

**Vitamin D in Human Health and Performance:
The Pursuit of Evidence-Based Practice in an Era of
Scientific Uncertainty**

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ABSTRACT

Background: Calciferol (vitamin D) is an essential nutrient that can be synthesized in the skin upon exposure to ultraviolet-B (UVB) light, or obtained through dietary and supplement sources. Traditionally known for its role in bone metabolism, vitamin D is currently described as a pleiotropic hormone with genomic and non-genomic roles in most body tissues. Clinical practice guidelines related to vitamin D are inconsistent and controversial. The purpose of this dissertation was to describe current patterns of vitamin D-related clinical care in a variety of settings, and to evaluate the impact of vitamin D supplements on the health and performance of collegiate athletes, a group with high prevalence of low vitamin D (LVD).

Methods: This dissertation consists of five studies: 1) a scoping review of the health services literature related to clinical management of vitamin D; 2) a retrospective analysis of clinical care following non-indicated vitamin D testing using electronic health record (EHR) data from a regional health system; 3) a survey study to assess vitamin D-related practices among National Collegiate Athletic Association (NCAA) Division I programs; 4) an open clinical trial to evaluate the efficacy of a specific vitamin D supplement protocol in treating collegiate basketball athletes with LVD; and 5) a randomized, double-blind clinical trial to determine health and performance effects of vitamin D supplements in collegiate swimmers participating in fall season training.

Results: Substantial inconsistency in vitamin D-related care was observed throughout the first three studies. Exponential increases in vitamin D testing and treatment, and associated costs, were identified in the U.S. and several other countries. A high proportion of this care was considered non-indicated (i.e., counter to professional guidelines). A lower rate of non-indicated vitamin D-related services was conducted within the health system we studied, but a cascade of low value services followed non-indicated vitamin D testing. Vitamin D testing was regularly performed by more than 65% of NCAA programs. In basketball athletes, 10 weeks of daily vitamin D₃ supplements (5000 or 10,000 IU based on initial vitamin D status) improved serum 25-hydroxyvitamin D [25(OH)D], the common biomarker of vitamin D status. In swimmers, a vitamin D supplement protocol (5,000 IU vitamin D₃ daily for 12 weeks) was efficacious in attenuating a seasonal decline in 25(OH)D compared with placebo. Swimmers taking vitamin D supplements also showed greater improvements in strength, power, and fat free mass. In both athlete studies, taking vitamin D supplements was associated with higher free testosterone concentration.

Conclusions: The provision of evidence-based care related to vitamin D is complicated by contradictory clinical practice guidelines, resulting in inconsistent and sometimes, non-indicated care. Focused research on specific populations at high-risk for LVD can inform best practices. Our results suggest that taking vitamin D supplements is an efficacious strategy for athletes to improve 25(OH)D, especially when UVB exposure is low, and to enhance strength and power in collegiate swimmers.

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GENERAL AUDIENCE ABSTRACT

Vitamin D is known as the “sunshine vitamin” since it can be synthesized by the human body when exposed to specific wavelengths of ultraviolet-B (UVB) light. Some foods and dietary supplements also contain vitamin D. A relationship between vitamin D and bone health is well-established, but emerging research has also associated vitamin D status with a number of different diseases and health problems, including cancer, cardiovascular disease, autoimmune conditions, and depression. Unfortunately, this research is currently inconclusive, and healthcare providers’ professional guidelines related to vitamin D are highly variable. Thus, providing evidence-based care related to vitamin D is complicated. This dissertation consists of a series of three research studies that describe healthcare providers’ vitamin D-related care considering the uncertain landscape, and two research studies that explore the role of vitamin D in collegiate athletes. We chose athletes since a high proportion of them have deficient or insufficient vitamin D status, and because some research has shown that this low vitamin D status affects athletic performance. Results of these studies showed that vitamin D-related health services such as blood testing have increased dramatically over the past 15 years, as have costs associated with these services. Opportunities to improve consistency and quality of care were observed in multiple settings. In the athlete studies, a high rate of vitamin D deficiency and insufficiency was observed among basketball athletes, and we identified vitamin D supplement treatment protocol effective in improving vitamin D status. In addition, swimmers who took vitamin D supplements performed better on strength and conditioning tests than those who took placebo supplements. A favorable relationship between testosterone concentrations and vitamin D status was shown in both basketball athletes and swimmers. Continuing to conduct research focused on specific populations can help healthcare providers develop consistent, high quality, evidence-based care related to vitamin D.

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ATTRIBUTION

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This manuscript was published in the Journal of Nutrients.

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Data from this study were also presented as a poster at the North American Primary Care Research Group's Annual Meeting (2018).

John Epling, MD, MSED, Professor and Medical Director of Research, Department of Family and Community Medicine, Virginia Tech Carilion School of Medicine, participated in study design and manuscript review. Matthew Hulver, PhD, Professor and Department Head, Department of Human Nutrition, Foods, and Exercise, Virginia Tech, and Co-Director, Center for Transformative Research on Health Behaviors, Virginia Tech Carilion Fralin Biomedical Research Center and Vivica Kraak, PhD, RDN, Assistant Professor, Human Nutrition, Foods, and Exercise, Virginia Tech edited the manuscript. Virginia Pannabaker, MS, Associate Director, Research Collaboration and Engagement, Virginia Tech Library Services, provided assistance with the literature search.

CHAPTER 4

Following additional data analysis, this manuscript will be submitted for potential publication in a scientific journal. Data from this study have been submitted for presentation at the North American Primary Care Research Group's Annual Meeting (2019).

Dr. Epling was involved in study design, approval, data analysis, and manuscript preparation. Marianna Salamoun, MS, Data Analyst, Health Analytics Research Team, Carilion Clinic, extracted data from the electronic health record.

CHAPTER 5

This manuscript has been accepted by the Journal of Athletic Training, and will be published in an upcoming issue.

Ernest Eugene, EdD, ATC, former Associate Director, Department of Intercollegiate Athletics, Virginia Tech, participated in survey development and reviewed the manuscript.

CHAPTER 6

This manuscript is in preparation for submission to a scientific journal. Data from this study were also presented as a poster at the Sports, Cardiovascular, and Wellness Nutritionists (SCAN) Dietetics Practice Group Annual Meeting (2018).

Dr. Hulver was involved in study design, data analysis, and manuscript review. Madlyn Frisard, PhD, Associate Professor, Department of Human Nutrition, Foods, and Exercise participated in study design, led approval and registration processes, and edited the manuscript. Mark Rogers, DO, Professor, Edward Via College of Osteopathic Medicine, and Team Physician, Department of Intercollegiate Athletics, Virginia Tech served as medical director for the study, and edited the manuscript. Jennifer Zabinsky, MAED, RDN, Associate Athletics Director for Sports Nutrition, Department of Intercollegiate Athletics, Virginia Tech, participated in study design, data collection, and dissemination of results. Ryan McMillan, PhD, Research Assistant Professor, Department of Human Nutrition, Foods, and Exercise, Virginia Tech, led blood analyses. Greg Donlon, BS, was involved in study design and data collection. Greg Werner, CSCS, CSCC, Associate Director of Athletics, Department of Intercollegiate Athletics, Virginia Tech, and Dr. Eugene helped coordinate supplement and questionnaire administration.

CHAPTER 7

This manuscript is in preparation for submission to a scientific journal. Data from this study were also presented as a poster at the Food and Nutrition Conference Expo (FNCE) of the Academy of Nutrition and Dietetics (2018).

Dr. Hulver was involved in study design, data analysis, and manuscript review. Dr. Frisard participated in study design, led approval and registration processes, and edited the manuscript. Kevin Davy, PhD, Professor, Department of Human Nutrition, Foods, and Exercise assisted with study design, data analysis, and edited the manuscript. Dr. Rogers participated in study design and served as medical director for the study. Wen You, PhD, Associate Professor, Department of Agricultural and Applied Economics, Virginia Tech, assisted with statistical analysis. Janet Rankin, PhD, Professor Emeritus, Department of Human Nutrition, Foods, and Exercise, Virginia Tech, edited the manuscript. Dr. McMillan led blood analyses and edited the manuscript. Ms. Zabinsky participated in study design, data collection, and dissemination of results. Adam Viet, MAED, ATC, Assistant Director of Athletics, Department of Intercollegiate Athletics, Virginia Tech, helped coordinate testing and administration of supplements.

APPENDIX P

Drs. Davy and Hulver participated in study design and data analysis. Jana Leotta, undergraduate student, Human Nutrition, Foods, and Exercise, Virginia Tech, assisted with data analysis.

LIST OF ABBREVIATIONS

1,25(OH)₂D= 1,25-dihydroxyvitamin D
25(OH)D= 25-hydroxyvitamin D
DEQAS= vitamin D external quality assessment scheme
DBP= vitamin D binding protein
DRI= dietary reference intakes
DXA= dual-energy x-ray absorptiometry
EHR= electronic health record
fT= free testosterone
IGF-1= insulin-like growth factor-1
IOM= Institute of Medicine
IRB= Institutional Review Board
IU= International Units
LVD= low vitamin D
NAM= National Academy of Medicine
NCAA= National Collegiate Athletic Association
NFL= National Football League
NHANES= National Health and Nutrition Examination Survey
NI= non-indicated
NOAEFL= No Adverse Effect Level
OTC= over-the-counter
PRISMA= Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTH= Parathyroid hormone
RDA= Recommended Dietary Allowance
SHBG= Sex hormone binding globulin
T= total testosterone
USPSTF= United States Preventive Services Task Force
UVB= ultraviolet-B light
VDR= vitamin D receptor

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Vitamin D is a hormone that is synthesized in human skin upon exposure to ultraviolet-B light, and found in some foods (ex: fatty fish, eggs, mushrooms, fortified dairy products) and dietary supplements. An association between vitamin D status and skeletal health is well-established.¹ Over the past 15-20 years, a large body of evidence has also linked LVD to the development and progression of a variety of health issues, including cardiovascular disease, cancer, diabetes, and autoimmune diseases, among many others.^{2,3} Although much of this evidence is contradictory and inconclusive, interest in the role of vitamin D in human health has soared.

Depending on the criteria used, more than half of adults in the U.S. have vitamin D deficiency or insufficiency, or low vitamin D (LVD), a condition that is on the rise worldwide.⁴ Unfortunately, clinical practice related to vitamin D is complicated. In addition to evolving research and intense public interest, healthcare providers must navigate conflicting professional guidelines and recommendations related to the prevention, evaluation, and treatment of LVD. For example, The National Academy of Medicine, formerly known as the Institute of Medicine, considers 25-hydroxyvitamin D [25(OH)D] (the common biomarker for vitamin D status) of 12 to 20 ng/mL to be adequate.⁵ In contrast, the U.S. Endocrine Society recommends 25(OH)D of 30 to 60 ng/mL for optimal health.⁶ Research to evaluate patterns of healthcare that have emerged as a result of this uncertain landscape is needed.

In order to establish clear and consistent professional practice guidelines, a better understanding of the functions of vitamin D in health and performance is also needed. Since LVD is especially common in collegiate athletes,^{7,8} and there is some evidence that LVD is associated with decreased athletic performance, impaired muscle recovery, and increased susceptibility to illness and injury, focused clinical trials in this population are warranted.^{9,10}

Vitamin D supplements are a safe, low-cost intervention that, if shown to impact athletic performance and health status, offer an appealing intervention for collegiate athletes.

The purpose of this dissertation is to describe current patterns of vitamin D-related clinical care in a variety of settings, and to explore health and performance effects of vitamin D supplements in collegiate athletes, a group with high prevalence of LVD.

1.2 RESEARCH OBJECTIVES

Study 1. Clinical Management of Low Vitamin D: A Scoping Review of Physicians' Practices

Primary Objective:

To review the global healthcare services literature regarding physicians' management of LVD, costs associated with clinical practices related to LVD, and efforts to constrain inappropriate clinical practices related to LVD.

Approach:

Scoping review of health services literature

Study 2. An Exploration into Patterns of Clinical Care Subsequent to Non-indicated Vitamin D Testing in Primary Care

Primary Objective:

To explore patterns of clinical management for two years following a non-indicated 25(OH)D test among Primary Care patients within a regional health system.

Approach:

Descriptive study using electronic health record (EHR) data

Study 3. Vitamin D Practice Patterns within NCAA Division I Collegiate Athletics Programs

Primary Objective:

To describe clinical care related to the evaluation, prevention, and treatment of LVD within NCAA Division I athletics programs.

Approach:

Survey

Study 4. Evaluation of a Protocol to Treat Low Vitamin D in Male and Female Collegiate Basketball Athletes

Primary Objective:

To evaluate the effectiveness of a specific treatment protocol in improving vitamin D status in basketball athletes with LVD

Approach:

Open clinical trial

Study 5. Vitamin D Supplementation Attenuates a Seasonal 25(OH)D Decline and Enhances Strength and Power in Collegiate Swimmers

Primary Objective:

To determine if 12 weeks of daily vitamin D supplementation attenuates a fall-season decline in 25(OH)D in collegiate swimmers, and if such supplementation impacts strength and power performance

Approach:

Randomized, double-blind clinical trial

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 BACKGROUND

Calciferol is an essential nutrient commonly known as vitamin D. Vitamin D can be synthesized from cholesterol in the skin when exposed to ultraviolet-B light (UVB), or obtained through dietary sources or supplements. The role of vitamin D in bone metabolism was discovered nearly 100 years ago when treatment with cod liver oil (a rich source of vitamin D) and exposure to sunlight were shown to cure rickets, a debilitating condition that causes bone softening, pain, and weakness.¹¹ Rickets, which was a widespread health concern in the early 1900's, was essentially eradicated in most developed countries by the 1940's through increased UVB exposure and the addition of vitamin D-fortified foods to the food supply.¹² In the U.S., milk, some dairy products, and a few other foods are fortified with vitamin D to this day.^{13,14}

An analysis of National Health and Nutrition Examination Survey (NHANES) data showed that 39% of community-dwelling adults in the U.S. had vitamin D deficiency in 2010, which was defined as 25(OH)D <20 ng/mL.¹⁵ Over the past 20 years, classification of vitamin D insufficiency, 25(OH)D higher than deficient levels, yet considered inadequate to support optimal health, has been more commonly described.^{16,17} The U.S. Endocrine Society defines vitamin D insufficiency as 25(OH)D of 20 to 29.9 ng/mL.⁶ Estimates of vitamin D insufficiency are as high as 20 to 50% of individuals in the U.S.^{4,17} Vitamin D deficiency and insufficiency, which will be referred to as low vitamin D (LVD) throughout this review of literature, are more common in non-Caucasian individuals and older adults, and in some special populations such as pregnant women and well-trained athletes.^{4,18} Additional risk factors for LVD include limited sun exposure, obesity, and other medical conditions such as malabsorption syndromes and kidney disease.¹⁹ Symptoms of LVD include fatigue, bone and muscle pain, generalized

weakness, recurrent fracture, bowed legs (with severe deficiency), and can be difficult to recognize since they are associated with many different causes.²⁰

Decreased time spent outdoors, increased use of sunscreen (blocking dermal vitamin D synthesis), and low dietary intake of vitamin D have contributed to rising occurrence of LVD.²¹ From 1994 to 2004, the number of Americans with LVD more than doubled, a trend that was observed globally.²² It has been estimated that over one billion individuals worldwide have LVD presently.²³ A dramatic increase in the occurrence of rickets was reported in the U.S., United Kingdom, and Australia beginning in 2000 – 2005, concurrent to rising rates of LVD.¹¹ This “resurgence of rickets”, as it was described by the media, contributed to increased attention to the topic of LVD.²⁴ Google searches for vitamin D increased nearly five-fold from 2004 to 2010, and vitamin D was the fourth most popular nutrition topic included in top 100 U.S. newspapers in 2008-2015.²⁵ The New York Times referred to this intense focus as the “vitamin D craze”.²⁶

Increased interest in vitamin D has also been fueled by rapidly evolving research. More than 300 new PubMed entries for “vitamin D” or a similar term have been made monthly since 2013. Much of this research involves new insights into vitamin D physiology and metabolism. In particular, it is now recognized that vitamin D interacts with almost every tissue in the human body via a complex vitamin D endocrine system.²⁷ As such, vitamin D is better described as a hormone than as a vitamin.²⁸

There are two forms of vitamin D: Ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃) (Figure 2.1).

Figure 2.1. Structure of Vitamin D₂ (Ergocalciferol) (1) & Vitamin D₃ (Cholecalciferol) (2)

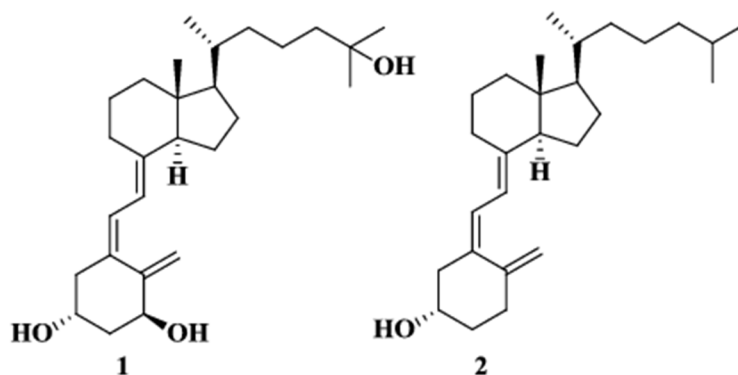


Image reprinted with permission from Tsiaras and Weinstock, 2011 (REF)

Vitamin D₃ is the form synthesized in the skin, and found in some animal products and fortified foods (Table 2.1). Vitamin D₂ originates in plant-based dietary sources, and is also found in some fortified foods (Table 2.1). Both forms of vitamin D travel through the bloodstream to the liver bound to vitamin D binding protein (DBP). Once in the liver, vitamin D is converted to 25(OH)D, an inactive metabolite. Active vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)₂D), is formed when 25(OH)D is hydroxylated in the kidneys. The enzyme responsible for activating 25(OH)D, CYP27B1(1- α -hydroxylase), has also been identified within more than 30 target tissues, including skeletal muscle, brain, and reproductive tissues.²⁸ 1,25(OH)₂D has been shown to initiate many rapid, non-genomic actions throughout the body.²⁹ It also interacts with vitamin D receptors (VDR) in bone, intestinal cells, and numerous other tissues to influence transcription of more than 1000 different genes.³⁰ An overview diagram of vitamin D metabolism is shown in Figure 2.2.

Table 2.1. Dietary Sources of Vitamin D

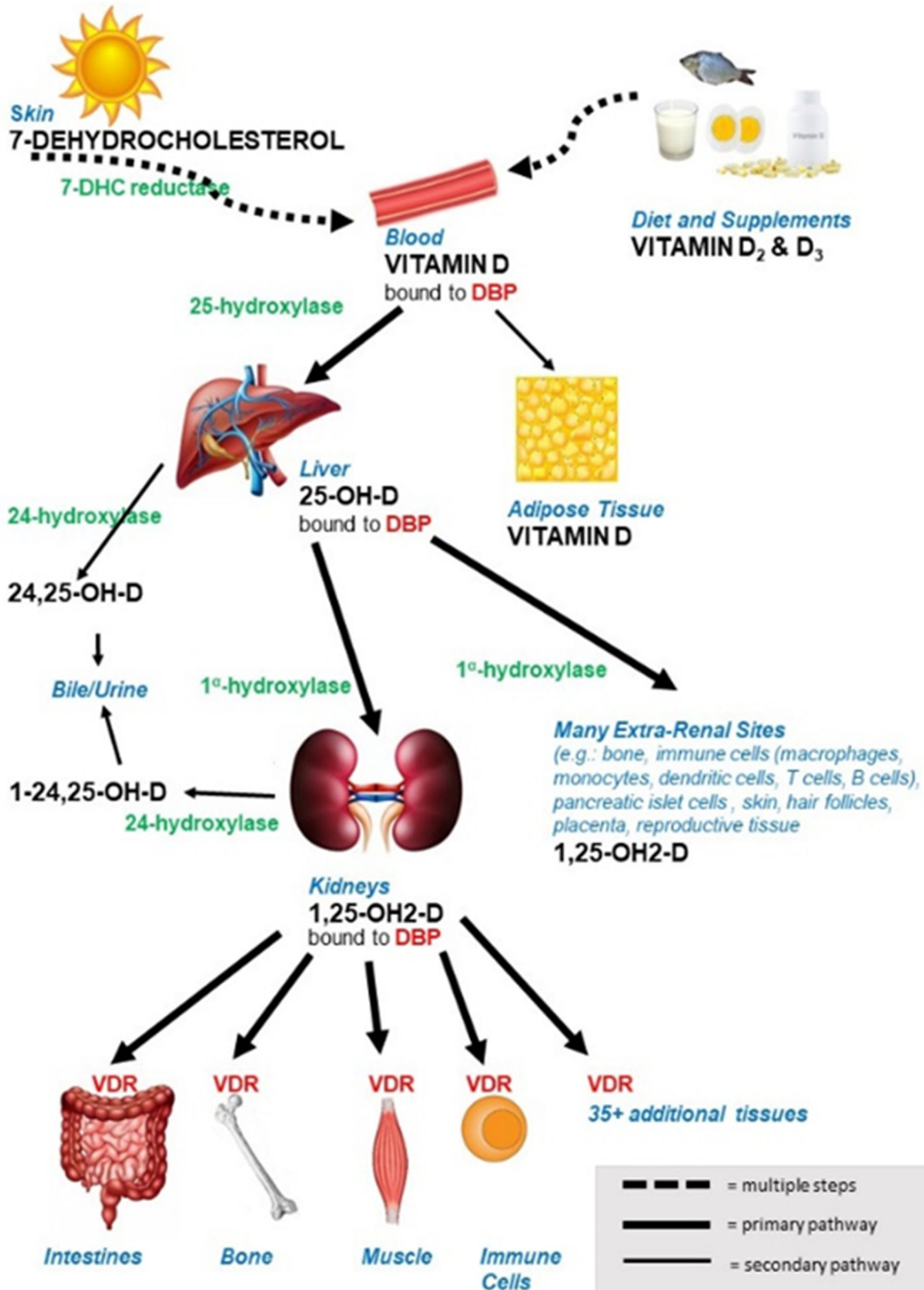
Food	Serving Size	Vitamin D (IU)
Cod liver oil	1 Tablespoon	1360
Salmon, cooked	3 ounces	810
Mackerel, cooked	3 ounces	340
Tuna, canned	3 ounces	180
Milk, fortified *	8 ounces	100
Sardines, canned	1.5 ounces	80
Margarine, fortified	1 Tablespoon	60
Egg	1 whole	20
Cheese	1 ounce	10

Reference: U.S. Department of Agriculture Food Composition Database

(<https://ndb.nal.usda.gov/ndb/search/list?home=true>)

*In the U.S., cow's milk is fortified with 100 IU of vitamin D per 8 ounces. Juices, cereals, and other products are sometimes fortified with vitamin D as well.

Figure 2.2. Overview of Vitamin D Metabolism



Considering the widespread role of vitamin D in human biology, involvement in many health problems such as cancer, cardiovascular disease, diabetes, autoimmune disorders, mental health disorders, and neurological conditions have been investigated.² Although numerous cross-sectional studies suggest a relationship between vitamin D status and these health issues, and multiple explanatory mechanisms have been identified, results of clinical trials in which vitamin D status is improved through sunlight or supplementation are primarily inconclusive and inconsistent at the present time.³¹

2.2 A LANDSCAPE OF UNCERTAINTY

Increasing occurrence of rickets, rapidly evolving research, substantial media attention, and proposed involvement in many health conditions have contributed to an intense focus on vitamin D in recent years. However, compared with other popular health and nutrition issues, extraordinary conflict and controversy surrounding clinical practice guidelines related to vitamin D make it a uniquely challenging issue. Beginning with the way vitamin D status is measured, a great deal of uncertainty exists.

Vitamin D status is typically measured via serum 25(OH)D. Unfortunately, the 25(OH)D biomarker has many limitations. For example, the multiple different assays and techniques used to measure 25(OH)D reflect notable intra- and inter-assay variability.³²⁻³⁴ Although quality and standardization initiatives such as the Vitamin D External Quality Assessment Scheme (DEQAS) have been implemented, interpretation and comparison of 25(OH)D laboratory values remains problematic.^{33,35} Concentrations of 25(OH)D are also influenced by existing illness or inflammation, use of some medications, adiposity, race/ethnicity, genetic variation, recent exercise, or UVB exposure. Some researchers have suggested alternate biomarkers, such as free

25(OH)D, as preferable for measuring vitamin D status,³⁶⁻³⁸ but this assay is rarely used in clinical practice at this time.

In 2014, the U.S. Preventive Services Task Force (USPSTF) recommended against population-based screening (i.e.: testing without cause or indication) for LVD due to insufficient evidence of benefits or harms.³⁹ The American Academy of Family Physicians, American Board of Internal Medicine, American Society for Clinical Pathology, U.S. Endocrine Society, and numerous other organizations support the USPSTF guideline, and recommend targeted 25(OH)D testing of only patients at high risk for LVD.⁴⁰ There is disagreement over what factors should be considered “high risk” and thus, indicate testing 25(OH)D in the clinical setting. For example, the Endocrine Society considers obesity and non-Caucasian race as indicators for 25(OH)D testing, but most others do not.⁴⁰ The Kidney Disease Outcomes Quality Initiative classifies Stage 3 kidney disease as high risk, while multiple others recommend 25(OH)D testing only in patients with more advanced kidney disease.⁴¹

Goal or target 25(OH)D is another source of controversy. The National Academy of Medicine (NAM), formerly known as the Institute of Medicine, considers 25(OH)D of 12 to 20 ng/mL adequate to support the bone health of 97.5% of American adults.⁴² The U.S. Endocrine Society, on the other hand, considers 25(OH)D of 20 to 29.9 ng/mL insufficient to support health, and recommends 30 to 60 ng/mL.⁶ Research published since the IOM and Endocrine Society reports were released has continued to investigate 25(OH)D concentrations needed to support health.⁴³⁻⁴⁵ Further research is needed to determine the relationship between vitamin D and non-skeletal conditions, identify a protective 25(OH)D concentration (if one exists), and inform professional recommendations and clinical practice guidelines.

There is also high variability in dietary vitamin D recommendations. The Recommended Dietary Allowance (RDA) for vitamin D, which was set by NAM in 2011, is 600 IU/day for individuals one to 70 years of age.⁵ In contrast, the Endocrine Society recommends 1500 to 2000 IU/day to achieve recommended concentrations of 25(OH)D.⁶ The NAM and Endocrine Society guidelines are based on intake required to achieve adequate vitamin D status assuming no UVB exposure. Individuals who are exposed to sunlight or other sources of UVB at intensities capable of stimulating vitamin D synthesis have lower dietary vitamin D needs. However, UVB exposure is difficult to quantify or include in guidelines and practical recommendations, especially because there is tremendous individual variability in vitamin D synthesis.⁴⁶⁻⁴⁸ Furthermore, guidelines for limited sun exposure and regular sunscreen use to prevent skin cancer complicate recommendations to improve vitamin D status through exposure to sunlight.

Finally, the plethora of strategies used to treat LVD also adds to an environment of uncertainty. Vitamin D supplements are available as vitamin D₂ or vitamin D₃, in multiple forms (ex: tablets, capsules, liquid, oral spray, nasal spray, and intramuscular injection), a variety of doses (100, 400, 800, 1000, 2000, 5000, or 10,000 IU over-the-counter and 5,000 to 300,000 IU as prescription), and taken in daily, weekly, monthly, and even yearly doses.^{49,50} Some vitamin D supplements also contain calcium or other micronutrients. Vitamin D₃ is significantly more effective in raising 25(OH)D than vitamin D₂.^{51,52} Since this is a fairly recent discovery, many professional guidelines (including NAM and Endocrine Society) do not currently differentiate between the two forms.^{5,6} Clinical practice guidelines differ in terms of recommended treatment strategies for LVD, and many do not provide specific strategies or vitamin D dosing.^{53,54}

Moreover, increased prevalence and detection of LVD, rapidly evolving research, and intense media focus have contributed to unprecedented public interest in vitamin D. Conflicting clinical practice guidelines related to vitamin D, and variability in the 25(OH)D biomarker, also contribute to an environment of uncertainty.

2.3 CLINICAL PRACTICE AMIDST UNCERTAINTY

In order to promote quality, safety, and consistency in healthcare, clinicians are tasked with providing evidence-based care to patients and clients.⁵⁵ Interpretation and application of the evidence related to vitamin D presents a challenge due to the uncertain and evolving environment that currently exists. Nearly 100% of primary care physicians in a three-country survey agreed with the statement that “clear and concise guidelines regarding LVD are needed”.^{56,57} In the meantime, clinicians report exposure to information about vitamin D from a variety of sources, including scientific journals, colleagues, their own medical providers, internet, popular media, and celebrities.⁵⁸⁻⁶⁰ Confidence about vitamin D recommendations was described as “poor” or “not at all confident” by 61% of dietitians and 40% of physicians in Australia.^{56,61}

Dramatic changes 25(OH)D testing patterns have been documented. Tarn et al.⁵⁹ observed that the topic of vitamin D was discussed in 15% of primary care visits in a California-based clinic, and that broad inter and intra-clinician variability in testing indicators and follow-up testing existed. Multiple studies report an exponential increase in 25(OH)D testing in the past 15 to 20 years.⁶²⁻⁶⁴ An 83-fold increase in 25(OH)D tests, for example, occurred among U.S. Medicare beneficiaries from 2000 to 2010.⁶⁵ In the state of Virginia, 25(OH)D testing increased 43-fold from 2010 to 2016.⁶⁶ These increases have occurred disproportionate to requisitions for other laboratory tests.^{65,67} In addition, some studies describe a rise in clinicians’ ordering of the

incorrect vitamin D test – typically 1,25(OH)₂D rather than 25(OH)D – which provides minimal insight into vitamin D status.^{68,69}

Inconsistency in normal or goal ranges for 25(OH)D have evolved, beyond what may be considered expected variation between laboratories. For example, in comparing health systems in Southwest and Central Virginia, the 25(OH)D considered “within normal limits” varies (Carilion Clinic: 30 to 100 ng/mL, Lewis Gale Medical Center: 20 to 150 ng/mL, Virginia Commonwealth University Health System: 32 to 120 ng/mL, and University of Virginia: 50 to 250 nmol/L) (personal communication). Several studies describe provider variation in 25(OH)D concentration at which treatment for LVD should be initiated.^{58,60,70}

Although less information about vitamin D prescribing patterns is available, a dramatic increase in vitamin D prescriptions has occurred simultaneous to increased 25(OH)D testing.^{71,72} The number of adults taking vitamin D supplements increased more than 100-fold from 2000 to 2014 in the U.S.⁷³ In 2016, approximately 20% of U.S. adults over 18 years of age, and 45% of those over 65, were taking vitamin D supplements.^{4,74} Not surprisingly, marked inconsistency in doses, types, and forms of supplements prescribed and purchased over-the-counter has been reported.^{75,76}

Together, exponential increases in 25(OH)D testing and vitamin D supplement use, and inconsistencies in vitamin D-related practices, have contributed to increased healthcare costs. To illustrate, Americans spent \$936 million on vitamin D supplements, and Medicare reimbursed over \$365 million for 25(OH)D tests in 2017.²⁶ It is unclear what benefits and harms are associated with this high rate of testing and supplementation. Further, there is substantial evidence that 25(OH)D tests are ordered as a screening of asymptomatic patients, rather than targeted testing of those at high risk for LVD.⁴⁰ This non-indicated 25(OH)D testing has been

cited as an example of low value care. Low value care is defined as patient care that provides no benefit in specific clinical scenarios.^{77,78} Low value care is problematic not only because it contributes to wasted financial resources, but also to decreased care quality and increased patient harm.⁷⁸ The Choosing Wisely Campaign was founded in 2012 with the goal of reducing low value care.⁷⁹ Choosing Wisely includes population-based, asymptomatic screening for LVD among its targets.⁸⁰

In order to inform clinical practice guidelines and interventions aimed at reducing non-indicated, low value care, a more complete understanding of current trends in vitamin D practice is needed. Further insight into outcomes and consequences associated with vitamin D screening and supplementation is also warranted. Finally, more high-quality clinical trial evidence is needed to clarify the impact of vitamin D status on human health and performance, and inform recommendations for clinical practice.

2.4 VITAMIN D IN SPORTS MEDICINE

A focused examination of population-specific vitamin D practice patterns is valuable in establishing best practices. Presently, no professional guidelines related to vitamin D screening, supplementation, or treatment of competitive athletes exist. In their 2016 Joint Position Statement on Nutrition for Athletic Performance, the Academy of Nutrition and Dietetics and the American College of Sports Medicine called for further empirical data to clarify the role of vitamin D in the health and performance of athletes.⁸¹

The topic of vitamin D is of particular relevance to competitive athletes. First, many functions known to be related to vitamin D – bone health, muscle, immune, and cardiovascular function, to name just a few – are especially important for athletes. Furthermore, LVD is common among athletes,^{7,8} athletes' diets are consistently found to be low in vitamin D,⁹ and

many have limited UVB exposure.⁸² Finally, there is a growing body of research that relates vitamin D to performance, health, and well-being of athletes. A review of the available evidence related to vitamin D and athletes can help inform best practices for working with this unique population.

Vitamin D and Athletes: Overview of the Best Available Evidence

Athletic Performance

A theorized relationship between vitamin D status and athletic performance dates back to the 1930's, when Russian and German researchers reported enhanced swimming and running performance following sun lamp treatments.⁸² Many others have observed seasonal fluctuation in performance, with elevated speed and strength in summer months when vitamin D exposure typically peaks.^{29,49} Vitamin D status has been positively correlated with muscular strength in healthy adult and elderly populations,⁸³⁻⁸⁵ but whether or not a similar association exists in competitive athletes is less clear.

Several observational studies report better strength and power in athletes with higher 25(OH)D⁴⁹ (Appendix A). Hildebrand et al.,⁸⁶ for example, observed a positive correlation between 25(OH)D concentrations and performance of maximal squat, vertical jump, shuttle run, and triple hop tests among 100 male and female collegiate athletes (from a variety of sports teams) in the mid-west U.S. Better hand-grip strength was also reported in elite Danish swimmers with higher 25(OH)D.⁸⁷ Caswell et al.,⁸⁸ on the other hand, did not observe a similar association between vitamin D status and strength or power performance in nearly 1000 male and female army recruits in the United Kingdom. However, 25(OH)D was positively correlated

with 1.5 mile run performance in the same subjects.⁸⁸ Specifically, every 0.4ng/mL increase in 25(OH)D equated to an approximately 0.5 second faster 1.5 mile run performance.⁸⁸

A relationship between 25(OH)D and athletic performance was not observed in all studies (Appendix A).⁸⁹⁻⁹¹ Although an explanation for the discrepancy in findings is not clear, one possibility is that performance detriment is more likely in athletes with the lowest 25(OH)D concentrations. The studies that did not identify an association between 25(OH)D and athletic performance⁸⁹⁻⁹¹ included athletes with higher 25(OH)D, and fewer with LVD. On the other hand, most studies that identified a positive association involved athletes with substantially lower 25(OH)D, and a high proportion of athletes with LVD, sometimes severe vitamin D deficiency (<12 ng/mL).^{92,93}

Multiple potential mechanisms underlie a relationship between vitamin D and skeletal muscle function.^{10,29,49,94} First, vitamin D receptors (VDR) have been identified within skeletal muscle, suggesting direct involvement of vitamin D within muscle tissue.³⁰ Vitamin D is believed to enhance skeletal muscle function through a direct impact on the contractile apparatus of muscles, by influencing calcium handling within muscle cells, and through interaction at the neuromuscular junction.^{30,87,95} Additionally, vitamin D supplementation increases the size and number of Type II muscle fibers in older adults, although this effect has not yet been explored in athletes.^{96,97} Finally, a relationship between vitamin D and testosterone, a hormone involved in muscle function and strength development, has also been suggested,^{98,99} but this also warrants further exploration in athletes.

Vitamin D status has been shown to influence muscle recovery and adaptation, another mechanism by which overall muscular performance may be impaired in athletes with LVD.¹⁰⁰ Two human studies^{101,102} showed faster return of muscle force after muscle-damaging exercise

in athletes with adequate 25(OH)D, while another ¹⁰⁰ showed that treatment with vitamin D supplements improved muscle recovery compared with placebo. These benefits may be due to the role that vitamin D plays in cell differentiation and proliferation,²⁹ and muscle protein synthesis.^{103,104} Recent studies have also suggested that 25(OH)D status influences muscle stiffness⁹³ and antioxidant capacity.¹⁰⁵

Although limited human studies link vitamin D status with enhanced performance of endurance exercise performance, several potential mechanisms explain such a benefit. Cardiac, vascular, and lung tissues all express VDR, again suggesting a direct role for vitamin D within these tissues.^{9,49} In addition, LVD has been shown to reduce oxidative capacity through effects on mitochondrial function, hemoglobin structure and function, and lung volume.^{49,94} Finally, some research suggests a relationship between vitamin D status and cardiac structure and function. For example, Allison et al.¹⁰⁶ observed that athletes with severe vitamin D deficiency had smaller hearts compared with athletes with adequate 25(OH)D.

The observational studies correlating vitamin D status with athletic performance, and the numerous mechanisms that may explain such an effect, raise the question as to whether or not taking vitamin D supplements can enhance strength, power, and cardiorespiratory performance in athletes. Of the 13 published studies that explored the impact of taking vitamin D supplements on athletic performance (Appendix A), six showed improvements in some or all of the performance outcomes evaluated.¹⁰⁷⁻¹¹² For example, male professional soccer athletes in the United Kingdom experienced greater improvements in vertical jump and 10 meter sprint tests after taking 5,000 IU vitamin D₃ daily for eight weeks compared with those who took placebo.¹¹³ Positive effects on speed, strength, and power were also observed in ballet dancers taking 2,000 IU vitamin D₃ daily for 16 weeks,¹¹¹ taekwondo athletes taking 5,000 IU vitamin D₃ for four

weeks,¹¹⁰ and judo athletes receiving one 150,000 IU dose of vitamin D₃¹¹² compared with athletes who received placebo or no treatment. Another study reported that youth soccer players who took 5,000 IU of vitamin D₃ daily for eight weeks showed greater improvements in VO_{2max} compared with athletes who took placebo.¹⁰⁹ Interestingly, three studies that reported positive findings involved athletes for whom balance is of particular importance (dance and martial arts). Although not confirmed in athletes, results of studies in older adults suggest a link between vitamin D status and balance or neuromotor function.^{114,115} Strength and power outcomes may have been indirectly influenced by improved balance in these dance and martial arts athletes.

In contrast, seven of the thirteen vitamin D supplement studies did not show positive performance outcomes in athletes.^{88,89,107,109,116-118} These studies involved military recruits or soccer, swimming, or basketball athletes taking various doses of vitamin D₃, ranging from 400 IU daily to 40,000 IU weekly. Synthesizing and comparing results of vitamin D supplement studies (Appendix A) is difficult due to tremendous heterogeneity in athlete characteristics, types and levels of exercise in which subjects routinely participated, and outcomes measured. However, most studies that show no statistically significant difference provided lower dose supplements (<2000 IU) for a shorter period of time^{88,89,116,118} compared with studies that reported positive effects of taking vitamin D supplements.^{107-109,112,119} Additionally, compared with studies that report performance benefits as a result of taking vitamin D supplements, studies showing no significant effects tended to include subjects with lower baseline 25(OH)D and who experienced a smaller increase in 25(OH)D as a result of taking vitamin D supplements. There was no clear post-treatment 25(OH)D concentration consistently associated with benefits in these studies. For example, post-treatment 25(OH)D was 40-42 ng/mL in six studies.^{108,109,113,116,117,119} Three of these studies reported benefits and three reported no benefit to athletic performance.

Conflicting study results may have also been related to differences in supplement type dose, or to subjects' compliance to the treatment protocol. Most studies did not report compliance, and some did not measure it. Finally, since detecting performance improvements in well-trained athletes is difficult, it's possible that neutral interventions may have been positive in different subjects, or if treatment was modified.

In summary, observational studies generally support a positive correlation between vitamin D status and physical performance in athletes, but only about half of longitudinal vitamin D supplement trials support a relationship. Additional research using experimental trials with vitamin D supplementation with control of supplement compliance, similar baseline vitamin D status, and realistic performance measures is necessary to fully understand the potential of this vitamin for athletic populations.

Illness and Injury

The role of vitamin D for athletes may extend beyond performance. Increasing evidence supports the involvement of vitamin D in prevention of illness and injury in athletes. For example, 25(OH)D concentration and frequency of illness were inversely correlated in a diverse group of collegiate athletes in Wyoming, U.S.¹²⁰ and recreational endurance athletes in the United Kingdom.¹²¹ In the Wyoming study, athletes with 25(OH)D >48 ng/mL were significantly less likely to present with upper respiratory illness (URI) than those with 25(OH)D <12 ng/mL.¹²⁰ It is not clear if the absolute 25(OH)D concentration is most important in the prevention of illness, or if a change in 25(OH)D is more influential. For example, Dubnab-Raz et al.¹²² showed that Israeli adolescent swimmers who experienced the greatest decline in 25(OH)D during fall and winter training reported greater URI severity and longer symptom

duration compared with swimmers whose 25(OH)D increased, stayed the same, or mildly decreased. Although most of the evidence regarding vitamin D and illness in athletes is observational and does not necessarily establish a cause-effect relationship, one study showed that a four-week supplement protocol (5000 IU vitamin D₃) resulted in decreased URI symptoms in collegiate taekwondo athletes compared with those using placebo.¹²³

There is less evidence of a relationship between vitamin D status and injury in athletes, but findings of one study suggested that occurrence of injury (i.e. bone, connective tissue, or muscle injury not associated with trauma) coincided with decreasing 25(OH)D concentration in collegiate swimmers in Kentucky, U.S.¹²⁴ National Football League (NFL) athletes who had experienced core or lower body soft tissue injury in the previous season were also more likely to have 25(OH)D <32 ng/mL than those who did not become injured.¹²⁵ Vitamin D's role in inflammation and immune function may underlie the observed reduction in injury and illness. Studies have shown that LVD is associated with higher concentrations of pro-inflammatory cytokines and lower concentrations of antimicrobial peptides in athletes¹²⁶⁻¹²⁸. Overall, the 2018 International Olympic Committee Consensus Statement on Dietary Supplements for High Performance Athletes found moderate evidence for the effect of vitamin D supplements on immune function.¹²⁹

There is also evidence that vitamin D status influences risk of developing non-traumatic bone fractures and stress fractures in athletes. A recent study showed that members of the Pittsburgh Steelers NFL team who had experienced fractures in the past year had significantly lower 25(OH)D than those who did not.¹³⁰ In addition, a meta-analysis of military studies found that personnel who experienced one or more stress fractures had lower 25(OH)D upon entry to the military and at the time of diagnosis than those who did not experience such injuries.¹³¹ In

female U.S. Navy cadets, 25(OH)D <20 ng/mL was associated with double the risk of tibial and fibular stress fractures than 25(OH)D >40 ng/mL.¹³² Finally, another study reported that female U.S. Navy recruits taking vitamin D and calcium supplements (800IU and 2000mg, respectively) were 20% less likely to experience a stress fracture during basic training than recruits taking placebo.¹³³ The role of vitamin D in calcium absorption and bone mineralization is the likely explanation for the observed benefit to reduced fracture and stress fracture risk.

In summary, a small number of randomized clinical trials have reported that taking vitamin D supplements enhances performance and reduces occurrence of injury and illness in competitive athletes. Athletes have tremendous interest in safe, effective strategies to enhance performance and prevent illness and injury. Thus, it is not surprising that the topic of vitamin D has gained great popularity among athletes and sports medicine clinicians. Many athletes and sports medicine clinicians have reported the use of vitamin D supplements not just to prevent or treat LVD, but as an ergogenic aid.¹⁰ Further research is needed to clarify the role of vitamin D in athletes' performance, health, and well-being. Research that aims to identify optimal 25(OH)D concentrations, viable strategies for preventing LVD, and effective treatment protocols for LVD have great practical value for athletes and sports medicine clinicians.

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CHAPTER 3

MANUSCRIPT

Clinical Management of Low Vitamin D: A Scoping Review of Physicians' Practices

ABSTRACT

Background: The role of vitamin D in the prevention and treatment of non-skeletal health issues has received significant research and media attention in recent years. Costs associated with clinical management of low vitamin D (LVD) have increased exponentially. However, no clear evidence supports vitamin D screening to improve health outcomes. Authoritative bodies and professional societies do not recommend population-wide vitamin D screening in community-dwelling adults who are asymptomatic or at low risk of LVD. The purpose of this study was to assess patterns of physicians' management of LVD in the current, conflicting environment.

Methods: A scoping review of three electronic databases and the gray literature was conducted in 2017.

Results: Thirty-eight records met inclusion criteria and were summarized in an evidence table. Thirteen studies published between 2006 and 2016 across seven countries showed a consistent increase in vitamin D lab tests and related costs. Many vitamin D testing patterns reflected screening rather than targeted testing for individuals at high risk of vitamin D deficiency or insufficiency. Interventions aimed at managing inappropriate clinical practices related to LVD were effective in the short term.

Conclusions: Variability and controversy were pervasive in many aspects of vitamin D management, shining a light on physicians' practices in the face of uncertainty. Interventions can be useful in reducing unnecessary vitamin D screening. Future research is needed to inform better clinical guidelines and to assess implementation practices that encourage evidence-based management of LVD in adult populations.

Keywords: vitamin D; 25-hydroxyvitamin-D; 25(OH)D; low vitamin D; screening; physician practices; low value care; test overutilization

3.1 INTRODUCTION

Vitamin D is an essential nutrient obtained by humans through sunlight exposure to ultraviolet B (UVB) light, dietary sources, and dietary supplements. Many factors influence the vitamin D status of individuals and populations including: latitude, season, time spent outdoors or in UVB light, clothing habitually worn, sunscreen use, weight status, skin color, and some medications and medical conditions.¹ People who are deficient in vitamin D may develop rickets, osteomalacia or other bone disorders.

Vitamin D is found naturally in only a few foods—fatty fish (i.e., salmon, tuna, and mackerel), egg yolks, certain mushrooms—and in dairy products, margarine, ready-to-eat cereals, and fruit juices that have been fortified. Supplemental vitamin D is available in a variety of over-the-counter (OTC) and prescription strengths, in both ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃) forms, and for administration orally or via intramuscular injection. Vitamin D is fat soluble; therefore, a risk of toxicity may exist with excessive vitamin D treatment.

Blood concentrations of vitamin D are most commonly evaluated through measurement of serum 25-hydroxyvitamin-D (25(OH)D). While 1,25-dihydroxyvitamin D (1,25(OH)₂D) is the active form of vitamin D, it has a shorter half-life than 25(OH)D (hours vs. weeks); thus, 25(OH)D is considered the best clinical indicator of vitamin D status. Estimates of the incidence of population-wide vitamin D deficiency and insufficiency, referred to as low vitamin D (LVD) throughout this paper, vary widely. Holick² has described LVD as reaching pandemic proportions in populations, whereas other clinicians and researchers have asserted that LVD rates are overestimated or exaggerated.^{3,4} Variability in estimates of LVD may be due to how it is defined, and 25(OH)D targets considered sufficient or optimal to support good health.^{1,5} In 2011, an expert committee convened by the U.S. Institute of Medicine (IOM) (changed to the

Health and Medicine Division of the National Academy of Medicine in 2016) reported that 25(OH)D of 20 ng/mL is sufficient to support bone health in 97.5% of the population.⁶ In contrast, the U.S. Endocrine Society considers <20 ng/mL indicative of LVD.⁷ Tables 3.1 and 3.2 summarize the vitamin D screening and testing guidelines and recommendations from several authoritative bodies and professional societies in North America and Europe. Variations in clinical diagnosis of LVD in individuals/patients occur for various reasons, including conflicting professional recommendations and practice guidelines, unfamiliarity with recommendations and guidelines, independent clinical judgement, or the tendency to default to laboratory reference ranges.

Daily requirements, treatment guidelines and protocols, and monitoring strategies for LVD are unclear, variable, contradictory, and sometimes poorly-defined. Additionally, many laboratory methods are used to quantify 25(OH)D (e.g., liquid chromatography-tandem mass spectrometry, enzyme linked immunosorbent assay, chemiluminescence immunoassay, and new point-of-care assays⁸) and notable intra- and inter-assay variability has been identified.

In recent years, the role of vitamin D in the prevention and treatment of numerous non-skeletal conditions and chronic diseases has gained attention. Cardiovascular disease, diabetes, some cancers, autoimmune disorders, infertility, and depression are among many conditions associated with LVD status.⁹⁻¹¹ More than 300 new PubMed entries for “vitamin D” or a similar term in the title have been made monthly since 2013. A majority of the research that links vitamin D status to non-skeletal issues or conditions is based on observational studies, theories, and newly discovered mechanisms rather than randomized controlled trials conducted in human populations. In 2011, the IOM revised the Dietary Reference Intakes (DRI) for vitamin D for populations (i.e., adequate intake for infants ages 12 months and younger (400 IU); estimated

average requirement (400 IU) and recommended dietary allowance (600 IU) for children ages 1 year and older through adulthood). The U.S. Endocrine Society also published clinical guidelines for the Evaluation, Treatment, and Prevention of Vitamin D Deficiency that same year. However, only skeletal health research was used to inform these recommendations because the available research on non-skeletal conditions was considered insufficient or conflicting.^{6,7} Debate exists regarding the role of vitamin D in non-skeletal conditions and the quality of data for some conditions has continued to evolve. Nevertheless, the U.S. Preventive Services Task Force (USPSTF), an independent panel of experts who issue evidence-based clinical practice recommendations, concluded in 2015 that there was insufficient evidence to support population-wide screening for individuals at low risk of vitamin D deficiency.¹² Improved health status has not been reported in asymptomatic individuals treated for LVD.¹³

Emerging research and inconsistencies in clinical guidelines have captured the attention of the media, public, and healthcare providers.¹⁴ Despite formal guidelines and recommendations suggesting otherwise, a significant increase in screening and testing for LVD has been reported.^{15,16} Laboratory test overutilization and over diagnosis are recognized problems since both impact healthcare costs and quality of care.^{17,18} A 2012 IOM report concluded that \$750 billion annually (representing over 30% of total U.S. healthcare spending) is used for unneeded care, such as non-indicated laboratory testing. Efforts to curb this overutilization include the Choosing Wisely campaign (www.choosingwisely.org), which outlines recommendations against vitamin D testing for low-risk patients.^{19,20}

The identification of existing and evolving clinical practice patterns associated with LVD in adult populations is necessary to design, implement, and evaluate interventions, such as

Choosing Wisely, to reduce low value care. Numerous research studies and reports have assessed physicians' practice patterns associated with LVD, but no overview or comprehensive summary of the clinical management of LVD and its implications has been published. This paper addresses this knowledge gap by reviewing the healthcare services literature regarding: 1). Physicians' management of LVD in community-dwelling adults, 2). costs associated with physicians' clinical practices related to LVD, and 3). efforts to constrain inappropriate clinical practices demonstrated by physicians related to LVD.

3.2 METHODS

The research question that guided this review was: How are clinical practices regarding vitamin D impacted by the changing guidelines and research base concerning the management of LVD in community-dwelling adults? Due to the broad nature of the research question, a scoping review was selected to systematically assess and describe the published literature for clinical management, associated costs, and attempts to constrain physicians' practices related to LVD in an unbiased and transparent manner, while identifying key themes and future research needs.²¹ Vitamin D screening was defined as testing asymptomatic individuals for the presence of LVD, whereas vitamin D testing was defined as evaluating selected symptomatic or at-risk individuals for LVD. As a scoping review, this study sought to describe the breadth of the literature rather than to emphasize quality of the studies, and to determine the value and feasibility of undertaking a systematic review for a more focused research question related to this topic.²²

Search Strategy

The Cochrane Library scoping review methodology²² and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Extension for Scoping Reviews²³ informed the conduct of this scoping review. A literature search was performed in consultation with a research librarian in November 2017. Three electronic databases (i.e., PubMed, EMBASE, and Cochrane) were searched between 1997 and 2017. The search start date was selected as 1997 when the previous U.S. recommended dietary allowance for vitamin D was established. The following MeSH search terms were used: “vitamin D” [title or abstract] AND (“physician” OR “healthcare provider” OR “manag*” OR “primary care” OR “general practice” OR “lab* test” OR “screen*” OR “prescri*” OR “cost” OR “economic” OR “attitude”) (all fields). An update search was conducted in January 2018 to identify any articles published since the original search. During this second search, the reference lists from included articles were scanned for additional relevant literature, and a gray literature search was conducted in January 2018 using Google and the search terms above.

Inclusion and Exclusion Criteria

Review involved scanning the title and abstract of each identified article for relevance to the research question. All articles written in the English language that related to vitamin D screening and testing in community-dwelling adults were included. Only articles focused on physicians were included because published articles related to vitamin D testing patterns for other health professionals and medical team members were limited (three were identified). However, in a few of the included articles, medical team members such as physicians’ assistants

or nurse practitioners were grouped with physicians for analyses. Articles were excluded that focused exclusively on children, individuals living in residential care facilities, and those with specific medical conditions (e.g., osteoporosis, kidney disease, or multiple sclerosis). Cost evaluations were included if they assessed outcomes directly resulting from physicians' management of LVD.

Data Extraction and Synthesis

Data were extracted and summarized in an evidence table that included population, setting, study methodology, and key findings. Articles were grouped by outcomes reported including: vitamin D laboratory testing patterns, costs associated with vitamin D testing, knowledge, attitudes and/or behaviors related to physicians' management of vitamin D, and attempts to change physicians' practices involving vitamin D. Some articles were grouped in more than one outcome. Specific quality assessments were not performed beyond noting the methodology in keeping with the purpose of this scoping review.

Throughout the study, vitamin D was reported as IU (1 IU = 0.025 µg) and serum 25(OH)D was reported as ng/mL (1 ng/mL = 2.5 nmol/L). When applicable, monetary data was reported in the currency used in the original source and converted to U.S. dollars using January 2018 exchange rates.

3.3 RESULTS

Figure 3.1 shows the PRISMA flowchart for the scoping review. Of the 688 articles identified by the search, 72 met the initial inclusion criteria. An additional 34 articles were excluded after title and abstract review because clinicians, patients, the setting, or outcomes did

not meet the inclusion criteria. The remaining 38 articles were included in this review.²⁴⁻⁵² Two gray literature documents were included within the final 38 articles.

Vitamin D Laboratory Testing

Trends in 25(OH)D laboratory tests are shown in Table 3.3. An increase in 25(OH)D testing was reported in six different countries: Australia, Canada, France, Saudi Arabia, United Kingdom, and the U.S. No articles reported that the rate of 25(OH)D testing decreased or stayed the same. A 94-fold increase in testing (over 4.5 million tests) was reported in Australia between 2006 and 2010,²⁴ 83-fold increase in tests in U.S. Medicare Part B recipients,²⁵ 11-fold increase among primary care patients in Liverpool, United Kingdom,²⁶ and nearly eight-fold increase (in 25(OH)D and/or 1,25(OH)D) in France based on nationally-representative health insurance data, totaling 18% of patient visits from 2008 to 2013.²⁷ The volume of 25(OH)D tests increased by six-fold in a National Health Service hospital in London, United Kingdom and more than doubled in a large Scottish hospital from 2008 to 2010, creating a substantial laboratory backlog.²⁸

Initial tests represented most recorded tests [32,39,41,42].^{26,29-31} One exception was reported by a U.S. Veterans Administration study in which over 70% of tests were repeat or follow-up tests.³² Of studies evaluating repeat tests over time, a quarter of French patients incurred three or more tests in a five year period²⁶ while 27% of Australian patients incurred three or more tests in a four year period²⁴ and three or more 25(OH)D tests were ordered for patients in a hospital in Saudi Arabia within one year, with some patients incurring more than six tests.²⁹ Khalifa et al.²⁹ described three trends in their analysis of 25(OH)D testing patterns: (1)

physicians ordered many initial tests in different patients; (2) physicians repeated tests in the same patient; and (3) some physicians demonstrated both 1 and 2.

Minimal data regarding characteristics of physicians who order 25(OH)D tests are available. However, Tapley et al.³³ reported that Australian physician trainees were more likely to order tests if they worked within a practice that completely bulk bills the national insurance plan (no out-of-pocket or private insurance charges) or if they were ordering other laboratory blood tests. In 2006–2010, 80% of the 25(OH)D tests ordered throughout Australia were ordered by general practitioners and 20% were ordered by specialists.²⁴ Caillet et al.²⁷ reported an increase in proportion of 25(OH)D tests ordered by general practitioners in France from 2008 to 2013 (54% to 66%) and a concurrent decrease in 25(OH)D tests ordered by specialists (30% to 13%).

Physicians in all countries were more likely to order 25(OH)D tests for female patients, older patients, and migrant patients.^{26,31-37} Gowda et al.³⁴ reported that 25(OH)D testing increased with age throughout adulthood. Lower socioeconomic status was associated with higher likelihood of being tested in one study³⁸ but had no impact on test likelihood in another study.³⁴ Individuals classified as “visible minorities” were more likely to have 25(OH)D tests in one study.³⁹

Medical diagnoses associated with 25(OH)D testing were most commonly “health maintenance”, “medical check-up”, and “tiredness/lethargy/fatigue” in a 2010-2013 Australian cohort.³³ Bilinski and Boyages²⁴ evaluated how the 94-fold increase in 25(OH)D testing from 2006 to 2010 in Australia compared to more routine testing—e.g., complete blood count (CBC) orders. Orders for CBC increased only 2.5-fold, indicating that 25(OH)D testing increased at a significantly greater rate than orders for other tests. The number of bone densitometry tests

ordered during the 2006-2010 timeframe increased just 2.5-fold. The same research team reported a 43.6-fold increase in 25(OH)D testing among 45–74-year-old females in Australia.⁴⁰ Because they noted only a concurrent 1.2-fold increase in bone densitometry testing, authors labeled this pattern “the Vitamin D Paradox”, as it appeared that 25(OH)D testing was not associated with evaluation of bone health.⁴⁰ Huang et al.⁴¹ reported that 97.2% of the 7.5 million 25(OH)D tests ordered within a national U.S. outpatient cohort were coded as ICD-9 268.9, *unspecified vitamin D deficiency*, with less than three percent coded as *vitamin D deficiency-related osteomalacia* or *general vitamin D deficiency*.

The proportion of 25(OH)D tests results categorized as vitamin D deficient or insufficient ranged from 42% to 67%.^{26,30-32,36} Of note, researchers used different cut-offs for *deficiency* and *insufficiency* and the *insufficiency* category was not always reported. For example, Zhao et al.²⁶ classified vitamin D deficiency as 25(OH)D <12 ng/mL and insufficiency as 12–20 ng/mL whereas Wei et al.³⁰ classified <20 ng/mL as deficiency and 20–30 ng/mL as insufficiency. Three studies did not include an insufficiency category in their analyses.^{31,32,35}

Five studies analyzed whether ordered 25(OH)D tests were medically indicated. It is difficult to compare the results of these studies because varying criteria and guidelines were used in analyses. Forty-eight percent of 25(OH)D tests ordered by physicians in an Australian health system during 2012 were not considered guideline-supported based on authors’ application of multiple professional guidelines.³¹ Over 40% of 25(OH)D tests ordered for patients were covered by a private insurance company in upstate New York, U.S. but did not meet the company’s criteria for medically indicated.⁴¹ Non-indicated tests comprised nearly 10% of 25(OH)D tests in a 2014 northeast U.S. analysis³⁵ and 8.2% of tests ordered by physicians in a

research and teaching hospital in Italy from 2012-2014,⁴² both based on respective national guidelines. In the later analysis, 1,25(OH)₂D was ordered for an additional 8% of patients, also deemed inappropriate by authors.⁴² Only a fraction (3%) of 25(OH)D tests ordered in a California, U.S. managed care health system were classified as “high risk” (if patients were diagnosed with fat malabsorption, chronic kidney disease, HIV, anti-epileptic drug use, or had a history of bariatric surgery).³⁰

Vitamin D Prescriptions

Assessing strategies for treating LVD is difficult because they may include either recommended dietary changes, increased UVB exposure, and/or vitamin D supplements obtained over-the-counter or by prescription. However, a 75-fold increase in vitamin D₃ prescriptions was observed in Tuscany, Italy from 2006 to 2013.⁴³ An eight-fold increase in vitamin D₂ prescriptions was reported in California, U.S. Kaiser Permanente patients from 2007 to 2010.⁴⁴

Prescribing patterns varied among physicians. For example, Caillet et al.³⁶ observed over 350 different treatment regimens administered to 1311 French patients in 2008 and 2009 while Pepper et al.⁴⁵ described 36 discrete vitamin D prescribing regimens within a Veterans Medical Center in Georgia, U.S. in 2003 to 2006. Vitamin D treatments varied by form (i.e., vitamin D₂ vs. D₃), mode of delivery (i.e., intramuscular injection vs. oral), dose and frequency, and length of treatment regimen.

Physicians’ Knowledge, Attitudes, and Behaviors Related to Management of LVD

Physicians’ knowledge, attitudes, and behaviors related to vitamin D testing were evaluated by six studies. Three studies⁴⁶⁻⁴⁸ administered adaptations of the same survey,

“Prescribing Sunshine”, aimed at assessing the attitudes, practices, and knowledge regarding vitamin D and sun exposure among primary care physicians in Australia, New Zealand, and Saudi Arabia, respectively. Epling et al.⁴⁹ assessed primary care providers’ practice patterns involving vitamin D using focus groups, while Tarn et al.⁵⁰ analyzed recordings of patient-physician office visits, and Bennett et al.⁵¹ explored physicians’ management of vitamin D through structured interviews.

-Knowledge

Physicians’ confidence in their vitamin D knowledge varied, with 9–40% responding “not at all confident” in their vitamin D knowledge.⁴⁶⁻⁴⁸ Information regarding vitamin D was obtained through multiple different sources and strategies. The study by Bennett et al.⁵¹ reported prevalence of both passive and active information-seeking strategies, with few physicians reporting interactive strategies in obtaining vitamin D knowledge. Physicians in the Epling et al.⁴⁹ study discussed informal conversations with colleagues (not necessarily recent), point-of-care resources, professional guidelines, and scientific literature as information sources. Physicians in Saudi Arabia stated that continuous medical education, Internet resources, and medical journals were their primary information sources.⁴⁸ Australia released a national position statement regarding vitamin D and sun exposure in 2009, but only about 20% of physicians reported having read it when responding to a 2010 survey.⁴⁷ Bovisnki et al.⁴⁷ and Reeder et al.⁴⁶ both reported that about half of surveyed physicians agree with the statement “information about vitamin D is not readily available to general practice physicians”. Regardless, more than half of physicians in these two studies reported that the amount of information they were exposed to

regarding vitamin D was “more than normal” in the previous year.^{46,47} Very few physicians agreed that this information influenced their practice. Physicians in the Tarn et al.⁵⁰ study provided information to patients that was inconsistent with clinical guidelines regarding vitamin D screening in asymptomatic adults, the definition of LVD, and the optimal range for 25(OH)D. Nearly 100% of “Prescribing Sunshine” respondents strongly agreed that clear and concise guidelines regarding LVD would be useful.⁴⁶⁻⁴⁹

-Communication

The topic of vitamin D was raised in more than 15% of patient encounters in the study of Southern California, U.S. physicians.⁵⁰ Despite a great deal of uncertainty regarding vitamin D information and guidelines, physicians conveyed over 95% of vitamin D-related statements with certainty.⁵⁰ For example, some patients were told that vitamin D screening was routinely recommended despite insufficient evidence to support screening.⁵² Bennett et al.⁵¹ described physicians’ employment of Uncertainty Management Theory in conversations with patients about vitamin D treatment.

-Testing and Treatment

Physicians varied in their beliefs and practices regarding testing for LVD, with some supporting screening for all their patients, others believing that testing should be based on risk factors (the definitions of these risk factors were highly variable), and others focusing minimally on testing.^{49,51} Epling et al.⁴⁹ found that patient demand was a primary driver for vitamin D

testing. However, only about 20% of “Prescribing Sunshine” respondents indicated that patients initiated testing.⁴⁷

The definition of deficient/adequate/optimal 25(OH)D concentration and recommended treatment regimens varied broadly.^{49,50} Treatment of LVD with dietary supplements was more commonly recommended than dietary changes or increased exposure to sunlight.⁴⁶⁻⁴⁸ Confusion about the amount of sunlight exposure required for optimal vitamin D synthesis was expressed, in addition to concern about the association between excess sun exposure and skin cancer risk.^{46,47} About 70% of responding physicians in Australia and New Zealand disagreed that “it is more important to stay out of the sun than get enough vitamin D”.^{46,47}

A variety of maladaptive responses to uncertainty surrounding vitamin D testing were reported. For instance, some physicians admitted manipulating diagnostic codes so vitamin D tests were more likely to be reimbursed by insurance.⁴⁹ Bennett et al.⁵¹ discussed physicians’ tendency to craft certain statements and stories even when uncertainty exists.

-Attitudes

Uncertainty, doubt, and skepticism regarding vitamin D management were themes in two studies.^{49,51} Some physicians discussed their desire for patients to be proactive in their own care, yet also expressed frustration about the influence and unreliability of accessed media sources.⁵¹ The issue of limited time for patient encounters was discussed, with some physicians mentioning that vitamin D management was not always the top priority in patient visits.^{49,51}

-Economic Impact

The economic impact of vitamin D testing is sizable and increasing. Table 3.4 includes studies and reports which have analyzed or estimated direct costs of vitamin D testing. For example, Bilinski and Boyages⁵³ reported that nearly \$100 million (Aus.)/ \$794 million (U.S.) was spent on vitamin D testing in Australia in 2010, a value that reflects 1% of national healthcare spending. In the U.S., \$224 million was spent on vitamin D testing for Medicare patients (individuals over 65 years of age or qualifying based on disability) and \$33 million was spent on 2014 vitamin D tests among privately insured patients in Upstate New York, U.S.⁴¹ Over \$20 million of “unnecessary” testing was identified in Virginia, U.S. in 2014 based on analysis using health waste calculator software.⁵⁴ The \$20 million represents approximately 0.9% of the state’s healthcare spending in 2014, up from 0.4% in 2013.⁵⁴ Non-indicated vitamin D tests were more common in U. S. Medicare patients than commercially insured patients based on Medicare guidelines for vitamin D testing (13% vs. 8% of patients seen from 2009-2011, respectively).⁴⁰ No studies identified a decrease in vitamin D testing.

Patients diagnosed with LVD in U.S. Veteran’s Medical Centers used more healthcare services and incurred higher medical costs than patients not diagnosed.^{32,55} Vitamin D status also correlated with increased hospitalization and medical costs in generally healthy German adults.⁵⁶ Decreased muscle relaxant and pain medication prescriptions were associated with vitamin D status and supplementation in French patients dealing with chronic pain.⁴¹

Efforts to Constrain Inappropriate Clinical Practice Related to Low Vitamin D

Interventions aimed at reducing inappropriate vitamin D test-ordering have been impactful. For example, the national health systems in France and Ontario, Canada restricted testing to only a subset of high-risk conditions.^{57,58} Through reimbursing 25(OH)D testing only for osteoporosis/osteopenia, rickets, malabsorption syndromes, renal disease, and concurrent medications which may affect vitamin D metabolism, officials in Ontario predict a savings of approximately \$65 million annually.⁵⁷ Deschasaux et al.⁵⁹ recommended a screening questionnaire, the vitamin D insufficiency prediction score, as an effective tool for identifying patients at high-risk for LVD and as a precursor for 25(OH)D testing while a Utah, U.S.-based team suggested benchmarking as an effective method of monitoring vitamin D testing.⁶⁰ Implementation of three clinical decision support tools in the electronic medical record of a large U.S.-based health system resulted in a 13% reduction in tests considered unnecessary by the health system's evidence-based guidelines.⁶¹ White et al.⁶² also showed a decrease in inappropriate test-ordering through electronic medical record modification in two U.S. medical facilities. Direct physician feedback reduced inappropriate repeat 25(OH)D testing by 25% in Italy.⁶³ For example, physicians received a phone call and computer message when ordering a repeat 25(OH)D test less than 90 days after the previous 25(OH)D test.⁶³ Finally, patient and clinician education were shown to be effective in reducing the ordering of 25(OH)D tests.^{64,65}

3.4 DISCUSSION

This scoping review identified literature related to physicians' clinical management of LVD, costs associated with physicians' clinical management of LVD, and efforts to constrain inappropriate clinical management of LVD by physicians in a variety of developed countries.

Vitamin D laboratory testing, prescriptions, and costs associated with these practices have increased, in some cases dramatically, over the past 10–15 years. Patterns of test overutilization were demonstrated throughout reviewed studies. Interventions designed to constrain inappropriate clinical management patterns have produced promising results.

Although a substantial volume of patients with LVD were identified through 25(OH)D testing, the odds of detecting LVD decreased. Reported increases in vitamin D testing were disproportionate to increases in other laboratory tests. Most articles reported testing patterns indicative of vitamin D screening. These patterns are inconsistent with the clinical guidelines and recommendations from USPSTF, IOM, U.S. Endocrine Society, and others (Tables 3.1 and 3.2) who recommend vitamin D testing only for symptomatic patients or those at high risk of LVD. Billinski and Boyages⁵³ showed that vitamin D testing was not associated with bone-related diagnoses, which are commonly considered indicative of vitamin D testing. It is unknown, however, what proportion of tests were associated with other problems or diagnoses which may be considered high risk for LVD, such as chronic renal disease or malabsorption. Ambiguity and inconsistencies in LVD treatment guidelines may explain the excessive number of repeat vitamin D tests ordered in a short timeframe in some analyses.

As noted in Table 3.4, the cost of rising 25(OH)D testing is significant. It could be argued that spending on 25(OH)D testing is trivial since it contributes marginally to total healthcare spending. However, achieving the global goal to contain healthcare spending, in part, by reducing low value care and medical waste will require collective effort at all levels of care and all levels of spending. Better management of vitamin D may serve as an example for future efforts to achieve higher value care.

Costs reported in Table 3.4 do not include downstream costs associated with increased testing such as increased laboratory personnel, time/personnel needed to communicate test results to patients, tests ordered as follow-ups to initial testing, and treatment expenses. Minimal information is available about resource utilization related to increased vitamin D prescriptions and the variation in treatment patterns identified by this review.

Although increased healthcare costs were associated with LVD, it is difficult to determine if patients in these studies incurred higher healthcare costs only due to LVD. Since numerous factors are related to both LVD and poor health, patients with LVD may have been sicker than those without LVD. Rather than LVD causing health problems (and thus, higher costs), it is feasible that other health problems resulted in LVD.

Authors of several reviewed articles concluded that the standardization of guidelines and procedures regarding vitamin D testing and medical management would be valuable. Almost all “Prescribing Sunshine” respondents agreed that clear and concise guidelines were needed, with over 50% indicating their perception that information about vitamin D is not readily available to general physicians.^{46,47} However, guidelines and recommendations from multiple expert bodies and professional associations exist (Tables 3.1 and 3.2). Data collection for some studies occurred before Tables 3.1 and 3.2 guidelines and recommendations were published, so it is possible that physicians may have changed their vitamin D management after reviewing revised professional guidelines. Inconsistency in published guidelines and recommendations coupled with the recent intense focus on the role of vitamin D in non-skeletal conditions may explain the wide variation in management of LVD. Physicians’ lack of awareness of existing guidelines may also contribute to inconsistencies.

A better understanding of the proportion of physicians who have reviewed the guidelines and recommendations included in Tables 3.1 and 3.2 is needed. Finally, perhaps some physicians were aware of guidelines but did not agree with them, preferred to make decisions based on their own clinical judgement, or were influenced by the high volume of reports related to non-skeletal effects of LVD.⁶⁶

Epling et al.⁴⁹ discussed physicians' practice patterns regarding vitamin D as set within clinical "mindlines". Mindlines have been defined as 'collectively reinforced, internalized tacit guidelines'⁶⁷ that arise from the interaction of knowledge, practice patterns and constraints, and the larger context of patient demand and the medical community. These mindlines may explain the noted contradictions in guidelines and physician practices. We found differences in the impact of patient demand on vitamin D test ordering.^{49,68} Overall, a better understanding of the factors that influence the clinical management of LVD is needed.

The issue of uncertainty was repeatedly cited as a highly influential contributor to excessive low value care, including 25(OH)D testing in low risk patients. Tarn et al.⁵⁰ reported that over 60% of surveyed physicians found uncertainty involved in providing care to be disconcerting. Bennet et al.⁵¹ described several communication and coping strategies employed by physicians in relation to uncertainty in vitamin D management. Other influences potentially include: defensive behavior/ fear of malpractice accusations, responding to patients' or family members' demands, ease of ordering and obtaining test results, profit for medical subspecialties, clinical performance measures, and lack of feedback regarding cost and prevalence of testing. The allure of identifying an easy-fix or "magic pill" for patient treatment (i.e., treating LVD, recommending vitamin D supplementation) may be appealing to patients and physicians alike, contributing to vitamin D lab test overutilization.

Some physicians noted conflict regarding multiple health goals and initiatives. For instance, the challenge of promoting UVB exposure to improve vitamin D status while recommending limited UVB exposure as a skin cancer precaution. Guidelines and tools for recommending appropriate sun exposure for different individuals in a variety of regions would be valuable to clinicians. Finally, with the average primary care visit lasting an average of 13-16 minutes,⁶⁹ time to adequately address topics such as vitamin D may be limited, particularly in complex patients. One physician expressed practical challenges in translating medical recommendations in clinical practice given multiple constraints, stating “In training, the most important lesson they teach you is when not to do something. But in real life, it’s all about staying out of trouble, surviving, and keeping it quick”.⁶⁶

Multiple interventions led to meaningful reductions in inappropriate 25(OH)D test-ordering in the short term. However, evidence of long-term effectiveness is needed, in addition to physicians’ acceptance of these interventions.

Future Research

High-quality evidence regarding whether or not vitamin D testing and/or treatment in asymptomatic adults improves health status or the economic bottom line is the priority for further research related to clinical management of vitamin D. Once this information is elucidated, methods for standardizing the 25(OH)D test, improving adherence to guidelines, and reducing the cost of testing would appropriately be considered. Understanding more about why healthcare practitioners provide increasing amounts of low value care—especially low cost, low value

care—and how they experience uncertainty and emerging information may provide perspective into effective intervention for vitamin D management in addition to other health services.

Study Strengths and Limitations

This study is the first review of literature related to clinical management of LVD. As is appropriate for the intent of a scoping review, the included evidence is heterogeneous in clinical setting, research methods, and analysis. Limitations of this review include the restriction to English language articles, and the lack of detailed critical appraisal of the included studies. Literature included in the review includes studies which took place at different points in time relative to published guidelines. Additionally, the researchers may have had different baseline assumptions for what constitutes appropriate management of LVD.

3.5 CONCLUSIONS

Evidence regarding the role of vitamin D in the prevention and treatment of non-skeletal conditions continues to evolve. The impact of vitamin D screening for asymptomatic or low-risk patients is unknown. Nevertheless, physician practice, as demonstrated in a variety of studies, is widely inconsistent, and includes many examples of non-indicated testing and overutilization. Clinical practice has surpassed available supporting evidence. Broad variability in physicians' knowledge, attitudes, and behaviors related to vitamin D testing are reflective of the landscape of uncertainty in research findings, recommendations, and guidelines. Future research is needed to inform better clinical guidelines in this area, and to assess implementation practices that will encourage evidence-based management practices for LVD in adult populations. Moreover,

greater understanding of physician management of uncertainty in clinical practice may help avoid overutilization and inconsistent practice in similar clinical situations.

TABLES AND FIGURES
(shown in order referenced within text)

Table 3.1. Vitamin D screening and testing guidelines and recommendations by authoritative bodies and professional societies

<i>Recommendation</i>	<i>Population-Wide 25(OH)D Screening Recommended?</i>	<i>25(OH)D Testing for Individuals at High Risk of Deficiency Recommended?</i>	<i>Definition of “High Risk”</i>
American Academy of Family Physicians ¹²	Current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency (I statement)	No	n/a
Canadian Medical Association ⁷⁰	No	Yes	Significant renal or liver disease Osteomalacia, osteopenia or osteoporosis Malabsorption syndromes Hypo or hypercalcemia/ hyperphosphatemia Hypo or hyperparathyroidism Patients on medications that affect vitamin D metabolism or absorption Unexplained increased levels of serum alkaline phosphatase Patients taking high doses of vitamin D (> 2000 IU daily) for extended periods of time (> 6 months), and who are exhibiting symptoms suggestive of vitamin D toxicosis (hypervitaminosis D)
Central European Scientific Committee on Vitamin D ⁷¹	No	Yes	Rickets, osteomalacia, osteoporosis, musculoskeletal pain, history of fracture or falls Calcium/phosphate metabolism abnormalities Hyperparathyroidism Malabsorption syndromes At-risk medications Dietary restriction, parenteral nutrition, eating disorder Kidney disease (stages 3–5) or transplant, liver disease, autoimmune disease, cardiovascular disease, some cancers, some infections

Kidney Disease Outcomes Quality Initiative (KDOQI) ^{72*}	No	Yes	Stage 3–5 kidney disease, particularly if on dialysis
U.S. Endocrine Society ⁷	No	Yes	Rickets, osteomalacia, osteoporosis Chronic kidney disease Hepatic failure Malabsorption syndromes Certain medications African-American and Hispanic children and adults Pregnant and lactating women Older adults with history of falls or non-traumatic fractures Obese children and adults Granuloma-forming disorders Some lymphomas
U.S. Preventive Services Task Force ⁷³	Current evidence is insufficient to assess the balance of benefits and harms of screening in asymptomatic adults (I statement)	n/a	n/a

*KDOQI changed diagnostic criteria for stage 3 kidney disease in 2003 resulting in more stage 3 kidney disease diagnoses and subsequent 25(OH)D tests.

n/a = not available or not applicable

Table 3.2. Serum 25-hydroxyvitamin D [25(OH)D] concentrations indicative of vitamin D deficiency, insufficiency, adequacy, and toxicity

Study	Vitamin D Deficiency (25(OH)D)	Vitamin D Insufficiency (25(OH)D)	Adequate Vitamin D (25(OH)D)	Toxicity (25(OH)D)
Australian and New Zealand Bone Mineral Society/ Endocrine Society of Australia and Osteoporosis Australia ⁷⁴	Mild deficiency: 12–19.5ng/mL Moderate deficiency: 5–12 ng/mL Severe deficiency: <5 ng/mL		20 ng/mL at the end of winter; 24–28 ng/mL at the end of summer to allow for seasonal decrease	Not defined
Central European Scientific Committee on Vitamin D ⁷¹	<20 ng/mL	20-30 ng/mL	30–50ng/mL	>100 ng/mL
National Academy of Medicine (formerly IOM) ⁶	<12.5 ng/mL	Not defined	12–20 ng/mL 25(OH)D of 20 ng/mL is sufficient to meet needs of 97.5% of the population	>50 ng/mL
Public Health England/ National Osteoporosis Society ⁷⁵	<10 ng/mL	10-19.5 ng/mL	>20 ng/mL	Not defined
U.S. Endocrine Society ⁷	<20 ng/mL	20-30 ng/mL	>30 ng/mL	>150 ng/mL

Figure 3.1. Preferred reporting items for systematic reviews and meta-analyses flow diagram for the scoping review

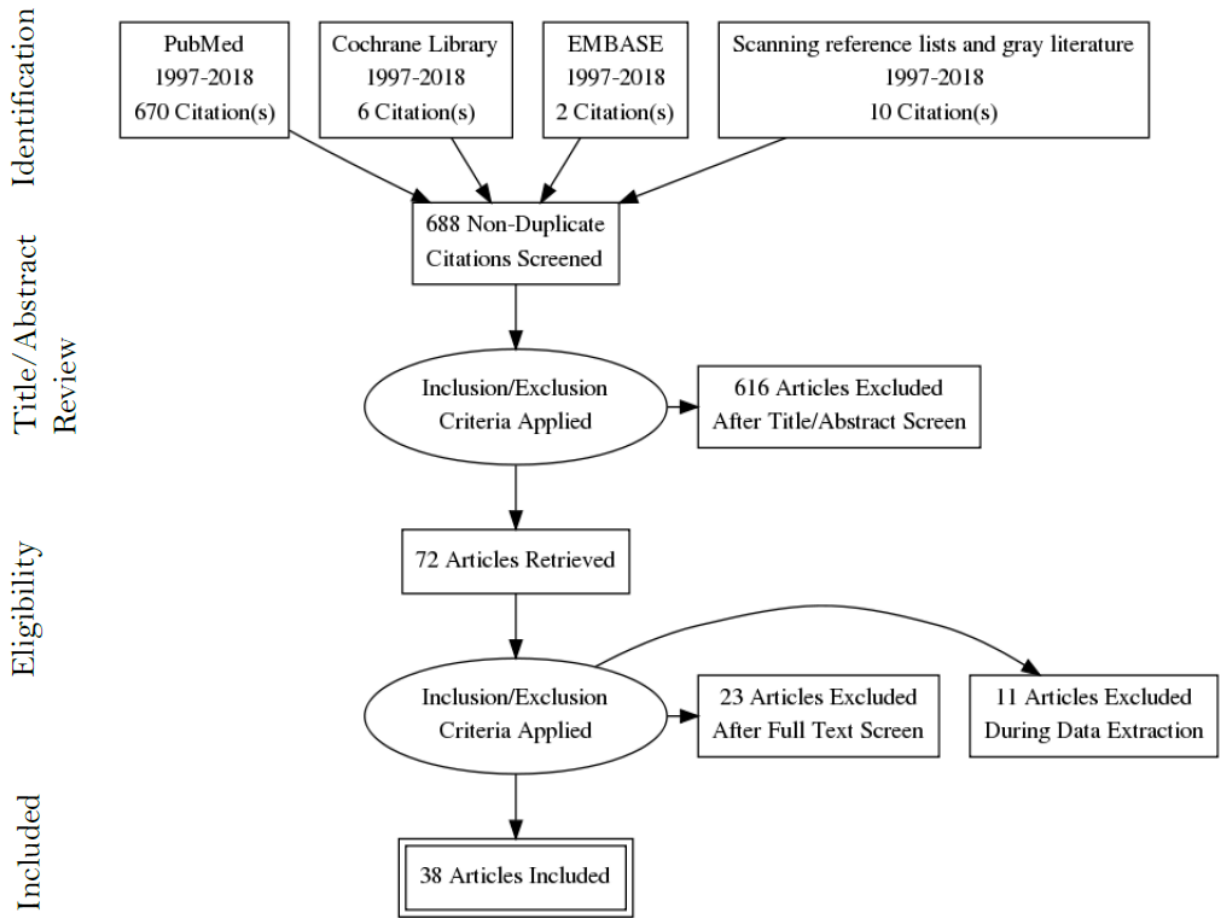


Table 3.3. Studies reporting trends in vitamin D testing patterns

Study	Population	Setting	Time Frame	Key Findings
Bilinski and Boyages A, 2013 ²⁴	2.4 million patients who received 25(OH)D tests (national health system data)	Australia	4-year period 2006–2010	94-fold increase in tests
Bilinski and Boyages B, 2013 ⁴⁰	Women, ages 45–74 (national health system data)	Australia	10-year period 2001–2011	44% increase in tests
Caillet et al., 2017 ²⁷	639,163 patients (national health insurance database)	France	1-year period 2008–2009	18.5% were tested
Colla et al., 2017 ⁷⁶	Medicare and commercially insured patients (Health Care Cost Institute database)	United States	2-year period 2009–2011	10-16% of Medicare patents and 5-10% of commercially insured were tested
de Koning et al., 2014 ³⁹	Adult residents of 1436 census regions	Alberta, Canada	1-year period 2010–2011	8% were tested
Gowda et al., 2016 ³⁴	2187 patients seen in community health center	Melbourne, Australia	2-year period 2010–2012	56% of patients were tested
Khalifa et al., 2016 ²⁹	Hospital patients (King Faisal Hospital and Research Center)	Jeddah, Saudi Arabia	1-year period 2014–2015	30% increase in tests
Tapley et al., 2015 ³³	General practice patients (Recent cohort study)	4 states in Australia	3-year period 2010–2013	1% of patients were tested
Wei et al., 2014 ³⁰	22,784 managed care patients	California, United States	2-year period 2011–2013	11% of patients were tested
Zhao et al., 2015 ²⁶	Primary care patients	Liverpool, United Kingdom	5-year period 2007–2012	11-fold increase in tests

Table 3.4. Cost of Vitamin D Testing

<i>Study/Report</i>	<i>Population</i>	<i>Setting</i>	<i>Timeframe</i>	<i>Key Findings</i>
Bartells, 2014 ³⁷	Commercially insured adult patients	Upstate New York, U.S.	1-year period 2014	\$33 million spent on 25(OH)D tests
Bilinski and Boyages A, 2013 ²⁴	Adults (national health system data)	Australia	4-year period 2006–2010	\$20 million (Aus.)/ \$16 million (U.S.) spent on “non-indicated” 25(OH)D tests
Bilinski and Boyages B, 2013 ⁴⁰	Women, ages 45–74 (national health system data)	Australia	10-year period 2001–2011	\$7 million (Aus.)/ \$555,492 (U.S.) spent on 25(OH)D tests in 2001 and \$40.5 million (Aus.)/ \$32 million (U.S.) in 2011
Caillet et al., 2016 ²⁷	All individuals (national health insurance database)	France	2-year period 2009–2011	€27 million/ \$33 million (U.S.) in 2009 to €65 million/ \$79 million (U.S.) on 25(OH)D tests
Cianferotti et al., 2015 ⁴³	Adults (20–90)	Tuscany, Italy	7-year period 2006–2013	€3.2 million/ \$3.9 million (U.S.) in 2006 to €8.2 million/ \$10.1 million (U.S.) in 2013 on 25(OH)D tests
Colla et al. 2015 ²⁰	Medicare patients (>65 years of age, qualify based on disability)	U.S.	5-year period 2006–2011	\$224 million in 2011, average of \$198 million/year 2006–2011 on 25(OH)D tests
Fairfield, 2017 ³⁵	All individuals without high risk diagnosis (ex: osteoporosis, malabsorption, liver disease, etc.)	Maine, U.S.	2-year period 2012–2014	\$9,596,000 spent on “non-indicated” on 25(OH)D tests

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CHAPTER 4

MANUSCRIPT

An Exploration into Patterns of Clinical Care Subsequent to Non-indicated Vitamin D Testing in Primary Care

ABSTRACT

Background: Low value care is defined as clinical care that offers minimal benefit, or leads to net harm. Overutilization of laboratory test services is a source of low value care. Although vitamin D testing is indicated for some patients, exponential increases in non-indicated testing have been reported over the past 15 to 20 years. Increased financial burden has resulted from this pattern of increased testing, but downstream effects of such testing patterns have not been well-quantified.

Methods: The purpose of this study was to describe patterns of non-indicated vitamin D [25(OH)D] testing within primary care of a large regional health system, and to quantify downstream health service utilization subsequent to such testing. An automated search of the electronic health record (EHR) of a regional health system was conducted to identify instances of non-indicated vitamin D testing conducted by primary care providers in 2015, and to track subsequent vitamin D-relevant health service utilization.

Results: There were 77,836 adult continuous primary care patient records in the system for 2015. In this cohort, vitamin D tests were conducted in 8042 patients (10.3%). Tests considered non-indicated comprised <1% of these tests. More than 4000 vitamin D-related services (laboratory tests, imaging, and prescriptions) were conducted during the 24 months following non-indicated vitamin D testing. Substantial variability in care was noted. For example, 26 different vitamin D prescription regimens were utilized. Forty-five percent of low vitamin D lab tests were not followed up with repeat vitamin D tests, and a less effective form of vitamin D (vitamin D₂) was the most common vitamin D prescription utilized.

Conclusions: Although less non-indicated vitamin D testing was observed within this health system than previously reported in other health systems, the tests that occurred seemed to trigger a cascade of low value care. Opportunities for improved consistency and quality of care exist. These results may inform clinical pathways related to the prevention, evaluation, and treatment of low vitamin D.

4.1 INTRODUCTION

In 2012, the Institute of Medicine estimated that as much as 30% (>\$700 billion annually) of U.S. healthcare spending is unnecessary and wasteful.¹ Low value care, which is defined as clinical services that confer minimal or no benefit or may cause harm, is a substantial component of unnecessary healthcare expenditures.^{2,3} Initiatives to constrain low value care aim to reduce healthcare spending, while improving quality of care and health outcomes.⁴

The Choosing Wisely Campaign is an initiative aimed at reducing low value care and highlighting clinical practices inconsistent with the evidence.^{5,6} Initially founded in 2012 by the American Board of Internal Medicine Foundation, Choosing Wisely presently includes recommendations from over 80 professional medical societies.⁵ Many Choosing Wisely recommendations focus on the overuse of diagnostic tests.⁷ For example, four medical societies have identified a Choosing Wisely recommendation of: *Do not order population-based screening for vitamin D.*⁸ This recommendation aligns with a 2014 U.S. Preventive Services Task Force (USPSTF) recommendation statement that advises against vitamin D screening in asymptomatic community-dwelling adults due to insufficient evidence of benefits and harms.⁹ Choosing Wisely and others do recognize, however, that vitamin D testing is indicated in patients at high risk for abnormal vitamin D, or related complications.^{10,11}

Vitamin D status is most commonly assessed via a laboratory serum 25-hydroxyvitamin D [25(OH)D] test.¹² Throughout this paper, “vitamin D test” refers to a serum 25(OH)D laboratory test. Healthcare providers have been documented as inappropriately ordering 1,25-dihydroxyvitamin D [1,25(OH)₂D] tests to evaluate vitamin D status.¹³⁻¹⁵ Although 1,25(OH)₂D is the active form of vitamin D, it has a short half-life, is closely regulated by parathyroid hormone (PTH), and has no relationship to vitamin D stores.¹⁶ It may be appropriate to conduct 1,25(OH)₂D testing when kidney or parathyroid problems are suspected,¹⁶ and a separate

Choosing Wisely recommendation is: *Do not routinely measure 1,25(OH)₂D unless the patient has hypercalcemia or decreased kidney function.*

Numerous studies have identified an exponential increase in vitamin D tests conducted in the U.S. and many other countries over the past 10 to 15 years.¹⁷⁻²¹ Much of these tests follow a pattern of non-indicated screening rather than targeted testing of high-risk patients.²² Unfortunately, the lack of consensus in criteria for what constitutes a clinically indicated vitamin D test complicates interpretation of these studies.²² For instance, the Choosing Wisely recommendations include osteoporosis, kidney or liver disease, malabsorption syndromes, or history of abnormal vitamin D among their criteria for indicated vitamin D tests.⁵ The Endocrine Society Clinical Guideline on Vitamin D (2011), on the other hand, adds African American and Hispanic individuals, pregnant and lactating women, and individuals with obesity to the list of criteria for indicated vitamin D testing.¹⁰ The use of specific medications has also been described by some as an indication for vitamin D testing.^{23,24}

At \$50 to \$250 per test, vitamin D testing is itself a low to moderately priced laboratory test.²⁵ However, the large volume of tests regularly conducted makes vitamin D testing a significant economic investment.^{22,26} For example, it is estimated that over one billion vitamin D tests are ordered annually worldwide.²⁷ More than six million vitamin D tests labeled as “low value” were conducted within commercial and Medicare-insured Americans in 2014, tallying over \$800 million.²⁷ Additionally, \$24 million was spent on vitamin D testing considered non-indicated in the state of Virginia in 2016.²⁸ Overall, the high-volume utilization of low cost services, such as non-indicated vitamin D testing, comprises over 90% of low value care expenditures.²⁸

Importantly, along with financial burden, overuse of screening and diagnostic tests has been associated with downstream effects, such as increased health services and greater patient harm.^{29,30,31} Further research is needed to examine downstream effects and consequences following non-indicated vitamin D testing. To date, much of the research related to low value care has been conducted using health claims data. Analysis of electronic health record (EHR) data offers opportunity for more detailed and focused analysis of low value care within health systems.³² Thus, the purpose of this study was to analyze EHR data to describe patterns of non-indicated vitamin D testing within primary care of a regional health system, and to quantify downstream health service utilization subsequent to such testing.

4.2 METHODS

Study Design

We performed an automated search of the EHR database for a regional health system to identify instances of non-indicated vitamin D testing and track subsequent vitamin D-related laboratory testing, imaging services, and prescriptions. For the purpose of this study, **non-indicated vitamin D testing was defined as vitamin D tests that do not meet Choosing Wisely criteria for indicated services.**⁸ The classification used to describe vitamin D status (Table 4.1) is based on Institute of Medicine and Endocrine Society clinical guidelines,^{10,33} and Carilion Clinic's established vitamin D reference range. This study was approved by the Institutional Review Board of Carilion Clinic (IRB-18-274).

Setting

Carilion Clinic is a non-profit comprehensive healthcare system that serves over one million patients in Southwest Virginia. Primary care services at Carilion Clinic are delivered by

194 Family Medicine providers and 43 Internal Medicine providers in 49 different facilities. Providers are physicians, physician assistants, and nurse practitioners. Epic (Epic Systems, Wisconsin, U.S.) has served as the EHR platform for Carilion Clinic since 2008.

Quest Diagnostics (New Jersey, U.S.) has served as the laboratory services provider for Carilion Clinic since 2014. Patient blood draws are conducted on-site at some primary care facilities, or at other laboratories, test centers, clinics, or the hospital. Vitamin D test analyses are conducted within one of two Quest Diagnostics laboratories (Chantilly, Virginia or Charlotte, North Carolina) using immunoassay methodology certified by the Centers for Disease Control and Prevention Vitamin D Standardization Certification Program. Providers also have the option to select liquid chromatography/ tandem mass spectrometry methodology for vitamin D tests. Carilion Clinic assigns 30 to 100 ng/mL as the normal reference range for 25(OH)D.

Data Extraction

From Carilion Clinic's EHR, we identified adult patients (≥ 18 years of age) who received ongoing care (>1 visit annually) from one or more primary care providers between January 1, 2013 and December 31, 2017. Data were extracted for any of these continuous primary care patients for whom an outpatient initial vitamin D test (i.e., first test within 12 months) was conducted between January 1, 2015 and December 31, 2015. In addition to vitamin D test date and test results, we also extracted the following data: age, gender, race/ethnicity, body mass index (BMI), insurer, and diagnoses codes associated with the vitamin D test.

In order to identify a subset of patients for whom the initial vitamin D test was deemed non-indicated, we excluded patients who had diagnoses shown in Table 4.2 in the 12 months prior to their vitamin D test. Patients who had one or more previous vitamin D test(s)

documented anywhere in their history were also excluded, as were patients who received a vitamin D prescription in the previous 12 months. For patients who had non-indicated tests in 2015, the following additional vitamin D-related service data were extracted: laboratory tests, prescriptions, imaging, and diagnoses documented in the 24 months subsequent to the non-indicated vitamin D test (Table 4.3). The additional vitamin D-related service data included results from other healthcare systems, which are inconsistently included in the record in an extractable format.

Data Analysis

Data were analyzed using GraphPad Prism 8.0 and Microsoft Excel (Office 2019). Descriptive statistics (means, standard deviation, and frequencies as applicable) were calculated on all data. Differences between means were analyzed using unpaired t-tests, or one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. For categorical data, chi-square and Fisher's exact tests were used. Multivariate linear regression was used to evaluate the contribution of patient factors to vitamin D test results. Mean Abnormal Results Rate (MARR) was calculated by dividing the number of abnormal vitamin D test results by the total number of vitamin D tests conducted.³⁴ Statistical significance was set at an alpha level of 0.05.

4.3 RESULTS

We identified 77,836 continuously-enrolled adult primary care patient records for 2015. Vitamin D tests were conducted on 8042 of these patients (10.3%) (Figure 4.1). Nearly all (n=8040) were analyzed via immunoassay. Non-indicated vitamin D tests were conducted on

574 patients (0.7% of total primary care patients), representing 7.3% of the vitamin D tests conducted (Figure 4.1). Figure 4.2 shows number of vitamin D tests conducted each month during 2015.

Demographic and clinical characteristics of patients who had vitamin D testing in 2015 are shown in Table 4.4. Patients who had non-indicated vitamin D tests were younger, more likely to be male, more likely to be self-pay or commercially insured, and less likely to be insured by Medicare compared with patients who had indicated vitamin D testing.

Vitamin D Test Results

Vitamin D test results did not differ by patient age. White patients had 18% higher vitamin D test results than black or African American, Asian, and Hispanic patients ($p < 0.0001$). Patients with underweight ($< 18.5 \text{ kg/m}^2$) or normal BMI (18.5 to 25 kg/m^2) had higher (17% and 15%, respectively) vitamin D test results than patients with BMI $> 25 \text{ kg/m}^2$ ($p < 0.001$) as did those with commercial insurance or self-pay status compared with other insurers (Medicare, Medicaid, Other) ($p < 0.001$).

Mean vitamin D test results were similar among non-indicated and indicated tests (41.1 ng/mL vs. 39.9 ng/mL). However, there was a greater proportion of *insufficient* test results [25(OH)D 20 to 29 ng/mL] ($p < 0.001$) and smaller proportion of *normal* test results [25(OH)D 30 to 99.9 ng/mL] among non-indicated tests compared with indicated tests ($p < 0.001$) (Table 4.5). The MARR was higher for non-indicated than indicated vitamin D tests (38.4% vs. 24.9%) ($p < 0.001$).

Downstream Services Following Non-Indicated Vitamin D Testing

A total of 4437 vitamin D-relevant laboratory, prescription, and imaging services were provided over the 24-month observation to patients who had non-indicated vitamin D testing (Table 4.6). Patients with *deficient* initial vitamin D test results had 54% more subsequent laboratory tests than those with *insufficient* test results and 51% more than those with *normal* test results ($p=0.037$) (Table 4.6). However, patients with *insufficient* initial vitamin D test results had 2-fold more follow-up vitamin D tests than patients with *deficient* test results and 5-fold more than those with *normal* test results ($p=0.022$). Twice as many total vitamin D-relevant services were provided to patients with *deficient* initial vitamin D test results than those with *insufficient* and normal test results ($p=0.1117$).

Vitamin D-Relevant Laboratory Testing Subsequent to Non-Indicated Vitamin D Testing

Follow-up vitamin D testing was conducted in 200 patients (34.8%) who had non-indicated initial vitamin D testing (Figure 4.3). The number of follow-up tests conducted over 24 months was: one (129 patients), two (37 patients), three (23 patients), four (9 patients), or five (2 patients). No follow-up vitamin D testing was conducted in 6.1% of patients with *deficient* initial vitamin D test results, 55.6% of patients with *insufficient* initial vitamin D test results, and 78.0% of patients with *normal* initial test results (Table 4.7). Significantly more first follow-up vitamin D tests were conducted in patients who had low (*deficient* or *insufficient*) non-indicated vitamin D test results compared with *normal* non-indicated vitamin D test results ($p<0.001$) (Figure 4.3). However, abnormal vitamin D test results were not associated with likelihood of having second, third, fourth, or fifth vitamin D tests. Time lapse between initial and follow-up

tests varied, but nineteen (5.9%) of follow-up vitamin D tests took place sooner than 12 weeks after the non-indicated test.

Within the 24-month observation period, 41 patients with *deficient* initial non-indicated vitamin D tests (83.6%) and 57 patients with *insufficient* initial non-indicated vitamin D tests (33.3%) attained improved vitamin D test results (Table 4.7). Of patients with *normal* initial non-indicated vitamin D test results, 57 (16.1%) later tests revealed *low* vitamin D test results (Table 4.7).

Other vitamin D-relevant laboratory tests included calcium (n= 2183), alkaline phosphatase (n= 1409), phosphorus (n= 123), 1,25(OH)₂D (n= 26), and PTH (n=18). The 26 1,25(OH)₂D tests were conducted in 24 patients. The MARR for these tests was 19.8% (calcium), 10.0% (alkaline phosphatase), 23.6% (phosphorus), 20.0% [1,25(OH)₂D], and 33.3% (PTH). There was no relationship between initial vitamin D status and likelihood of having a 1,25(OH)₂D test. Most 1,25(OH)₂D tests were conducted in patients who did not have abnormal PTH test results (n= 22/ 92%) or elevated calcium test results (n=20/ 83%).

Prescriptions Subsequent to Non-Indicated Vitamin D Testing

A total of 275 prescriptions for oral vitamin D supplements were provided to 112 (20%) patients. Nine different vitamin D prescriptions were provided in a total of 26 different treatment regimens (Table 4.8). Seventy-three (65%) prescriptions were for ergocalciferol (vitamin D₂) and 39 (35%) were for cholecalciferol (vitamin D₃). All vitamin D₂ prescriptions were for a weekly dose of 50,000 IU. Vitamin D₃ dosing varied broadly (Table 4.8). There was no relationship between vitamin D test result and type or dosage of vitamin D prescribed. For example, there were 11 25(OH)D test results <12 ng/mL. The following vitamin D prescriptions

were provided to these patients: high dose vitamin D₂ (n=2), high dose vitamin D₃ (n=2), moderate dose vitamin D₃ (n=2), and no vitamin D prescription (n=5).

Of the 112 patients who received a vitamin D prescription, 82 (73%) had at least one follow-up vitamin D test. Vitamin D₂ prescriptions were significantly less likely to result in a subsequent *normal* vitamin D during the analysis period compared with vitamin D₃ prescriptions (p= 0.0107) (Figure 4.4). On average, vitamin D test results decreased by 68% (range: -710% to 48%) following vitamin D₂ prescriptions and increased by 43% (range: -180 to 430%) following vitamin D₃ prescriptions. Half (50%) of patients with *low* initial vitamin D test results achieved *normal* test results during the 24-month observation. Likelihood of achieving *normal* vitamin D was significantly higher in patients prescribed moderate-dose (1000 to 2000 IU/day) or high-dose (\geq 2000 IU/day) vitamin D₃ compared with those prescribed low-dose (400 to 1000 IU/day) vitamin D₃ (p= 0.0301). There were no *high* vitamin D test results following provision of vitamin D prescriptions in this cohort.

Calcium (without vitamin D) was prescribed to 21 patients (5%), and bone resorption inhibitors were prescribed to 15 patients (3%) who had non-indicated vitamin D tests. Calcimimetic medications (e.g., Cinacalcet) were not prescribed to any patients in this cohort.

Imaging Subsequent to Non-Indicated Vitamin D Testing

Vitamin D-related imaging studies were conducted on 30 patients (5%) during the 24 months following non-indicated vitamin D testing. One patient underwent two imaging studies, while the remainder had a single imaging study. The majority of imaging studies (25) consisted of multi-site dual energy x-ray absorptiometry (DXA), and the remainder consisted of axial-only DXA. All imaging studies were conducted on white females.

Diagnoses Subsequent to Non-Indicated Vitamin D Testing

During the 24 months following a non-indicated vitamin D test, a diagnosis of vitamin D deficiency or vitamin D insufficiency was documented for 356 patients (62%). Of these, 107 (21%) had only *normal* vitamin D test results during the 24-month observation period. Other vitamin D-related diagnoses documented were osteoporosis (8%), osteopenia (1%), osteoporotic fracture (1%), pathological fracture (0.5%), stress fracture (0.5%), inflammatory bowel disease (5%), malabsorption (2%), and parathyroid/calcium/phosphate disorder (1%). There was no difference in likelihood of receiving one of these diagnoses based on initial vitamin D status.

DISCUSSION

Low cost, low value health services have been shown to contribute substantially to healthcare waste in the U.S., and also cause a variety of harms.^{2,4} The purpose of this study was to describe patterns of non-indicated vitamin D testing within primary care of a Southwest Virginia health system, and quantify some of the downstream service utilization related to such testing. Our key findings are that, although primary care providers within this health system conducted less non-indicated vitamin D testing than has been documented within other health systems, inconsistency in follow-up care contributed to a cascade of low value services.

Colla et al.³⁵ described occurrence of low value care in more than 300 health centers across the U.S. in 2009-2011. Lynchburg, Virginia and Morgantown, West Virginia (the two regions closest to our health system) recorded the highest rate of non-indicated vitamin D testing.³⁴ Other studies have reported that 43% of total vitamin D testing in Upstate New York in 2014 was non-indicated,³⁶ as was 35% in Washington in 2017,³⁷ 25% in Virginia in 2016,²⁵ and 10% in Maine in 2014.³⁸ In contrast, only 7% of vitamin D tests ordered by primary care providers within our health system were classified as non-indicated. We also observed less

overall vitamin D testing (10% of patients) than has been previously reported.^{27,38-40} Although comparison of these results is complicated by varying definitions of vitamin D testing indicators, a number of factors may explain the lower occurrence of non-indicated vitamin D testing. For example, our cohort was exclusively outpatient (some others included inpatients as well) and we studied non-indicated initial vitamin D tests ordered only by primary care providers (not specialists). Other differences in providers or patients may explain variation in findings as well. Factors such as provider age, gender, training, and years of experience have been shown to influence patterns of care,⁴¹⁻⁴³ although a recent study suggested that these characteristics only minimally influence provision of low value care services.⁴⁴ Little is known about the impact of patient differences on receipt of low value services, but patient demand and expectations have been shown to influence vitamin D test ordering patterns.^{45,46}

We observed higher average vitamin D test results (41.1 +/- 9.3 ng/mL) than other health systems. Average test results in the range of 23 to 29 ng/mL were reported among outpatients in New York and Iowa health systems, and a Southeastern Veteran's Association hospital.^{47,48} We also observed substantially fewer severely *deficient* (<1%) vitamin D test results compared with other U.S. health systems who have reported up to 30%.^{39,48,49} As there are numerous factors that influence vitamin D status, these differences could be based on variability in geography, age, race, adiposity, medication use, socioeconomic status, overall health status, or other factors. Our cohort did include almost exclusively white patients (who typically have higher vitamin D than individuals with darker skin), and patients with lower BMI compared with other cited studies (higher BMI is associated with lower vitamin D).^{24,29,30,32} However, we did not have BMI data for all patients. Laboratory and assay variability and differences in *normal* ranges for vitamin D among health systems, and even within our own (our laboratory services provider changed in

2014) may have also contributed to inconsistent observations. However, the very low occurrence of *high* vitamin D test results in our study is similar to that observed by others.^{29,30,32}

A few differences in characteristics of patients who had non-indicated initial vitamin D tests compared with those who had indicated vitamin D tests were identified. The difference in age (non-indicated test patients were younger and less likely to be insured by Medicare) may be due to the exclusion of patients taking vitamin D supplements. More than 50% of Americans over 60 years of age take vitamin D supplements.⁵⁰ The greater proportion of self-pay and commercially insured patients in the non-indicated test cohort is likely a factor of the younger cohort that is less likely to be insured by Medicare.

Health Services Cascade

Abnormal test results have been described as triggering a cascade process by which a number of referrals and additional investigations arise.⁵¹ The high volume of vitamin D-relevant services (more than 4000 services for 574 patients) that followed a non-indicated vitamin D test suggests the occurrence of a cascade process within our health system. We acknowledge that service chronology does not necessarily reflect downstream effects. However, the high volume of vitamin D-relevant services is suggestive of a causal association. Another consideration is that, during 2015, our health system had a limited endocrinology service and did not have a nephrology service, so referrals for these services were made outside of our health system. Thus, we are likely missing vitamin D-relevant health services that were not documented in our EHR.

Some of the services provided subsequent to a non-indicated vitamin D test can themselves be described as low value care. For example, the majority of 1,25(OH)₂D tests were conducted in patients who did not fulfill the Choosing Wisely indications for this test

(hypercalcemia and/or abnormal PTH).⁵ Additionally, up to five follow-up vitamin D tests were conducted for some patients. Some guidelines discourage more than two tests per year, even with an abnormal result.^{11,52} In fact, there are examples of insurers who have limited payment for vitamin D tests to ≤ 2 per year.^{35,53}

Based on the average cost of a vitamin D test in the state of Virginia (\$145),²⁸ the cost of all vitamin D testing within primary care of this health system in 2015 is estimated at \$1,166,090, with \$83,230 spent on non-indicated vitamin D testing. However, costs of downstream services and resources associated with these can result in a much greater financial burden. In addition, consequences such as increased false positive test results, enhanced health risk related to follow-up services, emotional impact to patients, increased resources required to administer services, and decreased opportunity for higher value care have been observed following low value health services.^{54,55}

Patterns of Care

A great deal of inconsistency in clinical care following non-indicated vitamin D testing was observed in the present study. For example, one-fifth of patients with low vitamin D test results were not assigned a diagnosis of vitamin D deficiency or insufficiency. Alternately, a vitamin D deficiency diagnosis was subsequently assigned to some patients with *normal* vitamin D test results. Additionally, although first follow-up vitamin D testing was more likely in patients with *low* initial vitamin D test results, follow-up vitamin D testing was also conducted on many patients with *normal* initial vitamin D test results and further follow-up testing vitamin D testing (a second, third, fourth, or fifth test) was not predicted by vitamin D test result (Figure 4.3). The time between initial and follow-up testing varied greatly. Inconsistency in vitamin D

prescriptions existed as well, as 26 different prescription regimens were utilized (Table 4.8). Furthermore, dosing of vitamin D prescriptions was not associated with vitamin D status. Inconsistency in vitamin D-relevant care has been observed by others,^{54,56,57} and is reflective of evolving research and conflicting guidelines related to clinical management of vitamin D status.

We identified some potential opportunities to improve quality of care related to vitamin D. For example, nearly half of *low* vitamin D test results were not followed up by repeat vitamin D testing. Monitoring 25(OH)D following *low* test results and at least 12 weeks of treatment is a common recommendation.^{10,11,52} Additionally, two-thirds of vitamin D prescriptions were for vitamin D₂ rather than vitamin D₃. Vitamin D₃ has greater bioavailability than vitamin D₂, and is more effective in raising 25(OH)D.^{58,59} Additionally, there is evidence that vitamin D₂ supplements have a shorter shelf-life than vitamin D₃ supplements.⁶⁰ However, these findings are fairly recent, and are not reflected in major vitamin D guidelines (Institute of Medicine and Endocrine Society) released in 2011, which describe both forms as suitable for raising vitamin D.^{10,61} Lesser availability of high-dose vitamin D₃ supplements may also impact form of vitamin D prescribed and dispensed.⁶⁰ Possible interventions to improve care and reduce inconsistency related to vitamin D services include provider education and feedback, development of clinical pathways related to the identification and treatment of vitamin D-related abnormalities, or modifications to EHR default settings and prompts.

A clinical pathway or health system guideline related to *vitamin D insufficient* test results may also be warranted. The definition of vitamin D insufficiency is highly controversial, and not even recognized by all practice guidelines. For instance, the Endocrine Society recognizes 25(OH)D of 20 to 29.9 ng/mL as *vitamin D insufficient*, but the National Academy of Medicine (formerly known as the Institute of Medicine) does not recognize a *vitamin D insufficient*

category, stating that 25(OH)D of 20 ng/mL meets the needs of 97.5% of the population. Although a similar proportion of *vitamin D deficient* test results existed in the indicated and non-indicated test cohorts, significantly more *vitamin D insufficient* results existed in the non-indicated group (30% vs. 17.5%). This may be due to our exclusion of patients prescribed vitamin D supplements in the non-indicated group, or the younger age of the non-indicated group. However, it may also reflect a pattern of “shotgun testing” for complaints of fatigue or vague musculoskeletal symptoms. Patients with *vitamin D insufficient* initial test results were significantly less likely to receive vitamin D prescriptions compared with patients with *vitamin D deficient* results. In fact, patients with *vitamin D insufficient* and *normal* test results were prescribed vitamin D supplements at the same (low) rate. Only one-third of patients with *vitamin D insufficient* test results showed improved vitamin D test results within the 24-month observation period. It is possible that over-the-counter vitamin D supplements were recommended for patients with *vitamin D insufficient* test results. If so, compliance was poor or they were ineffective in raising 25(OH)D. However, it should be noted that these results are difficult to interpret since more than half of *vitamin D insufficient* test results were not followed-up with subsequent vitamin D tests.

A strength of this study is the use of EHR data to explore downstream impacts of a common non-indicated laboratory test within a large health system. A few limitations to our study exist as well. Although EHR data offers good insight into patient care, we were unable to discern details of specific cases that may have contributed to provider decisions. Also, the number of non-indicated vitamin D tests was potentially underestimated. We classified vitamin D tests associated with a diagnosis of *vitamin D deficiency* as indicated, assuming a follow-up or repeat test. However, there is evidence that providers may assign a diagnosis of *vitamin D*

deficiency (or other diagnosis) in order to gain insurance coverage for the test.^{45,62,63} Next, since calcium and alkaline phosphatase laboratory tests are included as part of common laboratory test panels (basic metabolic panel and comprehensive metabolic panel, respectively), we may have captured tests that were conducted unrelated to vitamin D status or concern. Finally, in evaluating vitamin D treatment, we were unable to account for over-the-counter vitamin D supplements, diet, and sun exposure, which all affect vitamin D status. Future research using records from individual patients is a next step in evaluating care patterns related to non-indicated vitamin D testing, and for learning more about cascade processes, benefits, and harms that may result from such testing. In addition, investigations aimed at identifying provider and patient characteristics and decision-making related to non-indicated laboratory testing would be valuable in informing interventions to reduce low value care.

Conclusion

Less than 1% of initial vitamin D tests conducted in 2015 within primary care of our Southwest Virginia-based health system were considered non-indicated, which may be a reflection of provider awareness of recent guidelines, or a limitation of our exclusion criteria. However, patterns of care were highly inconsistent, and downstream vitamin D-relevant laboratory, prescription, and imaging services seem to have been triggered by these tests in 65% of patients. These findings suggest that low value health services not only increase costs, but may also initiate a cascade of unnecessary services and consequences. We identified some opportunities to improve patient care related to vitamin D within our health system.

Tables and Figures
(shown in the order they appear in the text)

Table 4.1. Vitamin D Status Classification used for this Study

Category	Vitamin D (ng/mL)
LOW	0 to 29.9
<i>Vitamin D Deficient</i>	0 to 19.9
<i>Vitamin D Insufficient</i>	20 to 29.9
NORMAL	30 to 99.9
HIGH	>100

Table 4.2. Indicators for Vitamin D Testing

	Diagnosis
1	Vitamin D Deficiency or Insufficiency, Osteomalacia, Rickets
2	Osteoporosis, Osteopenia
3	Pathological fracture or stress fracture
4	Malabsorption or Inflammatory Bowel Disease
5	Parathyroid, Calcium, or Phosphate Disorder
6	Chronic Kidney Disease

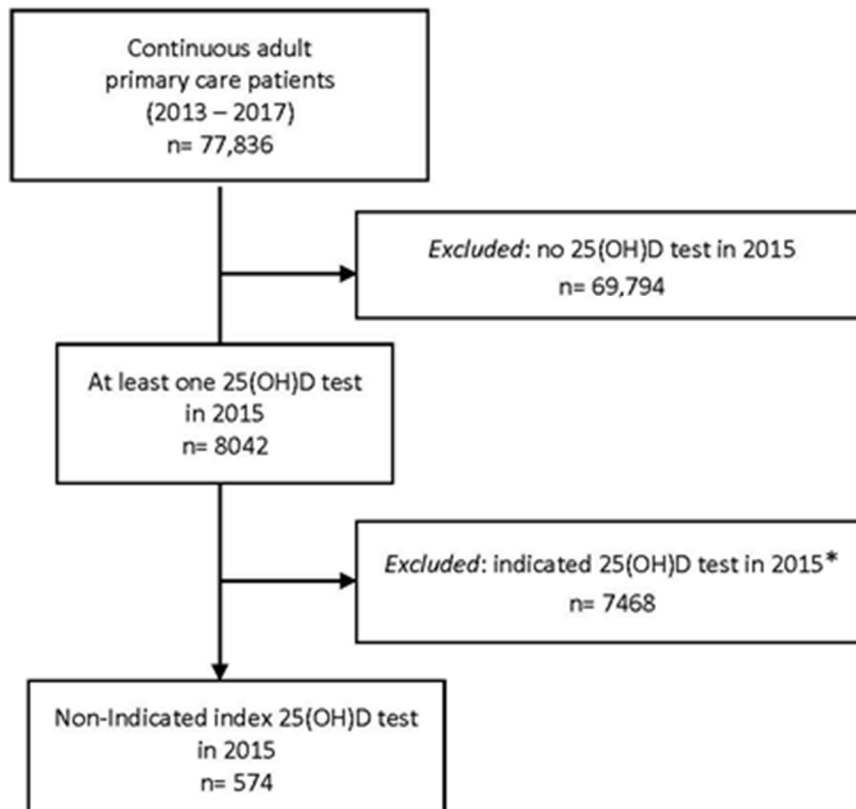
Based on Choosing Wisely criteria for indicated vitamin D testing

Appendix C shows International Classification of Diseases (ICD-10) codes associated with indicated vitamin D testing. Patients with a diagnosis of any of these ICD-10 codes were excluded from the subset of patients identified as having non-indicated vitamin D testing in 2015.

Table 4.3. Vitamin D-Relevant Service Data Extracted for 24 Months Following a Non-Indicated Vitamin D Test

Laboratory Tests	Prescriptions	Imaging	Diagnoses
-25(OH)D -1,25(OH) ₂ D -Calcium -Phosphorus -Alkaline Phosphatase	-Vitamin D Preparations (>400 IU) -Osteoporosis medications -Calcimimetics medications	- dual energy x-ray absorptiometry (DXA) and other bone mineral density imaging	-Categories 1-6 from Table 1

Figure 4.1. Diagram of Patient Selection



*Criteria for indicated vitamin D testing are described in Table 1.

Figure 4.2. 2015 Month in which Vitamin D Tests were Conducted

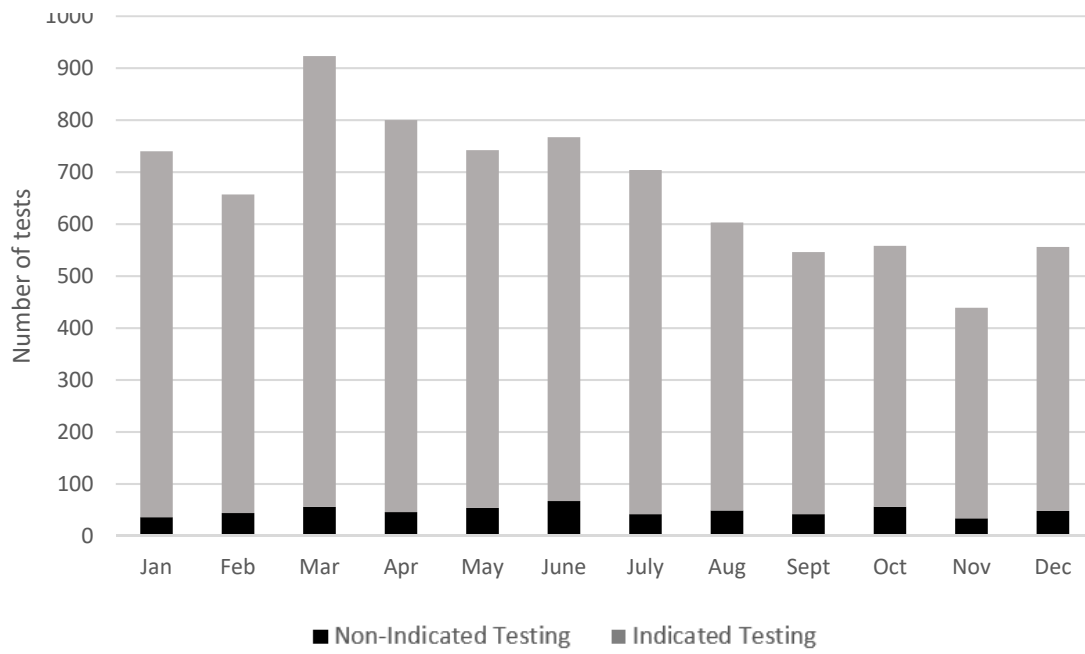


Table 4.4. Demographic and Clinical Characteristics of Patients who had Vitamin D Testing

	Indicated Vitamin D Testing n= 7468	Non-Indicated Vitamin D Testing n= 574
	n (%)	n (%)
AGE (years)		
Means/Range	65.7 +/- 13.5 (range: 19.8 to 101.3)	56.9 +/- 11.1 *** (range: 19.9 to 95.3)
18-29.9	55 (0.7%)	20 (3.5%)
30-39.9	244 (3.3%)	54 (9.4%)
40-49.9	658 (8.8%)	104 (18.1%)
50-59.9	1388 (18.6%)	165 (28.7%)
60-69.9	2154 (28.8%)	126 (22.0%)
70-79.9	1884 (25.2%)	68 (11.8%)
80-89.9	949 (12.7%)	31 (5.4%)
90-99.9	135 (1.8%)	6 (1.0%)
>100	1 (0.1%)	0 (0.0%)
SEX		
Male	1779 (23.8%)	227 (39.5%)
Female	5689 (76.2%)	347 (60.5%)
RACE/ETHNICITY		
White	6792 (90.9%)	526 (91.6%)
Black	522 (7.0%)	35 (6.1%)
Asian	53 (0.7%)	3 (0.5%)
Hispanic	22 (0.3%)	2 (0.3%)
Other	79 (1.1 %)	8 (1.4%)
BMI (kg/m²)		
Means/Range	30.1 +/- 7.5 @ (range: 9.9 to 74.4)	30.7 +/- 9.0 @@ (range: 17.2 to 56.8)
<18.5	73 (1.9%)	1 (0.3%)
18.5 to 24.9	899 (23.0%)	70 (22.4%)
25.0 to 29.9	1252 (32.1%)	99 (31.7%)
30.0 to 34.9	873 (22.4%)	71 (22.8%)
35.0 to 39.9	418 (10.7%)	44 (14.8)
>40.0	388 (9.9%)	27 (8.7)
Insurer		
Medicare	3564 (47.7%)	98 (17.1%)
Medicaid	108 (1.4%)	12 (2.1%)
Commercial	2092 (28.0%)	240 (41.8%)
Self-Pay	1675 (22.4%)	220 (38.3%)
Other	29(0.38%)	4(0.70%)

***p<0.001

@n = 3904, @@n = 312

Table 4.5. Results of Initial Vitamin D Tests in Primary Care Patients in 2015

	Indicated tests n=7468	Non-indicated tests n=574
	<i>n (% of indicated tests)</i>	<i>n (% of non-indicated tests)</i>
<i>Vitamin D Deficient</i> <i>(0 to 19.9 ng/mL)</i>	469 (6.3%)	49 (8.6%)
<i>Vitamin D Insufficient</i> <i>(20 to 29.9 ng/mL)</i>	1302 (17.5%)	171 (30.0%) **
<i>Normal Vitamin D</i> <i>(30 to 99.9 ng/mL)</i>	5658 (75.7%)	354 (61.6%) **
<i>High Vitamin D</i> <i>(>100 ng/mL)</i>	39 (0.5%)	2 (0.5%)

**p<0.01

Table 4.6. Vitamin D-Relevant Laboratory, Prescription, and Imaging Services Provided for 24 Months Following Non-Indicated Vitamin D Testing in 2015

	Total Laboratory Tests	Total Abnormal Laboratory Tests	Vitamin D Follow-Up Tests	Total Abnormal Vitamin D Follow-Up Tests	Total Prescriptions	Vitamin D Prescriptions	Imaging Services	Total Number of Services (Total Laboratory Tests + Total Prescriptions + Imaging)
	<i>n</i> (average tests per patient)	<i>n</i> (% of total laboratory tests)	<i>n</i> (average tests per patient)	<i>n</i> (% of vitamin D follow-up tests)	<i>n</i> (average prescriptions per patient)	<i>n</i> (average prescriptions per patient)	<i>n</i> (average services per patient)	<i>n</i> (average total services per patient)
<i>Vitamin D Deficient</i> 25(OH)D 0 to 19.9 ng/mL n=49	505 (10.3)	116 (23.0%)	125 (0.7)	50 (64.1%)	199 (4.1)	195 (3.9)	1 (0.02)	705 (14.4)
<i>Vitamin D Insufficient</i> 25(OH)D 20 to 29.9 ng/mL n=171	1147 (6.7) ^	208 (18.1%) ^	78 (1.6) ^	60 (48.0%) ^	58 (0.3) ^	35 (0.2) ^	11 (.07)	1216 (7.1) ^
<i>Normal Vitamin D</i> 25(OH)D 30 to 100 ng/mL n=354	2410 (6.8) ^,^^	316 (13.1%) ^,^^	118 (0.3) ^^	43 (36.4%) ^	87 (0.2) ^	45 (0.1) ^	19 (.05) ^	2516 (7.1) ^
Total	4062	640	321	153	344	275	31	4437

^= significantly different from vitamin D *deficient* (p<0.05)

^^= significantly different from vitamin D *insufficient* (p<0.05)

Figure 4.3. Vitamin D Follow-up Tests Relative to Vitamin D Test Results During the 24 Months Following a Non-Indicated Vitamin D Test in 2015

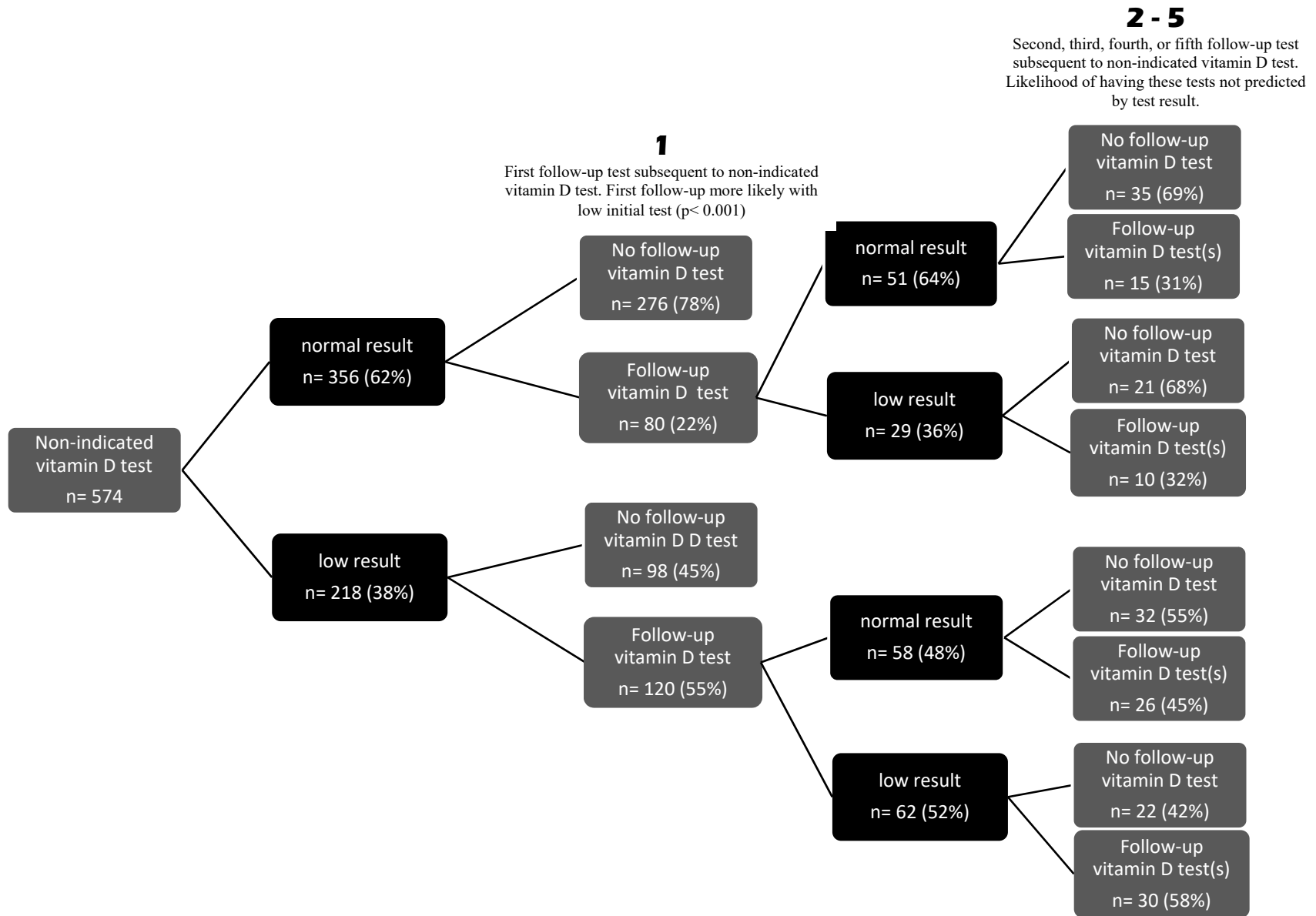


Table 4.7. Changes in 25(OH)D during 24 Months Following Non-Indicated Vitamin D Testing

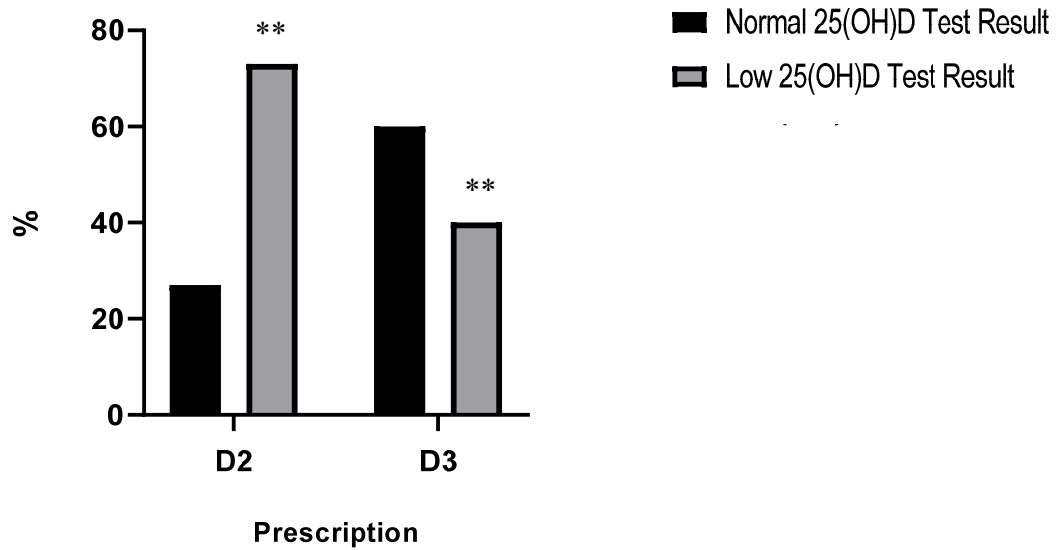
	Change in 25(OH)D during 24-month follow-up	Normal 25(OH)D achieved or maintained during 24-month follow-up	Increased 25(OH)D during 24-month observation	Decreased 25(OH)D during 24-month observation	No 25(OH)D follow-up available
	%	n (% of tests)	n (% of tests)	n (% of tests)	n (% of tests)
<i>Vitamin D Deficient</i> n=49	210 Range: -33 to 946	24 (49.0)	41 (83.6)	5 (10.2)	3 (6.1)
<i>Vitamin D Insufficient</i> n=171	47 Range: -44 to 181	47 (27.5)	57 (33.3)	19 (8.8)	95 (55.6)
<i>Normal Vitamin D</i> n=354	-9 Range: -52 to 140	54 (15.3)	21 (7.0)	57 (16.1)	276 (78.0)

These values are based on the highest 25(OH)D result during the 24-month observation.

Table 4.8. Vitamin D Prescriptions Provided to Patients who had Non-Indicated Index Vitamin D Tests in 2015

	Dose	Frequency	Duration	n	
Ergocalciferol/ vitamin D₂ (n= 73)					
	50,000 IU	weekly	4 weeks	2	High-Dose Vitamin D₂
	50,000 IU	weekly	8 weeks	35	
	50,000 IU	weekly	12 weeks	20	
	50,000 IU	weekly	16 weeks	9	
	50,000 IU	weekly	20 weeks	4	
	50,000 IU	weekly	24 weeks	2	
	50,000 IU	weekly	48 weeks	1	
Cholecalciferol/ vitamin D₃ (n= 39)					
	50,000 IU	daily	2 months	1	High-Dose Vitamin D₃
	50,000 IU	weekly	12 weeks	1	
	10,000 IU	daily	4 months	1	
	5000 IU	daily	3 months	5	
	5000 IU	daily	2 months	1	
	4000 IU	daily	3 months	1	
	2000 IU	daily	10 months	1	
	2000 IU	daily	6 months	3	Moderate-Dose Vitamin D₃
	2000 IU	daily	4 months	3	
	2000 IU	daily	3 months	4	
	2000 IU	daily	2 months	3	
	2000 IU	daily	1 month	1	
	1000 IU	daily	12 months	1	
	1000 IU	daily	8 months	1	
	1000 IU	daily	6 months	4	Low-Dose Vitamin D₃
	1000 IU	daily	4 months	2	
	1000 IU	daily	3 months	1	
	400 IU	daily	6 months	1	
	400 IU	daily	3 months	2	
	600 mg Calcium + 400 IU	daily	3 months	2	

Figure 4.4. Proportion of Low and Normal Vitamin D Test Results Following Vitamin D₂ and Vitamin D₃ Prescriptions



*p<0.05, **p<0.01

These values are based on the highest 25(OH)D result during the 24-month observation.

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CHAPTER 5

MANUSCRIPT

Vitamin D Practice Patterns within NCAA Division I Collegiate Athletics Programs

ABSTRACT

Background: Vitamin D status has been theorized to be related to performance, health, and well-being in athletic populations. Measurement of vitamin D status via 25-hydroxyvitamin D [25(OH)D] testing has increased in the general population, as has vitamin D supplement use. It is unclear if similar patterns exist within collegiate athletics programs. The purpose of this study was to describe clinical care related to the prevention, evaluation, and treatment of vitamin D deficiency and insufficiency provided by sports medicine providers with National Collegiate Athletic Association (NCAA) Division I programs.

Methods: We administered a survey related to vitamin D practice patterns to all NCAA Division I Head Athletic Trainers in 2018.

Results: Responses were received from 249 Head Athletic Trainers (72% response rate). Vitamin D testing was reported by 68% of participants, with the most common indicators being health status/history (78%) and injury status/history (74%). One-fifth of participants reported that vitamin D testing is completed as screening (without specific cause or indication). Target 25(OH)D concentration was highly variable, ranging from 20 to 30 ng/mL to >50 ng/mL. A range of 8 to 1660 annual vitamin D tests at a cost of <\$50 (8%), \$51-100 (51%), \$101-150 (20%), and >\$150 (10%) per test were reported. Forty-two percent of programs cover the cost of vitamin D supplements. More than half of participants stated that vitamin D testing and supplements were not a good use of program funds. Football Bowl Subdivision programs were significantly more likely to conduct vitamin D testing, pay for vitamin D supplements, and state that testing and supplements are a good use of program funds than Football Championship Subdivision programs.

Conclusions: A great deal of variability in vitamin D-related clinical practice (particularly in testing indicators, target 25(OH)D concentration, test cost, and likelihood of paying for vitamin D supplements) was reported among NCAA Division I athletics programs, reflecting existing contradiction and uncertainty in evidence, recommendations, and guidelines. Knowledge of current practice patterns is important in evaluating and establishing best practices, policies, and procedures for sports medicine and sports nutrition professionals in the collegiate setting.

Keywords: 25-hydroxyvitamin D, 25(OH)D, testing, athlete, sports medicine

5.1 INTRODUCTION

Vitamin D is a hormone primarily obtained by way of cutaneous synthesis following exposure to sunlight and other sources of ultraviolet B (UVB) light. Some foods and dietary supplements also provide vitamin D. Vitamin D deficiency and insufficiency, or low vitamin D (LVD), are prevalent among athletes.¹ For example, 20 to 85% of National Collegiate Athletic Association (NCAA) collegiate athletes has LVD.²⁻⁴

Symptoms of LVD may include bone and muscle pain and weakness and fatigue.⁵ There is also evidence that LVD impacts athletes' performance, health, and well-being. Observational studies suggest that muscle function, exercise recovery and adaptation, and occurrence of stress fracture, soft tissue injury, and illness are higher in athletes with LVD.^{3,4,6-13} However, investigations to evaluate the effects of vitamin D supplementation on these measures are limited in number, and complicated by contradictory results.^{6,14-23}

Presently, there is minimal consensus regarding the prevention, evaluation, and treatment of LVD for the general population²⁴ or athletes.²⁵ One example of this inconsistency is the very definition of LVD. The Endocrine Society defines vitamin D deficiency as having serum 25-hydroxyvitamin D (25(OH)D) <20 ng/mL, and insufficiency as 25(OH)D 20-30 ng/mL.²⁶ In contrast, the National Academy of Medicine, formerly known as the Institute of Medicine, describes 25(OH)D of 12-20 ng/mL as meeting the needs of 97.5% of adults in the U.S.²⁷ Recommendations for optimal 25(OH)D for athletes vary broadly, but 25(OH)D targets as high as 40 to 100 ng/mL exist.²⁸

In light of the high prevalence of LVD, and potential health and performance consequences of LVD, vitamin D has become a popular topic among athletic populations. Anecdotal evidence of substantial increases in vitamin D [25(OH)D] screening (testing without specific cause or indication) and testing in the collegiate athletics setting exists, mirroring

increases observed in the general population.²⁴ Use of vitamin D supplements for prevention and treatment of low vitamin D has increased in the collegiate setting as well.²⁹ Knowledge of existing clinical practice patterns in the collegiate setting is important in evaluating and establishing programmatic best practices, policies, and procedures for sports medicine providers. The purpose of this study was to describe clinical care provided by NCAA Division I sports medicine providers related to the evaluation, prevention, and treatment of LVD.

5.2 METHODS

Participants and Study Design

In Spring 2018, Head Athletic Trainers from the 347 athletics programs that sponsor NCAA Division I intercollegiate sports were invited via email to participate in an online survey about vitamin D-related clinical care within the athletics programs by which they are employed. Head Athletic Trainers were selected since they are well-situated to respond to questions about health and welfare of student-athletes. In the event that a program did not have an individual with the title of “Head Athletic Trainer”, the Director of Sports Medicine, Athletic Director for Sports Medicine, or Director of Sports Health was invited to participate. When more than one individual had the same title, a group email invitation was sent to each person possessing the title, with a request for only one response per program. Invited participants will be referred to as Head Athletic Trainers throughout this paper. In all, email invitations were sent to 361 Head Athletic Trainers. This study was reviewed by the Institutional Review Board of Virginia Tech and considered exempt (IRB# 17-1239).

Survey Development

The survey contained 20 questions (six of these were two-part questions) in the following domain areas:

1. Protocol for the evaluation, prevention, and treatment of LVD
2. Vitamin D [serum 25(OH)D] tests: procedures
3. Vitamin D [serum 25(OH)D] tests: indicators
4. Vitamin D [serum 25(OH)D]: results
5. Vitamin D [serum 25(OH)D]: costs
6. Vitamin D supplements
7. Characteristics of the athletics program
8. Demographic characteristics of the participant

A survey-development team of one athletic trainer and two registered dietitians with >12 years of experience in NCAA Division 1 athletics programs developed the survey that contained multiple choice and open-ended questions. Questions were formatted either based on those used in previous studies, or designed originally by the survey-development team. Table 5.1 provides more details about survey questions. A complete copy of the survey may be obtained by contacting the corresponding author (M.R.).

Questions were transferred into Qualtrics online survey tool (Qualtrics XM, Provo, Utah). A group of 27 athletic trainers working in NCAA Division 1 athletics programs pilot-tested the survey in online format. Modifications to survey questions were made based on pilot study feedback. For example, 0/27 pilot participants were able to provide details about the vitamin D supplements (dose, form, frequency and duration of treatment) prescribed to athletes within their

programs. Thus, we asked more general questions about vitamin D supplements, and allowed free response space for provision of more details. Pilot participants also provided feedback that the survey was too long and that some questions felt intrusive. Ten pilot participants stated that they would be unlikely to respond to the survey as written since they felt their institution was identifiable through data provided. Thus, questions and details not considered essential for the primary objective were eliminated or modified. The survey-development team tested and provided feedback on the revised survey.

A brief statement of study purpose was sent to participants along with a link to the online survey. Participants were given six weeks to respond to the survey. Reminder emails were sent to Head Athletic Trainers who had not responded by weeks four and five. Survey responses were considered “complete” if at least 50% of questions were answered.

Data Analysis

Data were downloaded from Qualtrics into a single spreadsheet, and imported into GraphPad PRISM 8 (GraphPad, San Francisco, CA) for statistical analysis. Descriptive statistics (frequency, %, and means +/- standard deviations, if applicable) were conducted on survey responses. One-way analysis of variance (ANOVA) was used to assess differences in responses based on program and participant characteristics. Chi-square tests were used for responses containing categorical responses. Significance was set at $p < 0.05$.

5.3 RESULTS

Survey responses were received from 249 Head Athletic Trainers, which reflects a 72% response rate. Participants were 65% male, 82% >45 years of age, and 77% had >16 years of

experience as an athletic trainer. Characteristics of athletics programs represented are shown in Table 5.2. Two responses were omitted from analyses since <25% of questions were complete.

Vitamin D Protocol or Policy

Fifty participants (20%) indicated that their athletics program has formal protocol or policy related to the evaluation, prevention and/or treatment of low vitamin D, while 119 (80%) participants indicated they do not or that they were unsure. No (0/0%) participants provided details about their vitamin D protocol or policy in the space provided.

Vitamin D Testing

In response to the question about whether or not vitamin D [25(OH)D] tests are conducted on any student-athletes within their athletics program, 169 (68%) participants responded “yes”, 70 (29%) “no”, and 8 (3%) “unsure”. About one quarter of participants (59/24%) indicated that student-athletes from “all sports” undergo vitamin D testing and 37 (15%) indicated that student-athletes from “all female sports” do. Other sports mentioned specifically by more than 10% of participants include: Men’s Basketball, Women’s Basketball, Women’s Cross Country, Women’s Gymnastics, Football, Men’s Track & Field, and Women’s Track & Field. Team physicians (163/66%) are most likely to order vitamin D tests, followed by Athletic Trainers (47/19%), Registered Dietitians or Nutritionists (25/10%), and Student Health personnel (12/5%).

The majority of participants responded that general health status or health history (193/78%), or injury status or injury history (183/74%) are indicators for vitamin D tests. Vitamin D screening (i.e., testing student-athletes without specific cause or indication) was

reported by 49 (20%) respondents, while 22 (9%) responded that vitamin D tests are conducted based on previous vitamin D test results. In a separate question, 85 (35%) indicated that student-athletes who have a diagnosis of LVD are likely to be followed up or re-tested in the future, while 146 (59%) indicated that no follow up or repeat vitamin D tests were likely to occur in student-athletes who have a LVD diagnosis (6% were unsure).

Table 5.3 shows target or goal 25(OH)D reported by participants. When asked their opinion about the appropriateness of their athletics program's target 25(OH)D concentration, 74 (30%) stated their program's target was appropriate, 57 (23%) expressed it should be lower and 22 (9%) higher, while 94 (38%) were unsure.

Vitamin D Supplements

Figure 5.1 shows responses related to whether or not the athletics program is responsible for the cost of vitamin D supplements. There was not a correlation between whether or not an athletics program covers vitamin D supplement costs and whether or not vitamin D supplements were rated as a good use of athletics program funds. In response to the final survey question, which was "If there is any additional information you would like to share about clinical practice related to vitamin D within your athletics program", 43 (17%) of participants described blanket or routine vitamin D supplementation patterns within their athletics program. Examples of statements provided include:

- "We used to get a vitamin D test on every athlete at physicals, but it seems like they were all coming back low, so now we just give out vitamin D supplements after practice."

- “For all of our teams that are primarily indoors for training, we provide vitamin D [supplements] prophylactically.”

Costs Related to Vitamin D Testing and Vitamin D Supplements

Participants who reported vitamin D testing within their athletics program indicated that an average of 58.6 tests per year (range: 8 to 1660) were ordered. Costs to athletics programs per vitamin D test are shown in Figure 5.2. An estimated total cost of vitamin D tests was calculated by multiplying number of annual tests X the mid-range of the costs summarized in Figure 5.2. According to this calculation, an average of \$7,250 was spent on 25(OH)D testing (range: \$600 to \$160,000). There was no correlation between the cost of vitamin D tests and the number of tests ordered. One hundred forty-three (58%) of participants stated that vitamin D testing and vitamin D supplements are not a good use of athletics program funds, while 77 (31%) responded that they are a good use of funds, 22 (9%) that they are sometimes a good use of funds, and 5 (2%) were unsure. There was no correlation between participants' perception of the use of athletics programs' funds and the likelihood of the program conducting vitamin D testing or funding vitamin D supplements.

Athletics programs that employ a Registered Dietitian/Nutritionist were significantly more likely to have a vitamin D protocol in place ($p < 0.05$). The 115 respondents who reported that their athletics program was part of the Football Bowl Subdivision were significantly more likely to conduct vitamin D testing, pay for vitamin D supplements, and state that vitamin D testing and supplements are a good use of athletics program funds compared with Football Championship Subdivision programs ($p < 0.05$). No other differences between responses based on characteristics of athletics programs or Head Athletic Trainers were identified.

5.4 DISCUSSION

The purpose of this study was to describe clinical care related to the evaluation, prevention, and treatment of LVD among NCAA Division I collegiate athletics programs. The strong response rate from experienced athletic trainers from diverse athletics programs leads us to believe that responses are representative of NCAA Division I athletics programs throughout the U.S. Our results showed that over two-thirds of NCAA Division I athletics programs regularly evaluate vitamin D status via 25(OH)D testing. More than half of programs sometimes or always pay for vitamin D supplements for student-athletes. To our knowledge, this is the first study to describe vitamin D-related clinical care within the collegiate athletics setting.

There are three primary reasons for ordering a blood test for a nutritional metabolite such as 25(OH)D: evaluation for deficiency, evaluation for toxicity, or monitoring treatment.³⁰ Screening for the evaluation of vitamin D deficiency and toxicity is typically not recommended in the general, healthy population due to insufficient evidence of benefits and harms.^{26,27,31} Some clinical practice guidelines do recommend vitamin D testing to monitor response to treatment eight to 12 weeks after initiating treatment.^{26,32} Different standards and specifications for biomarker testing in athletes are common, considering the desire to detect even minor physiological issues or changes that may impact performance, recovery, and risk of injury or illness.³³

In the absence of consistent athlete-specific vitamin D testing guidelines, observing practices that have evolved within the athletic community can help evaluate current practices, and move toward deliberate, evidence-informed care. One-fifth of NCAA Division I collegiate athletics programs reported vitamin D screening for all athletes, without particular cause. Future research or internal analyses should examine the utility and value of this testing. We observed that less than half of participants reported follow-up testing in athletes who are diagnosed with

LVD based on vitamin D testing. In order to evaluate athletes' response to treatment, effectiveness of treatment form/dose, and limit risk of vitamin D toxicity, monitoring 25(OH)D throughout treatment is recommended, particularly after an athlete has had a diagnosis of LVD.²⁸

Consequences of laboratory test over-utilization have been well-documented within the general population.³⁴ Specifically, non-indicated laboratory testing has been associated with minimal patient benefit, and increased harms.³⁵ As described, indicators for and expectations related to laboratory testing among some athletic populations differ from that of the general population. The American College of Sports Medicine and Academy of Nutrition and Dietetics Joint Position Statement on Nutrition and Performance advises that “athletes with a history of stress fracture, bone or joint injury, signs of overtraining, muscle pain or weakness, and a lifestyle involving low exposure to UVB” may require 25(OH)D assessment.³⁶ Athletics programs may benefit from the development of strategic vitamin D testing and follow-up testing guidelines and protocols, particularly when budget resources are limited.

Few participants (20%) reported that their athletics program has a vitamin D-specific protocol in effect. A complicating factor in establishing vitamin D policies and procedures is disagreement over many aspects related to evaluating, preventing, and treating LVD. Conflicting recommendations for target 25(OH)D exist,²⁴ as evidenced by the high variability in target 25(OH)D concentrations reported among athletics programs in this study (Figure 5.1). It should be noted that many factors are known to influence 25(OH)D. For example, non-Caucasian individuals typically have lower 25(OH)D compared with individuals who have darker skin pigmentation.³⁷ While some of this may be explained by decreased endogenous synthesis when exposed to UVB based on skin pigmentation differences, genetic variation is likely to also play a role.³⁸ Lower 25(OH)D may also be observed in athletes with higher body

fat, illness, infection, and recent muscle damage.^{39,40} Finally, variation within and between laboratories and assays that measure 25(OH)D is well-documented, so evaluating and comparing results is difficult.^{41,42} Variable guidelines for goal dietary vitamin D intake and treatment strategies for LVD exist as well.³² Furthermore, dosing strategies vary broadly in terms of form of vitamin D (D₂ vs. D₃), mode of delivery (ex: tablet or capsule, spray, intramuscular injection), frequency of dosing (ex: daily, weekly, monthly), and amount of vitamin D (from 400IU to 150,000IU is available).³² Although there is evidence that vitamin D₃ is preferable to vitamin D₂, and daily or weekly dosing seems to favor larger monthly doses, an optimal dosing strategy has not been described.⁴³⁻⁴⁵ In this study, athletics programs who employed a Registered Dietitian/Nutritionist were more likely to have an established vitamin D protocol. It is possible that designating a specific member of the sports medicine staff to managing such a protocol and navigating the emerging and variable evidence would be valuable.

An alternative to vitamin D testing is blanket or routine supplementation (i.e., providing vitamin D supplements broadly to athletes without individualization or knowledge of 25(OH)D). This method has become more common among athletic populations, as noted by more than 15% of participants in this study. The use of 1000-2000 IU of vitamin D₃ daily has been recommended as a strategy to prevent LVD or maintain 25(OH)D during autumn and winter months when UVB synthesis is low or non-existent.⁴⁶ However, a limitation of this strategy is that individual factors that influence 25(OH)D are not considered, and that choice of supplementation may be too low, or excessive. Vitamin D toxicity is uncommon, but symptoms can include nausea and vomiting, other gastrointestinal symptoms, bone loss, and kidney stones.²⁶

Based on our data, the option to conduct vitamin D testing may be dependent on budget, as evidenced by Football Bowl Subdivision programs having a higher rate of vitamin D testing than Football Championship Subdivision programs. Interestingly, more than half of participants responded that vitamin D testing and vitamin D supplements are not a good use of athletics programs funds. It is impossible to determine what factored into this response, but continued education about the potential consequences of LVD to athletes' health and performance may be beneficial to athletic trainers and other sports medicine providers.

Strengths of this study are an excellent response rate (72%), with nearly 250 head athletic trainers from NCAA Division I collegiate athletics programs offering insight into clinical practice related to vitamin D. Limitations of this study include not gaining information about participants' geographic location nor total number of student-athletes within each athletics program. Future research to learn more about specific vitamin D supplementation protocols used within athletics programs, in addition to outcomes of vitamin D testing is needed. Additionally, clinical trials investigating the impact of vitamin D supplementation on athletic performance, health, and well-being would be beneficial in continuing to establish best practices for sports medicine providers working with collegiate athletes.

5.5 CONCLUSIONS

Overall, vitamin D is an accessible, low-risk, and fairly low-cost intervention with potential to improve performance and health in athletes. Emerging research, controversial guidelines, and paucity of athlete-specific recommendations are reflected in the high variability in vitamin D-related clinical care demonstrated by NCAA Division I athletics programs. With 68% of athletics programs regularly testing 25(OH)D and 42% covering the costs of vitamin D supplements, development of policies and procedures informed by the best evidence available is

important. Knowledge of clinical practice patterns in the collegiate setting is critical for evaluating and establishing best practices, policies, and procedures for sports medicine and sports nutrition professionals.

TABLES AND FIGURES
(shown in order referenced within text)

Table 5.1. Synopsis of Vitamin D Practice Patterns Survey Questions

- Whether or not athletics program has policy/procedures dedicated to vitamin D testing (i.e., serum 25(OH)D testing) and treatment
- Whether or not vitamin D tests (i.e., serum 25(OH)D) are conducted on any student-athletes
- Which student-athletes have vitamin D tests (i.e., serum 25(OH)D)
- Indications for vitamin D tests (i.e., serum 25(OH)D)
- Whether or not follow-up vitamin D tests (i.e., serum 25(OH)D) are conducted if abnormal results are obtained in an initial test
- Target vitamin D (i.e., serum 25(OH)D) concentration
- Individuals responsible for ordering vitamin D tests (i.e., serum 25(OH)D)
- Cost of each vitamin D test (i.e., serum 25(OH)D)
- Whether or not athletics program covers the cost of vitamin D supplements
- Participants' opinions about appropriateness of program's target vitamin D (i.e., serum 25(OH)D) concentration and value of vitamin D tests (i.e., serum 25(OH)D)

Table 5.2. Characteristics of Athletics Programs Represented by Participants

Characteristic	Response Number of responses (% of total responses)
Number of sports sponsored by athletics program	24.8 sports (range: 13 - 28)
Sports considered highest revenue generator by participant	Football: 165 (69%) Men's Basketball: 53 (22%) Also mentioned by 1-3 participants: Baseball, Women's Basketball, Men's Ice Hockey, Men's Lacrosse
Athletics program sponsors Football	Yes: 179 (72%) No: 69 (28%)
Football subdivision (if applicable)	Football Bowl Subdivision (FBS): 115 (64%) ^a Football Championship Subdivision (FCS): 64 (36%)
Athletics program employs a full-time Registered Dietitian/ Nutritionist	Yes: 107 (43%) No: 139 (56%) Unsure: 2 (1%)

^aDivision I Football programs are classified into two subdivisions: FBS (which was called Division I-A until 2006) and the FCS (formerly I-AA).

Table 5.3. Athletic Programs' Target Serum 25(OH)D Concentrations

<20ng/mL	0 (0%)
20-30ng/mL	7 (3%)
30-40ng/mL	15 (6%)
40-50ng/mL	67 (27%)
>50ng/mL	32 (13%)
Unsure	126 (51%)

Figure 5.1. Athletics Program Covers Cost of Vitamin D Supplements

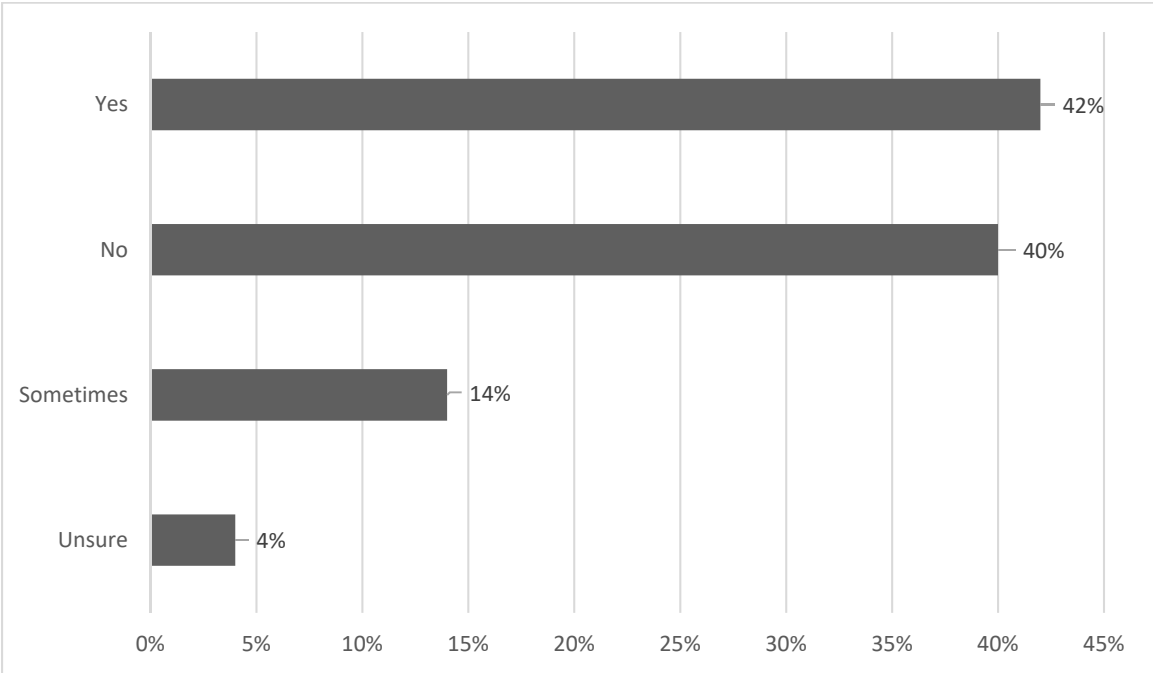
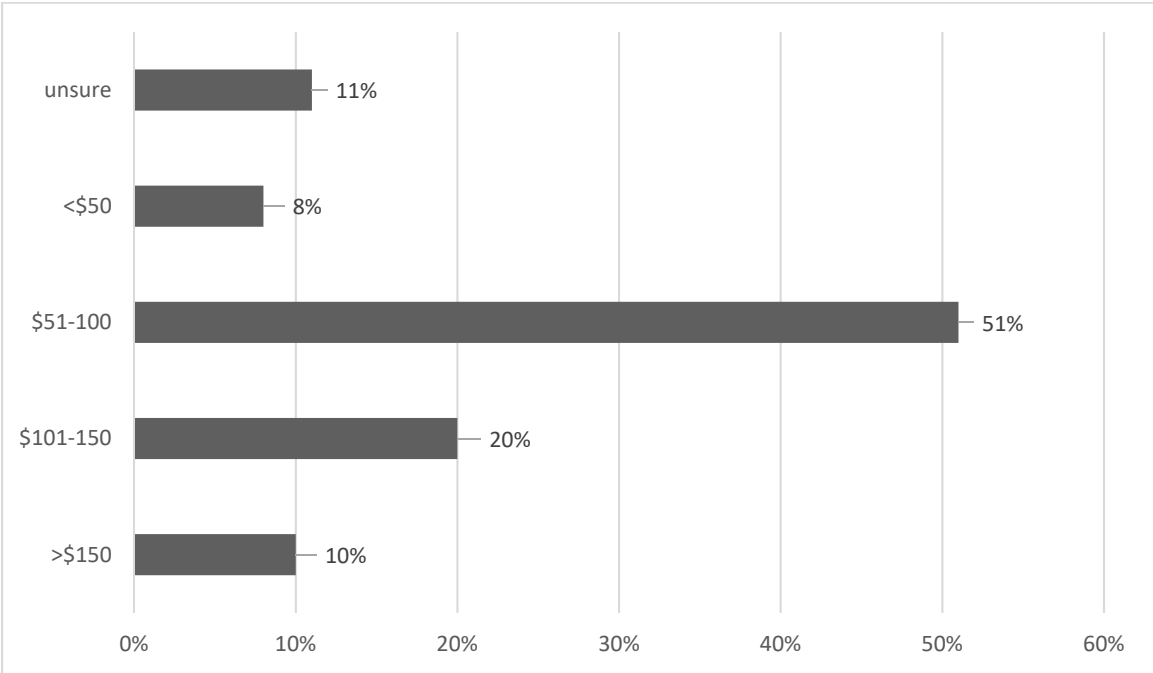


Figure 5.2. Cost Per Vitamin D [25(OH)D] Test Reported by Participants



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CHAPTER 6

MANUSCRIPT

Evaluation of a Protocol to Treat Low Vitamin D in Male and Female Collegiate Basketball Athletes

ABSTRACT

Background: Emerging research has linked vitamin D to muscle function, recovery and adaptation, and immune function, all of great importance to competitive athletes. Since vitamin D deficiency and insufficiency, or low vitamin D (LVD), is common in basketball athletes, effective treatment strategies are needed. The purpose of this study was to evaluate the effectiveness of a specific treatment protocol in improving vitamin D status in basketball athletes with LVD. We also assessed factors such as diet, lifestyle, body composition, and testosterone concentration that may influence or be influenced by vitamin D status.

Methods: Collegiate basketball players (n=24: 10 males and 14 females) at a NCAA Division I university in Virginia, U.S. volunteered to participate in this open clinical trial. A LVD treatment protocol was administered daily for 10 weeks based on springtime serum 25-hydroxyvitamin D (25(OH)D) status (10,000 IU supplemental vitamin D₃ for 25(OH)D <20 ng/mL, 5,000 IU for 25(OH)D 20-39.9 ng/mL, or no vitamin D supplements for 25(OH)D >40 ng/mL). Dual energy x-ray absorptiometry (DXA) scans, and blood sampling were completed before and after treatment.

Results: 79.2% of participants had LVD prior to treatment. The treatment protocol was effective in raising 25(OH)D (23.6 +/- 7.2 to 45.9 +/- 3.0 ng/mL). Only six (25%) participants had LVD post-treatment, three of whom did not receive treatment based on their baseline 25(OH)D. No significant changes in bone mineral density (BMD), percent body fat, fat free mass (FFM), parathyroid hormone (PTH), total testosterone (tT), and free testosterone (fT) were observed. 25(OH)D was positively correlated with fT and negatively correlated with percent body fat (p<0.05).

Conclusions: Ten weeks of daily supplemental 5,000 or 10,000 IU vitamin D₃ is an effective treatment for LVD in collegiate basketball athletes, who consumed low dietary vitamin D and typically spent <5 minutes per day in the sun, independent of season. Adiposity was negatively correlated with 25(OH)D. The observed association between 25(OH)D and fT warrants further study. These results may inform protocols and procedures aimed at the prevention and treatment of LVD in basketball athletes.

Key words: 25-hydroxyvitamin D, cholecalciferol, testosterone, BMD, NCAA

6.1 INTRODUCTION

Vitamin D is an essential nutrient produced endogenously in humans upon exposure to ultraviolet-B (UVB) light. Dietary sources such as fatty fish, egg yolks, or fortified dairy products also contain vitamin D. Deficiency of vitamin D is associated with idiopathic bone and muscle pain, fatigue, and bone disorders such as osteomalacia and rickets.¹ Emerging evidence suggests that consequences of vitamin D deficiency and insufficiency, or low vitamin D (LVD), are more far-reaching than traditionally described.² For example, LVD has been linked to decreased strength and muscle function, greater incidence of stress fracture, impaired immune function, and increased risk of several health problems.³ Research aiming to investigate the role of vitamin D in athletes' performance and health status has yielded conflicting and inconclusive results.⁴⁻⁶ Results of some studies suggest an influence of vitamin D status or vitamin D supplementation on strength and power, recovery, and risk of injury and illness among athletes, but other studies have observed no effect.^{3,4}

Occurrence of LVD is common in athletes.^{7,8} Factors such as skin pigmentation, sun exposure, season, and diet may influence vitamin D status.¹ Basketball athletes are at particular risk for LVD considering they train, practice, and compete primarily indoors. Additionally, many basketball athletes have darker skin pigmentation (61% of male and 48% of female U.S. collegiate basketball athletes, and 68% of female and 74% of male professional basketball athletes in the U.S. are black or African American).⁹⁻¹¹ Approximately 80% of male national basketball association (NBA) athletes had inadequate serum 25-hydroxyvitamin D (25(OH)D) in 2009-2013.^{12,13} High rates of LVD have also been observed in male and female collegiate basketball athletes,¹⁴⁻¹⁶ and adolescent basketball athletes.¹⁷

An effective protocol for the treatment of LVD in basketball athletes is needed. Thus, the purpose of this study was to evaluate the effectiveness of a specific LVD treatment protocol in

improving collegiate basketball athletes' vitamin D status. We also examined factors that have been shown to influence or be influenced by vitamin D status, including diet and UVB exposure, body composition, occurrence of injury and illness, and testosterone concentrations.

6.2 METHODS

Subjects

National Collegiate Athletic Association (NCAA) Division I men's and women's basketball athletes from the same university (Virginia, U.S., 37°N latitude) were invited to participate. A total of 24 participants (10 males and 14 females) volunteered and completed the study in its entirety.

Procedures

Participants underwent two testing sessions: one in May (after basketball season and prior to the start of summer training) and another in August (at the end of summer training). Included in both testing sessions were diet and ultraviolet-B (UVB) questionnaires, a fasting (10 hours) blood draw (6:00 to 7:00 am), and dual-energy x-ray absorptiometry (DXA) scan. A LVD treatment protocol was administered between testing sessions (10 weeks) based on (25(OH)D) concentration in May (Table 6.1).

Vitamin D₃ supplements (gel capsules produced by NatureMade, Pharmavite, Alabama, U.S.) were provided directly to participants daily before training sessions (Table 6.1). Supplements were provided in take-home pouches when participants had a day off from training. Participants were instructed to return any unconsumed supplements the following day. Compliance was recorded daily based on unconsumed supplements.

Outcomes Measures

25-Hydroxyvitamin D, Parathyroid Hormone, and Testosterone

Approximately 20ml of blood was obtained from the antecubital vein in one 10ml serum tube and one 10ml serum separator tube. The serum separator tube was set aside for analysis of 25(OH)D, which was performed by LabCorp (Burlington, North Carolina, U.S.), a Clinical Laboratory Improvement Amendments-certified commercial laboratory. Serum tubes were centrifuged at 4°C for 15 minutes at 3200rpms (Eppendorf 5810R, Hauppauge, New York, U.S.), and stored at -80°C for later analyses.

Following the 10-week treatment period, stored serum samples were thawed and analyzed for intact parathyroid hormone (PTH), total testosterone (tT), and free testosterone (fT). An Immulite immunoassay 1000 analyzer (Siemens, Germany) and commercially available kit (Siemens, Erlangen, Germany) were used to analyze PTH, while commercially available ELISA kits (Alpco, New Hampshire, U.S.) were used for testosterone assays.

Dietary Vitamin D and Ultraviolet-B Exposure

Participants completed a brief food frequency questionnaire that was previously validated to assess daily vitamin D and calcium intake¹⁸. They also completed an ultraviolet-B (UVB) exposure questionnaire based on a tool developed by Halliday and colleagues.¹⁹

Height, Weight, Body Composition, and Bone Mineral Density

Height was measured using a standard wall-mounted stadiometer, and weight was measured using a digital scale (Model 5002, Scale-Tronix, New York, U.S.). Percent body fat and fat free mass (FFM) were measured via DXA (General Electric, Lunar Prodigy Advance,

software version 8.10e (Madison, WI), as was bone mineral density (BMD) at the L1-L4 spine, both hips, and total body regions. All DXA scans were performed by a technician certified by the International Society of Clinical Densitometry, and DXA scanners were calibrated each morning prior to use.

Injury and Illness

Throughout the treatment period, participants were surveyed weekly regarding occurrence of injury and illness via online questionnaire (Qualtrics XP, copyright 2015). Injuries were defined as new musculoskeletal problems unrelated to an accident or trauma that limited full participation in team training for more than 72 hours. Illnesses were defined as new sicknesses that imposed the same restrictions on training.

Statistical Analysis

Descriptive statistics were reported as means +/- standard deviations. T-tests were used to evaluate group (male/female) and time (pre/post treatment) differences. Pearson correlation analysis was used to assess relationships between variables. Statistical analyses were performed using Graphpad Prism 8.0 and significance was set at $p < 0.05$.

Ethical Considerations

This study was approved by the Institutional Review Board of Virginia Tech (IRB #17-009) and is registered with ClinicalTrials.gov (NCT03151174). Each participant signed a written informed consent prior to the start of the study.

6.3 RESULTS

All participants (n = 24; 10 males and 14 females) completed the 10-week treatment and both testing sessions in their entirety. Participants were 20.1 years old (18.7 to 22.2 years) and racially diverse (15 black or African American, 5 white or Caucasian, and 4 other race). Compliance to supplements was 94.3%. No side effects of treatment were reported.

25-Hydroxyvitamin D, Parathyroid Hormone, and Testosterone

Males had significantly lower 25(OH)D than females at baseline (17.6 +/- 6.1 ng/mL, range 9.3 to 40.2 ng/mL and 36.5 +/- 5.7 ng/mL, range 20.0 to 61.3 ng/mL, respectively) (p<0.05) (Figure 6.1). Seven (70%) males were vitamin D deficient, two (20%) were insufficient, and one (10%) was adequate. Zero (0%) females were vitamin D deficient, 10 (71%) were insufficient, and four (28.6%) were adequate.

Following treatment, adequate 25(OH)D was achieved by 16 out of 19 participants (Figure 6.2). However, three out of five participants who did not receive treatment due to adequate 25(OH)D at the start of summer experienced a decline in 25(OH)D to <40 ng/L (Figure 6.2). Males experienced a 32.3 ng/mL (255.4%, range -16.4 to 520.8%) increase in 25(OH)D, while females experienced an 18.7 ng/mL (65.5%, range -33.1 to 214.4%) increase (p<0.01). The degree of change in 25(OH)D did not statistically differ between participants who were vitamin D deficient vs. insufficient at baseline (Figure 6.2).

There was no significant change in PTH, tT, or fT from pre to post treatment (Table 6.2). Elevated PTH (>65 pg/mL) was observed in two males (99.0 and 87.1 pg/mL) and one female (82.8 pg/mL) pre-treatment, and one female (103.0 pg/mL) post-treatment. There was no correlation between 25(OH)D and PTH. There was a significant positive correlation between 25(OH)D and fT (r= 0.3910, p= 0.031), but not 25(OH)D and tT (r= 0.1111, p= 0.740).

Diet and UVB Exposure

No participants reported consuming vitamin D supplements at baseline. Dietary vitamin D (23% of the RDA for males, 33% for females) and calcium (35% of the RDA for males, 46% for females) intake remained stable pre and post treatment (Table 6.3). Participants reported consuming an average of 0.6 cups of milk per day (range: 0 to 5 cups). Dietary vitamin D was not correlated with 25(OH)D. However, cups of milk consumed per day was positively correlated with 25(OH)D ($r= 0.5551$, $p= 0.04$).

Responses to the UVB-exposure questionnaire provided by males and females are shown in Table 6.4. All males ($n=10$) responded that they had not received a suntan in the past 12 months, that they do not typically wear sunscreen, and that they had not used a tanning bed in the past three months both pre and post treatment. Additionally, all males ($n=10$) reported that they spent <10 minutes outdoors training/exercising and in leisure time both pre and post treatment.

Body Composition and Bone Mineral Density

Anthropometric, body composition, and BMD results are shown in Table 6.5. Three males were excluded from percent body fat, fat free mass (FFM), and BMD analyses since their stature exceeded measurement capabilities of our DXA scanner. No pre/post treatment differences were observed. There was a significant negative correlation between 25(OH)D and percent body fat ($r= -0.487$, $p=0.05$), but not between 25(OH)D and any other measure in Table 6.3.

Injury and Illness

Two males and four females had a history of stress fracture prior to starting the study. Throughout the 10-week treatment period, 11 injuries (in 8 different participants: 3 males and 5 females) were reported. Seven of these were muscle strains or pulls, two were torn ligaments, and two were new stress fractures (both females). One stress fracture occurred in a participant with a positive history of stress fracture. Only one illness (respiratory infection in a female participant) was reported. Occurrence of injury and illness were not associated with baseline 25(OH)D or percent change in 25(OH)D throughout the study.

6.4 DISCUSSION

A 10-week treatment protocol (10,000 IU supplemental vitamin D₃ daily for vitamin D deficiency, 5,000 IU supplemental vitamin D₃ daily for vitamin D insufficiency) was effective in improving vitamin D status in male and female collegiate basketball athletes. Some participants not treated due to adequate 25(OH)D at baseline experienced a decline in 25(OH)D despite the study taking place during summer months. A relationship between 25(OH)D and bioavailable testosterone was observed.

A high rate of LVD was observed in participants in our study, with only 20.8% having adequate 25(OH)D at baseline. Similar to our findings, Fishman et al.¹³ also observed LVD in approximately 80% of basketball athletes. These rates are higher than the approximately 25-55% reported for collegiate athletes in other sports.^{7,20,21} Indoor training, a high proportion of athletes with darker skin pigmentation, and larger body size may explain these differences. Because LVD has been associated with decreased athletic performance, impaired recovery, and increased risk of injury and illness,^{4,22} identification and treatment of LVD is warranted in athletes, particularly basketball athletes who seem to have increased susceptibility to LVD.

It should be noted that the concentrations of 25(OH)D classified as deficient, insufficient, and sufficient or adequate vary among research studies, clinical practice guidelines, and real-world practice.^{23,24} For example, the National Academy of Medicine (NAM), formerly known as the Institute of Medicine, recommends that 25(OH)D > 20 ng/mL meets the needs of almost all adults.²⁵ The Endocrine Society, on the other hand, considers 25(OH)D of 20-29 ng/mL to be insufficient, and advises 30-60 ng/mL.²⁶ Optimal 25(OH)D concentration for athletes is controversial, but recommendations for minimum 25(OH)D of >30 ng/mL to >100 ng/mL are common.²⁷ Our classification of 25(OH)D >40 ng/mL as adequate is based on that used by other research teams,^{19,28} the theory that athletes have higher vitamin D needs than the general population,²⁹ evidence that 25(OH)D is not stored in muscle when <40 ng/mL,³⁰ and reports that 25(OH)D >40 ng/mL is protective against stress fractures³¹ and upper respiratory infection.³²

Sex difference in vitamin D status was observed at baseline, as females had significantly higher 25(OH)D compared with males. This variation cannot be explained by dietary differences. Although UVB exposure is not believed to contribute significantly to dermal vitamin D synthesis until May at our latitude (sub-optimal Zenith angle of the sun),⁸ 25(OH)D may have been higher in females due to increased time spent outdoors prior to baseline. Three females (and no males) also reported using tanning beds during the three months prior to baseline, and then again throughout the summer. Not all tanning beds emit UVB, but it is possible that tanning bed exposure increased synthesis of vitamin D in the skin in females. Additionally, use of estrogen-containing birth control pills, which was reported by three participants, may elevate 25(OH)D. Finally, since males in this cohort had a larger body size

than females, a dilutional effect may explain the differences observed in 25(OH)D.³³ Further research is needed to determine if risk of LVD varies by sex.

Our treatment protocol included relatively high doses of vitamin D₃ (5,000 or 10,000 IU/ day). These doses are higher than the Tolerable Upper Limit (4000 IU/ day), but lower than the No Observed Adverse Effects Level (NOAEL) established by NAM.²⁵ The 5,000 and 10,000 IU/ day doses provided to our participants for 10 weeks did not result in excessively high 25(OH)D concentrations. Furthermore, the protocol was not effective in achieving adequate 25(OH)D in three participants. This could be due to individual variation in response to supplementation, or may indicate that a different treatment protocol, or longer treatment, was needed to achieve adequate 25(OH)D. No side effects were reported by participants in this study. However, consequences of excessive vitamin D supplementation do exist.^{5,25} Consistent monitoring of 25(OH)D concentration is, thus, important when supplementing with vitamin D in higher doses. It is possible, though not tested, that lower dose vitamin D supplements could have been as effective as those used in this study. Further research is needed to identify the lowest vitamin D dose effective in treating LVD in basketball athletes.

Diet was not a significant contributor to vitamin D status in this study, as participants consumed just 23% (males) and 33% (females) of the Recommended Dietary Allowance (RDA) for vitamin D (600 IU/ day). Bescos-Garcia et al.³⁴ identified similar low dietary consumption (25% of RDA) in professional basketball athletes. Athletes from many different sports and backgrounds have been consistently observed to consume diets well short of the RDA.³⁵ Increasing dietary vitamin D is often recommended as a strategy for preventing and treating LVD. However, this may prove challenging to athletes since vitamin D is found in few foods, even high dietary consumption cannot match most supplemental doses, and dietary vitamin D

consumption is not commonly associated with 25(OH)D status. Nevertheless, we did identify an association between cups of milk consumed and 25(OH)D. Incorporation of vitamin D-fortified milk into basketball athletes' diets may contribute to improved vitamin D status.

Several studies have shown that athletes have higher 25(OH)D during summer months than fall and winter months, presumably due to increased UVB exposure^{19,36,37}. In contrast, 25(OH)D decreased in three of our participants who did not take vitamin D supplements during the summer (Figure 6.2). The two participants who experienced increased 25(OH)D without treatment also reported tanning bed use (and thus, probable increased UVB exposure) during the study. Participants in the present study spent very little leisure time outdoors during summer months, and males did not train or exercise outdoors in spring or summer. Encouraging sensible sun exposure and planning outdoor training sessions may also contribute to improved vitamin D status.

We observed a negative relationship between adiposity and 25(OH)D. Similar results have been observed in some,^{20,38} but not all,^{19,39,40} studies. Suggested mechanisms for this observation include: altered vitamin D metabolism with obesity, sequestering of 25(OH)D within adipose tissue, and a dilution effect.³³ Although the clinical relevance of lower 25(OH)D in individuals with greater adiposity is not clear,⁴¹ percent body fat may be considered as a risk factor for LVD in athletes.

A positive association between 25(OH)D and fT, a measure of bioavailable testosterone, was observed in this study. It is plausible that percent body fat may have been impacted by differences in fT in participants with lower compared to higher 25(OH)D. In addition to percent body fat, testosterone concentrations may impact muscle mass, strength, bone mineral density, mood, and drive to perform.⁴² Thus, the relationship between 25(OH)D and fT should be

explored further in athletes. One athlete study⁴³ has shown improvements in tT with vitamin D₃ supplementation, but another⁴⁴ did not. Neither of these studies measured fT.

A strength of this study is inclusion of racially diverse athletic participants, which is uncommon in the vitamin D literature. Individuals with darker skin pigmentation have higher incidence of LVD.^{21,35} Decreased dermal vitamin D synthesis with increasing melanin content is one explanation for this occurrence.⁴⁵ Genetic variation in vitamin D metabolism is another.⁴⁶ Some have suggested that black or African American individuals have higher vitamin D needs.²⁶ Others have questioned the clinical relevance of low 25(OH)D in black or African American individuals.⁴⁷ It is also possible that 25(OH)D is not the best biomarker for vitamin D status in some populations.⁴⁸ Further research aimed at identifying vitamin D needs and effects of LVD in racially diverse athletic populations is needed.

The primary limitation of this study is the lack of control group. We declined to use a control group since members of the research team felt it was unethical to withhold treatment from competitive athletes with known LVD. Additionally, because there is a strong NCAA and institutional emphasis on encouraging student-athletes to carefully consider all dietary supplements they wish to take, full transparency in supplementation was considered in the best interest of participants. Fortunately, data from the five participants not treated with vitamin D due to adequate 25(OH)D at baseline provides some insight into expected summer changes in 25(OH)D without supplementation in this cohort of athletes. Another limitation is that we did not control diet or training, which may have affected 25(OH)D. Likewise, we did not assess stage of maturation, nor did we exclude participants taking birth control pills (n=3). Both of these may potentially influence 25(OH)D and testosterone concentrations.

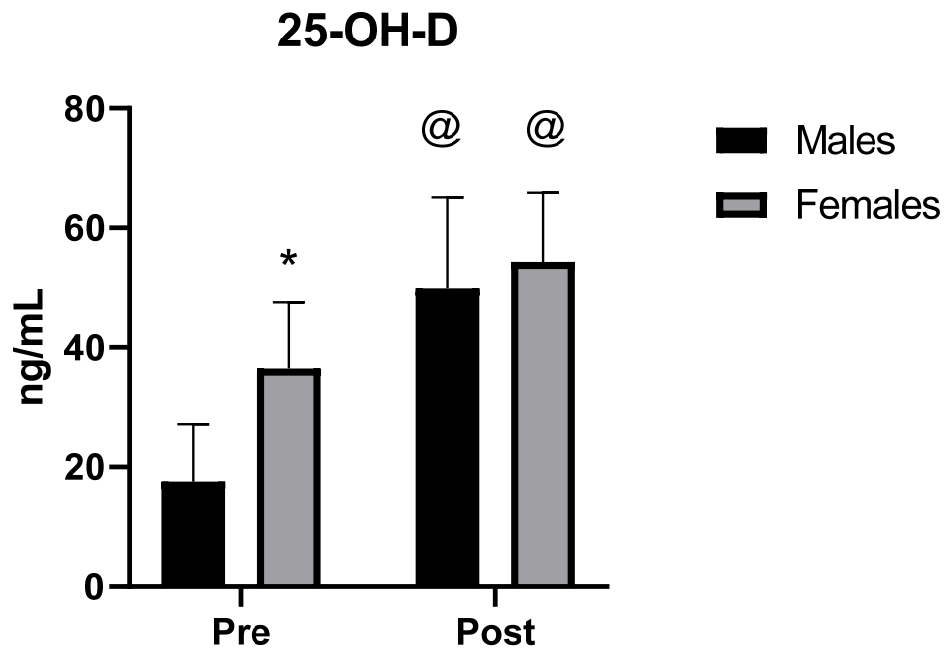
In conclusion, protocols aimed at the prevention and treatment of LVD in basketball athletes should take these athletes' high incidence of LVD into consideration, in addition to their limited habitual dietary vitamin D and UVB exposure. Treatment with 5,000 IU or 10,000 IU of vitamin D₃ for vitamin D insufficiency and deficiency, respectively, may serve as an effective treatment regimen for LVD in college basketball athletes, although more research on the lowest effective supplement dose is needed. Randomized controlled trials aimed at exploring the relationship between vitamin D and bioavailable testosterone in active populations are warranted.

TABLES AND FIGURES
(shown in order referenced within text)

Table 6.1. Low Vitamin D Treatment Protocol Administered for 10 Weeks

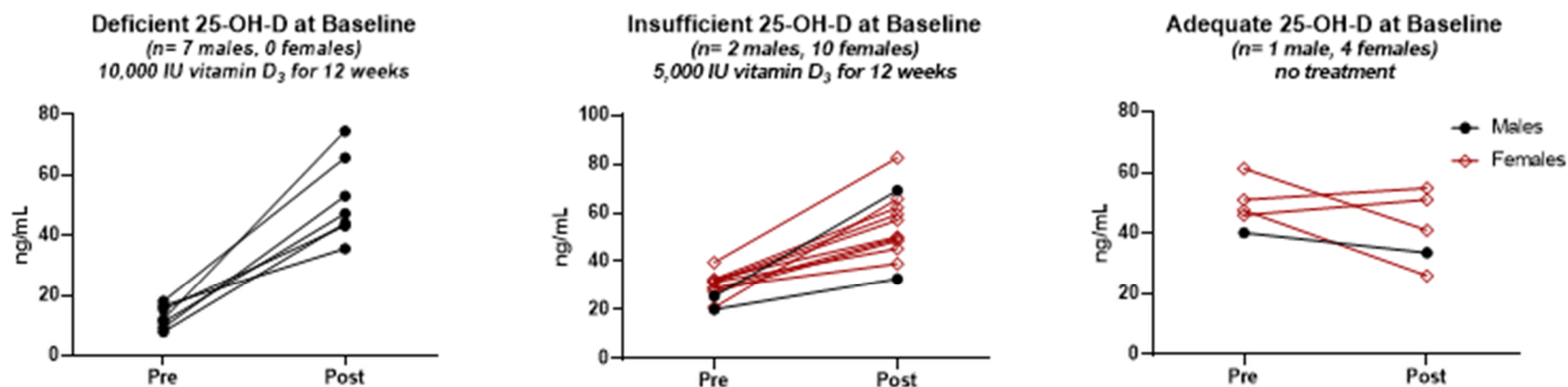
Vitamin D Status	25(OH)D	Daily Vitamin D₃ Supplement
Deficient	<20 ng/mL	10,000 IU
Insufficient	20 to 39.9 ng/mL	5,000 IU
Adequate	>40 ng/mL	None

Figure 6.1 25(OH)D Pre and Post Vitamin D₃ Treatment



25(OH)D in male and female collegiate basketball athletes at baseline (Pre) and following (Post) treatment with 10,000 IU vitamin D₃ supplements/ day for 25(OH)D <19.9 ng/mL (deficient), 5,000 IU vitamin D₃ supplements/ day for 25(OH)D 20-39.9 ng/mL (insufficient), and no vitamin D supplements for 25(OH)D >40 ng/mL (adequate). *= male/female difference (p<0.01), @= pre/post difference (p<0.01)

Figure 6.2. Individual 25(OH)D Concentrations



Individual 25(OH)D in male (n=10) and female (n=14) collegiate basketball athletes at baseline (Pre) and following (Post) treatment with 10,000 IU vitamin D₃ supplements/ day for 25(OH)D <19.9 ng/mL (deficient), 5,000 IU vitamin D₃ supplements/ day for 25(OH)D 20-39.9 ng/mL (insufficient), and no vitamin D supplements for 25(OH)D >40 ng/mL (adequate).

Table 6.2. Parathyroid Hormone, Total Testosterone, and Free Testosterone Pre and Post Vitamin D₃ Treatment

	MALES		FEMALES	
	<i>Pre-Treatment</i>	<i>Post- Treatment</i>	<i>Pre-Treatment</i>	<i>Post-Treatment</i>
PTH (pg/mL)	51.8 +/- 5.7	38.9 +/- 7.9	35.9 +/- 9.8	49.3 +/- 11.1
Total Testosterone (tT) (ng/dL)	973.1 +/- 202.4	806.6 +/- 329.1	64.0 +/- 17.2 *	68.8 +/- 11.9 *
Free Testosterone (tT) (pg/mL)	16.5 +/- 4.4	15.0 +/- 2.7	3.3 +/- 0.9 *	3.8 +/- 1.7 *

Data are presented as mean +/- SD. Parathyroid hormone, total testosterone, and free testosterone concentrations in male (n=10) and female (n=14) collegiate basketball athletes at baseline (Pre) and following (Post) treatment with 10,000 IU/ day for 25(OH)D <19.9 ng/mL (deficient), 5,000 IU/ day for 25(OH)D 20-39.9 ng/mL (insufficient), and no vitamin D supplements for 25(OH)D >40 ng/mL (adequate).
 *= male/female difference (p<0.01). No significant pre/post differences existed.

Table 6.3. Dietary Vitamin D and Calcium Intake

	Pre-Treatment	Post Treatment
Males	Vitamin D: 89 IU (21 to 444 IU) Calcium: 306 mg (47 to 998 mg)	Vitamin D: 191 IU (19 to 587 IU) Calcium: 480 mg (101 to 884 mg)
Females	Vitamin D: 202 IU (60 to 512 IU) Calcium: 556 mg (303 to 1103 mg)	Vitamin D: 197 IU (72 to 598 IU) Calcium: 555 mg (178 to 1010 mg)

Data are presented as means and ranges. Dietary vitamin D and calcium intake in male (n=10) and female (n=14) collegiate basketball athletes at baseline (Pre) and following (Post) treatment with 10,000 IU vitamin D₃ supplements/ day for 25(OH)D <19.9 ng/mL (deficient), 5,000 IU vitamin D₃ supplements/ day for 25(OH)D 20-39.9 ng/mL (insufficient), and no vitamin D supplements for 25(OH)D >40 ng/mL (adequate). No significant differences based on sex or time were observed.

Table 6.4. Results of UVB-Exposure Questionnaire

Have you received a suntan in the past 12 months?	Pre-treatment	Yes 0 (0%)	No 23 (96%)	Unsure 1 (4%)
	Post treatment	Yes 2 (8%)	No 21 (88%)	Unsure 1 (4%)
Do you typically wear sunscreen?	Pre-treatment	Yes 2 (8%)	No 22 (92%)	Unsure 0 (0%)
	Post treatment	Yes 2 (8%)	No 22 (92%)	Unsure 0 (0%)
Have you used a tanning bed in the past 3 months?	Pre-treatment	Yes 3 (12%)	No 21 (88%)	Unsure 0 (0%)
	Post treatment	Yes 3 (12%)	No 21 (88%)	Unsure 0 (0%)
Time spent outdoors training or exercising in the past 3 months:	Pre-treatment	More than 30 min/day 3 (12%)	10 to 30 min/day 0 (0%)	<10 min/day 21 (88%)
	Post treatment	More than 30 min/day 12 (50%)	10 to 30 min/day 2 (8%)	<10 min/day 10 (42%)
Leisure time spent outdoors in the past 3 months:	Pre-treatment	More than 30 min/day 4 (16%)	10 to 30 min/day 9 (38%)	<10 min/day 11 (46%)
	Post treatment	More than 30 min/day 1 (4%)	10 to 30 min/day 2 (8%)	<10 min/day 21 (88%)

Results of UVB-exposure questionnaire in male (n=10) and female (n=14) collegiate basketball athletes at baseline (Pre) and following (Post) treatment with 10,000 IU vitamin D₃ supplements/ day for 25(OH)D <19.9 ng/mL (deficient), 5,000 IU vitamin D₃ supplements/ day for 25(OH)D 25(OH)D 20-39.9 ng/mL (insufficient), and no vitamin D supplements for 25(OH)D >40 ng/mL (adequate).

Table 6.5 Anthropometric, Body Composition, and BMD Results

	Pre-Treatment	Post Treatment
Height (cm)	M: 196.9 (182.9 to 215.9) F: 175.0 (163.5 to 188.0)	M: 198.8 (182.9 to 215.9) F: 175.0 (163.5 to 188.0)
Body Weight (kg)	M: 97.9 (82.3 to 120.7) F: 72.0 (59.7 to 96.5)	M: 99.9 (85.0 to 123.3) F: 70.1 (59.3 to 96.4)
Body Fat (%) n=7 males, 14 females	M: 15.8 (10.1 to 20.7) F: 23.3 (17.1 to 31.0)	M: 16.2 (10.6 to 20.9) F: 22.5 (17.1 to 28.5)
Fat Free Mass (kg) n=7 males, 14 females	M: 82.4 (74.3 to 94.2) F: 55.7 (45.5 to 65.9)	M: 83.6 (74.6 to 94.3) F: 57.3 (45.8 to 67.1)
Total Body BMD (g/cm²) n=7 males, 14 females	M: 1.625 (1.465 to 1.783) F: 1.269 (1.121 to 1.278)	M: 1.618 (1.504 to 1.7830) F: 1.277 (1.121 to 1.282)
AP Spine BMD (g/cm²) n=7 males, 14 females	M: 1.618 (1.406 to 1.960) F: 1.412 (1.131 to 1.606)	M: 1.648 (1.406 to 1.998) F: 1.392 (1.131 to 1.630)
Total Hip BMD (g/cm²) n=7 males, 14 females	M: 1.554 (1.390 to 1.750) F: 1.242 (1.098 to 1.399)	M: 1.550 (1.402 to 1.800) F: 1.232 (1.098 to 1.446)

Data are presented as means and ranges. Anthropometric, body composition, and BMD in male (M) and female (F) collegiate basketball athletes at baseline (Pre) and following (Post) treatment with 10,000 IU vitamin D₃ supplements/ day for 25(OH)D <19.9 ng/mL (deficient), 5,000 IU vitamin D₃ supplements/ day for 25(OH)D 25(OH)D 20-39.9 ng/mL (insufficient), and no vitamin D supplements for 25(OH)D >40 ng/mL (adequate).

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CHAPTER 7

MANUSCRIPT

Vitamin D Supplementation Attenuates Seasonal 25(OH)D Decline and Enhances Strength in Collegiate Swimmers

ABSTRACT

Background: The purpose of this study was to determine whether vitamin D supplementation attenuates a seasonal decline in 25-hydroxyvitamin D [25(OH)D] in collegiate swimmers participating in indoor fall season training. We also evaluated the impact of such supplementation on strength and power outcomes and anabolic hormones.

Methods: Male and female NCAA Division I swimmers with adequate 25(OH)D (≥ 40 ng/mL) (n=19) were randomized to receive 5000 IU vitamin D₃ (VITD) or matched placebo (PLA) daily for 12 weeks while participating in swimming and strength and conditioning training (August – November). Before and after the intervention period, participants underwent testing that consisted of seven strength & conditioning tests (maximal bench press, squat, dead lift, standing broad jump, vertical jump, and dips and pull-ups to exhaustion), dual-energy x-ray absorptiometry (DXA) scan, and blood sampling for analysis of serum 25(OH)D, parathyroid hormone (PTH), total testosterone (tT), free testosterone (fT), sex hormone binding globulin (SHBG), and insulin-like growth factor-1 (IGF-1).

Results: 25(OH)D decreased by 44% in PLA ($p < 0.05$) and increased by 8% in VITD over the 12-week intervention ($p < 0.05$). Fat free mass (FFM) increased in VITD ($p < 0.05$), but not PLA. Improvements in strength and power test performance (compiled change score of all seven tests) were also greater in VITD compared with PLA (50.8% vs. 37.4%, respectively) ($p < 0.05$). tT decreased similarly in both groups, but fT decreased and SHBG increased only in PLA ($p < 0.05$). No significant changes or group differences in IGF-1 were observed.

Conclusions: These findings suggest that vitamin D supplementation may be an efficacious strategy to maintain 25(OH)D during fall season training, and enhance strength and power in swimmers. An effect on testosterone bioavailability may explain these benefits.

Key words (4-6):

25-hydroxyvitamin D, cholecalciferol, performance, testosterone, power

7.1 INTRODUCTION

Although initially classified as a vitamin, calciferol (vitamin D) is currently recognized as a hormone with widespread functionality in physiology and metabolism. There are a number of direct and indirect mechanisms by which vitamin D may influence skeletal muscle function and remodeling including improved calcium handling, enhanced protein synthesis, and action upon anabolic steroid hormones ^{4,5}.

Serum 25-hydroxyvitamin D (25(OH)D), the common biomarker for vitamin D status, has been reported to be positively correlated with muscular strength in healthy adult and elderly populations ⁴⁹⁻⁵¹. Whether or not this association exists in well-trained athletes is less clear. Several observational studies show better strength and power in athletes with higher 25(OH)D ⁵². The results of some ^{28,53-55}, but not all ^{37,56,57}, studies suggest that vitamin D supplementation increases muscular strength and power in athletes.

Improvement in skeletal muscle function following vitamin D supplementation may be limited to those with inadequate or low vitamin D concentrations ^{28,53,55}. Nevertheless, many athletes take vitamin D supplements regardless of, or without knowledge of, their vitamin D status, and without consideration of the numerous factors that influence 25(OH)D ⁵. Diet, adiposity, skin pigmentation, and habitual sun exposure exert an important influence on 25(OH)D concentrations. Several studies have also reported that 25(OH)D is lower in athletes who train primarily indoors, and during the fall and winter months ^{19,36}.

Strategic seasonal supplementation may mitigate the decline in 25(OH)D and its sequelae. There is limited evidence supporting the use of vitamin D supplementation to maintain end-of-summer 25(OH)D in athletes ^{58,59} and, to our knowledge, there are no studies that evaluate the impact on muscle function. Thus, the purpose of this study was three-fold: 1) to

determine if vitamin D supplementation during indoor fall training attenuates a seasonal decline in 25(OH)D in collegiate swimmers; 2) to evaluate the impact of vitamin D supplementation on strength, power, and body composition in swimmers with adequate baseline 25(OH)D; and 3) to explore a relationship between vitamin D and anabolic steroid hormones.

7.2 METHODS

Experimental Design

This randomized, double-blind trial took place in 2017. Participants (collegiate swimmers) completed testing sessions at the start (August) and end (November) of fall indoor training. Between testing sessions (12 weeks), participants with adequate 25(OH)D (>40ng/mL) were randomized to receive 5000IU vitamin D (VITD) or placebo (PLA) supplements daily. In order to gain further understanding of the seasonal variation in 25(OH)D in this population, blood was also sampled in April, at the start of outdoor summer training. There was no intervention between April and August. This study was approved by the Institutional Review Board of Virginia Tech (IRB# 17-009) and is registered with ClinicalTrials.gov (NCT03151174).

Participants

Male and female members of a National Collegiate Athletic Association (NCAA) Division I intercollegiate swim team located in Virginia, United States (latitude = 37°N) served as study participants. Sophomores, juniors, and non-graduating seniors were invited to participate. Freshmen and swimmers who had not been part of the team for at least one year were not invited to participate to minimize differences in outcomes based on novelty of training.

Procedures

Swimmers who volunteered to participate completed an eligibility screening form. Those who were not able to participate in team-prescribed training, strength and power testing, were taking supplements likely to influence outcome variables in the previous 30 days, or had 25(OH)D <40 ng/mL in August (prior to randomization) were excluded. Eligible participants completed a health screening and provided written informed consent before beginning the study.

Fasting blood samples were obtained from all participants for analysis of 25(OH)D and parathyroid hormone (PTH) in April and again in August and November. Participants completed diet and lifestyle questionnaires, body composition analysis, and strength and power tests within one week of the blood sampling in August and November. In August, participants were classified as vitamin D adequate (25(OH)D \geq 40 ng/mL), insufficient (25(OH)D 20-39.9 ng/mL), or deficient (25(OH)D <20 ng/mL). Vitamin D deficient or insufficient participants began 12 weeks of vitamin D supplementation (5000 IU vitamin D₃) and were excluded from remaining study analyses. Vitamin D adequate participants (25(OH)D \geq 40 ng/mL) were randomized to VITD or PLA daily for 12 weeks. A researcher not involved with the study conducted randomization using a computer program (www.randomizer.org), stratifying randomization to VITD and PLA based on sex.

Throughout the study, participants completed team-prescribed training, which included up to 10 swimming workouts per week, strength and conditioning sessions in a weight room, and other “dry land” training (running, plyometrics, and other exercises out of the water), for a total of up to 20 hours of training per week. Participants were instructed to maintain their typical diet throughout the study.

Vitamin D Supplements

Vitamin D supplements used for the study were vitamin D₃ gel capsules (5000 IU per capsule) produced in a single lot by NatureMade (Pharmavite LLC, Opelika, Alabama, U.S.), and verified to be within 10% of 5000 IU by third party testing commissioned by NatureMade. The 5000 IU daily dose was selected because it is a common dose prescribed in clinical athletic settings, and is similar to what was used in other vitamin D supplementation trials in athletes^{28,53,57}. Placebo capsules containing corn oil with maltodextrin casing that were identical in appearance to vitamin D capsules were produced by a local pharmacy (Roanoke, Virginia, U.S.).

Supplements were distributed to each individual participant by researchers following daily swim training sessions. In the event that participants would be away from the training facility for one or more days (typically Sundays), VITD or placebo capsules were provided to participants in travel pouches along with instructions to return any unconsumed capsules to researchers the following day. Supplement compliance was evaluated daily based on the number of remaining capsules. Double blinding was maintained until all study analyses were completed. Participants were interviewed at the conclusion of the study to determine which intervention they believed they received.

Diet and Lifestyle Questionnaires

Daily dietary vitamin D and calcium consumption were assessed using a previously cross-validated food frequency questionnaire¹⁸. An additional questionnaire based on the assessment tool used with collegiate athletes by Halliday et al.¹⁹ was also administered to assess characteristics and lifestyle patterns that may influence vitamin D status, including sun exposure, sunscreen use, and tanning bed use.

Blood Sampling and Analyses

Blood samples were taken between 6:00 and 7:30am following an overnight fast, and at least 12 hours after completion of the last training session. Approximately 20ml of blood was obtained from the antecubital vein in one 10ml serum tube and one 10ml serum separator tube (BD Vacutainer, Franklin Lakes, New Jersey, U.S.). The serum separator tube (10ml) was set aside for analysis of 25(OH)D. Serum tubes were allowed to clot in a vertical position for 20-30 minutes, centrifuged at 4°C for 15 minutes at 3200rpms (Eppendorf 5810R, Hauppauge, New York, U.S.), and stored at -80°C for later analyses. Measurement of serum 25(OH)D was conducted by a Clinical Laboratory Improvement Amendments certified commercial laboratory (LabCorp, Burlington, North Carolina, U.S.) using an immunochemiluminometric assay (DiaSorin Liaison, Saluggia, Italy) immediately following blood draws. Both LabCorp and the DiaSorin assay are certified by the Vitamin D External Quality Assessment Scheme (DEQAS).

All remaining analyses were conducted after the 12-week intervention. Serum intact parathyroid hormone (PTH), sex hormone binding globulin (SHBG), and insulin-like growth factor (IGF-1) were measured using an Immulite immunoassay 1000 analyzer (Siemens, Erlangen, Germany) and commercially available kits (Siemens, Erlangen, Germany). Free testosterone (fT) and total testosterone (tT) were measured using ELISA. The coefficient of variation was <8% for all analyses.

Body Composition Analyses

Height, weight, and body composition were measured within one week of blood sampling. Body weight was measured to the nearest 0.1 lbs. on a digital scale (Model 5002, Scale-Tronix, White Plains, New York, U.S.) and height was measured to the nearest 0.1 in.

using a standard stadiometer; values were then converted to kg and cm, respectively. Dual energy x-ray absorptiometry (DXA) scans (General Electric, Lunar Prodigy Advance, software version 8.10e Madison, WI) were used to quantify body composition (percent body fat and fat free mass). All DXA scans were conducted by a technician certified by the International Society for Clinical Densitometry and licensed by the Virginia Department of Health. The DXA scanner was calibrated each morning prior to beginning scans.

Strength and Power Tests

All strength and power tests were administered by a single strength and conditioning specialist certified by the National Strength and Conditioning Association (NSCA) and Collegiate Strength and Conditioning Association (CSCCa) using consistent, standardized procedures. The strength and conditioning specialists was blind to participants' treatment group. Participants completed a maximal effort one-repetition parallel back squat (starting with 85% previous maximum and increasing by approximately 10% upon successful completion), standing broad jump and standing vertical jump (three attempts for each, separated by two to five minutes, with the highest distance/height recorded), and pull-ups and dips to failure. Testing protocols described in detail by NSCA⁶⁰ were utilized and enforced for all tests except back squat which employed CSCCa exercise techniques⁶¹.

Illness and Injury

Vitamin D status has been negatively associated with the occurrence of illness and injury in athletes^{19,59,62,63}. Thus, online questionnaires (Qualtrics XP, copyright 2015) were administered to participants weekly to track occurrence of illnesses and injuries. Illness was defined as a new sickness that limited full participation in team-prescribed training for more than

72 hours. Injury was defined as a new musculoskeletal problem unrelated to an accident or trauma (i.e., primarily “overuse” type injuries) that limited full participation in team-prescribed training for more than 72 hours. Researchers followed up any affirmative illness/injury response on questionnaires with a personal interview to obtain more details about reported occurrences (i.e.: type of illness/injury, duration of illness/injury, etc.).

Data Analysis

Descriptive statistics are expressed as means and standard deviations. Strength and power test results are expressed in absolute units (i.e.: pounds, inches, or repetitions) and change scores (percent change from August to November). Total change score (sum of percent change for each of the seven strength and power tests) was also calculated. Paired t-tests were used to analyze group differences pre and post intervention. Welch’s correction was used when data were not normally distributed. Repeated measures analysis of variance (RMANOVA) was used to assess time and group differences in 25(OH)D and PTH at three time points. When a significant F value was observed, Bonferonni-corrected t-tests were used to identify differences between means. Categorical data (illness and injury) were analyzed using Chi-squared tests. Pearson correlations were used to analyze relationships between variables, and linear regression was used to determine predictive relationships between variables. Graphpad Prism 8.0 was used for statistical analysis, with significance set at $p < 0.05$.

7.3 RESULTS

Out of 35 swimmers who met established criteria for age and team experience, 32 volunteered to be screened for the study. Nineteen participants (n=10 VITD, n=9 PLA) completed the entire study and were included in all analyses (Figure 7.1). There were no between group differences in any descriptive characteristics (Table 7.1) or any other measures at baseline.

There was no significant change in vitamin D or calcium intake following the intervention in the two groups (Table 7.1). Groups were also similar with regard to whether or not they had experienced a suntan in the past 12 months (17/19 had), typically wore sunscreen (16/19 did not), and in time spent outdoors for reasons other than training (all reported more than 30 minutes per day at each time point).

There were five male participants (three white and two Asian) excluded from randomization due to inadequate 25(OH)D in August. All of these participants were classified as vitamin D insufficient (25(OH)D = 30.2mg/mL), and none were vitamin D deficient. These individuals consumed significantly lower dietary vitamin D in August (217.3 ng/mL) and November (191.4 ng/mL) ($p < 0.05$) compared with VITD and PLA, and 5/5 reported not receiving a suntan in the past 12 months.

Supplement compliance was 95.6% overall (94.6% VITD, 97.1% PLA). There were no side effects reported with the exception of one PLA participant who reported constipation during the first two weeks of the intervention. Participants were adequately blinded to the intervention (16% correctly identified which intervention they received, 37% incorrectly identified their intervention, and 47% stated they were unsure).

25-HydroxyvitaminD and Parathyroid Hormone

The proportion of total participants ($n=24$) with adequate 25(OH)D ($>40\text{ng/mL}$) was 33.3% in April and 79.2% in August following summer training (26.1 \pm 2.7 ng/mL and 47.9 \pm 4.2 ng/mL, respectively). Over the course of the 12-week intervention period (August to November), 25(OH)D increased by 8% in VITD and decreased by 44% in PLA (Figure 7.2). In November, all participants in the VITD group ($n=10$) had adequate 25(OH)D concentrations, while all but one participant in PLA ($n=8$) dropped to 25(OH)D <40 ng/mL.

PTH did not change throughout the study in either group (April: 38.4 \pm 15.1 pg/mL, August: 32.2 \pm 9.4 pg/mL in VITD and 39.9 \pm 11.7 pg/mL in PLA, and November: 29.2 \pm 9.4 pg/mL in VITD and 40.7 \pm 14.7 pg/mL in PLA). Three participants had elevated PTH (>65 pg/mL) in April, but none did in August or November. PTH and 25(OH)D were not correlated.

Leisure time spent outdoors and not wearing sunscreen were significant predictors of 25(OH)D ($R_2 = 0.5930$, $p < 0.05$ and $R_2 = 0.6591$, $p < 0.05$, respectively). Dietary vitamin D intake ($R_2 = 0.3294$, $p = 0.0944$) and whether or not participants had experienced a suntan in the past year did not predict 25(OH)D.

Body Composition

Fat free mass (FFM) increased by 13.6% in VITD ($p < 0.05$), but was unchanged in PLA (Table 7.1). Percent body fat decreased 6.2% in VITD and 4.0% PLA during the intervention, but these changes did not reach statistical significance. No correlation between body weight, FFM, or percent body fat and 25(OH)D was observed.

Strength and Power Tests

The overall change score (compiled percent change from August to November) was 35.8% higher in VITD compared with PLA ($p < 0.05$) (Table 7.2). There was a positive correlation between overall change score and 25(OH)D ($r = .3983$, $p < 0.05$).

Illness and Injury

Thirteen illnesses ($n = 12$ upper respiratory infection or common cold, $n = 1$ gastroenteritis) were reported during the intervention period. There were significantly fewer illness reported in VITD ($n = 2$) compared with PLA ($n = 11$) ($p < 0.05$). Two injuries ($n = 2$ muscle or other soft tissue injury) were reported, with one in each intervention group.

Anabolic Hormones

Males and females experienced a similar decrease in tT following the intervention period ($p < 0.05$) (Figure 7.3). However, fT decreased significantly in males and females only in PLA, but not VITD ($p < 0.05$). Regression analysis showed that 25(OH)D concentration predicted fT throughout the study ($R_2 = .4906$, $p < 0.05$). SHBG increased significantly from August to November in in PLA, but not VIT D ($p < 0.05$).

7.4 DISCUSSION

The present study is the first to investigate effects of routine or blanket vitamin D supplementation on strength and power in a group of athletes with adequate vitamin D status at baseline. Our results suggest that vitamin D supplementation may attenuate a seasonal decline in 25(OH)D concentration, and enhance strength and power in collegiate swimmers.

The inadequate vitamin D status observed in the majority of participants in the spring was reversed in all but five participants following outdoor summer training. However, there was a substantial decline in 25(OH)D following 12 weeks of fall season indoor training in participants who did not take a vitamin D supplement. This decrease occurred despite dietary vitamin D intake being above the Recommended Dietary Allowance (RDA) of 600 IU/day for 19 – 70 years of age. Others have observed similar seasonal variation in athletes^{19,37,64,65}, but a supplementation protocol effective in preventing a seasonal 25(OH)D decline in athletes has not been clearly established. Vitamin D supplementation (5000 IU daily for 12 weeks) attenuated the seasonal decrease in vit D observed in PLA in the current study. Targeted seasonal vitamin D supplementation protocols could be considered as a strategy to maintain vitamin D status year-round.

Future research aimed at identifying the lowest dose of vitamin D effective in preventing a seasonal decline in 25(OH)D status is needed. Caswell et al.³⁷ recommend 400 IU/day of vitamin D during fall and winter months to maintain normal 25(OH)D. Much higher recommendations for athletes also exist^{27,30}. The 5000 IU/day supplemental dose implemented in the current study raised 25(OH)D by approximately 7 ng/mL over 12 weeks during the fall season. This supplementation protocol was based on those used in other athlete studies^{28,53,57}, protocols commonly used by athletes in real-world practice⁵, and the fact that lower doses have not been effective in preventing a seasonal decline in 25(OH)D⁶⁶.

The 5000 IU/day dose we used is higher than the tolerable upper limit of 4000 IU/day established by the National Academy of Medicine (NAM, formerly known as the Institute of Medicine), but lower than the No Observed Adverse Effects Level (NOAEL) of 10,000 IU/day established by the same group²⁵. This dose was not associated with side effects in our study or other studies involving athletes^{28,53}. Additionally, the highest 25(OH)D achieved through supplementation was 76.0 ng/mL, which is well below the U.S. Endocrine Society's recommendation upper limit of 150 ng/mL²⁶, and is within the range recommended for athletes²⁷. Nevertheless, because consequences of vitamin D excess and toxicity exist^{8,67}, regular 25(OH)D monitoring is appropriate when vitamin D supplementation - especially higher dose supplementation - is used.

Improved strength and power following vitamin D supplementation has been previously reported in athletes. For example, vitamin D supplementation was associated with better strength outcomes in collegiate taekwondo athletes⁵³, professional judoka athletes⁵⁵, and a mixed group of professional athletes²⁸ compared to non-supplemented counterparts. Comparison of these studies to the current investigation is difficult, however, since subjects had inadequate vitamin D status at baseline (25(OH)D <12 ng/mL compared to 47 ng/mL in the current study). No participants experienced vitamin D deficiency (25(OH)D <20 ng/mL) in the current study. Our results support the ergogenic potential of raising 25(OH)D above a concentration typically considered "adequate". Alternately, it is possible that group differences in strength and power were associated with a lack of decline in 25(OH)D, which was observed in PLA.

We hypothesized that vitamin D supplementation would positively influence anabolic steroid hormone concentrations in athletes. Although tT decreased in both groups following 12

weeks of fall season training, vitamin D supplementation appears to have attenuated a decline in fT. Measurement of fT reflects the proportion (1-2%) of testosterone that is not bound to SHBG or albumin and is thus, biologically available to tissues ⁴². The concurrent decrease in fT and increase in SHBG observed in PLA, but not VITD, supports our hypothesis. Improvements in strength, power, and fat free mass experienced by VITD participants may be explained, at least in part, by maintenance of SHBG and fT. Since decreases in fT can impact performance and increase risk of overtraining syndrome in athletes ⁶⁸, further research on the effects of vitamin D supplementation on testosterone bioavailability is warranted.

Remaining healthy and free from injury is paramount to successful athletic training programs. Vitamin D is known to play a role in innate immune function and inflammatory processes ⁸. A positive association between vitamin D status and occurrence of illness was previously reported in swimmers ⁶² and a diverse group of collegiate athletes that included swimmers ¹⁹. Similarly, Jung et al. ⁶⁹ observed reduced symptoms of upper respiratory infection in athletes who took vitamin D supplements. Results of the current study are consistent with previous findings, as vitamin D supplementation was associated with lower rate of illness. These results may be explained by a positive effect of vitamin D on inflammatory cytokines ⁴⁰ and anti-microbial peptides ³², which have been observed in athletes.

Others have reported that vitamin D status or vitamin D supplementation reduced occurrence of injury in athletes ^{54,59,63}. We did not observe a similar effect, but only two total injuries were experienced by our study participants during the 12-week intervention period. It is possible that our participants' adequate vitamin D status at baseline was protective against injury.

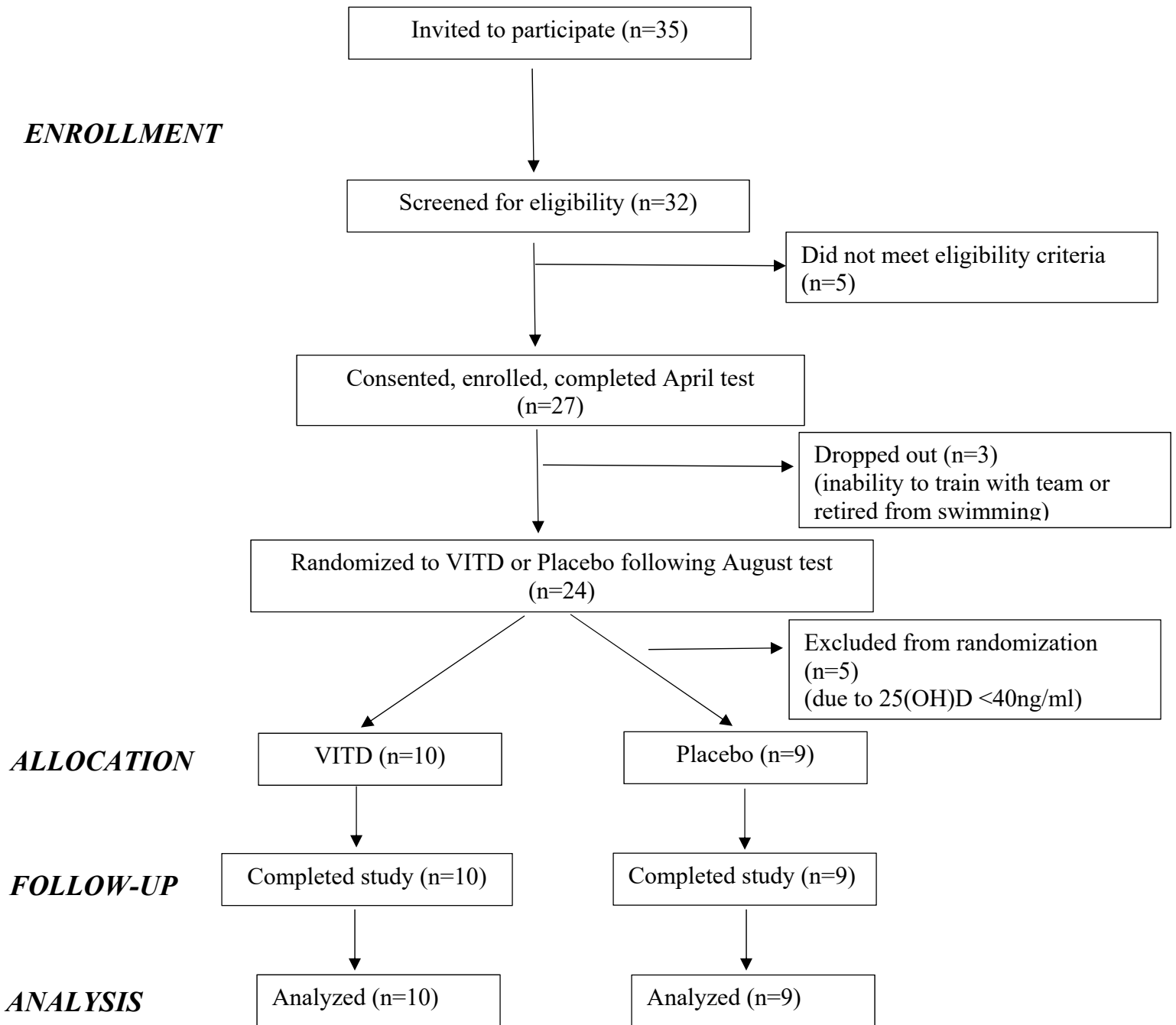
Strengths of the current investigation include a seven-month study duration that allowed for evaluation of seasonal effects on 25(OH)D in a randomized controlled design experiment,

excellent compliance with the intervention, and the use of a vitamin D supplementation protocol commonly adopted by athletes in everyday practice to explore outcomes of considerable importance to athletes. A limitation of this study is a relatively small sample size, although most of the swim team (91% of eligible swimmers) participated. We did not control for menstrual cycle or oral contraceptive use (n=2) among female participants, both factors that may potentially influence 25(OH)D and anabolic hormones. Finally, we measured strength and power in swimmers, but did not evaluate swimming performance itself.

In conclusion, we observed that supplementation with 5000 IU of vitamin D₃ for 12 weeks attenuated the fall-season decline in 25(OH)D, and was associated with increased strength, power, and FFM in collegiate swimmers. Testosterone bioavailability was maintained and the occurrence of illness decreased following vit D supplementation in the present study. Taken together, the results of the present study suggest that vitamin D supplementation is an accessible, low-cost intervention with potential to improve athletes' performance and health. Future research with larger and more diverse sample sizes is needed to further explore effects of vitamin D supplementation on anabolic hormones and immune function, and to identify the lowest effective dose of vitamin D in athletes.

TABLES AND FIGURES
(shown in order referenced within text)

Figure 7.1. Flow Diagram of Participant Enrollment



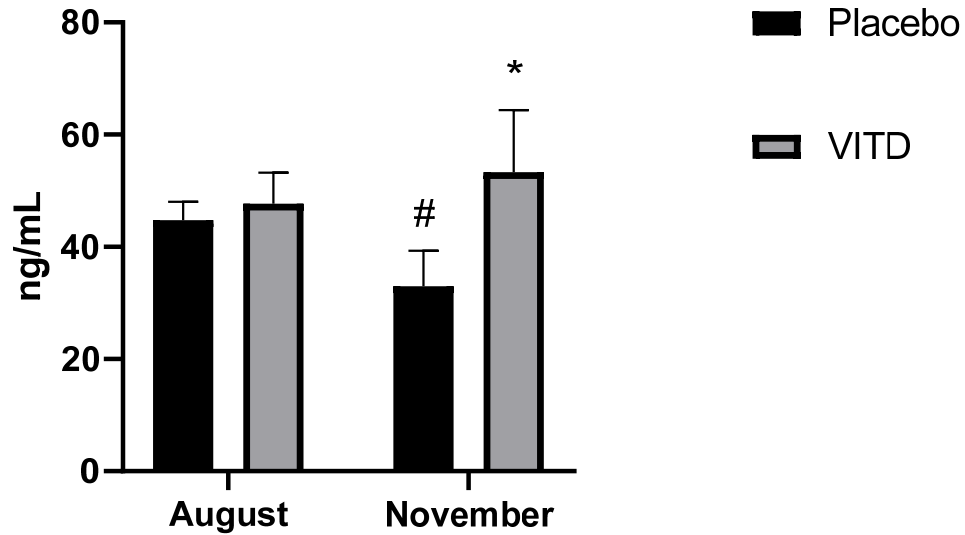
Non-freshman members of a collegiate swim team (n=35) were invited to participate in the study. Following eligibility screening, drop-outs after the April testing session, and exclusion due to 25(OH)D <40ng/mL, n=19 participants were randomized to VITD or placebo.

Table 7.1. Descriptive Characteristics of Participants

	Placebo		VITD	
	Aug.	Nov.	Aug.	Nov.
Age (years)	19.7 (0.8)	20.1 (0.8)	20.1 (1.6)	20.5 (1.6)
Sex	3 males 6 females	3 males 6 females	3 males 7 females	3 males 7 females
Race	8 white 1 black	8 white 1 black	10 white	10 white
Height (cm)	175.5 (9.4)	176.5 (9.1)	174.0 (6.6)	174.8 (5.3)
Weight (kg)	73.9 (11.3)	74.0 (10.7)	70.1 (9.8)	72.5 (8.3)
Body Fat (%)	23.9 (2.6)	19.9 (4.1)	25.3 (2.1)	19.1 (3.16)
FFM (kg)	59.4 (6.0)	59.7 (9.7)	54.6 (10.8)	62.0 (8.4) #
Dietary vitamin D (IU)	598 (142)	710 (443)	701 (140)	615 (191)
Dietary calcium (mg)	2218 (303)	2704 (185)	2118 (556)	3002 (732)

Descriptive characteristics of participants in August, and November. Data are expressed as mean (SD). # = pre/post intervention (p<0.05)

Figure 7.2. 25(OH)D Pre- and Post Vitamin D Supplementation



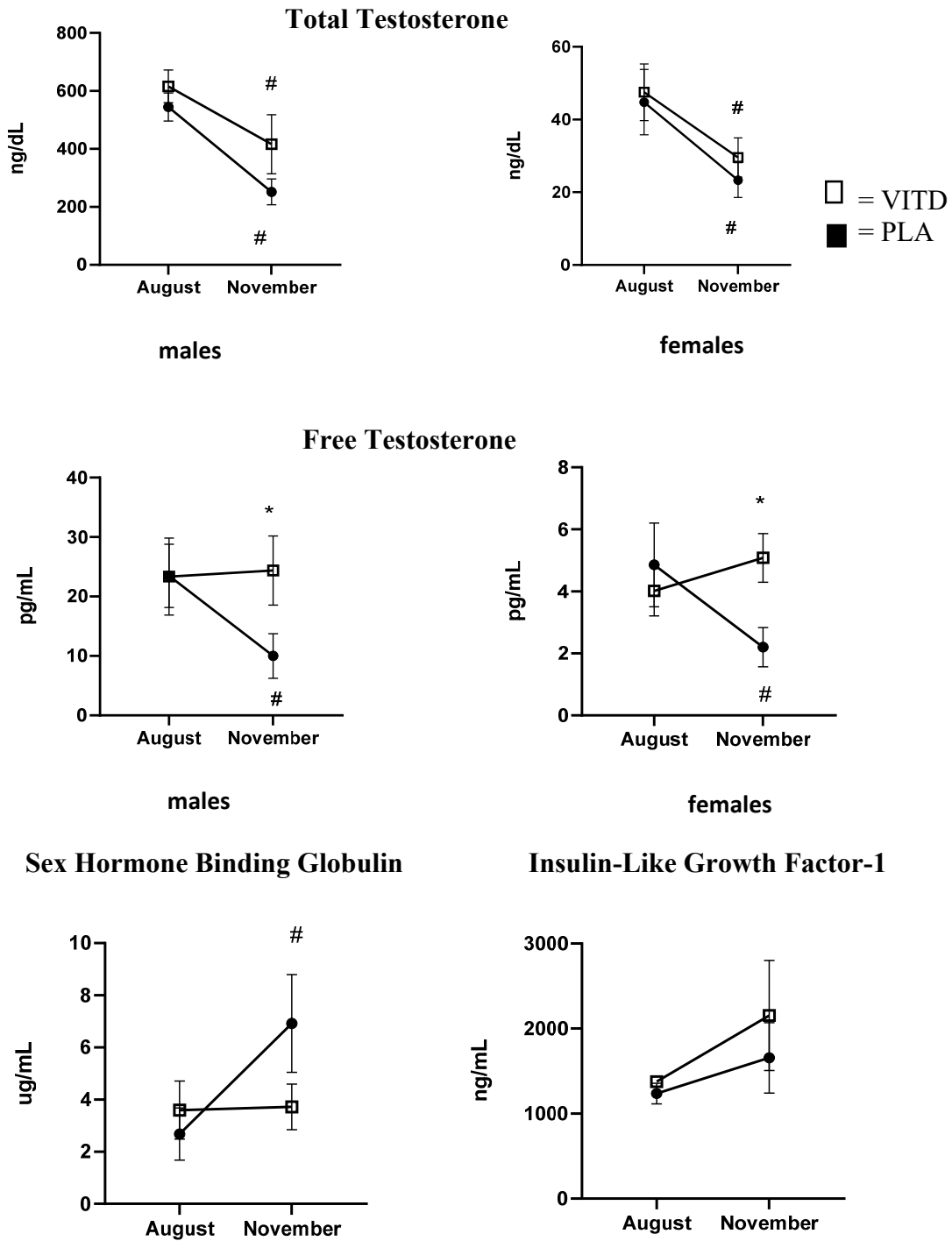
25(OH)D in collegiate swimmers before and after taking 5000IU vitamin D₃ (n=10) or placebo (n=9) for 12 weeks. All participants had 25(OH)D >40ng/mL in August. Data are expressed as mean +/- SD. * = VITD vs. PLA (p<0.05), # = pre/post intervention (p<0.05)

Table 7.2. Results of Strength and Conditioning Tests

	Placebo			VITD			p for Change (VITD vs. PLA)
	August	November	Change	August	November	Change	
Bench Press (lbs.)	167.2 (30.1)	175.0 (44.4)	4.67%	140.6 (17.8)	149.4 (31.7)	6.26%	0.883
Squat (lbs.)	205.6 (51.9)	220.0 (39.3)	7.00%	178.3 (20.1)	198.0 (16.1) #	16.27%	0.037 *
Dead Lift (lbs.)	227.5 (57.4)	233.3 (44.1)	2.55%	197.2 (37.3)	224.4 (24.5)	13.79%	0.048 *
Standing Broad Jump (feet)	7.6 (1.1)	7.7 (0.9)	1.05%	7.0 (1.4)	7.6 (0.5)	8.84%	0.240
Vertical Jump (inches)	22.8 (3.5)	23.3 (4.7)	2.19%	20.2 (1.0)	22.9 (2.9) #	13.37%	0.015 *
Dips (repetitions)	19.9 (6.1)	23.2 (3.0)	16.58%	11.3 (2.7)	13.3 (2.0)	17.70%	0.703
Pull-ups (repetitions)	10.9 (7.8)	11.8 (8.3)	8.26%	9.4 (7.3)	9.2 (6.9)	-2.17%	0.184
	Overall change score		37.4% (7.1)	Overall change score		50.8% (12.0)	0.029 *

Results of seven strength and conditioning tests in August and November, before and after supplementation with vitamin D (n=10) or placebo (n=9) for 12 weeks, and percent change from August to November. Data are expressed as mean (SD). * = VITD vs. PLA (p<0.05), # = pre/post intervention (p<0.05)

Figure 7.3. Anabolic Hormone Results



Anabolic hormones in August and November, before and after supplementation with vitamin D (n=10) or placebo (n=9). Males (n=6) and females (n=13) were analyzed and graphed separately for fT and tT since typical ranges are extremely different based on sex. Data are expressed as mean +/- SD. * = VITD vs. PLA (p<0.05), # = pre/post intervention (p<0.05)

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CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS

CONCLUSIONS AND FUTURE DIRECTIONS

The last 20 years have brought about tremendous discoveries related to the role of vitamin D in human physiology and metabolism. We now understand that vitamin D is part of a complex endocrine system that not only influences bone health and metabolism, but nearly every tissue in the body.¹ Research into effects of vitamin D status on human health and performance has yielded promising, yet inconclusive results.² Healthcare providers are faced with evolving and contradictory guidelines related to the management of vitamin D, and report substantial inconsistency in knowledge, attitudes, and practices related to vitamin D.³ Amidst this uncertainty, media and public interest in vitamin D have soared.⁴

This dissertation research identified high volumes of vitamin D-related care in many countries, a regional health system, and within NCAA Division I collegiate athletics programs. Some of this care was classified as non-indicated (i.e., contrary to professional guidelines and recommendations). Non-indicated, low value healthcare services contribute to rising healthcare costs, decreased quality of care, and potentially, harm.^{5,6} We identified a cascade of low value services that seemed to trigger more low value services within primary care of a Southwest Virginia health system.

In 2014, the USPSTF found insufficient evidence of benefits and harms of vitamin D screening in asymptomatic individuals. Yet, rates of vitamin D screening in asymptomatic individuals continue to rise. Further research on benefits and harms of vitamin D screening in various populations is needed. Analyzing patient charts along with EHR data would provide deeper insight into downstream patterns of care and health outcomes following vitamin D screening. Cascades of unnecessary health services could be considered a harm of vitamin D screening.

Investigations to reduce the occurrence of low value care should focus not only on non-indicated services themselves, but on subsequent cascades as well. Effective healthcare interventions involve multiple stakeholders. Future research and policy related to constraining low value care and subsequent healthcare cascades should focus on patients, providers, health systems, and insurers, in addition to unintended consequences that may result from such interventions. A better understanding of provider and patient decision-making related to low value care and response to medical uncertainty would be valuable in informing effective interventions.

Continued research on the role of vitamin D in health and performance of specific populations will provide evidence for use in the refinement of clinical practice guidelines and interventions. Competitive athletes are an especially interesting population to study since they have a high prevalence of LVD. Most vitamin D-athlete studies have used white male athletes as subjects. We were able to recruit both males and females and a racially diverse study population. Since race and sex are factors in vitamin D status and metabolism, further work with populations such as those we studied are needed. There is a small, yet emerging, body of evidence associating vitamin D status with improved athletic performance, enhanced recovery, and reduced occurrence of injury and illness.^{7,8} Although it is challenging to identify large groups of homogenous athletes participating in similar training, additional clinical trials with a greater number of participants are needed to confirm these results. Most studies that identify a positive effect of vitamin D supplements on athletic performance involve athletes with low or very low baseline 25(OH)D concentrations. Our research findings are novel because we observed seasonal strength and power improvements and favorable body composition changes following vitamin D supplementation in swimmers with normal baseline 25(OH)D. Additional research is needed to determine whether it is the overall 25(OH)D concentration, 25(OH)D bioavailability, or a decline in 25(OH)D that is most

influential to health and performance. Furthermore, we assessed strength, power, and body composition outcomes in swimmers, but not swimming performance itself. Research that evaluates sport performance outcomes would be beneficial. Finally, the identification of positive performance outcomes in elite athletes for whom physical performance changes are difficult to assess provides justification for deeper exploration of vitamin D and physical performance in other populations, such as elderly.

We also identified a positive association between vitamin D status and free testosterone concentrations. As athletes are continually seeking safe, effective strategies for improving anabolic hormone profiles, performance, and body composition, vitamin D is a promising intervention for competitive athletes. Further research on the relationship between vitamin D, testosterone, and other anabolic hormones is needed. Additionally, in our study, swimmers taking vitamin D supplements had a lower occurrence of illness during the fall season. Considering that over 60% of NCAA athletes report occurrence of respiratory infection or other illness each year, vitamin D may be an appealing intervention to promote healthy training.⁹ More research to investigate the role of vitamin in immune function is needed.

Moreover, evidence-based care related to vitamin D is complicated by rapidly evolving research, contradictory professional guidelines, and intense public focus. Healthcare providers have responded inconsistently, but overall, with exponential and costly increases in vitamin D-related care, especially 25(OH)D testing. A proportion of this care has been identified as non-indicated, which contributes to the low value care environment prevalent in U.S. healthcare. Improved knowledge of the role of vitamin D in health and performance of specific populations such as competitive athletes will inform clinical practice guidelines and interventions moving forward.

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APPENDIX A

Data Tables: Vitamin D and Athletic Performance

Table AA.1. Vitamin D Status and Athletic Performance (Observational Studies) (n=13)*

Study	Subjects	n	Location/ Season	25(OH)D measured by	25(OH)D	25(OH)D cutoff considered adequate or sufficient	Outcomes measures	Key findings	Effect
Carswell et al Med Sci Sports Ex (2018) INFLUENCE OF VITAMIN D SUPPLEMENTATION BY SUNLIGHT OR ORAL D3 ON EXERCISE PERFORMANCE (STUDY 1 of 2)	Army recruits Males and Females (age = 22) 95% Caucasian 45% smokers BF%=30%	967	UK (54 D N) Winter	Mass Spec	9% of men and 36% of women were "sufficient" (>20 ng/mL) at baseline	20 ng/mL	Endurance performance, strength, power (1.5 mile run, power clean simulator, VJ)	25(OH)D accounted for 4-6% of variance in 1.5 mile run time. Every .4 ng/mL increase in 25(OH)D = .42 s faster 1.5 mile run time in men and .57 in women after controlling for FM, smoking, season, and off days 25(OH)D not associated with strength or power	+ (mixed)
Dubnov-Raz et al Ped Ex Sci (2014) VITAMIN D CONCENTRATIONS AND PHYSICAL PERFORMANCE IN COMPETITIVE ADOLESCENT SWIMMERS	Competitive swimmers (training 17 hrs/week) Males and females (age 12-18) Caucasian	80	Israel (32 D N) Fall	Radio-immunoassay	27 ng/mL (66% insufficient, 14% deficient)	30 ng/mL = sufficient 20-29.9 ng/mL = insufficient <20 ng/mL = deficient	Handgrip strength, balance, swimming performance (6 different tests)	No association between 25(OH)D and outcomes	None
Fitzgerald et al J Str Cond Res (2014) VITAMIN D STATUS AND VO ₂ PEAK DURING A SKATE TREADMILL GRADED EXERCISE TEST IN COMPETITIVE ICE HOCKEY PLAYERS	Ice hockey athletes Males (age = 20) All Caucasian	52	Minnesota (45 D N) Summer	Mass Spec (capillary blood samples used)	36 (38% had "insufficiency"; 0 had "deficiency")	32 ng/mL	Cardiorespira tory fitness (VO ₂ peak)	No correlation between 25(OH)D and VO ₂ peak	None
Fitzgerald et al J Str Cond Res (2015) ASSOCIATION BETWEEN VITAMIN D STATUS AND MAXIMAL-INTENSITY EXERCISE PERFORMANCE IN JUNIOR AND COLLEGIATE HOCKEY PLAYERS	Ice hockey athletes Males (20 YO) All Caucasian <i>Same subjects as previous study</i>	52	Minnesota (45 D N) Summer	Mass Spec (capillary blood samples used)	36 (38% had "insufficiency"; 0 had "deficiency")	32 ng/mL	Max intensity performance Handgrip, VJ, Wingate	25(OH)D predicted handgrip performance after adjusting for level of play, FFM, FM, and self-reported total physical activity	+ (mixed)

Geiker et al IJSNEM (2017) VITAMIN D STATUS AND MUSCLE FUNCTION AMONG ADOLESCENT AND YOUNG SWIMMERS.	Elite Club swimmers (training 30 hours/week) Males and Females (19 YO) Race not reported	29	Denmark (55 D N) Spring	HPLC	21 ng/mL (45% had <20 ng/mL)	≥20 ng/mL = adequate	Hand-grip strength	Athletes with adequate 25(OH)D had better hand-grip strength compared with athletes with inadequate 25(OH)D; 25(OH)D positively correlated with strength	+
Griesrober et al Orth J Sports Med (2018) VITAMIN D INSUFFICIENCY AMONG PROFESSIONAL BASKETBALL PLAYERS A RELATIONSHIP TO FRACTURE RISK AND ATHLETIC PERFORMANCE	Basketball athletes at NBA Combine 2009-2013 Males (21.5 YO) Race not reported	279	Diverse locations Spring	Not specified	Mean 25(OH)D not reported. (74% had inadequate vitamin D)	>30 ng/mL = sufficient <20 ng/mL = deficient	Likelihood of being selected in first or second round of NBA draft	Athletes with vitamin D deficiency significantly less likely to be drafted. "Participants with higher vitamin D more likely to be drafted into the NBA. This supports the potential role of vitamin D in athletic performance."	+(No direct measure of performance)
Hamilton et al J Sci Med Sport (2013) VITAMIN D CONCENTRATION IN 342 PROFESSIONAL FOOTBALL PLAYERS AND ASSOCIATION WITH LOWER LIMB ISOKINETIC FUNCTION	Professional football (soccer) athletes Males Race not reported	342	Qatar (25 D N) for testing, but subjects were from many different regions Diverse region of origin (Asia, Middle East, Gulf, Africa, Persia) Fall	Immunoassay (Diasorin)	84% "inadequate" (<30 ng/ml), with 12% "severely deficient" (<10 ng/mL)	>30 ng/mL = sufficient 20–30 ng/mL = insufficiency <20 ng/mL = deficient <10 ng/mL = severely deficient	Body composition, isometric strength (DXA, Biodex)	Severely deficient 25(OH)D participants had lower hamstring strength	+
Hildebrand et al IJSNEM (2016) COMPROMISED VITAMIN D STATUS NEGATIVELY AFFECTS MUSCULAR STRENGTH AND POWER OF COLLEGIATE ATHLETES	College athletes from 8 sports representing 3 NCAA I and II athletic programs Males and females 80% Caucasian	103	Oklahoma (35-37 D N) Fall	Immunoassay (Diasorin)	36 ng/mL (32% inadequate)	>30 ng/mL = adequate 20-30 ng/mL = insufficient <20 ng/mL = deficient	Vertical jump, shuttle run, triple hop, max squat	Athletes with lower 25(OH)D had decreased performance in all four outcomes.	+

Koundourakis et al PLOS One (2014) VITAMIN D AND EXERCISE PERFORMANCE IN PROFESSIONAL SOCCER PLAYERS	Professional soccer players Males All Caucasian	67	Greece (39° N) Spring/Summer	Immunoassay (Diasorin)	34 ng/mL before off-season and 47 ng/mL after off-season	Not defined	Max squat, max countermovement jump (CMJ), 10m and 20m sprints, VO _{2max} before and after 6 week off-season period (no intervention during off-season)	Positive correlation between 25(OH)D and squat, CMJ, 10m and 20m sprints, and VO _{2max} before off-season and all but CMJ after (performance measures decreased with off-season but positive correlation remained)	+
Ksizak et al Biology of Sport (2018) RELATIONSHIP BETWEEN 25(OH)D LEVELS AND ATHLETIC PERFORMANCE IN POLISH JUDOISTS	Professional judo athletes Males All Caucasian	25	Poland (52° N) Season not described	Electrochemiluminescence (ECLIA) (Roche) Only measured 25(OH)D ₃ , not total 25(OH)D	17 ng/mL (80% deficient)	30-60 ng/mL = adequate 20-30 ng/mL = insufficient <20 ng/mL = deficient	Hand-grip strength, isokinetic lower limb strength, power (jump mat)	Positive correlation between 25(OH)D and all measures	+
Orysiak et al PLOS One (2018) VITAMIN D STATUS AND ITS RELATION TO EXERCISE PERFORMANCE AND IRON STATUS IN YOUNG ICE HOCKEY PLAYERS	Junior Hockey athletes Males (17 YO) All Caucasian	50	Poland (52° N)	ELISA (DiA Source)	30 ng/mL (40% insufficient and 22% deficient)	≥30 ng/mL = sufficient 20-29.9 ng/mL = insufficient <20 ng/mL = deficient	Isometric strength of various muscle groups, vertical jump, repeated sprint test	No relationship between 25(OH)D and outcomes	None
Zietler et al Int J Env Res Pub Health (2018) ASSOCIATION BETWEEN THE 25-HYDROXYVITAMIN D STATUS AND PHYSICAL PERFORMANCE IN HEALTHY RECREATIONAL ATHLETES	Recreational athletes Males and females (39 YO) All Caucasian	581	Austria (48° N)	Immunoassay (Elecys Vitamin D total II, Roche Diagnostics)	26 ng/mL (70% insufficient or deficient)	≥30 ng/mL = sufficient 20-29.9 ng/mL = insufficient <20 ng/mL = deficient	Maximal and submaximal physical performance (treadmill)		+

Table AA.2. Vitamin D Supplementation and Athletic Performance (Intervention Studies) (n=13)*

Study	Subjects	n	Location/ Season	Supplement Protocol	25(OH)D measured by	25(OH)D (BEFORE and AFTER supplementation)	25(OH)D cutoffs	Outcomes measures	Key findings	Effect
Carswell et al MSSE (2018) INFLUENCE OF VITAMIN D SUPPLEMENTATION BY SUNLIGHT OR ORAL D ₃ ON EXERCISE PERFORMANCE (STUDY 2 OF 2)	Military recruits Males (22 YO) All white (with skin types I to IV)	137	UK (55° N) Winter	1000 IU D ₃ daily for 4 weeks then 400 IU D ₃ daily for 8 weeks + simulated sun vs. Supplement only vs. Simulated sun vs. Placebo supplement -compliance >80%	Mass spec	PRE-TREATMENT: 10 ng/mL (74% <20ng/mL and 31% <12ng/mL) POST-TREATMENT: 26 ng/mL <i>160% increase</i> (3% <20 ng/mL) Placebo ↑ 60% to 16 ng/mL	20 ng/mL = adequate	1.5 mile run, dynamic lift, vertical jump	No group differences	None
Close et al J Sport Sci (2013) ASSESSMENT OF VITAMIN D CONCENTRATION IN NON- SUPPLEMENTED PROFESSIONAL ATHLETES AND HEALTHY ADULTS DURING THE WINTER MONTHS IN THE UK: IMPLICATIONS FOR SKELETAL MUSCLE FUNCTION	Professional soccer players Males (18 YO) Race not described	14	UK (53° N) Winter	5000 IU D ₃ vs. Placebo daily for 8 weeks -compliance not reported	Mass spec	PRE-TREATMENT: 16 ng/mL (1% >40 ng/mL) POST-TREATMENT: 41 ng/mL <i>156% increase</i> (60% >40 ng/mL) Placebo ↓ 25% to 12 ng/mL.	>40 ng/mL = optimal >30 ng/mL = adequate 20-30 ng/mL = insufficient <20 ng/mL = deficient	10m and 30m sprints, VJ, max squat, max bench press, Illinois agility test	Significantly greater improvement in 10m sprint and vertical jump in supplemented athletes; trend for squat and bench press	+ (mixed)
Close et al Br J Sports Med (2013) THE EFFECTS OF VITAMIN D ₃ SUPPLEMENTATION ON SERUM TOTAL 25[OH]D CONCENTRATION AND PHYSICAL PERFORMANCE: A RANDOMISED DOSE- RESPONSE STUDY	Club soccer players Males (21 YO) Race not described	30	UK (53° N) Late winter/ early spring	40,000IU D ₃ vs. 20,000 IU D ₃ vs. Placebo weekly for 12 weeks -compliance not reported	Mass spec	PRE-TREATMENT: 20 ng/mL (43% >20 ng/mL) POST-TREATMENT: 34 ng/mL (20,000 IU) 36 ng/mL (40,000 IU) <i>70-80% increase</i> (100% >20 ng/mL) Placebo ↓ 20% to 16 ng/mL	>40ng/mL = optimal >30ng/mL = adequate 20-30ng/mL = insufficient <20ng/mL = deficient	Max bench press, leg press, vertical jump	No group differences	None

Dubnov-Raz et al IJSNEM (2015) VITAMIN D SUPPLEMENTATION AND PHYSICAL PERFORMANCE IN ADOLESCENT SWIMMERS	Adolescent swimmers Males and females (14 YO) Race not described	52	Israel (32° N) Fall	2000 IU D ₃ vs. Placebo daily for 12 weeks -compliance not monitored	RIA (DiaSorin Inc., Stillwater, MN, USA)	PRE-TREATMENT: 24 ng/mL (0% >30 ng/mL) POST-TREATMENT: 29 ng/mL 21% increase (48% >30 ng/mL) Placebo ↓ 20% to 20 ng/mL	>30 ng/mL= sufficient	Balance, strength, swim performance	No improvements	None
Dunn and Robert-McComb Int J Res Stud Med Health Sci (2017) VITAMIN D DEFICIENCY IN COLLEGE-AGE MALE BASKETBALL PLAYERS: SPORTS MEDICINE PHYSICIANS CAN PLAY AN IMPORTANT ROLE	College basketball athletes Males (53% white, 47% black)	17	Texas, US (32° N) Time of year not specified	4000 IU/day for 8 weeks Vs. Placebo -compliance not reported	Mass spec	BEFORE: 19.9 ng/mL AFTER: 41.1 ng/mL	30 ng/mL	vertical jump, 20 m sprint time trial, and 5-10-5 agility drill time trial	No improvements	None
Fairbairn et al. IJSNEM (2018) VITAMIN D ₃ SUPPLEMENTATION DOES NOT IMPROVE SPRINT PERFORMANCE IN PROFESSIONAL RUGBY PLAYERS: A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND INTERVENTION STUDY	Professional rugby players	57	New Zealand (41° N) Late summer/early fall	50,000 IU D ₃ once every 2 weeks for 12 weeks v. placebo -compliance not reported	Mass spec	BEFORE: 37.6ng/mL (lowest = 20ng/mL) AFTER: 46.1ng/mL (vitamin D supplemented group 12.5 ng/mL higher than placebo)	>30ng/mL = adequate	Six tests (10m sprint, 30m sprint, bench press, bench pull, reverse chin-up, recovery test)	Slightly higher reverse chin-up in vitamin D supplemented group; no other differences	+ (mixed)
Jastrzebska et al J Str Cond Res (2016) EFFECT OF VITAMIN D SUPPLEMENTATION ON TRAINING ADAPTATION IN WELL-TRAINED SOCCER PLAYERS	Elite junior soccer players (avg age = 17.5) Males Race/ethnicity not specified	36	Poland (52° N) winter	5000 IU D ₃ daily for 8 weeks (liquid drops) -compliance not reported	Immunoassay (BIOMÈRIEUX/mini VIDAS analyzer)	BEFORE: 19ng/mL AFTER: 42ng/mL	≥20ng/mL = adequate ≥40ng/mL = optimal	Several speed and strength tests and simulated soccer match	No group differences	None

Jastrzebska et al J Human Kin (2018) CAN SUPPLEMENTATION OF VITAMIN D IMPROVE AEROBIC CAPACITY IN WELL TRAINED YOUTH SOCCER PLAYERS?	“Youth” soccer players (avg age= 17.5) Males Race/ethnicity not specified <i>Same subjects and supplementation period as previous study</i>	36	Poland (52° N) winter	5000 IU D ₃ daily for 8 weeks (liquid drops) -compliance not reported	Immunoassay (BIOMÈRIEUX/mini VIDAS analyzer)	BEFORE: 19ng/mL AFTER: 42ng/mL	≥20ng/mL = adequate ≥40ng/mL = optimal	VO _{2max} , lactate threshold (LT)	D supplemented group significant improvement in VO _{2max} , but not LT	+ (mixed)
Jung et al IJSNEM (2018) CORRECTING VITAMIN D INSUFFICIENCY IMPROVES SOME BUT NOT ALL ASPECTS OF PHYSICAL PERFORMANCE DURING WINTER TRAINING IN TAEKWONDO ATHLETES	Collegiate taekwondo athletes Males and females	35	South Korea (33° N) Winter	5000 IU D ₃ daily for 4 weeks (capsules) -compliance not reported	Immunoassay (Liaison)	BEFORE: 11ng/mL AFTER: 28ng/mL	≥20ng/mL = adequate	Wingate, isokinetic muscle strength and endurance, countermovement jump test, sit-ups, agility test, and 20-m pacer	D supplemented group showed greater improvement in Wingate and isokinetic knee extension	+
Skalska et al Nutrients (2019) VITAMIN D SUPPLEMENTATION AND PHYSICAL ACTIVITY OF YOUNG SOCCER PLAYERS DURING HIGH-INTENSITY TRAINING	Youth soccer players Males (17 YO) All Caucasian	36	Poland (55° N) Winter	5000 IU D ₃ drops vs. Placebo daily for 8 weeks -compliance not reported	Immunoenzymatic Method (Biomerieux)	BEFORE: 19ng/mL (61% were inadequate) AFTER: 42ng/mL in supplemented group; placebo ↓ by 8%	>20ng/mL = adequate <20ng/mL = inadequate	Distance covered in small-sided games (GPS analysis)	No group differences	None
Todd et al Eur J Nutr (2017) VITAMIN D ₃ SUPPLEMENTATION USING AN ORAL SPRAY SOLUTION RESOLVES DEFICIENCY BUT HAS NO EFFECT ON VO ₂ MAX IN GAELIC FOOTBALLERS	Gaelic soccer players Males and females (20 YO) All Caucasian	42	Ireland (55° N) fall to spring	3000 IU D ₃ spray vs. Placebo daily for 12 weeks -compliance >95%	Mass spec	BEFORE: 19ng/mL (50% insufficient and 22% deficient) AFTER: 33ng/mL in supplement group and 19ng/mL in placebo	>20ng/mL = adequate 12 to 20ng/mL = insufficient <12ng/mL = deficient	VO _{2max} , handgrip strength, VJ	No group differences	None

Wyon et al J Sci Med Sport (2013) THE INFLUENCE OF WINTER VITAMIN D SUPPLEMENTATION ON MUSCLE FUNCTION AND INJURY OCCURRENCE IN ELITE BALLET DANCERS: A CONTROLLED STUDY	Elite ballet dancers (training 6-8 hours/day) Females All Caucasian	24	UK (53° N) winter	2000 IU D ₃ daily for 16 weeks (tablets) Vs. Placebo <i>(treatment self-selected; no randomization)</i> -compliance not monitored	Not measured	Not measured	Not discussed	Isometric strength, vertical jump	VITD group had 18% improvement in isometric strength and 7% improvement in vertical jump; no significant changes in Placebo	+
Wyon et al Clin J Sports Med (2016) ACUTE EFFECTS OF VITAMIN D ₃ SUPPLEMENTATION ON MUSCLE STRENGTH IN JUDOKA ATHLETES: A RANDOMIZED PLACEBO-CONTROLLED, DOUBLE-BLIND TRIAL	Professional judoka athletes Males All caucasian	22	UK (53° N) winter	One 150,000IU D ₃ dose (tablets) vs. Placebo (retest after 8 days) -compliance 100%	Immunoassay (Tecan Infinite F500)	PRE-TREATMENT: 14 ng/mL <i>(VITD group had significantly lower 25(OH)D at baseline compared with the Placebo group)</i> POST-TREATMENT: 17 ng/mL (21% increase)	≥30ng/mL = adequate	isokinetic concentric quadriceps and hamstring muscle function assessments	VITD group had 13% increase in strength measures, while Placebo had a 3% increase	+

*A literature search was performed in October 2017 using PubMed and Google Scholar. Search terms included: [vitamin D OR vitamin D supplement OR 25-hydroxyvitamin D] AND [athlete OR exercise OR sport OR performance OR strength OR power OR cardiorespiratory]. MeSH terms were applied throughout. Only articles written (or available) in English, and those involving trained human athletes and physical performance outcomes were included. Reference lists of articles obtained via search were searched for additional references. 374 articles were obtained in the original search, and 15 articles were included in data tables.

A repeat search using the same parameters was performed in March 2019 to obtain recently published references. Of 101 articles obtained in the repeat search, 10 were included, for a total of 25 articles in data tables.

Within the tables, numerical data were rounded to the nearest whole number. Some numerical data (particularly those extracted from graphs) were estimated and may not be exact. Serum 25(OH)D was converted to ng/mL if necessary.

APPENDIX B

Institutional Review Board Approval, Carilion Clinic IRB-18-274



January 11, 2019

PI: John Epling

Re: IRB Approval for Protocol #IRB-18-274, An exploration into patterns of clinical care subsequent to non-indicated vitamin D testing in primary care

Approval Date: **01/11/2019**

Expiration Date: 01/10/2020

The Carilion Clinic Institutional Review Board (IRB) fully approved the above referenced study via expedited review under category Category 5: Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). This approval is limited to the activities conducted by the research team members as described in the approved version of the IRB Application. Modifications may not be initiated without prior IRB review and approval except where necessary to eliminate apparent immediate hazards to human participants.

All research activities must cease the day before the Expiration Date if the study has not been reapproved by the IRB. If this study is expected to extend beyond one year, please submit a continuing review request at least 45 days prior to the expiration date. HHS regulations at 45 CFR 46.109(e) require that continuing review of research be conducted by the IRB at intervals appropriate to the degree of risk but not less than once per year. The regulations make no provision for any grace period extending the conduct of the research beyond the Expiration Date. Once research activities have been completed, please submit a closure form least 30 days prior to the Expiration Date.

OBTAINING INFORMED CONSENT:

- The IRB determined that a waiver of consent for this retrospective chart review is justified under 45 CFR 46.116(d)

HIPAA WAIVER:

- The IRB has determined that a **full** HIPAA waiver of research subject authorization for this study is justified under 45 CFR 46 164.512.

In conducting this study, you are required to follow the requirements described in “INVESTIGATOR GUIDANCE: Investigator Obligations (HRP-800)”, located on the IRB website.

Please note that this letter conveys IRB approval only and does not grant institutional approval.

If your research involves any Carilion Clinic facilities, then separate arrangements must be made with the appropriate hospital or medical staff department or committees, along with the Carilion Clinic Department of Research and Development.

The Carilion Clinic Institutional Review Board would like to thank you for the opportunity to review this protocol. We wish you the best and look forward to learning of your results. If you have any questions, please do not hesitate to contact Janet McDowell at the IRB by email at jdmcowell@carilionclinic.org or 540-981-8015.

APPENDIX C

Electronic Health Record Data Extraction Details

Indicators for 25(OH)D Testing: ICD-10 Codes

E20.0	Idiopathic hypoparathyroidism
E20.9	Hypoparathyroidism, unspecified
E21.0-E21.5	Secondary hyperparathyroidism
E55.0	Rickets, active
E55.9	Vitamin D deficiency, unspecified
E64.3	Sequelae of rickets
E83.51	Hypocalcemia
E83.52	Hypercalcemia
K50	Crohn's disease [regional enteritis]
K51	Ulcerative colitis
K90.0-K90.4	Celiac disease, Tropical sprue, Blind loop syndrome, Pancreatic steatorrhea, Other malabsorption due to intolerance
K90.89	Other intestinal malabsorption
K90.9	Intestinal malabsorption, unspecified
K91.2	Postsurgical malabsorption, not elsewhere classified
M80	Osteoporosis with current pathological fracture
M81	Osteoporosis without current pathological fracture
M83	Adult osteomalacia
M83.3	Adult osteomalacia due to malnutrition
M84.3	Stress fracture
M84.4	Pathological fracture
M85.9	Disorder of bone density and structure, unspecified
M89.9	Disorder of bone, unspecified
N18.3-18.5; N18.6	Chronic kidney disease (CKD), Stage 3-5; End Stage Renal Disease

APPENDIX D

Institutional Review Board Approval, Virginia Tech #17-1239

TO: Michelle S Rockwell, Ernest Eugene, Matthew Wade Hulver
FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE: NCAA Division 1 Vit D Practice Patterns
IRB NUMBER: 17-1239

Effective March 7, 2018, the Virginia Tech Institution Review Board (IRB) approved the New Application request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at: <http://www.irb.vt.edu/pages/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Exempt, under 45 CFR 46.110 category(ies) 2,4**
Protocol Approval Date: **March 7, 2018**
Protocol Expiration Date: N/A
Continuing Review Due Date*: N/A

***Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.**

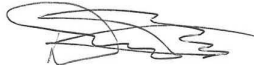
FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

APPENDIX E

Institutional Review Board Approval, Virginia Tech #17-009

MEMORANDUM

DATE: March 6, 2017 

TO: Matthew Wade Hulver, Madlyn Irene Frisard, Michelle S Rockwell, Mark Rogers, Gregory Thomas Donlon, Jennie Zabinsky, Janet T Rinehart, Megan Elizabeth Evans

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Vitamin D, Athletic Performance, and Health

IRB NUMBER: 17-009

Effective February 13, 2017, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the New Application request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at: <http://www.irb.vt.edu/pages/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **February 13, 2017**
Protocol Expiration Date: **February 12, 2018**
Continuing Review Due Date*: **January 29, 2018**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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MEMORANDUM

DATE: February 13, 2018

TO: Matthew Wade Hulver, Madlyn Irene Frisard, Michelle S Rockwell, Mark Rogers, Jennie Zabinsky, Janet T Rinehart, Megan Elizabeth Evans, Sarah Vaughan, Ernest Eugene, Abby Gail Bellows, et. al.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Vitamin D, Athletic Performance, and Health

IRB NUMBER: 17-009

Effective February 13, 2018, the Virginia Tech Institutional Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at: <http://www.irb.vt.edu/pages/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **February 13, 2018**
Protocol Expiration Date: **February 12, 2019**
Continuing Review Due Date*: **January 29, 2019**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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Office of Research Compliance
 Institutional Review Board
 North End Center, Suite 4120
 300 Turner Street NW
 Blacksburg, Virginia 24061
 540/231-3732 Fax 540/231-0959
 email irb@ut.edu
 website <http://www.irb.ut.edu>

MEMORANDUM

DATE: January 31, 2019
TO: Matthew Wade Hulver, Madlyn Irene Frisard, Michelle S Rockwell, Mark Rogers, Jennie Zabinsky, Janet T Rinehart, Megan Elizabeth Evans, Sarah Vaughan, Ernest Eugene, Abby Gail Bellows, et. al.
FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE: Vitamin D, Athletic Performance, and Health
IRB NUMBER: 17-009

Effective January 31, 2019, the Virginia Tech Institutional Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at: <https://secure.research.vt.edu/external/irb/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Expedited, under 45 CFR 46.110 category(ies) 8c**
 Protocol Approval Date: **February 13, 2019**
 Protocol Expiration Date: **February 12, 2020**
 Continuing Review Due Date*: **January 29, 2020**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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APPENDIX F

Informed Consent Form (VT IRB #17-009, Vitamin D and Basketball Study)

INFORMED CONSENT FOR PARTICIPANTS OF INVESTIGATIVE PROJECTS

Department of Human Nutrition, Foods and Exercise

Virginia Tech

TITLE: Vitamin D, Athletic Performance, and Health

**INVESTIGATORS: Mathew W. Hulver, Ph.D.
Madlyn Frisard, PhD.
Michelle Rockwell, MS, RD, CSSD
Jennie Zabinsky, MAEd, RD
Janet Rinehart
Megan Evans
Ernest Eugene**

MEDICAL DIRECTOR: Mark Rogers, D.O.

PURPOSE: Vitamin D is a hormone that is important for bone and muscle health. As such, not having enough vitamin D in your body is associated with increased risk of injury and reduced health and athletic performance. It is often recommended that individuals who have low levels of vitamin D take vitamin D supplements. However, the effects of vitamin D supplementation on health and athletic performance in athletes are not known. The goal of this study is to determine whether vitamin D supplementation improves your health, reduces your risk of injury, and improves your athletic performance. The information collected in this project will be published and will be used in theses and dissertations. The information collected in this project may also be shared with the coaches, sports medicine staff, and other interested parties within the athletic training department at Virginia Tech.

METHODS:

You are being asked to be involved in a study to determine whether vitamin D supplementation affects your health and athletic performance. If you agree to participate, a small sample of blood will be taken to determine your blood levels of vitamin D. You will not be told your levels of vitamin D until the end of the study. You will then be given either none, one vitamin D tablet (5000IU) or 2 vitamin D tablets (10000IU) to take for the duration of your off-season training period (depending on your vitamin D levels). This will be about 12 weeks. You will continue to eat your normal diet and maintain your regular physical activity/ training habits. If you leave the study early, you will be told your vitamin D levels from what has been measured up to the point of your departure from the study. You will be given tablets to take home over the weekends or if at any time you are out of town (or otherwise not reporting to campus for training sessions). You will meet with study investigators once per week to discuss any problems (side effects, etc.), or other issues you may be having with taking the supplements. You will also be asked to return any pills you have not taken. The information obtained in this study will be compared to your maximum performance test results (conducted as part of your regular training). We

will collect this information from your athletic record from the athletic training/ sports medicine staff. By signing this consent, you are agreeing to let us collect this information.

If you agree to be involved in this study you will first have to fill out an online screening questionnaire. The additional tests are described below under each testing session. The full details are outlined further below, but a small amount of blood will be taken to measure vitamin D levels as well as several other determinants of health. Your vitamin D results may be discussed with the study medical director to determine if you can be a subject. You may be able to be a subject if you are between 18 and 30 years of age and you are an athlete for a sanctioned Virginia Tech sport. If you are a woman, you will not be able to participate if you are pregnant or trying to become pregnant. You will not be eligible to participate in this study if you are currently taking vitamin D (>600IU/day), calcium (>1000mg/dl), or any performance enhancing supplements (example, creatine), or any other medication or nutritional supplements that might influence the study variables. You will not be eligible to participate in this study if you have any cardiac or thyroid problems, have diabetes, or epilepsy.

Study Session:

To be included in the study, you will complete all components of the study session one and two (except for the health history), two times, once before and once after your off-season training period. You will complete the injury/ respiratory illness questionnaire every week. You will also be asked to meet with study investigators once per week to make sure you are not having any problems as result of participating in the study.

Testing Session One: Approximate time required is 1 hour

This study will take place at the Virginia Tech Athletic facilities.

- **Overnight Fast:** You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Health History:** You will be asked to complete an online health history questionnaire. This questionnaire is used to screen for health problems or reasons you should not participate in this study. The study staff will go over your medical history during the study visit.
 - **Catheter and Blood Draw:** A small needle will be inserted in your arm to draw blood (approximately 3 tablespoons). We will measure your complete blood count (red blood cells, white blood cells, hemoglobin, hematocrit, and platelets), iron, vitamin D, testosterone, free testosterone, and other measures of health.
- **Vitamin D and Calcium Screener:** You will be asked to complete an online vitamin D and Calcium questionnaire to determine your dietary intake of vitamin D and calcium.

Testing Session Two: Approximate time required is 30 minutes

This study will take place at the War Memorial Hall.

- **Weight and Height:** Your height and weight will also be measured at this time. Your body weight will be measured on a standard digital scale. Your height will be measured with a standard stadiometer (ruler on the wall). Your waist, hip, and neck circumference will be measured using a measuring tape.
- **Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require

you to collect 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.

- **Body Composition:** This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 10 minutes and there is no pain associated with the procedure.
- **Bone Density:** This test is to measure your bone density. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. We will measure your bone density in your leg, hip, and lower back. This test takes approximately 5 minutes and there is no pain associated with the procedure.

Weekly Testing session three:

You will be asked to complete the following online questionnaire every week for 8 weeks. Approximate time required is 15 minutes.

- **Injury/ Respiratory Illness Questionnaire:** You will be asked to complete a questionnaire about any recent injuries or respiratory infections.

You will be asked to complete the following questionnaire once per week for 8 weeks. Approximate time required is 15 minutes.

- **Compliance Form and Side Effects Questionnaire:** You will be asked to complete an online questionnaire about whether you have missed taking any of the tablets and if you are having any side effects while taking the tablets.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the investigators of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend the scheduled experiments.
- Follow all participant instructions for each session.

RISKS OF PARTICIPATION

- **Catheter and Blood Draw:** Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this should persist for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant

blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff experienced in catheter placement and blood draws will minimize these risks.

- HIV/ Hepatitis B/ Hepatitis C: In the event a researcher or other staff person is inadvertently exposed to your blood, your blood will be tested for the presence of HIV, the Hepatitis B Virus, and the Hepatitis C Virus. There will not be any cost to you for this test. The research team will follow proper procedures for testing and reporting as outlined by Virginia State Law, which includes sending the sample to a certified laboratory. Please note that, should your blood require testing, you will be informed of your test results and provided with the opportunity to receive appropriate and timely counseling. In addition, positive test results will be sent to the local health department.
- DEXA Scan: The amount of radiation that you will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however, the exact increase in such risk is not known.
- Vitamin D Supplementation: The maximum amount of vitamin D that you may receive is less than what is expected to cause vitamin D toxicity. Vitamin D toxicity can result in symptoms such as weight loss, weakness, fatigue, sleepiness, headache, loss of appetite, nausea, and vomiting. An additional risk of Vitamin D supplementation may be that you are taking in less Vitamin D by eating/drinking differently as a result of knowing that you may be taking a vitamin D supplement. Therefore it is important to keep your food intake the same during the entire time you are participating in the study. Additionally, you will meet with study investigators each week to discuss any possible side effects.
- It is not possible to identify all potential risks in an experiential study. However, the study doctors and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory.
- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or study staff.

BENEFITS OF PARTICIPATION

There are no direct benefits of participating in this study. However, your participation will provide you with the following information at the end of the study. If you decide to stop your participation in the study before you finish the study, you will be provided with the information from the tests that have been conducted to date.

- Information on your body fat percentage.
- Information on your bone mineral density.
- Information on your blood vitamin D levels.
- Educational materials about Vitamin D.

COMPENSATION

You will not be monetarily compensated for participating in this study.

CONFIDENTIALITY

The data from this study will be kept strictly confidential. No data will be released to anyone but those working on the project without your written permission. Data will be identified by subject numbers, without anything to identify you by name. In the event that any of your information indicate that you are at increased risk for any disease, Dr. Rogers or investigators may want to share this information with your doctor but he will request your approval first.

FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. In addition, circumstances could arise which would lead to your exclusion from the study. For example, lack of compliance to instructions, failure to attend testing sessions, and illness could be reasons for the researchers to stop your participation in the study. Other reasons include an inability by the researchers to obtain muscle, body fat or other measurements that are necessary for the study.

APPROVAL OF RESEARCH

This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech. You will receive a copy of this form to take with you.

SUBJECT PERMISSION

I have read the informed consent and have had all my questions satisfactorily answered. I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:

- Principal Investigator: Matthew Hulver, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-7354; After hours: (540) 809-0584
- Personnel: Madlyn Frisard, Research Assistant Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-9994; After hours: (540) 818-9907

Should you have any questions or concerns about the study's conduct or your rights as a research subject, or need to report a research-related injury or event, you may contact the Virginia Tech Institutional Review Board at irb@vt.edu or (540) 231-3732.

Name of Subject (please print) _____

Signature of Subject _____ Date _____

Signature of Witness _____ Date _____

APPENDIX G

Vitamin D and Athletes- Screening Questionnaire

Vitamin D Study Screening
(administered online through Qualtrics)

Welcome to the online screening survey for the Vitamin D study being conducted in the Department of Human Nutrition, Foods, and Exercise. Please fill out the following questions to help us determine your eligibility to participate in the study. We appreciate your interest in our research study!

If it is determined that you are not eligible to participate in this research study, this data will be discarded.

*Note: If the question is multiple choice, please click on the box to highlight your desired answer.

For contact purposes, please enter the following information:

First and Last Name

Email Address

Preferred Phone Number

Preferred Mode of Contact (click on one of the boxes below)

Email (1)

Phone (2)

Home Address (City, State minimum)

The following questions are related to your personal and health information.

Gender

Male (1)

Female (2)

Age

Height - Feet

Height - Inches

Weight - Pounds

Are you taking any prescribed medications, over-the-counter medications and/or supplements or vitamins?

- Yes (1)
- No (2)

List your current medications and/or supplements or vitamins. Please include how long you have been on your current dose.

Have you been diagnosed with cardiac or thyroid problems (Example, Hypothyroidism)?

- Yes (1)
- No (2)

Have you been diagnosed with diabetes?

- Yes (1)
- No (2)

Have you been diagnosed with epilepsy?

- Yes (1)
- No (2)

Are you pregnant or nursing or planning on becoming pregnant?

- Yes (1)
- No (2)

Is there anything else you would like to tell us about your medical history?

- Yes (1)
- No (2)

Please tell us about your medical history.

Thank you! You will be contacted by study staff once your survey entry is reviewed.

APPENDIX H

Vitamin D and Athletes- Health History Questionnaire

Virginia Tech
Department of Human Nutrition, Foods, and Exercise

HEALTH HISTORY QUESTIONNAIRE (this form is filled out online in Qualtrics)

Please do not use a public computer to fill out this form. Also clear your internet browser and close out the browser once you have submitted the form. Please note that there is always a risk of filling out forms online.

STUDY _____ **DATE** _____

PLEASE PRINT

Name: _____

Address: _____

City: _____ State: _____ Zip Code _____

Home Phone: _____ Work Phone: _____

E-mail address: _____

Emergency Contact: _____ Phone: _____

Relation to you: _____

Age: _____ Sex: _____

Race and/or Ethnic Origin

- American Indian or Alaskan Native Asian or Pacific Islander Black, not of Hispanic Origin
 Hispanic White, not of Hispanic Origin
 Other

GENERAL MEDICAL HISTORY

Do you have any current medical conditions? YES NO If Yes, please explain:

Are you currently taking any medications or supplements, including aspirin, NSAIDS, or other over-the-counter products?
YES NO If Yes, fill out table below:

Medication/Supplement	Reason	Times taken per Day	Amount	Taken for how long?
------------------------------	---------------	----------------------------	---------------	----------------------------

Have you had any major illnesses in the past? YES NO If Yes, please explain:

Have you ever been hospitalized or had surgery?
date and type of surgery, if possible)

YES

NO

If Yes, please explain: (include

4. FAMILY HISTORY

	Age (if alive)	Age of Death	Cause of Death
Father	_____	_____	_____
Mother	_____	_____	_____
Brothers/Sisters	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

Do you have a family history of any of the following: (Blood relatives only; please give age at diagnosis, if possible)

	YES	NO	Relation	Age at Diagnosis
a. High blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	_____	_____
b. Heart Attack	<input type="checkbox"/>	<input type="checkbox"/>	_____	_____
c. Coronary bypass surgery	<input type="checkbox"/>	<input type="checkbox"/>	_____	_____
d. Stroke	<input type="checkbox"/>	<input type="checkbox"/>	_____	_____
e. Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	_____	_____
f. Obesity	<input type="checkbox"/>	<input type="checkbox"/>	_____	_____

6. TOBACCO/ALCOHOL HISTORY (check one)

- None
- Quit (when) _____
- Cigarette
- Cigar
- Pipe
- Chew Tobacco
- Snuff

CURRENT TOBACCO USE

- (if applicable)
per day
- Cigarette _____
 - Cigar _____
 - Pipe _____
 - Chew Tobacco _____
 - Snuff _____

Total years of tobacco use _____

Do you consume alcohol? Drinks per day ____ Drinks per week ____

7. CARDIORESPIRATORY/METABOLIC HISTORY

	YES	NO
Are you presently diagnosed with heart disease?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have any history of heart disease?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have a heart murmur?	<input type="checkbox"/>	<input type="checkbox"/>
Occasional chest pain or pressure?	<input type="checkbox"/>	<input type="checkbox"/>

Chest pain or pressure on exertion?	<input type="checkbox"/>	<input type="checkbox"/>
Episodes of fainting?	<input type="checkbox"/>	<input type="checkbox"/>
Daily coughing?	<input type="checkbox"/>	<input type="checkbox"/>
High blood pressure?	<input type="checkbox"/>	<input type="checkbox"/>
Shortness of breath?		
At rest?	<input type="checkbox"/>	<input type="checkbox"/>
lying down?	<input type="checkbox"/>	<input type="checkbox"/>
After 2 flights of stairs?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have asthma?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have a history of bleeding disorders?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have a history of problems with blood clotting?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have high cholesterol? Or, low good (HDL) cholesterol?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have thyroid problems?	<input type="checkbox"/>	<input type="checkbox"/>

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

8. MUSCULOSKELETAL HISTORY

	YES	NO
Any current muscle injury or illness?	<input type="checkbox"/>	<input type="checkbox"/>
Any muscle injuries in the past?	<input type="checkbox"/>	<input type="checkbox"/>
Do you experience muscle pain at rest?	<input type="checkbox"/>	<input type="checkbox"/>
Do you experience muscle pain on exertion?	<input type="checkbox"/>	<input type="checkbox"/>
Any current bone or joint (including spinal) injuries?	<input type="checkbox"/>	<input type="checkbox"/>
Any previous bone or joint (including spinal) injuries?	<input type="checkbox"/>	<input type="checkbox"/>
Do you ever experience painful joints?	<input type="checkbox"/>	<input type="checkbox"/>
Do you ever experience swollen joints?	<input type="checkbox"/>	<input type="checkbox"/>
Do you ever experience edema (fluid buildup)?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have pain in your legs when you walk?	<input type="checkbox"/>	<input type="checkbox"/>

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

11. OBSTETRIC/GYNECOLOGICAL HISTORY FOR FEMALES

Do you have a normal menstrual cycle (1 menses each ~1 month)?	YES	NO
	<input type="checkbox"/>	<input type="checkbox"/>

If no, please indicate frequency _____

Do you take any kind of contraceptive (oral, injectable, implant)?

If yes, please indicate type and name _____

Signature: _____

Date: _____

Witness: _____
Print Name Signature

Date: _____

Reviewer: _____

Date: _____

APPENDIX I

Vitamin D and Athletes- Vitamin D and Calcium Food Frequency Questionnaire & Lifestyle Questionnaire

Vitamin D and Calcium Screening

Please describe how often you have consumed the foods and beverages below in the last 30 days. Indicate your daily or weekly consumption of the food or beverage, making careful note of the serving size listed. For example, if you consume 20 ounces of chocolate milk each day, you would mark “1-3 servings/day” since 20 ounces = 2.5 servings of milk.

Food	Serving Size	Never	< 1 serving/ week	1-3 servings /week	4-6 servings /week	1 serving/ day	1-3 servings /day	> 4 servings /day	Comments
Cold Cereal	1 cup								
Milk (whole, lowfat, skim, chocolate)	8 ounces/ 1 cup								
Milk (any) over cereal	4 ounces/ ½ cup								
Milk (any) or cream in coffee	1 ounce/ 2 Tablespoons								
Yogurt (flavored or fruited)	8 ounces yogurt/ 6 ounces Greek yogurt								
Yogurt (plain)	8 ounces yogurt/ 6 ounces Greek yogurt								
Ice Cream	½ cup								
Frozen Yogurt	1 cup								
Ice Cream Bar/Frozen Fudge Bar	1 item								
Cheese: American or Mozzarella	1 ounce/ 1 slice								
Cheese (hard): Cheddar, Swiss, Provolone, etc.	1 ounce/ 1 slice								
Cottage cheese	1 cup								
Cheese dip or cheese spread	1 ounce								
Pudding made with milk	½ cup								
Creamy soup or sauce	1 cup								
Broccoli	½ cup								
Greens: mustard, turnip, collard, spinach, etc.	½ cup								
Calcium-fortified juice (orange, others)	8 ounces/ 1 cup								
Bread (white, wheat, pita, English Muffin)	1 sauce								

Bagel or muffin	1 medium								
Biscuit or cornbread	2" diameter								
Pancakes or waffles (frozen)	4" diameter								
Pancakes or waffles (homemade)	4" diameter								
Beans: red, pinto, lima, etc.	1 cup								
Tofu, regular	1 cup								
Pasta	1 cup								
Eggs, cooked any style	1								
Hamburger	4 ounces								
Cheeseburger	4 ounces								
Oysters, shrimp, crab, crawfish, herring	3 ounces								
Canned salmon (with bones)	3.75-ounce can								
Sardines	3.75-ounce can								
Cake	3"X3"X2" piece								
Almonds	¼ cup								
Milk Chocolate	1.6-ounce bar								
Recovery shake, nutrition shake, meal replacement formulas (please specify type and serving size)									
Sports bars (please specify type and serving size)									

Please answer the following questions to the best of your ability.

- 1) Have you received a suntan in the past 12 months? Yes or No
- 2) Do you use sunscreen? Yes or No
- 3) On average, how much sun exposure have you had in the past week? less than 5 minutes per day, 5–15 minutes per day, 15–30 minutes per day, more than 30 minutes per day
- 4) Have you used a tanning booth in the past year? Yes or No
- 5) How many servings of milk do you get daily? _____
- 6) Do you take multivitamins? Yes or No
If yes, how many multivitamin tablets do you take daily? _____
- 7) Do you take vitamin D supplements or calcium with vitamin D? Yes or No
If so, how many IU per day? _____
- 8) Do you take cod liver oil or omega-3 fatty acids (fish oil)? Yes or No
- 9) What is your ethnic background?
- 10) Have you been diagnosed with Crohn's disease, ulcerative colitis, or celiac sprue? Yes or No
- 11) Have you had diarrhea in the past 2 weeks? Yes or No

Taylor et al. Validation of a Food Frequency Questionnaire for Determining Calcium and Vitamin D Intake in Adolescent Girls with Anorexia Nervosa. J Am Diet Assoc. 2009; 109:479-485.

Papandreou et al. Validation of a Food Frequency Questionnaire for Vitamin D and Calcium Intake in Healthy Female College Students. Food and Nutrition Sciences. 2014; 5: 2048-2052.

Bolek-Berquist, et. Al. Use of a Questionnaire to Assess Vitamin D Status in Young Adults. Public health Nutrition. 2009; 12(2): 236

APPENDIX J

Vitamin D and Athletes- Injury and Illness Questionnaire

Injury/ Illness Questionnaire

Subject ID#: _____

Date: _____

Study: _____

- 1) Have you experienced a new fracture in the last week (Circle one)? Yes No
- 2) Have you experienced a new stress fracture in the last week (Circle one)? Yes No
- 3) Have you experienced a muscle-related injury in the last week (Circle one)? Yes No
- 4) Have you experienced any other soft tissue injury in the last week (Circle one)? Yes No

If you answered yes above, please include how the injury occurred, where on the body the injury occurred, and specify the injury.

- 5) Have you experienced an illness in the last week (Circle one)? Yes No

If you answered yes above, please describe symptoms, include when you first developed symptoms and how long they lasted):

APPENDIX K

Serum 25(OH)D Assay Pilot Test Data

Serum 25(OH)D Pilot Test

Our study design (Chapters 6 and 7) required rapid (<3 days) measurement of serum 25-hydroxyvitamin D (25(OH)D). Two 25(OH)D assays options that could meet this timeframe were available: LabCorp and Quest Diagnostics. They differed in cost (\$19 vs. \$84 per test, respectively) and accessibility to Virginia Tech Athletics (at the time, they were required to use LabCorp for all blood analyses).

Serum 25(OH)D was measured in three pilot samples using two different assays in February 2017. An Immunochemiluminometric assay (IA) using DiaSorin LIAISON® instrumentation was performed by LabCorp (Burlington, North Carolina, U.S.) and Chromatography/Mass Spectrometry (MS) was performed by Quest Diagnostics (Roanoke, Virginia, U.S.).

RESULTS:

Sample #	IA-1 (ng/mL)	IA-2 (ng/mL)	Intra-assay variability	MS-1 (ng/mL)	MS-2 (ng/mL)	Intra-assay variability	Inter-assay difference
101	24.1	25.0	3.6%	21.1	24.9	15.3%	6.5%
102	33.2	32.3	2.7%	31.8	27.4	13.8%	9.8%
103	13.3	11.1	16.5%	12.8	12.7	0.7%	4.3%

Intra and inter-assay variability in 25(OH)D assays is a common, well-documented issue (REF). Our goal was variability <10%. Inter-assay variability was <10%, but intra-assay variability exceeded this goal in one IA sample, and two MS samples. The decision was made to move forward with IA methodology since 1) two samples showed minimal variability, 2) VT Athletics would be able to compare 25(OH)D measurements obtained in this study to previous and former 25(OH)D measurements, and 3) the cost was substantially lower.

APPENDIX L

Informed Consent Form (VT IRB #17-009, Vitamin D and Swimmer Study)

Informed Consent for Participants of Investigative Projects

Department of Human Nutrition, Foods and Exercise

Virginia Tech

TITLE: Vitamin D, Athletic Performance, and Health

INVESTIGATORS: **Mathew W. Hulver, Ph.D.**
Madlyn Frisard, PhD.
Michelle Rockwell, MS, RD, CSSD
Jennie Zabinsky, MAEd, RD
Janet Rinehart
Megan Evans
Ernest Eugene

MEDICAL DIRECTOR: Mark Rogers, D.O.

PURPOSE: Vitamin D is a hormone that is important for bone and muscle health. As such, not having enough vitamin D in your body is associated with increased risk of injury and reduced health and athletic performance. It is often recommended that individuals who have low levels of vitamin D take vitamin D supplements. However, the effects of vitamin D supplementation on health and athletic performance in athletes are not known. The goal of this study is to determine whether vitamin D levels change over the course of the year (following winter and following the summer) and whether supplementation improves health, reduces risk of injury, and improves athletic performance. The information collected in this project will be published and will be used in theses and dissertations. The information collected in this project may also be shared with the coaches, sports medicine staff, and other interested parties within the athletic training department at Virginia Tech.

METHODS:

You are being asked to be involved in a study to determine whether vitamin D supplementation affects your health and athletic performance. If you agree to participate, a small sample of blood will be taken to determine your blood levels of vitamin D. This measurement will take place in April, August, and again in November. You will not be told your levels of vitamin D until the end of the study. Following the August measurement, you will then be given either vitamin D (5000IU or 10000IU) or placebo tablets to take for the duration of your fall training period. This will be about 16 weeks. You will receive two tablets per day. You will continue to eat your normal diet and maintain your regular physical activity/ training habits. You will not be told until the end of the study whether you were taking Vitamin D or placebo. However, if you leave the study early, you will be told your vitamin D levels from what has been measured up to the point of your departure from the study and whether or not you were taking Vitamin D or placebo. You will be given tablets to take home over the weekends or if at any time you are out of time (or otherwise not reporting to campus for training sessions). You will meet with study investigators once per week to discuss any problems (side effects, etc.), or other issues you may be having with taking the supplements. You will also be asked to return any pills you have not taken. The information obtained in this study will be compared to your maximum performance test results (conducted as part of your regular training). We will collect this information

from your athletic record from the athletic training/ sports medicine staff. By signing this consent, you are agreeing to let us collect this information.

If you agree to be involved in this study you will first have to fill out an online screening questionnaire. The additional tests are described below under each testing session. The full details are outlined further below, but a small amount of blood will be taken to measure vitamin D levels as well as several other determinants of health. Your vitamin D results may be discussed with the study medical director to determine if you can be a subject. You may be able to be a subject if you are between 18 and 30 years of age and you are an athlete for a sanctioned Virginia Tech sport. If you are a woman, you will not be able to participate if you are pregnant or trying to become pregnant. You will not be eligible to participate in this study if you are currently taking vitamin D (>600IU/day), calcium (>1000mg/dl), or any performance enhancing supplements (example, creatine), or any other medication or nutritional supplements that might influence the study variables. You will not be eligible to participate in this study if you have any cardiac or thyroid problems, have diabetes, or epilepsy.

Study Session:

To be included in the study, you will complete all components of the study session one and two (except for the health history and Vitamin D and Calcium Screener), three times; once in the spring (April), once at the end of the summer (August), and after the fall training period (November). You will complete the online injury/ respiratory illness questionnaire once per week during the training period. You will also be asked to meet with study investigators once per week during the training period to make sure you are not having any problems as result of participating in the study.

Testing Session One: Approximate time required is 1 hour

This study will take place at the Virginia Tech Athletic facilities.

- **Overnight Fast:** You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Health History:** You will be asked to complete an online health history questionnaire. This questionnaire is used to screen for health problems or reasons you should not participate in this study. The study staff will go over your medical history during the study visit.
 - **Catheter and Blood Draw:** A small needle will be inserted in your arm to draw blood (approximately 3 tablespoons). We will measure your complete blood count (red blood cells, white blood cells, hemoglobin, hematocrit, and platelets), iron, vitamin D, testosterone, free testosterone, and other measures of health.
- **Vitamin D and Calcium Screener:** You will be asked to complete an online vitamin D and Calcium questionnaire to determine your dietary intake of vitamin D and calcium.

Testing Session Two: Approximate time required is 30 minutes

This study will take place at the War Memorial Hall.

- **Weight and Height:** Your height and weight will also be measured at this time. Your body weight will be measured on a standard digital scale. Your height will be measured with a standard stadiometer (ruler on the wall). Your waist, hip, and neck circumference will be measured using a measuring tape.

- **Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require you to collect 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
 - **Body Composition:** This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 10 minutes and there is no pain associated with the procedure.
- **Bone Density:** This test is to measure your bone density. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. We will measure your bone density in your leg, hip, and lower back. This test takes approximately 5 minutes and there is no pain associated with the procedure.

Weekly testing session three:

You will be asked to complete the following online questionnaire every week for 12 weeks. Approximate time required is 15 minutes.

- **Injury/ Respiratory Illness Questionnaire:** You will be asked to complete a questionnaire about any recent injuries or respiratory infections.

You will be asked to complete the following questionnaire once per week for 12 weeks. Approximate time required is 15 minutes.

- **Compliance Form and Side Effects Questionnaire:** You will be asked to complete an online questionnaire about whether you have missed taking any of the tablets and if you are having any side effects while taking the tablets.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the investigators of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend the scheduled experiments.
- Follow all participant instructions for each session.

RISKS OF PARTICIPATION

- **Catheter and Blood Draw:** Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this should persist for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk

of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff experienced in catheter placement and blood draws will minimize these risks.

- **HIV/ Hepatitis B/ Hepatitis C:** In the event a researcher or other staff person is inadvertently exposed to your blood, your blood will be tested for the presence of HIV, the Hepatitis B Virus, and the Hepatitis C Virus. There will not be any cost to you for this test. The research team will follow proper procedures for testing and reporting as outlined by Virginia State Law, which includes sending the sample to a certified laboratory. Please note that, should your blood require testing, you will be informed of your test results and provided with the opportunity to receive appropriate and timely counseling. In addition, positive test results will be sent to the local health department.
- **DEXA Scan:** The amount of radiation that you will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however, the exact increase in such risk is not known.
- **Vitamin D Supplementation:** The maximum amount of vitamin D that you may receive is less than what is expected to cause vitamin D toxicity. Vitamin D toxicity can result in symptoms such as weight loss, weakness, fatigue, sleepiness, headache, loss of appetite, nausea, and vomiting. An additional risk of Vitamin D supplementation may be that you are taking in less Vitamin D by eating/drinking differently as a result of knowing that you may be taking a vitamin D supplement. Therefore it is important to keep your food intake the same during the entire time you are participating in the study. Additionally, you will meet with study investigators each week to discuss any possible side effects.
- It is not possible to identify all potential risks in an experiential study. However, the study doctors and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory.
- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or study staff.

BENEFITS OF PARTICIPATION

There are no direct benefits of participating in this study. However, your participation will provide you with the following information at the end of the study. If you decide to stop your participation in the study before you finish the study, you will be provided with the information from the tests that have been conducted to date.

- Information on your body fat percentage.
- Information on your bone mineral density.
- Information on your blood vitamin D levels.

- Educational materials about Vitamin D.

COMPENSATION

You will not be monetarily compensated for participating in this study.

CONFIDENTIALITY

The data from this study will be kept strictly confidential. No data will be released to anyone but those working on the project without your written permission. Data will be identified by subject numbers, without anything to identify you by name. In the event that any of your information indicate that you are at increased risk for any disease, Dr. Rogers or investigators may want to share this information with your doctor but he will request your approval first.

FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. In addition, circumstances could arise which would lead to your exclusion from the study. For example, lack of compliance to instructions, failure to attend testing sessions, and illness could be reasons for the researchers to stop your participation in the study. Other reasons include an inability by the researchers to obtain muscle, body fat or other measurements that are necessary for the study.

APPROVAL OF RESEARCH

This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech. You will receive a copy of this form to take with you.

SUBJECT PERMISSION

I have read the informed consent and have had all my questions satisfactorily answered. I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:

- Principal Investigator: Matthew Hulver, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-7354; After hours: (540) 809-0584
- Personnel: Madlyn Frisard, Research Assistant Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-9994; After hours: (540) 818-9907

Should you have any questions or concerns about the study's conduct or your rights as a research subject, or need to report a research-related injury or event, you may contact the Virginia Tech Institutional Review Board at irb@vt.edu or (540) 231-3732.

Name of Subject (please print) _____

Signature of Subject _____ Date _____

Signature of Witness _____ Date _____

APPENDIX M

Institutional Review Board Approval, Virginia Tech #17-1157, Virginia Tech

MEMORANDUM

DATE: January 8, 2018

TO: Kevin Davy, Michelle S Rockwell, Janet T Rinehart, Matthew Wade Hulver, Elaine Lynn Marinik

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: DXA Cross-Calibration

IRB NUMBER: 17-1157

Effective December 20, 2017, the Virginia Tech Institutional Review Board (IRB), at a convened meeting, approved the New Application request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

<http://www.irb.vt.edu/pages/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **January 4, 2018**
Protocol Expiration Date: **January 3, 2019**
Continuing Review Due Date*: **November 26, 2018**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Invent the Future

MEMORANDUM

DATE: January 2, 2019
TO: Kevin Davy, Michelle S Rockwell, Janet T Rinehart, Matthew Wade Hulver, Elaine Lynn Marinik, Jana Leotta
FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE: DXA Cross-Calibration
IRB NUMBER: 17-1157

Effective January 2, 2019, the Virginia Tech Institutional Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at: <https://secure.research.vt.edu/external/irb/responsibilities.htm>

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **January 4, 2019**
Protocol Expiration Date: **January 3, 2020**
Continuing Review Due Date*: **November 25, 2019**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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APPENDIX N

Informed Consent Form (VT IRB #17-1157, DXA Cross Calibration Study)

Consent Document
Department of Human Nutrition, Foods, and Exercise
Virginia Tech

TITLE: **Dual-Energy X-ray Absorptiometry (DXA): Interdevice Variability**

INVESTIGATORS: Elaina Marinik PhD; Janet Rinehart; Michelle Rockwell MS, RD; Matt Hulver PhD; and Kevin Davy PhD

PURPOSE

Dual-energy X-ray absorptiometry (DXA) is considered the gold standard technique for assessing bone mineral density (BMD) and is an accurate and broadly utilized method of measuring body composition. A DXA scan employs low-dose X-ray technology to differentiate tissue types (ex: bone, fat mass, lean body mass).

The Human Integrated Physiology Laboratory within the Department of Human Nutrition, Foods, and Exercise at Virginia Tech utilizes DXA in several research studies. There are currently two DXA scanners in the lab. The International Society for Clinical Densitometry (ISCD) estimates that interdevice variability may be up to +/- 5-7% for BMD and 3-5% for body fat%. Thus, it may not be possible to compare subject or study measurements performed on different machines.

The aim of the current study is to assess the extent of agreement in BMD and body composition between two DXA scanners.

SUBJECTS

One hundred 18-65 year-old individuals, 50 males and 50 females of all races and ethnic backgrounds, will serve as subjects. In order to participate, individuals must be shorter than 6'1" and weigh less than 400 pounds due to scanner capabilities. Individuals who are unable to lie motionless on their backs for 30 minutes will not be able to participate in the study. Those with metal implants or devices that cannot be removed, and jewelry that cannot be removed will be excluded from participation. Women will be excluded from the study if they are pregnant.

PROCEDURES

You are invited to participate in a study that involves comparing results of two DXA scanners. You will have two DXA scans separated by a 10-15 minute break.

Before beginning DXA scans, females will be asked to provide a urine sample for a pregnancy test. All females will be required to have the pregnancy test except those who have been post-menopausal for more than one year (i.e.: more than one year since your last menstrual period). If you are pregnant you will not be able to participate in the study.

Height and weight will then be measured using a stadiometer and digital scale. You will then be asked to remove all metal clothing and jewelry.

To begin your first scan, you will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle, and fat in your body (bone density and body composition). These scans take about 15-20 minutes in all. The technician will reposition you in between scans. You will need to lay still during the scans, but you will not feel anything. There is no pain associated with the procedure.

Following the 10-15 minute break, your second scan will be on a different scanner, across the hall and will involve a repeat of the scan process described above. The order of your scans will be randomized (a procedure similar to flipping a coin).

SUMMARY OF SUBJECT RESPONSIBILITIES

- Follow subject instructions.
- Be still during DXA scans.

RISKS OF PARTICIPATION

- DXA Scan: The amount of radiation that you will receive in the DXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray per DXA scan, for a total of 1/10 of a chest x-ray for two scans. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however, the exact increase in such risk is not known.

- It is not possible to identify all potential risks. However, the staff will take all possible safeguards to minimize any known and potential risks to your well-being. All of the procedures are well established and used routinely in the investigator's research program.

COMPENSATION

- There will be no compensation for your participation.

BENEFITS OF PARTICIPATION

Your participation will provide you with:

- Information about your body composition and bone density and how it relates to health.

Information gained from the study will be used to calibrate DXA scanners in the lab.

CONFIDENTIALITY

The data from this study will be kept strictly confidential. No identifiable data will be released to anyone but those working on the project. Subject numbers without anything to identify your name will identify data.

FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time for any reason. Simply inform the researchers of your intention to cease participation. Circumstances may come up that the researcher will determine that you should not continue as a subject in the study. For example, lack of compliance to instructions, failure to attend testing sessions and illness could be reasons for the researchers to stop your participation in the study.

INJURY DURING PARTICIPATION

Neither the researchers nor the university have money aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long-term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

APPROVAL OF RESEARCH

This research has been authorized, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech. You will receive a copy of this form to take with you.

SUBJECT PERMISSION

I have read the informed consent and I have had all my questions answered to my satisfaction. I hereby give my voluntary consent to be a participant in this research study. I understand that I may withdraw from the study at any time.

If you have any questions about the study, you may contact:

- Kevin Davy, PhD, Professor, Department of Human Nutrition, Foods, and Exercise. (540) 230-0486 or (540) 231-3487.
- Michelle Rockwell, Doctoral Student, Department of Human Nutrition, Foods, and Exercise. (540) 231-9572.

If you have any questions about your rights as a participant, you may contact:

- Institutional Review Board, Office of the Vice President for Research and Innovation, Virginia Tech: (540) 231-3732

Name of Subject (please print) _____

Signature of Subject _____ **Date** _____

APPENDIX O

Dual-Energy X-Ray Absorptiometry Cross Calibration Study

AP1. INTRODUCTION

Dual energy x-ray absorptiometry (DXA) uses low-dose x-ray technology to differentiate types of tissue. It is considered the gold standard technique for assessing bone mineral density (BMD) and body composition.¹ The International Society for Clinical Densitometry (ISCD) recommends that a cross-calibration analysis be performed whenever altering scanner machinery, replacing scanner technology, or comparing results from multiple scanners.² According to ISCD, maximum acceptable variance between scanners is < 2% for body composition measurements (e.g., percent body fat and fat free mass) and less than 1% for BMD.² If unacceptable variance exists, adjustments to scanner calibration may be needed. Mathematical equations may also be used to correct for data with unacceptable variance.

The Human Integrated Physiology Laboratory (HIPL) at Virginia Tech houses two GE Lunar Healthcare Prodigy Advance DXA scanners. Typically, the same scanner is used consistently throughout each research protocol. However, the need may arise for the alternate scanner to be utilized (ex: equipment maintenance, scheduling conflict, etc.). Thus, the purpose of this study was to perform a cross calibration analysis of measurements made by two DXA scanners within HIPL.

AP2. METHODS

Male and female participants between the ages of 18 and 65 years were recruited for the cross-calibration study. Participation was limited to individuals who met scanner size criteria (height \leq 73 inches and weight <300 pounds), did not have metal implants or irremovable jewelry, were not pregnant, and could lie still for 30 minutes. This study was approved by the Virginia Tech Institutional Review Board (#17-1157, Appendix P) and all participants provided written informed consent before beginning the study.

Participants reported to the laboratory for a single study visit during February or March 2018. At the start of the study visit, female participants provided a urine sample upon which a pregnancy test was conducted. Each participant's height was measured using a general stadiometer and weight was

measured using a digital scale with +/- 0.01 kg accuracy (Model 5002, Scale-Tronix, White Plains, New York, U.S.).

Participants then underwent two sets of DXA scans, one on each HIPL DXA scanner (in a randomized order). Both DXA scanners were calibrated daily prior to the start of testing, and a fifteen minute break was provided between sets. Each set of scans included BMD at the L1-L4 spine and both hips, in addition to a total body scan for body composition. Percent fat (body fat %) and fat free mass (FFM) were extracted from body composition scans. All scans were completed by a DXA technician certified by the International Society of Clinical Densitometry and licensed by the Virginia Department of Health. A total of four different technicians performed scans during this study, but the same technician completed both scans for each individual participant. One researcher adjusted measurement regions, if needed, and compiled all scans.

In order to compare results from both scanners, data were analyzed two ways: ISCD's Cross Calibration Tool, which uses the Greatest Least Significant Change Calculator (REF), and Bland-Altman Method Comparison. Descriptive and Bland-Altman tests were performed using GraphPad Prism 8.0, and significance was set at $p < 0.05$.

AP.3 RESULTS

All scans were well-tolerated and 48 participants completed the study. Descriptive characteristics of participants are shown in Table A1.1.

Table AP.1. Descriptive characteristics of participants (n=48) who completed the DXA cross calibration study. Data expressed as means and standard deviations where appropriate.

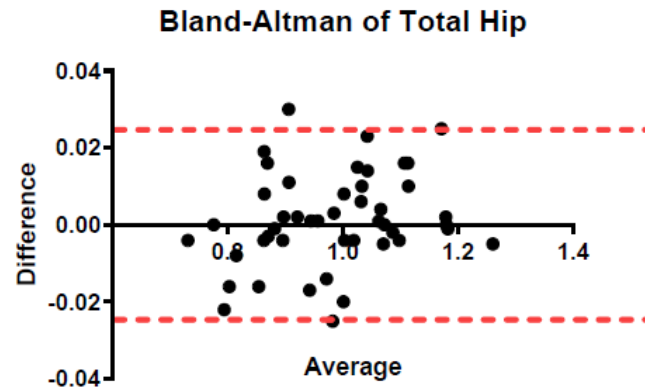
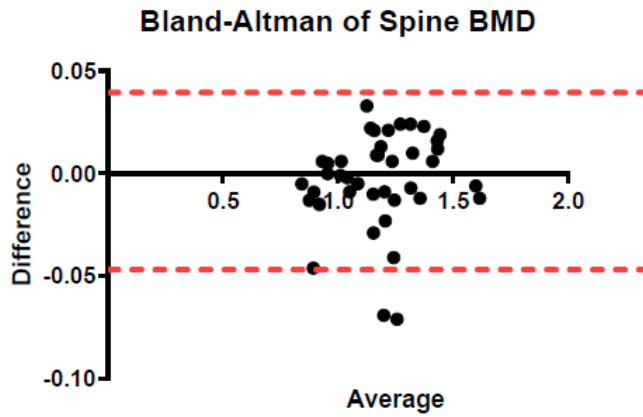
Sex	15 males, 33 females
Race	44 white, 3 Asian, 0 black, 1 other race
Age (years)	49.3 +/- 11.7 (range: 19.1 to 64.8)
Height (inches)	68.3 +/- 2.4 (range: 60.4 to 70.8)
Weight (pounds)	184.3 +/- 20.0 (range: 110.2 to 296.7)
Body Fat (%)	34.9 +/- 8.5 (range: 16.0 to 54.1)

Some scans were excluded from analyses because the participant's body width exceeded the scanner's measurement capacity (n=1), temporary equipment failure (n=1), or because required regions were absent from scans (n=4 L1 spine missing and n=1 part of the greater trochanter in the hip region missing).

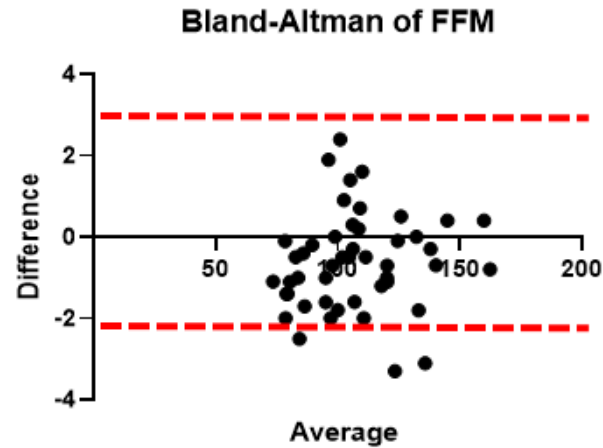
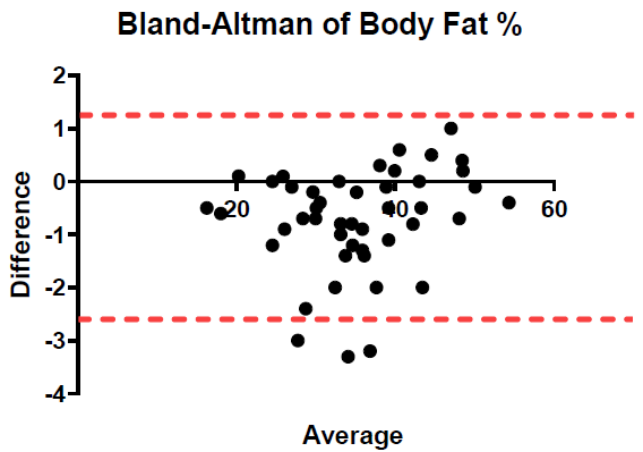
In considering ISCD's recommended maximum variance between scanners (<1% for BMD scans and <2% for % body fat and FFM), 23/42 (55%) of spine BMD scans showed >1% variance, 19/47 (40%) of total hip BMD scans showed >1% variance, 24/47 (51%) of % body fat and 11/47 (23%) of FFM showed >2% variance. Using the ISCD Cross Calibration Tool, the number of scan pairs that exceeded the range of acceptable variation were: 3/42 (7%) for spine BMD, 0/47 (0%) for total hip BMD, 8/48 (17%) for % body fat and 8/48 (17%) for FFM. Bland-Altman Comparison Method results are shown in Figure AP.1.

It was noted that two technicians' (#1 and #4) results produced zero values outside of the acceptable variance range when analyzed by both the ISCD Tool and the Bland-Altman Comparison Method. All variance was associated with the other two technicians' (#2 and #3) results.

Figure AP.1 Bland-Altman Method Comparison Plots of Spine BMD, Total Hip BMD, % Body Fat, and FFM for Difference in Measurements Recorded by Two DXA Scanners



--- = 95% limits of agreement



AP4. CONCLUSION

In a cross calibration of two DXA scanners, some unacceptable variance in scans for spine BMD, total hip BMD, % body fat, and FFM were revealed by two separate analyses. Overall, 0-20% of scan pairs showed unacceptable variance. Total hip showed the lowest rate of variation in both analyses, while % body fat showed the highest. One limitation of the study is that we estimated technician precision for the ISCD Tool analysis since this information was not available. Interestingly, degree of variation appeared to be impacted by which technician conducted the scans. Review and standardization of scanning protocol, and measurement of technician precision may be recommended, followed by a repeat of the cross-calibration study.

Implications for Vitamin D and Basketball Study (Chapter 6)

Baseline BMD and body composition measurements were completed using the DXA scanner in 228 War Memorial Hall. However, when the male basketball athletes' 12-week intervention concluded, this DXA scanner was not functional due to mechanical problems. Thus, post-intervention measurements were completed on the DXA scanner in 231 War Memorial Hall. Both baseline and post-intervention scans were completed by the same technician. Since 22 out of 22 (100%) of scans conducted by this technician yielded consistently well-calibrated results in the DXA cross calibration study, results were considered acceptable for analysis.

REFERENCES

1. Burke L. Dual X-Ray Absorptiometry (DXA) for measurement of body composition in athletes: Experiences that underpin the importance of optimising the reliability of measurement. *J Sci Med Sport*. 2017;20(S3):76-80.
2. International Society for Clinical Densitometry Official Position- Adults. 2017.