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Chronic inflammation, vitamin D status and body composition in college-aged individuals

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by

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Introduction

Chronic inflammation has been linked to the development of many of the more costly and common diseases that are prevalent in our society today (1). Over the course of the past 20 years, low grade, systemic inflammation has been identified as a significant risk factor associated with the development of cardiovascular disease (2). C-reactive protein (CRP), an acute-phase protein found in the human blood, is an established marker of inflammation. Presently, CRP concentrations are frequently used to assess risk for cardiovascular disease (3). While this biomarker is commonly used in the clinical and research environments, some have suggested that CRP may directly interact with damaged vessels or ischemic myocardium to promote inflammation and thrombosis (3).

Both clinicians and research scientists alike have studied various interventions that can reduce the risk for chronic inflammation. Although a number of drugs are effective in reducing this risk, a number of side effects are associated with long-term use. Consequently, more natural and alternative therapies present a more attractive approach. Vitamin D, or the sunshine vitamin, has received increased attention over the years. Although vitamin D is better known for its role in bone health maintenance, studies show vitamin D may help protect the body against the development of many age related chronic diseases including diabetes, cancer, obesity, osteoarthritis, and cardiovascular disease (4). Interestingly, those with vitamin D deficiency have been shown to correspond with higher levels of inflammation in the body than those individuals with adequate vitamin D intake (5). For example, a recent study showed that a relationship exists between serum vitamin D status and vascular endothelial inflammation in middle-aged and older adults (6). Vascular endothelial cell expression of the pro-inflammatory

transcription factor nuclear factor κB was greater in deficient versus sufficient subjects (0.59±0.07 versus 0.44±0.05; P<0.05) (6).However, more research is necessary to completely understand the relationship between vitamin D status and inflammation.

Vitamin D status has also garnered attention due to its association with body composition. Studies have recently shown that decreased vitamin D levels are related to obesity (7). This relationship is provocative given that higher levels of body fat are also linked to high plasma levels of CRP in otherwise healthy people. Elevated CRP concentration has also been linked to obesity (8).

Although vitamin D and CRP have both been reviewed extensively as separate studies in relation to inflammation, few studies have compared both vitamin D and CRP together (9, 10). Accordingly, the primary aim of this study is to determine whether levels of vitamin D as assessed by 25-hydroxyvitamin D (25OHD), are related to levels of CRP. I hypothesize that plasma 25OHD will be inversely related to CRP in a younger population.

Literature Review:

Cardiovascular Disease

Cardiovascular disease (CVD) has rapidly become a central cause of globally declining health and increasing mortality (11). Cardiovascular disease is a class of conditions that involve the heart or blood vessels, affecting the cardiovascular system. Currently, CVD accounts for almost half of noncommunicable diseases and is the leading global cause of death (11). Cardiovascular disease accounts for 17.3 million deaths per year and is predicted to increase to over 23.6 million deaths by the year 2030 (11). There are many conditions related to the development of CVD but atherosclerosis and

hypertension are the most common (12). Although the progression of atherosclerosis is multi faceted, it is currently accepted that inflammation within lesions in the blood vessels greatly contributes to the initiation and progression of the condition (13).

C-Reactive Protein

C-reactive protein is an acute phase reactant, the levels of which rise in response to inflammation (14). CRP consists of 5 identical subunits, each with a single binding site for phosphocholine (PC) (14). CRP is synthesized by hepatocytes in the liver (15). In response to infection or tissue inflammation, production of CRP is stimulated by various cytokines such as interleukin 6 (IL-6), interleukin 1 (IL-1), and tumor necrosis factor, in particular (15). Once released into circulation, CRP undergoes calcium-dependent binding to the phosphocholine on the surface of dead or dying cells and some types of bacteria, thus activating the C1Q complement complex which promotes the inflammatory immune response (14). CRP also has the ability to modulate the function of phagocytic cells (15). CRP has been shown to enhance the tumoricidal activity of monocytes, induce the production of pro-inflammatory cytokines by macrophages, trigger the shedding of IL-6 receptors from neutrophils, and stimulate the synthesis of tissue factors by monocytes (16).

CRP as a Marker of Inflammation

The rapid increase in synthesis of CRP immediately following tissue injury or infection suggests its contribution to host defense and the innate immune response (17). A strong relationship has been established between elevated levels of CRP and cardiovascular disease (17). Specifically, high plasma concentrations of CRP are

associated with a 2-fold increase in risk of stroke, a 3-fold increase in risk of myocardial infarction, and a 4-fold increase in risk of developing peripheral vascular disease (18).

Normal levels of CRP in healthy individuals are usually less than 10 mg/L, with the mean level increasing slightly with age (15). In diseased individuals afflicted with an acute infection, however, levels of CRP can begin to rise in the first 6 to 8 hours and can peak at approximately 350-400 mg/L after 48 hours (15). Elevation of CRP in the 10-40 mg/L range generally accompanies mild inflammation and viral infections, while active inflammation and bacterial infection typically produce elevated levels around 40-200 mg/L (15). Severe bacterial infections and burns usually produce CRP levels over 200 mg/L (15). In addition, CRP levels have been divided into ranges that assess the amount of risk an individual has for future cardiovascular disease events such as heart attack and stroke (19).

CRP and Cardiovascular Disease

Local inflammation can predispose the vessel to plaque instability and possible rupture (13). Because CRP is a marker for inflammation, it is often used as a diagnostic tool and is also used for exploration in predicting cardiovascular disease risks. Other large scale studies have also revealed that among apparently healthy men and women, high sensitivity CRP analysis is a convincing predictor of future myocardial infarction and stroke based on low (<1 mg/L), moderate (1-3 mg/L), and high risk categories (>3 mg/L) for cardiovascular disease (2, 19).

CRP and Body Composition

A strong positive relationship exists between CRP and obesity (8, 20). It is known that CRP is largely regulated by circulating levels of IL-6, and it is well established that

subcutaneous adipose tissue secretes IL-6 *in vivo*(8). The production of IL-6 has been shown to increase with adiposity and results estimated that in healthy individuals approximately 30% of total circulating IL-6 is derived from adipose tissue (8). Therefore, concentrations of IL-6 and CRP were both strongly related to measures of total obesity in subjects.

Additionally, recent studies have also shown visceral adipose tissue as a strong element in connection to elevated CRP concentrations (21, 22). Visceral fat has also been found to be positively related to CRP independently of total adiposity (22). Thus, both subcutaneous and visceral adipose tissues are significant in their contributions to CRP concentration. An additional study, which included a tri-ethic population of non-Hispanic whites, African-American, and Mexican-Americans, reported a strong correlation between CRP and body mass index, waist circumference, and adipose body mass and that the relationship between CRP and measures of body composition (with the exception of waist to hip ratio) were generally stronger in women than men (23).

Vitamin D Sources

Vitamin D is a fat-soluble compound that can act as secosteroid hormone and is primarily produced in the skin upon exposure to ultraviolet B radiation (4). Other sources of vitamin D in the body include diet and supplements. Sunlight exposure supplies the majority of an individual's vitamin D requirement because few foods naturally contain vitamin D (24). Two forms of vitamin D are utilized in the body, vitamin D_2 and vitamin D_3. Vitamin D_2 (ergocalciferol), considered the plant form, comes from the UV irradiation of ergosterol obtained from yeast (25). Vitamin D_2 has been considered the most effective way to treat and prevent vitamin D deficiency in children and adults for

more than 80 years and is the form found in many supplements (25). Vitamin D_3, the animal form, is produced when the skin is exposed to sunlight (25). In this process, the body converts 7-Dehydrocholesterol, which functions in the serum as a cholesterol precursor, and is converted to vitamin D_3 in the skin, to cholecalciferol through a series of steps (25). When ingested, both forms of vitamin D undergo metabolism in the liver to form 25OHD (25). Recent studies have shown that vitamin D_3 is the more potent form and is able to raise and maintain the circulating serum 25OHD levels more efficaciously (26, 27). Cod liver oil and oily fish such as salmon and sardines are good sources of vitamin D_3(24). Adequate intake of vitamin D can be obtained by consuming these oily fish 3-4 times per week (24). Some milk, orange juices, cereals, and breads are also fortified with vitamin D to help to decrease the prevalence of vitamin D deficiency (24). In late 2010, the Institute of Medicine (IOM) released new Recommended Dietary Allowance (RDA) values for vitamin D in the Dietary Reference Intakes data tables (28). The new recommendation increased the RDA from 400 IU for healthy adults, with higher values for the elderly and youth, to 600 IU per day for individuals between the ages of one and 71 years old, but this value is still debated in the research community as being insufficient (28). In order to reach the necessary serum level of 30 ng/mL, the estimated average vitamin D requirement for older adults is suggested to be increased to 800-1,000 IU/day (29). For example, one tablespoon of cod liver oil contains 1,360 IUs of vitamin D per serving, while salmon has 447 IUs per serving and fortified whole milk supplies 124 IUs per serving (30). Interestingly, vitamin D produced in the skin as a result of sun exposure can last at least twice as long in the blood as compared to vitamin D ingested in food and supplements (31).

Vitamin D Synthesis

As displayed in Figure 1, after exposure to UVB light a series of steps occurs to synthesize vitamin D. After a precursor compound stored in the skin known as 7-dehydrocholesterol (7DHC) comes into contact with a photon from UVB light, it is converted to previtamin D_3(32). Vitamin D_3 is synthesized in human skin from wavelengths that are ideally less than 315 nm (32). After initial photoisomerisation the previtamin D is then converted to vitamin D in the skin with body heat (32).

After vitamin D enters the blood, it circulates bound to vitamin D binding protein (DBP) and is rapidly converted to its major circulating form, 25-hydroxyvitamin D 25OHD by the liver (9). Under the influence of parathyroid hormone (PTH), 25OHD is converted by the 1-alpha-hydroxylase (1α-OHase) in the kidney to form the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25 $(OH)_2D$) (9). It is thought that the presence of the extra-renal 1α-OHases allows 25OHD to be converted to 1,25 $(OH)_2D$ to work as a paracrine or autocrine hormone (9). Circulating 1,25 $(OH)_2D$ enters the target cell and binds to the vitamin D receptor (VDR) in the cytoplasm which then translocates to the nucleus and binds with the retinoic x receptor (RXR). Vitamin D receptors (VDRs) are present on a large variety of cell types, including myocytes, cardiomyocytes, pancreatic beta-cells, vascular endothelial cells, neurons, immune cells, and osteoblasts (33). The 1,25 $(OH)_2D$-VDR-RXR complex then binds to vitamin D response elements (VDRE) on DNA to increase transcription of vitamin D regulated genes (9). Vitamin D status is best determined by a serum 25OHD as opposed to 1,25 $(OH)_2D$ (9).

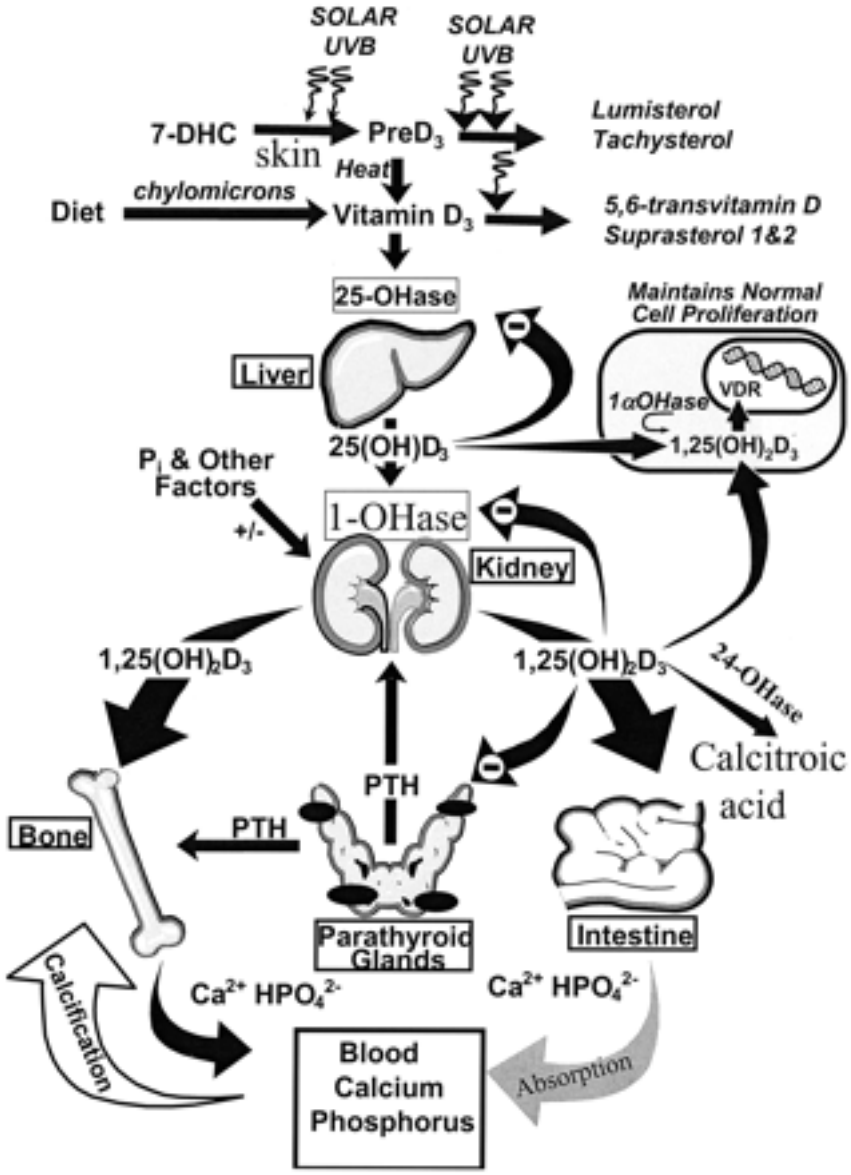

Figure 1. Diagram of cutaneous production of vitamin D and its metabolism and regulation of calcium homeostasis and cellular growth. 7-dehydrocholesterol (7-DHC) in the skin absorbs UVB radiation upon exposure to sunlight and is converted to previtamin D_3 ($preD_3$). Once formed, pre D_3 undergoes heat-induced transformation to vitamin D_3. Additional sunlight exposure converts $preD_3$ and vitamin D_3 to biologically inert products. Vitamin D entering the body through the diet or from the skin enters the circulation and is metabolized in the liver by vitamin D-25-hydroxylase (25-OHase) to [$25(OH)D_3$], which then reenters the circulation and is converted in the kidney by 25-hydroxyvitamin D_3 -1α-hydroxylase (1-OHase) to 1,25-dihydroxyvitamin D_3 [$1,25(OH_2)D_3$]. Reprinted with permission (24).

Assessment of Vitamin D Status

Vitamin D made in the skin or consumed through dietary intake is biologically inactive and must complete two consecutive hydroxylations prior to use by the body (34).

The first hydroxylation occurs in the liver on carbon 25 to form 25- hydroxyvitamin D (25OHD) and the second takes place in the kidney for a hydroxylation on carbon 1 to form the biologically functioning form of vitamin D, 1-25-dihydroxyvitamin D (1,25 $(OH)_2D$) (34).

Serum 25OHD is the major circulating form of vitamin D and the indicator used to determine vitamin D status; serum 1,25 $(OH)_2D$ provides no information on vitamin D status (34). Currently, 25OHD is used as a combined measure of both vitamin D ingested in the diet and vitamin D that is produced from exposure to the sun (34). Also, 25OHD is used to determine vitamin D status for several other reasons including its long circulating half life, the concentration of 25OHD is 1000x higher in circulation compared to 1,25 $(OH)_2D$, and the production of 1,25 $(OH)_2D$ is mainly under the influence of PTH which tightly regulates calcium levels (9). Consequently, 1,25 $(OH)_2D$ levels have the possibility of elevation in individuals suffering from severe vitamin D deficiency in order to maintain normal serum calcium levels (9). Although widely debated, most researchers agree that a 25OHD serum level of about 30 ng/ml or more is necessary for favorable calcium absorption and good health (5). Recently, the Dietary Reference Intake (DRI) suggests that a Reference Daily Intake (RDA) and Estimated Average Requirement (EAR) of vitamin D between 12-20 ng/mL may also be sufficient (35).

Serum 25OHD is the most valid approximation for determining vitamin D status in humans (36). There are a variety of different assays used to measure 25OHD, but radioimmunoassays using competitive protein binding assays are particularly useful in detecting vitamin D deficiency and sufficiency (34). Direct detection methods such as high-performance liquid chromatography (HPLC), liquid chromatography-mass

spectrometry (LC-MS) and automated direct chemiluminescent assays may be used as well, depending on the reason for being measured(37). Vitamin D status can also be estimated from dietary intake using the US Department of Agriculture's Nutrient Database to analyze the vitamin D content of foods (36). This method is not heavily relied on, as it can be inaccurate and vitamin D status is greatly influenced by sunlight exposure. Sunlight exposure data obtained through questionnaires or recalls is also an additional way of obtaining information about an individual's vitamin D status (36).

Prevalence of Vitamin D Deficiency

While extensively disputed, many researchers define vitamin D deficiency as a 25OHD below 20 ng/ml (50 nmol/liter) and vitamin D insufficiency is a 25OHD of 21-29 ng/ml (525-725 nmol/liter) (31). Because very few foods naturally contain or are fortified with vitamin D, vitamin D deficiency can be mainly attributed to inadequate exposure to sunlight (31). Many people are not obtaining the adequate amount of sunlight due to working indoors daily and the heightened awareness and application of sunscreen every day. Vitamin D synthesis in the skin is reduced by over 95% when wearing a sunscreen with a sun protection factor of 30 (31).

Vitamin D and Inflammation

Other than CRP levels, research has also shown that vitamin D levels can be related to inflammation in the body. Insufficient 25OHD levels are related to an increase in systemic inflammation, as shown by elevated levels of CRP (33). This is shown in a study in which down-regulation of inflammatory markers (CRP, in particular) occurred after administration of 1,25 $(OH)_2D$ to vitamin D deficient individuals (33).

A separate study in which elderly individuals were given either a high-dose (14

μg) or low-dose (0.14 μg) vitamin D_3 supplementation for one year, showed a 40% decrease in mean CRP levels in the high group and a 5% decrease in the low group (38). Furthermore, researchers in Belgium have shown that vitamin D lowers the CRP and IL-6 levels in critically ill patients in the Intensive Care Unit (39). Over a ten-day period of time in the hospital, plasma concentrations of CRP and IL-6 serum concentrations decreased in both a low-dose (200 IU) vitamin D group and a high-dose (500 IU) vitamin D group (39). The drop in CRP levels was significantly more pronounced in the high-dose vitamin D group between days 3 and 7 as compared to the low-dose vitamin D group (39). Another additional study has also shown that vitamin D deficiency was associated with increased risk of inflammation in otherwise healthy people (5). Evidence has shown that vitamin D regulates directly or indirectly the development and function of T cells. T cells-mainly Th1-control the pathology of autoimmune diseases (5). The Th1 cell mediated autoimmune disease is characterized by T cells that express TNF-α and IF-ϒ that are located in the tissue and induce inflammation in that particular location (5). Thus, when there is a vitamin D deficiency, the pro-inflammatory cytokines present mediate this inflammation.

Direct and indirect immunomodulating effects involving the T cells, B cells, and antigen- presenting cells (dendritic cells and macrophages) and effects on innate and adaptive immune responses have been shown to respond to changes in vitamin D levels (4). These immunomodulating effects of vitamin D may help to explain the associations between vitamin D levels in the body and autoimmune and inflammatory disease occurrence. Increased interest in the study of vitamin D as an immune system regulator has resulted after the recent discovery of VDR in the peripheral blood mononuclear cell.

Vitamin D and Body Composition

Increasing evidence has shown associations between obesity and low levels of serum 25OHD (7). One study revealed that total body fat was inversely related with serum 25OHD status in both sexes in adults over the age of 55 (7). This relationship has also been demonstrated in healthy adults as well (40). Additionally, a separate study indicated an inverse correlation between body mass index and serum 25OHD concentration in children of both sexes aged 11-18 (41). The underlying causes that have been suggested in favor of this trend can be attributed to low levels of sun exposure in obese subjects due to mobility limitations, a sedentary lifestyle or clothing choices that tend to cover more of the body, and a higher storage of vitamin D in adipose tissue (7). Because vitamin D is fat-soluble and can be deposited in fat, its lipid solubility modifies the bioavailability from dietary and cutaneous sources (41). Presently, the increased sequestration of vitamin D in fat is a preferred theory, but more research to investigate this relationship is necessary.

Methods:

Study Subjects

Forty subjects (n= 20 males, n= 20 females; ages 18-40 years) were recruited to participate in the study. Participants were required to be physically active in moderate to vigorous intensity activities for at least 3 days per week to be eligible for the study. Participants must also have sustained a consistent body weight for three months prior to the study. Outside of the amount of vitamin D included in a daily multivitamin (400 IU), the subjects had no history of vitamin D supplementation. This project was approved by the Louisiana State University Institutional Review Board.

Study Design

Subjects reported to the lab three times throughout the study. During the first visit all subjects signed an informed consent document, completed physical activity and health assessment questionnaires, and height and weight were measured. Next, over the course of two additional visits that took place over a 14-day period, subjects completed tests to determine plasma vitamin D and CRP status and body composition. Due to influence from hormonal cycles, blood collection for females took place on days 5 to 7 of the menstrual cycle.

Blood Collection and Analysis

During the testing period, each subject reported to the lab for a single blood draw. Subjects were asked to fast for 12 hours and withhold from strenuous exercise for 24 hours prior to collection. Blood collection was conducted in the exercise biochemistry lab at Louisiana State University. A registered nurse collected 20 mL blood samples from each subject in vacutainers containing no additive. Samples were chilled for at least one hour at 8-10 °C, then the samples were centrifuged at 10 °C (10 minutes, 1000 rcf) and serum was aspirated, aliquoted, and stored at -80 °C until used in analysis. Vitamin D (25OHD) status was determined using ELISA (Alpco Diagnostics, Salem, NH) with a BioTekmicroplate reader (BioTek Instruments, Model MQX200, Winooski, Vt., USA). C-reactive protein status was also determined using ELISA (Alpco Diagnostics, Salem, NH) with a BioTekmicroplate reader (BioTek Instruments, Model MQX200, Winooski, Vt., USA).

Sun Exposure and Dietary Intake

One week before blood collection, subjects were provided with two different

surveys that accounted for their overall endogenous vitamin D production and their exogenous dietary intake of vitamin D. Both completed surveys were returned at the time of the blood collection. The first survey quantified the amount of time spent outdoors and in sunlight via a sun exposure questionnaire. This survey measures endogenous vitamin D that is produced by exposure to ultraviolet light. This method, set forth by Hanwell et al., combines the time spent outdoors or in exposure to ultraviolet light into a numerical scale (42). The second survey was a seven day dietary log that was examined for dietary vitamin D content using the USDA database (43).

Body Composition- Dual-Energy X-ray Absorptiometry (DXA)

A trained technician performed whole-body DXA scans using a General Electric Lunar iDXA (General Electric; Milwaukee, WI). The data were then analyzed using enCORE software version 13.40. Each subject removed all metal objects from his or her body and changed into a hospital gown before the test was performed. The subject was then centered and secured on the surface of the machine. The subject was instructed to lie completely stationary throughout the duration of the scan, which lasted approximately 10 minutes.

Statistical Analysis

Data were analyzed using SPSS (Version 19, IBM, Armonk, NY). Descriptive statistics, including mean and standard error (SE), were calculated for all outcome variables. A one-way ANOVA was used to compare the subjects by gender and vitamin D status, either above (HIGH; >35 ng/mL) or below (LOW; <35 ng/mL) the normal value for physically active individuals (44). C-reactive protein data were log transformed prior to analysis. Pearson correlation analysis was used to determine relationships

between all measurements. Significance was set at $p < 0.05$. All values presented are mean ± SE.

Results:

A total of forty subjects were recruited into the study. One female was excluded from all final analyses due to failure to complete testing. One male subject was excluded from 25OHD serum analysis, as his value was considered a statistical outlier. Subjects were between the ages of 20 and 38 years and descriptive data are displayed in Table 1. Overall, males had a significantly higher dietary intake of vitamin D; however, there were no other significant differences in descriptive measures aside from those expected based on variation of body size and composition between genders (Table 1). Males showed significant correlations between CRP levels and BMI ($p \leq 0.05$), CRP and percent body fat ($p \leq 0.05$), and BMI and percent body fat ($p \leq 0.05$).

Vitamin D Status: Measures of Intake and Serum Content

Twenty subjects (9 females, 11 males) exhibited serum 25OHD levels below 35 ng/mL, which is considered to be lower than normal in young, physically active individuals (44). Although the recommended daily intake of vitamin D for this range of ages is 600 IU per day (45), totaling 4200 IU per week, only one individual in the study met this condition. Despite supplementation, this subject's serum 25OHD level still fell short of the normal 25OHD level. The mean dietary intake of vitamin D for subjects was just above 1000 IU per week, and these values did not correlate with serum levels (Figure 2). Dietary intake was significantly higher in males than in females ($p= 0.002$); however, there was no significant intake difference between the HIGH and LOW vitamin D groups

(p= 0.93) nor was there an association between vitamin D intake and serum 25OHD content (r= 0.003).

Sun Exposure

Using the charting procedure for sun exposure, scores ranged from 11 to 52. In the study in which the survey measurement was first proposed, the scores varied from 0 to 41 (42). Furthermore, no significant differences were observed in sun exposure between men and women, or the HIGH and LOW vitamin D groups (p= 0.66 and p= 0.81, respectively) (Table 1). There were no significant correlations in the complete set, nor were there any correlations when divided by gender or between the HIGH and LOW 25OHD groups.

Body Composition

Apart from the expected distinctions in percent body fat between genders ($P \leq 0.001$), there were no other significant findings when body size and composition were compared between HIGH and LOW groups (Table 1). A significant positive correlation was found between CRP and percent body fat and BMI in males ($r= 0.38$, $p \leq 0.05$; $r= 0.37$, $p \leq 0.05$, respectively); higher percent body fat and BMI in males accompanied elevated CRP.

Correlational Analysis

Correlations were performed between 25OHD and CRP concentrations and all outcome variables for the complete group of subjects, by gender, and by vitamin D status (LOW and HIGH groups). No statistically significant relationships were discovered.

<u>Discussion:</u>

In this study there were no significant relationships found between 25OHD status and CRP. Research has shown that decreased serum 25OHD levels are associated with increased CRP concentrations in the elderly (5, 38); however, this was not observed in the current study which included college-aged individuals.

Overall, the subjects in this study presented with lower levels of CRP. All participants were below the 10 mg/L cutoff for normal, healthy levels of CRP. A total of 23 of the 39 subjects had CRP values in the low (<1 mg/L) risk for future cardiovascular events category, 12 subjects presented with values in the moderate (1-3 mg/L) risk category, and 4 subjects had CRP values in the high (>3 mg/L) risk classification. This study showed no significant correlations between CRP and gender or CRP and 25OHD status. Although not statistically significant, CRP concentrations were slightly higher in females than in males. This has been noted in several other studies as well (46, 47). C-reactive protein was also slightly higher in the HIGH 25OHD group than the LOW 25OHD group (Figure 1). This trend is opposite of what is expected, but could be attributed to higher amounts of error and a low sample size with only a small variability in CRP values. Low levels of 25OHD and high levels of CRP are associated with higher levels of body fat (7, 8, 48). In this study there were significant positive correlations between BMI and percent body fat, BMI and CRP, and percent body fat and CRP in males. There were no other significant relationships observed between CRP levels and the other variables.

In this study, half of the healthy, physically active participants presented with insufficient or deficient levels of serum 25OHD. Studies have defined vitamin D deficiency as a serum 25OHD below 20 ng/ml and vitamin D insufficiency as a serum

25OHD of 21-29 ng/ml (31); however, depending on the research environment the values may vary slightly. The normal reference value of 35 ng/mL was considered for participants in this study because this cutoff has been used as the reference value in a previous study with physically active individuals (49). Heavy reliance on dietary intake alone may make it difficult to attain adequate vitamin D status (50). This becomes especially difficult without consideration of supplementation with a multivitamin. In the United States, many foods such as orange juice, dairy products, and cereals are required by the Food and Drug Administration to be fortified with vitamin D (51). While the fortification of these foods aids in increasing vitamin D intake, they cannot be considered a sufficient means to obtain the recommended amount of vitamin D (50). Because of these factors, it is not a surprise that the dietary intake of vitamin D was exceptionally low in the college-aged individuals enrolled in this study. The Institute of Medicine recommends that 600 IU of vitamin D per day be consumed, and only one of the 39 participants in this current study met this condition with an average daily intake of 700 IU per day (4,644 IU per week). This trend in young, physically active individuals has been recently observed in several other recently published studies (44, 52). Additionally, males had over double the amount of dietary intake of vitamin D as females. This finding may be a function of daily caloric intake differences (53). Males tend to consume more calories per day than females (53). Consequently, this discrepancy may have allowed the males to eat more of the foods that contain vitamin D.

Levels of 25OHD are not solely dependent on dietary intake, but are also associated with exposure to UV light. The participants in this study resided in the Southeast United States where it is estimated that there is sunlight 218 days per year. The

data for this study were collected during the summer and early fall. Current recommendations advise that ten to fifteen minutes in sunlight is enough to solicit a large production of vitamin D (32). But, the production and conversion of vitamin D to its useable form is affected by many different factors including age, geographical location, ethnicity, adiposity, and behaviors such as sunscreen use (32, 54). In this particular study, sun exposure was assessed through a survey that accommodated for direct combination of time spent in the sun as well as overall sunlight exposure (42). This survey was used for this study because many of the participants enjoyed outdoor recreational activities, but did so under different conditions including time of day or wearing various styles of clothing, therefore making it difficult to analyze time under UV exposure. The data from the survey in the current study differed significantly as both a function of time spent outdoors and the amount of skin exposed to UV light from the sun. No relationship was found between sun exposure or dietary intake and serum 25OHD. Contrary to belief, there was also not found to be any significant relationship between length of sun exposure and serum 25OHD, although most subjects reported infrequent use of sunscreen, which greatly expanded the potential for endogenous vitamin D production. Previous studies and the present results suggest that individuals with substantial sun exposure may possibly still be at risk for vitamin D deficiency (55). Many studies on vitamin D and inflammation have examined the relationship in the older, sicker populations, but few have looked at college-aged students. It would be expected that this younger, more active population would have adequate vitamin D levels, but this is not the case. This study has shown that college-aged individuals may also be at risk for vitamin D deficiency.

This study includes several limitations. First, it is crucial to point out that the subject population included only 40 participants. This small pool of subjects limited the variability of 25OHD and CRP levels, thus limiting the extent to which I could investigate the relationship between CRP and 25OHD. Half of the participants presented with insufficient or deficient levels of serum 25OHD and the individuals did not display a wide range of vitamin D levels. Because the relationship with CRP could not be explored on a wide array of vitamin D levels, the data analysis is limited. Additionally, the CRP values measured in the subjects were lower and fell within the healthy range. When both values are low, correlating CRP and 25OHD becomes difficult. A wider range of values for both CRP and vitamin D may show other correlations. Secondly, the current study recruited mostly Caucasian subjects (n=36) with a few Hispanics (n= 3), but included no African Americans. The observed levels of vitamin D insufficiency would have potentially been greater had more minorities been recruited because they are at a higher risk for insufficiency or deficiency due to melanin's role in blocking the production and circulation of 25OHD (56).

In conclusion, this study revealed no significant relationships between CRP and vitamin D status. A secondary finding was that a large percentage of young, healthy, and physically active individuals had low serum levels of 25OHD and low CRP. Furthermore, sun exposure and dietary intake did not appear to influence 25OHD status. C- reactive protein levels in this young, active population were mostly in the low to moderate risk category and there was a positive relationship between BMI, percent body fat, and CRP in males.

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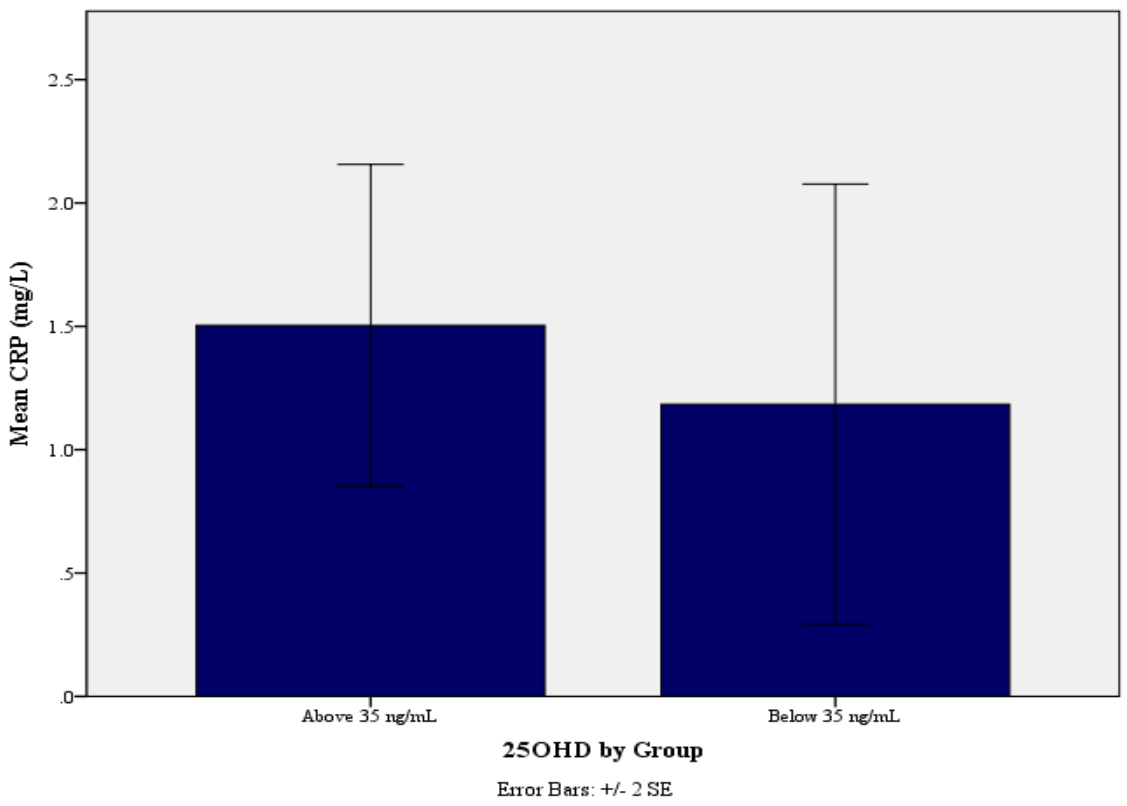
APPENDIX 1

Table 1. Descriptive Measures

		Gender		25OHD Status	
	All Subjects (n=39)	**Male (n=20)**	**Female (n=19)**	**LOW (n=20)**	**HIGH (n=19)**
Age	23.26 ± 0.7	23.75 ± 1.1	22.74 ± 0.7	22.75 ± 0.5	23.79 ± 1.2
Height (cm)	167.77 ± 1.9	174.37 ± 2.1*	160.82 ± 2.3	169.29 ± 2.5	166.17 ± 2.9
Weight (kg)	68.85 ± 2.1	77.29 ± 2.4*	59.79 ± 1.8	71.96 ± 3.1	65.40 ± 2.6
BMI	24.34 ± 0.6	25.45 ± 0.8	23.17 ± 0.7	24.99 ± 0.9	23.65 ± 0.7
Body Fat %	24.19 ± 1.3	18.65 ± 1.5*	30.02 ± 1.0	24.66 ± 1.7	23.69 ± 2.0
Lean Body Mass (g)	49957.13 ± 1514.5	59725.90 ± 1689.0*	39674.21 ± 1340.0	51972.95 ± 2807.1	47835 ± 2679.1
Dietary Vitamin D Intake (IU/week)	1117.16 ± 169.2	1601.90 ± 273.6*	606.90 ± 112.4	1131.55 ± 254.1	1102.00 ± 228.6
Sun Exposure	28.82 ± 1.8	29.60 ± 2.9	30.02 ± 1.0	28.40 ± 2.7	29.26 ± 2.3
25OHD (ng/mL)	34.83 ± 1.9	33.02 ± 2.1	36.73 ± 3.2	25.97 ± 1.2	44.15 ± 2.2†
CRP (mg/L)	1.34 ± 0.3	1.11 ± 0.3	1.58 ± 0.5	1.18 ± 0.4	1.50 ± 0.3

Data are presented as means ± SE. BMI, body mass index; 25OHD, 25-hydroxyvitamin D; CRP, C-reactive protein. * Indicates significant difference between males and females ($p < 0.05$); † Indicates significant difference between HIGH and LOW vitamin D status ($p < 0.05$).

Figure 1.



Data represent inflammation (CRP mg/L) expressed as mean (±SE) for individuals above (HIGH) or below (LOW) 35 ng/mL 25-hydroxyvitamin D (25OHD).