamin C (50 mg/kg) demonstrated numerous hyperlipidemic effects such as lowered high-density lipoprotein (HDL); and elevated triacylglycerides (TAG), total and very-low-density lipoprotein (VLDL)/low-density lipoprotein (LDL) cholesterol, and cholesteryl ester transfer protein (CETP). Authors propose that the potential mechanism behind these observed effects is due both to an increased secretion of VLDL from the liver into circulation as well as a decreased removal of TAG from circulation as a result of changes in lipolytic enzymes in the liver (Montano et al., 1998).

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are a commonly used marker of lipid peroxidation and overall circulating oxidation. Johnston et al. examined the differential effect of consuming orange juice versus a vitamin C supplement on TBARS formation. Study participants either drank 8 ounces of orange juice or took about 70 mg of supplemental vitamin Cs, each treatment providing comparable amounts of vitamin C. Results show that the orange juice and the supplement were equally effective at reducing plasma TBARS formation. One surprising finding is that one cup of orange juice was equally effective than two cups at inhibiting lipid peroxidation in plasma, perhaps due to a leveling effect (Johnston et al., 2003).

Multiple Organ Failure

Another serious health concern resulting in low concentrations of vitamin C is multiple organ failure (Borrelli et al., 1996). Interestingly, vitamin E

concentrations in patients with multiple organ failure were not significantly different from patients who were at risk of developing multiple organ failure but did not do so. Authors explain these findings by appealing to ascorbic acid's early intervention in the free radical scavenging process; thus, it makes sense that its concentrations would decrease before those of vitamin E. Although this study was not an intervention, the finding that vitamin C concentrations were significantly lowered in these patients is important so that practitioners can be aware that extra supplementation is likely necessary to restore them to healthy values.

Pregnancy

Ramirez et al. studied how various levels of ascorbic acid supplementation in pregnant and virgin rats affected a number of physiological markers. Notably, plasma concentrations of the oxidative stress marker malondialdehyde (MDA) were significantly elevated in both virgin and marginally supplemented pregnant rats (receiving 0.25 mg/mL ascorbic acid in fluid) compared with fully supplemented ones (receiving 1 mg/mL ascorbic acid in fluid). Birth weights of fetuses from marginally supplemented pregnant rats were significantly less than those from fully supplemented ones. These data indicate the importance of consuming adequate vitamin C during pregnancy (Ramirez et al., 2006).

Vitamin C also appears to protect embryonic development and maternal health during pregnancy. Endothelial cells can phagocytose dead trophoblasts (which make up the outer layer of the ectoderm that nourishes a developing fetus).

However, this process may be accompanied by oxidation, which can either damage or activate the endothelial phagocyte and lead to preeclampsia. An *in vitro* study by Chen et al. showed that treating phagocytosing endothelial cells with vitamin C led to increased phagocytosis of dead trophoblasts while protecting the endothelial cells from activation and preventing an increase in interleukin-6 (IL-6), which often accompanies phagocytosis of trophoblasts. Thus, vitamin C supplementation may be beneficial in preventing the development of preeclampsia (Chen et al., 2009).

Insulin

Supplementing with megadoses of vitamin C has been shown to delay the insulin response to a glucose challenge in non-diabetic individuals. It is hypothesized that this is due to competition between glucose and vitamin C for cellular uptake by glucose transporters (Johnston & Yen, 1994). While research would have to be conducted among diabetics before definitive conclusions can be drawn, these results hold promise as a means to help control blood glucose.

Patients with insulin-dependent diabetes appear to have impaired tissue storage of ascorbic acid, even if their intake is sufficient. This may lead to intracellular scurvy, which may be responsible for some of the degeneration typical of this disease. Cunningham et al. analyzed blood samples from diabetics and nondiabetics and compared trends. Although vitamin C consumption exceeded recommendations among diabetics and their plasma AA concentrations were normal, storage of AA (as seen in mononuclear leukocytes) was

significantly reduced by 33%. These data support the theory that glucose is inhibiting cellular uptake of ascorbic in insulin dependent diabetes (Cunningham et al., 1991)

Mood

An interesting finding that may affect the way doctors treat patients with clinical depression in the future is that patients with major depression have lower concentrations of plasma ascorbic acid compared to control subjects (Dakhale et al., 2005). Authors propose that this suggests depressed patients have higher oxidative stress and lipid peroxidation than non-depressed individuals. Interestingly, AA concentrations rose significantly above baseline values for patients with depression when they were treated with the selective serotonin reuptake inhibitors fluoxetine and citalopram. This further suggests that the plasma ascorbic acid concentrations are altered in depression.

Ascorbic acid appears to operate synergistically with conventional antidepressants in addition to producing antidepressant effects of its own. Ascorbic acid's antidepressant effect appears to be related to its interaction with the monoaminergic system (Binfare et al., 2009). Monoamines include serotonin, norepinephrine, epinephrine, and dopamine which are integral to mood regulation.

Brody et al. noted a direct correlation between ascorbic acid supplementation of 3,000 mg daily and a decrease in Beck Depression scores among his subjects. Interestingly, female subjects were more responsive to the supplementation than the males in this study. Brody noted that supplementation

at this level is actually low compared to levels used in animal studies, when they are compared on a mg per kg basis (Brody, 2002)

Eren et al. also found that concentrations of vitamin C, reduced glutathione, and glutathione peroxidase (GSH-Px) activity were all lowered in the cortex of the brains of depressed rats compared to controls. Their study induced depression in rats by exposing them to various stressors collectively referred to as chronic mild stress (CMS). Depressed rats were then treated with one of three antidepressant medications, or with nothing. None of them were supplemented with vitamin C, although concentrations of vitamin C in the brain cortex were measured to note changes in response to the induction of depression and treatment with antidepressant medications (Eren et al., 2007).

Jaser et al. examined if a relationship exists between depression and healthy behaviors. Although this study cannot draw causative conclusions (that is, it is unclear whether the depression caused poor health behaviors or if these behaviors subsequently led to the depression), it was clear that depressed youths had higher BMIs and fasting insulin concentrations than those who were not depressed. They also had lower levels of physical activity, self-efficacy for diet and movement as reported in METs (Metabolic Equivalent Task), poorer dietary intention and choice, and less support for physical activity. While this study did not measure plasma concentrations of vitamin C, the poor dietary choices reported among depressed youth indicate that they likely would have low concentrations of plasma vitamin C too (Jaser et al., 2009).

Pain

Ascorbic acid appears to interact with the glutamatergic system to ameliorate pain sensation. Rosa et al. studied this pattern in mice, and concluded that ascorbic acid attenuated pain induced by formalin, glutamate, and ionotropic glutamatergic-induced pain perception in mice. Authors propose that inhibiting *N*-methyl-D-aspartate (NMDA) receptors is largely responsible for this effect (Rosa et al., 2005)

Vision

Vitamin C appears to be protective against the development of cataracts, both in the form of ascorbate and DHA. It is hypothesized that DHA may be reduced to ascorbate in this situation (Martensson & Meister, 1991). Dherani et al. conducted a cross-sectional study of 1,112 people from North India, looking at plasma concentrations of antioxidants and incidence of cataracts. They found that plasma vitamin C was inversely associated with having cataracts (Dherani et al., 2008)

Fletcher et al. studied the relationship between blue light exposure (part of the spectrum of visible light, not UV light, from the sun) and age-related macular degeneration (AMD), and the influence of dietary antioxidants on the development of AMD. They found that while in general there was no association between blue light exposure and incidence of early AMD, among individuals in the lowest quartile of antioxidant status there was a significant association between blue light exposure and early AMD development (Fletcher et al., 2008).

Mortality

It may follow, intuitively, that since vitamin C lowers the risk of developing so many serious health issues that it would also be linked to a reduced mortality. This association was studied by Loria et al. They found that although no relationship was observed between serum vitamin C and mortality among women, there was a significant association among men, even when controlling for smoking status. Men in the lowest quartile of serum ascorbate were 57% more likely to die of any cause and 62% more likely to die from cancer. After controlling for fruit and vegetable consumption the association was still significant, implying that vitamin C exerts an independent effect on mortality risk apart from general produce consumption (Loria, Klag, Caulfield, & Whelton, 2000).

Physical Activity

Recommendations

It is well known that physical activity is important for health and quality of life; however, many Americans are unsure how much exercise is recommended by health professionals. The American College of Sports Medicine (ACSM) currently recommends that in order to maintain health and reduce disease risk, healthy adults under age 65 should participate in moderately intense aerobic activity for at least 30 minutes 5 days per week, or vigorously intense cardio activity for at least 20 minutes 3 days per week. Additionally, adults should perform 8-10 strength training

exercises of 8-12 repetitions each, twice a week (American College of Sports Medicine & the American Heart Association.,). Moderately intense physical activity is defined as being vigorous enough to raise one's heart rate and to begin sweating, but the individual is able to converse. In order to lose weight and maintain weight loss, adults may need to engage in 60 to 90 minutes of physical activity.

Benefits

Diabetes risk reduction

A large study by Laaksonen et al. examined the link between physical activity and preventing Type 2 Diabetes. Their findings show that people in the upper third quadrant of those who accumulated the most leisure-time physical activity were 80% less likely to develop diabetes compared to those in the lower quadrant. This finding was true even after adjusting for age, sex, group, smoking status, and major risk factors for diabetes. Those who followed current American College of Sports Medicine (ACSM) guidelines of engaging in moderate-to-vigorous leisure time physical activity at least 2.5 hours per week during the follow-up period in this study were 44% less likely to develop diabetes than those who remained sedentary (Laaksonen et al., 2005). These findings indicate that physical activity is intrinsically beneficial in the prevention of developing type 2 diabetes, irrespective of associated weight loss.

How to increase likelihood of exercising

There are numerous, varied motivators for people to engage in physical activity. These may differ based on age, sex, and health of the individual. Multiple types of interventions have been studied to determine what methods are most effective at encouraging people to begin, and more importantly, to continue to adhere to engaging in regular physical activity for the long-term.

One study led by Wilcox examined the efficacy of telephone-based and group-based programs aimed at increasing the physical activity level of participants. Since the two programs consisted of different demographics, it is difficult to draw comparisons between the two studies as to which is more effective. In general, for both groups results indicate that the least active group showed the greatest improvement. Larger increases effects were observed among people with stronger self-reported social support than those without such a support network. The group-based program also reported more significantly increased physical activity concentrations among females, Latinos/Hispanics, overweight and obese persons, and those limited in physical activity due to osteoporosis (Wilcox et al., 2009).

Heshka et al. investigated whether self-help or a structured commercial weight loss program would produce greater weight loss results. Participants in the self-help group received nutritional counseling for 20 minutes from a dietitian at the start of the study and again at week 12. They were also provided with printed material directing them to general dietary and exercise recommendations for weight loss. The other group were given vouchers for weekly Weight

Watchers meetings and informed about local sites. They were put on a food plan with a moderate caloric deficit built in, an exercise plan, and behavior modification plan. While both groups were able to lose and successfully keep weight off for two years, the commercial group had higher average losses (4.3-5.0kg) than the self-help group (1.3-1.4kg) (Heshka et al., 2003).

Another technique used to increase likelihood of increasing physical activity is participating in motivational interviewing (MI). In a study led by Bennett cancer survivors who had completed at least six months of treatment were assigned either to the intervention group or a control one. Those receiving motivational interviewing counseling had an initial meeting with the counselor immediately following group assignment, then three subsequent telephone conversations (2 weeks, 2 months, and 4.5 months after the initiation of the trial). Phone conversations consisted of about 20 minutes of MI, during which time the counselor tried to help participants figure out ways to overcome barriers to increasing physical activity levels. The control group did not receive any MI. After the six month trial, participants in the MI group participated in significantly more physical activity than the control group, even after controlling for time since cancer treatment ($p < 0.05$) (Bennett, Lyons, Winters-Stone, Nail, & Scherer, 2007). This study affirms the importance of emotional support and problem solving for increasing levels of physical activity.

Similarly, Coble et al studied the theory of planned behavior (TPB) on the likelihood of increasing levels of physical activity among Westbank First Nation

members (an Indian tribe in Canada). Findings of the study indicate that intention to engage in physical activity explained 16% of variance in reported levels of physical activity. Affective behavior and perceived behavioral control predicted reports of intention (Coble, Rhodes, & Higgins, 2009).

Reasons why people do not exercise

Despite the well-documented benefits of exercise, few Americans actually meet the recommended daily levels of physical activity. While much of this decision is fairly controllable by the individual, it is certainly more easy to be active when resources are readily available and in safe locations. Estabrooks et al. examined available resources to promote physical activity in numerous neighborhoods of varying socioeconomic status (SES). Neighborhoods were classified as high, medium, or low SES based on information from the U.S. Census Bureau. Not surprisingly, low- and medium-SES neighborhoods had fewer free resources (for example, parks). However, the neighborhoods did not vary in terms of number of pay-for-use fitness facilities (Estabrooks, Lee, & Gyurcsik, 2003). Nonetheless, the cost may be too much of a barrier for some of these families to take advantage of the resources available. Thus, the cost of fitness facilities may be a deterrent for some people to exercise.

Although people may think that they need to hire a personal trainer in order to adopt an exercise program, Rooks et al. demonstrated that self-paced resistance training and walking were effective at improving functional performance and neuromuscular efficiency in adults age 65 and older living

independently (Rooks, Kiel, Parsons, & Hayes, 1997). This study was unique in that it shows that fitness can be improved through a self-paced intervention not directed by a fitness professional.

Correlation with mood

Not only has physical activity been linked to improved physical health, it also has benefits for mental wellbeing. Singh et al. conducted a study examining the different effects of high intensity weight training, low intensity weight training, and general practitioner care for treating clinical depression in the elderly. Their results show a significant, dose-responsive effect for high intensity weight training in reducing depression. Not only did this exercise ameliorate the depression, it also led to an improved quality of life, better sleep, and greater muscle strength than either other type of treatment (Singh et al., 2005).

Aerobic activity has also been documented to improve mental wellbeing. Sakuragi and Sugiyama studied the effect of walking for one hour daily for four weeks on subject's emotional state, as assessed by the POMS test, compared to controls who did not walk. They found that following the intervention subjects who walked had significantly lower scores on the Anger-Hostility (A-H) subsection of POMS compared to controls, suggesting that walking may improve mood (Sakuragi & Sugiyama, 2006).

Berger and Owen studied the effect of swimming, another type of aerobic exercise, on mood. They found that both beginner and intermediate level swimmers experienced significant reductions in tension, depression, anger, and

confusion, and they increased in vigor on the POMS scale after exercising compared to before. Controls who simply sat in a classroom lecture did not experience these reductions (Berger & Owen, 1983).

Relationship with mental performance

Another documented benefit of physical activity is that it can enhance cognitive functioning. Williamson et al analyzed data from the Lifestyle interventions and Independence for Elders pilot (LIFE-P) study and concluded that exercise benefits mental operation of the elderly (Williamson et al., 2009).

The elderly are not the only ones to benefit mentally from exercising. Davis et al. studied the effect of low dose exercise (20 minutes daily, 5 days per week) and high dose exercise (40 minutes daily, 5 days per week) compared to a control group (sedentary) in overweight children. They found that there was a significant difference between the control and high dose exercise groups when assessed on the Cognitive Assessment Scale (CAS) in the area of executive function (brain functions such as planning, organizing, and goal-setting). Since both intervention groups exercised at about the same intensity (keeping heart rates above 150 beats per minute), it appears that the length of exercise session was important in achieving the mental benefits (Davis et al., 2007).

An interesting retrospective cohort study by Aberg et al. found a significant relationship between cardiovascular fitness and better cognitive scores. They reviewed data for all Swedish men born between 1950 and 1976 who enlisted in the military at age 18. Fitness and intelligence data were gathered

from initial conscription tests for military service. Fitness level was also positively linked to educational achievements later in life, as well as socioeconomic status (SES). Information on education and SES was garnered from other national databases (Aberg et al., 2009).

Quality of life

Exercise has been shown to improve overall quality of life. This encompasses a number of different aspects, such as sleep quality, ability to carry out functions of daily life, and general mobility- a concern particularly salient among the elderly. As mentioned earlier, Singh et al found that high-intensity weight training improved sleep quality (Singh et al., 2005). Manty et al analyzed whether a single session of physical activity counseling, followed by supportive telephone contact every four months for two years would improve mobility. Their results indicate that this is, in fact, the case (Manty et al., 2009).

Cress et al. used the Continuous Scale-Physical Functional Performance test (CS-PFP) to analyze the effects of exercise on physical capacity, health status, and physical functioning in a population of older adults (mean age 76 \pm 4). Results showed an improvement among the exercisers in maximal oxygen consumption, muscle strength, and overall CS-PFP score. These findings demonstrate that physical activity can not only prevent functional decline associated with aging, but can actually reverse it (Cress et al., 1999).

Correlation with vitamin c

It is interesting to study plasma ascorbic acid as it relates to physical activity. Camoes et al. studied the relationship between diet and physical activity of participants. Results showed that leisure time activity energy expenditure was significantly positively associated with vitamin C consumption in both men and women (Camoses & Lopes, 2008).

As generally beneficial as it is to exercise, one negative effect is the production of free radicals and consequent oxidative damage. Ascorbate has been shown to counteract oxidation specifically in this context. Ferrer et al. studied induced oxidative damage in lymphocytes and neutrophils following an intense (75-80% maximal capacity) one hour long swim in male and female swimmers. Following the swim session ascorbate concentrations in neutrophils were decreased in both males and females, and oxidative damage was observed in neutrophils. Neutrophilia was also documented. Ascorbate concentrations in lymphocytes did not decrease following swimming (Carrillo, Murphy, & Cheung, 2008a; Ferrer et al., 2009). This study suggests that ascorbate is influential in responding to oxidative stress induced by exercise.

Not only does exercise produce free radicals, it also transiently elevates cortisol concentrations. Cortisol, commonly known as “the stress hormone”, can lead to health problems when concentrations remain elevated for prolonged periods of time. Carrillo et al. studied the effect of ingesting 500 mg of vitamin C daily for 12 days prior to a 2-hour long exercise session in a hot and humid environment compared to taking a placebo. Results indicate a significant

attenuation in postexercise cortisol among study participants taking the supplement versus those taking the placebo (Carrillo, Murphy, & Cheung, 2008a).

Davidson et al. explored the relationship of exercise-induced immunoendocrine responses and antioxidant supplementation. Subjects either consumed a placebo or an antioxidant comprised of 1,000 mg L-ascorbic acid and 400 IU RRR-alpha-tocopherol daily for 4 weeks prior to cycling at 60% of maximal oxygen uptake for 2.5 hours. Following the exercise, subjects taking the placebo had significantly higher concentrations of plasma cortisol. Furthermore, participants consuming the antioxidant supplement had lower concentrations of thiobarbituric acid reactive substances (TBARS, a commonly recognized marker of oxidative damage), after exercising (Davison et al., 2007).

Kirby et al. studied the effect of an ascorbic acid infusion in the forearm during rhythmic handgrip exercise in older adults (mean age 65 +/- 2). Blood flow was found to increase 30% during the continuous exercise due to vasodilation. Furthermore, when they transitioned from rest to continuous exercise with simultaneous ascorbic acid infusion, steady-state blood flow improved significantly, and vasodilator responses to acetylcholine were no longer observed (Kirby et al., 2009).

Apparently these dilation effects are dependent upon the baseline status of the individual receiving the treatment. Eskurza et al. studied the effect of ascorbic acid infusion on flow-mediated dilation among young sedentary men (mean age 25 +/- 1), older sedentary men (64 +/- 2), and older endurance trained men (64 +/-

2). The infusion significantly improved flow-mediated dilation in older sedentary men, but not in the other two groups (Eskurza et al., 2004)

These findings collectively suggest that ascorbic acid status can influence one's level of physical activity, as well as the physiological response to exercise due to several mechanisms. Ascorbic acid appears to attenuate post-exercise cortisol response and to combat free radicals that are produced during exercise. It can improve vasodilation and is a vital mediator in energy production, specifically as a co-factor in beta-oxidation (fat metabolism) as discussed in the section *Cofactor*. Also, observational studies have noted correlations between body fatness, BMI, and plasma vitamin C status (Canoy et al., 2005; Johnston et al., 2007; Watters et al., 2008). Based upon the existing evidence and theory in the literature, we chose to investigate if daily physical activity would vary between subjects supplemented with vitamin C and those depleted of vitamin C.

Chapter 3

Materials and Methods

Participants

Thirty-one young men were recruited from the Polytechnic Campus of Arizona State University via flyers, e-mails, announcements on Blackboard, and word of mouth. Interested individuals were screened for age (18-40 y), sedentary behavior (no more than three bouts of cardiovascular exercise weekly), general health (free of active illness and medical conditions), and vitamin C supplement use (<100 mg/d). Individuals meeting these criteria who were willing to comply with the dietary protocol for eight weeks were invited to the test site following a 5-h fast. Twenty-nine men completed the whole study, but one was a vitamin C outlier at data analysis; therefore, my final sample size was 28. At the test site, participants provided written consent (Appendix A), and a blood sample was collected. Participants completed the POMS survey (Appendix B), and a validated physical activity questionnaire that was designed to assess exercise behavior change in intervention trials (Appendix C). Participants then completed the YMCA 3 minute step test (Appendix D). Subjects were given instructional sheets explaining the study protocol and a list of restricted and permitted foods (Appendix E) and shown how to use the pedometer and record steps (Appendix F). All research was conducted in accordance with the guidelines established by the University Human Subjects Review Committee at Arizona Sate University

(Appendix G) and took place at the Nutrition Department building on Arizona State University's Polytechnic campus.

Study Design

Study Protocol

A randomized, double blind, placebo controlled, parallel arm study was implemented for this 8 week long study. At the initial visit, participants filled out the POMS questionnaire and a questionnaire about food consumption and physical activity; subjects' height, weight, and body fat percentage were measured; they completed the YMCA 3 minute step test; and a fasting (5 hour fast) blood sample was drawn. Pedometers were customized for each subject based on their stride length and given to them to use for the duration of the study. Subjects were instructed to begin consuming the study capsule daily (starting the day of their visit) and to wear their pedometer daily. Step logs were recorded on paper for submission to researchers at each visit and also stored in the memory of the pedometers to be downloaded onto the researchers' computer at each visit.

For study weeks 1-2, all subjects consumed a placebo capsule. At the end of week 2, participants fasted for at least five hours and reported to the test site for visit #2. Subjects again completed the POMS questionnaire and the same questionnaire about recent dietary intake and physical activity as at the first visit. They were measured for weight and body fatness using the Tanita scale and performed the YMCA 3 minute step test prior to having a blood sample drawn. The Tanita scale has been found to give results very close to other methods that

are more time consuming {{183 Spencer,C.E. 2003; }}. Based on the data from the first visit subjects were stratified by vitamin C status, BMI, and age, and randomly assigned to the vitamin C group (VC) or the placebo group (CON). For the next six weeks of the study (weeks 3-8) subjects continued to adhere to the same dietary restrictions and consumed one study capsule daily. VC subjects consumed a vitamin C capsule containing 500 mg, and CON subjects consumed a similar placebo capsule containing white flour once daily. I chose to use 500 mg as the supplement dose for vitamin C since theoretically it could be attained through dietary consumption and a study by Johnston et al. found that optimal levels of consumption (as determined by Heinz body neutralization) is between 500 and 1000 mg of vitamin C {{48 Johnston,C.S. 2001; }}

Blood draws, the YMCA 3 minute step test, weight and body fat measurements, the food and activity questionnaire, and the POMS questionnaire were repeated at study week 8. Five dollar gift certificates to Target were given to participants at study week 5 and 20 dollar gift certificates to Target were given to participants at study week 8.

I conducted a power calculation which determined that I should have 34 subjects, assuming 0.05 probability and 0.80 power. I used a change of 0.2 mg/dL for plasma vitamin C and 2.5 hours for vigorous activity in the calculation.

Dietary Intake

For the duration of the study subjects were asked to comply with the dietary restrictions provided on a handout (a list of selected fruits, vegetables and

fortified foods containing >40 mg vitamin C per serving; see Appendix E).

Furthermore, they were not to consume any vitamin supplements aside from a basic multi-vitamin and mineral tablet during the 8-week trial.

Plasma Vitamin C Assay

Plasma vitamin C was analyzed using the colorimetric method by Omaye et al. Fasting venous blood samples were collected in sodium K3 EDTA-anticoagulated vacutainer tubes; ascorbic acid was oxidized in the presence of copper and reacted with 2,4-dinitrophenylhydrazine to yield bis-2,4-dinitrophenylhydrazone; and a chromophore was produced in the presence of sulfuric acid. The resulting chromophore was read colorimetrically at 520nm.

Physical Activity Questionnaire

Subjects answered questions pertaining to the number of hours per week they engaged in light, moderate, and vigorous physical activity (see Appendix C). These answers were used to calculate their total MET expenditure (“light” activity was multiplied by 3, moderate by 5, and vigorous by 9). This questionnaire was designed by Gionet and Godin (Gionet & Godin, 1989) to assess changes in exercise behavior that may occur during an intervention trial .

YMCA 3 minute step test

Equipment needed for the YMCA three minute step tests includes a 12 inch step, stopwatch, and metronome set at 96 beats per minute. The subject steps up first with one foot, then with the second, then steps down with the first, and finally down with the second to the beat of the metronome. This pattern is

repeated for three minutes, at which time he or she immediately sits down.

Within five seconds the tester is to count the subjects' pulse for one minute. The subject's score on the test is his or her heart rate at the conclusion. This test is conducted as a means to assess physical fitness. See Appendix D for further details.

Profile of Mood States (POMS)

The Profile of Mood States tests subjects on 6 factors: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. Subjects are asked to complete the test based on how they felt overall during the last week. The test can be either administered to groups or individuals, and it should be understandable even by those with only a seventh grade reading level ability. The test is generally able to be completed in 3-5 minutes and is intended for use in non-clinical populations (that is, those without mental illness). The test was developed by Drs Douglas M. McNair, Maurice Lorr, and Leo F. Droppleman (1992). This test was validated through a study by Nyenhuis et al (Nyenhuus, Yamamoto, Luchetta, Terrien, & Parmentier, 1999).

Statistical analyses

Data were reported as mean values \pm the standard error, and were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Mean physical activity levels of the supplemented compared to the unsupplemented group were analyzed using independent t-tests. A p value of ≤ 0.05 was

considered significant. The Statistical Package for the Social Sciences (SPSS, version 16.0) was used for all analyses.

Chapter 4

Results

Fifty men initially indicated interest in being study participants. Thirty-one of these men qualified and remained interested after description of the study. One subject broke his foot in a car accident during the first week and could not complete the study. Another decided that he did not want to comply with the dietary restrictions and dropped out of the study during the first week. Hence, twenty-nine men completed my study in its entirety. At data analysis, one subject was a plasma vitamin C outlier (>3 standard deviations from the mean) and his data were removed from my dataset, leaving me with twenty-eight subjects in my dataset for statistics. At baseline there were no significant differences between subjects in the placebo or vitamin C groups with regards to age, height, weight, body mass index (BMI), body fat percentage, or waist circumference (see table 1), and all data were normal. Our participants were healthy individuals- they did not have any major health problems and had fairly typical activity levels representative of most Americans.

Measure	Vitamin C group	Placebo group	P
Sample size	14	14	
Age (years)	23.9 \pm 0.6	22.9 \pm 0.7	0.310
Height (inches)	70.0 \pm 0.7	69.2 \pm 0.8	0.452
Weight (pounds)	166 \pm 8	169 \pm 8	0.809
BMI	24.9 \pm 0.7	24.7 \pm 1.0	0.893

Body fat percent	17.8±1.1	19.0±1.7	0.567
Waist (inches)	33.4±0.9	33.7±0.9	0.802

Table 1. Baseline values for participants

Descriptives (p value represents independent t-test)

$$SE=SD/\sqrt{n}$$

At the first visit participants were given pedometers to measure their steps. Despite having no significant differences in demographics, the placebo group averaged significantly fewer steps than the vitamin C group at week 2, based on either the two week average or the 7 day prior to the second visit average (see table 2).

Measure	Week 0	Week 8	P*
Steps (2-wk average)			
Placebo	5092±438†	6002±710	0.083
Vitamin C	7374±794	7207±652	
Steps (7-day average)			
Placebo	5148±480†	6920±894‡	0.017
Vitamin C	7343±816	7166±599	

Table 2. Steps at weeks 2 and 8 for participants consuming a vitamin C-restricted diet and randomized to receive placebo capsule (n=14) or vitamin C (500 mg/d) capsule (n=14).

*denotes P value for repeated measures ANOVA analysis for interaction.

†denotes significant difference from vitamin C group

‡denotes significant difference from baseline week 2 value

Contrary to our hypothesis, the placebo group, and not the vitamin C group as hypothesized, significantly increased their steps at week 8 compared to week 2 (p=0.017), and the step difference between the placebo and vitamin C group was no longer significant at week 8 (see figure 1).

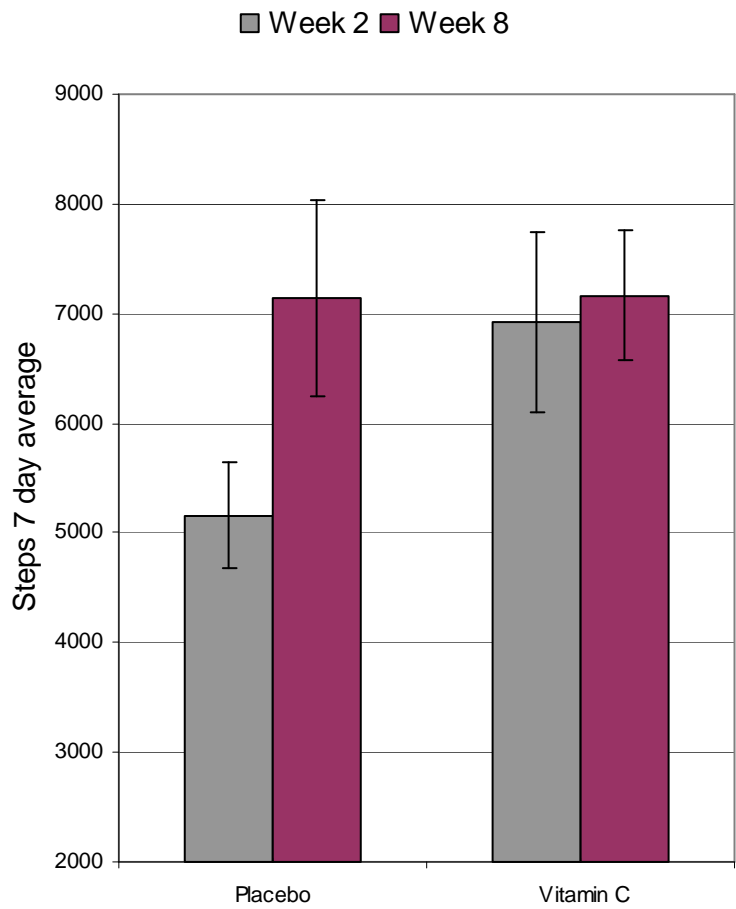


Figure 1. 7 day step average at weeks 2 and 8

Plasma vitamin C, activity levels, and heart rate at weeks 0, 2, and 8 are displayed in table 3. At baseline, there were no differences between groups for any of the measures. At the completion of the trial, the only significant difference between groups (controlling for the pill numbers missed) was for plasma vitamin C ($p=0.035$) (see figure 2). However, some interesting trends were noted.

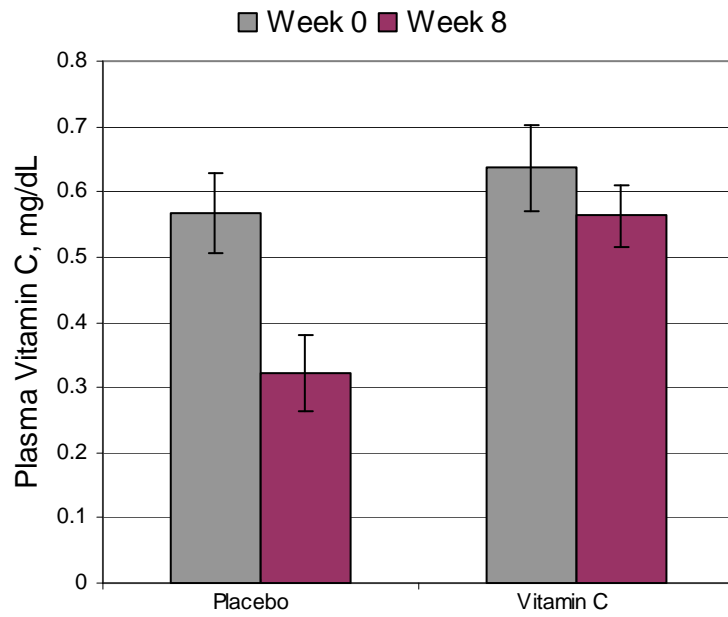


Figure 2. Plasma vitamin C at weeks 0 and 8

Study Group	Week 0	Week 2	Week 8	P*
Plasma vitamin C, mg/dL				
Placebo	0.567±0.227	0.557±0.216	0.321±0.217‡	0.035
Vitamin C	0.637±0.246	0.602±0.222	0.563±0.181	
Light Activity				
Placebo	4.08±0.67	6.15±3.16	6.31±2.90	0.331
Vitamin C	5.38±0.94	6.21±3.29	6.64±2.10	
Moderate				

Activity				
Placebo	4.43±0.64	5.23±2.65	4.31±1.93	0.994
Vitamin C	4.00±0.45	5.82±3.17	4.00±2.45	
Vigorous Activity				
Placebo	2.64±0.60	2.31±1.93	2.15±1.95	0.082
Vitamin C	2.92±0.47	3.07±1.27	3.71±3.07	
METS				
Placebo	58.8±8.1	65.4±7.6	59.8±6.2	0.262
Vitamin C	62.5±6.1	75.4±5.7	73.4±3.9	
Heart Rate (beats per minute for one minute following the 3 minute step test)				
Placebo	93.6±4.7	99.7±4.4	100.1±4.6	0.421
Vitamin C	84.4±4.2	90.8±4.0	85.4±3.9	

Table 3. Plasma vitamin C, activity levels, and heart rate at weeks 0, 2, and 8 for participants consuming a vitamin C-restricted diet and randomized to receive placebo capsule (n=14) or vitamin C (500 mg/d) capsule (n=14).

*denotes P value for repeated measures ANOVA analysis for interaction (weeks 0 and 8)

‡ denotes significant difference from vitamin C value (p=.004) controlling for number of pills remaining at completion of trial

Light activity levels were modestly elevated in both the vitamin C and placebo groups during the study, although vitamin C subjects still recorded

slightly higher levels of light activity compared to placebo values ($p=0.331$).

Vigorous activity levels tended to increase in the vitamin C group and to decrease in the placebo group ($p=0.082$). Recovery heart rate appeared to increase to some extent in the placebo group during the study. While the vitamin C group saw a minimal increase in heart rate following the initial 2 week depletion period, this was reversed back to the starting point after 6 weeks of vitamin C supplementation ($p=0.421$).

MET values increased very slightly for the vitamin C group and remained static for the placebo group ($p=0.262$) (see figure 3). MET values were calculated by multiplying the number of hours of “light” activity by 3, “moderate” activity by 5, and “vigorous” activity by 9 and summing these scores. Thus, higher MET values reflect higher energy output.

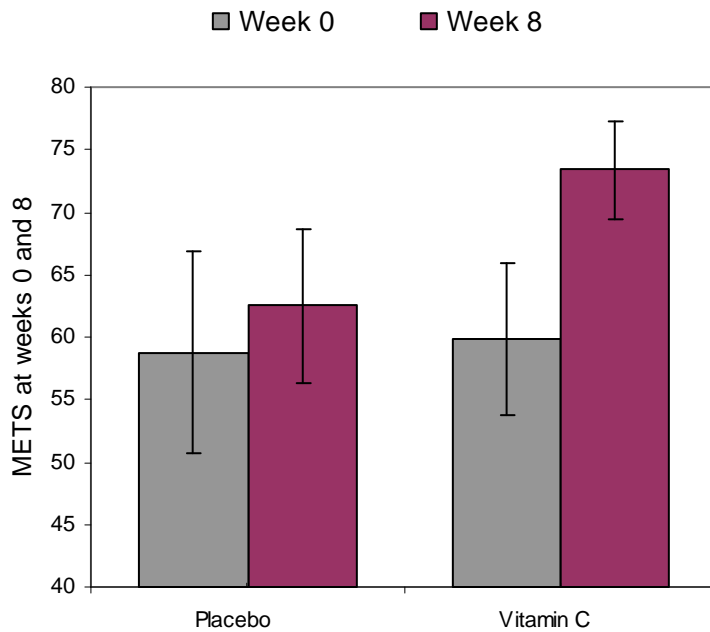


Figure 3. METS at weeks 0 and 8

Of all the subsections of POMS, the only significant difference in mood between vitamin C and placebo groups occurred at week 8 in depression (see table 4 and figure 3). Placebo subjects reported significantly more depression than vitamin C subjects ($p=0.041$). There were some notable trends that did not reach statistical significance. Tension tended to increase among placebo subjects and to decrease among vitamin C subjects ($p=0.088$), and total POMS score increased very slightly among subjects receiving the placebo and to decrease somewhat among subjects receiving vitamin C ($p=0.107$). Subjects receiving vitamin C reported modest attenuation in fatigue ($p=0.280$) and confusion ($p=0.190$) as the study progressed, although this was not significant. Low POMS

scores reflect less mood disturbance and are thus desirable. POMS scores are manually calculated by following a rubric provided by the company.

Measure	Week 0	Week 2	Week 8	P*
POMS Tension				
Placebo	8.7±1.0	9.7±0.8	11.1±1.6	0.088
Vitamin C	9.9±1.1	9.4±1.4	7.4±1.5	
POMS Depression				
Placebo	11.8±1.5	13.1±1.6	14.4±2.0‡	0.048
Vitamin C	11.5±2.6	9.54±1.86	8.5±1.90	
POMS Anger				
Placebo	11.6±1.1	13.3±1.9	12.6±1.9	0.744
Vitamin C	10.3±1.4	9.08±1.69	10.5±1.9	
POMS Vigor				
Placebo	21.1±1.2	20.1±1.0	20.8±1.1	0.606
Vitamin C	23.0±1.1	24.2±1.7	24.0±1.9	
POMS Fatigue				
Placebo	12.1±1.1	12.4±1.5	12.5±1.7	0.280
Vitamin C	12.5±1.6	11.5±1.7	10.9±1.6	
POMS Confusion				

Placebo	8.3±1.0	8.36±0.82	9.6±0.96	0.190
Vitamin C	7.1±1.1	6.38±1.12	6.1±1.03	
POMS total				
Placebo	31.4±4.1	36.7±5.8	39.4±7.8	0.107
Vitamin C	28.2±5.6	21.6±7.1	19.5±7.0	

Table 4. POMS at weeks 0, 2, 8 for participants consuming a vitamin C-restricted diet and randomized to receive placebo capsule (n=14) or vitamin C (500 mg/d) capsule (n=14).

*denotes P value for repeated measures ANOVA analysis for interaction (weeks 0 and 8).

‡ denotes significant difference from vitamin C value (p=.041)

Chapter 5

Discussion

While we hypothesized that subjects in the vitamin C group would increase their physical activity levels, as measured by the number of steps recorded on the pedometer, the opposite was true. Subjects in the placebo group increased their steps and subjects in the vitamin C group decreased their steps. This coincides with the reported increase in depression and tension among placebo subjects. Although contrary to what we expected, a study by McKercher et al. also found that, in young men who recorded <10,000 steps/day, step counts were higher in men with a higher prevalence of depression. However, young women did not show this pattern of step counts; rather, as the prevalence of depression increased in young women, step counts decreased. (McKercher et al., 2009). These puzzling results make it clear that future research must probe how gender impacts depression and physical activity, especially the psychological mechanisms and reasons behind why men and women behave as they do when depressed. It would also be important to differentiate between leisure time physical activity and structured exercise programs to see if participation rates in these activities vary based on depression status.

In addition to the pedometer data, we recorded activity intensity using a validated questionnaire designed to capture exercise behavior change for intervention trials (Gionet & Godin, 1989). This uncomplicated, quick to administer instrument had subjects quantify over a 7-d period, how many hours

they participated in light, moderate, and vigorous activities. Each category was assigned an average metabolic rate (MET) allowing for an estimated quantification of activity intensity for each subject, subjects receiving vitamin C reported marginal increases in light activity, vigorous activity, and overall METS. Thus, while total steps did not increase, overall energy output due to increased vigorous activity seems to have increased somewhat among the vitamin C group. This should not be surprising since adequate vitamin C status is documented to help exercise performance (Johnston, Corte, & Swan, 2006a). Johnston et al. conducted a submaximal exercise test on subjects at the start of an intervention trial and after 8 weeks of supplementing half of the participants with vitamin C (the others were the control group). Participants supplemented with vitamin C had significantly lower plasma free carnitine, higher fat energy expenditure, and lower RER during exercise than controls. Fat oxidation during the exercise test was also inversely related to fatigue as measured by POMS. In summary, it appears that marginal vitamin C status hinders fat oxidation during submaximal exercise, which increases feelings of fatigue and leads to diminished exercise ability.

Marshall et al. found that pedometer step measurement is actually a poor proxy for METS and determining physical activity output (Marshall et al., 2009). In his study he analyzed pedometer step counts by multiple regression analysis, mixed-model analysis, and ROC-curve analysis. The differences between these three modes of analysis indicate that steps per minute account for 15-41% of

variance in METS. Thus, only 50-60% of participants were accurately classified as walking at moderate intensity based on step counts alone. It appears that about 100 steps per minute translates into “moderate” activity necessary for health benefits. This would be accomplished by walking 3,000 steps in 30 minutes 5 days per week or three daily bouts of 1,000 steps in 10 minutes per day 5 days per week to meet current exercise recommendations. Therefore, perhaps more weight should be given to our METS findings than our pedometer records. In this case, our findings would actually support our hypothesis, albeit weakly. Additionally, health practitioners might be wise to make exercise recommendations pertaining more to intensity as measured by METS than to simple step counts alone.

Improved mood and increased MET output due to vitamin C supplementation may be related. Mood exerts a strong influence on people’s daily decisions, including whether to exercise, and how intensely to exercise. It is possible that if people feel more positive and full of vigor they would be more likely to want to exercise and to have more emotional energy available to do so. Or it is possible that if they feel more full of vigor, they would be more likely to exercise, which may improve mood. At any rate, these two outcomes appear to exert a bit of a synergistic relationship on each other.

While I cannot draw definitive clinical implication conclusions since our study did not use clinically depressed subjects, it is interesting to reflect on the possibility of extending our findings into the clinical realm. Further studies ought to be conducted among clinically depressed individuals to see if they would

experience improved mood through higher intakes of vitamin C as our subjects did. If so, mental health practitioners would be wise to begin including vitamin C supplementation in their recommendations to depressed patients.

Given I saw improved mood and higher MET output among subjects consuming 500 mg of vitamin C daily compared to my controls, I would like to see the current RDA increased to 500 mg for both men and women. I realize that this is improbable since the RDA is set to prevent deficiency symptoms, not to optimize health. I think that this is a flaw and recommended intake levels ought to be set to optimize health. Certainly this would require new RDA levels for most nutrients, and such a change may fuel the public's perception that nutrition experts change their minds constantly. Nonetheless, I believe such changes are needed.

There are limitations to this research including the lack of compliance. At the conclusion of the study subjects were asked if they took all of their supplements. Subjects reported skipping on average 3-4 pills with no significant difference between groups. My sample size was also smaller than desired, based on my power calculation. Also, subjects voluntarily reported occasionally removing pedometers during crucial times (such as during a basketball game) which would have accrued many steps. Furthermore, the researcher saw many of the subjects outside of the research office and observed them twirling the pedometers, apparently out of boredom, which likely would skew step records. Finally, the method in which the vitamin C was taken may have prevented

maximal absorption. That is, since subjects took the supplement in one dose and were told that it did not matter if they took it with or without food, they may not have been able to absorb the full amount of vitamin C. Had the dosage been split between a couple of smaller pills and taken with food it is possible that their plasma levels of vitamin C would have been far higher.

It is difficult to determine the best way to promote compliance among subjects; however, we sent out weekly e-mails, provided subjects with the researcher's private cell phone number to answer questions about the study whenever they might arise, and gave gift cards twice during the study.

This study provides exciting support for existing literature documenting an association between higher levels of plasma vitamin C and lower levels of depression, and it provides groundbreaking research on the connection between higher levels of plasma vitamin C and increased levels of MET output. Future studies ought to probe the discrepancy between step counts and METS, particularly among subjects reporting increased depression using the POMS test.

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APPENDIX A
CONSENT FORM

ARIZONA STATE UNIVERSITY

CONSENT FORM

Vitamin status and Physical Activity Levels

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

Drs. Carol Johnston, a Nutrition professor, and Pam Swan, an Exercise Wellness professor, at Arizona State University, and Heidi Netland, a nutrition master student, have requested your participation in a research study.

STUDY PURPOSE

The purpose of the research is to determine if vitamin status is related to levels of physical activity as measured using pedometers.

DESCRIPTION OF RESEARCH STUDY

If you decide to participate, then as a study participant you will join a study examining the relationship between vitamin status and physical activity. At your first visit you will be randomly assigned to one of two research groups. You will be assigned to take either a vitamin supplement or a placebo daily for 6 weeks. Then you will complete the YMCA 3 minute step test to assess baseline fitness and have a blood sample drawn to assess baseline plasma vitamin C. The initial visit will take approximately one hour. You will fill out the Profile of Mood States (POMS) survey, have blood drawn, complete the YMCA 3 minute step test to assess fitness, and have use of the pedometer explained to you. You will be given the pedometer to wear daily and the supplements to take daily. Both research groups will follow dietary restrictions for the duration of the study as indicated on the Restricted Foods List handout. The second week you will wear a pedometer and record daily steps taken. At the end of the first two weeks you will come back to retake the YMCA 3 minute step test and to have your blood drawn again. During the final 6 weeks you will record total daily steps taken, as recorded by the pedometer, in a spreadsheet provided. At the end of weeks 5 and 8, you will return and complete the POMS survey, have blood drawn, complete the YMCA 3 minute step test, and submit your record of steps taken daily.

If you say YES, then your participation will last for 8 weeks. There will be an initial visit with a blood draw and three follow-up visits at Arizona State University's Polytechnic Campus, located at 7001 E. Williams Field Rd., Mesa, AZ 85212. Approximately 20 subjects will be participating in this study.

RISKS

If you decide to participate in this study, then you may face a risk discomfort during blood draws. The researcher tried to reduce these risks by having a qualified phlebotomist conduct the blood draws.

And as with any research, there is some possibility that you may be subject to risks that have not yet been identified.

There are no feasible alternative procedures available for this study

BENEFIT

The possible/main benefit of your participation in the research are that you will contribute to the understanding of the role of vitamins in physical activity participation.

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. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on

all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

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 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 inconvenience. The only costs to you will be time and travel. In order to thank you, you will receive one \$20 gift card to Target at the end of the study.

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; 7001 E. Williams Field Rd., Mesa, AZ 85212; 480-727-1713.

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

Other Signature

Printed Name

Date

(if appropriate)

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 B

PROFILE OF MOOD STATES


ID:

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		NOT AT ALL A LITTLE MODERATELY QUITE A BIT EXTREMELY	NOT AT ALL A LITTLE MODERATELY QUITE A BIT EXTREMELY
	Col <input type="radio"/> O.P. <input type="radio"/>	21. Hopeless	45. Desperate
		22. Relaxed	46. Sluggish
1. Friendly		23. Unworthy	47. Rebellious
2. Tense		24. Spiteful	48. Helpless
3. Angry		25. Sympathetic	49. Weary
4. Worn out		26. Uneasy	50. Bewildered
5. Unhappy		27. Restless	51. Alert
6. Clear-headed		28. Unable to concentrate	52. Deceived
7. Lively		29. Fatigued	53. Furious
8. Confused		30. Helpful	54. Efficient
9. Sorry for things done		31. Annoyed	55. Trusting
10. Shaky		32. Discouraged	56. Full of pep
11. Listless		33. Resentful	57. Bad-tempered
12. Peeved		34. Nervous	58. Worthless
13. Considerate		35. Lonely	59. Forgetful
14. Sad		36. Miserable	60. Carefree
15. Active		37. Muddled	61. Terrified
16. On edge		38. Cheerful	62. Guilty
17. Grouchy		39. Bitter	63. Vigorous
18. Blue		40. Exhausted	64. Uncertain about things
19. Energetic		41. Anxious	65. Bushed
20. Panicky		42. Ready to fight	MAKE SURE YOU HAVE ANSWERED EVERY ITEM.
		43. Good natured	
		44. Gloomy	 POM 021

ID:

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		NOT AT ALL A LITTLE MODERATELY QUITE A BIT EXTREMELY	NOT AT ALL A LITTLE MODERATELY QUITE A BIT EXTREMELY
	Col <input type="radio"/> O.P. <input type="radio"/>	21. Hopeless	45. Desperate
		22. Relaxed	46. Sluggish
1. Friendly		23. Unworthy	47. Rebellious
2. Tense		24. Spiteful	48. Helpless
3. Angry		25. Sympathetic	49. Weary
4. Worn out		26. Uneasy	50. Bewildered
5. Unhappy		27. Restless	51. Alert
6. Clear-headed		28. Unable to concentrate	52. Deceived
7. Lively		29. Fatigued	53. Furious
8. Confused		30. Helpful	54. Efficient
9. Sorry for things done		31. Annoyed	55. Trusting
10. Shaky		32. Discouraged	56. Full of pep
11. Listless		33. Resentful	57. Bad-tempered
12. Peeved		34. Nervous	58. Worthless
13. Considerate		35. Lonely	59. Forgetful
14. Sad		36. Miserable	60. Carefree
15. Active		37. Muddled	61. Terrified
16. On edge		38. Cheerful	62. Guilty
17. Grouchy		39. Bitter	63. Vigorous
18. Blue		40. Exhausted	64. Uncertain about things
19. Energetic		41. Anxious	65. Bushed
20. Panicky		42. Ready to fight	MAKE SURE YOU HAVE ANSWERED EVERY ITEM.
		43. Good natured	
		44. Gloomy	 POM 021

APPENDIX C
PHYSICAL ACTIVITY QUESTIONNAIRE

Considering a 7-day period (a week), how many times on average do you do the following kinds of **exercise for more than 15 minutes** during your free time?

[Write the appropriate number on each line.]

STRENUOUS exercise (heart beats rapidly) [i.e. running, jogging, hockey, football, soccer, squash, basketball, cross-country skiing, judo, roller blading, vigorous swimming, vigorous long distance bicycling]

Times per week _____

MODERATE exercise (not exhausting) [i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing]

Times per week _____

MILD exercise (minimal effort) [i.e. yoga, archery, fishing, bowling, horseshoes, golf, snow-mobiling, easy walking]

Times per week _____

Considering a 7-day period (a week), during your leisure-time, how often do you engage in any regular activity long enough to **work up a sweat** (heart beats rapidly)?

____ 1. Often

____ 2. Sometimes

____ 3. Never /

Rarely

APPENDIX D

YMCA 3 MINUTE STEP TEST

Equipment required: one stopwatch, one 12 inch step, one metronome set to a cadence of 96 beats per minute

The tester shall demonstrate to subjects proper protocol by stepping completely onto the step first with one foot, then the other, then stepping down first with one foot, then the other. Each step shall be to the beat of the metronome. The tester will time the subject for 3 minutes, immediately after which the subject shall sit down and have his pulse counted manually by the tester for one minute. The heart rate is recorded.

Reference: <http://www.topendsports.com/testing/tests/step-ymca.htm>

APPENDIX E
RESTRICTED AND APPROVED FOODS LIST

Restricted Foods List

Limit consumption of these foods to no more than once per week:

- **Fruits and Fruit Juices**

- Orange, grapefruit, tangerine, V8, pineapple, cranberry
- Fortified fruit drinks: cranberry, grape, fruit punches, Kool-Aid
- Strawberries
- Melons: cantaloupe, honeydew, watermelon
- Other: papaya, mangos, kiwi, plantains

- **Vegetables**

- Broccoli, kale, Brussels sprouts, cauliflower, cabbage, collard greens, asparagus, turnip greens
- Peppers: sweet green, red, yellow; hot green chili; hot red chili; jalapeno

- **Highly Fortified Breakfast Cereals**
including
 - Total Cereal, Nabisco 100% Bran, All bran, Honey BucWheat Crisp, Bran buds, Product 19 etc.
 - Ovaltine, Maypo, Instant Breakfast etc.
- **Fortified Energy/Fitness Bars and Drinks**
 - Power bars, Powerade, etc.

APPENDIX F
PEDOMETER STEP LOG

Directions:

1. Enter the day's date. Note you need to wear the pedometer on 2 weekdays and 1 weekend day -- for a total of 3 consecutive days.
2. Record the time you put on the pedometer in the morning.
3. Zero pedometer and check for accuracy by performing brief walking trial (walking 20 steps and comparing that number to pedometer display window)--
4. Record the time the pedometer was removed for the day
5. Record the number of hours the pedometer was worn.
6. Record the cumulative steps indicated on pedometer.
7. Record any Comments about the
8. Circle the Main activity done during the day. In other words record the activity you did more than 50% of your day.

Example if you were on your feet walking or hiking or exercising greater than 50% of the total hours then you would circle WALK.
9. Note the return date -- Return the pedometer & Log Receive Your Gift Card

Date	Ped.		Calibration	Time	Time	Total	# Steps	Comments
	ID #	#	Check	On	Off	Time		
2-5 weekday (day 1)	011	025	√	11:00 AM	10:30 PM	11.5 hrs	8,000	Forgot to wear 11am
_____ weekday (day 1)								
_____ weekday (day 2)								
_____ weekend (day 3)								
_____ weekday (day 4)								
_____ weekday (day 5)								
_____ weekend (day 6)								
_____ weekday (day 7)								
_____ weekday (day 8)								


_____weekend (day 9)								
_____ weekday (day 10)								
_____ weekday (day 11)								
_____ weekend (day 12)								
_____ weekday (day 13)								
_____ weekday (day 14)								

APPENDIX G
IRB APPROVAL



Office of Research Integrity and Assurance

To: Carol Johnston
HSC

From: Carol Johnston, Chair 
Biosci IRB

Date: 01/15/2010

Committee Action: Amendment to Approved Protocol

Approval Date: 01/15/2010

Review Type: Expedited F12

IRB Protocol #: 0907004162

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

Expiration Date: 07/16/2010

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.

