Vitamin D Deficiency and Physical Performance in Athletes

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Vitamin D deficiency is now characterized as a pandemic (1-4) and estimates of the prevalence within populations are raising concern (5, 6). Recent reports of vitamin D deficiency have placed the prevalence in the United States at approximately 42% (7). Insufficiency estimates in the United States have been reported to be 75% in Caucasians and upwards of 90% in populations with pigmented skin (8). Global insufficiency estimates are in the proximity of one billion people (3). This deficiency/insufficiency is not without potential consequences. The most well-known vitamin D deficient consequences are rickets in children and osteomalacia in adults (3). However, the list of possible adverse effects with deficient levels of vitamin D is not limited to the typical role of vitamin D, such as calcium control and bone formation (6, 9-12). The active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25[OH]_2D$), is a steroid hormone. Therefore, it acts through a vitamin D receptor (VDR) to signal metabolic action. Tissues containing the VDR are influenced by $1,25(OH)_2D$. The VDR has been identified in a vast and diverse list of human tissues (3, 11, 13). This rather expansive list reinforces the broad impact that vitamin D has on human physiology and the wide-ranging potential for adverse consequences associated with its deficiency. Of specific interest to this research is that the VDR is found in skeletal muscle (3). Since the VDR is found in muscle tissue, $1,25(OH)_2D$ should have some role in its homeostasis. It is possible that vitamin D insufficiency or deficiency could elicit a negative effect on muscle homeostasis, and this detrimental influence could potentially compromise muscle function.

The causal pathway in which vitamin D deficiency may compromise skeletal muscle function in humans has yet to be established; however, numerous researchers have offered theories attempting to substantiate the biological relevance of vitamin D in regards to muscle function (2, 14-18). It has been proposed that vitamin D influences muscle tissue through both genomic and non-genomic pathways (14-17). Through these pathways, it is believed that

$1,25(OH)_2D$ is involved in myogenic regulation (18), proliferation, differentiation, phosphate and calcium modulation (14), and protein synthesis in skeletal muscle (2, 17). Disruptions in these vital processes in muscle may compromise the functional ability of the tissue (6). Poor vitamin D status has also been associated with muscle atrophy (19) and increased muscle fat infiltration (20), both of which may influence physical performance.

Beyond the biological theory, physical performance data regarding the effect of vitamin D status appear to indicate that vitamin D influences skeletal muscle function. Vitamin D status has been associated with measures of physical performance in numerous cross-sectional investigations (21-28). Interventional trials involving vitamin D supplementation in vitamin D deficient populations have documented increases in measures of physical performance with improved vitamin D status at the end of the trials (29-32). Research investigating the impact of vitamin D receptor polymorphisms on prevalence of falling (33) and measures of strength (34, 35) and power (36) in senior populations also seem to indicate that vitamin D plays a role in skeletal muscle's functional ability. Recent investigations have also linked vitamin D deficiency with reduced physical performance in tasks predominantly involving aerobic metabolism (29, 37, 38).

There is mounting evidence that vitamin D deficiency compromises physical performance in aging populations (22, 24-32, 39, 40); however, investigations in other populations are lacking (21, 23, 29, 41), which is creating a vacuum in the literature. At this point, there appear to be only six studies that incorporated non-elderly populations in their study design investigating physical performance outcomes (21, 23, 29, 41-43). Of these, four investigations involved adolescent populations, which were female-only cohorts (21, 23, 41, 42). Only two studies were found, one cross-sectional (43) and one interventional trial (29), which evaluated the effects of vitamin D status on measures of physical performance in men and women with a broad age range. However, none of these investigations have utilized sample populations

of athletes. Nor do they concurrently employ a battery of tests measuring physical performance that have a meaningful relationship to physical performance in athletic competition.

Investigating the impact of vitamin D status on measures of physical performance in athletes would further build on the scientific literature and address the current population-specific void. Utilizing measures of physical performance that are associated with various tasks involved in sport performance would further bolster the relevance of this data to athletes. Collection and interpretation of data pertaining to this issue may also impact the decision to conduct more expensive randomized controlled trials. This data is pertinent to the practice of sporting clubs, as vitamin D status may become an important observational variable for sports physicians to evaluate in their athletes. A physician could evaluate and effectively address vitamin D deficiency in athletes, which may equate to increased physical performance. The results of this investigation would also impact the current discussion concerning vitamin D and athletic performance. At this time, the basis for suggesting vitamin D as a potential ergogenic substance for athletes is substantiated merely through biological plausibility and extrapolation of data from other populations. In order to address the question of whether vitamin D status has any impact on physical performance in athletes, investigations must begin to explore this question in these populations.

The intent of this cross-sectional study is to investigate the relationship between vitamin D status and laboratory measures that are relevant to physical performance in Division I and Division III collegiate hockey players which include: 1) power production during vertical jump performance and the Wingate test; 2) maximal oxygen consumption during a graded exercise test; and 3) body composition as evaluated by underwater weighing. It is hypothesized that a low blood serum level of 25(OH)D is associated with less desirable testing outcomes in this population.

CHAPTER 2: Literature Review

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2.1 Introduction

The following section is a review of the literature pertaining to vitamin D and physical performance. Vitamin D physiology, issues around vitamin D status determination and correction, and the prevalence of deficiency will be discussed first. The focus of the review will then shift toward the literature regarding the biologically plausible way in which skeletal muscle may be influenced by vitamin D. Establishing biological relevance will entail reviewing the following topics: proposed mechanisms of action in skeletal muscle, the VDR knockout mouse model, skeletal muscle tissue repair and the involvement of the immune system, vitamin D and the cardiovascular system, vitamin D status and muscle fat infiltration, VDR polymorphisms, vitamin D status and measures of physical performance, and seasonality of physical performance. The plausibility of vitamin D impacting adipose tissue accumulation and androgen production will also be examined.

2.2 Vitamin D Physiology

Vitamin D is typically known for its role in calcium regulation (9, 13), bone formation, and curing the medical disease rickets (10, 11). More recently the involvement and importance of this vitamin has expanded to encompass physiological processes in numerous body tissues (10-12). The following section will describe vitamin D, depict its metabolism, and discuss how it achieves such a broad impact on human physiology.

Vitamin D is a prohormone and not a vitamin, and numerous researchers are calling for this to be recognized (3, 11, 13, 44). In its prohormone state, vitamin D is believed to be impotent and lacking any physiological impact (11, 13). It is not until the prohormone undergoes various hydroxylations that it yields physiological impact on target tissues (11, 13, 16). Furthering its distinction from other vitamins, it does not need to be consumed in the diet; in fact, the predominant source of vitamin D is through sunlight exposure (UVB radiation) (3, 11, 13).

Endogenous vitamin D production is achieved through exposing skin to UVB radiation possessing a wavelength characteristic range of 290-315nm (3, 13). Skin contains a cholesterol derivative, 7-dehydrocholesterol (provitamin D). Upon UVB exposure (290-315nm range), the double bonds in the molecule absorb the radiation (3), which break the 9,10 carbon-carbon bond (13). Following the breaking of the 9,10 carbon-carbon bond, the previtamin D undergoes a heat transformation to form vitamin D_3 (cholecalciferol) (13). Vitamin D_3 starts its migration from the epidermis to the blood circulation upon formation and it is picked up by vitamin D binding protein in the capillary beds (3). The vitamin D binding protein is then responsible for the transportation of vitamin D_3 to the liver (3).

Vitamin D is also obtained through the diet. In the diet, two forms of vitamin D exist; vitamin D_2 (ergocalciferol) from irradiation of plant sources and vitamin D_3 (cholecalciferol) from animal sources (16). Dietary sources include oily fish, egg yolk, and fortified foods such as milk and other dairy products (3). Once Vitamin D_2 or D_3 has been obtained through the diet, it is picked up and transported by chylomicrons through the lymphatic system (3). Once in venous circulation, similar to endogenous production, vitamin D is bound to a carrier protein (vitamin D binding protein) and ultimately reaches the liver (3).

Once vitamin D enters circulation it must undergo two hydroxylations before it becomes the active steroid hormone 1,25-dihydroxyvitamin $D_{2 \text{ or } 3}$ ($1,25[OH]_2D$) (11, 13, 16). The liver is the site of the first hydroxylation that vitamin D undergoes on its transformation from prohormone to hormone (3). At the liver, vitamin D-25-hydroxylase adds a hydroxylation at the 25^{th} position on vitamin D, forming 25-hydroxyvitamin D (25[OH]D) (3). Serum 25(OH)D itself is not believed to impart a physiological impact (11); however, there remains some controversy concerning the biological activity of 25(OH)D (10). Serum 25(OH)D is the circulating form of vitamin D used to evaluate vitamin D status (3). The final hydroxylation occurs at the kidneys via D-1-hydroxylase, adding a hydroxylation at the 1-alpha position, forming 1,25-dihydroxyvitamin

D (1,25[OH]$_2$D) (3). This process can also take place in other tissues besides the kidney (13), which will be discussed later.

After conversion to its active form, 1,25(OH)$_2$D's most recognized role is that of participating in calcium regulation (10, 13). As calcium and phosphate levels decrease, parathyroid hormone increases, thereby triggering an upregulation of 1,25(OH)$_2$D (11, 16, 44). Low levels of calcium and phosphate can be remedied by 1,25(OH)$_2$D through its ability to increase calcium and phosphate absorption in the intestine (11, 16, 44). Together, 1,25(OH)$_2$D and parathyroid hormone can initiate calcium mobilization from bone and provide the signal to the distal renal tubule to allow for calcium reabsorption (11, 44). Once calcium levels have been adequately raised, parathyroid activity is down-regulated, FGF23 is upregulated (45, 46), 1-alpha hydroxlyase activity slows (13), and 1,25(OH)$_2$D clearance becomes dominant.

Clearance of the steroid hormone 1,25(OH)$_2$D is also achieved through a hydroxylation step. The enzyme responsible for 1,25(OH)$_2$D clearance, cytochrome P-450 (CYP24A), is in control of D-24-hydroxylase activity and metabolic degradation of both 25(OH)D and 1,25(OH)$_2$D (3, 11, 44, 46). An antagonist of 1,25(OH)$_2$D signaling, 1,25(R)(OH)D23(S),26-lactone may also be the product of D-24-hydroxylase activity (47). Disposal of calcitroic acid, the metabolic product of 1,25(OH)$_2$D degradation, is accomplished via bile and the lower gastrointestinal tract (44). Regulation of D-24-hydroxylase activity seems to be governed by the circulating concentration of 1,25(OH)$_2$D (11, 47). The rate of D-24-hydroxylase activity is directly proportional to circulating 1,25(OH)$_2$D; when circulating levels of 1,25(OH)$_2$D are elevated, so is the rate of its removal by way of escalating D-24-hydroxylase activity (11).

The upregulation of 1,25(OH)$_2$D can occur in the absence of disrupted calcium homeostasis to meet the needs of other tissues (11, 44). This can be achieved through the secretion of calcitonin from the thyroid's C-cells (11, 44). Calcitonin reduces bone and renal calcium sequestering functions, and stimulates 1-alpha hydroxlyase activity in the kidney (11,

44). In addition, various other tissues also express 1-alpha hydroxylase and thus can convert 25(OH)D to $1,25(OH)_2D$ on demand (13). To date, at least 10 tissues have been identified with D-1-hydroxylase and 1-alpha hydroxylation capability (13). These tissues are able to covert 25(OH)D to $1,25(OH)_2D$ as needed, giving rise to vitamin D's paracrine activity (13). It has been suggested that $1,25(OH)_2D$ kinetics in these tissues are influenced by the 25(OH)D status of the individual (45). Thus, poor vitamin D status may alter optimal $1,25(OH)_2D$ signaling in these tissues and compromise function.

The VDR itself is influenced by $1,25(OH)_2D$ signaling (48). The VDR is a class II nuclear steroid hormone receptor and is essential in the $1,25(OH)_2D$ signaling process (11), which will be discussed later in further detail. Vitamin D receptor mRNA expression fluctuates based on the exposure to $1,25(OH)_2D$ (48). It seems that VDR tissue concentration is augmented with increased exposure to $1,25(OH)_2D$ (48). This regulatory feature may have important implications when examining normal signaling in VDR containing tissues.

As previously mentioned, $1,25(OH)_2D$ is a steroid hormone which is biologically active and capable of influencing all tissues which possess a vitamin D receptor (3, 11, 13, 16). The VDR has been identified in a vast and diverse list of human tissues (3, 11, 13). These tissues are influenced by $1,25(OH)_2D$. This hormone has the ability to induce tissue specific responses (45). Therefore, its functional role may be very different from tissue to tissue. The wide-ranging tissues that express the VDR underscore the broad impact and importance of this hormone in many physiological processes. It is believed that this hormone influences 100-1000 genes (13, 49, 50), equating to approximately 3% of the human genome (51). The potential of the hormone for such a broad-spectrum impact on human physiology underscores the importance of accurate methods of determining vitamin D status.

2.3 Evaluating Vitamin D Status

Vitamin D status is assessed using serum 25(OH)D (10). The use of 25(OH)D in the evaluation of vitamin D status has been selected because it is believed to respond to both endogenous production in the epidermis and dietary consumption of vitamin D_2 and D_3 (52). Hydroxlyation of ergocalciferol or cholecalciferol in the liver is thought to be largely unregulated and therefore reflects vitamin D yields (13, 52). This deregulated circulating value, in theory, is minimally affected by other physiological processes, is relatively stable, and is believed to properly categorize vitamin D status (1, 13, 52, 53). This is not true for the active form $1,25(OH)_2D$ which is influenced by 25(OH)D, calcium and parathyroid hormone (4, 53, 54). For instance, low levels of 25(OH)D cause increased parathyroid hormone, which triggers $1,25(OH)_2D$ production in the kidneys and masks a deficiency if $1,25(OH)_2D$ is the biomarker for vitamin D status (4, 53-55). Serum 25(OH)D has a long half-life, approximately 2-8 weeks (4, 52, 53), whereas $1,25(OH)_2D$ has a half-life of up to four hours (53). Lastly, many tissues possess the D-1-hydroxylase enzyme that is required to perform the final hydroxylation of 25(OH)D (13). The hydroxylation activity in those tissues is dependent on the amount of circulating 25(OH)D and not $1,25(OH)_2D$ (52, 53). Quantifying serum 25(OH)D provides a useful indicator of vitamin D status and metabolism that reflects both upstream intake and synthesis, and downstream hydroxylase and $1,25(OH)_2D$ activity.

Setting a normal or optimal level for circulating 25(OH)D is problematic (52, 55, 56). Adequate levels of vitamin D are dependent on the functional marker used to evaluate vitamin D status (57, 58). Some of the functional markers currently used to evaluate reference ranges for vitamin D status include: clinical symptoms, $1,25(OH)_2D$ kinetics, parathyroid hormone, and health outcomes (57, 58). Each of the criteria stated are associated with differing 25(OH)D levels that induce the desired effect (57). Beyond the problem associated with biomarkers, accuracy of the various assays has been called into question (52, 54, 59-61).

Numerous assays are available for the assessment of vitamin D status and an assay-specific bias in reported results appears to be an issue (62, 63). According to Snellman et al. (62), currently utilized methods for the assessment of 25(OH)D include, "high-pressure liquid chromatography (HPLC) and mass spectrometry (MS), radioimmunoassay (RIA), enzyme immunoassays (EIA), competitive protein binding assays (CPBA), automated chemiluminescence protein-binding assays (CLPBA) and chemiluminescence immunoassays (CLIA)" (p.1). These assay techniques seem to exhibit substantial interassay variability (62, 63). Snellman et al. (62) found interassay variation when they evaluated the performance of HPLC-APCI-MS, RIA, and CLIA. Interassay variation may complicate the interpretation of various research findings when trying to establish a demarcation value for vitamin D sufficiency (62, 63). This variation may also mischaracterize an individual's vitamin D status when the assay-specific results are compared to the current recommended 25(OH)D sufficiency level (63). The majority of the research investigating normative vitamin D levels has used the DiaSorin RIA when measuring 25(OH)D status (58, 63). Thus, the DiaSorin RIA is the "gold standard" or reference assay by default (63). When interpreting the results from a specific assay, the performance of that assay in comparison to the DiaSorin RIA must be assessed and corrected for bias in order to use the current 25(OH)D status recommendations for sufficiency (63).

Due to problems in assay accuracy, Cavalier et al. (64) proposed assuming a margin of error in the 25(OH)D measurement and correcting for that error by increasing the target value for sufficiency. Cavalier et al. (64) reported a "measurement uncertainty" of 16.4% to 31.3% when comparing the DiaSorin RIA, DiaSorin liaison, Roche Elecsys, and HPLC. This uncertainty in 25(OH)D measurement poses problems when classifying an individual's vitamin D status. To manage assay accuracy issues, Cavalier et al. (64) suggest adding a correction factor of 20 $nmol \cdot L^{-1}$ to the target 25(OH)D level sought.

Another problem to consider is the temporal characteristic of the sample (52). Season, activity patterns, geographical location, and sunlight exposure are all influential factors in vitamin D endogenous production (52, 54). Commonly, endogenous production of vitamin D via sunlight exposure and subsequent synthesis in the skin provide upwards of 80% of total vitamin D status (52, 65, 66). Sampling may be varied depending on the time of year the sample was taken and may not accurately reflect a level that is maintained throughout the year (52, 66). Variability of within-individual 25(OH)D assessments has been reported to be as high as 13-19 % (59). This observation may be attributed to endogenous synthesis-based seasonal fluctuations, or it may be an indicator of problematic vitamin D assay reliability (59). Other problematic variables that may influence 25(OH)D levels are fat mass, BMI, 24-hydroxlyase activity (52), medications (67) and genetics (68).

At present time, the accepted normative values and thresholds for circulating 25(OH)D are largely based on calcium control and bone health (13, 69, 70). According to a majority of the literature, sufficient levels of circulating 25(OH)D are currently considered to be 30-32 $ng \cdot mL^{-1}$ (75-80 $nmol \cdot L^{-1}$) (1, 3, 53, 58, 59). Values below 30-32 $ng \cdot mL^{-1}$ are considered to be the start of insufficiency (3, 4, 59). The deficiency threshold is regarded as a 25(OH)D level below 20 $ng \cdot mL^{-1}$ (3, 4). Reports of 25(OH)D thresholds are not all consistent in the literature (4, 44, 53, 57). Recommendations for an optimal 25(OH)D level are still being debated (56, 58, 59, 71). Optimal 25(OH)D concentrations may be as much as 40-70 $ng \cdot mL^{-1}$ (4, 49, 50).

The Institute of Medicine recommendation for sufficient vitamin D status is more conservative. There appears to be a disagreement between the Institute of Medicine (IOM) and the majority of researchers investigating vitamin D and non-skeletal outcomes when it comes to setting the 25(OH)D level indicating sufficiency. The IOM's 25(OH)D level indicating sufficiency is 20 $ng \cdot mL^{-1}$ (72). The functional marker chosen by the IOM to evaluate vitamin D status was bone health (72). This decision was made because the panel deemed sufficient

evidence for causality was only met for vitamin D and bone health (72). Since much remains unknown regarding vitamin D and other outcomes, the IOM (72) and others endorse a conservative approach to setting optimal 25(OH)D levels and vitamin D requirements (73).

A possible consequence of excess vitamin D intake is toxicity. Reports of vitamin D intoxication are sparse (3, 50, 71, 74). The vitamin D status associated with intoxication is a 25(OH)D level of 150 $ng \cdot mL^{-1}$ (3, 50). If a high level of 25(OH)D is accompanied by hyperphosphatemia and hypercalcemia, a vitamin D intoxication diagnosis will be indicated (3, 44). Calcification of soft tissues can result from chronic exposure to toxic levels of vitamin D (44). Vitamin D toxicity is a possible consequence of dietary supplementation, but not from sun exposure (50). However, vitamin D supplementation of 10,000 IU/day or below is not likely to result in toxicity (50, 75).

When evaluating vitamin D status, it is important to note the inter-reliant nature of vitamin D, calcium and parathyroid hormone (PTH). Parathyroid hormone regulates $1,25(OH)_2D$ synthesis in the kidneys in order to maintain plasma calcium concentrations (54). In response to low plasma calcium concentrations, PTH levels increase, which in-turn increases $1,25(OH)_2D$ synthesis (54). The resulting elevation of these two hormones initiates calcium mobilization from bone and also provides the signal to the distal renal tubule to allow for calcium reabsorption (11, 44). Moreover, $1,25(OH)_2D$ increases calcium and phosphate absorption in the intestine (11, 16, 44). Concurrently, $1,25(OH)_2D$ is also responsible for signaling renal phosphate excretion (54). Prentice (54) has hypothesized that a calcium deficiency could lead to excessive phosphate wasting and an increased requirement for vitamin D. In this model, a diet deficient in calcium leads to persistently elevated PTH and $1,25(OH)_2D$. Habitually elevated $1,25(OH)_2D$ results in hypophosphatemia. Vitamin D status may also be compromised due to the increased synthesis of $1,25(OH)_2D$, which also triggers its own degradation via upregulation of 24-hydroxylase activity (11). This synthesis-degradation loop would result in an increased vitamin D demand and

secondary vitamin D deficiency (54). Calcium status should not be overlooked when evaluating vitamin D status because of the co-dependent nature of the two.

2.4 Prevalence of Insufficiency and Deficiency

Vitamin D deficiency is now being depicted as a world-wide problem (10). However, most of the general population prevalence data regarding national vitamin D status comes from a limited number of countries (54). Two sources of national vitamin D status data are the National Diet and Nutrition Survey (NDNS) in the United Kingdom and the National Health and Nutrition Examination Survey (NHANES) in the United States (54). It appears that all populations are at risk for becoming vitamin D deficient (76). The old RDA for vitamin D appeared inadequate (65), needing revision (77), and was recently addressed by the IOM (72).

Vitamin D status reflects both dietary sources and endogenous synthesis. Vitamin D sufficiency can be achieved through a combination of dietary intake and sunlight exposure, or achieved independently through either means. However, there is a paucity of foods that are rich sources of vitamin D (54). The main dietary sources are oily fish and fortified foods (54). Supplemental forms of vitamin D have gained some popularity in the United States (54). The current RDA for vitamin D has been revised upward from 200 IU/day to 600 IU/day for individuals from 1-70 years of age and 800 IU/day for those above 70 years (72). The upper limit (UL) for vitamin D has also been adjusted from 2000 IU/day to 4000 IU/day (72). An estimate for the total daily vitamin D requirement has been stated to be between 3000-5000 IU (10). It has been proposed that without adequate sunlight exposure, dietary consumption needs to be minimally 1,000 IU (76). Without sufficient UVB exposure, supplemental vitamin D may be necessary. It has been estimated that upwards of 80% of an individual's vitamin D status is acquired through endogenous synthesis via sunlight exposure (52, 65, 66); albeit, this estimate is subject to variability due to geographic location (54). Nevertheless, vitamin D status is highly dependent on environmental factors.

Numerous factors impact endogenous synthesis of vitamin D. Endogenous vitamin D production is achieved through exposing skin to UVB radiation possessing a wavelength characteristic range of 290-315nm (3, 13). Factors that impact the magnitude of UVB exposure and vitamin D synthesis include the following: latitude, season, time of day, cloud cover, pollution, exposure duration, clothing, sunscreen, skin pigmentation, and age (54). Factors that influence endogenous synthesis can be divided into two categories: features that limit the amount of UVB (290-315nm) exposure and features that limit the vitamin D synthesis rate when adequate UVB exposure occurs. For example, latitude is a commonly cited factor that influences the amount of UVB radiation possessing a wavelength characteristic range of 290-315nm (1, 54, 57, 76). Few UVB photons reach the earth during the winter months in geographic localities above 35 degrees latitude, which reduces or eliminates the possibility for vitamin D synthesis (76). On the other hand, a feature that limits the vitamin D synthesis rate when adequate UVB exposure takes place is skin pigmentation. Melanin effectively absorbs UVB radiation, which reduces the amount that is available to break the 9,10 carbon-carbon bond on 7-dehydrocholesterol (10). Thus, individuals with greater skin pigmentation will require a greater duration of UVB radiation exposure to synthesize a stated vitamin D quantity when compared to individuals with less skin pigmentation.

Other factors related to vitamin D metabolism may affect an individual's status. Adiposity, calcium intake, genetic differences, renal function and disease all have been reported to be possible factors that may impact vitamin D status (54). Regardless of the origin of influence, aspects that restrict vitamin D sources or increase the body's demand may potentially result in deficiency.

Estimates of the prevalence of vitamin D deficiency/insufficiency were found to be highly variable depending on the level of 25(OH)D used to designate status. According to the 1992-2001 NDNS data, average vitamin D deficient status was reported at approximately 5-20%

across the majority age group stratum in the UK when 10 ng·mL^{-1} 25(OH)D was defined as deficiency (54). A few select populations demonstrated high occurrence rates in the range of 20-40% and interestingly, young men and women 19-24 years of age were among this group (54). When the demarcation of vitamin D deficiency was set to 20 ng·mL^{-1}, the generally accepted 25(OH)D deficiency level, the general population prevalence rose to 20-60% across stratum (54). Using this demarcation value, vitamin D deficiency was estimated at roughly 75% in young men and women ranging in age from 19-24 years (54). Only 10% of the sample population in the UK demonstrated vitamin D sufficiency when the 25(OH)D cut-point value was adjusted to account for insufficiency (32 ng·mL^{-1}) (54). Vitamin D status seasonal fluctuations were also reported in this population (54).

In the United States, the prevalence of vitamin D deficiency in the general population has been cited at approximately 25% (65), but more current reports place the overall prevalence at 42% (7). Using the 1988-1994 NHANES data, vitamin D deficiency (10 ng·mL^{-1}) occurrence range was estimated at 1-5% (54). Incidence climbed to 20-40% when the demarcation of deficiency was 20 ng·mL^{-1} (54). However, the accuracy of the prevalence estimates in the United States using the NHANES data are suspect due to the sampling strategy employed. Serum 25(OH)D samples were collected from April-October in the northern states and November-March in the southern states. This sampling design may underestimate the prevalence of deficiency in the northern states, as vitamin D status is influenced by latitude and season (54). Using 2005-2006 NHANES data, Forrest and Stuhldreher (7) estimated the prevalence of vitamin D deficiency at 42% when defining deficiency as 20 ng·mL^{-1} or less (7). Recent reports of vitamin D insufficiency have placed the prevalence in the United States at approximately 75% in Caucasians and upwards of 90% in populations with pigmented skin in the general population (8).

Although few studies have explored the prevalence of vitamin D deficiency in sporting populations, the existing evidence suggests they are not exempt regarding deficiency

susceptibility. In a population of 93 Middle Eastern athletes with a mean age of 21.3 years, vitamin D deficiency (20 ng·mL^{-1}) prevalence was reported at 91% (78). Fifty-eight percent of this sample population exhibited severe deficiency (< 10 ng·mL^{-1}). Not one Middle Eastern athlete possessed a 25(OH)D level above 30 ng·mL^{-1} (78). In a cohort obtained through sampling individuals at a sport medicine clinic in Israel, vitamin D insufficiency was reported to be 77% in a sample of 98 athletes (79). Eighteen female gymnasts (10-17 years of age) from Australia underwent a 25(OH)D evaluation in Lovell (80) and only three gymnasts demonstrated vitamin D sufficiency (> 30 ng·mL^{-1}). However, not all investigations into vitamin D status have yielded such staggering results. The prevalence of vitamin D deficiency was no more than 4% when seasonal samples were collected from athletes at the University of Wyoming (81). Vitamin D insufficiency in this population demonstrated more fluctuation with the highest prevalence in the winter (60.6%) followed by a marked improvement in the spring (16%) and fall (9.8%) (81). Athletes that practice and compete indoors may be at increased risk for poor vitamin D status (82). Both Halliday et al. (81) and Constantini et al. (79) cited better vitamin D status in outdoor athletes when compared to indoor athletes. However, this phenomenon was only apparent in the fall when seasonal sampling was used in Halliday et al. (81).

2.5 Correcting Vitamin D Deficiency

If vitamin D insufficiency is present, oral supplementation or sun exposure are potentially effective means to raise vitamin D levels. Brief exposure to sunlight, provided an adequate UVB spectrum (290-315 nm) is present, allows for large amounts of vitamin D synthesis (3). This occurs even with low quantities of exposed skin (83). However, there are a variety of factors that interfere with subcutaneous vitamin D synthesis, and clinical trials investigating sun exposure prescriptions for improving vitamin D status have reported poor effectiveness (84). Vitamin D supplementation is effective at augmenting vitamin D levels. The most commonly cited dose response to vitamin D supplementation is 100 IU for every 1 ng·mL^{-1} increase in 25(OH)D (45,

55, 75). This dose response relationship fluctuates based on individual factors (85). The most prominent factor affecting this relationship appears to be the baseline vitamin D status of the individual (71, 83, 85).

Aloia et al. (83) set out to determine the appropriate vitamin D supplemental dose to achieve serum 25(OH)D levels above 80 $nmol \cdot L^{-1}$ and below 200 $nmol \cdot L^{-1}$ in a six month trial. The population sample included black and white men and women ranging in age from 18-65 years (83). The vitamin D dose was adjusted three times during the trial on an individual basis (83). The reported mean supplemental dose that achieved the specified desired vitamin D status was 3440 IU (83). When mean dose was stratified based on race, the mean supplemental dose was higher in black participants (3916 IU) when compared to white participants (3040 IU) (83). However, the differing mean dose needed to achieve the desired vitamin D status was not attributed to a race-specific response to vitamin D supplementation; it was suggested that the high mean dose observed in blacks was likely the product of lower baseline vitamin D status (83). Cherniak et al. (86) cited a mean increase of 14.3 $ng \cdot mL^{-1}$ $25(OH)D_3$ in response to 2000 IU/day for six months in a veteran population with a baseline mean level of 28.4 $ng \cdot mL^{-1}$. According to Bischoff-Ferrari et al. (71) a vitamin D supplemental dose of approximately 1,800 to 4,000 IU daily was needed to achieve sufficient vitamin D status in most participants in studies reviewed. When evaluating the risk of vitamin D toxicity with oral supplementation, Bischoff-Ferrari et al. (71) concluded minimal risk of toxicity with daily supplemental doses below 10,000 IU. Intermittent high dose supplemental protocols can also raise vitamin D level and not result in toxicity (87). However, it has been suggested that low-frequency, high-dose supplementation may not be an appropriate means of raising vitamin D levels (88). The rationale provided by Dawson-Hughes and Harris (88) was that high dose vitamin D may upregulate D-24-hydoxylase activity and subsequently reduce circulating 25(OH)D levels.

2.6 Vitamin D and Mechanisms of Action in Skeletal Muscle

Muscle was identified as a target tissue of the biologically active form of vitamin D when Simpson et al. (89) first identified the presence of a vitamin D receptor (VDR) in muscle using a chick myoblast cell line. Sixteen years later, the VDR was discovered in human skeletal muscle (90), and this finding was later duplicated (91). However, the presence of the VDR in human muscle tissue has recently been called into question (92).

In its biologically active form, $1,25(OH)_2D$ exerts its influence on muscle through an interaction with the VDR. First, $1,25(OH)_2D$ binds with the VDR in skeletal muscle (14). As a subsequent result of this binding, the VDR undergoes a conformational change because the repressor can no longer bind to the VDR (11). Next, a heterodimer is formed between the VDR and retinoic receptor (14). This heterodimerization of the VDR and the retinoic receptor along with other protein interactions allow for the formation of a transcription complex (11). The transcription complex interacts with the DNA, which leads to gene dependent transcription (11, 14). This process represents the genomic pathway in which $1,25(OH)_2D$ elicits action in a target tissue. Proliferation, differentiation, and phosphate and calcium modulation in muscle cells are all proposed genomic effects of $1,25(OH)_2D$ (14). The genomic pathway may also affect phospholipid metabolism (93).

It is believed that $1,25(OH)_2D$ can also influence muscle in a more rapid fashion which is referred to as the non-genomic pathway. These rapidly induced effects occur too swiftly to be accomplished through the gene transcription process (15). The exact manner in which the non-genomic signaling is accomplished remains unclear (14, 15). Two proposed mechanisms are present in the literature. One explanation is the presence of an alternative cell membrane receptor (11). The alternative rationale is that $1,25(OH)_2D$ initiates a process that translocates the nuclear vitamin D receptor to the cell membrane (94). Calcitriol binding to the VDR in the cell membrane (novel to translocated VDR) and initiating non-genomic effects is thought to occur through

mitogen-activated protein kinase signaling (MAPK) (15) or cyclic adenosine monophosphate (AMP) pathways (16). Muscle cell apoptosis, proliferation, and differentiation appear to be affected through MAPK pathways (14). Muscle cell calcium control may also be influenced by MAPK signaling pathways due to altered activity of calcium channels (93, 95). This may impact calcium concentrations in the sarcoplasmic reticulum, which, in turn, may influence muscle function (95, 96). Myogenic regulation seems to be modulated by the non-genomic pathway (14). Muscle growth may be influenced by the effects associated with the non-genomic action of $1,25(OH)_2D$ (2).

The impact of vitamin D on protein synthesis and muscle contraction has been evaluated using animal models. Muscle contraction and relaxation characteristics were found to be compromised in vitamin D depleted animals (17). Troponin C and actin protein reductions have also been documented (17). All of these effects were reversed when the animal's vitamin D status was normalized (17). Calcitriol has also been shown to reduce myostatin expression *in vitro* (97). Myostatin is a myoregulator that inhibits muscle cell proliferation and growth (98). Myoregulation disruptions are expressed in mice devoid of a vitamin D receptor (18). These mice also have reductions in muscle size and increased expression of immature muscle fibers when compared to their wild type counterparts (18).

2.7 The VDR Knockout Mouse Model

Attempting to establish skeletal muscle tissue as a physiological target of $1,25(OH)_2D$, Endo et al. (18) utilized the VDR knockout mouse model. This experimental animal model allows for developmental comparisons to be made between VDR knockout mice (mice devoid of VDR) and wild type controls (18). In mice devoid of the VDR, the impact of $1,25(OH)_2D$ is negated due to the absent VDR which is needed for signaling (11, 14). The VDR knockout mice begin to express rickets symptoms after weaning which occurs approximately three weeks after birth (18). This symptom expression temporal characteristic is valued, because it enables the evaluation of

morphological discrepancies independent of secondary influences associated with rickets symptoms (18). Using the VDR knockout model, Endo et al. (18) demonstrated that the VDR is necessary for normal skeletal muscle development in mice.

Atypical skeletal muscle developmental was reported in the VDR knockout mice (18). Muscle samples from the quadriceps femoris were obtained three weeks after birth (18). When compared to the wild type controls, VDR knockout mice demonstrated reduced muscle cell diameter (20%) and a wider cell diameter distribution pattern (18). Morphological expression differences were even more apparent at eight weeks (18). Endo et al. (18) reported atypical skeletal muscle development in the VDR knockout mice that did not display a predisposition for a specific skeletal muscle fiber type (type I, type IIa, type IIx).

To delve into the mechanisms responsible for the observed differences in skeletal muscle, Endo et al. (18) evaluated myogenic regulatory factors (MRFs) and myosin heavy chain isoforms. The MRFs are expressed exclusively in skeletal muscle tissue and are essential in directing skeletal myogenesis (99, 100). The MRFs are expressed in a sequential pattern during myocyte maturation and this temporal expression seems to allow for a normal developmental program (99, 100). The myocyte maturation sequence begins with an undifferentiated mesodermal cell; transitions to a myoblast; multiple myoblasts fuse forming a myotube; and further maturation of the myotube results in a mature skeletal muscle fiber (100). Disruptions in the temporal characteristic of MRFs may alter myocyte maturation leading to abnormal skeletal muscle development (99, 100). Myogenic regulation disruptions were reported when VDR knockout mice were compared to the wild-type controls: persistent elevation of myf5, E2A, and myogenin (18). Disrupted myogenic regulation was coupled with abnormal skeletal muscle development (18). Myosin heavy chain (MHC) also displayed altered expression in the VDR knockout mice: increased embryonic and neonatal myosin MHC was detected when compared to wild-type controls (18). The disrupted myogenic regulation and abnormal muscle development reported by

Endo et al. (18) provides evidence to indicate a role of the VDR in the myocyte maturation process. It appears that the VDR's role in myocyte maturation is sequence-specific down-regulation of myf5, E2A and myogenin (18).

If the VDR does modulate the expression of MRFs during the myocyte maturation developmental program, a possible role in muscle regeneration and repair may deserve consideration. Satellite cells are responsible for the majority of postnatal skeletal muscle growth (100). Muscle hypertrophy and regeneration are also mediated by satellite cells (100). These cells become active in response to skeletal muscle stress such as injury and mechanical loading (100). In essence, satellite cells recapitulate the process of skeletal muscle myogenesis that was previously depicted (100). Satellite cells respond to a trigger stimulus (skeletal muscle stress), begin to divide, and fuse with myofibers (100). Of specific interest, satellite cells are signaled for activity via MRFs (100). Moreover, the following satellite cell developmental program is also influenced by MRFs (100). Since the sequential expression of MRFs are critical for normal muscle development (99, 100) and the VDR is associated with altered expression of both, consideration may be warranted for a potential role of the VDR in skeletal muscle growth, repair, and adaptability (18).

2.8 Skeletal Muscle Tissue Repair, the Immune System, and Vitamin D

The sequence of events that enables skeletal muscle tissue regeneration involves the immune system (101). When skeletal muscle incurs structural injury, the injured muscle tissue must be degraded and cleared before satellite cell mediated myogenesis can replace the damaged tissue (102). An immune response is triggered (innate immune system) in such an event and it involves a balance between proinflammatory and anti-inflammatory activity (101, 103). Focusing on the macrophage, the following section will review the immune system's involvement in skeletal muscle repair and how vitamin D may affect this process.

The macrophage appears to play a crucial role in achieving the appropriate inflammatory response (101) and may be a modulating factor in the time needed for skeletal muscle repair (102). It seems that macrophages have a dual role in skeletal muscle repair: degradation and regeneration (101-103). Type I macrophages are proinflammatory, releasing cytokines, and have been associated with degradation of damaged tissue (101); type II macrophages favor the regenerative process and possess the ability to dampen the inflammatory response (101). Both populations of macrophages may influence satellite cell mediated myogenesis via release of growth factors (101, 102). Reinforcing the importance of macrophages in the skeletal muscle repair process, Summan et al. (102) demonstrated that this process could be slowed by pharmacologically reducing the macrophage population in damaged muscle tissue. Summan et al. (102) noted a disruption in necrotic tissue clearance in the pharmacologically treated muscle tissue. A trend for fat infiltration in muscle tissue was also reported in the treatment group (102). This demonstrates that disruption to the macrophage population in injured skeletal muscle may result in disruption to the regenerative process.

Skeletal muscle macrophage populations may also influence an individual's response to exercise (102). By comparing macrophage populations and cytokine expression in young (31.9 years) and aging (71.4 years) healthy participants, Przybyla et al. (103) demonstrated that aging impacts macrophage and cytokine response to a single bout of hypertrophy-oriented resistance exercise. Both type I and type II macrophage populations increased in response to the training stimulus in the young group, but this increased expression was not mimicked in the aging group (103). Przybyla et al. (103) noted that in both groups type II macrophages were the predominate macrophage in skeletal muscle. Cytokine expression also differed between groups (103). Prior to the resistance training exposure, elevated levels of cytokines (IL-1B, IL-1RA, IL-10) were present in the aging group (103). A blunted cytokine expression was cited after the exercise exposure in the aging group as various cytokines (IL-1B, AMAC-1, IL-10) were upregulated in

the young group, but no change occurred in the aging group (103). Przybyla et al. (103) attributed the disrupted cytokine expression in the aging group to the altered macrophage response to the exercise exposure. Aging is associated with declining muscle mass and physical performance (104). It is possible that the differences in the inflammatory response may contribute to the documented age related decline in muscle mass and physical performance (103). The cytokine and macrophage disruptions documented in Przybyla et al. (103) may have a deleterious impact on skeletal muscle tissue homeostasis, regeneration, and adaptability.

It appears that vitamin D is an influential factor in the functioning of the macrophage. Considering innate immunity, much focus has been placed on the role of vitamin D in macrophage functioning (105). Macrophages have the ability to produce and respond to $1,25(OH)_2D$ as they possess both the VDR and 1-alpha hydroxylase (106). Calcitriol is involved in macrophage functioning through its influence on monocyte maturation, phagocytosis, cathelicidin, and reactive oxygen species production (105, 107-109).

Vitamin D not only impacts macrophages, it is involved in the normal functioning of the immune system. Cells of both innate and adaptive immunity are frequently discussed targets of $1,25(OH)_2D$ (105, 107-109), as the vast majority of immune cells possess a VDR (107). The maturation, signaling (105), and survival of dendritic cells are impacted by $1,25(OH)_2D$ (108). Recently, seasonal differences in innate immune response have been detected and linked to seasonal changes in vitamin D status (110). Vitamin D has bearing on adaptive immunity as well; it has been implicated in the modulation of immunoglobulins, T-helper cells, and lymphocyte populations (105, 109).

It is possible that vitamin D may impact skeletal muscle regeneration due to its role in immune function. The macrophage both produces and responds to the active hormone (106). Poor vitamin D status may negatively influence macrophage functioning. This has been the case for macrophage-produced cathelicidin (109). If macrophage function is negatively altered due to low

vitamin D status, muscle regeneration could be impacted due to the macrophage's role in phagocytosis and signaling.

2.9 Vitamin D and Cardiovascular Function

Along with the previously described impact on the immune mediated inflammatory response, vitamin D may also play a beneficial role in other factors that contribute to cardiovascular function and health (111, 112). It is not the intent of this review to discuss in depth the mechanisms in which vitamin D may influence cardiovascular function, but rather provide a brief overview. The relationship between vitamin D and the cardiovascular system may provide important insight when evaluating the possible mechanisms in which vitamin D could possibly impact physical performance during tasks primarily dependent on aerobic metabolism.

It appears that vitamin D is an essential regulator of multiple tissues and systems involved in the cardiovascular system (111, 112). The VDR and 1-alpha hydroxylase activity have been identified in both cardiac and vascular tissue (111, 113) allowing these tissues the capability to produce $1,25(OH)_2D$ from 25(OH)D and respond to it. Calcitriol has been implicated as a possible factor affecting the structural remodeling of cardiac muscle (111, 113) and endothelial tissue (114). Matrix-metalloproteinases (MMP) are also thought to be involved in cardiac and vascular remodeling and $1,25(OH)_2D$ may alter MMP expression (111). Cardiac muscle contractility appears to be modulated by $1,25(OH)_2D$ *in vitro* (115) and improvement in cardiac muscle function has been reported in hemodialysis patients when vitamin D deficiency was remedied (111). Improvements in flow-mediated dilation and blood pressure have been linked to correcting vitamin D deficiency (112, 116). Vitamin D may support nitric oxide production in endothelial tissue, which is involved in blood pressure control and endothelial dilation (111). The renin-angiotensin system which is also involved in blood pressure regulation appears to be regulated by $1,25(OH)_2D$ (113, 115). The overexpression of renin is associated with increased blood pressure and $1,25(OH)_2D$ may ameliorate renin expression (113, 115). Vitamin D

may also have a beneficial impact on atherosclerotic progression due to its participation in immune function and associated inflammatory response (111).

Vitamin D appears to have a substantial role in the maintenance of the cardiovascular system. Recent investigations have linked vitamin D deficiency to reduced performance in tasks predominately involving aerobic metabolism (29) and cardiorespiratory fitness measures (37, 38). These investigations will be reviewed, in detail, in a later section. The mechanisms by which vitamin D may influence aerobic task performance are not known (38). It is possible that vitamin D deficiency compromises the cardiovascular system, which subsequently results in reduced aerobic physical performance.

2.10 Vitamin D and Muscle Fat Infiltration and Lean Body Mass

An emerging theory suggests that insufficient vitamin D status may be related to increased muscle fat infiltration (19, 20). It is postulated that vitamin D status plays a role in sarcopenia progression and prevention (96, 104, 117). Sarcopenia typically refers to the loss of skeletal muscle (104, 117). Increased muscle fat infiltration also occurs in the progression of sarcopenia (117). Observational investigations have reported an inverse association between 25(OH)D status and the progression of sarcopenia (118-120). In a cohort of 845 men ranging in age from 45-85 years, Szulc et al. (118) documented decreased relative skeletal muscle mass scores in men with 25(OH)D levels below 10 $ng \cdot dL^{-1}$ when compared to those with sufficient status (118). Higher levels of 25(OH)D were protective against sarcopenia independent of parathyroid hormone in a cohort of men and women ranging in age from 55-85 years (119). In another prospective cohort of older men and women, Scott et al. (120) found baseline 25(OH)D status to be associated with a relative measure of skeletal muscle mass. Baseline 25(OH)D was also positively associated with a change in leg strength and an estimate of leg muscle specific force in a 2.6 year follow-up (120). When evaluating 20 men and women with a mean age of 77.6 years, Tagliafico et al. (19) found an inverse relationship between 25(OH)D status and fatty

degeneration of thigh skeletal muscle. Tagliafico et al. (19) also documented complete selective atrophy of one thigh muscle only in participants with a 25(OH)D status below 20 $ng \cdot mL^{-1}$. Thus, vitamin D status may impact age-related skeletal muscle atrophy that leads to muscle fat deposition (19).

Interestingly, the association between 25(OH)D and muscle fat infiltration has not only been observed in aging populations. Gilsanz et al. (20) also cited an association between 25(OH)D status and thigh muscle fat. An inverse association between 25(OH)D status and percent muscle fat was found in this population of 90 females, devoid of clinical symptoms of vitamin D deficiency, with an age range of 16-22 years (20). This association was independent of measures of physical activity, BMI, subcutaneous fat, and visceral fat (20). When vitamin D status was dichotomized into sufficiency or insufficiency, an effect size of 24% was reported for increased muscle fat in the insufficient group when compared with the sufficient group (20). Vitamin D status was not associated with muscle cross-sectional area in Gilsanz et al (20).

Increases in lean mass have been reported with vitamin D supplementation (42) and cross-sectional studies have also documented this association (121, 122). Stewart et al. (121) found vitamin D status to be associated with lean body mass in postmenopausal women; however, vitamin D status accounted for only 1% of the variance in lean body mass. The association between vitamin D status and lean body mass was also reported in a population of Chinese adolescent girls (122). Fuleihan et al. (42) investigated the effects of low (200 IU/day) and high dose (2000 IU/day) vitamin D supplementation in 179 adolescent girls ranging in age from 10-17 years. Increases in lean mass after a one-year intervention were cited in both low ($p = 0.04$) and high dose ($p = 0.001$) supplementation groups when compared to the control (42). However, no significant difference was found when evaluating strength measures (42). Not all investigations have reported results consistent with these findings (43, 123). Marantes et al. (43) found no relationship between 25(OH)D and muscle mass in a cohort of men and women ranging

in age from 21-97 years. However, when looking at younger participants (under 65 years), 25(OH)D was associated with muscle mass in women only (43). Interestingly, $1,25(OH)_2D$ was positively associated with muscle mass regardless of age (43). Moreover, when the analysis was restricted by age, participants with higher $1,25(OH)_2D$ levels possessed greater muscle mass and this relationship remained significant after adjustment for confounders in both men and women (43).

Both muscle cross-sectional area reductions and degree of muscle fat may influence an individual's physical performance (19, 20). Muscle cross-sectional area is associated with muscle strength and higher levels of muscle fat have been associated with disruptions in muscle metabolism (20). However, it is not clear if vitamin D influences either muscle cross-sectional area or muscle fat accumulation (19, 20).

2.11 Vitamin D and Body Adiposity

Moving beyond muscle, the VDR is also found in adipose tissue and its presence may indicate that vitamin D signaling could participate in fat metabolism (124). It appears that $1,25(OH)_2D$ has both genomic and non-genomic signaling in adipose tissue (124). This signaling in adipose tissue seems to modulate calcium channels, uncoupling protein 2 (UCP2) and glucocorticoid expression (124). Exposure to $1,25(OH)_2D$ increases calcium channel activity in adipocytes and subsequently increases intracellular calcium (124). Increased intracellular calcium in adipocytes impedes lipolysis and activates fatty acid synthase (124). Calcitriol inhibits uncoupling protein (UCP) expression, which is thought to play a role in thermogenesis by favoring a reduced mitochondrial potential (124, 125). Adipose apoptosis is stymied by $1,25(OH)_2D$ (124). Glucocorticoids, which promote cortisone production, are upregulated by calcitriol (124). Calcitriol appears to be involved in adipocyte-related production of reactive oxygen species and various cytokines that may also influence metabolism (124). Calcitriol's signaling in adipose tissue appears to promote the conservation and storage of energy in mature

adipocytes. In the pre-adipocyte, $1,25(OH)_2D$ exposure may inhibit maturation (126). It is not clear if vitamin D status is causally associated with adipocyte accumulation *in vivo*; however, the association between vitamin D status and body fat can be found in the literature (127-130).

An association between vitamin D and body fat has been reported in a few cross-sectional studies (127-131). Vitamin D status was correlated with subcutaneous and visceral adiposity measures in a cohort of male and female high school students ranging in age from 14 to 18 years (128). Kremer et al. (129) also reported an association between vitamin D status and subcutaneous and visceral adiposity in young women (16-22 years). When vitamin D status was dichotomized, women who were vitamin D insufficient had increased subcutaneous and visceral fat when compared to women with sufficient status (129). Using the Framingham Heart Study cohort, Cheng et al. (130) also reported vitamin D status to be inversely related to subcutaneous and visceral adiposity in both men and women. Caron-Jobin et al. (131) replicated these findings in a population of female participants. Stewart et al. (121) found central adiposity related to vitamin D status in postmenopausal women. The results from Looker (127) suggest that the relationship between vitamin D status and adiposity may be modulated by race and age in women. The strength of the relationship was stronger in white women when compared to black women (127). Looker (127) also found that age attenuated the relationship between vitamin D status and body fat percentage in both white and black women, but this effect was more pronounced in black women. Whether sex is a modulating variable in the relationship between vitamin D status and adiposity has yet to be discerned; however, investigations targeting female populations appear to be more prevalent in the literature.

It appears that there is enough preliminary evidence supporting the link between vitamin D and adiposity to warrant randomized control trials. However, evidence from the few trials that have been conducted does not support a causal relationship between vitamin D and adipose accumulation (132). It is possible that thresholds for calcium and vitamin D status should be

considered when selecting study participants (132, 133). Only in a subgroup analysis composed of women who consumed little calcium did Major et al. (133) find significant weight loss in the treatment (400 IU vitamin D and 1200 mg calcium) group compared to the control during a weight loss intervention. More interventional trials are needed to evaluate if vitamin D is causally associated with adiposity (132).

The exact manner in which improving vitamin D status may promote fat loss is unknown and likely complex (127, 130). It may be that sufficient vitamin D status normalizes parathyroid hormone production which results in reduced $1,25(OH)_2D$ exposure in adipocytes (130). Cheng et al. (130) proposed that vitamin D insufficiency might favor increased adipogenesis. Reverse causality cannot be ruled out until temporality is established (129). It may be that low vitamin D status is associated with adiposity because adipose tissue is a storage site and sequesters vitamin D (130, 132). It is also possible that the association between vitamin D and adiposity is due to levels of physical activity (132). Persons with higher levels of adiposity are less likely to participate in outdoor physical activity, thereby limiting sunlight exposure and endogenous vitamin D synthesis (132).

2.12 VDR Polymorphisms

Another observation connecting vitamin D to physical performance is the association of VDR polymorphisms to physical performance measures in senior populations (33-36). The VDR is one of the few genes to be associated with physical performance measures in multiple investigations (134). Reduced incidents of falling have been reported in individuals possessing the bb BsmI VDR genotype (33, 36). The bb BsmI VDR genotype has also been associated with higher leg muscle power (36). Reduced isometric quadriceps strength was reported in individuals with a VDR Fokl polymorphism, CC genotype. The results from Windelinckx et al. (35) also demonstrated an association between quadriceps strength and VDR polymorphisms; however, specific VDR polymorphisms linked to higher quadriceps strength varied by sex. The data

involving VDR polymorphisms and differences in physical performance outcomes seem to indicate that vitamin D is involved, to some extent, in muscle's functional capacity and that VDR polymorphisms may influence that relationship.

2.13 Vitamin D Status and Measures of Physical Performance

The vast majority of the data regarding the influence of vitamin D on measures of physical performance is skewed toward senior populations. Six studies were found that investigated the topic, using physical performance endpoints, which did not utilize populations with only advanced age participants. Of these, four investigations involved adolescent populations, which were female-only cohorts (21, 23, 41, 42). One cross-sectional study was found that sampled men and women with a broad age range (43). One study has been conducted that employed an interventional study design with the intent of evaluating the impact of vitamin D levels on physical performance in a young and healthy population that included both male and female participants (29). At present, there seems to be substantial evidence from cross-sectional studies (21, 23-28, 135-137) and interventional trials (29-32, 39-41, 123, 138) that vitamin D may play a role in muscle function, which is expressed and subsequently evaluated using physical performance outcome measures. These outcome measures include both measures of upper-body and lower-body muscle function. However, not all controlled trials have detected a relationship between 25(OH)D levels and muscle function (42, 139-143) and a recent meta-analysis supports the null (144). Study replication of randomized control trials is needed that investigate the effect of vitamin D supplementation on physical performance as a primary endpoint (145). The extent to which outcomes relating to vitamin D status and physical performance in aging populations will be mirrored in younger populations is not yet established. If vitamin D status does play a role in physical performance in these individuals, the levels at which it elicits deterioration in performance need to be determined. Although sex-specific outcome differences concerning

vitamin D status have been documented and deserve further exploration (22, 43, 146, 147), the majority of the research does not appear to support sex as a moderating variable.

The only measure employed to quantify upper-body extremity function relative to vitamin D status and muscle function has been grip strength. Grip strength has been positively associated with levels of 25(OH)D (23, 25, 26). Mowe et al. (26) examined an aging population (70-90 years) and reported reduced grip strength in participants with lower 25(OH)D levels. These results were replicated by Houston et al. (25), who incorporated a larger population in their cross-sectional study design, with individuals ranging in age from 65 to 102 years. In a population of Chinese adolescent girls, Foo et al. (23) also demonstrated a relationship between 25(OH)D status and handgrip strength. Adolescent girls with a 25(OH)D status greater than 50 $nmol \cdot L^{-1}$ (20 $ng \cdot mL^{-1}$) demonstrated high grip strength values when compared to girls who possessed 25(OH)D levels that fell below the 50 $nmol \cdot L^{-1}$ stratification value (23). This association held after numerous adjustments were made in the statistical model including pubertal stage, physical activity, and body size (23). Improvements in grip strength have also been documented in interventional vitamin D supplementation trials (29). In a population of vitamin D deficient, but otherwise healthy, subjects ranging in age from 20 to 40 years, Gupta et al. (29) utilized a two-stage oral supplementation protocol with the treatment group receiving 60,000 IU D_3 per week for the first 2 months and 60,000 IU D_3 per month for the remaining four months of the study. The treatment was effective at increasing 25(OH)D levels (29). Increased 25(OH)D levels in the treatment group resulted in increased grip strength along with other measures of physical performance when compared to the subjects who received the placebo (29).

Not all investigations have found associations or improvements in grip strength with increases in vitamin D status (27, 32, 41-43, 140, 146). Ceglia et al. (147) did not detect an association between 25(OH)D levels and grip strength in a multi-ethnic cohort of 1219 males living in Massachusetts. Smedshaug et al. (140) failed to find improvements in physical

performance when using grip strength to quantify upper-body muscle function in their supplementation intervention. Daily vitamin D_3 (10 μg) was given to the treatment group and the control group received a placebo (140). Grip strength was evaluated at baseline and after one year (140). Smedshaug et al. (140) concluded that 10 μg (400 IU) of vitamin D_3 taken daily is not effective at improving grip strength in advanced age nursing home residents. Possible explanations for a lack of effect of vitamin D supplementation on grip strength included lack of vitamin D deficiency in the population examined, and a vitamin D dosing protocol that was too conservative (140). Marantes et al. (43) concluded that 25(OH)D was not associated with grip strength or other strength measures in a cohort of men and woman ranging in age from 21-97 years. Conversely, the active hormone, $1,25(OH)_2D$, was positively associated with grip strength and knee extensor strength, though in women only (43). Dam et al. (146) did not find an association between grip strength and vitamin D status in older adults; however, 25(OH)D status was associated with other measures of physical performance in women, but not men.

It is possible that grip strength is not as sensitive as other measures of physical performance when evaluating the effects of vitamin D on physical performance. Bunout et al. (32), LeBoff et al. (27) and Ward et al. (41) have all demonstrated relationships between measures of physical performance and vitamin D status, but none of these investigations found improvements or associations between vitamin D status and grip strength. In a population of female hip fracture patients, LeBoff et al. (27) reported improved lower-body function composite score (LEGS performance) during the 12-month evaluation period in women with 25(OH)D above 22.5 $nmol \cdot L^{-1}$ (9 $ng \cdot mL^{-1}$) when compared to women below that value. Women with 25(OH)D above 22.5 $nmol \cdot L^{-1}$ also experienced fewer falls during the 12-month post-fracture observation period (27). Grip strength remained unchanged during the duration of the evaluation period in this population, while lower-body extremity function improved and was associated with vitamin D status (27). When subjects with a 25(OH)D level of 16 $ng \cdot mL^{-1}$ or below were

supplemented with 800 mg of calcium and 400 IU of vitamin D per day for nine months, gait speed, timed-up-and-go test, and body sway all increased while grip strength remained unchanged (32). In a recent vitamin D supplementation interventional trial involving vitamin D deficient (< 15 ng·mL^{-1}) adolescent females (postmenarchal, 12-14 years of age), Ward et al. (41) reported increases in vertical jump velocity in the treatment group when compared to the control group after a period of one year. This intervention did not detect any change in grip strength (41). The trend of detecting an association between 25(OH)D status and lower-body physical performance, but not in grip strength, may suggest a reduced sensitivity of grip strength as a measure. Sensitivity of lower-body measures of physical performance could potentially be increased due to the quantification of measures involving larger muscle cross-sectional area, corresponding force capacity, and more complex movement tasks. It could also point to the pathophysiology of vitamin D deficiency manifesting as deterioration of functional ability in the larger muscles of the lower body.

Numerous investigators have found positive associations between vitamin D status and lower-body extremity performance (21, 24-26, 28, 135-137). In an older population of men and women, Mowe et al. (26) documented a relationship between 25(OH)D status, stair climbing ability and a reduced incidence of falling. Thirty-meter gait speed has also been associated with levels of 25(OH)D (136). Gerdhem et al. (136) found that elderly women with 25(OH)D levels below 30 ng·mL^{-1} had reduced 30-meter gait speed when compared to women with 25(OH)D levels above 30 ng·mL^{-1}. A positive association between 25(OH)D status, timed sit-to-stand, and the 8-foot walk test have been described in both men and women who were 60 years of age and older (28). Lower-body extremity composite scores and 25(OH)D levels have also been linked (24, 25). Wicherts et al. (137) tracked lower-body functional changes (walking test, chair stands, tandem stands) across a three-year time period in a population of 1234 men and women with a mean age of 75.3 years. Subjects were stratified into four groups based on 25(OH)D status (137).

The four strata were composed of individuals who met the following 25(OH)D criteria: < 10 $ng \cdot mL^{-1}$, 10-20 $ng \cdot mL^{-1}$, 20-30 $ng \cdot mL^{-1}$, ≥ 30 $ng \cdot mL^{-1}$ (137). All participants in the first and second strata displayed reduced physical performance when compared to the ≥ 30 $ng \cdot mL^{-1}$ 25(OH)D group (137). After a three-year follow-up period, the first two strata groups were also more susceptible to a decline in physical performance (137). In a population of 12 to 14 year-old post-menarchal girls from the United Kingdom, Ward et al. (21) concluded that muscle function was influenced by 25(OH)D status. Utilizing vertical jump performance as the measure of lower-body physical performance, Ward et al. (21) documented a relationship between 25(OH)D status and force, power, velocity and jump height.

Interventional study designs investigating the effect of vitamin D supplementation on lower-body muscle function in vitamin D deficient aging populations have also demonstrated that vitamin D levels may influence muscle function (29-32, 39-41, 123, 138). Bunout et al. (32) incorporated both a supplement and exercise exposure in their investigation. Subjects were selected from an elderly community and had to meet the inclusion criteria of a 25(OH)D level of 16 $ng \cdot mL^{-1}$ or less (32). The supplement exposure consisted of 800 mg of calcium and 400 IU vitamin D_3 (32). After nine months, subjects who received the supplement and exercise protocol exposure saw the greatest improvement in the timed up-and-go test (32). Performance in both gait speed and body sway improved in the supplemental group independent of the exposure to the training protocol (32). Pfeifer et al. (31) also investigated the effects of a calcium and vitamin D preparation on lower-body muscle function in an aging population of both men and women. Inclusion criteria concerning baseline 25(OH)D status was set at below 78 $nmol \cdot L^{-1}$. The placebo group received 500 mg of calcium carbonate daily while the treatment group received both 500mg of calcium carbonate and 400 IU of vitamin D_3 daily (31). At the 12-month follow up, the treatment group demonstrated increased mean 25(OH)D levels, increased quadriceps strength (8 %), and improved timed up-and-go test (11%) (31). In a six-month vitamin D and calcium

interventional trial, Moreira-Pfrimer et al. (30) observed increased isometric hip flexor (16.4%) and knee extension (24.7%) strength in institutionalized elderly participants. Subjects were vitamin D deficient at the start of the trial (< 50 $nmol \cdot L^{-1}$) (30). The treatment protocol consisted of once a month 150,000 IU vitamin D_3 and 1,000 mg calcium doses for the first two months and a reduced dose of 90,000 IU vitamin D_3 and 1,000 mg calcium monthly for the remaining four months (30). This supplementation protocol effectively increased the treatment group's 25(OH)D levels by 84% and they were no longer considered vitamin D deficient (< 50 $nmol \cdot L^{-1}$) (30). The improved vitamin D status resulted in increased lower-body muscle function in institutionalized elderly whose activity patterns were unchanged during the trial (30). Sato et al. (39) reported an improvement in muscle strength of the hip and thigh using the British Medical Research Council scale in female hospitalized Alzheimer's patients with exposure to sunlight. Both the treatment and control group had deficient vitamin D status (39). Sunlight exposure was used as the treatment while the control group remained sunlight deprived (39). The sunlight exposed group increased group mean 25(OH)D levels from 24 $nmol \cdot L^{-1}$ to 52.9 $nmol \cdot L^{-1}$ while the deprived group mean 25(OH)D levels dropped from 24 $nmol \cdot L^{-1}$ to 10.7 $nmol \cdot L^{-1}$ (39). After the 12-month evaluation period, increased levels of 25(OH)D led to increased scores on the British Medical Research Council scale (lower-body muscle function assessment) and decreased falling incidents when compared to the sunlight deprived group (39). Using supplemental calcitriol ($1,25[OH]_2D$), Gallagher (40) cited reduced incidence of falling and reduced decay of both timed rising and timed walk performance measures in older women after a three-year observation period. Interestingly, these observations were made in a population that did not possess deficient vitamin D levels in either group (mean serum 25[OH]D 31 $ng \cdot mL^{-1}$) (40).

Control trials using supplementation protocols in non-aging populations have also demonstrated positive effects of vitamin D supplementation on lower-body physical performance. Ward et al. (41) investigated the effects of vitamin D_2 (ergocalciferol) supplementation on various

characteristics of vertical jump performance in 69 post-menarchal girls ranging from 12 to 14 years of age. Subjects needed to meet the inclusion criteria of a 25(OH)D level below 37.5 $nmol \cdot L^{-1}$ (41). The treatment group received a 150,000 IU ergocalciferol dose every three months for a duration of one year (41). At the one-year follow-up, the treatment group's mean 25(OH)D status was 56 $nmol \cdot L^{-1}$ and the control group's 25(OH)D status remained deficient (15.7 $nmol \cdot L^{-1}$) (41). When compared to the control, the treatment group had an increased efficiency regarding vertical jump performance (41). Efficiency of movement was defined as "maximum power relative to peak force" (41). Ward et al. (41) stated a probable rationale for this effect to be the product of non-significant increases in jump height, jump velocity, and power (41). Ward et al. (41) also found an association between 25(OH)D status and jump velocity in their baseline data. Girls with the most compromised 25(OH)D status at baseline had greater increases in jump velocity after vitamin D treatment (41).

In another trial, Gupta et al. (29) explored the effect of vitamin D supplementation on measures of physical performance in a population of 40 vitamin D deficient, but otherwise healthy, subjects ranging in age from 20 to 40 years. Participants either received a 1,000 mg calcium placebo or oral vitamin D_3 with 1,000 mg calcium (29). The treatment group's vitamin D_3 dosing consisted of 60,000 IU D_3 per week for the first 2 months and 60,000 IU D_3 per month for the remaining four months of the study (29). The supplementation protocol increased 25(OH)D levels in the treatment group from a mean of 25.4 $nmol \cdot L^{-1}$ to 94.5 $nmol \cdot L^{-1}$ at the end of the first two-month period (29). During the second four-month period, 25(OH)D mean values for the treatment group declined to 56 $nmol \cdot L^{-1}$ with the reduced vitamin D_3 dose (29). Note that a vitamin D_3 dose of approximately 2,000 IU per day was not enough to maintain vitamin D sufficiency in this population if the normal baseline value of 30-32 $ng \cdot mL^{-1}$ (75-80 $nmol \cdot L^{-1}$) was used to indicate sufficient 25(OH)D status (1, 3, 53, 58, 59). Nevertheless, the treatment group (56 $nmol \cdot L^{-1}$) expressed significantly higher 25(OH)D levels when compared to the control group

(29.7 nmol·L^{-1}) at the conclusion of the trial (29). Improvements in the strength of the gastro-soleus muscle and increases in the distance traveled during the 6-minute walk test were noted in the treatment group when compared to the control (29).

There is data that indicates that 25(OH)D status may be an influential factor in physical performance that is heavily dependent on aerobic metabolism (29, 37, 38, 148, 149). The evidence is primarily correlational data from cross-sectional investigations (37, 38, 148, 149); however, one randomized controlled trial indicated positive effects of vitamin D supplementation on aerobic performance (29). Vitamin D status has been found to be correlated with maximal oxygen consumption during maximal treadmill exercise tests in population samples including both men (38) and women with a population mean age of 40 and 49.6 years, respectively (37). The association between 25(OH)D and maximal oxygen consumption remained significant in both studies after adjusting for obesity, physical activity, and other potential confounders (37, 38). The results from Ardestani et al. (37) indicate hours spent participating in physical activity may attenuate the relationship between 25(OH)D status and maximal oxygen consumption. If this is the case, 25(OH)D status may have a more potent influence in the sedentary (149). Using a cross-sectional study design, Dong et al. (128) found 25(OH)D levels to be significantly associated with cardiorespiratory fitness that was evaluated using a submaximal treadmill test (128). Baseline measurements of 25(OH)D and 400-meter walk performance were correlated in an aging population (mean age 76.7 years) (148). In a large cohort of men and women (NHANES 2001-2006 data), Scragg et al. (149) found deficient 25(OH)D status associated with increased resting myocardial work, which was indirectly measured using rate pressure product. Boxer et al. (24) documented an association between 25(OH)D status and the distance traveled in the 6-minute walk test in 169 men and women undergoing outpatient heart failure treatment. Higher 25(OH)D status was associated with greater distance traveled (24). As previously mentioned, Gupta et al. (29) reported a significant effect of vitamin D supplementation on 6-minute walk

performance. The treatment group's mean distance traveled during the test was 15.9 meters greater than the control (29). The possible pathways through which vitamin D may influence aerobic capacity are not known at this time (24).

Not all interventions have detected a relationship between 25(OH)D levels and physical performance (139-143) and this notion is echoed by a recent meta-analysis (144). Kenny et al. (141) concluded that supplementation with 1000 IU vitamin D_3 and 500 mg calcium per day for six months in 65 healthy older men was not effective at improving physical performance. A possible explanation for the lack of effect of vitamin D_3 supplementation observed in Kenny et al. (141) is that the sample population of men was not vitamin D deficient. However, at the end of the trial the mean 25(OH)D status in the control group could be classified as insufficient according to the most accepted classification values (3, 4, 59). The findings of Kenny et al. (141) may be evidence that improving status from 25(OH)D values that are merely classified as insufficient will not result in increased physical performance. Lathamet et al. (139) evaluated the effectiveness of a single 300,000 IU calciferol dose in 243 frail elderly and concluded the treatment did not affect measures of physical performance. As previously mentioned in the discussion concerning 25(OH)D status and grip strength, Smedshaug et al. (140) found that supplementing 60 nursing home residents with 10 μg (400 IU) per day had no effect on grip strength after one year. The findings may have been influenced by the lack of vitamin D deficiency in the sample population and the dose of vitamin D_3 administered, which came from cod liver oil (140). Lastly, Brunner et al. (142) also failed to find any effect of supplementing 400 IU vitamin D_3 and 1,000 mg calcium carbonate on ameliorating the decline in physical performance in 3,137 women. Once again, this population was not selected on the basis of having a vitamin D deficiency and it is possible the daily vitamin D_3 dose may not have been adequate (142).

Stockton et al. (144) conducted a meta-analysis which included 17 randomized control trials involving vitamin D supplementation and measures of physical performance. Although numerous studies included in the analysis reported significant results independently, vitamin D supplementation was ineffective at improving various measures of physical performance when data was categorized and pooled (144). A review of randomized control trials involving vitamin D and measures of physical performance expressed concerns regarding the heterogeneity of current trials, making comparisons across trials difficult (145). Rejnmark (145) also articulated reservations regarding the statistical power of some trials since the majority of studies were not specifically designed to addresses vitamin D and measures of physical performance: these questions were secondary endpoints. Study replication of randomized control trials that investigate the effect of vitamin D supplementation on physical performance as a primary endpoint is needed (145).

Sex-specific outcome differences regarding vitamin D status and measures of physical performance have been documented (22, 43, 146, 147). However, the influence of sex on an individual's physical responsiveness to vitamin D appears to be unclear. Sex-specific outcomes associated with vitamin D have pointed to both female (146) and male (22) sex-specific outcome differences. Recently, Ceglia et al. (147) proposed that men may be less responsive to vitamin D treatment in regards to changes in physical performance. The responsiveness of an individual to improvements in 25(OH)D status and associated improved physical performance appears to be related to the degree of initial vitamin D deficiency and the magnitude of the 25(OH)D improvement. Whether or not sex is a moderating variable needs further investigation (147).

At this time, the data regarding vitamin D status and muscle function does not support a specific 25(OH)D level delineating sufficiency for optimal muscle function. Sufficient levels of circulating 25(OH)D are currently considered to be 30-32 $ng \cdot mL^{-1}$ (75-80 $nmol \cdot L^{-1}$) (1, 3, 53, 58, 59). However, it is not known if 30-32 $ng \cdot mL^{-1}$ represents optimal vitamin D status (58, 59).

In general, the correlation data dealing with muscle function and physical performance appears to support a delineating 25(OH)D level in the proximity of the proposed normative value: 30-32 ng·mL^{-1} or 75-80 nmol·L^{-1}. Gerdhem et al. (136) found decrements in gait speed and balance in older women whose 25(OH)D levels were below 30 ng·mL^{-1} when compared to women whose levels were above 30 ng·mL^{-1}. The data collected by Wicherts et al. (137) indicated that the majority of physical performance deterioration occurred below 16 to 20 ng·mL^{-1} 25(OH)D. Above the value of 20 ng·mL^{-1}, the association between 25(OH)D and physical performance deteriorated (137). Wicherts et al. (137) also reported that subjects with 25(OH)D levels below 10 ng·mL^{-1} were more likely to exhibit decreased physical performance after the three year follow-up period (137). Bogaerts et al. (138) and Verschueren et al. (123) reported increases in physical performance measures with vitamin D supplementation. These investigations also compared the effects of different vitamin D dosing protocols (880 IU and 1600 IU), both reporting no difference (123, 138). Almost all participant 25(OH)D levels were 20 ng·mL^{-1} or greater in the treatment group regardless of dosing protocol (123). This may indicate that a threshold 25(OH)D level may have been reached in both treatment groups or that the dosing protocols lacked distinction. Bischoff-Ferrari et al. (28) established a positive association between 25(OH)D status and physical performance. The degree of physical performance improvement with increased 25(OH)D status began to decrease at roughly 40 nmol·L^{-1}, but performance improvements with advancing 25(OH)D levels continued until 94 nmol·L^{-1} (28). The data collected by Houston et al. (25) gives rise to the notion that different measures of physical performance may be differentially affected by 25(OH)D status. Older men and women scored lower on both lower-body physical performance tasks and grip strength when 25(OH)D values were below 25 nmol·L^{-1} and 50 nmol·L^{-1}, respectively (25). In men, grip strength was found to be higher with 25(OH)D levels above 75 nmol·L^{-1} (30 ng·mL^{-1}) (25).

2.14 Seasons and Physical Performance

Cannell et al. (49) have recently broached the topic of seasonality of physical performance in a review. Since Cannell et al. (49) was published, the topic has been revisited by another review on vitamin D and physical performance (2), and others have characterized the possible phenomenon of physical performance being influenced by the changing seasons and vitamin D status as a "reasonable hypothesis" (51).

Seasonal changes in physical performance are proposed to be induced by the seasonal variation in 25(OH)D levels (49). Since exposure to sunlight has such a profound effect on vitamin D status, seasonal variation in 25(OH)D is commonly observed (2, 3, 57). First, seasonal weather patterns impact an individual's activity patterns and time spent outdoors. Time spent outdoors is associated with vitamin D status (54). Secondly, depending on geographical location and time of year, endogenous vitamin D synthesis may not be possible (54). This is the product of the trajectory of the UVB radiation and the inability of the radiation to penetrate the earth's atmosphere (54). In relation to calendar year, vitamin D status usually reaches a peak around August, begins to decline thereafter, and approaches a nadir in approximately March (2, 49, 51). It has been suggested that physical performance trends should follow this same seasonal pattern if vitamin D does affect measures of physical performance (49). Whether this phenomenon exists has yet to be elucidated in the literature. Wehr et al. (51) have expanded the seasonal performance trends and vitamin D discussion to include testosterone as a potential mediating variable.

2.15 Vitamin D and Androgens

It is known that androgens fluctuate on an hourly, daily, monthly and yearly basis (150, 151). Of these hormones, testosterone has received significant attention regarding its seasonal variation (51, 150-154). Testosterone is an anabolic steroid hormone and has been associated with muscle protein accretion (155) and measures of physical performance (156). It has been hypothesized that seasonal variation of testosterone could, to some extent, explain seasonal

athletic performance trends (51). It has been proposed that physical performance also exhibits seasonal fluctuations with peak performances being obtained in August (49). Seasonal performance trends would indicate that late summer should correspond with peak testosterone levels. Not all of the literature supports this speculation (151-153). However, some of the research does appear to correspond with the stated seasonal performance trend (51, 150, 154). The rationale and pattern for the observed seasonal variations in testosterone is not clear (150-153). Possible explanations for the seasonal variation in testosterone are mean air temperature (150), duration of daylight, body weight, and physical activity (153). Age may also impact observed seasonal variations in testosterone (151). Recently, a new explanatory variable has been examined as a possible influential factor regarding seasonal testosterone fluctuation: 25(OH)D. Levels of 25(OH)D have been reported to exhibit seasonal fluctuations (51) that also correspond to seasonal variations in testosterone (51, 150, 154) and physical performance trends (49).

The associations between vitamin D and serum androgens have been documented in men (51, 157). Wehr et al. (51) investigated this relationship in 2299 male coronary angiography patients using a cross-sectional study design. Eighteen percent of the sample population met the criteria for hypogonadism (testosterone levels < 11.3 nmol/L) (51). The men categorized as hypogonadal demonstrated significantly lower ($p< 0.001$) mean 25(OH)D levels when compared to men who met the criteria for sufficient gonad function (51). Wehr et al. (51) demonstrated associations among 25(OH)D, testosterone, free androgen index (FAI), and serum hormone-binding globulin (SHBG), and these associations were not significantly altered after various adjustments were performed using multiple regression. A seasonal trend was reported among 25(OH)D, testosterone, and FAI (51). All three variables peaked in August and reached their lowest values in March (51). When evaluating the seasonal difference of levels of 25(OH)D, testosterone, and FAI, nadir to peak differences were recorded at 64%, 16%, and 18%, respectively (51). Although an association among 25(OH)D, testosterone, and FAI was apparent,

no distinct mathematical relationship among these variables existed (51). However, Wehr et al. (51) established a possible link among vitamin D, testosterone, and FAI. Wehr et al. (51) also demonstrated that 25(OH)D insufficiency/deficiency corresponded with an increased odds ratio for hypogonadism.

In a subgroup analysis of older overweight male participants enrolled in a weight loss and vitamin D supplementation trial, Pilz et al. (157) investigated the effect of 3332 IU cholecalciferol per day on testosterone levels. Total testosterone, bioactive testosterone, and free testosterone increased in the vitamin D supplementation group when comparing baseline to the conclusion of the 12-month trial (157). Testosterone indices in the control group did not change during the intervention and neither the treatment nor the control group had significantly different weight loss outcomes (157). It must be noted that the participants enrolled in Pilz et al. (157) possessed a mean total testosterone level of 10.7 $nmol \cdot L^{-1}$ in the treatment group and 11.8 $nmol \cdot L^{-1}$ in the control group. The testosterone value for meeting the criteria for hypogonadism is 11.3 $nmol \cdot L^{-1}$ (51). Thus, participants in this study possessed testosterone levels at the low end of the normal range and some individuals met the criteria for hypogonadism (157). Participant mean baseline 25(OH)D levels were also low, below 20 $ng \cdot mL^{-1}$, which is the criteria for vitamin D deficiency (157). Although the results of Pilz et al. (157) should be interpreted with caution, it is the first study to suggest that vitamin D supplementation may influence indices of testosterone in men with both low 25(OH)D and testosterone levels.

The exact means in which 25(OH)D may influence androgen levels is currently unknown; however, the conformation of the VDR in human ovarian (158) and testicular tissue (159) supports the notion that $1,25(OH)_2D$ may play a role in gonadal function. Parikh et al. (158) identified VDR mRNA in ovarian cells and demonstrated that $1,25(OH)_2D$ added to a cell culture had no effect on testosterone, but increased progesterone, estrone, and estradiol. Parikh et al. (158) concluded that the increased steroid hormone production by the ovarian cells was likely the

result of upregulated aromatase activity. In human testes, the VDR (159) and various vitamin D hydroxylation enzymes have been identified (159, 160). Jenson et al. (159) identified both the 25-hydroxlase and 1-alpha hydroxlase in the leydig cells of the testes, which gives these cells the ability to metabolize vitamin D at will. Foresta et al. (160) documented the concentration of 25-hydroxlase mRNA in the leydig cells to be substantially greater than all other tissues, including the liver. Thus, the leydig cells of the testes could be a contributing factor in circulating 25(OH)D concentrations (160). Foresta et al. (160) also reported that testiculopathic participants expressed lower levels of 25-hydroxalase mRNA and 33% lower 25(OH)D levels when compared to controls. Despite the testiculopathic patient's lower vitamin D status, testosterone levels were comparable to the control group. However, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were significantly elevated, which is likely a compensatory reaction to a disruption in testicular function acting to preserve normal testosterone levels (160). In conjunction with the ability to metabolize vitamin D, it is likely that the leydig cells respond to it being that they also appear to possess the VDR (159). This combination of metabolizing enzymes and response elements in the leydig cells of the testes has led to the notion that 25(OH)D may influence androgen production (159), as the leydig cells are the major site of testosterone production (161). However, reverse causality cannot be ruled out. It may be that testicular dysfunction in males may lead to lower 25(OH)D levels.

Another proposition stated is that vitamin D may have an influential role in androgen regulation by modulating estrogen production (162). Estrogen synthesis involves the aromatization of testosterone and androstenedione (C 19 androgens) (161). Aromatization of the C 19 androgens is achieved through aromatase cytochrome p450 activity, which is coded for by the gene CYP19 (161, 163). Estrogen production is dependent on the C 19 androgens and the aromatase enzyme. Estrogen is believed to have a role in hypothalamus and pituitary hormonal control (161). Evidence suggests that estrogen is involved in the gene regulation of LH, which is

secreted by the pituitary along with FSH, and both are hormones that are necessary for normal testicular function (161). Evidence from the VDR knockout model suggests that the absence of the VDR may reduce cytochrome p450 activity and CYP19 gene expression in reproductive tissues, thereby leading to reductions in aromatase activity and subsequent estrogen production (163).

The knockout mouse model was used to investigate the role of vitamin D and estrogen control. Kinuta et al. (163) reported that the VDR knockout mice expressed decreased aromatase cytochrome p450 activity (58.5% of control), which was accompanied by a decrease in the CYP19 gene expression in the reproductive tissues of both male and female mice. Sperm count and motility were described as significantly compromised in the VDR knockout mice (163). The VDR knockout mice also possessed some testicular abnormalities and altered hormone profiles (163). Luteinizing hormone and FSH levels were significantly elevated in the VDR knockout mice when compared to the controls (163). It has been suggested that this observation indicates hypergonadotropic hypogonadism (51). Estradiol levels were lower in the VDR knockout mice when compared to the control; however, significance was only reported in the female VDR knockout mice (163). Kinuta et al. (163) further demonstrated that the previously stated abnormalities expressed in the VDR knockout mice hormonal profile, gonadal function and histology could be reversed with estrogen supplementation (163). Calcium supplementation could also revert most of the VDR knockout mice abnormalities (163). However, calcium supplementation could not fully reverse the disruptions in aromatase activity, LH, and FSH (163). Being that estrogen supplementation restored a normal hormone profile in the VDR knockout mice, vitamin D may play a role in estrogen regulation beyond that of calcium homeostasis in reproductive tissues (163). Evidence from the VDR knockout model suggests that vitamin D may impact estrogen synthesis through aromatase cytochrome p450 activity and CYP19 gene

expression (163). Thus, it may be that disrupted aromatase activity induced by the absence of the VDR leads to estrogen insufficiency and subsequently causes disrupted gonad function (51, 163).

Using another animal model, Hirai et al. (164) found 1,25(OH)D to upregulate various genes and protein synthesis in mouse testes. The specific target for 1,25(OH)D appeared to be the sertoli cells (164). Once again, vitamin D was implicated with the normal functioning of the testes (164).

2.16 Summary

The prevalence of vitamin D deficiency in populations all over the world has drawn the scientific community to investigate the overall effect of this deficiency (1-5). Physiological consequences can result from a vitamin D deficiency (3, 9-11). Since the VDR has been isolated in skeletal muscle tissue (3), it seems logical to ponder the potential impact of vitamin D status on muscle homeostasis, function, and physical performance. The literature pertaining to proposed mechanisms of action in muscle (11, 14-17, 93, 94) and the VDR knockout mouse model (18) appear to depict the notion of vitamin D status impacting skeletal muscle homeostasis as being biologically plausible. The preponderance of the vitamin D and physical performance data appears to sustain this premise (21, 23-32, 39-41, 123, 135-138). However, evidence from randomized control trials does not consistently support a positive effect of vitamin D supplementation on measures of physical performance (42, 139-143). Rejnmark (145) raised two important calls to action that are needed to determine if vitamin D is causally associated with physical performance: 1) replication of previous randomized control trials; and 2) randomized control trial primary endpoints that are physical performance measures. It appears that there is sufficient evidence to support further investigation of the influence of vitamin D status on cardiorespiratory fitness (29, 37, 38) and adiposity (127-130). Both of these measures have the potential to influence physical performance. At this time, the basis for suggesting vitamin D as a potential ergogenic substance for athletes is substantiated merely through biological plausibility

and extrapolation of data from other populations (2, 4, 49, 53). If these questions are to be appropriately addressed, investigations must begin to explore these questions in athlete populations (4). Another deficiency in previous studies is the physical performance testing tasks utilized often yield little relevance to athletic performance. Employing measures associated with athletic performance, such as the vertical jump test, the Wingate anaerobic power test, graded exercise testing, and underwater weighing would further strengthen the relevance of this research to athletes.

CHAPTER 3: Vitamin D Status is Associated with Body Adiposity in Competitive Ice Hockey Players

CHAPTER 3: Vitamin D Status is Associated with Body Adiposity in Competitive Ice Hockey Players (for submission to Medicine and Science in Sports and Exercise)

Vitamin D Status is Associated with Body Adiposity in Competitive Ice Hockey Players

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Abstract

The prevalence of insufficient 25(OH)D status is of concern and may negatively impact health and physical performance. **Purpose:** The purposes of this study were to evaluate the association between 25(OH)D status and percent body fat after adjusting for covariates, and assess the prevalence of insufficiency in a sample population of athletes. **Methods:** Data was collected for 53 junior and collegiate ice hockey players residing near Minneapolis, Minnesota (44.9° N) during the offseason (May 16-June 28). Liquid chromatography-tandem mass spectrometry was used to assess 25(OH)D levels and hydrostatic weighing was used to determine % body fat. Dietary intake and endogenous synthesis of vitamin D were evaluated via questionnaire. **Results:** Twenty (37.7%) athletes possessed insufficient 25(OH)D levels (< 32 $ng \cdot mL^{-1}$). Log-transformed 25(OH)D status was inversely associated with (log of) % body fat in the athletes ($r = -0.50$, $n = 51$, $p = 0.00$). After controlling for measured covariates using hierarchal linear regression, the adjusted R^2 change value indicated that 10% of the variability in (log of) 25(OH)D status is predicted by (log of) % body fat ($p = 0.002$). **Conclusion:** The findings of this study demonstrate an inverse association between % body fat and 25(OH)D status, after adjusting for environmental, dietary, and anthropometric predictors in a sample population of athletes. Athletes with higher levels of adiposity may be at increased risk of poor 25(OH)D status.

Introduction

Estimates of the prevalence of 25-hydroxyvitamin D (25[OH]D) deficiency and insufficiency are raising concern all over the world (3). Adequate levels of 25(OH)D are necessary for skeletal health (3). Albeit, it is believed that the role of the vitamin D system extends beyond that of calcium control and bone formation to encompass a variety of non-skeletal related outcomes (11). Vitamin D status has been associated with multiple factors related to physical performance including skeletal muscle function (95), cardiovascular fitness (38), and body adiposity (132). Although evidence for causal inference is still lacking, these associations have garnered attention from those interested in public health and sport.

Cross-sectional investigations have repeatedly demonstrated an inverse association between 25(OH)D status and degree of body adiposity (165). The observed association between 25(OH)D and body adiposity is thought to be due to the lipophilic nature of 25(OH)D and its precursors (81, 166). Adipose tissue is a known storage site for these compounds (166, 167), though the exact mechanism responsible for the association between 25(OH)D and body adiposity remains unclear (165).

It is currently unknown if the inverse association between 25(OH)D status and degree of body adiposity exists in athletes. Halliday et al. (81) assessed 25(OH)D status and body adiposity in athletes participating in a variety of intercollegiate sports and only detected an association in the fall after adjusting for sex, but not in the winter or spring. The authors speculated that the low range of participant adipose levels and small sample size likely contributed to the lack of an association. Morton et al. (168) and Willis et al. (169) also failed to find an association between 25(OH)D and body adiposity in small samples of athletes. Investigations incorporating larger sample populations of athletes are needed in order to properly address this question.

If 25(OH)D status is associated with degree of adiposity in athletes, body fat may be considered an independent risk factor for insufficient or deficient 25(OH)D status in this

population. Recently in the United States, the prevalence of 25(OH)D deficiency and insufficiency in the general population have been estimated at 42% (7) and upwards of 75% (8), respectively. Limited reports exist characterizing 25(OH)D status in athletes (170). Population specific prevalence estimates are needed to determine if athletes from a variety of sports are equally at risk for poor 25(OH)D status.

The purpose of this study was to investigate 1) the cross-sectional associations between 25(OH)D status and percent body fat after adjusting for covariates and 2) the prevalence of vitamin D deficiency and insufficiency in a sample population of competitive ice hockey players. We hypothesized that 25(OH)D status would be inversely associated with % body fat.

Methods

Participants. Division I, Division III and Junior A male ice hockey players residing in Minneapolis, Minnesota (44.9° N) and the surrounding metropolitan area during the off-season were recruited to participate in the study. The procedures and risks associated with study participation were communicated to all interested athletes. Informed written consent was obtained before potential participants were enrolled in the study. Approval for this study was granted by the institutional review board at the University of Minnesota. Hockey players were excluded from study participation for any of the following: self-reported evidence of vitamin D metabolism disruption, corticosteroid use, injury that inhibits performing physical performance tasks, and self-reported playing position of goaltender.

Study Design. During this cross-sectional study, participants attended three consecutive testing sessions at the Human and Sport Performance Laboratory and the Mariucci and Ridder Arenas at the University of Minnesota. Basic background information was collected during session one, along with anthropometric measurements, hydrostatic weighing and assessments of physical performance. The physical activity and vitamin D-specific questionnaires were administered at the beginning of session two. Next, a blood spot sample was collected followed

by a graded exercise test. Session three was conducted on ice and consisted of acceleration, top speed, and repeated-shift performance tests. All testing sessions for each participant were usually completed within a 9-day period and no longer than 14 days. At least two days separated each testing session. The data collection period began on May 16, 2012 and ended on June 28, 2012. Participants were instructed to refrain from training the morning prior to their scheduled testing sessions. Participants were advised to refrain from eating at least two hours before testing sessions and avoid carbonated and caffeinated beverages on testing days. Participants were instructed to maintain normal dietary, hydration, and physical activity habits for the duration of the study.

Anthropometric assessment and hydrostatic weighing. Weight was measured using a Detecto mechanical scale with the participant wearing a spandex undergarment. Standing height was measured using a wall mounted Accustat Genentech Stadiometer. Percent body fat was determined by hydrostatic weighing using Exertech Body Densitometry Systems software (Dresbach, MN). The hydrostatic weighing procedure adopted for this study has been described previously (171). Residual lung volume was estimated (172). The Brozek et al. (1963) equation was used to estimate percent body fat (171). Favorable test-retest reliability has been reported for hydrostatic weighing ($r > .95$) (171).

Vitamin D-specific Questionnaire. The questionnaire was completed at the beginning of session two. The vitamin D-specific Diet and Lifestyle Questionnaire was adopted from Halliday et al. (81), which was designed to assess dietary intake and endogenous synthesis of vitamin D in collegiate athletes. Athletes were instructed to complete the questionnaire in the context of average consumption and exposure in recent months. Using a structured response format, the questionnaire consists of questions related to the frequency of consumption of foods containing vitamin D. Vitamin D intake from multivitamins and vitamin D supplements was also assessed. The vitamin D content of each food listed on the questionnaire was determined using the National

Nutrient Database for Standard Reference (173). Product labels and self-reported doses were used to assess the vitamin D content in multivitamins and supplements, respectively. Daily vitamin D (IU) consumption for each item was determined by multiplying the vitamin D content by the frequency of consumption (81). Endogenous synthesis of vitamin D was estimated from frequency of outdoor leisure time between the hours of 10AM and 3PM (81). Average outdoor exposure was reported as hours per week.

25(OH)D assay. The clinically relevant measure of vitamin D status is 25(OH)D (58). 25(OH)D status was assessed from a capillary blood sample. After sterilizing the area with an alcohol prep pad, a contact activated lance was use to puncture the distal lateral region of the participant's ring finger. After lancing, gentle pressure was applied to the participant's finger to promote a bead of blood to form on the fingertip. The first bead of blood was wiped away with a sterile gauze pad, while the subsequent beads where guided to one of 12 circles on a participant's blood spot card. All 12 blood spot circles were filled for each participant. Blood spot cards were allowed to dry for approximately 14 hours. They were then individually placed in a sealed plastic bag along with a 5-gram Tyvek covered silica gel packet. Blood samples were placed in a -20° C freezer until the end of the data collection period. At this time, all samples were placed in a Styrofoam mailer with cold packs and shipped overnight to ZRT Laboratory for analysis. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine 25(OH)D status from the dried blood spot cards. ZRT Laboratory claims that their assay has been calibrated to yield results for total 25(OH)D that are strongly correlated ($r^2 = .97$) with the DiaSorin RIA. The agreement between serum and dried blood spot techniques is strong for both $25(OH)D_3$ ($r = .91$) and $25(OH)D_2$ ($r = .90$) (174) . Favorable precision has also been documented for dried blood spot techniques ($CV \leq 13\%$, reference range: 14-81 $ng \cdot mL^{-1}$) (174). ZRT laboratory participates in the Vitamin D External Quality Assessment Scheme.

Statistical procedures. Statistical analyses were performed using SPSS version 20.0 (IBM, Armonk, NY). Data were inspected to determine if the criteria for normal distributions were met. The Kolmogorov-Smirnov test of normality was used. 25(OH)D status and % body fat were positively skewed with a few suspected outliers. Logarithmic transformations were performed on both variables, improving the normal distribution of the data and removing the presence of outliers. Descriptive characteristics are presented as means and standard deviations. Pearson and Spearman correlation coefficients were used to evaluate the associations between continuous and non-continuous variables. Potential covariates were identified through theoretical considerations and by assessing the correlations between variables. Hierarchal linear regression was used to model the relationship between 25(OH)D status and % body fat after adjustment of potential covariates. Independent variables such as body mass (175), age, sex, race, season and sun exposure have been identified previously to be associated with 25(OH)D status (54). The sample population characteristics preclude the need to adjust for age, sex and race. The brief data collection period likely mitigates the impact of season. The study enrollment period was binned at its midpoint, creating a dichotomous variable, to examine the influence of date of data collection on 25(OH)D status. Study enrollment date (binned) and body mass were not significantly associated with 25(OH)D status, but were included in the final model on theoretical grounds. Frequency of sun exposure and total dietary vitamin D intake were logarithmically transformed and included in the final model. Residual plots from the regression analysis were examined for multivariate outliers and normality. Alpha was set at 0.05 and two-sided p-values were used for all calculations.

Results

Fifty-seven men participating in competitive ice hockey leagues volunteered to participate in the study. Three athletes dropped out of the study after the first testing session. One athlete was excluded from study participation due to a self-reported injury to the lower body. The

study sample used to report 25(OH)D status included 53 Caucasian male competitive ice hockey players (33 forwards, 20 defensemen) from collegiate (25 Division I, 11 Division III) and Junior A (n = 17) ice hockey leagues. Hydrostatic weighing data was not available for two Junior A hockey players because they were unable to schedule an additional testing session after an equipment malfunction did not allow hydrostatic weighing during their scheduled appointment. 25(OH)D status and % body fat data were available for 51 athletes.

25(OH)D status. Athletes' mean 25(OH)D status was 35.7 ± 8.9 $ng \cdot mL^{-1}$ and ranged from 20.3 to 65.4 $ng \cdot mL^{-1}$. Thirty-three (62.3%) athletes had sufficient 25(OH)D levels (≥ 32 $ng \cdot mL^{-1}$) (58). Twenty (37.7%) athletes possessed insufficient 25(OH)D levels (< 32 $ng \cdot mL^{-1}$) (58). None of the participants in the study had a 25(OH)D status low enough to be considered vitamin D deficient (< 20 $ng \cdot mL^{-1}$) (58).

25(OH)D status, dietary vitamin D intake and sun exposure. Athlete characteristics and descriptive statistics for estimated dietary vitamin D intake and hours of sun exposure are presented in Table 1. Correlations between 25(OH)D status, dietary vitamin D intake and sun exposure are presented in Table 2. Figure 1 depicts the positive relationship between total dietary vitamin D intake and 25(OH)D status in our athletes.

Table 1. Demographic, anthropometric, body composition, dietary and sun exposure characteristics for the athletes (n = 53).

	Mean (SD)	Min-Max
Age (years)	20.1 (1.5)	18-23
Height (cm)	182.0 (6.3)	169.5-197.8
Body mass (kg)	84.3 (6.9)	71.2-98.5
BMI ($kg \cdot m^{-2}$)	25.4 (1.3)	21.5-27.7
Percent body fat (%) (n = 51)	12.5 (3.9)	7.1-28.9
Dietary vitamin D from food ($IU \cdot d^{-1}$)	405 (246)	67-1005
Dietary vitamin D from multivitamin ($IU \cdot d^{-1}$)	152 (368)	0-2400
Dietary vitamin D from supplement ($IU \cdot d^{-1}$)	226 (522)	0-2000
Dietary vitamin D from multi and supp ($IU \cdot d^{-1}$)	378 (647)	0-2400
Total dietary vitamin D ($IU \cdot d^{-1}$)	782 (687)	80-2602
Sun exposure between 10AM and 3PM ($h \cdot wk^{-1}$)	5.9 (4.6)	0.5-18.8

Table 2. Correlations between potential predictor variables and (log of) 25(OH)D status.

	r	*p*-value
Age (years)	-0.213*	0.125
Height (cm)	0.029	0.834
Body mass (kg)	-0.20	0.159
BMI ($kg \cdot m^{-2}$)	-0.334	0.014
Percent body fat (log) (n = 51)	-0.501	0.000
Dietary vitamin D from food ($IU \cdot d^{-1}$)	0.308*	0.025
Dietary vitamin D from multivitamin ($IU \cdot d^{-1}$)	0.271*	0.049
Dietary vitamin D from supplement ($IU \cdot d^{-1}$)	0.346*	0.011
Dietary vitamin D from multi and supp ($IU \cdot d^{-1}$)	0.451*	0.001
Total dietary vitamin D (log)	0.586	0.000
Sun exposure (log)	0.332	0.015
Study enrollment date (binned)	0.185*	0.184

Note. * indicates Spearman rho correlational test. n = 53.

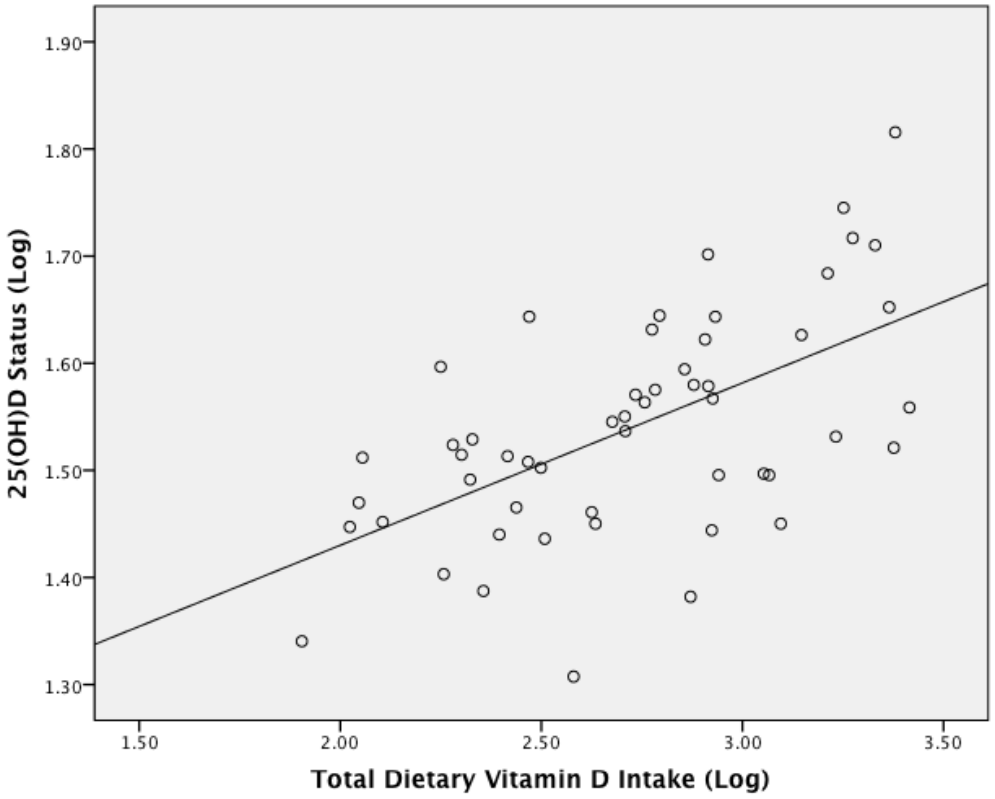

Figure 1. The positive relationship between total dietary vitamin D intake and 25(OH)D status. Data available for 53 athletes (n = 53). R^2 linear = 0.343, p = 0.000.

25(OH)D status and % body fat. Hierarchal linear regression was employed to determine if the addition of information regarding % body fat improved prediction of 25(OH)D status beyond that provided by sun exposure, enrollment date, total dietary vitamin D intake, and body mass. Figure 2 depicts the inverse relationship between % body fat and 25(OH)D status in our athletes. The correlations between the variables and the final step in the regression model are presented in Table 2 and Table 3, respectively. After both steps in the model, R was significantly different from zero. After step two, the final step in the model, $R^2 = 0.57$, $F(5, 45) = 11.72$, p = 0.000. The adjusted R^2 value indicates that 52% of the variability in (log of) 25(OH)D status was predicted by the model.

After step one, with (log of) sun exposure, (binned) enrollment date, (log of) total dietary vitamin D intake, and body mass in the model, $R^2 = 0.46$, $F_{inc}(4, 45) = 9.89$, $p = 0.000$. After step two, with (log of) % body fat added to the prediction of the control variables, $R^2 = 0.57$, $F_{inc}(1, 45) = 10.70$, $p = 0.002$. Addition of (log of) % body fat to the model resulted in a significant increase in R^2. The adjusted R^2 change value indicates that 10% of the variability in (log of) 25(OH)D status was predicted by (log of) % body fat.

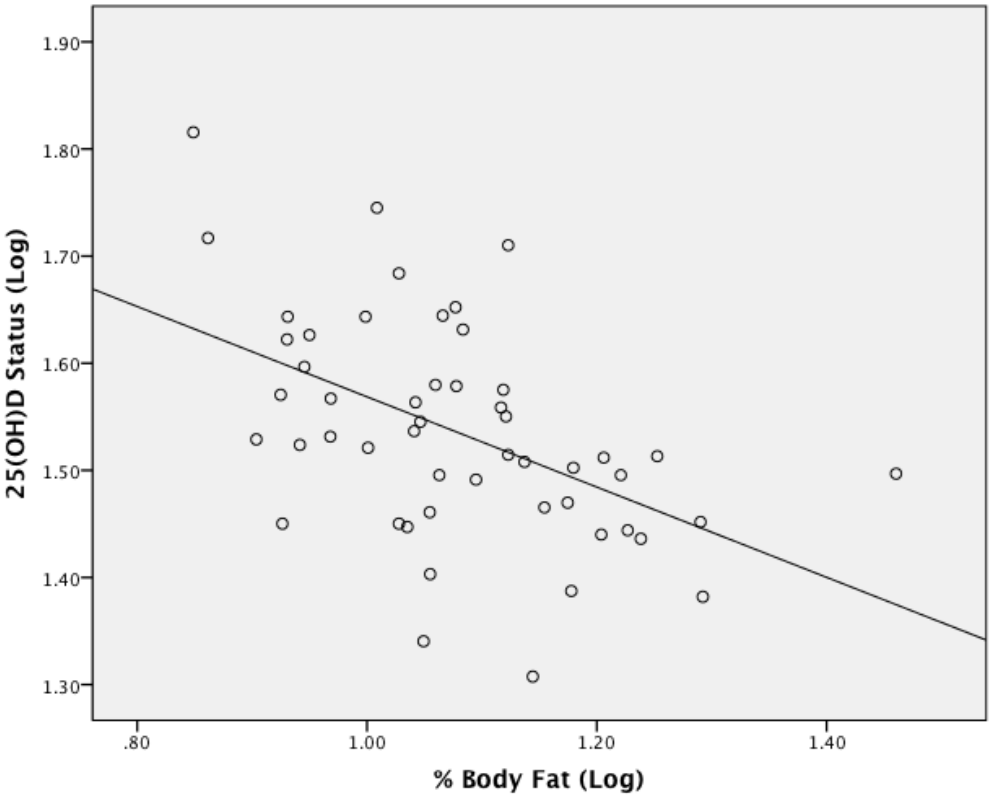

Figure 2. The inverse relationship between % body fat and 25(OH)D status. Data missing in 2 of 53 athletes (n = 51). R^2 linear = 0.251, $p = 0.000$.

Table 3. Hierarchal linear regression of potential covariates and (log of) % body fat on (log of) 25(OH)D status.

	Unstandardized Coefficients		Standardized Coefficients			Correlations	
	Beta	Std. Error	Beta	t	*p*-value	partial	part
Step 2							
Sun exposure	0.101	0.036	0.285	2.841	0.007	0.390	0.279
Enrollment date	0.009	0.022	0.041	0.409	0.658	0.061	0.040
TDVD	0.131	0.026	0.519	5.011	0.000	0.598	0.492
Body mass	-0.001	0.002	-0.037	-0.366	0.716	-0.054	-0.036
% Body fat	-0.287	0.088	-0.341	-3.271	0.002	-0.438	-0.321

Note. TDVD = total dietary vitamin D. Sun exposure, TDVD and % body fat were log-transformed; Enrollment date was binned at the midpoint of the study enrollment period. Data was missing for 2 athletes (n = 51).

Discussion

The primary purpose of this cross-sectional study was to evaluate the association between 25(OH)D status and % body fat after adjusting for previously identified covariates in a sample population of athletes. In this sample of competitive ice hockey players, % body fat was inversely associated with 25(OH)D status, independent of other environmental, dietary, and anthropometric predictors. Although consistent with investigations in general populations (175-177), this finding is not in agreement with the results from three previous investigations incorporating sample populations of athletes (81, 168, 169). Halliday et al. (81) did not find an association between 25(OH)D and % body fat during the fall (n = 41), winter (n = 33), or spring (n = 25) in Division I colligate athletes; however, a significant association was detected in the fall after adjusting for

sex. It appears that the heterogeneity of the sample – for example, including males and females – may suppress the association between 25(OH)D status and % body fat in studies with small samples. Sex-related differences in 25(OH)D status have been reported after adjusting for fat mass (177). Dissimilarities in vitamin D metabolism and behavioral practices associated with sun exposure may account for the sex-related variance in 25(OH)D status (177). The inability of Halliday et al. (81) to find significance after adjusting for sex in the winter (n = 33) and spring (n = 21) was likely hindered by attrition and the restricted range in body fat of the athletes tested. Moreover, the association between 25(OH)D status and % body fat may be attenuated during nadir seasonal sampling due to greater stores of 25(OH)D and its precursors in adipose tissue (178). Although not a primary endpoint of either study, % body fat was not associated with 25(OH)D status in male and female endurance runners or triathletes (n = 19) training in Louisiana (169) or male professional soccer players (n = 20) residing in the United Kingdom (168). Both of these investigations suffered from at least two of the previously stated issues.

After controlling for measured predictors, % body fat explained 10% of the variability in 25(OH)D status in our sample population of athletes. This observation is likely explained by the influence of adipose tissue on 25(OH)D status. Adipose tissue is a known storage site for cholecalciferol and 25(OH)D (166). It appears that adipose tissue sequesters calciferol compounds when concentrations of 25(OH)D and its precursors are elevated in the serum (176) and this process may be enhanced when endogenously synthesized cholecalciferol migrates from the epidermis to the capillary beds (175). A strong, positive association ($r = 0.68$) between serum and adipose tissue cholecalciferol concentrations has been reported in obese individuals (167). This appears to support the hypothesis that adipose sequestration of the calciferol compounds is, in part, dependent on concentrations in the serum. Additionally, a 57% reduction in 24-hour peak serum cholecalciferol was documented after a single suberythemic dose of UV-B in obese individuals (BMI > 30) when compared to normal weight individuals (BMI $\leq$ 25), despite no

difference in cholecalciferol production in the skin (175). Regardless of its derivation, it is thought that increased adipose accumulation is accompanied by an increased capacity to sequester calciferol compounds (178).

Body adipose accumulation may negatively impact 25(OH)D status through other mechanisms. It has been proposed that adipose accumulation may upregulate $1,25(OH)_2D$ production (175), which subsequently increases D-24-hydroxylase activity and the metabolic degradation of both 25(OH)D and $1,25(OH)_2D$ (46). Several other factors associated with obesity, such as reduced D-25-hydroxylase activity in the liver, increased inflammation, reduced outdoor activity, and increased skin coverage when outdoors may adversely affect 25(OH)D status (165). Percent body fat was not associated with sun exposure frequency in our athletes ($p = 0.43$) and was accounted for in our regression model. The extent to which the other aforesaid factors may have influenced the association between 25(OH)D status and % body fat is unknown. Not one of our athletes possessed a BMI high enough to be considered obese (BMI ≥ 30) and only one athlete had greater than 19.6% body fat.

Reverse causality cannot be ruled out. The identification of the VDR in adipose tissue and subsequent *in vitro* investigations have led to the hypothesis that poor vitamin D status may contribute to adipose accumulation (179). However, the role of the calciferol hormones in adipose tissue functioning *in vivo* has yet to be discerned (165, 179). The few randomized controlled trials investigating the effect of vitamin D supplementation on weight loss do not support a causal association between vitamin D and adipose accumulation (132).

The secondary purpose of this investigation was to estimate the prevalence of vitamin D insufficiency in this population. This is the first investigation to report the vitamin D status in competitive ice hockey players. Approximately 38% of the athletes had insufficient 25(OH)D levels (< 32 ng·mL^{-1}) (58). The prevalence of vitamin D deficiency/insufficiency in our competitive ice hockey players was not as high as the majority of reports in athletes living outside

of the United States, citing combined deficiency and insufficiency from 49-100% (78-80, 180, 181). While not one athlete in our cohort was vitamin D deficient (< 20 $ng \cdot mL^{-1}$), the prevalence of insufficiency (< 32 $ng \cdot mL^{-1}$) was similar to that in 19 endurance runners training in Louisiana (169). Although not in the winter (61%), a greater percentage of our athletes possessed insufficient 25(OH)D status when compared to Division I collegiate athletes in Wyoming in the spring (16%) and fall (9.8%) (81) .

Making comparisons between studies is complicated by the temporal characteristics of data collection with respect to season. 25(OH)D status usually peaks in late summer and reaches its nadir in late winter (49) due to the fluctuation of the presence of the UV-B radiation spectrum that is necessary for endogenous synthesis and the profound effect endogenous synthesis has on 25(OH)D status (3). Very few UV-B photons reach the earth during the winter months in geographic locations above 35 degrees latitude, which reduces or eliminates the possibility for vitamin D synthesis during this time (76). We recruited hockey players residing near the Minneapolis metropolitan (44.9° N) area during the off-season. The majority of the hockey players in this study also competed near this location. Therefore, it is reasonable to assume that during the data collection period, our athletes' 25(OH)D levels were likely on the rise from a nadir in late winter. We speculate that the prevalence of 25(OH)D insufficiency in our athletes would have been substantially higher in late winter.

The descriptive and behavioral characteristics of the sporting population along with the geographical location of in-season and off-season residence and travel likely contribute to the observed variability in vitamin D deficiency/sufficiency prevalence between studies. The variability in 25(OH)D status is thought to be largely induced by factors that influence endogenous vitamin D synthesis: latitude, season, time of day, cloud cover, pollution, exposure duration, clothing, sunscreen, skin pigmentation, and age (54). Other factors related to vitamin D metabolism such as calcium intake, genetics, sex (54) and body mass (175) may also contribute to

variability in 25(OH)D status. Athletes who practice and compete indoors may be at increased risk for developing poor 25(OH)D status at some point throughout the year (79, 81).

The strong, positive correlation between 25(OH)D status and total dietary vitamin D intake ($r = 0.59$, $n = 53$, $p = 0.000$) in our athletes was not expected. Although vitamin D intake from food was associated with 25(OH)D status ($r = 0.31$, $n = 53$, $p = 0.025$), the strength of this association appears to be largely mediated by the combined effect of multivitamin and vitamin D supplement use ($r = 0.45$, $n = 53$, $p = 0.001$). From food alone, only 18.9% of athletes met the RDA for vitamin D (600 IU). The prevalence of multivitamin and vitamin D supplement use in our athletes was 41.5% and 22.6%, respectively. The percentage of athletes consuming the RDA for vitamin D increased to 47.2% when combining vitamin D intake from food, multivitamins and supplements. Sun exposure frequency was moderately correlated with 25(OH)D status ($r = 0.33$, $n = 53$, $p = 0.015$). This is to be expected due to the strong influence of endogenous synthesis on 25(OHD status (3). Interestingly, total dietary vitamin D intake explained more of the variance in 25(OH)D status when compared to sun exposure frequency indicated by the part correlations of $r = 0.49$ and $r = 0.28$, respectively, in our regression model. The strength of the association between sun exposure frequency and 25(OH)D status is likely minimized due to the accuracy of the self-reported questionnaire employed in this study (81) and the various factors that influence endogenous synthesis of vitamin D (54).

Developing deficient/insufficient 25(OH)D status at any point during the year is considered problematic (178). Due to the seasonal variation, the risk of poor 25(OH)D status during the winter months appears to be elevated (81, 168, 176-178, 182). This may be especially problematic for athletes that compete during the winter months. Although the evidence for causality in athlete populations is lacking, poor vitamin D status may influence physical performance in tasks related to strength, power, and aerobic fitness (49). Recently, a small randomized, placebo controlled trial found improvements in 10-meter sprint times and vertical

jump performance after eight weeks of vitamin D_3 supplementation (5,000 IU) in mostly vitamin D deficient UK soccer players (180). Vitamin D has also been associated with immune system functioning. Frequency of illness (81), injury (53) and TNF-alpha (169), a biomarker of inflammation, have been associated with 25(OH)D status in athletes. Correcting poor 25(OH)D status may positively impact the health and physical performance of athletes.

This investigation has several limitations. The cross-sectional study design does not allow for the determination of causality. A capillary blood sample was utilized for the determination of 25(OH)D status. Comparing the 25(OH)D values obtained in studies using a different assay should be done with caution recognizing the reported inter-assay variation (58). The results of this investigation are not generalizable outside of the scope of our sample population of hockey players.

The main strengths in this study include the relatively large, homogenous sample population of athletes. The homogeneity of the sample population, in theory, reduces the factors that influence 25(OH)D status. The short study enrollment period during the offseason may have reduced the influence of the yearly training cycle on physiological variables.

In conclusion, this is the first study to find an inverse association between % body fat and 25(OH)D status, after adjusting for environmental, dietary, and anthropometric predictors in a sample population of athletes. It appears that elevated body fat may be an independent risk factor for poor 25(OH)D status in athletes. This finding also has implications for cross-sectional studies evaluating physical performance in athletes. Associations between 25(OH)D and measures related to physical performance may be confounded by body adiposity and should be adjusted accordingly. Future studies should examine the strength of association in female athletes and athletes participating in other sports. The causal relationship between 25(OH)D and body adiposity needs to be elucidated. The prevalence of vitamin D insufficiency in our ice hockey players support the assertion that vitamin D supplementation may be needed for some athletes to

achieve or maintain sufficient vitamin D status throughout the year (81, 168, 180-182). Interventional trials in populations of athletes are needed to determine if 25(OH)D status is causally related to the performance, illness and injury outcomes of interest. If so, appropriate 25(OH)D demarcation values indicating sufficiency for these outcomes need to be determined.

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CHAPTER 4: Association Between Vitamin D Status and Physical Performance Measures in Junior and Collegiate Hockey Players

CHAPTER 4: Association Between Vitamin D Status and Physical Performance Measures in Junior and Collegiate Hockey Players (for submission to the Journal of Sport Sciences)

Association Between Vitamin D Status and Physical Performance Measures in Junior and Collegiate Hockey Players

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Abstract

Recent evidence suggests that athletes are at risk for poor vitamin D status. The current study utilized a cross-sectional design to investigate the strength of association between 25(OH)D status and laboratory measures of physical performance in a sample population of competitive hockey players. Fifty-three collegiate and junior male ice hockey players training near Minneapolis, Minnesota (44.9° N) participated in the study during the offseason (May 16-June 28). Despite no athletes demonstrating a 25(OH)D concentration indicative of deficiency (< 20 $ng \cdot mL^{-1}$), significant bivariate correlations were detected between 25(OH)D status and measured physical performance outcome variables. After adjusting for potential covariates, (binned) 25(OH)D status was only significantly associated with handgrip strength ($p = 0.025$) and trended with PP during the WAnT ($p = 0.089$). Representing a novel finding of this study, 25(OH)D status was inversely related to jump execution time ($p = 0.035$), time to peak power ($p = 0.040$) and positively associated with force gradient ($p = 0.020$) during the squat jump after adjusting for potential covariates. Interventional trials should investigate the impact of vitamin D supplementation on physical performance outcomes and jump execution variables in vitamin D deficient and insufficient athletes.

Introduction

Recent investigations in athletes appear to indicate that poor 25-hydroxyvitamin D (25[OH]D) status is not only a general population concern (79, 169, 181). Classically known for

its role in skeletal health, the vitamin D system is now considered to influence a vast and diverse list of human tissues and processes (13). Pertinent to athletes, it is thought that vitamin D plays a role in skeletal muscle homeostasis (183). The vitamin D system achieves its impact on target tissues via a ligand and vitamin D receptor (VDR) interaction (13). The active form of vitamin D ($1,25[OH]_2D$) binds with the VDR in the cell membrane and cell nucleus which leads to rapid non-genomic signaling and genomic signaling, respectively (13). Through this signaling it is believed that the vitamin D system influences numerous processes, which may impact muscle function: calcium and phosphate control, phospholipid metabolism, myogenic regulation and contractile and regulatory protein concentrations (183).

The majority of the research investigating the impact of 25(OH)D status on measures of physical performance has been conducted in aging populations. Of these investigations, evidence from cross-sectional studies consistently supports a positive association between 25(OH)D status and scores on physical performance evaluations, whereas, the results from interventional trials are mixed (145). Evidence characterizing the association between 25(OH)D status and measures of physical performance in athletes is lacking. Therefore, the purpose of this study was to investigate the cross-sectional associations between 25(OH)D status and measures of physical performance in a sample population of competitive ice hockey players.

Methods

Participants. Junior and collegiate male ice hockey players participating in offseason training near the Minneapolis, Minnesota (44.9° N) metropolitan area were recruited to participate in this study. The decision to recruit this sample of convenience was due to the relatively homogenous population in terms of anthropometric characteristics, training exposure and susceptibility to low vitamin D status. During the informed written consent process, study participation details and risks were communicated, in an accessible manner, to the potential study participants. Athletes who voluntarily consented to participate were enrolled in the study. The

institutional review board at the University of Minnesota approved this study. The exclusion criteria for this study were as follows: self-reported evidence of vitamin D metabolism disruption, corticosteroid use, injury that inhibits performing physical performance tasks, and self-reported playing position of goaltender.

Study Design. Testing sessions were conducted at the Human and Sport Performance Laboratory, Mariucci arena and Ridder arena at the University of Minnesota. Participants attended three testing sessions which included the following: 1) pretest questionnaire, anthropometric and body composition evaluation and physical performance assessments; 2) vitamin D and physical activity questionnaires, vitamin D assay and a graded exercise test; and 3) on-ice skating performance evaluation, which will not be presented in this paper. Testing sessions took place between the hours of 7:00AM and 3:30PM. Testing sessions for each participant were separated by a minimum of two days and all testing sessions were completed within 9-14 days. Participants were instructed to abstain from training prior to testing on testing days. Data was collected for all participants between May 16 and June 28, 2012.

Anthropometric assessment and hydrostatic weighing. Standard measurements of height and weight were recorded. The hydrostatic weighing technique was used to determine total body density, residual lung volume was estimated (172) and % body fat was calculated using the Brozek et al. (1963) equation. See Graves et al. (171) for a description of the hydrostatic weighing procedure used. All measurements and calculations were performed using Exertech Body Densitometry Systems software (Dresbach, MN).

Grip strength evaluation. A Jamar hydraulic dynamometer was used to determine the grip strength of the athlete's dominant hand. Grip strength testing was performed using the standard testing position approved by the American Society of Hand Therapists (184). The grip diameter was set at 3.8 cm. After one submaximal attempt and a brief rest period, the athlete was instructed to maximally grip the device with their dominant hand for three seconds. The test was

repeated two more times with a one-minute rest period between attempts. The scores were averaged for the three attempts and reported in kilograms. Test-retest and inter-rater reliability for the Jamar dynamometer are favorable ($r > .9$) (184).

Vertical jump evaluation. Vertical jump performance was assessed simultaneously by jumping mechanography and the Vertec™ (Huntington Beach, CA). The force platform (Kistler 9286AA, Switzerland) was located directly beneath the vanes of the Vertec™. Jump height and jump execution variables were measured by jumping mechanography. Jump height was also assessed using the Vertec™ by the number of vanes contacted.

Before each test, ground force reaction data was verified for accuracy using calibration weights. The athlete's body mass while wearing athletic shoes, shorts, and a T-shirt was measured using the weight function in BioWare® software (Kistler 2812A, Switzerland). The force plate sampling frequency utilized during data collection was 1,202 Hz (185). Athletes performed the squat jump (SJ) after a five-minute aerobic treadmill warm-up. Athletes were instructed to squat down to a self-selected squat depth (approximately 90 degrees knee flexion), which was held for approximately three seconds before a vertical jump for maximal height was attempted (186). To eliminate arm swing, participants were instructed to place one arm on their hip and the other above their head in a reaching position. Prior to vertical jump data collection, athletes performed two submaximal practice jumps. Next, 3-5 SJs were performed allowing at least 30-second rest periods between jumps. The test was terminated when the athlete failed to increase jump height, as measured by the Vertec™, in two consecutive trials or after the fifth trial. The best performance in regards to jump height was used in the data analysis.

The raw data was collected using BioWare® software (Kistler 2812A, Switzerland). Force-time data were filtered using a fourth-order Butterworth low-pass filter with a cutoff frequency of 50 Hz. Filtered data was exported and analyzed in Excel (Microsoft Corp.). A macro program was created using Visual Basic (Microsoft Corp.), which was used to automate jump

commencement and takeoff identification along with the calculation of jump height and jump execution variables. Jump commencement was identified when the vertical net force trace exceeded 2.5% of the participant's body mass. A block moving average and peak residual were calculated from a 0.3 second data sample selected from the middle of the flight phase when the force plate was unloaded. Takeoff was identified at the first point the net force trace equaled the average unloaded vertical force trace plus the peak residual. A similar procedure for takeoff identification has previously been employed (185). The forward dynamics approach was used to ascertain the vertical velocity of the center of mass (COM) from the ground force reaction data (187). A description of the calculation of jump height and jump execution variables is presented in Table 4. Measuring jump height from ground reaction forces using force plates is strongly correlated ($r = .96$) with camera data and demonstrates favorable reliability ($R_{tt} = .986$) (188).

Table 4. Descriptions of the calculation of jump height and jump execution variables from ground force reaction data.

Jump height (m)	Vertical velocity of center of mass at takeoff ^{2} / 2 gravity (185)
Jump execution time (s)	Time from jump commencement to takeoff
Peak force (N)	Highest vertical force trace prior to takeoff
Time to peak force (s)	Time from jump commencement to peak force
Time to half peak force (s)	Time from jump commencement to half peak force
Peak rate of force development ($N \cdot s^{-1}$)	Peak time derivative of the vertical force trace
Average rate of force development ($N \cdot s^{-1}$)	Peak force / time to peak force
Force gradient ($N \cdot s^{-1}$)	Half peak force / time to half peak force (189)
Acceleration gradient ($N \cdot s^{-1}$)	Half peak force / time to peak force – time to half peak force (189)
Peak power (W)	Highest product of vertical force trace · velocity of center of mass
Time to peak power (s)	Time from jump commencement to peak power

Wingate Anaerobic Test. The Wingate Anaerobic Test (WAnT) was used to assess lower-body power production (190) and was performed on a Monarch 874 E cycle ergometer (Langley, WA). Testing procedures for the WAnT were adopted from Maud et al. (190). Athletes were instructed to pedal at a maximum velocity in an unloaded condition for approximately two seconds, when a flywheel resistance load equaling 7.5% body mass was applied, and continue to pedal maximally for the 30-second duration of the test. Participants' peak power (PP) and mean power (MP) were calculated as the highest average power recorded for intervals of five and 30 seconds, respectively (190). Power values during the test were recorded in watts and normalized for body weight. Fatigue during the test was quantified by calculating the fatigue index. The

reliability for both PP and MP during the WAnT is favorable (r = .91-.93) when using the 7.5% body mass loading parameter (191).

Physical activity assessment. Self reported physical activity was assessed using the Short Questionnaire to Assess Health-Enhancing Physical Activity (SQUASH) (192). See Wendel-Vos et al. (192) for a complete description of the SQUASH. In the leisure domain, athletes entered in activities specific to being a competitive hockey player and recreational activities including, but not limited to: 1) on-ice activity; 2) weight training; 3) anaerobic and aerobic conditioning; 4) recreational sports. MET scores for the added activities in the leisure section were derived from Ainsworth et al. (193) and adjusted for participant rated effort. A total physical activity score was calculated by multiplying weekly activity duration by adjusted activity score and was reported as MET minutes per week. The validity and reliability for the SQUASH are within normal parameters for physical activity questionnaires (192).

25(OH)D assay. Circulating 25(OH)D status was assessed from a capillary blood sample. ZRT Laboratory's instructions for collecting capillary blood samples were closely followed. The dried blood spot cards were packaged appropriately and shipped to ZRT Laboratory where 25(OH)D concentrations were determined using Liquid chromatography-tandem mass spectrometry (LC-MS/MS). The ZRT laboratory dried blood spot technique is strongly correlated to traditional serum measurement of $25(OH)D_3$ (r =.91) and $25(OH)D_2$ (r =.90) and demonstrates favorable precision (CV ≤ 13%, reference range: 14-81 $ng \cdot mL^{-1}$) (174). According to the manufacturer, the ZRT Laboratory dried blood spot assay is strongly correlated (r^2 = .97) with the DiaSorin RIA. ZRT laboratory participates in the Vitamin D External Quality Assessment Scheme.

Statistical procedures. Data were screened at the univariate and multivariate levels for deviations from normality. The Kolmogorov-Smirnov statistic was used to evaluate univariate data. Vitamin D status and % body fat were logarithmically transformed to deal with positive

skew and suspected outliers. If transformation of a variable did not improve the distribution of the data, it was left untransformed. Percent body fat data was missing for two participants due to the failure of hydrostatic weighing equipment to calibrate correctly during the testing session and the inability of those participants to schedule an additional testing session. The missing values for those participants were replaced using mean substitution specific to level of play. A procedural error or failed calibration of the force plate was suspected for one participant's squat jump data. The linear relationship between the two vertical jump height devices was modeled using linear regression. Values for Casewise Diagnostics standardized residual, Mahalanobis Distance, and Cook's Distance were above critical values, which confirmed our original suspicion and the data point was removed.

Means and standard deviations are used to present descriptive characteristics. Pearson's *r* was used to evaluate associations between normally distributed continuous variables and dichotomous variables. Spearman rho was utilized when a nonparametric test was needed. Vitamin D status was analyzed as both a continuous and dichotomous variable. To investigate a threshold effect, 25(OH)D status was binned at its midpoint (33.8 $ng \cdot mL^{-1}$). Theoretical relationships and correlational matrices were evaluated to identify potential covariates. Physical performance variables demonstrating significant associations with continuous or dichotomous 25(OH)D status were selected for further analysis. Handgrip strength was included in the regression analysis despite lacking a significant bivariate association with 25(OH)D status to allow for the statistical adjustment of weight and height (184). Hierarchal linear regression (HLR) was used to model the relationships between 25(OH)D status and physical performance variables while adjusting for potential covariates. Levels of play (194), weight, height, % body fat and physical activity have been previously reported to be associated with physical performance tests (195). Potential covariates were entered sequentially into step one in the HLR model while 25(OH)D status entered the model in step two. Multivariate normality was evaluated using

residual plots from the regression analysis. An alpha of 0.05 and two-sided p-values were used for all calculations. SPSS version 20.0 (IBM, Armonk, NY) was used for all statistical analyses.

Results

Of the 57 male junior and collegiate hockey players who volunteered to participate in the study, data on 53 athletes (Mean ± SD: age = 20.1 ± 1.5 yr, height = 182 ± 6.3 cm, body mass = 84.3 ± 6.9 kg, body fat = 12.5 ± 3.9%, 53 Caucasian, 25 Division I, 17 Junior A, 11 Division III, 33 forwards, 20 defensemen) were available for analysis. One athlete reported sustaining a severe injury to the lower body prior to the study that limited physical performance and was excluded from study participation. After the first testing session, three athletes withdrew from study participation. Mean 25(OH)D status for the athletes was 35.7 ± 8.9 $ng \cdot mL^{-1}$. The percentage of the athletes with sufficient ($\geq$ 32 $ng \cdot mL^{-1}$), insufficient (< 32 $ng \cdot mL^{-1}$) and deficient (< 20 $ng \cdot mL^{-1}$) 25(OH)D status (58) was 62.3%, 37.7% and 0%, respectively. Physical performance and jump execution variable characteristics are presented in Table 5 and Table 6, respectively.

Table 5. Physical performance characteristics for the athletes (n = 53).

	Mean (SD)	Min-Max
Handgrip strength (kg)	64 (9.6)	38-81
Wingate – peak power ($W \cdot kg^{-1}$)	12.6 (1.0)	10.8-14.7
Wingate – mean power ($W \cdot kg^{-1}$)	9 (0.6)	7.4-10.7
Wingate – fatigue index (%)	49.1 (7.0)	32.3-61.3
Jump height – Vertec™ (in)	20.2 (2.8)	14.5-25
Jump height – force platform (m) *	0.40 (0.06)	0.27-0.52

Note. * Indicates n = 52.

Table 6. Jump execution variable characteristics during the squat jump for the athletes (n = 52).

	Mean (SD)	Min-Max
Jump execution time (s)	0.413 (0.069)	0.292-0.631
Peak force (N)	1158 (148)	779-1461
Time to peak force (s)	0.314 (0.076)	0.141-0.502
Time to half peak force (s)	0.138 (0.084)	0.039-0.412
Peak rate of force development ($N \cdot s^{-1}$)	9337 (3002)	3642-17907
Average rate of force development ($N \cdot s^{-1}$)	3926 (1352)	2180-8364
Force gradient ($N \cdot s^{-1}$)	5708 (3009)	1364-13782
Acceleration gradient ($N \cdot s^{-1}$)	3789 (1620)	1428-8756
Peak power (W)	2805 (447)	1810-3792
Time to peak power (s)	0.342 (0.067)	0.228-0.547

25(OH)D status and handgrip strength. The bivariate associations between either continuous or dichotomous 25(OH)D status and handgrip strength were not significant ($p = 0.22$; $p = 0.09$). In HLR, (log of) 25(OH)D status was positively associated with handgrip strength after controlling for weight, height ($p = 0.019$), level of play, % body fat and total physical activity ($R^2 = 0.39$, $F_{inc}(1, 45) = 4.46$, $p = 0.040$). A similar result was found when 25(OH)D status was analyzed as a dichotomous variable ($R^2 = 0.40$, $F_{inc}(1, 45) = 5.34$, $p = 0.025$). The adjusted R^2 change value indicates that 6.5% of the variability in handgrip strength is predicted by (binned) 25(OH)D status.

25(OH)D status and the Wingate Anaerobic Test. When analyzed as a continuous variable, (log of) 25(OH)D status was not associated with any performance component during the WAnT. There was a small, positive correlation between (binned) 25(OH)D status and PP during the WAnT, $r = 0.29$, $n = 53$, $p = 0.035$. In HLR, (binned) 25(OH)D status was significantly

associated with PP after adjusting for level of play ($p = 0.013$), but not after adjusting for % body fat and total physical activity ($R^2 = 0.20$, $F_{inc}(1, 47) = 3.01$, $p = 0.089$).

25(OH)D status and Vertical Jump Performance. There was a medium, positive correlation between either continuous or dichotomous 25(OH)D status and jump height as measured by the Vertec^{TM} ($r = 0.30$, $n = 53$, $p = 0.028$; $r = 0.38$, $n = 53$, $p = 0.005$). Both (log of) 25(OH)D status and (binned) 25(OH)D status were not significantly associated with jump height after adjusting for level of play, % body fat and total physical activity ($p = 0.616$; $p = 0.149$) in HLR. The HLR results for (binned) 25(OH)D status and physical performance outcome variables after adjustment for potential covariates are presented in Table 7.

Neither continuous nor dichotomous 25(OH)D status were associated with jump height calculated from ground force reaction data ($r = 0.18$, $n = 52$, $p = 0.21$; $r = 0.21$, $n = 52$, $p = 0.14$). Bivariate associations between (log of) 25(OH)D status and jump execution variables are presented in Table 8. Jump execution variables were more strongly correlated to 25(OH)D status as a continuous variable. After modeling the relationship between (log of) 25(OH)D status and force gradient, we suspected that level of play may suppress the association between (log of) 25(OH)D status and the jump execution variables. Therefore, jump execution variables that exhibited significant and trending bivariate correlations were analyzed using HLR. A significant bivariate correlation existed between (log of) 25(OH)D status and time to half peak force ($r = -0.28$, $n = 52$, $p = 0.046$); however, HLR was not used for this variable due to its departure from normality that could not be rectified through transformation. The HLR models for the jump execution variables are presented in Table 9.

Table 7. Hierarchal linear regression results for (binned) 25(OH)D status and physical performance outcome variables after adjustment for potential covariates.

	Unstandardized Coefficients		Standardized Coefficients			Correlations	
Variable	Beta	Std. Error	Beta	t	*p*-value	partial	part
Handgrip strength (kg)	6.082	2.632	0.320	2.311	0.025	0.326	0.267
Wingate PP ($W \cdot kg^{-1}$)	0.546	0.315	0.267	1.736	0.089	0.245	0.226
Jump height (in)	1.135	0.774	0.209	1.466	0.149	0.209	0.177

Note. Potential covariates; weight, height, level of play, % body fat and total physical activity. Wingate PP and jump height were not adjusted for weight and height due to a lack of body size dependence. n = 53.

Table 8. Correlations between jump execution variables and (log of) 25(OH)D status.

	r	*p*-value
Jump execution time (s)	-0.231	0.100
Peak force (N)	-0.016	0.908
Time to peak force (s)	-0.212	0.131
Time to half peak force (s)	-0.278*	0.046
Peak rate of force development (sqrt) ($N \cdot s^{-1}$)	0.216	0.126
Average rate of force development ($N \cdot s^{-1}$)	0.059*	0.678
Force gradient ($N \cdot s^{-1}$)	0.323	0.020
Acceleration gradient ($N \cdot s^{-1}$)	-0.101	0.477
Peak power ($W \cdot kg^{-1}$)	0.031	0.825
Time to peak power (s)	-0.230	0.100

Note. * indicates Spearman rho correlational test. n = 52.

Table 9. Hierarchal linear regression of potential covariates and (log of) 25(OH)D status on jump execution variables.

Control variables	RC	PCC	*p-value*	RC	PCC	*p-value*
	Jump execution time (s)			Time to peak force (s)		
Level of play	-0.228	-0.318	0.018	-.0221	-0.283	0.037
Total physical activity	-0.207	-0.282	0.035	-0.193	-0.242	0.071
	Time to peak power (s)			Peak RFD (sqrt) ($N \cdot s^{-1}$)		
Level of play	-0.217	-0.313	0.020	29.7	0.189	0.189
Total physical activity	-0.196	-0.275	0.040			
	Force gradient ($N \cdot s^{-1}$)					
Level of play	10419	0.335	0.012			
Weight, height, % body fat	11506	0.325	0.014			
Total physical activity	10967	0.307	0.020			

Note. RC indicates regression coefficient; PCC, part correlational coefficient; RFD, rate of force development. Models with the jump execution variables as the dependent variable and (log of) 25(OH)D status as the independent variable of interest. Potential covariates were sequentially entered into the control block using HLR guided by theoretical considerations. The addition of weight, height and % body fat into the control block did not significantly impact the relationship between (log of) 25(OH)D status, jump execution time, time to peak force and time to peak power. n = 52.

Discussion

The primary aim of this investigation was to evaluate the strength of association between 25(OH)D status and laboratory measures of physical performance in a sample population of athletes. The results from this investigation in competitive hockey players are mixed. Significant bivariate associations were found between 25(OH)D status and two physical performance outcome measures: PP during the WAnT ($p = 0.035$) and jump height ($p = 0.005$) when measured

by the Vertec™. However, 25(OH)D status was not a significant predictor of either variable after adjusting for measured covariates in HLR. Only for handgrip strength was 25(OH)D status a significant predictor after adjusting for potential covariates in HLR, explaining 6.5% of the variability in handgrip strength, with higher levels of 25(OH)D associated with increased handgrip strength. This finding is consistent with previous investigations that found cross-sectional associations between 25(OH)D status and measures of physical performance (21, 23, 25, 26, 28, 135, 137). However, these investigations have primarily been carried out in aging populations and very few adjusted for body adiposity. We are not aware of any cross-sectional investigations in athletes in which to make comparisons. To our knowledge, this is the first study to demonstrate significant associations between 25(OH)D status and time-related jump execution variables: jump execution time, time to half peak force, force gradient and time to peak power.

It appears that the bivariate associations between 25(OH)D status, PP during the WAnT and jump height when measured by the Vertec™ are confounded by body adiposity. Vitamin D status (binned) was significantly associated with WAnT PP and jump height; however, neither association remained significant after % body fat was entered into the control block in HLR ($p = 0.084$, $p = 0.146$). Vitamin D status was inversely associated with % body fat in our athletes. The causal pathway responsible for this association needs clarification (165), but it appears that body adiposity may sequester vitamin D (167).

The association between 25(OH)D status and multiple jump execution variables represents a novel finding of this study. After adjusting for level of play and self-reported total physical activity, 25(OH)D status was inversely associated with jump execution time ($p = 0.035$), time to peak power ($p = 0.040$) and a trend was found for time to peak force ($p = 0.071$). Further adjustment for weight, height and % body fat did not significantly impact the models, which support the notion that these variables are body size independent (195). Vitamin D status was also associated with time to half peak force ($r = -0.28$, $n = 52$, $p = 0.046$); albeit, this relationship was

not modeled in HLR. In our athletes, it appears that higher levels of 25(OH)D positively influenced time to develop force during the squat jump. The positive impact of 25(OH)D status on time to develop force seems to manifest predominantly during the start of the movement. Further supporting this notion, 25(OH)D status was a significant predictor of force gradient after adjusting for potential covariates ($p = 0.020$), with higher levels of 25(OH)D associated with increased rate of force development in the initial portion of squat jump execution. Nearly 9% of the variability in force gradient was explained by 25(OH)D status in our model (adjusted R^2 change = .089). Task-specific neuromuscular recruitment, muscle contractile properties and strength likely influence RFD during the start of the squat jump (196). Specific to muscle, numerous factors may account for the variability in RFD: muscle fiber type, cross-sectional area, muscle-tendon visco-elastic properties, sarcoplasmic calcium kinetics and propagation of action potentials (196, 197). It is possible that 25(OH)D status influences one or more of the aforementioned physiological factors.

The exact mechanism responsible for the association between 25(OH)D status, handgrip strength and the jump execution variables in our athletes is not known. The vitamin D system achieves its influence on target tissues when $1,25(OH)_2D$ is derived from circulating 25(OH)D and binds with the VDR, initiating non-genomic and genomic signaling (13). The VDR has been identified in skeletal muscle tissue and numerous non-genomic and genomic effects of $1,25(OH)_2D$ have been documented (14). Experimental studies in animals have reported reductions in sarcoplasmic reticulum (SR) volume, T tubule size, troponin C, and actin content in vitamin D depleted animals (183). Furthermore, these investigations demonstrate that $1,25(OH)_2D$ modulates SR calcium kinetics, phosphate control, phospholipid metabolism and myogenic regulation (14). Disruptions in muscle contractile properties have also been noted. Time to peak tension after electrical stimulation was increased in vitamin D deficient rats (183). It appears that type II muscle fibers are more susceptible to the effects of severe 25(OH)D

deficiency in humans (14). Secondary hyperparathyroidism associated with 25(OH)D deficiency may also negatively influence skeletal muscle (14). Vitamin D may also impact the functioning of the central nervous system as the VDR has been identified in neurons and glial cells (198). The impact of 25(OH)D status on physical performance at the circulating concentrations observed in our athletes is unclear.

Interestingly, the bivariate associations were stronger when 25(OH)D status was analyzed as a dichotomous variable for physical performance outcome measures, but not for measured jump execution variables. Although difficult to interpret with our small sample, this may indicate that a threshold effect for 25(OH)D status may exist near our binned midpoint for physical performance outcomes. A linear model better fit the data for the jump execution variables. This may indicate that 25(OH)D status may have a differential impact on select components of task execution. It should be noted that no athlete in our cohort had deficient 25(OH)D levels, which may have contributed to the lack of association between 25(OH)D status and physical performance outcome measures after statistical adjustment.

This investigation has several limitations. The study population may not be representative of athletes participating in other sports and female athletes were not recruited. Vitamin D status was measured from a capillary blood sample. Due to inter-assay variation, caution should be taken when interpreting and comparing 25(OH)D levels between studies. Reliability data pertaining to the jump execution variables is scarcely documented in the literature. Future investigations should address the reliability concerns of theses variables before they are used in prospective trials. Our study has approximately 80% power to detect moderate correlations (0.37). Failure to detect relationships that are potentially relevant to sport may be due to type II error. Causal relationships cannot be determined due to the cross-sectional study design. Residual confounding may exist after adjusting for self-reported total physical activity due to the likely failure of MET-minutes to account for the task-specific adaptations of training. However, the

impact of the yearly training cycle on physical performance variables may have been mitigated due to the short study enrollment period during the offseason. Since all of our athletes were competitive hockey players, physical activity demands associated with sports participation and training exposure were likely similar. All athletes were participating in offseason conditioning programs at the time of study. However, the duration of the offseason conditioning participation and specific content of the conditioning programs were not evaluated.

In conclusion, 25(OH)D status was positively associated with handgrip strength, trended with PP during the WAnT, but not with vertical jump height after adjusting for measured covariates in a sample population of athletes. A novel finding of this study is the association between 25(OH)D status and jump execution variables in our athletes. It appears that 25(OH)D status may positively impact time to develop force and rate of force development during the initial portion of the squat jump. The impact of time to develop force and force gradient on physical performance outcomes associated with hockey performance is unknown. Future investigations incorporating interventional study designs are needed to determine if 25(OH)D status is causally related to jump execution variables and measures of physical performance in athletes with deficient and insufficient 25(OH)D status.

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CHAPTER 5: Vitamin D Status and VO_{2peak} during a Skating Treadmill Graded Exercise Test in Competitive Ice Hockey Players

CHAPTER 5: Vitamin D Status and VO_{2peak} during a Skate Treadmill Graded Exercise Test in Competitive Ice Hockey Players (for submission to the Journal of Strength and Conditioning Research)

Vitamin D Status and VO_{2peak} during a Skate Treadmill Graded Exercise Test in Competitive Ice Hockey Players

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Abstract

Vitamin D status has been associated with cardiorespiratory fitness (CRF) in cross-sectional investigations in the general population. Data characterizing the association between 25(OH)D status and CRF in athletes is lacking. Junior and collegiate ice hockey players were recruited from the Minneapolis, Minnesota (44.9° N) area during the offseason (May 16-June 28). The purpose of this study was to examine the cross-sectional association between 25(OH)D status and CRF in a sample population of competitive ice hockey players. Circulating 25(OH)D level was assessed from a capillary blood sample analyzed using liquid chromatography-tandem mass spectrometry. VO_{2peak} during a skate treadmill graded exercise test (GXT) was used to characterize cardiorespiratory fitness. Data on both 25(OH)D status and VO_{2peak} was available for 52 athletes. Insufficient 25(OH)D concentrations were found in 37.7% of the athletes (< 32 $ng \cdot mL^{-1}$). Vitamin D status was not significantly associated with any physiological or physical parameter during the skate treadmill GXT.

Introduction

Vitamin D is obtained through the diet or endogenously produced in the skin from sunlight exposure (46). Despite this duel source, 25-hydroxyvitamin D (25[OH]D) deficiency and insufficiency are commonly reported in the general population (7) and evidence of insufficiency

in populations of athletes is starting to accumulate (79, 169, 181). Insufficient circulating 25(OH)D levels may negatively impact the kinetics of the active form, 1,25-dihydroxyvitamin D, which is a steroid hormone synthesized as needed by the kidneys or in tissues that possess 1-alpha hydroxylase activity (45). The altered kinetics of 1,25-dihydroxyvitamin D may negatively impact the non-genomic and genomic signaling in tissues that express the vitamin D receptor (13).

The presence of the vitamin D receptor in cardiac muscle, vascular tissue and skeletal muscle (112) supports the notion that the vitamin D system may impact the cardiovascular system's ability to transport oxygenated blood and the skeletal muscles' ability to utilize oxygen. In its active form, 1,25-hydroxyvitmain D is believed to influence the structural remodeling of cardiac and vascular tissue (111). Improved cardiac muscle function has been reported in clinical populations after severe 25(OH)D deficiency was treated (111). Improvements in flow-mediated dilation and blood pressure have been linked to correcting vitamin D deficiency (112, 116). Recently, treating severe 25(OH)D deficiency has been associated with improved mitochondrial oxidative capacity in skeletal muscle (199). These converging lines of evidence indicate that poor 25(OH)D status could negatively influence cardiorespiratory fitness (CRF).

CRF is commonly characterized by the maximal oxygen consumption during a graded exercise test (GXT) and is used to evaluate the functioning of the cardiovascular system (200). Oxygen consumption during a GXT is dependent on cardiac output and arteriovenous oxygen difference. The established relationship between CRF and health-related outcomes underpin the importance of this measure in all populations (200). Specific to sporting populations, CRF is a valuable measure due to its association with endurance performance potential (201, 202), repeated sprint ability (203) and training adaptation (204).

It has been proposed that 25(OH)D status may impact CRF measures in athletes (49). Evidence from early European studies investigating the impact of ultra-violet B exposure and

seasonality on measures of CRF has been primarily used to support the link (2, 49). Recently, poor vitamin D status has been associated with reduced CRF in cross-sectional epidemiologic investigations (37, 38, 205, 206). However, these investigations are not generalizable to athletes. To our knowledge, the association between 25(OH)D status and CRF in athletes has not been previously reported. Therefore, the purpose of this study was to examine the cross-sectional association between 25(OH)D status and CRF in a sample population of athletes.

Methods

Experimental approach to the problem. A cross-sectional study design was utilized to evaluate the association between 25(OH)D status and peak oxygen consumption (VO_{2peak}) during a skate treadmill GXT. An observational approach to the problem is justified due to the absence of data regarding the influence of 25(OH)D status on CRF in athletes. During a 9-14 day period, athletes participated in three testing sessions at the Human and Sport Performance Laboratory and the Mariucci and Ridder Arenas at the University of Minnesota. Hydrostatic weighing, anthropometric assessments, laboratory physical performance tests and a skate treadmill familiarization session were conducted in session one. The skate treadmill GXT and 25(OH)D assay were completed during session two, along with a physical activity questionnaire. On-ice skating performance was evaluated in session three; however, this data will not be presented in this paper. At least two days separated each testing session. Before testing commenced, participants were given instructions to avoid training on testing days. Study enrollment began on May 16 and data collection concluded on June 28, 2012.

Subjects. Fifty-seven Caucasian male competitive ice hockey players volunteered to participate in this study. Athletes were recruited during the off-season from the Minneapolis, Minnesota (44.9° N) area. Informed written consent was obtained after the procedures and risks associated with study participation were explained in detail. The Institutional Review Board at the University of Minnesota approved this study. Exclusion criteria were self-reported evidence of

vitamin D metabolism disruption, corticosteroid use, injury that inhibited performing physical performance tasks. Goaltenders were also excluded from study participation.

Data were available for both 25(OH)D status and VO_{2peak} for 52 ice hockey players (25 Division I, 17 Junior A, 10 Division III; 32 forwards, 20 defensemen) after dropout, exclusion and equipment issues. One athlete was excluded from participating in the study due to a previous injury and three athletes dropped out after the first testing session. One athlete had an equipment issue concerning his skates and was not able to participate in the skate treadmill GXT. The descriptive characteristics for the athletes are presented in Table 10.

Table 10. Athlete characteristics (n = 53).

	Mean (SD)	Min-Max
Age (years)	20.1 (1.5)	18-23
Height (cm)	182.0 (6.3)	169.5-197.8
Weight (kg)	84.3 (6.9)	71.2-98.5
BMI ($kg \cdot m^{-2}$)	25.4 (1.3)	21.5-27.7
Percent body fat (%) (n = 51)	12.5 (3.9)	7.1-28.9

Equipment. The skate treadmill GXT was conducted on a motor-driven skate treadmill (Acceleration Products Inc., Fargo, ND). Oxygen consumption was assessed using indirect calorimetry via open-circuit spirometry using an Ultima CPX^{TM} (Medical Graphics, St. Paul, MN). Heart rate (HR) was measured with Polar S810 HR monitor (Kempele, Finland). Percent body fat was determined via hydrostatic weighing using Exertech Body Densitometry Systems software (Dresbach, MN). Standard measures of height and weight were taken using a wall-mounted stadiometer (Genentech Inc.) and mechanical scale (Detecto Scale Co.).

Procedures. Standard measures of height and weight were recorded at the beginning of session one. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively.

Next, the hydrostatic weighing technique was used to determine body density (171). The procedure used for hydrostatic weighing is described in Graves et al. (171). Briefly, residual lung volume was estimated (172) and percent body fat was determined using the Brozek et al. (1963) equation. At the conclusion of the first testing day, athletes underwent a skate treadmill familiarization trial. The intent of the familiarization trail was to allow the athletes to become accustomed to skating on the treadmill and the protocol. During the familiarization trial, the athletes performed the exact protocol that would be used during the GXT.

Upon arrival to the laboratory for session two, a non-fasted capillary blood sample was collected from each athlete for the determination of 25(OH)D status. The capillary blood sample was collected, stored, packaged and shipped in strict accordance to ZRT Laboratory's specifications. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine 25(OH)D concentrations from dried bloodspot samples. The ZRT laboratory dried blood spot technique is strongly correlated to traditional serum measurement of $25(OH)D_3$ ($r = .91$) and $25(OH)D_2$ ($r = .90$) and demonstrates favorable precision ($CV \leq 13\%$, reference range: 14-81 $ng \cdot mL^{-1}$) (174). According to the manufacturer, the ZRT Laboratory dried blood spot assay is strongly correlated ($r^2 = .97$) with the DiaSorin radioimmunoassay. ZRT laboratory participates in the Vitamin D External Quality Assessment Scheme.

Following the 25(OH)D assay, athletes performed a skate treadmill GXT. The skate treadmill GXT protocol used in this study was adopted from Koepp and Janot (207). The skate treadmill GXT was selected because it allows for a sport-specific assessment of VO_{2peak} in hockey players (207-209). The results from our pilot testing indicated that this protocol was appropriate for our sample population of hockey players. The protocol was continuous in nature with 1-minute stages, with an initial speed and grade of 6.5 mph and 2%, respectively. Workload progression was achieved by a 0.5 mph increase in speed after stages 1-7 and 1% increase in grade after each stage thereafter. The test was terminated upon the athlete's voluntary cessation.

Athletes wore a harness connected to an overhead support system as a safety precaution. The subjects were not unweighted to any degree during the performance of the test.

The Ultima CPX^{TM} was calibrated prior to each test. The athlete was fitted with a Polar HR monitor. The athlete's safety harness was inspected to ensure that it was secured correctly and attached to the overhead track appropriately. Just prior to the commencement of the test, the athlete was fitted with a neoprene facemask (Medical Graphics, St. Paul, MN) and the umbilical unit was attached. Collection lines were cleared after each test to remove moisture and were replaced after every third trial.

Statistical analysis. SPSS version 20.0 (IBM, Armonk, NY) was used for all statistical analyses. Data were inspected at the univariate level for normality and the Kolmogorov-Smirnov test was also utilized prior to further analysis. A logarithmic transformation was performed on 25(OH)D status, which successfully eliminated the positive skew and reduced the influence of a few suspected outliers. Descriptive characteristics are presented as means and standard deviations. Pearson moment-product correlation coefficients were used to evaluate the association between (log of) 25(OH)D status and physiological and physical performance variables during the skating treadmill GXT. Statistical significance was determined using two-sided p-values and an alpha of 0.05. This exploratory investigation had approximately 80% power to detect moderate correlations (0.37).

Results

The mean 25(OH)D concentration for the athletes was 35.7 ± 8.9 ng·mL^{-1}. None of the athletes' 25(OH)D concentrations were below the threshold for deficiency (< 20 ng·mL^{-1}) (58). Insufficient 25(OH)D concentrations were found in 37.7% of the athletes (< 32 ng·mL^{-1}) (58). Table 11 provides the descriptive data for the physiological and physical parameters during the skating treadmill GXT. Figure 3 depicts the distribution of skate treadmill GXT duration for the athletes. The bivariate associations between (log of) 25(OH)D status and these parameters are

presented in Table 12. Vitamin D status (log) was not significantly associated with any parameter during the skate treadmill GXT.

Table 11. Descriptive characteristics for the skate treadmill graded exercise test for the athletes (n = 52).

	Mean (SD)	Min-Max
Absolute VO_{2peak} ($ml \cdot min^{-1}$)	4604 (431)	3729-5843
Relative VO_{2peak} ($ml \cdot min^{-1} \cdot kg^{-1}$)	54.6 (4.3)	44.9-64.4
Max heart rate ($b \cdot min^{-1}$)	199 (10)	179-221
Peak RER	1.20 (0.07)	1.07-1.37
Final stage completed (stage)	9.5 (2.2)	4-14
Total exercise time (min)	9.94 (2.16)	4.38-14.13

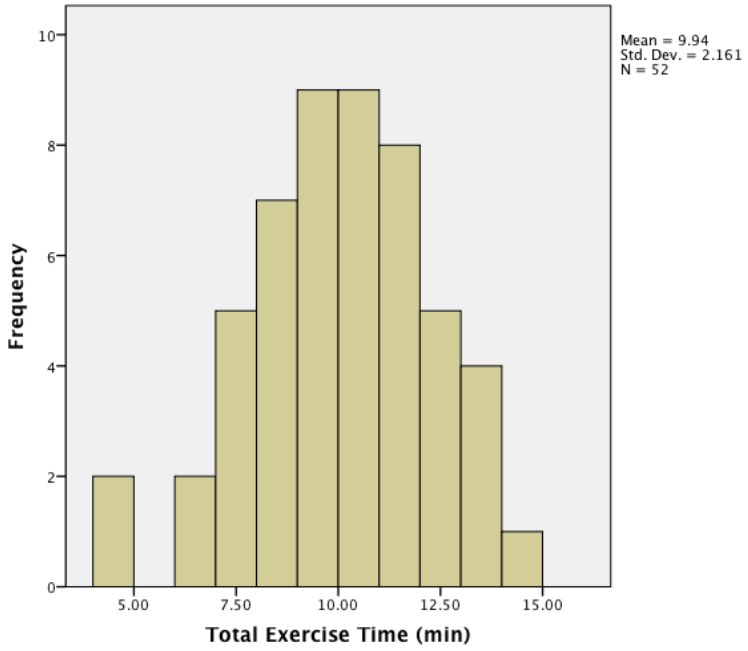

Figure 3. Distribution of skate treadmill GXT duration for the athletes

Table 12. Correlations between (log of) 25(OH)D status and physiological and physical performance parameters during the skate treadmill graded exercise test (n = 52).

	r	*p*-value
Absolute VO_{2peak} ($ml \cdot min^{-1}$)	-0.103	0.469
Relative VO_{2peak} ($ml \cdot min^{-1} \cdot kg^{-1}$)	0.052	0.713
Max heart rate ($b \cdot min^{-1}$)	0.059	0.689
Peak RER	0.151	0.284
Final stage completed (stage)	0.154	0.277
Total exercise time (min)	0.173	0.220

Discussion

The purpose of this investigation was to examine the association between 25(OH)D status and CRF in male hockey players. We did not detect a significant bivariate association between 25(OH)D status and any measured physiological or physical parameter during the skating treadmill GXT. We are unaware of any other investigations in athletes in which to compare our results. Findings from previous studies in non-athlete populations have reported significant associations between 25(OH)D status and CRF (37, 38, 128, 205, 206, 210, 211). Of the studies that reported bivariate correlations between 25(OH)D status and CRF, the strength of the unadjusted associations denoted as significant ranged from r = 0.07 to r = 0.36 (37, 128, 206, 210, 211).

The strongest bivariate correlation was detected in a population of 59 health young females ranging in age from 16 to 24 years (210). Interestingly, the majority (72%) of the study participants possessed sufficient 25(OH)D levels and no participant's levels were below 15 $ng \cdot mL^{-1}$ (210). No further analysis was conducted in this investigation to examine whether body adiposity mediated the relationship between 25(OH)D status and CRF. Since body adiposity was inversely associated with CRF (r = -0.70) and 25(OH)D status (r = -0.44) in this study, calciferol

compound sequestering by body adiposity (166, 167, 175) likely contributed to the strength of the reported bivariate association. It is possible that the restricted range of body fat in our athletes (12.5% ± 3.9) reduced confounding created by body adiposity, contributing to the lack of an association in our athletes.

Adjusting for the heterogeneous characteristics inherent in large population samples tends to reduce the reported bivariate associations between 25(OH)D status and CRF. In a large cohort of adolescents, 25(OH)D status was associated with CRF before ($r = 0.21$) and after ($r = 0.10$) adjusting for covariates; although, body adiposity was not included as a covariate (128). Vitamin D status was associated with CRF ($r = 0.29$) in 200 adults participating in a clinical trial investigating the impact of statins and physical performance (37). Interestingly, they reported an interaction between 25(OH)D status and hours spent participating in moderate and vigorous physical activity (37). When data were analyzed by percentiles of physical activity, 25(OH)D status was associated with CRF when physical activity was low and moderate ($p < 0.001$), but not high ($p = 0.9$). In an analysis of the National Health and Nutrition Examination Survey, 25(OH)D status was associated with CRF in men ($r = 0.11$) and women ($r = 0.07$) with a mean age of 28.9 ± 0.2 years, but neither association remained significant after adjusting for age, race/ethnicity, season, self-reported physical activity and BMI in general linear regression (211). A small effect was detected (1.7 $ml \cdot kg^{-1} \cdot min^{-1}$, $p = 0.001$) in men when data was analyzed based on deficiency (211). Beyond demonstrating that the association between 25(OH)D status and CRF is weak in a representative sample of the United States population, this investigation appears to indicate that the severity of 25(OH)D insufficiency in the study sample may also influence the strength of association between 25(OH)D status and CRF.

It is possible that the homogenous population of competitive hockey players in this study reduced the influence of covariates that tend to augment the association between 25(OH)D status and CRF. The sample population characteristics and short data collection period preclude the

need to adjust for many covariates: age, sex, race/ethnicity and season. It is possible that age-related changes in 25(OH)D status and metabolism strengthen the relationship between 25(OH)D and CRF (211). Compared to the general population, it is reasonable to assume that these athletes participated in more moderate and vigorous physical activity. It appears that higher levels of physical activity may attenuate the association between 25(OH)D status and CRF (37). The mean $MET \cdot min \cdot week^{-1}$ for moderate and vigorous physical activity was 6278 ± 3245 in our athletes as assessed using the Short Questionnaire for Health Enhancing Physical Activity (192) (data not presented). As previously mentioned, the restricted range of body fat in these athletes (12.5 ± 3.9) may reduce the potential confounding produced by body adiposity. Furthermore, none of our athletes had deficient 25(OH)D levels. While 37.7% of our athletes possessed insufficient 25(OH)D levels, deficiency may be necessary to result in compromised CRF (211). Analyzing the data based on insufficiency did not significantly impact the correlations between 25(OH)D status and any parameter during the skating treadmill GXT (data not shown).

In our athletes, relative VO_{2peak} during a skating treadmill GXT was used to characterize CRF. The mean relative VO_{2peak} in our athletes was similar to the reported VO_{2max} in comparative populations of competitive ice hockey players obtained during GXTs employing other modalities of exercise (212). The decision to use the skating treadmill GXT was guided by the principle of specificity. In athletes, it is generally recommended that the GXT modality used should reflect the primary locomotion involved in their sport (213). Utilizing sport-specific GXTs may improve the relevance of the testing data in hockey players (214). The optimal duration of a GXT is thought to be within 8-12 minutes (215). Approximately 17% of our athletes were below and 19% were above the stated optimal total test duration for a GXT. Only four athletes ended the GXT before 7.5 minutes. Two of the three secondary criteria for reaching VO_{2max} during a GXT were also achieved in the overwhelming majority of our athletes: a respiratory exchange ratio of greater than 1.10 (213) was not achieved in two athletes and one athlete failed to reach 90% of his age-

predicted maximum HR during the skating treadmill GXT (215). This data confirms our pilot testing that the skating treadmill protocol designed by Koepp and Janot (207) is well suited for use in junior and collegiate hockey players.

This investigation has several limitations. The homogenous characteristics of the sample population limit the generalizability of this data. A capillary blood sample was used for the determination of 25(OH)D status. Interassay variation may complicate the comparison of 25(OH)D status between studies. This exploratory investigation was powered to detect moderate correlations and may fail to detect meaningful associations relevant to hockey performance. The validity and reliability of the skating treadmill GXT has not been well characterized. However, no significant difference in VO_{2max} was found when comparing the continuous skating treadmill GXT and that obtained on a running treadmill (207). The results from our investigation indicate that the ideal parameters for a GXT were met in the majority of the athletes. The major strength of our study is the relatively large, homogenous sample population of athletes, which reduces the confounding by other factors. The short data collection period may ameliorate the impact of the training cycle on physical and physiological variables.

Practical Application

Evidence from large epidemiologic cross-sectional investigations appear to indicate that 25(OH)D status is associated with CRF. However, these investigations are not generalizable to athletes, the strength of the association between 25(OH)D status and CRF is generally weak (37, 128, 206, 211), and the association appears to be attenuated by time spent participating in moderate and vigorous physical activity (37). Vitamin D status was not associated with VO_{2peak} or end stage completed during a skating treadmill GXT in junior and collegiate male hockey players. Compared to the large epidemiologic studies, our investigation lacks statistical power to detect associations that are potentially relevant to sport. However, our exploratory investigation

indicates that the magnitude of the effect in athletes may be weaker than that observed in epidemiologic studies. The practical significance of such a weak association is unknown.

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CHAPTER 6: References

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CHAPTER 7: Appendices

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7.1 Informed consent

CONSENT FORM
Vitamin D and Measures Related to Physical Performance Consent Form
University of Minnesota

You are invited to participate in a research study assessing vitamin D levels, physical performance, and body composition. You were selected as a possible participant because you are an NCAA Division I or Division III hockey player. We ask that you read this form and ask any questions you may have before agreeing to be in the study.
The principal investigator (person in charge) of this study in Stacy Ingraham, Ph.D. The study co-investigators are John Fitzgerald, B.S., and Ben Peterson, M.Ed. The researchers work in the Department of Kinesiology at the University of Minnesota. The study is funded by the Human and Sport Performance Laboratory.

Study Purpose
The purpose of the study is to determine if vitamin D levels influence physical performance, aerobic capacity, and body composition in hockey players.

Study Procedures
If you agree to participate in this study, we would ask you to participate in two research visits to the Laboratory of Physiological Hygiene and Exercise Science, located in 27 University Recreation Center on the campus of the University of Minnesota. One of the two visits will also include a skating treadmill exercise test that will be conducted at Mariucci arena on the University of Minnesota campus. Total testing time will be approximately 120 minutes or less per session. The two testing sessions will include the following:

Laboratory of Physiological Hygiene and Exercise Science:

Session 1 (approximately 120 minutes)

- Standard measurements of height and weight will be taken.
- Body composition (fat mass and fat free mass) will be taken using underwater weighing. You will be asked sit on a chair that is under water. While seated on the chair, the water level will be at approximately shoulder level. You will be asked to take a deep breath and then maximally exhale, and then submerse your head below the water for a brief period.
- Basic background history, physical activity, dietary habits, and sun exposure will be assessed. You will be asked to fill out a brief questionnaire for each topic.
- Jumping ability will be assessed. You will be asked to jump as high as possible using two different jumping techniques.
- Hand grip strength will be assessed. You will be asked to maximally squeeze a gripping device for approximately two seconds.
- Power production while cycling will be measured. You will be asked to pedal as fast as possible on a cycle ergometer (exercise bike) against a resistance for 30 seconds.
- A skating treadmill familiarization session will be preformed after testing. The skating treadmill familiarization session will be performed on a Frappier skating treadmill. This oversized treadmill has a surface that enables you to skate naturally. The familiarization

session will require you to skate on the treadmill at different speeds and grades that you will experience in the skating treadmill GXT during session 2. The purpose of the familiarization session is to allow you to become comfortable with skating on the treadmill.

Session 2 (approximately 60 minutes)

- Vitamin D levels will be assessed. Your finger will be pricked and a small blood sample will be taken. The amount of blood needed for the test is approximately 13 drops or 1/5 of a teaspoon.
- Aerobic capacity will be assessed during a skating treadmill graded exercise test (GXT). The skating GXT will be performed on a Frappier skating treadmill. This oversized treadmill has a surface that enables you to skate naturally. The exercise intensity (skating speed and treadmill grade) will begin at a low level and will be advanced in stages depending on your fitness level. During the test you will wear a facemask and breathing valve that allows for exhaled air to be analyzed. We may stop the test at any time because of signs of fatigue or changes in your heart rate or symptoms you may experience. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort. For your safety, you will be wearing a safety harness that is attached to a steel structure on the celling. If you where to lose your balance while skating, the harness will prevent you from falling.

If you agree to participate in a secondary study, further testing will involve two additional visits to Mariucci and Ridder Arena on the University of Minnesota campus and include the following:

Session 3 (approximately 35 minutes)

- At the start of the session, you will complete a 10-minute general warm-up. This includes a 5-minute bike warm-up and a 5-minute dynamic warm-up of the lower extremities. During the dynamic warm-up, each participant will be taken through a series of motions that work to activate and warm-up the muscles that will be used during the testing exercise. The dynamic warm-up will involve 6 low-intensity bodyweight exercises performed in series.
- Lower-body power will be assessed. You will be asked to perform two attempts in a broad jump test. A piece of tape will be laid on the floor, marking where you will place your toes. You will be instructed to jump as far as you can.
- Lower-body strength will be assessed. You will be asked to perform the barbell back squat exercise. You will be asked to perform multiple sets of the exercise, starting with a low weight and gradually progressing to a maximal weight that you can perform while maintaining correct exercise form. The last successful attempt will be recorded as your one repetition maximum (1RM). Your barbell back squat 1RM is the maximal amount of weight that you can lift during this exercise while maintaining correct exercise form. We may stop the test at any time, because of signs of fatigue or changes in your exercise form or symptoms you may experience. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort. A National Strength and Conditioning Association certified strength and conditioning specialist (CSCS) will conduct all 1RM testing. In addition, a 3-point person spot will be provided on every maximal attempt for your safety.

Session 4 (approximately 50 minutes)

- You will be required to wear full hockey gear to the rink. Before testing begins, you will be instructed to go through your normal team, pre-game warm-up to ensure you are loose and prepared for high intensity activity.
- Skating acceleration and speed will be assessed on ice. You will be asked to skate as fast as possible from a standing position to a cone 15 meters away. You will then coast around the perimeter of the ice rink three times, building in speed on the last lap as you re-approach the starting line. You will be instructed to skate as fast as possible and maintain your speed for 15 meters after you reach the starting line. You will be required to wear all of your hockey equipment during the assessment. We may stop the test at any time, because of signs of fatigue or changes in your heart rate or symptoms you may experience. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort.
- Repeated sprinting ability will be assessed on-ice. The assessment involves skating around a series of cones as fast as possible for approximately 26 seconds. After a rest period (110 seconds), you will be asked to complete the course again. You will be asked to repeat the course eight times. You will be required to wear all of your hockey equipment during the assessment. We may stop the test at any time, because of signs of fatigue or changes in your heart rate or symptoms you may experience. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort.

Risks of Study Participation

The study has the following risks. The physical performance testing in this study involves maximal effort. There exists the possibility of certain changes during this testing. These include abnormal blood pressure, fainting, irregular, fast, or slow heart rhythm, and in rare instances, heart attack, stroke, or death. In addition, there is a maximal lower-body strength test if you participate in the secondary study, which will load the spine during a period of maximal exertion. While very rare, the possibility of serious bodily injury can occur in the form of a pulled or strained muscle, disc herniation, sports hernia, or broken bone. Every effort will be made to minimize these risks by evaluation of preliminary information related to your health and fitness and by careful observation during testing. We may stop the test at any time because of signs of fatigue or changes in your heart rate or symptoms you may experience. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort. This study involves a capillary blood test in which a small quantity of blood is taken. This procedure involves minimal discomfort. You may experience slight discomfort during the finger prick and you may experience pain, tenderness at the puncture site after the procedure and a small, but possible chance of infection. Every effort will be made to minimize the risks associated with this procedure by properly selecting the puncture site, sterilizing the selected site, and using sterilized equipment. After the procedure is completed, the puncture site will be cleaned and appropriately bandaged. This study also involves underwater weighing. You may not be comfortable maximally exhaling and submerging yourself underwater for a brief period. Every effort will be made to clearly explain the procedure and minimize any anxiety associated with the procedure. Emergency equipment and trained personnel are available to deal with unusual situations that may arise.

Benefits of Study Participation
There may be no direct benefit to you for participating in this study. The benefit to study participation is that you will receive physiological testing results, relevant to your sport, which may allow you to evaluate or modify your current training practices.

Study Costs/Compensation
You will not be compensated for your participation in this study. You may incur costs due to participation in this study. The cost of travel will vary depending on the distance you live from the Laboratory of Physiological Hygiene and Exercise Science. If you will be traveling by automobile, you will have to pay to park. Parking costs will range from $4 to $9 per visit. You will not be reimbursed for either travel or parking costs associated with participation in the study.

Research Related Injury
In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study investigators know right away.

Confidentiality
The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by research staff and by departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute. Study information will not be recorded in your medical record. Study data will be encrypted according to current University policy for protection of confidentiality. Only the previously stated research personnel will have access to the data. Neither coaches nor anyone else will be granted access to any information unless permission is given by the participant. The data/records will be destroyed after a five year time period.

Voluntary Nature of the Study
Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University of Minnesota. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions
The researchers conducting this study are Stacy Ingraham, John Fitzgerald, and Ben Peterson. You may ask any questions you have now, or if you have questions later, **you are encouraged to** contact them at:

Stacy Ingraham, PhD
University of Minnesota School of Kinesiology
(612) 626-0067

John Fitzgerald
University of Minnesota School of Kinesiology
(612) 210-5599
Advisor: Stacy Ingraham, PhD

Ben Peterson
University of Minnesota School of Kinesiology
(763) 300-8545
Advisor: Stacy Ingraham, PhD

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this office in writing or in person at *Fairview Research Administration, 2433 Energy Park Drive, St. Paul, MN 55108.*

You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Printed Name of Subject_____

Signature of Subject_____

Date_____

Printed Name of Person Obtaining Consent_____

Signature of Person Obtaining Consent_____

Date_____

7.2 Preparticipation Screening Questionnaire

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Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming more physically active.

Please read carefully and check all that apply to you at this time.

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all *true* statements.

History

You have had:
___ A heart attack
___ Heart Surgery
___ Cardiac catheterization
___ Coronary angioplasty (PTCA)
___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ Heart valve disease
___ Heart failure
___ Heart transplantation
___ Congenital heart disease

Symptoms

___ You experience chest discomfort with exertion.
___ You experience unreasonable breathlessness.
___ You experience dizziness, fainting, blackouts.
___ You take heart medications.

If you marked any of the statements in this section, consult your physician or other appropriate healthcare provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.

Other health issues

___ You have diabetes
___ You have asthma or other lung disease
___ You have burning or cramping in your lower legs when walking short distances
___ You have musculoskeletal problems that limit your physical activity
___ You have concerns about the safety of exercise
___ You take prescription medication (s)
___ You are pregnant

Cardiovascular risk factors

___ You are a man older than 45 years
___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal
___ You smoke or quit within the previous 6 mo.
___ Your BP is greater than 140/90
___ You don't know your BP
___ You take BP medication
___ Your blood cholesterol level is >200 mg/dL
___ You don't know your cholesterol level
___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister)
___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week)
___ You are more than 20 pounds overweight

If you marked two or more of the statements in this section, you should consult your physician or other appropriate healthcare provider before engaging in exercise. You might benefit by using a facility with a **professionally qualified exercise staff** to guide your exercise program.

___ None of the above is true

You should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your exercise program needs.

Balady et al. (1998). AHA/ACSM Joint Statement. Recommendations for Cardiovascular Screening, Staffing, and Emergency Policies at Health/Fitness Facilities. *Medicine & Science in Sports & Exercise, 30(6)*. (Also in: ACSM's Guidelines for Exercise Testing and Prescription, 7th Edition, 2005. Lippincott Williams and Wilkins http://www.lww.com)

www.acsm-msse.org/pt/pt-core/template-journal/msse/media/0698c.htm