VITAMIN D STATUS IN COLLEGIATE FEMALE ATHLETES: RELATIONSHIP TO INDOOR VS. OUTDOOR SPORTS

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To the Dean of Graduate School:

I am submitting herewith a thesis written by Bethany Bloom entitled "Vitamin D Status in Collegiate Female Athletes: Relationship to Indoor vs. Outdoor Sports." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Exercise and Sports Nutrition.

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We have read this thesis and recommend its acceptance:

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Accepted:

Dean of the Graduate School

DEDICATION

To my parents, James and Joy Bloom, thank you for your continued support through the years. I fail to often express my gratitude for your spiritual, emotional and financial support. Without you, I would not have been able to pursue a career in sports nutrition. I love you two very much.

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ABSTRACT

BETHANY BLOOM

VITAMIN D STATUS IN COLLEGIATE FEMALE ATHLETES: RELATIONSHIP TO INDOOR VS. OUTDOOR SPORTS

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Vitamin D is important for bone metabolism, muscle function and possibly athletic performance. Low serum 25(OH)D concentrations among adolescents and adults have been observed. Limited research exists on vitamin D status in female athletes living in the southern United States or on differences in serum 25(OH)D concentrations between athletes who play indoor or outdoor sports. The purpose of this study was to determine differences in fall 2009 serum 25(OH)D concentrations between indoor and outdoor sport athletes living at 33.2 °N latitude and to examine changes in serum 25(OH)D concentrations of indoor sport female athletes from fall 2009 to spring 2010.

Athletes were recruited from a NCAA division II college by purposive convenience sampling. Vitamin D intakes were estimated using 3-day food records and analyzed using Nutritionist Pro software. Body composition and bone mineral density were measured by DXA scanning. Serum 25(OH)D concentrations were obtained using blood spot testing and measured by liquid chromatography-tandem mass spectrometry. Sun exposure and use of tanning beds were assessed with self-reported questionnaires. Independent-t-tests were used to compare serum 25(OH)D concentration differences

between indoor and outdoor sport athletes. A paired-t-test was used to determine if there was a significant change from fall to spring in serum 25(OH)D concentrations of indoor sport athletes. A p value < .05 was significant.

Estimated vitamin D intakes for all athletes were below the recommended intake value (5 μg/d). In the fall, there was no significant difference (p = .52) found between indoor (n=17) and outdoor (n=19) sport athletes in serum 25(OH)D concentrations (mean ± SD) of 50 ± 20 and 46 ± 15 ng/ml, respectively. There was also no significant change (p = .31) in mean serum 25(OH)D concentrations in indoor athletes (n=11) from fall to spring.

The mean serum 25(OH)D concentrations of female athletes living at 33.2°N latitude were within optimal concentrations of 40-70 ng/ml. Although indoor athletes do not obtain similar sun exposure as outdoor athletes, no significant differences in serum 25(OH)D concentrations were observed. Summer UV exposure indicated on self-reported questionnaires may be a reason. No significant change in serum 25(OH)D concentrations was observed in indoor athletes from fall to spring, and may be due to UVB exposure from tanning bed use.

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CHAPTER I

INTRODUCTION

Vitamin D, specifically 25-hydroxyvitamin D [25(OH)D], has been a focus of current nutrition-related research. It is most known for its role in bone metabolism but now has been linked to other physiological processes in the immune, nervous, cardiovascular, and muscular systems (Hamilton, 2010; Nagpal, Na, & Rathnachalam, 2005; Norman, 2008; Wang et al., 2008; Ward et al., 2009). Vitamin D has been found to play an integral role through vitamin D receptors in regulating several pathways involved in cell proliferation, differentiation and immunomodulation (Nagpal et al., 2005). Low concentrations of 25(OH)D have been linked to an increased risk of cardiovascular disease (Wang et al., 2008). In addition, muscle function including force output, power, velocity and jump height in female adolescents has been shown to be positively associated with greater serum 25(OH)D concentrations (Ward et al., 2009). It has also been suggested that higher concentrations of 25(OH)D are positively associated with athletic performance (Cannell, Hollis, Sorenson, Taft, & Anderson, 2009). Athletic performance has appeared to peak during the season coinciding with a peak in serum 25(OH)D concentrations and a decrease in performance coinciding with nadir concentrations (Cannell et al., 2009).

Considering the dynamic roles of vitamin D, several suggestions and observations have been made that there is a greater need of vitamin D intake than what is currently

recommended in order to maintain sufficient serum 25(OH)D concentrations (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Cashman et al., 2008). Current recommendations ascertained by the United States Dietary Reference Intake panel in 1997 are 200 International Units per day (IU/d) for young adults, 400 IU/d for those between the ages of 51 – 70 years and 600 IU/d for those older than 70 years; however, it has been recommended that individuals may need twice the amount of vitamin D that was established as the adequate intake (Cashman et al., 2008). Following a meta-analysis of the scientific literature, Bischoff-Ferrari and colleagues (2006) recommended that the intake of vitamin D should be $\geq$ 1000 IU/d.

Not only has vitamin D been found to play a more integral role in maintaining health, but it also has been found that many individuals worldwide are vitamin D deficient or insufficient (Genuis, Schwalfenberg, Hiltz, & Vaselenak, 2009; Ginde, Liu & Camargo, 2009; Gozdzik, et al., 2008; Jacobs et al., 2008; Lehtonen-Veromaa et al., 1999; Looker et al., 2009; Nesby-O'Dell et al., 2002; van der Mei et al., 2007). Finnish adolescent girls were observed to be vitamin D deficient in the winter season and also that their vitamin D intakes were inadequate to maintain normal circulating serum 25(OH)D concentrations (Lehtonen-Veromaa et al., 1999). While low 25(OH)D concentrations are often expected in higher latitudes (Genuis et al., 2009), low concentrations occur in individuals living in southern latitude areas due to a greater opportunity for sun exposure. However, people living in geographical locations < 35° N latitude also were shown to have low circulating 25(OH)D concentrations (Jacobs et al., 2008).

To date, little is known about vitamin D status in female athletes, especially those living in states below 35° N latitude. There is also limited available research that reports changes in 25(OH)D concentrations among athletes. Due to the recent findings regarding functions and needs of vitamin D, it is imperative to determine the current 25(OH)D concentrations of female athletes.

Purpose of the Study

The purpose of this quasi-experimental and descriptive study was twofold: 1) to determine if there was a change in serum 25(OH)D concentrations of indoor sport female athletes living at a latitude < 35° N from the fall 2009 season to the spring 2010 season; and, 2) to determine if there was a difference in fall 2009 season serum 25(OH)D concentrations between indoor sport athletes and outdoor sport athletes.

Statement of the Problem

Vitamin D is an important micronutrient for female athletes due to its role in bone metabolism, muscle function and possibly athletic performance (Cannell et al., 2009; Nagpal, et al., 2005; Ward et al., 2009). Insufficient 25(OH)D concentrations among adolescents (Lehtonen-Veromaa et al., 1999) and adults, even in southern latitudes, have been observed (Jacobs et al., 2008).

To date, there is no current research reporting vitamin D status in female athletes living in southern latitude regions in the United States. There is also no research indicating if there is a difference in 25(OH)D concentrations between athletes who play indoor sports, including gymnastics and volleyball, and those who play outdoor sports, including softball.

Hypotheses

This study examined the following null hypotheses:

1. There will be no significant change in serum 25(OH)D concentrations in indoor sport female athletes between fall 2009 and spring 2010.
2. There will be no difference in fall 2009 serum 25(OH)D concentrations between indoor sport and outdoor sport female athletes.

Definitions

25-hydroxyvitamin D_3 [$25(OH)D_3$]. First hydroxylated form of vitamin D_3 and the primary circulating metabolite that is used to determine vitamin D status (Holick, 2006)

Bone Mineral Density (BMD). Bone mass as measured by x-ray absorptiometry as an areal density

Fall. Months between September 23 and December 21

Female College Athlete. Female college student who participates in a National Collegiate Athletics Association (NCAA) sanctioned sport

Ideal Vitamin D Concentrations. 25(OH)D concentrations between 40 and 70 ng/ml (Cannell & Hollis, 2008)

Indoor Sports. For this study, gymnastics and volleyball were considered indoor sporting events

Nanograms Per Milliliter (ng/ml). Commonly used unit of measurement for expressing 25(OH)D concentrations (Cannell et al., 2008)

Nanomoles Per Liter (nmol/L). Another common used unit of measurement for expressing 25(OH)D concentrations. To provide consistency for the this study, all measurements were converted to ng/ml by dividing nmol/L by 2.5 (Cannell et al., 2008)

Northern Latitude States. States that are north of 35° N latitude (Rajakumar, Greenspan, Thomas & Holick, 2007)

Outdoor Sports. For this study, softball was considered an outdoor sporting event

Hypovitaminosis D. Circulating serum 25(OH)D concentrations < 20 ng/ml (Rajakumar, et al., 2007)

Southern Latitude States. States that are south of the 35° N latitude (Rajakumar, et al., 2007)

Spring. Months between March 20 and June 20

Vitamin D. A 9,10-seco steroid that exists in two bioactive forms, 1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3 (Norman, 2008)

Vitamin D Deficiency. Serum 25(OH)D concentrations < 40 ng/ml (Cannell et al., 2008)

ZRT Laboratory. A Clinical Laboratory Improvement Amendments certified hormone testing facility located in Beaverton, Oregon, that emphasizes Research and Technological innovation as well as celebrates the founder, David T. Zaza (http://www.zrtlab.com)

Assumptions

There were several assumptions made for this study. Such assumptions included participant honesty when recording food intake, indicating the use of supplements, and answering questions about sunlight exposure.

Limitations and Delimitations

The main limiting factor in this study was purposive method of sampling. Another limitation of this study was that direct sunlight exposure was not quantitatively determined, but instead personal sunlight exposure behavior was determined via a self-reported questionnaire. This investigation did not measure parathyroid hormone concentrations or any other markers of bone metabolism.

Significance of the Study

This study provided information about vitamin D status among female college athletes, especially those who live in states where sun exposure is considered adequate enough for production of vitamin D. It was also an addition to the literature that describes some nutritional behaviors of college female athletes as well as indicates average BMD and body composition measurements among this population.

CHAPTER II

REVIEW OF LITERATURE

History of Vitamin D

In the mid 1600s in some European countries, it was observed that children were developing bone deformities as well as weak muscles and that their growth was stunted. The first individuals to document these clinical observations were Daniel Whistler in 1645 and Francis Glisson in 1650, and from that time the bone deformity disease was termed rickets. Even though this disease was recognized during that particular timeframe, it was not until the 19th century that rickets was associated with a lack of sunlight exposure (Rajakumar, Greenspan, Thomas & Holick, 2007).

Jedrzej Sniadecki and Theobald Palm (as cited in Holick, 1994) were among the first to make the connection between sunlight exposure and rickets, and both individuals realized the importance of sunlight exposure to prevent this bone disease. This concept was later supported at the turn of the 20th century when Kurt Huldschinski found that exposing children to a sun quartz lamp or to a carbon arc lamp led to better bone development and prevented the deformities associated with rickets. Alfred Hess and Lester Unger also found that sun exposure improved the conditions in children showing signs of rickets (Holick, 1994).

Cod liver oil supplementation was also found to prevent rickets; however, the antirachitic activity of cod liver oil was initially thought to be from vitamin A. It wasn't

until 1922 that Elmer McCollum identified the antirachitic properties of cod liver oil were due to anther nutrient he termed "vitamin D". He found that after the vitamin A in cod liver oil was destroyed, it still maintained its ability to reverse the symptoms of rickets (Holick, 1994).

The irradiation of food was also thought to be a method to prevent or cure rickets. Experiments of Hess & Mildred Weinstock as well as Harry Steenbock & Archie Black found that rats fed irradiated food did not develop symptoms of rickets (Rajakumar, et al., 2007). With this advancement, the vitamin D compound was identified, synthetically produced and added to milk products. This led to a near eradication of rickets in the 1930s.

However, there have been recent reports of rickets and vitamin D deficiency among children (Biser-Rohrbaugh & Hadley-Miller, 2001; Kreiter, et al., 2000; Weisberg, Scanlon, Li & Cogswell, 2004) as well as vitamin D deficiency and insufficiency in adolescents and adults (Genuis et al., 2009; Ginde, Liu & Camargo, 2009; Gozdzik, et al., 2008; Jacobs et al., 2008; Lehtonen-Veromaa et al., 1999; Looker et al., 2009; Nesby-O'Dell et al., 2002; van der Mei et al., 2007). In Finland, Lehtonen-Veromaa and colleagues (1999) observed that adolescent girls were vitamin D deficient in the winter season and also that vitamin D intakes were inadequate to maintain normal circulating serum 25(OH)D concentrations. While lower 25(OH)D concentrations are often expected at higher latitudes (Genuis, Schwalfenberg, Hiltz, & Vaselenak, 2009), Jacobs and associates (2008) found that people living in geographical locations < 35° N latitude also have low circulating 25(OH)D concentrations (Jacobs et al., 2008).

Specifically in the United States, nonpregnant women between the ages of 13-56 years had circulating 25(OH)D concentrations of approximately 24.6 ng/ml (Looker et al., 2008). This concentration is < 40 ng/ml, which is the lower value for what is considered optimal concentrations. In comparing women (age 18-49 years) of different ethnicities, the prevalence of hypovitaminosis D was greater in African American women (~42%) as compared to white women (~4%; Nesby-O'Dell et al., 2002); hypovitaminosis D was defined as 25(OH)D concentrations ≤15 ng/ml.

Speculations have been made regarding the reasons behind such a resurgence of rickets and vitamin D deficiency. Inadequate maternal supplementation while breastfeeding has been suggested as a possible cause of the reemerging disease in children (Rajakumar et al., 2007). Other authors have suggested a possible link between a greater amount of visceral adiposity and vitamin D deficiency (Cheng et al., 2010). Yet others have proposed a decreased consumption of dairy products as well as a greater use of sun protection (Looker et al., 2008). Other reasons suggested include place of residency in urban locations, minimal consumption of milk and fortified cereals, and a higher BMI (Nesby-O'Dell et al., 2002). As with many other health-related issues, the cause is most often multi-factorial and associated with several different underlying mechanisms as to the cause.

While identification of vitamin D deficiency has been resurging, further advances in science have also led to the identification of a vitamin D receptor. This receptor has been located in over 36 tissues (Bouillon, Okamura & Norman, 1995). Identification of such receptors led to the hypothesis that vitamin D functions in many more physiological

processes than previously thought. Vitamin D plays an integral role through vitamin D receptors in regulating several pathways involved in cell proliferation, differentiation and immunomodulation (Nagpal et al., 2005). Vitamin D deficiency has been linked to a myriad of diseases such as hypertension, cardiovascular disease, rheumatoid arthritis, inflammatory bowel disease, depression and certain types of cancer. Specifically for athletes, vitamin D has recently been found to influence muscle functioning and also regulate the immune system and inflammatory modulation.

Metabolites of Vitamin D

Vitamin D is a seco-steroid that exists primarily as two forms, vitamin D_2 and vitamin D_3. Vitamin D_2, also known as ergocalciferol, is a plant derivative of the sterol, ergosterol, and is a 28-carbon molecule. Vitamin D_3, also called cholecalciferol, is the biologically active vitamin form in animals and is a 27-carbon molecule. The main chemical structural differences are that vitamin D_3 has one more methyl group and has a double bond between carbons 22 and 23 (Hollis, 2008). Aside from these two forms, there have been over 50 metabolites identified (Zerwekh, 2008).

Endogenous production of vitamin D_3 through sunlight exposure is the primary source of the vitamin for humans. The process of skin synthesis begins when the precursor, 7-dehydrocholesterol, in the epidermal layer of skin absorbs ultraviolet-B (UVB) radiation and precholecalciferol is formed. Since precholecalciferol is biologically inert, another conformational change occurs to form cholecalciferol. This final vitamin D_3 form is incorporated into the vitamin D binding protein and transported to the liver

and kidneys for further conversion to its biologically active form (MacLaughlin, Anderson & Holick, 1982).

On average, UVB exposure to arms and legs for approximately 5 to 10 min will produce about 3000 IU of vitamin D_3 (Holick, 2007). Excessive sun exposure will not produce vitamin D_3 intoxication; when approximately 10-15% of initial 7-dehydrocholesterol in the skin is converted to precholecalciferol, there is a mechanism by which precholecalciferol undergoes conformational change into inert active lumisterol and tachysterol (Holick et al., 1980). In the event that cholecalciferol does not enter the circulation and remains in the skin, further sun exposure breaks the cholecalciferol into 5,6-trans-cholecalciferol, supersterol I and supersterol II, all which are inactive forms (Webb, deCosta & Holick, 1989).

Vitamin D_3 can also be obtained through dietary intake. Natural food sources of vitamin D_3 and food sources that are fortified are presented in Table 1. When consumed, vitamin D_3 is incorporated into chylomicrons and enters the lymphatic system. This complex is then bound to vitamin D binding proteins and lipoproteins and transferred to the liver where the first hydroxylation occurs by way of vitamin D-25-hydroxylase. This enzyme produces the major circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D], which is the primary form used to determine vitamin D status for reasons to be further discussed. Once produced, 25(OH)D binds to the vitamin D binding protein to be transported to the kidney, where it is converted to its biologically active form 1,25-dihydroxyvitamin D, [$1,25(OH)_2D$]. This is accomplished by 25-hydroxyvitamin D-1$\propto$-hydroxylase (1-OHase). From the kidney, it is transported by binding proteins to tissues

expressing the vitamin D receptor, and there $1,25(OH)_2D$ interacts with the receptors along with retinoic acid X receptor in the nucleus of the tissue cell to form the $1,25(OH)_2D$-VDR-RXR complex. This complex combines with the vitamin D-responsive element to promote genetic expression and regulate physiological functions (Bishop, Collins, Okamura & Norman, 1994).

Table 1

Natural and Fortified Food Sources of Vitamin D

Source	Amount	Vitamin D Content (IU)
Salmon		
	Fresh, wild (3.5 oz)	600-1000
	Fresh, farm (3.5 oz)	100-250
	Canned (3.5 oz)	300-600
Sardines	Canned (3.5 oz)	300
Tuna	Canned (3.5 oz)	230
Cod liver oil	(1 tsp)	400-1000
Shiitake mushrooms		
	Fresh (3.5 oz)	100
	Sun-dried (3.5 oz)	1600
Egg yolk	1	20
Fortified milk	(8 oz)	100
Fortified yogurt	(8 oz)	100
Fortified breakfast cereals	Serving per box	100

Note. Adapted from Holick, M. F. (2007). Vitamin D deficiency. *The New England Journal of Medicine*, *357*, page 270.

As mentioned earlier, vitamin D_2, also known as ergocalciferol, is also another form of vitamin D that humans are able to metabolize. This metabolite is produced when foods are irradiated by exposure to UVB radiation and undergoes some of the same metabolic pathways as vitamin D_3; however, ergocalciferol has been identified as less potent of a biological factor than cholecalciferol vitamin D_3 (Aramas, Hollis & Heaney,

2004; Houghton & Vieth, 2006; Trang, et al., 1998). In fact, vitamin D_2 was observed to be one-third as potent as vitamin D_3 in increasing and maintaining 25(OH)D concentrations in the study conducted by Aramas and colleagues (Aramas et al., 2004). Some explanations for a decreased potency include the following (Houghton et al., 2006):

- Vitamin D_2 has a lower affinity for vitamin D binding protein, which may lead to a shorter half-life than D_3.
- There may be differences in enzymatic activity in the first hydroxylation in the liver.
- There are different metabolic endpoints between vitamin D_2 and D_3.

Determinants of Vitamin D_3 Synthesis and of Circulating 25(OH)D Concentrations

One of the most influential characteristics in the process of vitamin D_3 synthesis is the source of radiation and its qualities. As mentioned earlier, UVB radiation must be present for 7-dehydrocholesterol to undergo the necessary conformational change to precholecalciferol. For optimal conversion of 7-dehydrocholesterol, UVB photons must be between 295-300 nm; the angle at which these photons reach the biosphere also influences the conversion process, and that angle is dependent upon latitude, season, altitude and time of day (MacLaughlin, et al., 1982).

Season

Season is one of the primary factors influencing circulating levels of 25(OH)D concentrations. In the winter months, individuals choose to spend more time indoors and

wear more clothing, resulting in less sunlight exposure. Also, the angle at which photons reach the surface of the earth increases, thereby, decreasing the amount of UV radiation reaching the earth. These factors contribute to a decreased amount of 7-dehydrocholesterol that is converted to precholecalciferol. Decreases in serum concentrations from end of summer to end of winter have been observed in numerous studies (Carnevale et al., 2001; Harris & Dawson-Hughes, 1998; Kim & Moon, 2000; Tangpricha, Pearce, Chen & Holick, 2002). A significant seasonal fluctuation ($p < .05$) was observed in both males and females from southern Italy (Carnevale et al., 2001) in a study evaluating the effects of season and sex on serum 25(OH)D concentrations.

A seasonal variation of serum 25(OH)D concentrations was also observed in young adults (18-29 years) living in Boston, Massachusetts. Winter concentrations were 30% less than summer concentrations. Tangpricha and colleagues found the difference to be 28 ± 10 and 36 ± 10 ($M \pm SD$) ng/ml, respectively (Tangpricha, et al., 2002). The difference observed in the 18-29 year group could have been attributed to spending time indoors for classes in the fall and spring while being outdoors in the summer during vacation even at 42°N latitude (Tangpricha et al., 2002).

A significant decrease in 25(OH)D from summer to winter was observed in Korean women between the ages of 20 and 75 years (Kim & Moon, 2000). Despite the marked observed changes, direct correlation between Korean women and US women should be cautioned because Korea does not routinely fortify dairy products with vitamin D like the US.

Seasonal changes were observed in both white and black premenopausal women from Boston, Massachusetts. From spring to the fall, mean plasma 25(OH)D concentrations increased by 8 and 4.4 ngl/ml for white and black women, respectively (Harris et al., 1998).

Latitude

Latitude affects the photon angle of ultraviolet radiation. Regions of latitude closer to the equator have a greater amount of sunlight exposure during the year. Some studies have assessed the differences of vitamin D status in individuals from different latitude and regions, while others have assessed vitamin D status in individuals from only one location.

After a winter season in four northern European countries between 52 and 64 °N, serum 25(OH)D concentrations were assessed in adolescent and elderly females. Ninety-two and 37% of adolescents and elderly, respectively, had concentrations that were < 20 ng/ml (Andersen et al., 2005). This value is well below what is now considered to be normal serum 25(OH)D concentrations (Bischoff-Ferrari et al, 2006; Cannell & Hollis, 2008; Heaney, 2004; Hollis, 2008).

An increase in prevalence of vitamin D deficiency and insufficiency was observed with an increase in latitude in a study comparing serum 25(OH)D concentrations among three different regions in Australia. The latitudes of the three different regions were 27, 38 and 43 °S (van der Mei et al., 2007). Serum 25(OH)D concentrations significantly decreased (p= .02) with an increase in latitude in Caucasian populations but not in non-

Caucasians (p= .14) in a meta-analysis drawing data from studies assessing 25(OH)D status globally (Hagenau et al., 2009).

Even though these studies indicated that individuals living at lower latitudes are not as prone to have deficient or insufficient 25(OH)D concentrations, a study assessing concentrations of individuals living in southern Arizona (latitude between 32 and 33 °N) observed approximately 25% of residents had serum 25(OH)D concentrations < 20 ng/ml. In fact, 75% of residents had concentration values < 30 ng/ml, which is below 34 ng/ml, the value associated with optimal calcium absorption (Heaney, Dowell, Hale & Bendich, 2003).

Additional Determinants

Skin pigmentation. Skin pigmentation is also known to greatly influence the production of vitamin D_3. Melanin is thought to mimic the effects of sunscreen and block UVB radiation (Holick, 1995) thus preventing the conversion of 7-dehydrocholesterol to precholecalciferol (Clemens, Henderson, Adams & Holick, 1982).

Several studies have noted that individuals reporting a different ethnicity other than Caucasian had lower serum 25(OH)D concentrations both in spring and fall (Gozdzik et al., 2008; Hagenau et al., 2009; Harris et al., 1998). Caucasian participants had greater concentrations than non-Caucasian participants (27 ± 1.3 and 19 ± 1.6 ng/ml, respectively) in a meta-analysis of 151 cross-sectional studies (Hagenau et al., 2009). Young black women had significantly lower concentrations than white women in both the spring (12 ± 8 and 24 ± 9 ng/ml, respectively) and fall (16 ± 7 and 34 ± 13 ng/ml, respectively) in a study completed in Boston, Massachusetts (Harris et al., 1998). In yet

another study assessing 25(OH)D concentrations in a diverse Canadian population during the winter, individuals reporting East Asian or South Asian ancestry had lower circulating concentrations than those reporting European ancestry (14, 12 and 22 ng/ml, respectively; Gozdzik et al., 2008).

Caucasian skin (type II skin) converted more than black skin (type V skin) when exposed to sunlight in a study observing epidermal conversion of 7-dehydrocholesterol in different skin types. If fact, there was about a 5-fold greater increase in serum 25(OH)D concentrations in type II skin when exposed to 12 weeks of simulated UVB radiation as compared to type V skin (Chen et al., 2007).

Age. Another characteristic that determines vitamin D_3 synthesis is age. As a person ages, there is a tendency towards a decreased amount of 7-dehydrocholesterol that is available for conversion to precholecalciferol. Skin samples from individuals of varying ages were exposed to ultraviolet radiation and there was an age-dependent decrease in precholecalciferol that was produced in the skin samples (MacLaughlin & Holick, 1985).

However, there have been recent studies assessing age differences with conflicting data. Some have observed lower concentrations in older individuals when compared to younger (Hagenau et al., 2009; Kim et al., 2000), while other studies have documented observations of the opposite (Andersen et al., 2005; Bischof, Heinze & Vierhapper, 2006). Those between the ages of 15-65 years had higher levels than those $\leq$ 15 years or > 75 years (22.8, 15 and 19 ng/ml, respectively; Hagenau et al., 2009). Yet, one study indicated that the age differences have diminished since 1988 in the United

States (Ginde, et al., 2009). Regression analysis revealed a significant decrease ($p < .05$) in concentrations with age on circulating 25(OH)D concentrations in Korean women between the ages of 20 and 75 years (Kim et al., 2000).

Andersen et al. (2005) compared vitamin D status in teenage girls (12.6 ± 0.5 years) and elderly women (71.8 ± 1.4 years) in the winter. Participants were residences of countries between the 51.9 and 60.1 °N latitudes. Women had higher median values of 25(OH)D concentrations than those of girls (16 and 12 ng/ml, respectively). A positive association ($p = .001$) between concentrations and age was observed assessing serum 25(OH)D concentrations in relation to BMI and age (Bischof et al., 2006).

The Third National Health and Nutrition Examination Survey (1988-1994) reported mean serum 25(OH)D concentrations that ranged from 32 to 28 ng/ml with the higher value being the mean of 12-19 year olds and the lower being the mean of individuals $\geq$ 60 years. In comparison, the NHANES 2001-2004 data revealed that serum 25(OH)D concentration values equalized around 24 ng/ml spanning over the age groups (Ginde, et al., 2009).

Body composition. Whether or not obesity has a direct effect on circulating 25(OH)D concentrations or an indirect effect has yet to be determined. The indirect effect that obesity can have on 25(OH)D concentrations possibly arises from obese individuals being outdoors less than lean individuals, and therefore, exposed to less sunlight. Direct effect hypotheses include the following with more evidence supporting the first (Looker, 2007):

1. Vitamin D may be sequestered and stored in fat tissue since it is a fat soluble vitamin (Wortsman, Matsuoka, Chen, Lu & Holick, 2000).
2. There is an increased rate of clearance from circulation due to larger pool of body fat (Looker, 2007).
3. There is negative feedback control of 25(OH)D production in the liver due to enhanced production of 1,25-dihydroxyvitamin D concentrations in obese individuals (Bell et al., 1985).

In addition, body composition can be defined and explored using several different methods of measurement. The most commonly examined is BMI (Bischof, et al., 2006; Botella-Carretero, et al., 2007), and other methods such as body fat percentage measured via DXA and visceral adipose tissue measured via computed tomography (Lenders et al., 2009; McKinney, Breitkopf & Berenson, 2008).

The possible link between adiposity and vitamin D deficiency has been explored in several different studies (Bell et al., 1985; Bischof, et al., 2006; Botella-Carretero, et al., 2007; Lenders et al., 2009; McKinney, et al., 2008). One of the first studies to observe a link was conducted by Bell and associates (Bell, et al., 1985). Serum 25(OH)D concentrations were measured in 2 groups, obese individuals (mean weight of 106 ± 6 kg) and nonobese individuals (mean weight of 68 ± 2 kg). Mean concentrations were 8 ± 1 and 20 ± 2 ng/ml, respectively (Bell et al., 1985). No other method of measurement quantifying fat mass or body composition was used.

Over 50% of morbidly obese participants (BMI $\geq$ 40 kg/m^2) were considered vitamin D deficient with serum 25(OH)D concentrations below 20 ng/ml in a study to determine if there was an association between 25(OH)D concentrations and the metabolic syndrome (Botella-Carretero, et al., 2007).

A greater percentage of participants with a BMI > 30 kg/m^2 (87%) were observed to have 25(OH)D concentrations considered deficient and insufficient when compared to participants with BMI < 30 kg/m^2 (78.5%). In this study, a concentration < 8.8 ng/ml was considered deficient, while a concentration value between 8.8 and 32 ng/ml was considered insufficient. Overall, there was a weak negative correlation (partial R^2 4.3%, $p < .001$) between 25(OH)D and BMI (Bischof et al., 2006).

Even after similar doses of UVB radiation in participants with similar baseline serum 25(OH)D concentrations, individuals with a BMI $\geq$ 30 kg/m^2 had lower concentrations of 25(OH)D when compared to individuals with a BMI $\leq$ 25 kg/m^2 (7 $\pm$ 1.4 vs. 15 $\pm$ 2.2 ng/ml) (Wortsman et al., 2000).

A link between body fat percent measured via DXA and serum 25(OH)D concentrations has been observed (Lenders et al., 2009; McKinney, et al., 2008). In obese adolescents with average body fat percent of 40.0 $\pm$ 5.5%, those who had a greater percentage of fat mass were more likely to have deficient levels of serum 25(OH)D concentrations. A moderate significant ($p < .05$) inverse relationship ($r = -.26$) was observed between fat mass percentage and serum 25(OH)D concentrations (Lenders et al., 2009).

A moderate significant ($p < .01$) inverse relationship ($r = -.28$) was also observed between total body fat percentage and serum 25(OH)D concentrations in females between the ages of 16 and 33 years. There was also a moderate significant ($p < .01$) inverse relationship ($r = -.36$) between BMI and serum 25(OH)D concentrations in the same study (McKinney, et al., 2008).

Medical conditions and medications. Some medications and medical complications have been identified as possible influences on serum 25(OH)D concentrations. Hepatic diseases can impact the conversion of cholecalciferol to 25(OH)D, whereas, renal disorders can influence the production of $1,25(OH)_2D$ from 25(OH)D. This is due to the decreased functioning of liver and kidney enzymes involved in converting precholecalciferol to its active vitamin D agent.

A significant difference ($p = .007$) in circulating 25(OH)D concentrations between oral contraceptive users and nonusers has been observed (Harris et al., 1998). Mean 25(OH)D concentrations of oral contraceptive users and nonusers were 33 ± 16 and 24 ± 9 ng/ml, respectively. The possible underlying mechanism may be related to an alteration in vitamin D binding protein levels. Adami and associates (2005) also observed a significant difference ($p < .001$) in serum 25(OH)D concentrations in premenopausal women between those taking oral contraceptives and those who were not (31.5 and 26.1 ng/ml, respectively). Even though the exact mechanism has not yet been identified, it is important to consider oral contraceptive use when assessing vitamin D status in women.

Use of statin medications has been shown to increase 25(OH)D concentrations. Individuals with acute coronary syndrome who received atorvastatin experienced a slight

increase in serum 25(OH)D concentrations from 16 $\pm$ 8 ng/ml at baseline to 19 + 8 ng/ml 12 months later (Perez-Castrillon et al., 2007). Perez-Castrillon and colleagues (2007) postulate that the mechanism causing the increase in 25(OH)D concentrations is due to the inhibition of 3-hydroxy-3 methylglutaryl coenzyme A reductase by atorvastatin. This inhibition possibly increases the availability of 7-dehydrocholesterol to be converted to precholecalciferol via UVB photons since the reductase is not as available to convert 7-dehydrocholesterol to cholesterol.

Sunscreen and clothing. There has been an increased awareness of the negative aspects of sun exposure; long-term exposure to sun can cause loss of skin integrity and can also lead to an increased risk of developing skin cancer. Coinciding with the increased awareness, the promotion of sunscreen with a sun protection factor (SPF) $\geq$ 15 has increased. Since the same ultraviolet radiation that increases the risk of sunburn is the same as that which is needed for conversion of 7-dehydrocholesterol to precholecalciferol (Holick, 1995), there is cause for concern with the increased promotion of sunscreen use. In fact, topical application of sunscreen with a SPF of 8 has shown to prevent the synthesis of precholecalciferol (Matsuoka, Ide, Wortsman, MacLaughlin & Holick, 1987).

Extra clothing worn outdoors also poses as an inhibitor of 7-dehydrocholesterol conversion. As the number of threads per inch became greater, there was a greater attenuation of sunlight with concomitant decreased conversion of 7-dehydrocholesterol to precholecalciferol in an *in vitro* study (Salih, 2004). A group of adolescent females who wore covered dress had significantly ($p < .001$) lower 25(OH)D concentrations than the

suburban and urban female groups who did not wear the same dress covering. In fact, 50% of the group had 25(OH)D concentrations < 10 ng/ml, which was considered deficient in that study (Hatun et al., 2005).

Assessing Vitamin D Status

Of the > 50 metabolites of vitamin D that have been identified, the two most clinically relevant are 25(OH)D and $1,25(OH)_2D$. Many of the other metabolites, as previously mentioned, are not as physiologically bioactive and do not regulate as many functions as $1,25(OH)_2D$ does. In fact, the chemical structures of many of the metabolites are susceptible to oxidation, free radical damage and conformational changes from exposure to excess UVB radiation and heat (Hollis, 2008).

As mentioned previously, serum 25(OH)D concentrations are now considered to be the best metabolite to measure in order to determine vitamin D status. Some suggested reasons for using this metabolite as a measurement of status include the following (Zerwekh, 2008):

- The half-life of 25(OH)D is approximately 3 weeks versus the half-life of $1,25(OH)_2D$ which is approximately 4 hr.
- The biological form 1,25-dihydroxyvitamin D is very tightly regulated by calcium needs.
- Liver conversion of cholecaliferol to 25(OH)D is dependent on substrate concentration and, therefore, not as tightly regulated as $1,25(OH)_2D$.

- The measurement of 25(OH)D is a better reflection of that which is ingested and that which is produced dermally over long periods.

Even though serum 25(OH)D concentrations have been identified as the best measurement of vitamin D status, there are still some difficulties in measuring 25(OH)D. There are two forms of 25(OH)D that can be measured: $25(OH)D_2$ and $25(OH)D_3$. As mentioned earlier, these metabolites are formed depending on the source of either ergocaliferol or cholecalciferol. Some of the assay methods today can measure both forms, but total 25(OH)D concentrations are the most beneficial to report unless the purpose of a study is to determine the potency of ergocaliferol in increasing total 25(OH)D concentrations (Zerwekh, 2008).

Another aspect to consider in measuring 25(OH)D concentrations is whether or not free 25(OH)D should be measured instead of that which is bound to a protein. Most of the metabolite is bound to either vitamin D binding protein (80-90%) or albumin (10-20%), while a small fraction (approximately 0.02-0.05%) is not bound to a protein for transportation (Zerwekh, 2008).

Methods of Assessment

Methods to assess serum 25(OH)D concentrations began in 1971 with competitive protein-binding assay (Haddad & Chyu, 1971). Since that time, different methodologies have been introduced as a means to provide more accurate results. These methods include radioimmunoassay, automated chemiluminescence, high-performance liquid chromatography and liquid chromatography coupled with mass spectrometry. The

positive and negative aspects of each method will be further discussed as a means to describe why ZRT laboratory was used for the purpose of this study.

Competitive protein-binding assay. In order to assess 25(OH)D concentrations, competitive protein-binding assay method utilizes vitamin D binding protein as a binding agent. It is considered to be a very valid method of measurement but a burdensome process (Hollis, 2008), especially for completing a high volume of assessments as would be required in a clinic or hospital setting. Also, since 25(OH)D is a lipophilic compound, matrix effect factors can interfere with 25(OH)D binding to the binding protein. This eventually causes a decrease in the validity of the assessment. Due to these matrix effect factors, the method is rarely used today, and comparisons between the results from competitive protein-binding assay and from the current methods of today are cautioned (Hollis, 2008).

Radioimmunoassay. Introduction to radioimmunoassay method of analyzing 25(OH)D concentrations was first made in the 1980s. Immunonuclear Corp., DiaSorin, developed a commercial kit that used ^{3}H-based radioimmunoassay, but it was not until the development of ^{125}I-labeled tracer did mass assessment become possible (Hollis, 2008; Hollis, Kamerud, Selvaag, Lorenz & Napoli, 1993). It was this assessment of vitamin D that the Food and Drug Administration approved for determining nutritional vitamin D deficiency. Currently, this method of radioimmunoassay is the only one of its nature that provides a total 25(OH)D concentration value (Hollis, 2008).

Automated chemiluminescent immunoassay. In an attempt to make assessment of vitamin D status more accessible to the practitioner, DiaSorin Corporation developed

and validated the Liaison 25 OH Vitamin D assay (Ersfeld et al., 2004). This method utilizes the automated chemiluminescent immunoassay technique in which an antibody is used as the primary-binding agent, and it reports a total 25(OH)D concentration value. A newer version of this method has been developed by DiaSorin and is considered to be the most commonly used assay throughout the world in clinical settings (Hollis, 2008). This version of chemiluminescent immunoassay provides results in approximately 40 min and has a correlation coefficient of r = .95 when compared to liquid chromatography-tandom mass spectrometry (Zerwekh, 2008).

Direct detection methods. High-performance liquid chromatography and liquid chromatography coupled with mass spectrometry are considered direct detection methods of assessing 25(OH)D concentrations. These methods individually measure $25(OH)D_2$ and $25(OH)D_3$ and also report a total 25(OH)D concentration value. Even though the methods are considered cumbersome, high-performance liquid chromatography without and with mass spectrometry are regarded as the gold standard for measuring 25(OH)D concentrations (Hollis, 2008; Zerwekh, 2008). One issue with liquid chromatography coupled with mass spectrometry is that is has difficulties distinguishing between $25(OH)D_3$ and 3-epi-$25(OH)D_3$, which is its inactive isomer.

In 2009, ZRT Laboratory (Beaverton, Oregon) developed a noninvasive method to measure 25(OH)D concentrations utilizing liquid chromatography coupled with tandem mass spectrometry in dried blood spots (Newman, et al., 2009). This method requires a finger prick to produce several drops of blood that are collected onto a filter paper. Concentrations of 25(OH)D obtained through this method have been compared

concentrations that were measured in blood samples obtained via venipuncture (Newman, et al., 2009). The interassay coefficients of variation were 13, 13 and 11% at concentrations of 14, 26 and 81 ng/ml, respectively. Strong positive correlation values (r = .90 and .91) were also observed for both 25(OH)D_2 and 25(OH)D_3, respectively (Newman, et al., 2009).

Recently proposed methods of assessing vitamin D status. Some researchers have also explored the idea of measuring other functional biomarkers or endpoints to assess vitamin D status. In a recent meta-analysis, the affects of vitamin D supplementation on different biomarkers were explored. Those biomarkers included 25(OH)D, parathyroid hormone, bone turnover markers, BMD, and rate of calcium absorption. The authors ultimately concluded that serum 25(OH)D concentrations are a valid method of measuring vitamin D status; however, more studies need to be conducted in order to determine if the other biomarkers could potentially be used as a means to complement 25(OH)D measurements (Seamans & Cashman, 2009).

Optimal Circulating 25(OH)D Concentrations

Normal serum 25(OH)D concentration values were first reported by plotting serum 25(OH)D concentrations gathered from healthy individuals and then using the Gaussian distribution to determine normative values (Haddad et al., 1971). However, it has recently been suggested that normal and optimal values should be standardized and based upon biomarkers of physiology and disease (Bischoff-Ferrari et al., 2006; Hollis, 2008).

Due to the role 1,25-dihydroxyvitamin D plays in skeletal integrity, some of the first biomarkers that were explored in relation to vitamin D status were parathyroid hormone, intestinal calcium absorption and bone mineral density (Adami et al., 2009; Bischoff-Ferrari, Dietrich, Orav & Dawson-Hughes, 2004; Chapuy et al., 1997; Kinyamu, Gallagher, Rafferty & Balhorn, 1998; Heaney et al., 2003; Vieth, Ladak & Walfish, 2003). With the recent developments in learning that vitamin D regulates several other physiologic pathways involved in cell proliferation, differentiation and immunomodulation (Nagpal et al., 2005), optimal circulating concentrations of 25(OH)D are now being investigated in relation to cancer prevention, diabetes and cardiovascular health, infectious diseases and autoimmunity.

The definition that was most commonly used to describe normal circulating 25(OH)D concentrations was based on concentrations that optimally suppressed parathyroid hormone levels in the blood. However, Bischoff-Ferrari and coworkers challenged that definition and determined that certain endpoint markers should be considered in determining optimal circulating 25(OH)D (Bischoff-Ferrari et al., 2006). The authors suggest that optimal concentration values be determined based on such endpoint outcomes such as BMD, lower-extremity function, dental health, and the risk of falls, fractures, and colorectal cancer. However, consensus on the validity and reliability for these measurements would have to be drawn as well. With measuring those endpoints, Bischoff and associates recommend that the optimal circulating 25(OH)D be 36 – 40 ng/ml or a target of at least 30 ng/ml (Bischoff et al., 2006).

Bone Metabolism Biomarkers

Parathyroid hormone. In the presence of insufficient calcium absorption due to inadequate amounts of either calcium or vitamin D, the parathyroid-calcitriol axis is stimulated in order to regulate serum calcium concentrations. Regulation occurs through an increase in parathyroid hormone which acts upon three different tissues – bone, kidney and intestine. In bone, parathyroid hormone stimulates the release of calcium from its reservoirs. Reabsorption of calcium and magnesium is enhanced in the kidney tubules with a concomitant increase in parathyroid hormone. Lastly, an increase in parathyroid hormone stimulates enzymes in the kidneys to produce the activated form of vitamin D [$1,25(OH)_2D$] in order to enhance the intestinal absorption of calcium.

Due to these physiological roles of parathyroid-calcitriol axis, several studies have investigated and observed the presence of an inverse relationship between parathyroid hormone levels and serum 25(OH)D concentrations (Chapuy et al., 1997; Kinyamu, et al., 1998; Vieth et al., 2003). Chapuy and colleagues observed a plateau in serum intact parathyroid hormone when serum 25(OH)D concentrations were greater than 31 ng/ml, but when 25(OH)D concentrations dropped below that value, intact parathyroid hormone increased (Chapuy et al., 1997). A significant yet moderate negative correlation ($r = -.33$, $p < .001$) was observed between serum parathyroid hormone and 25(OH)D concentrations (Kinyamu et al., 1998). These authors recommended that minimum serum 25(OH)D concentrations should be approximately 49 ng/ml.

A significant yet moderate negative relationship ($r = -.314$) was observed between 25(OH)D and parathyroid hormone concentrations in a study aimed to identify

at which 25(OH)D concentrations would a rise in parathyroid hormone be prevented in postmenarcheal females (12-18 years). When 25(OH)D and parathyroid hormone concentrations were graphed, there was also an observed change in slope when 25(OH)D were 36 ng/ml and parathyroid hormone concentrations were 30 ng/ml (Harkness & Cromer, 2005).

Heaney and coworkers concluded that most analyses observed that 25(OH)D concentrations between 28-44 ng/ml (70-110 nmol/l) promoted the lowest levels of circulating parathyroid hormone (Heaney et al., 2004); while, concentrations between 8 and 32 ng/ml produced potential long-term bone loss (Bischof et al., 2006).

Calcium absorption. As mentioned previously, the activated form of vitamin D, 1,25-dihydroxyvitamin D, is required for adequate absorption of calcium. Heaney and colleagues set out to quantify the 25(OH)D concentrations that are needed for optimal calcium absorption (Heaney, Dowell, Hale & Bendich, 2003). In comparing calcium absorption between two groups, those who had mean 25(OH)D concentrations of 34 ± 10 ng/ml had a 65% greater calcium absorption rate than that which was observed in the group with mean concentrations of 20 ± 6 ng/ml (Heaney et al., 2003). Barger-Lux and Heaney (2002) observed a slight decline in calcium absorption with a congruent decline in serum 25(OH)D concentrations; this was observed in white males who reported above average sun exposure in the summer and returned for the study to determine changes of 25(OH)D concentrations and calcium absorption in the winter.

Heaney (2004) compiled his findings as well as that of Bischoff and colleagues (2003) and Barger-Lux and Heaney (2002). He concluded that the findings indicate

fractional calcium absorption reaches its maximum and begins to plateau when serum 25(OH)D concentrations are approximately 32 ng/ml.

Bone mineral density. One of the first studies to assess an association between BMD and 25(OH)D concentrations was in a review of the Third National Health and Nutrition Examination Survey (NHANES III). From the data gathered on younger adults (20 – 49 years), all different ethnic backgrounds who were categorized in the highest quintile of 25(OH)D had significantly greater percentages of BMD than when compared to those who were categorized in the lowest quintile. Young whites, Mexican Americans and blacks displayed respectively the following greater BMD percentages above what is considered normal in their age cohort: 4.1% ($p < .0001$), 1.8% ($p < .004$) and 1.2% ($p < .08$). Using regression plot analysis, greater BMD values were observed in a 25(OH)D concentration range between 9 and 38 ng/ml (Bischoff-Ferrari et al., 2004).

In young premenopausal women (20-49 years), a positive correlation ($p = .043$) was observed between 25(OH)D concentrations and lumbar spine BMD when adjusted for age and body mass index (Adami et al., 2009).

Fracture Prevention

In regards to fracture prevention and optimal 25(OH)D concentrations, most literature focuses on fracture prevention in the elderly. Even though a direct comparison should not be made between the elderly and young premenopausal female athletes, it is still important to consider. In fact, this could be a potential area for research in the future since female athletes do run the risk of fractures, especially those with the female athlete triad.

Bischoff-Ferrari et al. (2006) concluded that optimal prevention of fractures in the elderly was achieved when serum 25(OH)D concentrations were approximately 40 ng/ml. In order to reach those concentrations, the authors further concluded from a meta-analysis review (Bischoff-Ferrari, et al., 2005) that individuals with a baseline 25(OH)D concentration < 18 ng/ml achieved this optimal value by supplementing with > 700-800 IU of vitamin D/day.

Muscular Strength and Lower Extremity Functioning

Only of recent interest is the relationship of neuromuscular functioning and serum 25(OH)D concentrations. The proposed relation to muscle strength and functioning is that 1,25-dihydroxyvitamin D binds to a vitamin D specific nuclear receptor in muscle tissue, which leads to de novo protein synthesis, muscle cell growth and improved muscle function (Bischoff-Ferrari et al., 2006).

Concentrations of 25(OH)D > 16 ng/ml have been considered to be advantageous; however, the same authors who made that conclusion stated that muscular functioning may be optimal when concentrations are 36-40 ng/ml (Bischoff-Ferrari et al., 2006). It is important to note, though, that the research evaluated by these authors was completed in the elderly populations. So, a direct correlation to a young female population may not be appropriate.

One recent research article (Ward et al., 2009) has observed an association between 25(OH)D concentrations and neuromuscular functioning in young females. After adjustment for weight, serum 25(OH)D concentrations were moderately related to muscle power ($r = .22$, $p = .004$), velocity ($r = .31$, $p = .002$), jump height ($r = .28$, $p = .006$),

Esslinger Fitness Index ($r = .32$, $p = .003$) and force ($r = .25$, $p = .04$). One interesting observation in this particular study population was that mean serum 25(OH)D concentrations were 11.6 ± 8.3 ng/ml, which is definitely below what is considered as the normal value (32 ng/ml); so, another possible area of further research would be to determine if there is a greater difference observed in individuals with values that are considered normal.

General Consensus for Optimal Concentrations

Based on endpoints for BMD, fracture risk reduction, neuromuscular functioning, periodontal health and colorectal cancer prevention, it was concluded that the optimal concentrations for 25(OH)D would be between 36-40 ng/ml with the lowest advantageous recommended amount of 30 ng/ml (Bischoff-Ferrari et al., 2006).

In a professional opinion article, Hollis (2008) explained that the current accepted normal values for 25(OH)D concentrations range between 32-100 ng/ml (based on clinical data from DiaSorin). The author stated that the lower value, 32 ng/ml, should not be considered optimal but rather a 'minimum normal value' (Hollis, 2008). He ultimately concluded that there is no optimal assigned value at this time and that there might be different optimal values for different physiological endpoints.

Heaney (2004) supported that normal concentrations should also be above 32 ng/ml based on optimal calcium absorption and fracture risk. The author also stated that concentrations between that which is considered deficient (8 ng/ml) and the lower value of what has been considered normal by some clinical labs (16 ng/ml) has been termed

vitamin D insufficiency. However, if considering the recommendations of the current literature, the lower value of normal would be 32 ng/ml.

Cannell & Hollis (2008) provided the following recommendations in their review of the literature: deficiency is < 40 ng/ml, ideal is between 40-70 ng/ml, excessive > 100 ng/ml, and toxic > 150 ng/ml. The authors concluded that when 25(OH)D concentrations reach > 40 ng/ml, the pharokinetics stabilize while the reactions involving $1,25(OH)_2D$ become saturable and controlled.

Controversy amidst the scientific community still exists, and there is no exact professional consensus as to what the optimal values of serum 25(OH)D should be. What can be concluded from the recent literature is that the lower end of normal should be between 32-40 ng/ml. It is still recognized that severe clinical vitamin D deficiency is defined as serum concentrations of 25(OH)D < 4-10 ng/ml, at which these levels can manifest into rickets in adolescents or osteomalacia in adults. As research continues to define what is considered normal, the lower value of normal for this study will be 40 ng/ml.

Vitamin D Intake Recommendations

The last revision of the Dietary Reference Intake (DRI) for vitamin D was completed by the Food and Nutrition Board of the Institute of Medicine in 1997. Since there was inadequate research at that time to determine estimated average requirements, adequate intake recommendations were reported for age and gender groups. Adequate intake recommendations were established with the assumption of minimal sun-mediated cutaneous synthesis. Vitamin D intake recommendations reported for selected groups are

presented in Table 2 (Institute of Medicine, 1997). At the same time, the Food and Nutrition Board indicated a need for more research focused on determining more precise vitamin D recommendations based on optimal serum 25(OH)D concentrations. Since then, there has been a drive to understand the biochemistry, physiology and roles of vitamin D.

Table 2

Adequate Intake Recommendations for Vitamin D

Age group	Adequate Intakes (μg/d)
7 mo to 3 y	5
4 – 18 y	5
19 – 50 y	5
51 – 65 or 70 y	10
> 65 or 70 y	15
Pregnant or lactating female	5
Tolerable upper intake for all persons ≥ 1 y	50

Note. Vitamin D reference intakes established in 1997 by Institute of Medicine

Yetley and colleagues (2009) described the process by which a working group of US and Canadian scientists systematically reviewed the vitamin D literature published after 1997 in order to determine if the vitamin D recommendations should be reviewed (Yetley et al., 2009). The scientific group formed several questions to address the newest research and then completed a systemic review to answer those questions, which include the following:

- What is the effect of circulating concentrations of 25(OH)D on health outcomes?
- What is the effect of vitamin D intakes on circulating concentrations of 25(OH)D?
- What is the effect of vitamin D intakes on health outcomes?
- What levels of vitamin D intakes are associated with adverse effects?

Based on the literature, the working group concluded that there was enough evidence to justify a review of vitamin D intake recommendations (Yetley et al., 2009). They also identified that there are very few published studies addressing vitamin D needs of populations other than the elderly, and the working group concluded further research addressing those populations is also warranted. This research concern was another reason for this research project.

In addition to the summary report of the "Vitamin D and Health in the 21st Century: an Update" conference, key areas in which there should be further research conducted were identified (Brannon et al., 2008). One of these areas again was a lack of research in groups other than the elderly and postmenopausal women. There was also a conclusion for a need for further research in the validation of using the biomarker 25(OH)D in relation to functional outcomes.

Vitamin D Intakes Needed to Achieve Optimal 25(OH)D Concentrations

Considering the dynamic roles of vitamin D, several suggestions and observations have been made that there is a greater need of vitamin D intake to maintain sufficient serum 25(OH)D concentrations (Bischoff-Ferrari, et al., 2006; Cashman et al., 2008). Cashman and colleagues (2008) concluded that individuals need twice the amount of

vitamin D that was established as the adequate intake (AI) ascertained by the United States Dietary Reference Intake panel in 1997 (refer to Table 2). After meta-analysis, Bischoff-Ferrari et al. (2006) recommended that the intake of vitamin D should be $\geq$ 1000 IU/d. If this is the case, the tolerable upper intake recommendations (2000 IU/d) should also be reviewed.

Vitamin D Intake Recommendations for Athletes

Currently, there is limited research identifying if athletes should have greater requirements for vitamin D intake due to increased physical training. In the most recent manual for sports nutrition (Volpe, 2006), the authors concluded that vitamin D intake recommendations for athletes are the same as that which were established for the general population (refer to Table 2). It was also recommended that everyday sun exposure for 15 minutes for light-skinned individuals and 30 minutes for darker-skinned individuals should be sufficient to obtain what is considered normal 25(OH)D concentrations. However, vitamin D research in athletic populations was warranted by the authors, yet another reason for the purposes of this study. As the Institute of Medicine reviews the recommendations for vitamin D intake, sports dietitians and physicians must take into consideration individual variations of vitamin D status and assess factors related to diet, endogenous synthesis and storage.

Nutrition Concerns for the Collegiate Female Athlete

Being a collegiate athlete encompasses a wide variety of demands, responsibilities and challenges. Academic schedules in combination with training schedules can create unique nutrition habits. A busy schedule and food availability greatly dictate when and

what an athlete will and can eat. Finances also greatly influence the type and quantity of food a collegiate athlete will consume. Other factors influencing the nutritional habits of a collegiate athlete include living situation, access to cooking facilities and/or grocery stores, and other challenges typically associated with attending college.

Collegiate female athletes deal with the added stresses from training and performance demands (Skinner, et al., 2001), and as society becomes more focused on thinness as being an indicator of success and happiness, they are also constantly struggling with issues surrounding weight, self-image and body shape (Berry & Howe, 2000). The most commonly identified health and nutritional concerns for the collegiate female athlete include low energy intake, eating disorders, and the female athlete triad (Beals & Manore, 2002; Gabel, 2006; Hassapidou & Manstrantoni, 2001; Nattiv, et al., 2007). One of the most alarming facts is that an estimated one-third or more of college athletes are at risk for some form of a clinical eating disorder (Beals et al., 2002); some data have reported a range of prevalence from 1-62% (Nattiv et al., 2007; Rosen & Hough, 1988; Rosen, Mceag, Hough & Curley, 1986; Wilmore, 1991).

Gabel (2006) outlined some of the most important nutritional concerns for female athletes. Inadequate dietary intake was identified as the chief concern, and the female athlete triad was also discussed as being of utmost importance. The female athlete triad is considered to be a continuum of three interrelated components: energy availability, bone mineral density and menstrual status (Nattiv, et al., 2007). Energy availability, defined as the dietary energy intake minus exercise energy expenditure, seems to be the cornerstone

of the triad (Ihle & Loucks, 2004; Loucks & Thuma, 2003; Loucks, Verdun & Heath, 1998; Nattiv et al., 2007).

Energy Intakes

Variability in energy and macronutrient intakes has been observed among female athletes in the same sport as well as among different sports (Hassapidou et al., 2001; Hinton, Sanford, Davidson, Yakushko & Beck, 2004; Mullins, Houtkooper, Howell, Going & Brown, 2001). Elite female athletes between the ages of 18 – 26 years from Greece competing in sports such as volleyball, middle distance running, ballet dancing and swimming were found to be in negative energy balance; average total energy intake during training was estimated at 1816 $\pm$ 537 kcal/d, while energy expenditure during training was estimated at 2311 $\pm$ 340 kcal/d. Those with the greatest energy deficit also reported menstrual irregularities (Hassapidou & Manstrantoni, 2001). In U.S. elite female heptathletes between the ages of 22 – 31 years, average energy intake was estimated at 2357 $\pm$ 897 kcal/d with a range between 1553 – 5276 kcal/d (Mullins et al., 2001).

Hinton and colleagues (2004) observed an average energy intake of 2141 $\pm$ 781 kcal/d with a protein intake of 87.8 $\pm$ 32.1 g/d, carbohydrate intake of 298 $\pm$ 112 g/d, and fat intake of 67.8 $\pm$ 29.8 g/d in collegiate female athletes (19 $\pm$ 1 years) who competed in a wide variety of sports. Estimations were based on utilizing the Youth Assessment Questionnaire, and energy intakes were compared to that which is recommended for young adult women who compete in light-to-moderate activity (2200 kcal/d; Hinton et al., 2004). In yet another study assessing nutrition behaviors via 3-day food records in collegiate female athletes, average energy intakes for pre-season and post-season were

2290 ± 310 kcal/d and 1865 ± 530 kcal/d, respectively (Clark, Reed, Crouse & Armstrong, 2003).

Eating Disorders and the Female Athlete Triad

The prevalence of disordered eating, menstrual irregularities and musculoskeletal injuries in female collegiate athletes from 15 different sports was assessed by Beals & Manore (2002). The athletes were grouped according to the nature of their sport: aesthetic, endurance or team/anaerobic. Even though there was a small percentage (5.5%) that reported clinical diagnosis of an eating disorder, the percentage of athletes considered "at risk" for developing either anorexia or bulimia were 25 and 38%, respectively. Of greatest nutritional concern, 67% reported eliminating certain food groups to control weight, and 42% indicated restricting calories to control weight. Other methods for weight control that were identified include fasting, very low-calorie diets, laxatives, diet pills and vomiting. Athletes from aesthetic sports were more likely to practice very low-calorie diets and scored higher on the Eating Attitudes Test-26, which indicates a greater risk for developing disordered eating (Beals & Manore, 2002).

In yet another study, 25.5% of the collegiate female athletes were classified as symptomatic (some symptoms of an eating disorder but insufficient to be classified as an eating disorder), and 2.0% were classified as having an eating disorder using the Questionnaire for Eating Disorder Diagnosis (Mintz, O'Halloran, Mulholland & Schneider, 1997). Other identified methods of weight control included fasting or going on strict diet plans at least two times in the past year and vomiting.

The prevalence of disordered-eating behaviors between undergraduate collegiate female athletes and nonathletes was compared by Reinking & Alexander (2005). Females participating in sports that emphasized leanness were at a greater risk for developing disordered-eating behaviors than those competing in non-lean sports; however, there was not a significant greater prevalence of disordered eating in athletes when compared with the nonathlete group. In fact, the athletes scored better than the nonathletes in the Body Dissatisfaction and Ineffectiveness subscales of the Eating Disorders Inventory – 2 (Reinking & Alexander, 2005).

Assessment and prevalence of the female athlete triad is challenging since it involves three interrelated yet separate health issues. Menstrual irregularities, bone mineral density and energy availability would all need to be assessed. Much of the research thus far has focused on addressing one of the three issues; further research assessing all three is needed in order to determine the incidence of the female athlete triad in collegiate female athletes.

With a combination of negative energy balance and disordered eating behaviors, the opportunity for consuming optimal amounts of micronutrients becomes challenging, especially when certain food groups such as dairy are avoided. In addition, access to affordable nutrient-dense food choices is limited for collegiate athletes.

Importance of Vitamin D in the Collegiate Athlete

As mentioned previously, vitamin D deficiency can impact not only bone health of an athlete but also influence muscular performance and proper functioning of the

immune system and inflammation modulation. Disturbances in the normal functioning of one of these systems can impact performance negatively.

In two recent studies in active military personal, the relationship of fracture risk and vitamin D status was explored. One study indicated that the risk of stress fractures significantly increased when 25(OH)D concentrations were < 30 ng/ml (Ruohola et al., 2006). In a randomized double-blind, placebo controlled trial, female naval recruits receiving an 8-week supplementation of 800 IU of vitamin D plus 2000 mg of calcium per day had a 20% lower incidence of stress fractures when compared to the control group (Lappe, et al., 2008).

An impairment of muscle functioning in association with low 25(OH)D concentrations has also been documented, primarily in the elderly population. A recently published article, though, assessed the relationship in adolescent females. Force output, power, velocity, jump height and Esslinger Fitness Index were associated with serum 25(OH)D concentrations (Ward et al., 2009). Average 25(OH)D concentrations of these adolescents was 11.6 ng/ml indicating that low concentrations may possibly impair muscle functioning. Some possible explanations given for this phenomenon were that vitamin D deficiency impairs sarcoplasmic calcium uptake prolonging the time to peak contraction of a muscle or atrophy of fast-twitch muscle fibers (Hamilton, 2010).

The immune system has also recently been associated with vitamin D status. Vitamin D has been found to be primarily involved in the innate immunity through the regulation of the production of antimicrobial peptides, specifically cathelicidin. Antimicrobial peptides act upon the cell membrane of pathogens and alter its integrity

(Larson-Meyer & Willis, 2010). What has been suggested is that antimicrobial deficiencies due to vitamin D deficiency could increase the occurrences of influenza and infections. In light of this, a study was conducted to determine if there was an association between low 25(OH)D concentrations and the incidences of self-reported illnesses in collegiate athletes. Poor status was associated with the number of reported illnesses, which included the common cold, influenza and gastroenteritis (Halliday, et al., 2010).

Vitamin D Intakes of Athletes

Research reporting vitamin D intake along with estimations of total caloric intake among collegiate female athletes is limited. The available data to report has been gathered using different methods of assessment.

In utilizing the Youth Assessment Questionnaire, female athletes from a wide variety of collegiate sports were found to have an average vitamin D intake of 6.7 ± 4.3 μg/d with a mean energy intake of 2141 kcal/d (Hinton, et al., 2004). However, when collegiate female soccer players used a 3-day food record for assessment, the mean vitamin D intake was 2.4 ± 1.7 and 2.5 ± 2.6 μg/d during the pre-season and post-season, respectively. These same athletes had an average energy intake of 2290 ± 310 kcal/d and 1865 ± 530 kcal/d, respectively (Clark et al., 2003). In younger (14.1 ± 1.7 years) U.S. female national figure skaters, average vitamin D and energy intakes were 2.7 ± 5.5 μg/d and 1536 ± 620 kcal/d, respectively (Ziegler, Nelson & Jonnalagadda, 1999).

To compare two methods of assessment, 4-day food records and Food Frequency Questionnaire results were compared. In young (9-15 years) female athletes competing in gymnastics and competitive running, vitamin D intake was estimated to be 2.9 ± 1.5 μg/d

utilizing the Food Frequency Questionnaire and 4.3 $\pm$ 2.1 μg/d using the 4-day food record.

Serum 25(OH)D Concentrations in Athletes

As is the case with vitamin D intake in athletes, there is limited research reporting serum 25(OH)D concentrations in collegiate athletes. There has been some recent literature published addressing vitamin D status in active individuals of varying ages. In adolescent girls (9-15 years) from Finland (64 °N latitude) who were either competitive gymnasts, runners or non-athletes, baseline 25(OH)D concentrations in the winter were 13.6 ngl/ml; 68% had concentrations below 15 ng/ml. Average summer concentrations were 25.2 ng/ml, and 12% of the participants had concentrations < 15 ng/ml. An intervention of a vitamin D supplement providing 10 μg/d (twice the RDA for that specific age group) for 3 months during the winter did not seem to prevent hypovitaminosis D. The authors reported that participants who consumed > 5 μg/d of vitamin D had a higher 25(OH)D concentrations than those who had < 5 μg/d, as estimated by food frequency questionnaire (Lehtonen-Veromaa et al., 1999).

Lovell (2008) assessed the vitamin D status in elite female gymnasts (10-17 years) residing in Australia (35.27 °S latitude). The mean 25(OH)D concentration in fall for Australia was 22.4 ng/ml with a range of 3.6-33.7 ng/ml; 83% of the participants were found to have concentrations below that which is recommended for optimal bone health (< 30 ng/ml), and 33% had concentrations below 20 ng/ml. In addition, calcium intake for 13 of the participants was below the recommendation of 1000 mg/d for 9-11 years and 1300 mg/d for 12-18 years.

A recent study has assessed seasonal changes in serum 25(OH)D concentration in collegiate male and female athletes living in Laramie, Wyoming (41.3 °N latitude). During the fall season, average concentration was 49 ng/ml, and 12% of the participants had values < 32 ng/ml. The average concentration value decreased to 30.5 ng/ml in the spring, and 64% of the athletes had concentrations < 32 ng/ml (Halliday, 2010).

CHAPTER III

METHOD

The purpose of this quasi-experimental and descriptive study was twofold: 1) to determine if there was a change in serum 25(OH)D concentrations of indoor sport female athletes living at 33.21 °N latitude from the fall 2009 season to the spring 2010 season; and, 2) to determine if there was a difference in fall serum 25(OH)D concentrations between indoor sport and outdoor sport athletes.

Overview

Data for this study were collected at Texas Woman's University (TWU) in Denton, TX, in the Exercise and Sports Nutrition Clinic, which is located in the Human Development Building, room 011. The Lunar Prodigy dual-energy x-ray absorptiometry (DXA) scans were performed in the Institute for Women's Health (IWH) Laboratory, which is located in the Human Development Building, room 017B.

The clinic offers fitness and health assessments throughout the year for the TWU athletics department, TWU students/faculty/staff and Denton, TX, community members. Information has been gathered for a larger research study with the consent of each participant. For the purpose of this study, information was gathered from the questionnaires, DXA scans and food record analyses; vitamin D blood spot tests were added to assess 25(OH)D concentrations. The assessment data were used to describe baseline characteristics and health status of the study participants. In the fall, the

questionnaires and previously stated assessments were administered; while in the spring, only the blood spot tests and sunlight exposure questionnaires were administered and used for data collection.

The individuals involved in collecting data were the principal investigator, IWH technicians and college graduate students in the TWU Exercise and Sports Nutrition or Exercise Physiology graduate programs.

Participants

A purposive sampling method was used by recruiting female students who participated in National Collegiate Athletic Association (NCAA) Division II athletics. These athletes participated in one of the following sports: volleyball, softball or gymnastics. Athletes were grouped according to the nature of their sport, indoor or outdoor. Volleyball and gymnastics were considered indoor sports. Softball was considered an outdoor sport. Athletes were excluded from the study if they had preexisting medical conditions that impact serum vitamin D concentrations. Such conditions included compromised kidney and/or liver functions. Each participant was informed of the purposes, procedures, risks and benefits through a written informed consent form (Appendix A) and was asked to sign it before participating in the study. A numerical code was assigned to each participant and the corresponding data to maintain confidentiality. Approval from the TWU Institutional Review Board was obtained (Appendix B) before recruiting the athletes.

Equipment and Procedures

Questionnaires

A demographic questionnaire (Appendix C) and health history questionnaire (Appendix D) were administered and used to report general demographic and health characteristics. Of particular interest in the health history questionnaire were questions addressing the use of multivitamin supplements and the use of oral contraceptives (Harris & Dawson-Hughes, 1998). A questionnaire (Appendix E) regarding ambient sun exposure, personal sun protection, and tanning bed use was added to provide background information about average sun exposure among female college athletes. Questions were adapted from McCarty (2008). The demographic and health history questionnaires were completed in the fall before initiation of any assessments. The sunlight exposure questionnaire was completed in both the fall and the spring either before or after the participant completed the blood spot test.

BMD and Body Composition

In the fall, participant BMD and body composition were measured using DXA scanning (Lunar DPX-IQ software version 4.6 c; Lunar, Madison, WI). Total body, lumbar spine (L1 – L4), and femoral neck scans were measured in the same scanning session. Whole body scans for body composition and total BMD were completed with the participant lying in supine position. The technician ensured the arms and legs of the participant were within the scanning boundaries and loosely tied the feet together with a Velcro band to keep them in position. Lumbar spine was measured from L1 – L4 while the participant was in supine position with legs resting on a box; knees and hips were

flexed to ensure that the lower back was flat on the table. Left femoral neck scans were measured at the top of the femur including part of the hip. The participant was instructed to lie supine in the center of the table with both legs straight while the technician turned the right leg medially approximately 30 degrees. In order to keep the legs in that position, the right and left feet were held in place by attaching both to a pyramid shaped object with a Velcro strap. Scans were completed by the same registered technician, and analyses were completed by either the registered technician or trained staff. Before the morning of each use, the scanner was calibrated using Lunar quality assurance procedures.

Nutrition Analysis

Each athlete was also asked to complete a 3-day food record in the fall and the spring. It was requested that two week-days and one weekend-day be recorded. The 3-day food records were analyzed using Nutritionist Pro analysis software (version 1.3; Axxya Systems, Stafford, TX) to determine daily average intakes for the following: Calories, macronutrient distributions, vitamin D (IU), calcium (mg), phosphorus (mg) and magnesium (mg). Analyses were completed by IWH technicians and Exercise and Sports Nutrition graduate students.

Serum 25(OH)D Concentrations

Blood spot test kits were obtained from ZRT Laboratory to measure serum 25(OH)D concentrations. Blood spot tests were completed in the fall and spring months by the same technician. Per instructions from ZRT Laboratory, the same standard protocol (Appendix F) was followed for collecting blood samples from each participant.

Test kits were mailed to ZRT Laboratory for analysis using liquid chromatography/tandem mass spectrometry (Maunsell, Wright, & Rainbow, 2005; Newman, et al., 2009). This assay has an inter-assay coefficient of variation of approximately 12%. Results were sent back to the clinic, and serum 25(OH)D values were entered into an Excel database worksheet.

Statistical Analysis

All analyses were conducted using SPSS statistical software program (version 16.0; SPSS Inc, Chicago, IL). Descriptive data and baseline characteristics were reported as mean $\pm$ standard deviation (M $\pm$ SD). Student t tests were used to determine differences in baseline descriptive characteristics between indoor and outdoor athletes. Since outdoor athlete data was not obtained for the spring, a paired t test was used to determine if there was a change in indoor athlete serum 25(OH)D concentrations. Levene's test was used to test assumption of equal variance. A p value $\leq .05$ was considered significant. For exploratory statistical analysis, Pearson r correlations were used to analyze associations between serum 25(OH)D concentrations and each of the following: body fat percentage, vitamin D intake and BMD. Also, a multiple regression analysis was used to determine if vitamin D intake, ethnicity, body fat percentage, supplement use and tanning bed use were significant predictors of fall 25(OH)D concentrations.

CHAPTER IV

RESULTS

In fall 2009, a total of 40 collegiate female athletes were recruited for assessments between August and the beginning of November. No participants were excluded for any medical conditions that had the potential for influencing 25(OH)D concentrations. Of those 40 athletes, four did not complete the blood spot tests for measuring 25(OH)D concentrations in the fall, so data from those athletes was not included in reporting baseline results. Of the 36 athletes, 97% completed 3-day food records, and one athlete did not complete DXA scans; 19 were categorized as outdoor athletes (including only softball players), and 17 were categorized as indoor athletes (including gymnasts and volleyball players).

In spring 2010 between April and May, only 11 of the 40 participants returned for reassessments of serum 25(OH)D concentrations. All 11 participants were indoor athletes, and three of those athletes did not have 25(OH)D concentrations measured in the fall. Along with blood spot tests, it was requested that these athletes complete sunlight exposure questionnaires and 3-day food records. However, only two returned food records, and 10 completed the questionnaire.

Recruitment for this study did not produce the desired participation in the spring. Both the softball and gymnastics teams were at the height of their seasons, and it was challenging to schedule appointments that would accommodate traveling and competition

as well as educational obligations. Since no outdoor athletes returned for reassessments of 25(OH)D concentrations, baseline descriptive data are presented and compared between indoor and outdoor athletes along with exploratory analysis that was completed on fall baseline data. Changes from fall to spring in individual serum 25(OH)D concentrations for the indoor athletes are presented.

Fall Baseline Characteristics

Anthropometrics

Average self-reported age for athletes in the fall was 19.3 ± 1.2 years with a range of 18-22 years. Baseline fall anthropometric measurements as well as total, lumbar (L1-L4) and femoral neck BMD are presented in Table 3.

Table 3

Baseline Descriptive Characteristics

Variable	Outdoor Athletes (n = 19)	Indoor Athletes (n = 17)	p value
Age (y)	19.3 ± 1.2	19.3 ± 1.1	.92
Height (cm)	164.3 ± 8.0	163.2 ± 7.9	.67
Weight (kg)	68.7 ± 11.8	62.7 ± 6.8	.07
BMI (kg/m^2)	25.5 ± 4.6	23.5 ± 1.9	.10
Body Fat (%)	35.0 ± 8.6	24.9 ± 3.5	.00
Total BMD (g/cm^2)	1.230 ± 0.054	1.245 ± 0.067	.47
Lumbar BMD (L1-L4; g/cm^2)	1.375 ± 0.1	1.388 ± 0.1	.78
Hip BMD (g/cm^2)	1.241 ± 0.1	1.224 ± 0.1	.60

Note. Measurements expressed as M ± SD. Level of significance was set at $p \leq .05$.

There were no significant differences in mean height, weight and BMI between indoor and outdoor athletes. However, indoor athletes had lower body fat percentage compared to outdoor athletes (24.9 ± 3.5 vs. 35.0 ± 8.6%, respectively; p = .00). Nine athletes, six of whom were indoor athletes, indicated a different ethnicity other than Caucasian including African American and Hispanic.

Dietary Records

Total energy, macronutrient, vitamin D, calcium and phosphorus intakes are presented in Table 4.

Table 4

Baseline Dietary Intakes

Variable	Outdoor Athletes (n = 18)	Indoor Athletes (n = 17)	p value
Energy intake (kcal/d)	1461 ± 274	1446 ± 313	.88
Total fat intake (g/d)	54.1 ± 17.1	50.0 ± 13.7	.44
Total protein intake (g/d)	60.0 ± 10.2	63.7 ± 15.3	.42
Total carbohydrate intake (g/d)	185.4 ± 37.9	186.7 ± 51.5	.89
Vitamin D intake (μg/d)	1.4 ± 1.1	1.8 ± 1.2	.36
Calcium intake (mg/d)	595 ± 140	692 ± 196	.10
Phosphorus intake (mg/d)	695 ± 217	703 ± 249	.92

Note. Analyses did not include supplement use. Intakes reported as M ± SD. Level of significance was set at $p \leq .05$.

There was no significant difference in vitamin D intakes between indoor and outdoor athletes (1.8 ± 1.2 and 1.4 ± 1.1 μg/d, respectively). It was noted that only one athlete met or exceeded the unrevised recommended vitamin D Adequate Intake for this population (5 μg/d; Institute of Medicine, 1997). Also, no significant differences in

calcium and phosphorus intakes were observed between the two groups. All athletes were noted to have daily calcium intakes below the Adequate Intake recommendations of 1000 mg/day and 1300 mg/day for females of the ages 19-50 years and 14-18 years, respectively (Institute of Medicine, 1997). Also, these female athletes did not meet the recommended RDA values for phosphorus intakes for females 14-18 years and ≥ 19 yrs, which are 1250 and 700 mg/d, respectively (Institute of Medicine, 1997).

Serum 25-hydroxyvitamin D Concentrations

Fall serum 25(OH)D concentrations are presented in Table 5.

Table 5

Baseline 25-hydroxyvitamin D Concentrations

	All athletes (n=36)	Outdoor Athletes (n=19)	Indoor Athletes (n=17)	p value
25(OH)D (ng/ml)	48 ± 18	46 ± 15	50 ± 20	.52
Range (ng/ml)	20 – 101	23 – 74	20 – 101	

Note. Measurements expressed as M ± SD. Recommended 25-hydroxyvitamin D concentrations: 40 – 70 ng/ml (Cannell & Hollis, 2008)

For this study, serum concentrations ≤ 40 ng/ml were considered to be insufficient. There was no significant difference between indoor and outdoor athletes at baseline (50 ± 20 vs. 48 ± 15 ng/ml, respectively; p = .52). The ranges of serum 25(OH)D concentrations for the indoor and outdoor athletes were 20-101 and 23-74 ng/ml, respectively. Of the 36 athletes, 13 (36%) had concentrations ≤ 40 ng/ml. Five of those 13 (38%) athletes reported either Hispanic or African-American ethnicity. While another

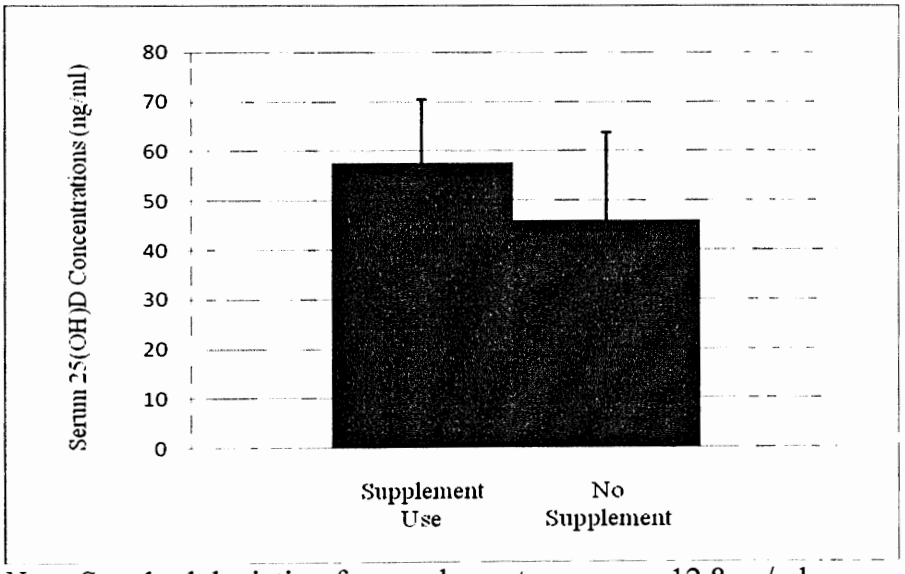
5 of the 13 (38%) who reported Caucasian ethnicity had a body fat percentage $\geq$ 35%. Of the remaining 3 Caucasian athletes with 25(OH)D concentrations $\leq$ 40 ng/ml, two had body fat percentages < 22%, while one did not complete DXA scans to determine body fat percentage. One athlete had a 25(OH)D concentration of 20 ng/ml, and this athlete reported African-American ethnicity and Canadian residency prior to the fall assessment.

Health Questionnaire

Commonly reported health conditions included allergies and asthma by 13 of 36 participants (36%). Hypertension (3%), back injury (3%), ulcers (3%), and rheumatoid arthritis (3%) were among the other conditions reported. There were no medical conditions or medications other than oral contraceptives indicated that would have influenced 25(OH)D concentrations. Fourteen athletes reported use of oral contraceptives; these athletes had a mean 25(OH)D concentration of 53 $\pm$ 15 ng/ml. Three outdoor athletes and 4 indoor athletes reported use of a multi-vitamin/mineral supplement; the mean 25(OH)D concentration for these athletes was 58 $\pm$ 13 ng/ml (Figure 1). There was no indication as to supplemental regimen or specific type of supplement taken; therefore, vitamin D content of supplements was not assessed and was not included in estimating vitamin D intake.

Figure 1

Comparison of Mean Serum 25-hydroxyvitamin D Concentrations: Supplement Users vs. Non Supplement Users



Note. Standard deviation for supplement users was 12.8 ng/ml compared to a standard deviation of 17.8 ng/ml for non supplment users.

Sunlight Exposure Questionnaire Results

Thirty-two of the 36 athletes (89%) completed the sunlight exposure questionnaire in the fall. Regular sunlight exposure between 10 AM and 4 PM was reported by 91% of the responders; 81% of these athletes reported $\geq$ 2 hrs each exposure. During time spent outdoors, 69% responded that $\geq$ 50% of their skin was exposed, and 59% reported that they use sunscreen $\leq$ 50% of the time.

Of the 14 indoor athletes who completed the sunlight exposure questionnaire, 9 (64%) reported use of tanning beds; while, 7 of the 18 outdoor athletes (39%) indicated such. Use varied between 1-2 times per wk with reported amount of time between 11-20 min.

Exploratory Statistics

Exploratory statistics were completed to determine if there was a correlation between serum 25(OH)D concentrations and each of the following: body fat percentage ($r = -.204$; $p = .24$), vitamin D intake ($r = -.227$; $p = .19$) and total BMD ($r = -.172$; $p = .32$). No significant correlations were observed. For multiple regression analysis, the only statistical significant ($p = .01$) predictor of fall vitamin D status was supplement use.

Changes in Individual Serum 25-hydroxyvitamin D Concentrations

Only 11 athletes completed assessments of serum 25(OH)D concentrations in the spring. Three of those 11 (27%) did not have fall assessments. Individual fall and spring serum 25(OH)D concentrations as well as ethnicity, body fat percentage and tanning bed use are presented in Table 6.

Table 6

Changes in Individual Serum 25-hydroxyvitamin D Concentration

Indoor Athlete ID Number	Fall 25(OH)D Concentration (ng/ml)	Spring 25(OH)D Concentration (ng/ml)	Ethnicity	Body Fat %	Tanning Bed Use
112	40	30	Caucasian	19.9	NA
185	51	56	Caucasian	23.3	1-2x/wk
117	20	15	African-American	20.9	No
48	74	63	Caucasian	19.9	2-3x/wk
33	47	50	Caucasian	27.7	2-3x/wk
110	48	55	Caucasian	27.9	3x/wk
182	47	42	Caucasian	23.2	4-5x/wk
184	33	28	Caucasian	21.4	No
106	NA	73	Caucasian	21.7	2x/day
187	NA	59	Caucasian	32.1	4x/wk
180	NA	29	Caucasian	24.3	No

Note. Shaded values are below the recommended optimal concentrations of 25-hydroxyvitamin D: 40 – 70 ng/ml (Cannell & Hollis, 2008). NA = not available.

Four of the athletes with spring assessments had concentrations ≤ 40 ng/ml. The athlete with a serum 25(OH)D concentration of 15 ng/ml was the same athlete who had a concentration value of 20 ng/ml in the fall. Of the 8 athletes with fall and spring assessments, 5 (63%) had a decrease in serum 25(OH)D concentrations; whereas, 3 (37%) had an increase. A paired t-test was completed to determine if there was a significant change in serum 25(OH)D concentrations from the fall to the spring among the indoor athletes; no significant difference was found ($p = .31$).

CHAPTER V

DISCUSSION

To date, little is known about vitamin D status, particularly trends in serum 25(OH)D concentrations, in collegiate female athletes. This study is among the first to assess such concentrations in collegiate female athletes, to explore possible influential factors affecting trends, and to determine if there was a difference in concentrations between indoor and outdoor athletes.

The hypothesis that there will be no significant change in serum 25(OH)D concentrations in indoor sport female athletes between fall 2009 and spring 2010 was accepted. The hypothesis that there will be no difference in fall 2009 serum 25(OH)D concentrations between indoor sport and outdoor sport female athletes was also accepted.

Serum 25-hydroxyvitamin D Concentrations

Blood spot testing using liquid chromatography coupled with tandem mass spectrometry has been shown to be a valid and reliable method for measuring 25(OH)D concentrations (Newman, Brandon, Groves, Gregory, Kapur & Zava, 2009). This method of analysis was chosen for this study due to the stability of 25(OH)D in serum (Hollis, 2008) and the accuracy of liquid chromatography coupled with mass spectrometry (Zerwekh, 2008). This study is one of the first to report such a method used to analyze 25(OH)D concentrations in female athletes. Other studies in female athletes have reported analysis methods such as DiaSorin vitamin D radioimmunoassay (Lehtonen-

reported analysis methods such as DiaSorin vitamin D radioimmunoassay (Lehtonen-Veromaa et al., 1999; Lovell, 2008; Maïmoun et al., 2006), and both methods have been reported to be correlated (r = .74 to .96; Zerwekh, 2008).

There was not a significant difference in serum 25(OH)D concentrations between outdoor and indoor athletes when measured in the fall. This is most likely due to the measurements being made after summer vacations during the off-season; 94% of athletes, both indoor and outdoor, indicated spending ≥ 30 min outdoors on average per day. Depending on skin pigmentation and UVB wavelength, UVB exposure to arms and legs for approximately 5 to 10 min will produce about 3000 IU of vitamin D_3 (Holick, 2007); therefore, sun exposure for about 30 min would theoretically produce approximately 9000 IU of vitamin D_3, which is well above the currently recommended vitamin D intake of 400 IU/d.

The mean serum 25(OH)D concentrations of both indoor and outdoor female athletes were within currently suggested optimal concentrations of 40-70 ng/ml (Cannell & Hollis, 2008). However, one-third of the study population had concentrations below the lower value of that range. In fact, 8 of the athletes had concentrations below that which is considered optimal for adequate calcium absorption (34 ng/ml; Heaney, Dowell, Hale & Bendich, 2003). Four of those athletes were outdoor athletes, all of whom had body fat percentage values ≥ 34.9%. Three of the four indoor athletes with concentrations < 34 ng/ml reported African-American ethnicity.

Among the many factors known to impact serum 25(OH)D concentrations, ethnicity and body fat percentage (Bell, et al., 1985; Bischof, et al., 2006; Botella-

Carretero, et al., 2007; Gozdzik et al., 2008; Hagenau et al., 2009; Harris & Dawson-Hughes, 1998; Lenders et al., 2009; McKinney, et al., 2008) were found to be potential confounding variables to consider in this study sample. Female athletes of either African-American or Hispanic ethnicity or those with a higher body fat percentage as assessed with DXA were found to have lower concentrations than those of Caucasian ethnicity or those who had lower body fat percentages.

In this study, mean serum 25(OH)D concentrations were greater than what has been observed in other studies reporting concentrations in female athletes (Lehtonen-Veromaa et al., 1999; Lovell, 2008). In Australian female gymnasts between the ages of 10-17 years, Lovell (2008) reported a mean value of 22 ng/ml; this value is about half the mean concentration value (48 $\pm$ 18 ng/ml) that was observed in this study. One explanation for the difference may be related to the time of year measurements were made; Lovell (2008) reported concentrations from the beginning of May, whereas this study reported mean measurements from the fall. Another reason for the observed difference between the studies may be that there might be a change in plasma volume concentrations of 25(OH)D due to the finger prick method.

Lehtonen-Veromaa and associates measured serum 25(OH)D concentrations in female gymnasts and competing runners between the ages of 9-15 years who lived in Turku, Finland (Lehtonen-Veromaa et al, 1999). The mean concentration at baseline was 14 ng/ml, while after supplementation, the mean concentration increased to 25 ng/ml. These values are considerably lower than the mean concentration observed in this study. One possible explanation for the observed differences is that Turku, Finland is at 60°N

latitude, while Denton, TX, is at 33°N latitude. It has been observed that individuals living at a greater latitude have lower 25(OH)D concentrations (Hagenau et al., 2009; van der Mei et al., 2007).

Changes in Serum 25-hydroxyvitamin D Concentrations

There was no significant change ($p = .31$) observed in serum 25(OH)D concentrations in the athletes assessed in both the fall and the spring. It was expected that serum 25(OH)D concentrations would decrease from fall to spring, especially in indoor athletes, since they would be exposed to less sunlight. However, that was not observed in this study. In fact, some athletes had an increase in 25(OH)D concentrations. One explanation for this observation may be the fact that those individuals indicated weekly use of tanning beds. The athletes with concentrations < 40 ng/ml indicated that they did not use tanning beds. One of the athletes with a spring concentration value of 73 ng/ml indicated use of a tanning bed twice daily. Even though tanning beds emit approximately only 5% UVB radiation (Scarlett, 2003), a significant increase ($p < .001$) in 25(OH)D concentrations has been observed with the use of tanning beds (Devgun, Johnson & Paterson, 1982).

Anthropometric Results

Baseline weight and BMI for both the indoor and outdoor female athletes was similar to what has been observed in other studies reporting such data for female collegiate athletes (Hinton, et al., 2004; Reinking & Alexander, 2005). There was a significant difference observed in body fat percentage between the indoor and outdoor athletes (24.9 + 3.5 and 35.0 + 8.6%, respectively). This observation was likely attributed

to the fact that most of the indoor athletes were gymnasts and most of the outdoor were softball players. Gymnastics is an aesthetic sport, as compared to softball; aesthetic sport athletes tend to be leaner than athletes from teams that do no emphasize aesthetics as much (Reinking & Alexander, 2005).

There was no significant difference observed in BMD between the indoor and outdoor athletes. This finding coincides with what has been observed in recent literature. Mudd and colleagues have previously compared the BMD of athletes in different sports (Mudd, Fornetti & Pivarnik, 2007). Mudd and associates reported similar total BMD between gymnasts (n = 8) and softball athletes (n = 14); total BMD for those athletes was 1.173 ± 0.036 and 1.163 ± 0.061 g/cm^2, respectively.

Energy and Nutrient Intakes of Female Athletes

Overall estimated energy intake was lower than that which has been reported in other studies assessing collegiate female athlete nutritional behaviors (Hinton, et al., 2004). Mean energy intake of approximately 2150 kcal/d was reported by Hinton and co-workers (Hinton et al., 2004) as compared to the mean energy intake estimate of approximately 1450 kcal/d observed in this study. Differences could be related to utilization of different nutrition analysis methods as well as individual tendencies to underreport when completing 3-day food records. Hinton and colleagues estimated energy and nutrient intakes using the Youth Assessment Questionnaire (Hinton et al., 2004), whereas individual 3-day food records were used for this study.

Also, for the female athletes studied by Hinton and colleagues (2004), average vitamin D and calcium intakes (6.7 ± 4.3 µg/d and 1085 ± 480 mg/d, respectively) were

greater than that which was observed in this study (1.6 + 1.1 μg/d and 643 + 160 mg/d, respectively). Low vitamin D and calcium intakes were also observed in a study assessing nutritional behaviors of collegiate female soccer athletes using a 3-day food record (Clark, Reed, Crouse, & Armstrong, 2003). Nonetheless, calcium intakes in this study were well below the recommended intake for females older than 19 years (1000 mg/day); and, vitamin D intakes for all participants were considerably below the recommended intake value (5 μg/d).

Exploratory Results

Ethnicity, multi-vitamin/mineral supplements and oral contraceptive use have been associated with serum 25(OH)D concentrations (Adami et al., 2005; Harris & Dawson-Hughes, 1998). However, in this study, there was no observed significant correlation between 25(OH)D concentrations and ethnicity due to the small population of different ethnic backgrounds other than Caucasian. Oral contraceptive and multi-vitamin/mineral supplement use was not significantly associated with serum 25(OH)D concentrations; however, in multiple regression analysis, supplement use was a statistically significant predictor of serum 25(OH)D concentrations. There was not a statistically significant correlation observed between serum 25(OH)D concentrations and BMD among the athletes in this study.

Summary

In summary, indoor and outdoor athletes living at 33.23°N latitude did not have different mean serum 25(OH)D concentrations as measured in the fall. It would have been ideal to measure the difference in the spring after finishing competitive seasons.

However, due to lack of volunteers for this study, the difference between indoor and outdoor athletes was not measured in the spring; so, there is still a need for determining if location of training potentially impacts vitamin D status particularly in the spring season.

Even though there was a visual trend observed between body fat percentage and serum 25(OH)D concentrations, there was not a significant correlation between the two. However, there was a small sample size for this study, and most of the athletes were fairly lean. Further investigation among a greater sample size including athletes and nonathletes would be warranted to determine if body fat percentage in this age group affects vitamin D status.

Indication of tanning bed use seemed to prevent the predicted decrease in serum 25(OH)D concentrations in indoor athletes from fall to spring. As mentioned, tanning bed use has been observed to significantly increase ($p < .001$) 25(OH)D concentrations (Devgun, Johnson & Paterson, 1982).

Another idea for research identified by the investigators included comparisons of serum 25(OH)D concentrations in male and female collegiate athletes, especially the differences between indoor and outdoor sports. The use of tanning beds was found to be an influential factor in the indoor athletes in this study, so it would be warranted to determine if indoor male athletes use tanning beds as well and if that would influence vitamin D status.

Indication of supplement use was a statistically significant predictor of fall serum 25(OH)D concentrations in this study. Since the vitamin D content of supplements was

not analyzed in this study, supplement use and regimens would also be an area of further research in athletic populations.

This study was not without limitations. The main limiting factor in this study was purposive method of sampling. Another limitation of this study was that direct sunlight exposure was not quantitatively determined, but instead personal sunlight exposure behavior was determined via a self-reported questionnaire. Lastly, this investigation did not measure parathyroid hormone concentrations or any other markers of bone metabolism.

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APPENDIX A

Consent Form

TEXAS WOMAN'S UNIVERSITY

CONSENT TO PARTICIPATE IN RESEARCH

Title of Study:	Wellness and Sport Evaluation Program
Investigator's Name:	Nancy DiMarco Institute for Women's Health
Investigator's Phone:	(940) 898-2792 or (940) 898-2799 (clinic)
Investigator's Email:	ndimarco@mail.twu.edu

Explanation and Purpose of the Research

The purpose of the Wellness and Sport Evaluation Program is to provide health and fitness assessments for the Denton community. The program is designed to offer both health-related information and sport performance-related information. The data collected in the Wellness and Sport Evaluation Program may be used for research purposes. The purpose of the research is to investigate the physiological and psychological etiology of cardiovascular and metabolic disease and obesity, as well as, athletic performance potential.

You may perform one or more physiologic assessments, which include a submaximal exercise test to estimate cardiorespiratory fitness test, electrical activity of the heart (ECG), resting metabolic rate, anaerobic power (sport testing only), muscular fitness and flexibility, lung function, body composition, bone density, blood glucose and lipid panel. In addition, you may complete a nutrition and psychological assessment.

Research Procedures

- **Time Involvement and Testing Requirements:** The total time for all testing to be completed will be approximately 3-4 hours. All test procedures will be conducted according to the criteria established by the American College of Sports Medicine for medical examination, physician supervision, and exercise testing and prescription. Participants must be at least 18 years old to participate in this program.

- **Cardiorespiratory Fitness:** During this test, the treadmill speed and grade (slope) or cycle ergometer workload will be gradually increased until you reach 85% of your age-predicted maximal heart rate (HRmax = 220 – age in years). You will also be expected to wear a noseclip and breathe through a mouthpiece for the duration of this test, which will likely last up to 20 minutes. The nose clip and mouthpiece may be uncomfortable. The purpose of this test will be to estimate your maximal aerobic capacity, or VO_2max, which measures the ability of your body to use oxygen and is a way to assess your level

of fitness. Heart responses will be monitored through an electrocardiograph device (ECG). The primary investigator or a technician will perform the prepping and placement of ECG electrodes and lead wires. You will be asked if you prefer a technician of the same sex to perform these procedures. You will be given a pre-exercise screening involving a resting ECG and blood pressure measurement. Blood pressure and ECG will be monitored during and following the treadmill test. Standard cool-down procedures will be encouraged after exercise.

- **Resting Metabolic Rate:** During this test, you will be asked to rest in the supine (on your back) position for approximately 30 to 45 minutes in a quiet room. The test will be performed after a 10 to 12 hr overnight fast. You will be expected to wear a respiratory mask that you will breath through for the duration of the test. The mask may be uncomfortable. The purpose of this test is to estimate resting caloric expenditure over a 24 hr period. This test will take approximately one hour to complete.

- **Anaerobic Power Test:** This test will be completed as part of the sport performance evaluation only. The purpose of this test is to determine your peak and mean anaerobic power, which measures the ability of your body to perform work over a short period of time. You will perform a 30-second power test on a cycle ergometer with the resistance set/based on a percentage of your body weight. You will be asked to pedal as fast as possible while the repetitions are counted and the data are assessed by a computer software package. You will be given a pre-exercise screening involving a resting ECG and blood pressure. Blood pressure will be monitored following the exercise test. After the exercise test, to cool-down you will be asked to continue pedaling at 50 to 60 rpm for 5 to 6 min. In addition, you will be given a carbohydrate-electrolyte drink following the test to avoid any hypoglycemic response post-test.

- **Muscular Fitness and Flexibility:** The purpose of the following tests is to determine your level of muscular fitness. You will complete a handgrip test, push-up test and curl-up (crunch) test. The push-up test involves the male participants to begin in the standard "down" position (hands pointing forward and under the shoulder, back straight, head-up, using the toes as the pivotal point) and female participants in the modified "knee-push-up" position (legs together, lower leg in contact with mat, back straight, hands shoulder width apart, head up, using knees as a pivotal point). You will perform push-ups consecutively without rest until failure. The curl-up test involves you to begin in the supine position (on back) on a mat with knees at 90 degrees. Curl-ups will be performed without pausing at a pace of 25 per minute until failure or 25 repetitions. Right and left grip strength will be evaluated by maximally squeezing a hand dynamometer. Three trials will be completed for each hand. This battery of tests can be completed in approximately 10 minutes.

- **Pulmonary Function:** The purpose of this test is to evaluate how well your lungs work using spirometry. The tests determine how much air your lungs can hold, and how quickly you can move air in and out of your lungs. Spirometry measures how much and how quickly you can move air out of your lungs. The test involves you breathing into a mouthpiece attached to a recording device (spirometer) following a deep breathe. Three to five trials may be completed. This test can be completed in approximately 5 minutes.

- **Body Composition and Anthropometric Measurements:** Body composition will be determined by skinfold thickness measurements. The purpose of this test will be to determine sum of seven skinfold thicknesses and determination of percent body fat. Skinfold thickness will be measured at seven anatomical sites on the right side of the body with a skinfold caliper. These seven anatomical sites are the biceps, triceps, subscapular (back), supraspinale, abdomen, calf, and thigh. Body weight will be determined using an electronic scale and height with a stadiometer. Waist and hip circumference will be measured with a tape measure placed directly on the skin while you stand balanced on both feet, with the feet touching each other and both arms hanging freely. For the measurement of waist circumference, the tape measure will be placed immediately above the iliac crest (hip bone). For the measurement of hip circumference, the tape measure will be placed at the maximal circumference of the hip. Following normal expiration, the measurements will be taken and repeated three times at the waist and hip. You will be asked if you prefer a technician of the same sex to perform these procedures. These assessments will be completed in approximately 20 minutes.

- **DEXA Procedures:** Body composition will also be determined by using a FDA-approved dual energy x-ray absorptiometer, the Lunar DEXA. You will be asked to lie face up, fully clothed, on a padded table for the total body scan. You will be asked to remove all jewelry or metal before the scan is performed. A registered technician will perform all x-ray scan measurements. This test can be completed in approximately 20 minutes.

- **Blood Spot Test:** A blood spot test will be performed to determine individual Vitamin D status. This test consists of a finger prick and will be taken in the morning before eating. You will be asked to wash your hands with soap and warm water and then dry them with a clean towel. While you are sitting, a lancet will be used to nick either the ring or middle finger. Approximately twelve blood drops will be collected on a blood spot card. If needed, blood from the palm will be gently massaged to the tip of the finger. A bandage will then be applied to the finger upon completion of the test. This test can be completed in approximately 10 minutes.

- **Blood collection:** Blood will be drawn from a forearm vein by a phlebotomist (a person trained to draw blood) after a 10 to 12 hour overnight fast. The purpose of the blood draw is to determine your blood glucose and lipid (triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol) concentrations. Three tubes of blood will be drawn totaling 10 mL of blood. Prior to each blood draw, you will be asked if you are allergic to latex. If you inform the phlebotomist that you are allergic to latex, non-latex gloves and tourniquet will be used. Each blood draw will be performed using a standard venipuncture technique. This test can be completed in approximately 10 minutes.

- **Dietary Records:** You will be asked to record all foods, beverages, and dietary supplements that you consume for a 24-hour period. The purpose of the dietary records will be to assess your average total caloric consumption, and carbohydrate, protein and fat intake, as well as vitamin, mineral, and fluid consumption. This test can be completed in your own time and turned in prior to your clinic appointment.

- **Questionnaires:** You will be asked to complete a health history questionnaire, demographic questionnaire, and a customer satisfaction survey. These questionnaires will take approximately 20 minutes to complete. The purpose of these questionnaires is shown below.

  Health History Questionnaire: The purpose of the health history questionnaire is to identify individuals that will need physician clearance prior to participation. Additionally, the medical history questionnaire will be used to optimize safety during exercise testing and participation in the program.

  Demographic Questionnaire: The purpose of the demographic questionnaire is to characterize who is participating in the program.

  Sun Exposure Questionnaire: The purpose of the sun exposure questionnaire is to describe the behavior of personal sunlight exposure.

  Physical Symptoms Questionnaire: The purpose of the physical symptom questionnaire is to provide ZRT laboratory appropriate information for a comprehensive assessment of vitamin D concentrations.

  Customer Satisfaction Survey: The purpose of the customer satisfaction survey is to how participants responded to the program. The responses from this survey will be used to make necessary changes to improve the program.

In addition, you will be asked to fill out two widely accepted, self-reported psychometric evaluations. The purpose of these tests is to determine your health self-efficacy and readiness to change. The tests can be completed in approximately 15 minutes. The battery of tests include:

Self-Rated Abilities of Health Practices (SRAHP) - developed by Becker, Stuifbergen, Soo Oh, and Hall (1993), consists of a 28-item 5-point Likert-type scale that measures four dimensions of health self-efficacy. These four dimensions comprise the subscales of exercise, psychological well-being, nutrition, and health practices.

Stages of Change – developed by Prochaska (1992) is a questionnaire designed to asses where on the continuum of change an individual is, from pre-contemplation to maintenance. This questionnaire will help both you and the professional you work with to determine behavior change strategies. It consist of a 7-item, 5-point Likert-type scale.

Potential Risks

Loss of Confidentiality: There exists the possibility of the loss of confidentiality as a potential risk of participation in this study. Confidentiality will be protected to the extent that is allowed by law. To minimize this risk, all data will be kept in a locked file cabinet in 011 Human Development Building, Texas Woman's University. Data collection forms will be coded with a numerical system rather than your name. A single identification form will be used to link names with numerical code. This will be the only way to connect data with a name. This form will be kept in a separate file than all other data in the principal investigator's office.

There exists the possibility of certain changes and risks occurring during maximal exercise tests, exercise sessions and blood collection. They include muscle fatigue or soreness, abnormal blood pressure, nausea and fainting, irregular, fast or slow heart rhythm, heart attack, stroke, death, hypoglycemia (low blood sugar), bruising and infection, falling on the treadmill, latex allergies, and mouthpiece discomfort during exercise.

Muscular Fatigue or Soreness: You will be monitored for signs of muscular fatigue. If you do not appear capable of maintaining adequate coordination, testing will be terminated. To minimize the risk of muscle soreness, you will be asked to stretch prior to and following all exercise sessions. If muscle soreness does occur, participants will be instructed to perform additional stretching and take an over the counter nonsteroidal anti-inflammatory medication.

Abnormal Blood Pressure: According to the American College of Sports Medicine, guidelines for exercise testing and blood pressure will be monitored during the submaximal treadmill test and exercise sessions. If blood pressure exceeds 260/115 mm Hg, systolic blood pressure falls more than 20 mm Hg, or signs of lightheadedness develop, the test will be terminated.

Nausea and Fainting: If you feel nauseous or faint, you will be encouraged to perform cool-down exercises. You will also be asked to lie on your back on the floor with your feet elevated to alleviate these symptoms.

Irregular, Fast or Slow Heart Rhythm: Cardiac responses will be monitored by an ECG device. If you have prior knowledge of an irregular, fast, or slow heart rhythm, you will not be admitted into the study. If you report these problems during the exercise test, or they are noted on the ECG, the test will be terminated and you will not be allowed to continue. If these problems persist, emergency medical assistance will be called immediately.

Skin Irritation Due to ECG Preparation: The surface of the chest will be prepared by roughing the skin in 10 specified areas with a piece of gauze and alcohol to optimize adhesion and conduction of the electrodes. The preparation for the ECG may cause slight discomfort in the areas of electrode placement, which may sting slightly, similar to a rug burn, but the discomfort should subside within two days.

Heart Attack, Stroke and Death: Serious risks like heart attack, stroke, and death are possible, however these risks are extremely rare in healthy adults. All technicians will be certified in CPR and AED (automated external defibrillators). If you are at high risk of these serious cardiovascular events, you will not be admitted into the study. Signs and symptoms for high risk include, but are not limited to ECG abnormalities; pain or discomfort in the chest, neck, jaw, arms, or other areas that may result from decreased blood flow; shortness of breath at rest or with mild exertion; dizziness or loss of consciousness; dyspnea (abnormally uncomfortable awareness of breathing); ankle edema, palpitations or tachycardia (forceful or rapid beating of heart); known heart murmur, or unusual fatigue or shortness of breath with usual activities. If it is suspected that any of these serious risks are occurring, emergency medical help will be called immediately. Every effort will be made to minimize these risks inherent to exercise through preliminary examination and observations during testing by trained personnel according to the American College of Sports Medicine guidelines for testing procedures. In addition, an AED is available in the Exercise and Sport Nutrition Clinic (HDB 011E).

Hypoglycemia: Hypoglycemia (low blood sugar) may result from prolonged fasting. If you have signs of hypoglycemia during a testing session, the test will be terminated. Signs of hypoglycemia include tremors, cold sweat, low body temperature, headache, confusion,

hallucinations, bizarre behavior, convulsions, and coma. You will be given a glass of orange juice and monitored until the signs of hypoglycemia subside.

Bruising: The risk of bruising resulting from blood draws is minimal due to this procedure being performed by trained personnel. Universal precautions will be used during all blood draw procedures. To minimize bruising, pressure will be applied to the site for approximately five minutes after each blood draw.

Infection: The risk of infection resulting from blood draws is minimal due to the procedures being performed by trained personnel. Also, the risk of infection from blood spot tests is minimal. Universal precautions will be used during all blood draw and blood spot test procedures. Sites for blood draws and blood spot tests will be cleaned with alcohol immediately prior to each venipuncture and finger prick. Each new needle that is opened will be disposed of in biohazard boxes immediately after use. Additionally, oral infection resulting from breathing through a mouthpiece is minimal. All mouthpieces and nose clips will be sterilized prior to use and handled with gloves.

Falling on the Treadmill: It is possible that you may fall on the treadmill. You will be given an orientation to the treadmill prior to exercise. The treadmill is equipped with handrails on three sides. These handrails should be used if you lose your balance. A technician who will assist you if you fall will be located behind the treadmill while you are exercising at high intensities. Another technician will be located close to the treadmill controls should the treadmill need to be stopped.

Latex Allergy: The phlebotomist will wear latex gloves during all blood draws. Prior to each blood draw, you will be asked if you are allergic to latex. If you inform the phlebotomist that you are allergic to latex, a non-latex type of glove and tourniquet will be used.

Mouthpiece Discomfort: During procedures that require the collection of CO_2 and O_2, you will be expected to wear a nose clip and to breathe through a mouthpiece. The nose clip and mouthpiece may be uncomfortable. To minimize discomfort, a headgear will be used to support a mouthpiece sized for your mouth.

Emotional Discomfort in Sharing Personal Information: During the collection of personal information you may feel emotionally uneasy. To minimize emotional discomfort with the collection of this information, you will have the option to share this information with a research team member of the same gender.

Radiation Exposure: During body composition assessment with the x-ray scan, there will be a small amount of radiation exposure to each participant. The total amount of radiation that each participant will receive during the study is 0.26 mrem (whole body) using the Lunar DEXA scan. The radiation exposure for each participant will be approximately the same amount received during a 2-hour airplane flight and less than normal background radiation an individual is exposed to in one day.

Embarrassment: During the ECG electrode placement, and measurement of body composition, height and weight, you may feel embarrassed. To minimize embarrassment, you will have the option to have measurements taken by a research team member of the same sex. Additionally, to ensure privacy, ECG preparations, body composition, height and weight measurements will be conducted in a small private room located in the Exercise and Sport Nutrition Clinic (HDB 011).

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Participation and Benefits

Participation in this study is voluntary and as a participant, you have the right to withdraw from the study at any time without penalty. Should you desire to withdraw from the study at any point, you are entitled to be informed of any data collected from you that has been analyzed at any time point.

All data with any personal identifiers will be destroyed on 1/1/2014. All identifiable data on paper will be shredded and data stored on the primary investigator's computer and the IWH server will be deleted from the hard drive.

You will benefit from participation in this study by learning your current risk for heart disease, diabetes and obesity and assist with the development of an appropriate nutrition and exercise program. You will be informed of your results following tabulation.

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you should ask the researchers; the phone numbers of the Institute for Women's Health and Clinic are at the top of this form. If you have any questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at (940) 898-3378 or via email at IRB@twu.edu.

_____ _____

Signature of Participant Date

If you would like to receive a summary of the results of this investigation, please provide an address to which this summary should be sent:

_____

_____

_____

APPENDIX B

IRB Letter of Approval

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378 Fax 940-898-3416
email: IRB@twu.edu

October 15, 2010

Bethany Bloom
Institute for Women's Health

Dear Ms. Bloom:

Re: Filing of consent forms for Bethany Bloom's Master's Thesis, *"Vitamin D Status in Collegiate Female Athletes: Relationship to Indoor vs Outdoor Sports"*

The TWU Institutional Review Board (IRB) has received the materials necessary to complete your portion of Dr. Nancy DiMarco's study, *"Wellness and Sport Evaluation Program"*. As applicable, signatures of your participants have been placed on file.

Sincerely,

Kathy DeOrnellas, PhD

Dr. Kathy DeOrnellas, Chair
Institutional Review Board – Denton

cc: Dr. Nancy DiMarco, Institute for Women's Health

APPENDIX C

Demographic Questionnaire

IWH Wellness & Sport Evaluation Program
Demographic Questionnaire

Name: _____ _____ Date: ____/____/20____
(Last) (First) (Middle)

Phone: (____) _____ Email: _____

Address: _____ City _____ ST _____ Zip _____

How would you prefer we contact you? ❑ Phone ❑ Email ❑ Mail

Date of Birth: ____ / ____ / _____ Gender: ❑ Male ❑ Female

Ethnicity: (Check all that apply)
❑ African American
❑ American Indian
❑ Asian/Pacific Islander
❑ Caucasian (non-Hispanic)
❑ Hispanic
❑ Scandinavian
❑ Other: _____

What is the highest level of education you have attained? (Please mark only one)
❑ Less than a high school diploma
❑ High school graduate
❑ Some college or technical training
❑ Associate's degree or equivalent
❑ Bachelor's degree
❑ Graduate degree

What is your present work situation? (Check all that apply)
❑ Employed full-time
❑ Employed part-time
❑ Semi-retired
❑ Fully-retired
❑ Self-employed
❑ Unemployed
❑ Homemaker
❑ Student
❑ On disability
❑ Other: _____

Are or were you a student at Texas Woman's University?
❑ Yes, I am a current student
❑ Yes, but I am not currently enrolled in any courses
❑ Yes, I am a TWU alumnus
❑ No

Are you a current employee of Texas Woman's University?
❑ Yes ❑ No

Please provide the name of a close relative or friend that we may contact, if necessary.

Name: _____ Phone: (____) _____

Please provide the name of your physician.

Name: _____ Phone: (____) _____

For office use:
Is physician clearance required?
❑ Yes ❑ No
Proof of physician clearance provided:
❑ Yes ❑ No
Approved by: _____

Proof of TWU employee/student status provided:
❑ Yes ❑ No
Approved by: _____

APPENDIX D

Health History Questionnaire

IWH Wellness & Sport Evaluation Program
Health Questionnaire

Name _____ Date ___/___/20_____
(Last) (First) (Middle)

		Circle One	Notes
(1)	Have you been under the care of a physician during the past 2 years?	Yes No	
(2)	Are you allergic to penicillin, any drugs, medicine, or latex?	Yes No	
(3)	Have you ever had excessive bleeding which required special treatment?	Yes No	
(4)	Women: Is there a chance you might be pregnant?	Yes No	
(5)	Women: Are you taking any birth control medication?	Yes No	
(6)	Have you had adverse reaction to local anesthetics?	Yes No	

(7) Do you use recreational drugs?_____ If yes, what?_____

(8) Do you use tobacco? _____ If so, what form? _____

(9) Date of last medical exam _____

(10) Circle Yes to any of the following which you have had or have at present. Circle No to those that you have not had.

Yes	No	High Blood Pressure	Yes	No	Unexplained Shortness of Breath
Yes	No	High Blood Cholesterol	Yes	No	Chronic Cough or Bronchitis
Yes	No	Chest Pain or Pressure (Angina)	Yes	No	Tuberculosis (TB)
Yes	No	Heart Disease or Attack	Yes	No	Emphysema
Yes	No	Heart Pacemaker	Yes	No	Asthma
Yes	No	Heart Failure	Yes	No	Hay Fever
Yes	No	Heart Surgery	Yes	No	Allergies or Hives
Yes	No	Fainting or Lightheadedness	Yes	No	Sinus Trouble
Yes	No	Artificial Heart Valve	Yes	No	Cancer
Yes	No	Congenital Heart Lesions	Yes	No	Leukemia or Lymphoma
Yes	No	Mitral Valve Prolapse	Yes	No	Radiation or Chemotherapy
Yes	No	Stroke	Yes	No	Anemia
Yes	No	Transient Ishemic Attack	Yes	No	Bruise Easily
Yes	No	Lupus	Yes	No	Bleeding Disorders
Yes	No	Rheumatic Fever	Yes	No	Sickle Cell Disease
Yes	No	Scarlet Fever	Yes	No	Alcoholism
Yes	No	Chronic Fatigue	Yes	No	Drug Addiction
Yes	No	Artificial Joints	Yes	No	Blood Transfusion
Yes	No	Kidney Dialysis	Yes	No	Liver Disease
Yes	No	Kidney Disease	Yes	No	Yellow Jaundice
Yes	No	Eating Disorder	Yes	No	Hepatitis
Yes	No	Rheumatoid Arthritis	Yes	No	AIDS / HIV Infection
Yes	No	Arthritis	Yes	No	Cold Sores / Fever Blisters
Yes	No	Chronic Head, Neck, or Back Pain	Yes	No	Psychiatric Treatment
Yes	No	Diabetes Requiring Insulin	Yes	No	Depression / Bipolar
Yes	No	Diabetes Not Requiring Insulin	Yes	No	Nervousness / Anxiety
Yes	No	Hypoglycemia	Yes	No	Dizzy Spells
Yes	No	Hyperthyroidism (High)	Yes	No	Epilepsy or Seizures
Yes	No	Hypothyroidism (Low)	Yes	No	Condition Requiring Cortisone Medicine
Yes	No	Ulcers	Yes	No	Glaucoma
Yes	No	Pulmonary Disease	Yes	No	Spine or Hip Fractures

List all prescription medications that you are currently taking.

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Please list all non-prescription medication or vitamins or nutritional supplements you are currently taking.

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

List all surgical procedures that you have had in the past.

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

List all hospitalizations of 24 hours or more for any reason.

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Other Health Information

Please use this space to record any other personal health information that was not listed above.

_____

_____

"I Attest To The Fact That The Information Given Above Is Correct And I Consent To Receive Clinical Services."

_____

(Parent or Guardian must sign for patient under age 18.)

This section for office use only:

Comments:

_____

_____

_____

_____

_____

APPENDIX E

Sun Exposure Questionnaire

1. In what state did you live this past summer?

   _____

2. Did you spend time outside in the sun? Yes No

   a. If yes…

      i. When were you typically outside?

         _____

      ii. On average, how much time did you spend outside?

         _____

      iii. What percent of the time were you in shaded areas?

         _____

      iv. What percent of the time was spent by or on a body of water?

         _____

      v. How would you describe the typical weather conditions?

         _____

      vi. Approximately, what percent of your skin was exposed to the sun? _____

      vii. What percent of the time did you wear a brimmed hat?

         _____

      viii. What percent of the time did you wear sunscreen?

         _____

         1. Did you purposefully NOT wear sunscreen?
            Yes No

         2. What was the Sun Protection Factor (SPF)?
            _____ Don't know

         3. Did it have both UVA and UVB protection?

            Yes No Don't know

3. Do you use tanning beds? Yes No

    a. If yes…

        i. How often?

        _____

        ii. How much time for each use?

        _____

APPENDIX F

Blood Spot Testing Protocol

Pre-test guidelines:

All study participants were encouraged to be well-hydrated before administration of the test.

Testing procedures for the technician:

1. All contents from the kit were made easily available. Latex gloves were placed on hands to prepare for the test.
2. The blood spot card was labeled appropriately with the date, time and identification number of the study participant.
3. The blood spot card was opened with the flap folded back to expose the collection circles. The card was placed on a table that was below waist level of the participant so that gravity will assist in the flow of blood.
4. The ring or middle finger of the non-dominant hand of the participant was selected to be the finger from which the blood was drawn.
5. The chosen finger was cleaned with an alcohol prep pad, and proper disposal methods were used to rid the pad in a biohazard container.
6. The lancet was prepared by removing the cap and positioned it so that the red end of the lancet was off-centered of the chosen fingertip.
7. The lancet was pressed down firmly until it clicked.
8. A sterile gauze pad was used to wipe away the first drop of blood.
9. The hand was gently massaged from the palm to the fingertip to produce blood drops if needed.

10. As blood drops formed, the finger was placed over the first collection circle. Care was used in order to avoid the finger from touching the filter paper or smearing blood drops on the card. One drop of blood was applied per circle.
11. Step 10 was repeated for the remaining 11 circles. The hand was massaged again if blood flow stopped.
12. A bandage was placed on the finger when finished.
13. The blood drop card was allowed to dry for at least 30 minutes before closing the flap.
14. All paperwork and blood spot card was placed into its original kit, and each kit mailed to ZRT lab for analysis.

Testing procedures for the study participant:

1. The study participant was directed to wash hands thoroughly with soap and warm, running water.
2. A clean towel was provided for the participant to dry hands.
3. To encourage blood flow, the participant was asked to rub hands together before the finger prick.