VITAMIN D STATUS AND BODY COMPOSITION OF FEMALE COLLEGIATE VOLLEYBALL PLAYERS

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ABSTRACT

VITAMIN D STATUS AND BODY COMPOSITION OF FEMALE COLLEGIATE VOLLEYBALL PLAYERS

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This pilot cross-sectional study was conducted to assess vitamin D status and body composition of collegiate indoor female volleyball players at Rice University in Houston, TX. Serum 25-hydroxyvitamin D [25(OH)D] concentrations and body composition was determined using air displacement plethysmography. Participants completed a 24-hour food record, vitamin D food frequency questionnaire, and sunlight habit questionnaire to assess dietary vitamin D intake and sunlight exposure habits. This study also piloted the use of La-Roche-Posay MyUV Patch to estimate ultraviolet light A (UVA) and ultraviolet light B (UVB) exposure. No significant relationship was found between vitamin D status, percent body fat, dietary vitamin D intake, or UVA and UVB exposure. However, the study did find that the majority of participants (64%) were vitamin D deficient or insufficient based on serum 25(OH)D. This study contributes to the knowledge of vitamin D status of indoor college athletes and can serve as a template for future studies.

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LIST OF ABBREVIATIONS

1,25-dihydroxyvitamin D	$1,25(OH)_2D$
25-hydroxyvitamin D	25(OH)D
AMP	Adenosine Monophosphate
BMD	Bone Mineral Density
BMI	Body Mass Index
DEXA	Dual X-ray Absorptiometry
IU	International Unit
NDSA	Nutrition Data System for Research
PTH	Parathyroid Hormone
RDA	Recommended Daily Allowance
TNA-∝	Tumor Necrosis Factor-alpha
UL	Upper Levels
UV	Ultraviolet Radiation
UVA	Ultraviolet A Radiation
UVB	Ultraviolet B Radiation
VDR	Vitamin D Receptor

CHAPTER I

INTRODUCTION

Vitamin D is a fat soluble vitamin that functions as a secosteroid hormone when hydroxylated to its active form (Moran, McClung, Kohen, & Lieberman, 2013). Active vitamin D functions in many physiological processes, including calcium absorption, parathyroid hormone (PTH) homeostasis, bone health, immune heath, and skeletal muscle function, and has been shown to have cardiovascular benefits (DeLuca, 2004). Vitamin D can be obtained through dietary sources or in the skin when 7-dehydrocholesterol is exposed to ultraviolet light B (UVB), which is converted to previtamin D_3 that isomerizes to vitamin D_3, cholecalciferol (DeLuca, 2004). Dietary sources of vitamin D include ergocalciferol, vitamin D_2, and cholecalciferol, vitamin D_3 (DeLuca, 2004). It is nearly impossible to meet needs through diet alone due to the limited number of foods containing high concentrations of ergocalciferol (Shuler, Wingate, Moore, & Giangara, 2012). Foods containing significant concentrations of vitamin D include salmon and fortified products such as milk, fruit juices, breads, and cereals (Moran et al., 2013).

The general consensus among researchers defines vitamin D deficiency as serum 25(OH)D concentrations < 20 ng/mL, insufficiency 20-29 ng/mL, and sufficient status ≥ 30 ng/mL (Moran et al., 2013). Serum 25(OH)D is the preferred marker of vitamin D status due to the stability of 25(OH)D, its longer half-life and higher concentration present in the serum (Lips, 2007). Several factors influence vitamin D status, including

high melanin levels in the skin, high body fat, frequent sunscreen use, extensive clothing coverage, reduced kidney function, and old age, all of which are inversely related to 25(OH)D levels. Geographical location also greatly affects how much vitamin D is synthesized. As UVB strength decreases the further away from the sun, the amount of vitamin D_3 (cholecalciferol) that can be converted from 7-dehydrocholesterol in the dermis is lessened (Moran et al., 2013). Those living above 37° North or below 37° South of the equator have reduced serum 25(OH)D concentrations compared to those living below 37° North and above 37° South due to decreased cutaneous synthesis. Levels are further decreased in the fall and winter months in the northern and southern hemispheres as there is less sunlight (Von Hurst & Beck, 2014).

Vitamin D and its role in athletic performance is a topic of interest. Studies have shown vitamin D influences bone density, cardiovascular fitness, endurance, muscle strength, recovery, and inflammation (Houston et al., 2007). Athletic performance has been theorized to be enhanced by vitamin D supplementation in vitamin D insufficient athletes compared to vitamin D sufficient athletes (Moran et al., 2013). Some researchers recommend athletes maintain serum 25(OH)D concentrations near 40 ng/mL (Farrokhyar et al., 2015). Adequate serum 25(OH)D concentration should be further explored in the indoor athlete population.

Few studies have assessed eating behaviors, vitamin D status, and body composition of female indoor volleyball players. Body composition of athletes is not frequently assessed in sports where leanness and weight are not closely monitored such as volleyball and softball (Beals, 2002). Currently, limited cross-sectional research exists

on the relationship of vitamin D status to body composition of female indoor athletes. Studies have shown an inverse relationship between adiposity and 25-hydroxyvitamin D [25(OH)D] levels with multiple proposed theories as to the occurrence (McCarty, 2003).

A growing body of research suggests vitamin D deficiency and insufficiency is more common than previously thought. Reports suggest the general population of the United States includes over 75% of Caucasians and 90% of African-Americans who are vitamin D deficient (Shuler et al., 2012). Vitamin D deficiency in the athlete population is also a topic of interest to researchers because studies have shown a high prevalence of vitamin D deficiency in athletes. A 2008 study found that 83% of elite gymnasts were vitamin D deficient while a similar study reported 94% of indoor basketball players were vitamin D deficient (Lovell, 2008; Schuler et al., 2012).

Purpose of the Study

The purpose of this pilot cross-sectional study was to evaluate vitamin D status and body composition of indoor female collegiate volleyball players. This study examined the association between serum 25(OH)D concentrations, UVB/UVA exposure, vitamin D dietary intake, and body composition of indoor collegiate female athletes.

Hypotheses

The null hypotheses of this study are:

1. Serum 25(OH)D concentrations of female volleyball players will not be inversely related to percent body fat and body mass index (BMI).
2. Serum 25(OH)D concentrations of female volleyball players will not fall in the deficient or insufficient ranges.

3. Serum 25(OH)D concentrations of female volleyball players will not be associated with UV exposure assessed by UVB questionnaire and MyUV Patch.
4. Serum 25(OH)D concentrations will not be associated with dietary intake of vitamin D.

CHAPTER II

REVIEW OF THE LITERATURE

Vitamin D

Vitamin D is a fat soluble vitamin that functions as a secosteroid hormone when hydroxylated to its active form (Moran et al., 2013). Vitamin D is best obtained through skin exposure to Ultraviolet B radiation (UVB), via the conversion of 7-dehydrocholesterol in the dermis to vitamin D_3, cholecalciferol (DeLuca, 2004). Vitamin D can also be obtained through diet via the form of cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) when yeast and fungi produce vitamin D_2 from previtamin D_2 UVB exposure (Jäpelt & Jakobsen, 2013). It is nearly impossible to meet needs through diet alone due to the limited number of plant foods containing high concentrations of vitamin D_2, ergocalciferol (Shuler et al., 2012). Foods containing significant concentrations of vitamin D, which includes both D_2 and D_3, include salmon and fortified products such as milk, fruit juices, breads, and cereals (Moran et al., 2013).

Once vitamin D is ingested from food and supplements as vitamin D_2, or formed via UVB exposure as vitamin D_3, it undergoes hepatic metabolism to form 25-hydroxyvitamin D [25(OH)D]. The accepted marker of vitamin D status is serum 25(OH)D concentrations (Moran et al., 2013). Further hydroxylation of 25(OH)D in renal tissue and extra-renal tissue produces 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], the active form of vitamin D (Angeline, Gee, Shindle, Warren, & Rodeo 2013). Serum 25(OH)D has a significantly higher concentration circulating in the plasma compared to

$1,25(OH)_2D$, with the concentration of 25(OH)D approximately 1000 times greater than $1,25(OH)_2D$ (Lips, 2007). Serum 25(OH)D also has a longer half-life of 25 days compared to the 7-hour half-life of $1,25(OH)_2D$ (Lips, 2007). Measuring $1,25(OH)_2D$ is not an accurate indicator of vitamin D status as $1,25(OH)_2D$ represents the amount produced by the kidney, not the overall storage of vitamin D in the form of 25(OH)D (Lips, 2007). The stability of 25(OH)D and its longer half-life and higher concentration present in the serum makes 25(OH)D the preferred marker of vitamin D status (Lips, 2007).

Vitamin D Receptors (VDRs) are responsible for vitamin D function in its target tissue (DeLuca, 2004). Active vitamin D_3 [$1,25(OH_2D$] must bind to its VDR receptor to activate the vitamin D hormone's gene expression on the target tissue (DeLuca, 2004). VDRs have been identified in many cells and tissues including enterocytes, osteoblasts, distal renal tubules, cells of the parathyroid gland, skin keratinocytes, promyelocytes, lymphocytes, colon cells, pituitary gland cells, ovarian cells, skeletal muscle tissue, cardiac, and liver tissue (DeLuca, 2004).

Activation of the vitamin D_3 hormone is vital in the regulation of calcium and bone mineralization. Vitamin D functions to maintain calcium homeostasis and increases serum calcium concentrations when plasma calcium concentrations are low, resulting in bone demineralization (Moran et al., 2013). When serum concentrations of vitamin D are low, plasma calcium concentrations are decreased resulting in increased PTH secretion from the parathyroid gland. This signals the vitamin D hormone to increases serum calcium through stimulating intestinal calcium and phosphate absorption, releases

calcium from bone and stimulates renal reabsorption of calcium to increase serum calcium concentrations (DeLuca, 2004). Active vitamin D functions in many other physiologic processes besides calcium and PTH homeostasis, including muscle synthesis and recovery, inflammation, bone health, cardiovascular benefits, and neuronal function (Shuler et al., 2012).

Factors Affecting Vitamin D Status

Several factors influence vitamin D status. High melanin levels in the skin, high body fat, frequent sunscreen use, extensive clothing coverage, reduced kidney function, and old age are all inversely related to 25(OH)D levels (Cannell, Hollis, Sorenson, Taft, & Anderson, 2009). Melanin, the dark brown or black skin pigment present in African Americans, is responsible for decreased cutaneous vitamin D production as melanin blocks 99% of UVB radiation responsible for the conversion of 7-dehydrocholesterol to vitamin D_3 in the dermis (Armas, 2007). Multiple hypotheses exist as to the relationship between high body fat and lower concentrations of vitamin D. One hypothesis suggests high body fat may sequester vitamin D in adipose tissue resulting in low serum concentrations of vitamin D (Looker, 2007). Frequent sunscreen use and extensive clothing coverage also block the skin from UVB exposure, thus reducing sun induced vitamin D biosynthesis (Cannell et al., 2009). Reduced kidney function related to old age or kidney disease affects the metabolism of vitamin D and hinders the production of $1,25(OH)_2D$ from 25(OH)D (Cannell et al., 2009).

Geographical location also greatly affects how much vitamin D your skin is able to synthesize. As UVB strength decreases with increasing distance from the sun, the

amount of vitamin D_3 (cholecalciferol) that can be converted from 7-dehydrocholesterol in the dermis to vitamin D_3 (cholecalciferol) is lessened (Moran et al., 2013). Those living above 37° North or below 37° South of the equator have reduced serum 25(OH)D concentrations compared to those living close to the equator due to decreased cutaneous synthesis. Levels are further decreased in the fall and winter months in the northern and southern hemispheres as there is less sunlight (Von Hurst & Beck, 2014).

Vitamin D Adequacy

Reports suggest over 75% of Caucasians and 90% of African-Americans of the United States general population are vitamin D deficient (Shuler et al., 2012). Vitamin D adequacy is the current focus of research and debate. The general consensus among researcher's classification of vitamin D nutritional status is based on serum 25(OH)D concentrations with deficient < 20 ng/mL, insufficient 20-29 ng/mL, and sufficient ≥ 30 ng/mL (Moran et al., 2013). The optimal sufficient range is based on research when the 25(OH)D concentration reaches a threshold inhibiting elevation of PTH and achieves the greatest intestinal calcium absorption and optimizes the bone health of older Caucasian people (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006)

The basis of determining adequacy and deficiency levels or ranges from the serum PTH of older Caucasian people leaves room for speculation if this is the best method to determine adequate levels or ranges of vitamin D sufficiency and deficiency (Bischoff-Ferrari et al., 2006). Vitamin D level variation can be attributed to numerous factors including age, diet, body composition, renal function, time of day, location, physical activity, and melanin production in people from a variety of racial backgrounds

(Bischoff-Ferrari et al., 2006). Bischoff-Ferrari et al. suggest desirable levels of serum 25(OH)D based on optimal bone mineral density (BMD) may be a more accurate evaluation than based on PTH threshold levels for younger adults and individuals from other non-Caucasian ethnic backgrounds (Bischoff-Ferrari et al., 2006).

Bischoff-Ferrari et al. conducted a meta-analysis assessing serum 25(OH)D adequate levels based on factors including BMD, lower extremity function, rate of falls, oral health, and colorectal cancer in people of varying age and racial backgrounds to determine serum 25(OH)D concentration most advantageous for overall health (Bischoff-Ferrari et al., 2006). The researchers found 36-40 ng/mL 25(OH)D to be most beneficial in enhancing and providing optimal BMD, lower extremity function, oral health, decreased rate of falls and reduced colorectal cancer incidence (Bischoff-Ferrari et al., 2006).

Vitamin D Adequate Intake

Currently, the Recommended Daily Allowance (RDA) for vitamin D is 600 International Units (IU) daily for those between 1-70 years of age. Nevertheless, the RDA for vitamin D remains controversial. Some researchers recommend a daily intake as high as 3000-5000 IU of vitamin D (Farrokhyar et al., 2015); however, the current safe upper limit (UL) for vitamin D set by the Institute of Medicine is 4000 IU/day for individuals 9 years of age or older (IOM, 2011). Serum 25(OH)D concentrations of 35-40 ng/mL in younger and older adults can be achieved with an intake of 700 to 1000 IU vitamin D per day, suggesting a higher RDA is needed to obtain adequate vitamin D concentrations in the population (Bischoff-Ferrari et al., 2006). An intake of 700-1000 IU

Vitamin D per day remains below the upper level (UL) of 10,000 IU of vitamin D per day. Vitamin D levels greater than 10,000 IU/day are potentially toxic with the potential to induce damage to the kidneys and other tissues (Ross, 2011). Little information exists on vitamin D toxicity as studies have not assessed vitamin D_2 or vitamin D_3 intoxication in humans (Jones, 2008),

Vitamin D and UVB Exposure

Obtaining vitamin D without supplementation is primarily achieved by UVB exposure. The amount of UVB exposure required to increase concentrations of serum vitamin D and its metabolites was studied by Adams et al. (Adams, Clemens, Parrish, & Holick, 1982). The researchers monitored levels of serum 25(OH)D and $1,25(OH)_2D$ for three weeks in subjects with adequate and inadequate levels of vitamin D (Adams et al., 1982). Researchers found that after a single dose of ultraviolet radiation (UV) exposure of both adequate and deficient subjects increased concentrations of serum vitamin D depending on the amount of UV dosage exposure. Concentrations of $1,25(OH)_2D$ peaked within 48 hours of irradiation and began to decrease after the 48 hours. Concentrations of 25(OH)D increased steadily and peaked at day 7 through 14, then decreased. A net increase of 0.03 mg $1,25(OH)_2D$ per square meter of body surface area in normal subjects was produced after UV exposure. The vitamin D-deficient subjects experienced 3-4 times the increase in serum $1,25(OH)_2D$ with the same dose of UV. In contrast, concentrations of serum $1,25(OH)_2D$ in subjects with adequate vitamin D did not change (Adams et al., 1982). Findings demonstrate a positive relationship between serum vitamin D concentrations and UV exposure, with vitamin D deficient subjects showing a more

dramatic increase in serum 1,25$(OH)_2$D after UVR exposure compared to those with adequate levels of vitamin D (Adams et al., 1982).

Vitamin D and Adiposity

Studies have shown an inverse relationship between adiposity and 25(OH)D levels (McCarty & Thomas, 2003). A possible theory suggests that decreased physical activity, associated with increased body weight and adiposity, leads to decreased sun exposure due to clothing choices to hide excess body fat resulting in reduced cutaneous vitamin D exposure and synthesis (Dekker et al., 2005). However, Dekker et al. found serum 25(OH)D concentrations to be negatively associated with the degree of adiposity and not necessarily body weight or BMI (Dekker et al., 2005). In this study, researchers examined older men and women in the Netherlands and assessed serum 25(OH)D, PTH, BMI, waist circumference, waist to hip ratio, sum of skin folds, and percent body fat measured by dual energy x-ray absorptiometry (DEXA). In addition, the researchers found stronger correlations between 25(OH)D concentrations and percent body fat than BMI or weight (Dekker et al., 2005). The researchers reported 25(OH)D concentrations were negatively associated with percent body fat and positively associated with (PTH) levels regardless of age, sex, season, study region, or smoking habits (Dekker et al., 2005).

An excess of adipose tissue has been proposed to modify the vitamin D endocrine system and inhibit the synthesis of active vitamin D (Bell, Epstein, Greene, Shary, Oexmann, & Shaw, 1985; Salehpour et al., 2012). Bell et al. suggested obesity and excess body fat can lead to hyperparathyroidism and modifies the vitamin D- endocrine

system as a result of secondary hyperparathyroidism (Bell et al., 1985). In a metabolic in-patient study conducted by Bell et al., 12 obese and 14 non-obese male and female participants between the ages of 20-35 years of age were provided a consistent diet which included 400 mg of calcium and 900 mg phosphorus. Lab measures included fasting blood samples of serum calcium, ionized calcium, phosphorus, magnesium, creatinine, bone galactosidase protein, 25(OH)D, $1,25(OH)_2D$, and immunoreactive PTH (Bell et al., 1985). Twenty-four-hour urine samples were also collected to assess calcium, phosphorus, sodium, potassium, magnesium, creatinine, and cyclic adenosine monophosphate (AMP) (Bell et al., 1985). Researchers found obese participants to have a lower concentration of serum 25(OH)D compared to non-obese participants (Bell et al., 1985) The obese participants showed increases in mean serum immunoreactive PTH, serum $1,25(OH)_2D$, urinary cyclic AMP, and decreased urinary calcium. Researchers proposed these results were related to a disruption in the vitamin-D endocrine system as a result of secondary parathyroidism mediated by obesity and enhanced tubular resorption of calcium and increased renal production of $1,25(OH)_2D$ (Bell et al.,1985).

Salehpour et al. (2012) examined the effect of vitamin D3 supplementation on body fat in a double-blind placebo controlled study with 77 overweight and obese women (BMI 29.8 ± 4.1 kg/m^2). The subjects were randomly divided into a placebo group consuming 25 μg lactose per day and experimental group consuming 25 μg (1000 IU) cholecalciferol per day for twelve weeks. After the twelve weeks, researchers found serum 25(OH)D concentrations higher in the experimental group (38.2± 32.7 nmol/L) compared to the placebo group (4.8 ± 14.8 nmol/L) ($p<0.001$) (Salehpour et al., 2012).

While there was no change in body weight or waist circumference in both groups, there was a significant decrease in body fat in the cholecalciferol supplement trial group (-2.7± 2.1 kg) compared to the placebo group (-0.47± 2.1 kg;$p<0.001$) (Salehpour et al., 2012). A 7% reduction in fat mass was associated with a 103% increase in serum 25(OH)D concentrations of participants (Salehpour et al., 2012). The results of this study suggest vitamin D concentrations are correlated to body fat mass and agree with current evidence that higher levels of serum 25(OH)D are positively associated with lean body mass which may inhibit adipocyte development (Salehpour et al., 2012). Alternative theories suggest excess adipose tissue decreases the concentration of serum 25(OH)D due to the sequestration and storage of the fat soluble vitamin in adipose tissue (Moran et al., 2013).

Vitamin D and Bone Density

Vitamin D functions to aid in calcium absorption to maintain bone density (Cannell et al., 2009). An inverse relationship between serum 25(OH)D and PTH levels related to vitamin D deficiency is hypothesized to correlate with poor bone health by increasing osteoclastic activity, thereby, increasing the risk for stress fractures (Angeline et al., 2013). A 2010 study conducted by Halliday et al. assessed the vitamin D status in collegiate athletes to determine if concentrations of 25(OH)D were linked to bone density, as well as sun exposure and adiposity. 25(OH)D concentrations of 41 National Collegiate Athletic Association Division I male and female athletes at the University of Wyoming were measured. Both indoor and outdoor athletes were included in the study and analyzed together. The study measured the 25(OH)D concentrations over the course of the academic year. Dual energy x-ray absorptiometry (DEXA) was used to measure

bone density during the spring at the end of the study. The researchers found that vitamin D status was not correlated with total body bone density ($p \geq 0.05$) (Halliday et al., 2010). The frequency of injury and stress fractures was also not related to 25(OH)D status but was negatively correlated with total body bone density ($p = 0.02$) (Halliday et al., 2010).

Other studies, however, have found positive correlations between vitamin D and bone density. Bone density is in part affected by calcium absorption needed to build and maintain bone (Cannell et al., 2009). Greater calcium absorption occurs when serum 25(OH)D concentrations ≥ 30 ng/mL (Larson-Meyer & Willis, 2010). The fractional calcium absorption in those with serum 25(OH)D ≥ 30 ng/mL equaled 30% compared to 10-15% fractional calcium absorption when 25(OH)D was less than 30 ng/mL (Larson-Meyer & Willis, 2010).

A 2006 prospective study involving 800 young healthy Finnish male military recruits studied the occurrence of stress fractures in relation to vitamin D status over a 90-day period (Ruohola et al., 2006). Baseline blood was drawn and serum 25(OH)D concentration was determined using an enzyme immunoassay. Height, weight, BMI, 12-minute running test, and muscle strength were also measured. At the end of the 90 day period, 22 of 756 recruits completing the study were diagnosed with a stress fracture. Participants with stress fractures had a significantly lower median serum 25(OH)D level (25.7 ng/mL) compared to the overall group median (30.5 ng/mL). Risk for stress fractures in the lower extremities was significantly higher when 25(OH)D was less than 30.5 ng/mL ($p=0.017$) (Ruohola et al., 2006). Those with stress fractures also had poorer scores in the 12-minute running test ($p=0.007$) and muscle strength test ($p=0.025$)

(Ruohola et al., 2006). These findings suggest that a vitamin D deficiency is correlated with an increased incidence of stress fractures in young, healthy Finnish male military recruits (Ruohola et al., 2006).

Vitamin D and Muscle

A 2010 study by Shubert and DeLuca assessed whether muscle weakness in rats was primarily a result of vitamin D-deficiency or rather a result of hypophosphatemia (Shubert & DeLuca, 2010). The researchers measured muscle force, peak contraction, time-to-half contraction, time-to-peek contraction, and time-to-half recovery in rats bread to have deficient levels of 25(OH)D. The results show that vitamin D-deficient rats displaying hypophosphatemia had a significant reduction in muscle force. To determine if the muscle force was a result of hypophosphatemia or vitamin D deficiency, the serum phosphorus levels were corrected which resulted in a return of muscle force. With ongoing maintenance of serum phosphorus and serum calcium in the vitamin D-deficient rats, there was no reduction in muscle force (Shubert & DeLuca, 2010). Results of this study suggest the decrease in muscle strength was a result of hypophosphatemia and not vitamin D deficiency (Shubert & DeLuca, 2010).

Vitamin D has been associated with Type II muscle fiber size and strength. Type II muscle fibers are fast-twitch muscle fibers which are important for athletic performance (Shuler et al., 2012). Type II muscle fibers atrophy in vitamin D deficient subjects. Supplementation with vitamin D produces hypertrophic effects on the Type II muscle fibers (Angeline et al., 2013). In a vitamin D deficient state, athletic performance is impaired due to Type II fiber atrophy with the rate of muscle contraction decreasing,

muscle relaxation slowing, and musculoskeletal pain increasing (Bartoszewska, Kamboj, & Patel, 2010). Muscle myopathy in the elderly population is also extremely common and has been linked to vitamin D deficiency and improved through supplementation with vitamin D_3 (Forney et al., 2014). Vitamin D_3 supplementation of the elderly is proposed to increase the synthesis of type II muscle fibers and inhibit myopathy (Forney et al., 2014).

Vitamin D Receptors in Muscle

Vitamin D receptors have been isolated in smooth and cardiac muscle, skeletal muscle, liver, lungs, and other tissues and are classified as a steroid hormone receptor (Moran et al., 2013; Wang & DeLuca, 2011). Active vitamin D, $1,25(OH)_2D$, binds to the VDR which in turn, acts as a transcription factor and promotes gene expression, mRNA transcription, protein synthesis, intestinal calcium uptake, and muscle fiber differentiation (Haussler et al., 2013; Moran et al., 2013). A 2001 study by Bischoff et al. identified the presence of VDRs in human skeletal muscle tissue *in situ*. The researchers used immunohistochemistry and determined that VDR is present in the human skeletal muscle tissue, however, it was noted that the VDR 9A7 antibody, also reacts with proteins not related to VDR, raising questions regarding the study findings (Wang & DeLuca, 2011).

A 2011 study by Wang and DeLuca sought to determine if VDR is present in skeletal, cardiac, and smooth muscle tissue. The researchers selected highly specific VDR antibodies. Using immunoblotting and *in situ* immunohistochemical staining, the researchers found VDRs to be present in rat duodenal tissue and rat gut epithelial cells but not in mature rat skeletal muscle, human skeletal muscle, cardiac muscle, and smooth

muscle. The researchers concluded that a secondary explanation of why VDRs were undetected in skeletal, cardiac, and smooth muscle was due to the low concentrations of VDR transcripts (Wang & DeLuca, 2011). Other studies have shown vitamin D supplementation corrects and improves muscle weakness at times of deficiency. More research is needed to determine if this improvement in muscle weakness is directly or indirectly related to vitamin D status (Wang & DeLuca, 2011).

Vitamin D and Athletic Performance

Athletic performance has been theorized to be enhanced by vitamin D supplementation in athletes with vitamin D insufficiency compared to athletes with sufficient vitamin D levels. A 2009 study found increased serum 25(OH)D concentrations were associated with increased muscle power, force, velocity, and jump height in girls ages 12-14 years (Ward et al., 2009). Improvement of athletic performance was achieved when serum 25(OH)D levels reached 40-50 ng/mL, with no significant improvement above this threshold level (Moran et al., 2013). Another study reported athletes may require higher levels of serum 25(OH)D of at least 40 ng/mL due to increased activity and use of vitamin D in metabolic pathways utilized during athletic performance (Farrokhyar et al., 2015).

A 2014 cross-sectional study conducted by Forney et al. assessed the relationship between serum 25(OH)D levels and resting metabolic rate, body composition, maximal cardiorespiratory fitness (VO2 max), anaerobic power and strength on healthy male ($n = 20$) and female ($n = 20$), college-aged students attending a university in the southern United States (Forney et al., 2014). Three of the participants were Hispanic and 37 were

Caucasian and all were engaged in moderate to vigorous physical activities 3 days per week or more while maintaining a consistent body weight for 3 months. Participants did not take vitamin D supplements over 400 IU per day (Forney et al., 2014). Data collection occurred during the months of July-September.

Results of the ergometer study found 9 women and 11 men considered to be vitamin D deficient with serum 25(OH)D concentrations below 35 ng/mL (Forney et. al, 2014). All subjects consumed less than the recommended amount of 15 μg (600 IU) vitamin D per day according to dietary intake records (Forney et al., 2014). Dietary intake, sun exposure, and resting metabolic rate did not significantly correlate with serum 25(OH)D levels (Forney et al., 2014). Researchers did notice a significant negative trend between serum 25(OH)D levels and BMI in both male and female groups. There was also no significant correlation between 25(OH)D concentration and anaerobic power or jump height between insufficient and sufficient 25(OH)D concentration study groups. (Forney et al., 2014). However, researchers found VO_2 max had a significant, positive correlation with serum 25(OH)D levels in men, but not in women. Males with higher levels of serum vitamin D (≥ 35 ng/mL) had a 20% higher VO_2 max compared to males with lower serum vitamin D levels (< 35 ng/mL) (Forney et al., 2014). This study suggests VO_2 max and cardiorespiratory fitness are increased and enhanced by higher levels of vitamin D (≥ 35 ng/mL) in males (Forney et al., 2014). More studies are warranted assessing VO_2 max and cardiorespiratory fitness in women.

Vitamin D is also believed to have anti-inflammatory effects. Vitamin D has been shown to elicit anti-inflammatory effects aiding in recovery and athletic performance

(Halliday et al., 2011). Vitamin D status and expression of tumor necrosis factor-alpha (TNF-α) and interleukin 6 are inversely related due to vitamin D's role in down-regulating these inflammatory cytokines (Halliday et al., 2011). A study involving long-distance runners found (TNF-α) was significantly elevated in vitamin D deficient runners, classified as having serum 25(OH)D concentrations < 32 ng/mL, supporting the hypothesis that vitamin D deficiency results in an increased risk for overuse inflammation injuries (Willis, Broughton, & Larson-Meyer, 2009).

Balance is another area of athletic performance investigated in relation to serum 25(OH)D status. VDRs are found in the cerebellum and spinal cord, requiring vitamin D for optimal neurological performance. Studies suggest that impaired balance and stability are affected much earlier than muscle impairments related to vitamin D deficiency (Moran et al., 2013).

Rate of Deficiency in Athletes

Vitamin D status and deficiency has been a topic of interest in the athlete population (Shuler et al., 2012). A 2008 study found that 83% of elite female gymnasts were vitamin D deficient (Lovell, 2008). A similar study reported that 94% of indoor basketball players were vitamin D deficient (Shuler et al., 2012). A 2011 longitudinal study by Halliday et al. assessed vitamin D status of indoor and outdoor athletes during the school year and found indoor athletes had significantly lower 25(OH)D serum levels compared to outdoor athletes during the fall months (Halliday et al., 2011).

Vitamin D and Female Volleyball Players

Few studies have assessed vitamin D status, body composition, and eating behaviors of female volleyball players. Body composition of athletes is not regularly assessed in sports where leanness and weight are not closely monitored such as volleyball and softball (Beals, 2002). Body composition and somatotype characteristics for volleyball players vary across different positions. For example, liberos, the back-court position player, often are not as tall as opposites and centers, who have to have long legs and arms to jump and attack the ball (Malousaris et al., 2008). Limited cross-sectional research of vitamin D status related to body composition of young female indoor athletes exists and more research is necessary to identify vitamin D adequacy and its relation to athletic performance and body composition in young female indoor athletes.

The purpose of this pilot cross-sectional study was to evaluate vitamin D status and body composition of indoor female collegiate volleyball players. This study examined the association between vitamin D status, UVB/UVA exposure, dietary intake, and body composition in indoor collegiate female athletes.

The null hypotheses of this study are:

1. Serum 25(OH)D concentrations of female volleyball players will not be inversely related to percent body fat and BMI.
2. Serum 25(OH)D concentrations of female volleyball players will not fall in the deficient or insufficient ranges
3. Serum 25(OH)D concentrations of female volleyball players will not be associated with UV exposure assessed by UVB questionnaire and MyUV Patch.

4. Serum 25(OH)D concentrations will not be associated with dietary intake of vitamin D.

CHAPTER III

METHOD

This pilot cross-sectional study was conducted to assess the relationship between vitamin D status of female collegiate indoor volleyball players and body composition, sun exposure, and vitamin D dietary intake. Data collection occurred during the month of August, 2016 at Texas Woman's University and Rice University in Houston, TX. The study was approved by the Institutional Review Boards of Texas Woman's University and Rice University (Appendix A).

Participants

Female participants were recruited from the women's volleyball team at Rice University in Houston, TX. A total of 16 participants provided consent to participate in the study during the study visit at Rice University in August, 2016. Participants were instructed to not take vitamin D supplements during the study. A vitamin D food frequency questionnaire (Appendix E) captured typical usage of multi-vitamin supplement usage. Inclusion criteria consisted of all ethnic and racial groups.

Procedure

Recruitment of Participants

Participants were recruited by email to the Rice University women's indoor volleyball team coach and trainer. Each female volleyball player who had been cleared to participate in her sport and play in games was invited to participate.

Questionnaires

On the initial study visit, researchers reviewed the study and consent forms with participants and gave the participants opportunity to ask and answer any questions. After explanation of the study and consent form, participants signed the consent forms (Appendix B). Participants completed questionnaires including a demographic questionnaire (Appendix C) to obtain baseline information including age, ethnicity, and dietary supplement usage. Participants were instructed on how to complete a 24-hour dietary recall (Appendix D) and a vitamin D food frequency questionnaire (Appendix E) to estimate dietary intake of vitamin D. Food models and portion size examples were available and explained to the participants to help the participants estimate portion size as they were completing the dietary questionnaires. Dietary recalls were analyzed utilizing the University of Minnesota 2016 Nutrition Data System for Research (NDSR) program (Nutrition Data System for Research, Version 2016) NDSR is a comprehensive nutrient calculation software program utilizing a database of 160 nutrients that includes more than 18,000 foods. A Sunlight Questionnaire (Appendix F) was completed to estimate UVB exposure and sun habits.

LaRoche-Posay MyUV Patch

LaRoche-Posay, a division of L'Oreal cosmetic and skin care manufacturing company located in San Francisco, CA, provided a newly developed MyUV Patch to estimate sun exposure. On the initial day of the study visit, participants downloaded the LaRoche-Posay MyUV Patch application on their personal smart phones and applied the LaRoche-Posay MyUV Patch to the outer portion of their hand or wrist. Participants were

instructed to complete the skin type demographic questions prompted by the MyUV Patch app to determine the participant's skin type. The five skin Types ranging from 1= pale to 5= dark. Participants were instructed to synchronize their patch with the app on their smart phone.

Photos of the participant's applied MyUV Patch and her study ID code sticker were applied next to the patch. Photos were taken under direct sunlight and emailed, along with the participant's phone number, to L'Oreal Headquarters in San Francisco, CA to be paired with the synchronized data from mobile phones. The participants were instructed to scan their patch with their mobile app under direct sunlight to record the first sun exposure reading. Participants were encouraged to repeat this process throughout the day whenever they were prompted by the reminders on the My UV Patch app for the next two days. The MyUV Patch app also sent participants reminders to re-scan their patch on their smart phone regardless of their current status of sun exposure or non-exposure.

Time stamps were recorded by LaRoche-Posay researchers for each scan completed by the participant for the two days the MyUV Patch was applied. Corrected calculations were performed by L'Oreal researchers in San Francisco, CA. using time stamps and UVA/UVB index for that day were completed for participants when a patch had fallen off to estimate total UVA/UVB sun exposure. Time stamps were compared to UVA/UVB exposure of the participant's MyUV Patch scans and used in the final analysis of total UVA/UVB MyUV Patch sunlight exposure. UVA and UVB exposure were estimated and calculated using an estimate of 60% of the participant's body surface

area being exposed to sunlight to account for clothing coverage. The 60% estimate was based on the mean body surface area exposed to sunlight based on the results of the completed sunlight exposure questionnaire. Students typically wore tee shirts and shorts during sunlight hours.

Serum 25(OH)D Blood Collection and Analysis

After consent forms and questionnaires were completed, subjects were assigned blood draw appointment times at the Texas Woman's University Human Nutrition Laboratory in Houston, TX. A trained phlebotomist collected one tube of blood (< 4 mL) in vacutainers. Samples were centrifuged and plasma separated into a plastic storage vial, labeled, and stored in a locked freezer at -80°F until analysis. Serum 25(OH)D concentration (from vitamin D_2 and vitamin D_3) was measured using enzyme-linked immunosorbent assay (ELISA) (Alpco Diagnostics, Salem, NH, USA) with a BioTek microplate reader (BioTek Instruments, Winooski, VT, USA). The standard curve of sample concentrations coefficient of determination was R^2 =0.89188. The average coefficient of variation (CV) of samples equaled 8.08.

Bod Pod Procedure

Participants (n=15) completed body composition analysis testing using a Bod Pod (Cosmed, Bod Pod GS), that utilizes air displacement plethysmography technology. The Bod Pod had completed a successful calibration before testing. Bod Pod participants were instructed to wear spandex shorts, non-padded sports bra, and a spandex swim cap to cover all hair. A trained researcher performed each Bod Pod test, instructing the participant to sit still and breathe normally during the two, 45-second measurements.

Results were printed immediately and provided to the researcher and participant after each test. The researchers reviewed findings and answered any questions with the participants.

Statistics

Descriptive frequency statistics were conducted to analyze demographic data, skin type data, serum 25(OH)D status, dietary intake of vitamin D, UVA/UVB exposure, as well as the sunlight habits questionnaire. Spearman's Correlations and Kruskal-Wallis Tests were used to assess the relationship between UVB Exposure, UVA Exposure, serum 25(OH)D, percent body fat, and dietary vitamin D intake. Serum vitamin D levels were categorized with vitamin D status groups according to defined parameters and were coded as 0= deficient, 1= insufficient, or 2=sufficient with a level of significance set at $p < 0.10$. Statistical Package for the Social Sciences (SPSS Version 24) software was used to perform all statistical analysis.

CHAPTER IV

RESULTS

Demographics

The study population consisted of 16 female collegiate volleyball players. The mean age was 19 years old ranging between 17-23 years of age. Ethnic distribution of participants (Table 1) included 11 Caucasian, 3 African American, and 2 Hispanic participants. The mean height of the 16 participants was 180.34 cm and mean weight of participants was 76.65 kg. The mean BMI of the 16 participants at the lab visit was 23.47 ± 2.21 kg/m^2 (Table 1).

Biochemical Data

Percent Body Fat

Percent body fat was determined by the Bod Pod procedure during the initial lab visit and was completed by 15 participants (Table 1). One participant declined to participate. Mean percent total body fat of the 15 participants was 26.11 ± 4.47 %. Spearman correlations indicated serum 25(OH)D was not significantly correlated to percent body fat ($p=0.469$). The Kruskal-Wallis test also indicated there was no significant relationship of percent body fat to serum 25(OH)D ($p = .855$) with the level of significance set at $p < 0.10$ (Table 7).

Percent Lean Body Mass

Percent lean body mass was determined by the Bod Pod procedure during the lab visit and was completed by 15 participants (Table 1). One participant declined to participate. The mean percent lean body mass of participants was 73.87 ± 4.46 %.

Serum 25(OH)D Status

Fourteen participants were able to successfully provide blood for the analysis of serum 25(OH)D concentrations. Two participants were excluded from the analysis of 25(OH)D due to one participant's inability to provide an adequate quantity of serum and because the other participant declined to participate in the blood draw. The mean serum 25(OH)D level of participants was 32.09 ± 28.7 ng/mL. Of the 14 participants, 6 were vitamin D deficient, 3 were vitamin D insufficient, and 5 participants were vitamin D sufficient (Table 2).

Dietary Vitamin D Intake

24-Hour Recall Vitamin D Dietary Intake

Results of the 24-Hour Dietary Intake of vitamin D completed during the initial study visit are presented in Table 2. The estimated mean vitamin D dietary intake was 6.40 ± 3.9 μg/d. Only one participant met the recommended dietary allowance (RDA) for vitamin D intake of 15μ/d with an intake of 17.3 μg/d.

Vitamin D Food Frequency

Results of the vitamin D food frequency questionnaire completed during the initial study visit are presented in Table 2. Percentage of participants consuming one or

more serving of the following vitamin D containing foods per week were: (63%) fortified cereal, (75%) fortified milk, (25%) fortified orange juice, (88%) eggs, (19%) Swiss cheese, (31%) fortified soy/almond milk, (6%) cod-liver oil, (12.5%) tofu, (63%) yogurt, (19%) mushrooms.

Skin Type and Sunlight Exposure

LaRoche-Posay My UV Patch skin type's ranged from 1= lightest skin tone, most likely for skin to burn to 5=darkest skin tone, least likely to burn (Table 3). Estimated UVB exposure by the LaRoche-Posay My UV Patch was .000159 ± .000198 MJ (n=16). Estimated UVA exposure by the LaRoche-Posay My UV Patch was .00867± .00747 MJ (n=15). One participant's UVA results was excluded from the analysis due to being an outlier.

Sunlight Habits Questionnaire

The sunlight habit questionnaire (Appendix F) was designed to provide the researchers a better understanding of time spent outdoors as well as sun protection and clothing coverage used. A summary of the results can be found in Table 4 and complete results can be found in Table 5. The questionnaire was completed by 16 participants. Results showed a majority of the participants (88%) spent their time outdoors with 50-70% of skin exposed and that 50% of participants used sunscreen during a school day including makeup with UVB protection.

Spearman Correlations and Kruskal-Wallis Test

Dietary vitamin D intake was not significantly correlated to serum 25(OH)D status ($p = .288$) or Kruskal-Wallis test ($p= .431$) (Table 7). Spearman Correlations (Table 6) did reveal that UVA exposure was significantly related to dietary vitamin D intake ($p= 0.041$) although no plausible explanation can be given. Level of significance was set at $p < 0.10$. UVA exposure was not significantly related to serum 25(OH)D according to Spearman's Correlations ($p=.455$) and Kruskal-Wallis Test ($p=.324$). UVB exposure was also not significantly related to serum 25(OH)D according to Spearman's Correlations ($p=.681$) and Kruskal-Wallis Test ($p=.810$).

Table 1

Demographics and Characteristics of Female Volleyball Players

Characteristics	n	
Age (yr)	16	19.81 ± 1.60
Height (cm)	16	180.34 ± 7.65
Weight (kg)	16	76.65 ± 10.45
BMI (kg/m^2)	16	23.47 ± 2.21
Ethnicity Percent - African American - Caucasian - Hispanic	16	 19% 69% 13%
Body Fat (%)	15	26.11 ± 4.47
Lean Body Mass (%)	15	73.87 ± 4.46
[1] Data Presented as Mean ± Standard Deviation or Percentage		

Table 2

Serum 25-hydroxyvitamin D Status and Dietary Intake

	n	Mean ± SD
Serum 25(OH)D ng/mL[1]	14	32.1 ± 28.7
Vitamin D Status[2]		**Percentage**
% Deficient	6	43%
% Insufficient	3	21%
% Sufficient	5	36%
		Mean ± SD
Dietary Intake (μg/d)	16	6.4 ± 3.9
Vitamin D Food Frequency[3]		**Percentage**
Fortified Cereal	10	63%
Fortified Milk	12	75%
Fortified Orange Juice	4	25%
Eggs	14	88%
Swiss Cheese	3	19%
Fortified soy/almond milk	5	31%
Cod-liver oil	1	6%
Tofu	2	12.5%
Yogurt	10	63%
Mushrooms (raw)	3	19%

[1] Serum 25(OH)D measured using enzyme-linked immunosorbent assay (ELISA) (Alpco Diagnostics, Salem, NH, USA) with a BioTek microplate reader (BioTek Instruments, Winooski, VT, USA).
[2] Serum 25(OH)D Status defined as: deficient (<19 ng/mL), insufficient (20-29 ng/mL) and sufficient (≥ 30 ng/mL)
[3] Dietary vitamin D frequency of players eating ≥ 1 serving per week.

Table 3

Skin Type and Sunlight Exposure

Skin Type[1]	n	%	Mean ± SD
1 2 3 4 5	0 1 5 9 1	0 6 31 56 6	- - - - -
Total Sunlight Exposure			
UVA Exposure (MJ) UVB Exposure (MJ)	15 16		.00867 ± .00747 .000159 ± .000198
[1] Skin Type based on My UV Patch App ranges from 1 (burns easily, never tans), 2 (usually burns, then tans), 3 (may burn, tans well), 4 (rarely burns, tans well), 5 (very rarely burns, tans well, brown-very dark skin)			

Table 4

Sunlight Habits of Female Volleyball Players Summary[1,2]

	n	%
Seek sunshine "sometimes"	11	69%
Seldom avoid the sun	7	44%
Never use tanning facilities	11	69%
Wear makeup with UVB protection	8	50%
Wear sunscreen during a school day/work day	8	50%
Spend time outdoors during the hours of 10am-3pm	9	56%
50-70% of skin exposed to sunlight during the school day	14	88%
50-70% of skin exposed to sunlight during recreational activities	9	56%
[1] n= 16 [2] Data obtained from the Sunlight Habits Questionnaire (Appendix F)		

Table 5

Sunlight Habits of Female Volleyball Players

	n	%
How often do you seek sunshine?[1]	16	
- Never	1	6.3
- Seldom	2	12.5
- Sometimes	10	62.5
- Often	13	81.3
How often do you avoid the sun?	16	
- Never	4	25
- Seldom	7	43.7
- Sometimes	3	18.7
- Often	1	6.3
- Always	1	6.3
How often do you wear a hat when it is sunny?	16	
- Never	2	12.5
- Seldom	11	68.8
- Sometimes	3	18.7
How often do you cover your arms and legs when it is sunny?	16	
- Never	9	56.2
- Seldom	6	37.5
- Sometimes	1	6.3
How often do you use tanning facilities?	16	
- Never	11	68.8
- Seldom	2	12.5
- Sometimes	3	18.8
How long do you tan per session?	16	
- 0 minutes	11	68.8
- 6-10 minutes	3	18.7
- 11-15 minutes	2	12.5
Do you wear anything while tanning?	16	
- Tanning Accelerators	1	6.3
- UV Protection	2	12.5
- None	13	81.2

Do you wear makeup that contains UVB protection?	16	
- Yes	9	56.2
- No	7	43.8
What Sunscreen Protection Factor is in your Makeup? [2]	14	
- No SPF	7	43.8
- 10 SPF	1	6.3
- 15 SPF	4	25
- 20 SPF	1	6.3
- 30 SPF	1	6.3
Do you wear sunscreen in general?	16	
- Never	2	12.5
- Seldom	8	50
- Sometimes	5	31.3
- Always	1	6.2
What SPF do you wear in general?	16	
- No SPF	3	18.7
- 15 SPF	3	18.7
- 30 SPF	5	31.3
- 50 SPF	4	25
- 70 SPF	1	6.3
How often do you wear sunscreen on a school day including makeup?	16	
- Never	8	50
- Seldom	5	31.2
- Sometimes	1	6.3
- Often	2	12.5
What SPF do you use during a school day? [2]	14	
- No SPF	8	50
- 10 SPF	1	6.3
- 15 SPF	3	18.7
- 20 SPF	1	6.3
- 50 SPF	1	6.3
How often do you use sunscreen during recreational activities? [2]	15	
- Never	5	31.3
- Seldom	5	31.3
- Sometimes	3	18.7
- Often	2	12.5
What SPF do you use during recreational activities?[2]	14	
- No SPF	4	25
	2	12

- 15 SPF		
- 30 SPF	4	25
- 50 SPF	3	18.8
- 70 SPF	1	6.3
When do you spend time outside during a typical school day?[2]	12	
- Midnight-7:00 a.m.	0	0
- 7:00 a.m.- 10:00 a.m.	3	18.8
- 10:00 a.m. – 3:00 p.m.	8	50
- 3:00 p.m.- 6:00 p.m.	1	6.3
- 6:00 p.m.- midnight	0	0
What percentage of your skin is exposed to sunlight when you are at work/school?[2]	15	
- 30% or less	1	6.3
- 50-70%	14	87.5
How many hours per week do you spend doing outdoor recreational activities?	16	
- 0 Hours	7	43.8
- 1 Hour	2	12.5
- 2 Hours	4	25
- 3 Hours	3	18.7
When do you engage in recreational activities?[1]		
- Early Morning	3	18.7
- Morning	5	31.3
- Midday	5	50
- Late Afternoon	6	37.5
- Late Night	0	0
When is your recreational activity spent outdoors?[2]		
- Midnight- 7:00 a.m.	1	6.3
- 7:00 a.m.- 10:00 a.m.	1	6.3
- 10:00 a.m. – 3:00 p.m.	4	25
- 3:00 p.m.- 6:00 p.m.	3	18.8
- 6:00 p.m.- midnight	1	6.3
How much of your skin is exposed during recreational activities?[2]		
- 0-30%	0	0
- 30-50%	1	6.3
- 50-70%	9	56.3
- 70-90%	1	6.3
- 90-100%	0	0

[1] Total percentage equals over 100% due to participants selecting more than one answer
[2] Total percentage equals less than 100% due to participants leaving some answers blank.

Table 6

Spearman Correlations[1,2]

	UVB Exposure	UVA Exposure	% Body Fat	Serum 25(OH)D	Dietary Vitamin D
Serum 25(OH)D	.681	.455	.469	-	.288
Dietary Vitamin D	.957	.041	.815	.288	-
% Body Fat	.708	.459	-	.469	.815
UVB Exposure	-	0.128	.708	.681	.957
UVA Exposure	.515	-	.835	.455	.161

[1] Percent body fat was controlled as a cofactor using ANCOVA Statistics
[2] Significance level is set at $p < .10$

Table 7

Kruskal-Wallis Test[1,2]

	UVB Exposure	UVA Exposure	Dietary Vitamin D Intake	% Body Fat
X^2	.421	2.252	1.684	.855
p	.810	.324	.431	.652
[1] Grouping variable: Serum 25(OH)D [2] Significance level is set at $p < .10$				

Table 8

Spearman Correlations and Kruskal-Wallis with Serum 25(OH)D

	Spearman Correlations	Kruskal-Wallis
Percent Body Fat	0.469	0.855
Dietary Vitamin D	0.288	0.431
UVA Exposure	0.455	0.324
UVB Exposure	0.681	0.810
Significance level is set at $p < .10$		

CHAPTER V

DISCUSSION

The purpose of this study was to assess the vitamin D status and body composition of women indoor volleyball athletes residing in Houston, Texas. The study also analyzed the relationship of serum 25(OH)D to dietary vitamin D, UVA exposure, UVB exposure. Biochemical data, vitamin D dietary intake, vitamin D food frequency, skin type, and sunlight questionnaire data were also analyzed.

Biochemical Data

BMI was calculated for all 16 participants using their stated height and measured weight. The mean BMI of the 15 participants was 23.47 kg/m^2, which is categorized as within the recommended range for women athletes. The participants BMI ranged from 19.1-27.3 kg/m^2. According to the American College of Sports Medicine, women athletes should aim for a BMI of less than 25 kg/m^2, but ≥ 18.5 kg/m^2 (Kruschitz et al., 2013). It

should also be noted that BMI may not be an accurate predictor of body composition for athletes because an increased muscle mass can skew BMI to upper ranges (Kruschitz et al., 2013).

We measured percent body fat and percent lean body mass using the Bod Pod. Average percent body fat of the 15 participants was 26.11% with a range of 15.8- 32.2%. The average percent lean body mass of participants was 73.87% with a range of 67.8-84.2%. The American College of Sports Medicine recommends women athletes have a body fat percentage between 20 and 33% (Kruschitz et al., 2013). All of the 15 participants who completed the Bod Pod assessment fell within the recommended range of less than 33% body fat. Only one participant had a percent body fat of < 20. In comparison, our study yielded similar results to a study involving 19 female physically active college students with a mean percent body fat of 30.02 ± 1.0, mean BMI of 23.17 ± 0.7 kg/m^2, and mean 25(OH)D concentration of 34.83 ng/mL (Forney et al., 2014). A significant relationship between percent body fat and serum 25(OH)D status was not found (Forney et al., 2014).

The mean serum 25(OH)D level of participants was 32.09 ng/mL with a range of 4.2 – 83.5 ng/mL. Of the 14 participants, 43% had deficient serum 25(OH)D levels of 19 ng/mL or less, 21% had insufficient serum 25(OH)D levels of 20-29 ng/mL, and 36% of participants had sufficient serum 25(OH)D status of 30 ng/mL or greater. The majority of the participants (64%) were vitamin D deficient or insufficient. Three African-American participants had low serum 25(OH)D concentrations ranging from 4.2 ng/mL – 8.9 ng/mL which correlates with the knowledge that increased melanin production by the

skin blocks UV radiation by 99%, limiting the amount of vitamin D_3 that can be produced by the skin (Armas, 2007). These results are similar to a trial conducted in the Netherlands where 58 male and 70 female Dutch indoor and outdoor athletes with a mean age of 22 ± 3 years and mean BMI of 22 ± 2 kg/m^2 (Backx et al., 2016). This Dutch study found 70% of athletes to be deficient (< 20 ng/mL) or insufficient (20-29 ng/mL) serum 25(OH)D concentrations (Backx et al., 2016). Our results also compare similarly to a study conducted in the Southern United States involving 39 male and female physically active college students. Researchers identified 20 participants (51%) with deficient concentrations of serum 25(OH)D (< 35 ng/mL), further identifying the high prevalence of vitamin D deficiency in physically active college students residing in warm climates with plenty sunshine (Forney et al., 2014). The results of our study also revealed the high prevalence of vitamin D deficiency (43%) and inadequacy (21%) in a young, healthy, active population residing in a warm environment with adequate UVB rays throughout the year. Athletes residing in a sub-tropical climate could be expected to have sufficient levels of vitamin D, due to the warm climate with plenty of sunlight. The fact that the athletes in our study spend a majority of their training indoors as an indoor-sport athlete could possibly contribute to lower serum vitamin D levels as a result of inadequate sun exposure.

Dietary Recalls

All 16 participants completed a 24-hour dietary recall and a vitamin D food frequency questionnaire to assess the amount and frequency of dietary vitamin D consumed. The mean vitamin D dietary intake of the participants, as assessed by the 24-

hour dietary recall, was 6.40 μ/d with a range of 2.2-17.3 μg/d. The current RDA for dietary vitamin D is 15μg/d (IOM, 2011). Only one participant met this recommended amount with a vitamin D intake of 17.3 μg/d based on the dietary recall due to consuming salmon. These findings illustrate the inadequacy of vitamin D obtained from food in the average diet of female athletes. Similarly, the Dutch study previously mentioned reported a mean dietary vitamin D intake of 4.2± 2.6 μg/d in male and female athletes and also did not identify a significant relationship between dietary intake of vitamin D to serum 25(OH)D concentrations (Backx et al., 2016). This Dutch study further illustrates how significant intake of dietary vitamin D is hard to achieve in the daily diet even in males and females of a different cultural background compared to the participants in our study (Backx et al., 2016).

To examine further the frequency of consumption of vitamin D containing foods, participants answered a vitamin D food frequency questionnaire. Foods frequently consumed that provided significant vitamin D through dietary intake of female volleyball players in order of most consumed to least consumed included: eggs, fortified milk, yogurt, fortified cereal, fortified soy/almond milk, fortified orange juice, mushrooms, Swiss cheese, tofu, and cod liver oil. It should be noted that participants may have underreported portion size and frequency of vitamin D containing foods. Nutrition epidemiological studies have reported measurement errors by participants completing 24-hour dietary recalls and food frequency questionnaires (George et al., 2012). Furthermore, the 24-hour dietary recall only represents one day of intake thus vitamin D intake could vary from day-to-day.

Sun Exposure

Participants piloted the use of LaRoche-Posay's MyUV Patch to estimate UVA/UVB exposure. Mean two day UVA exposure of participants (n=15) was .00867 MJ, while mean two day UVB exposure of participants (n=16) was .000159 MJ. The sunlight questionnaire showed a majority of the participants (88%) spent their time outdoors with 50-70% of skin exposed and that 50% of participants used sunscreen during a school day including makeup with UVB protection. Many participants (25%) recorded they spend the most time outdoors during a school day and during recreational activities between the hours of 10:00 am-3:00 p.m. However, it is also interesting that seven of the participants stated they spend zero hours outdoors for recreational activities. Because Rice University volleyball players reside most of the year in a warm and sunny environment, they would be expected to have adequate vitamin D status and spend many hours outdoors. However, according to the study findings, although the participants attend university in a southern state, with warm weather and strong sunlight year-round, volleyball players spend very few hours outdoors for recreational activities, limiting their sun exposure. This observation is likely due to the students participating in an indoor sport and spending a large portion of time in class and studying at their university.

Limitations

Several factors of this pilot study may have impacted findings. The sample size (n=16) was small. Two participants were unable to provide blood for 25(OH)D analysis. One student was unable to provide enough blood for analysis and another student declined to participate in the blood draw. One participant also declined to have her body

composition measured by the Bod Pod. In addition, this was a one-time study without a follow-up or other group to compare results due to the nature of the pilot study design. Possible reporting errors of the 24-hour diet recall and vitamin D food frequency questionnaires also may have not reflected actual dietary vitamin D intake.

It should be noted that the participants participated in volleyball team practice two times a day in a hot and humid environment which contributed to, in some instances, the LaRoche-Posay My UV Patch losing adhesiveness and falling off wrists of participants. Four My UV Patches fell off between the first and second day of application. A second patch was provided and data collection was restarted. Three other participants' My UV Patch fell off during the night of the second day before the final scan. Nevertheless, to correct for these instances, results were analyzed by the technical team at L'Oreal and UVA and UVB exposure estimates were adjusted accordingly in the few cases when the My UV Patch had fallen off during the study.

CHAPTER VI

CONCLUSIONS AND IMPLICATIONS FOR FURTHER RESEARCH

Findings of this pilot study provided cross-sectional data on the body composition, vitamin D status, dietary vitamin D intake, sun exposure, and sunlight behaviors of collegiate female volleyball players residing in Houston, TX. The LaRoche-Posay MyUV Patch was also successfully piloted as a new way to estimate UVA and UVB exposure. Although the study found no significant relationship between serum 25(OH)D status and percent body fat, dietary vitamin D intake, or UVA and UVB exposure, the study does contribute to the growing body of knowledge related to vitamin D and female collegiate athletes. The prevalence of vitamin D deficiency and insufficiency was confirmed in a young and active population residing in a subtropical climate. A majority of participants were categorized as having insufficient or deficient levels of serum vitamin D. It is also interesting that only one participant's dietary intake of vitamin D was greater than the RDA, contributing to the conclusion that it is difficult to achieve the recommended amount of vitamin D dietary intake. In this study, percent body fat was not correlated with serum 25(OH)D concentrations which has been a topic of interest of other researchers. However, the small study sample size may have influenced the results.

Further research can utilize this pilot study as a reference to base future studies with a larger sample size of female athletes or other populations. Data from this study can

be compared to larger studies of populations such as athletes representing a variety of outdoor or indoor sports, male athletes, or athletes residing in varying climates and geographic locations. Future studies can also include the use of the La-Roche Posay MyUV Patch for assessing UVA and UVB exposure, as our study found it to be feasible when used correctly.

This pilot study provides preliminary data for additional research to be conducted in this area. Although the relationship of vitamin D status with percent body fat, vitamin D dietary intake, UVA and UVB exposure was not significant, findings provided further insight and knowledge regarding the vitamin D status, body composition, vitamin D dietary intake, and sun exposure and sunlight behaviors of collegiate female indoor volleyball players.

REFERENCES

Adams, J. S., Clemens, T. L., Parrish, J. A., & Holick, M. F. (1982). Vitamin-D synthesis and metabolism after ultraviolet irradiation of normal and vitamin-D-deficient subjects. The New England Journal of Medicine, 306(12), 722-725. doi:10.1056/NEJM198293253961206

Angeline, M.E.,Gee, A.O.,Shindle,M., Warren, R.F., & Rodeo, S.A. (2013). The effects of vitamin D deficiency in athletes. *The American Journal of Sports Medicine,*41 (2), 461-464.

Armas, L. (2007). Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: The effect of UVB dose and skin color. *J Am Acad Dermatol, 57*(4), 588-593. doi:10.1016/j.jaad.2007.03.004

Backx, E. M. P., Tieland, M., Maase, K., Kies, A. K., Mensink, M., van Loon, L J C, & de Groot, L C P G M. (2016). The impact of 1-year vitamin D supplementation on vitamin D status in athletes: A dose-response study. *European Journal of Clinical Nutrition, 70*(9), 1009-1014. doi:10.1038/ejcn.2016.133

Bartoszewska, M., Kamboj, M.,& Patel, D.R. (2010). Vitamin D, muscle function, and exercise performance. *The Pediatric Clinics of North America*, 57(3), 849-861. doi: 10.1016/j.pcl.2010.03.008

Beals, K.A. (2002). Eating behaviors, nutritional status, and menstrual function in elite female adolescent volleyball players. *Journal of the American Dietetic Association,* 102(9), 1293-1296. doi: 10.1016/s0002-8223(02)90285-3

Bell, N. H., Epstein, S., Greene, A., Shary, J., Oexmann, M. J., & Shaw, S. (1985). Evidence for alteration of the vitamin D-endocrine system in obese subjects. *The Journal of Clinical Investigation, 76*(1), 370-373. doi:10.1172/JCI111971

Bischoff-Ferrari, H. A., Giovannucci, E., Willett, W. C., Dietrich, T., & Dawson-Hughes, B. (2006). Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *The American Journal of Clinical Nutrition*, 84(1), 18.

Cannell, J. J., Hollis, B. W., Sorenson, M. B., Taft, T. N., & Anderson, J. J. B. (2009). *Athletic performance and vitamin D. Medicine and Science in Sports and Exercise*, 41(5), 1102.

Dekker, J.M., Snider, M.B., Deeg, D.J.H., Lips, P.T.A.M., Visser, M., Bouter, L.M., Seidell, J.C. (2005). Adiposity in relation to vitamin D status and parathyroid hormone levels: A population-based study in older men and women. *Journal of Clinical Endocrinology and Metabolism,* 90(7), 4119-4123. doi:10.1210/jc.2005-0216

DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. *The American Journal of Clinical Nutrition, 80*(6 Suppl), 1689S.

Farrokhyar, F., Tabasinejad, N.J., Dao, D., Peterson, D., Ayeni, O.R., Hadioonzadeh, R., & Bhandari, M. (2015). Prevalence of vitamin D inadequacy in athletes: A systematic-review and meta-analysis. *Sports Medicine*, 45(3), 365-378. doi: 10.1007/s40279-014-0267-6

Forney, L. A., Earnest, C. P., Henagan, T. M., Johnson, L. E., Castleberry, T. J., & Stewart, L. K. (2014). Vitamin D status, body composition, and fitness measures in college-aged students. *Journal of Strength and Conditioning Research, 28*(3), 814-824. doi:10.1519/JSC.0b013e3182a35ed0

George, S. M., Thompson, F. E., Midthune, D., Subar, A. F., Berrigan, D., Schatzkin, A., & Potischman, N. (2012). Strength of the relationships between three self-reported dietary intake instruments and serum carotenoids: The observing energy and protein nutrition (OPEN) study. *Public Health Nutrition, 15*(6), 1000-1007. doi:10.1017/S1368980011003272

Halliday, T.M., Peterson, N.J., Thomas, J.J., Kleppinger, K., Hollis, B.W., & Larson-Meyer, D.E. (2011). Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Medicine and Science in Sports and Exercise*, 43(2), 335-343. doi: 10.1249/ MSS.0b013e3181eb9d4d

Haussler, M. R., Whitfield, G. K., Kaneko, I., Haussler, C. A., Hsieh, D., Hsieh, J., & Jurutka, P. W. (2013). Molecular mechanisms of vitamin D action. *Calcified Tissue International, 92*(2), 77-98. doi:10.1007/s00223-012-9619-0

Houston, D. K., Cesari, M., Ferrucci, L., Cherubini, A., Maggio, D., Bartali, B., . . . Kritchevsky, S. B. (2007). Association between vitamin D status and physical performance: The InCHIANTI study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 62*(4), 440-446. doi:10.1093/gerona/62.4.440

Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, et al., editors. Dietary Reference Intakes for Tolerable Upper Intake Levels: Calcium and Vitamin D. Available from: http://www.ncbi.nlm.gov/books/NBK56058/

Jäpelt, R. B., & Jakobsen, J. (2013). Vitamin D in plants: A review of occurrence, analysis, and biosynthesis. *Frontiers in Plant Science, 4*, 136. doi:10.3389/fpls.2013.00136

Jones, G. (2008). Pharmacokinetics of vitamin D toxicity. *The American Journal of Clinical Nutrition, 88*(2), 582S.

Kruschitz, R., Wallner-Liebmann, S. J., Hamlin, M. J., Moser, M., Ludvik, B., Schnedl, W. J., & Tafeit, E. (2013). Detecting body fat-A weighty problem BMI versus subcutaneous fat patterns in athletes and non-athletes. *PloS One, 8*(8), e72002. doi:10.1371/journal.pone.0072002

Larson-Meyer, D. E., & Willis, K. S. (2010). Vitamin D and athletes. *Current Sports Medicine Reports, 9*(4), 220.

Lips, P. (2007). Relative value of 25(OH)D and 1,25(OH)2D measurements. *Journal of Bone and Mineral Research, 22*(11), 1668-1671. doi:10.1359/jbmr.070716

Lovell, F. Vitamin D status of females in an elite gymnastics program. *Clinical Journal of Sports Med.* (2008). 18(2):159-161

Looker, A. C. (2007). Do body fat and exercise modulate vitamin D status? *Nutrition Reviews, 65*(8 Pt 2), S124-S126. doi:10.1111/j.1753-4887.2007.tb00339.

Malousaris, G.G., Bergeles, N.K., Barzouka, K.G., Bayios, I.A., Nassis, G.P., & Koskolou, M.D. (2008). Samatotype, size and body composition of competitive female volleyball players. *Journal of Science and Medicine in Sport*, 11(3), 337-344. doi: 10.1016/j.jsams.206.11.008

McCarty, M.F., & Thomas, C.A. (2003). PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. *Medical Hypotheses*, 61(5), 535-542. doi: 10.1016/S0306-9877(03)00227-5

Moran, D.S., McClung, J.P., Kohen, T., & Lieberman, H.R. (2013). Vitamin D and Physical performance. *Sports Medicine*, 43(7), 601-611. doi: 10.1007/s40279-013-0036-y

Nutrition Data System for Research (Version 2016) [computer software] Minnesota: Minneapolis.

Ross, A. C. (2011). *Dietary reference intakes for calcium and vitamin D* National Academies Press.

Ruohola, J., Laaksi, I., Ylikomi, T., Haataja, R., Mattila, V. M., Sahi, T., . . . Pihlajamäki, H. (2006). Association between serum 25(OH)D concentrations and bone stress fractures in finnish young men. *Journal of Bone and Mineral Research, 21*(9), 1483-1488. doi:10.1359/jbmr.060607

Salehpour, A., Hosseinpanah, F., Shidfar, F., Vafa, M., Razaghi, M., Dehghani, S., . . . Gohari, M. (2012). A 12-week double-blind randomized clinical trial of vitamin D3supplementation on body fat mass in healthy overweight and obese women. *Nutrition Journal, 11*(1) doi:10.1186/1475-2891-11-78

Schubert, L., & DeLuca, H. F. (2010). Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. *Archives of Biochemistry and Biophysics, 500*(2), 157-161. doi:10.1016/j.abb.2010.05.029

Shuler, F.D., Wingate, M.K., Moore, G.H., & Giangarra, C. (2012). Sports health benefits of vitamin D. *Sports Health: A Multidisciplinary Approach,* 4(6), 496-501. doi: 10.1177/1941738112461621

von Hurst, P.R., & Beck, K.L. (2014). Vitamin D and skeletal muscle function in athletes. *Current Opinion in Clinical Nutrition and Metabolic Care,* 17(6), 539-545. doi: 10.1097/MCO.0000000000000105

Wang, Y., & DeLuca, H. F. (2011). Is the vitamin D receptor found in muscle? *The Journal of Clinical Endocrinology & Metabolism, 96*(2), 555-555. doi:10.1210/jcem.96.2.zeg555b

Ward, K.A., Das, G., Berry, J.L., Roberts, S.A., Rawer, R., Adams, J.E., & Michael, Z. (2009). Vitamin D status and muscle function in post-menarchal adolescent girls. *The Journal of Clinical Endocrinology and Metabolism*, 94(2), 559-563. doi: 10.1210/jc.2008-1284

Willis, K. S., Broughton, K. S., & Larson-Meyer, D. E. (2009). Vitamin D status and immune system biomarkers in athletes. *Journal of the American Dietetic Association, 109*(9), A15-A15. doi:10.1016/j.jada.2009.06.382

APPENDICES

APPENDIX A

IRB Approval Forms

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research
6700 Fannin, Houston, TX 77030
713 794 2480
irb-houston@twu.edu
http://www.twu.edu/irb.html

DATE: June 13, 2016

TO: Ms. Haley Nagel
Nutrition & Food Sciences - Houston

FROM: Institutional Review Board (IRB) - Houston

Re: Approval for Vitamin D Status and Body Composition of Female Collegiate Volleyball Players (Protocol #: 19047)

The above referenced study has been reviewed and approved by the Houston IRB (operating under FWA00000178) on 6/9/2016 using an expedited review procedure. This approval is valid for one year and expires on 6/9/2017. The IRB will send an email notification 45 days prior to the expiration date with instructions to extend or close the study. It is your responsibility to request an extension for the study if it is not yet complete, to close the protocol file when the study is complete, and to make certain that the study is not conducted beyond the expiration date.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Ms. Rose Bush, Nutrition & Food Sciences - Houston
Dr. Carolyn Moore, Nutrition & Food Sciences - Houston
Graduate School

Institutional Review Board (IRB) Authorization Agreement

Name of Institution Providing IRB Review:	Texas Woman's University
IRB Registration #:	IRB00000845
Federalwide Assurance (FWA) #:	FWA 00000178
Name of Institution Relying on Designated IRB:	Rice University
FWA #:	FWA00003890

The Officials signing below agree that Rice University may rely on the designated IRB for review and continuing oversight of its human subjects research described below:

IRB Protocol #: 19047

Study Title: Vitamin D Status and Body Composition of Female Collegiate Volleyball Players

PIs: Ms. Haley Nagel and Dr. Carolyn Moore

The review performed by the designated IRB will meet the human subject protection requirements of Rice University's OHRP-approved FWA. The IRB at TWU will follow written procedures for reporting its findings and actions to appropriate officials at Rice University. Relevant minutes of IRB meetings will be made available to Rice University upon request. Rice University remains responsible for ensuring compliance with the IRB's determinations and with the Terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

Signature of TWU Signatory Official:

Robert Neely

Robert K. Neely, PhD
Provost

Date: 7/5/16

Signature of the Rice University Signatory Official:

Yousif Shamoo, PhD
Vice Provost for Office or Research

Date: 7/1/16

TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research
6700 Fannin, Houston, TX 77030
713-794-2480
irb-houston@twu.edu
http://www.twu.edu/irb.html

DATE: October 4, 2016

TO: Ms. Haley Nagel
Nutrition & Food Sciences - Houston

FROM: Institutional Review Board - Houston

Re: *Notification of Approval for Modification for Vitamin D Status and Body Composition of Female Collegiate Volleyball Players (Protocol #: 19047)*

The following modification(s) have been approved by the IRB:

The purpose of the amendment is to add a questionnaire to the study that would better classify the participants skin types using the Fitzpatrick Skin Scale. The original procedure involved collecting skin type data using the La Roche- Posay My UV Skin Patch App downloaded on the participant's cell phones. This approach proved to be unreliable and not clearly classify the participant's skin type in in a way that could be used in a scientific study. To gain a more scientific and accurate skin type measurement, the use of a questionnaire developed using Fitzpatrick Skin Scale would allow us to specifically classify each participant's skin type on the Fitzpatrick Skin Scale.
Participants would be contacted via email to complete an online questionnaire created by the researcher from Google Forms. Participants will click on the link provided in the email for the specific Fitzpatrick Skin Type Questionnaire created by the researcher and will type their name into the online Google Forms Fitzpatrick Skin Type Questionnaire and complete the questions. Each questionnaire answer is assigned a number (0-4). The researcher will add and total each participant's score and compare the score with the key to determine Skin Type. Results will be viewable immediately after the participant's completion of the questionnaire on the researcher's Google account. The results will only be viewable by the researcher and will be username and password protected. The researcher will take all necessary steps to ensure confidentiality. Potential Risks include loss of confidentiality in the setting of data sharing from researcher to researcher and researcher to participant. All necessary precautions will be taken to keep confidentiality.

cc. Dr. Carolyn Moore, Nutrition & Food Sciences - Houston

APPENDIX B

Consent Form

TEXAS WOMAN'S UNIVERSITY
CONSENT TO PARTICIPATE IN RESEARCH

Title: Vitamin D Status and Body Composition of Female Collegiate Volleyball Players

Investigator: Haley Nagel.....hnagel@twu.edu 417/262-3268
Advisor: Carolyn Moore, PhD, RD..... cmoore8@twu.edu 713/794-2377

Explanation and Purpose of the Research

You are being asked to participate in a research study by the Department of Nutrition and Food Sciences at Texas Woman's University for Ms. Haley Nagel's thesis work. The purpose of this research is to evaluate vitamin D status and body composition in indoor female collegiate volleyball players. This study will determine if there is an association between vitamin D status, sunlight exposure, and body composition in indoor collegiate female athletes. Vitamin D status is negatively associated with body composition and has many functions related to athletic performance including muscle synthesis and recovery, balance, reducing inflammation, bone health, and cardiovascular benefits. Vitamin D can be synthesized in the skin by Ultraviolet B radiation (UVB) exposure and can be obtained through dietary intake. Female volleyball players spend less time outdoors exposed to sun light and are at a higher risk for vitamin D deficiency/insufficiency.

Description of Procedures

You will be asked to complete a demographic questionnaire, a vitamin D food frequency questionnaire, sunlight habit questionnaire, and a 24-hour dietary recall. On the day of the study clinic visit, your weight, height, and waist circumference will be measured by the investigator. You will be asked to complete a Bod Pod assessment to measure your body composition performed by a trained investigator. You also will be asked to provide a sample of one tube of blood that will be taken by a trained investigator to measure circulating blood vitamin D levels. You will be provided with a La Roche-Posay My UV patch, if available, to wear on your arm for 2-3 days to measure sunlight exposure. The varying photosensitivity dye squares of the patch determine the amount of UV exposure when uploaded to a mobile phone app.

Potential Risks

There exists some risk for participants to experience nausea when completing the blood draw and body composition procedures. You will be instructed to inform the researcher if you are feeling nauseated and will be asked to lie on the floor with your feet elevated. You may also feel faint. If this occurs, the procedure will be stopped and then you will be asked to lie on your back on the

floor with your feet elevated to alleviate the symptom. The risk of infection resulting from blood draw is minimal. Standardized precautions will be used during all blood test procedures. Sites will be cleaned with alcohol immediately prior to the blood draw. During data collection of anthropometric measurements and the Bod Pod procedure, you may feel emotionally uneasy or embarrassed. To minimize emotional discomfort, data will be collected by a research member of the same sex and other participants will not be allowed to be present in the testing room.

_____
Initials

There exists the risk of accidental loss of personal information. All procedures to minimize risk according to law will be followed. All records of data will be stored in a limited access, locked file cabinet in room 7017 of the Nutrition and Food Sciences Department, Texas Woman's University Institute of Health Sciences Building. There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions. The researchers will try to prevent any problem that could happen because of this research. Confidentiality will be protected to the extent that is allowed by law.

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

Your involvement in this study is completely voluntary and you may withdraw from the study at any time. If you would like to know the results of this study we will mail them to you.* Results will include your blood vitamin D level, your personal sunlight exposure, your dietary vitamin D intake, and your body composition results (% body fat). This study will contribute knowledge to the field of vitamin D research in a young, athletic, female population.

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; their phone numbers are at the top of this form. If you have 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 at 713-794-2480 or via e-mail at IRB@twu.edu.

_____ _____
Signature of Participant Date

*If you would like to know the results of this study tell us where you want them to be sent:

Email: _____
or
Address:

_____

_____

_____

APPENDIX C

Demographic Questionnaire

TWU Vitamin D and Body Composition of Female College Volleyball Players
Demographic Questionnaire

Name: _____ Date: _____
(Last) (First) (Middle)

Phone: (_____) _____ Email: _____

Address: _____ City: _____

State: _____ Zip: _____

How would you refer we contact you?
- ❒ Email
- ❒ Mail
- ❒ Phone

Date of Birth: _____/_____/_____

Age: _____ Height: _____ Ft _____ in. Weight: _____ lbs.

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

APPENDIX D

24-Hour Food Record

INSTRUCTIONS FOR FOOD RECORDS

General Guidelines

- You will be asked to keep a record of your intake of food for ONE DAY (24 Hours)
- Please bring the completed food record on the day of the study clinic visit.
- Record everything you eat and drink – include your snacks and all drinks – even alcohol.
- Write down foods as you eat them. Try not to wait until the end of the day because it is easy to forget things. We recommend you keep your food record with you during the day.
- Details are needed – tell us everything you can about the food and its packaging, how it was cooked, and what you may have added to it.

How to Record Your Food and Drinks:

- **Put one food or drink item on each line of the record**
- **Record the TIME you ate the food or drink.**
- **Food description – proved a detailed description of each food you consume:**
    - Form of the food – fresh, frozen, canned, etc.
    - Cooking method – fried, baked, boiled, etc.
    - Include fat and oil that the food is cooked in.
    - Include types of fat such as low fat yogurt, 2% milk, etc.
    - Include sauces that were added to the food during cooking or while you were eating the food such as ketchup, mustard, pico de gallo, etc.
    - Include brands such as Kellogg's Frosted Flakes, Diet Coke, etc.

Portion Size:

- Use "household measures" to describe the food and drinks you consume. Estimate using measuring cups, measuring spoons, weight (oz. or fluid oz.).
- How many? Was it 1 cup or 2 cups? 1 or 2 slices of pizza?
- Size? Was it a small, medium or large muffin? 1 slice of a large pizza……

24-Hour Food Record

SUBJECT ID:_____ DATE: _____PAGE:_____ of_____

Please bring your food records to your clinic visit.

TIME	FOOD DESCRIPTION	HOW MANY/PORITON

APPENDIX E

Vitamin D Food Frequency Questionnaire

Vitamin D Food Frequency Questionnaire

- **How often do you eat the following foods each week? Please list number of times each week if applicable.**
- **Please give specific serving sizes when possible (ounces, cups, tablespoons, etc.)**
- **List the type of food (Chex cereal, 2% milk, cheddar cheese, salmon, oysters, cashews, romaine lettuce, etc.)**

FOOD	NUMBER AND TYPE OF SERVINGS PER WEEK
Fortified cereal	
Fortified milk (Vitamin A + D)	
Fortified orange juice (calcium + Vitamin D)	
Eggs	
Swiss Cheese	
Fortified soy/almond milk	
Fish (salmon, tuna, mackerel, sushi)	
Liver (Beef, Pork)	
Cod-liver oil	
Tofu	

Yogurt (specify brand)	
Mushrooms (raw)	
Multi-vitamin/Multi-mineral Supplement	

APPENDIX F

TWU Sunlight Questionnaire

TWU Sunlight Questionnaire

This questionnaire is designed to assess your sunlight exposure. Your honesty with the questions will help to validate the questionnaire, and give us an easy and effective way to determine attitudes towards sunlight. For the questions please choose the closest match, and only choose one answer.

For the questions that include specific time frames use these guidelines. Check if you spend at least 75% of that time engaged in the activity, and check all that are appropriate, for these questions only if you may have more than one answer.

For example: If you spend the hours of 8 am through 4 pm outdoors you would check the boxes for

7 am- 10am, 10am-3pm. Since 3 pm-4pm is not a significant portion of the 4 pm-6pm time frame, you would not check that box. It would look like this:

In a typical day, what hours would you spend outdoors?	❒ Midnight-7 am ✓ 7am-10am ✓ 10am-3 pm ❒ 3pm-6pm ❒ 6pm-midnight

As general guidelines, remember that most windows are treated to prevent UV exposure, and are considered to be indoors, even with very large windows. Many make up brands also include UV protection.

Thank you for your participation.

Participant Name: _____ Date: _____

ID Code: _____

TWU Sunlight Questionnaire

These questions will tell us about your habits in relation to sunlight. Please indicate how often you engage in the following activities.

How often do you seek sunshine?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
How often do you avoid the sun?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
How often do you wear a hat when it is sunny?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
How often do you cover your arms and legs when it is sunny?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
How often do you use tanning facilities?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
How long do you tan per session?	Minutes _____
Do you wear anything while tanning?	❒ Tanning Accelerators ❒ UV Protection ❒ None

TWU Sunlight Questionnaire

This series of questions is designed to assess your use of sunscreen in various situations. Consider how you would act in a normal situation, i.e. a normal planned weekend outing or day at school.

Do you wear makeup that contains Ultraviolet B radiation (UVB) protection?	❒ Yes ❒ No ❒ Not applicable
What Sunscreen Protection Factor (SPF) is it?	SPF _____
Do you wear sunscreen in general?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
What SPF is it?	SPF _____
How often do wear sunscreen on a school day, including makeup?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
What SPF do you use?	SPF _____
How often do you use sunscreen during recreational activities?	❒ Never ❒ Seldom ❒ Sometimes ❒ Often ❒ Always
What SPF do you use?	SPF _____

TWU Sunlight Questionnaire

These questions are related to your work habits. These questions will help us to estimate your time spent outdoors. If you are a student, you may consider your time studying in class as work. Please consider as a workday those days in which a majority of your time is spent working. Please consider as a recreational day those days in which a majority of time is spent engaged in recreational activities or running errands.

School Days:

In a typical school day, what hours would you spend outdoors?	❒ Midnight- 7 am ❒ 7 am-10am ❒ 10am-3pm ❒ 3pm-6pm ❒ 6pm-midnight
What percentage of your skin is exposed to sunlight while you work?	❒ (30% or Less) Hands and Face, or Less ❒ (30%-50%) Short Sleeves ❒ (50%-70%) Shorts and a T-Shirt ❒ (70%-90%) Shirtless or Bikini and shorts ❒ (90% or more) Small swimsuit or nothing

Recreational Habits:

How many hours per week do you spend in recreational activities?	_____Hours
When do you engage in recreational activities?	❒ Early Morning ❒ Morning ❒ Midday ❒ Late Afternoon ❒ Late Night
How much of this recreational activity is spent outdoors?	❒ Midnight-7am ❒ 7am-10am ❒ 10am-3pm ❒ 3pm-6pm ❒ 6pm-midnight

What percentage of your skin is exposed to sunlight during recreational activities?	❒ (30% or Less) Hands and Face, or Less ❒ (30%-50%) Short Sleeves ❒ (50%-70%) Shorts and a T-Shirt ❒ (70%-90%) Shirtless or Bikini and shorts ❒ (90% or more) Small swimsuit or nothing