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Vitamin D and Stress Fractures in Collegiate and Professional Athletes

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VITAMIN D AND STRESS FRACTURES IN COLLEGIATE AND PROFESSIONAL
ATHLETES

by

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ABSTRACT

An important aspect of orthopedics and sports medicine is to provide quality care and oversight for the overall health and wellbeing of athletes. Research in these fields aims to understand the underlying mechanisms of sport-induced injuries in order to improve treatment plans and prevent future complications. Overuse bone stress injuries are prominent among elite athletes and can cause detrimental setbacks to training and performance. Thus, prevention of these injuries is of primary concern. Vitamin D has been known to play an integral role in skeletal metabolism. Current research suggests vitamin D status may be indicative of bone density, structural integrity, and overall bone health. Results from recent studies evaluating the correlation of vitamin D to stress fracture development are providing insight into this emerging topic. Measuring vitamin D status provides a quick and inexpensive way to evaluate bone health in athletes. In addition, vitamin D supplementation has been shown to substantially increase vitamin D levels. Implementing standardized supplemental treatment options for athletes with suboptimal vitamin D status can potentially reduce the risk of stress fracture formation, keeping athletes healthy and performing at maximum levels.

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CHAPTER 1

INTRODUCTION

Current biomedical research seeks to find, treat, and prevent diseases and disorders. In the fields of orthopedics and sports medicine, clinicians and researchers focus on treatment and medical oversight to keep athletes healthy and injury free. Elite athletes with rigorous training schedules are constantly working towards the highest level of physical fitness and competition. Unfortunately, many athletes are affected by injuries that hinder their ability to train and perform.¹ By understanding both the mechanical and physiological underlying mechanisms, researchers and physicians can provide better care and insight to their athlete patients in order to prevent many sports-related injuries.

One area of recent research interest has been the role vitamin D has in skeletal health and in the prevention of overuse skeletal injuries in elite athletes.^{2,3} Vitamin D is known to be critically important to the proper development and function of the human skeletal system, as well as with multiple other processes in the body. Because of the close tie it has to bone health, researchers are interested in finding a correlation between vitamin D status and the rate of stress fracture injuries.³ Through this research, the importance of vitamin D's effects on skeletal health and injury prevention in both healthy and deficient athletes is beginning to emerge. This thesis encompasses pre-existing knowledge about bone physiology, vitamin D function, the etiology of stress fractures, and a review of the current literature on vitamin D and skeletal injuries in athletes.

Together, they may help to provide a clearer understanding of this relationship so that clinicians and athletes can work to ensure injuries are properly treated and avoided.

CHAPTER 2

BONE ANATOMY AND PHYSIOLOGY

2.1 ORIGINS AND STRUCTURE

The human skeletal system is the framework and infrastructure on which every organ system can be built and anchored to. It serves as a protective shield for many organs while also influencing physiological processes throughout the body.⁴ Bone itself is complex and dynamic, constantly changing as the body experiences physical and chemical stressors. This section provides a brief overview of bone development and metabolism.

During fetal development, mesenchymal cells lay out an embryonic skeleton, which will act as the blueprint for every bone in the body.⁵ From this blueprint are two distinct pathways that lead to bone development. The first is called intramembranous ossification, which is the primary way flat bones are formed. In this process, mesenchymal cells differentiate directly into osteoblasts upon activation of the core-binding factor alpha gene, or *Cbfa-1*.⁶ These newly differentiated osteoblasts begin to secrete a matrix of collagen and proteoglycans, which then combine with calcium salts in the process known as calcification.⁷ Alternatively, long bones of the appendicular skeleton, as well as the rest of the vertebral column and pelvis, are created mainly by a process known as endochondral ossification.

Unlike the intramembranous pathway, endochondral ossification first involves the formation of a cartilaginous template that becomes calcified later to form bone tissue.⁷

The mesenchymal precursor cells differentiate into chondroblasts, which then proliferate rapidly and secrete the cartilage matrix, creating the primary ossification center.⁵ As the template continues to lengthen, older chondroblasts grow in size and eventually die, leaving behind the cartilage matrix. Finally, osteoblasts begin the process of mineralization by depositing calcium on the cartilage template. In long bones, a secondary ossification center forms in the epiphysis. The cartilaginous region between this and the diaphysis is known as the epiphyseal growth plate, which adds to the length of the bone.

The process of ossification and mineral acquisition begins around eight weeks into fetal development.⁸ The highest rates of bone mineral acquisition occur during pubescent years and generally decline starting around age 30.⁹ Dual-energy x-ray absorptiometry scans (DEXA) are commonly used to determine bone density. Analyzing the attenuation of two photon beams of varying intensities and comparing these values to known materials provides a measurement of the bone's mineral content.¹⁰ Measuring bone density is an accurate way of evaluating overall skeletal health.

Bones are separated into one of four categories based on size, shape, and function.⁴ Long bones are found in the extremities and are longer than they are wide. Short bones are also found in the appendages and make up the hands and feet. Flat bones, like those in the skull, thoracic cage, and the scapulae, are relatively thin compared to other bones types. Lastly, irregular bones, like those found in the vertebral column, generally have multiple processes and vary greatly in shape compared to the other bone types. The functional properties of each bone is represented by its specific shape. While

long and short bones are generally used for locomotion, flat and irregular bones typically offer support and protection for vital organs and the body as a whole.⁴

Two subcategories of mature bone tissue are aptly named based on their physical and functional properties. The first type, called cortical or compact bone, is found in the periphery of the bone and is so named because of its thick histological appearance. The second type is called cancellous or trabecular bone. This type is found in the interior of the bone and has a spongy appearance. Cancellous bone differs from cortical bone in that cancellous has many normal open spaces. The typical human skeleton is comprised of roughly 80% cortical bone and 20% cancellous bone, with varying ratios depending on the specific bone function and its location in the body.¹¹

The structural subunits of both cortical and cancellous bone are called osteons or Haversian systems.¹² Here, the mineralized extracellular matrix is organized into concentric rings called lamellae. The collagenous organization of each successive ring is opposite that of rings before and after it.⁴ This series of interconnected rings provides the majority of a bone's compressive and torsional strength.¹² Within the center of each concentric lamella is a Haversian canal, providing an extensive pathway for capillaries to travel in order to bring nutrients to deeper regions of bone. Lying within the compact bone are the trabecular osteons. Compared to compact bone, trabecular osteons are only composed of concentric lamellae, and the superstructure resembles that of a honeycomb.⁴ Bone marrow is located within the interior open spaces of trabecular bone. The amount and type of marrow is dependent on age and bone shape. Red bone marrow is found in flat bones and the epiphyseal regions of long bones, while yellow bone marrow is found in the hollow medullary cavity of the diaphysis of long bones. Surrounding the outermost

layer of cortical bone is the periosteum, which is a sheet of thin fibrous connective tissue.⁴ It is primarily composed of collagenous fibers and is highly vascularized and innervated.¹²

2.2 PROMINENT CELL TYPES

A mature adult skeletal system has many roles in the body, including structural support and hormone-controlled mineral balance.¹² The three prominent cell types that are responsible for maintaining both the structural integrity of bone and blood mineral homeostasis are the osteoblasts, osteoclasts, and osteocytes. As mentioned earlier, osteoblasts are derived from mesenchymal precursor cells called osteoprogenitor cells.⁴ Osteocytes are terminally differentiated osteoblasts that become trapped within the calcified bone matrix.¹³ The third cell type, the osteoclast, belongs to a hematopoietic stem cell lineage.⁴ While each of these three cell types have a unique function, they are all closely linked and are in constant communication with each other.

The main function of an osteoblast is to lay down new bone. There are several different lineages that are responsible for altering a bone's microstructure depending on its local environment and the cellular signals it receives. However, the primary secretory product of an osteoblast is type I collagen, which accounts for up to 90% of the organic bone matrix.⁴ Additional osteoblast secretory products include: 1) the enzyme alkaline phosphatase, a protein that affects the bone mineralization process, 2) osteocalcin, a bone turnover marker that is thought to inhibit bone formation, and 3) osteonectin, a glycoprotein that acts to regulate osteoblast growth and proliferation.⁴

During bone development, osteoblasts become surrounded by the calcified matrix and differentiate into osteocytes. Although these cells share the same lineage, the

osteocytes role in bone metabolism is vastly different than osteoblasts. Within its lacuna, an osteocyte sends out multiple extensions of its cell membrane through the interconnected canaliculi. These processes allow the static osteocytes to detect changes in the bone microenvironment and communicate to other cells.⁴ In terms of the remodeling process, mechanical loads on bone cause a change in the interstitial fluid pressure gradient. Osteocytes detect this change and secrete cytokines and other signaling molecules to induce a mechanosensory response that stimulates other osteocytes as well as osteoblasts and osteoclasts.¹⁴ In general, osteocytes act as directors of resorption and mineralization of the bone matrix, thereby altering its structure in an adaptive manner. This process is crucial for maintaining the structural integrity of bone.

The third bone cell type, the osteoclast, is derived from a hematopoietic stem cell lineage.¹⁵ Osteoclasts are created from the same progenitor cells that give rise to dendritic cells, monocytes and macrophages.¹⁶ The osteoclast precursor cell requires two specific ligands to become fully mature and activated. Receptor activator of nuclear factor kappa ligand, or RANKL, is a peptide that is secreted by osteoblasts that functions to activate osteoclast precursor cells.¹¹ Macrophage-colony stimulating factor, or M-CSF, is a peptide secreted by marrow stromal cells and osteoblasts that functions both in precursor development as well as mature osteoclast activity.⁴ Unlike its immune cell relatives, mature osteoclasts begin as mononuclear cells that undergo fusion with other osteoclasts to form a single multinuclear cell. Macrophage-colony stimulating factor acts on the mononuclear cells to cause proliferation. Then, under the influence of RANKL, these cells differentiate into various phenotypes and fuse together to form multinucleate cells that can contain up to 10 nuclei.¹⁵ The osteoclast's responsibility is to degrade and digest

both mineralized and organic components of the bone matrix, contrasting the function of the osteoblast.

2.3 THE BONE REMODELING PROCESS

As the body experiences environmental stressors, such as the mechanical forces of locomotion or changes in blood chemistry due to metabolic processes, the skeletal system works to ensure the body is kept within its homeostatic range. This concept was first described by the German anatomist and orthopedic surgeon Julius Wolff in the late 19th century.¹² Bone is a very dynamic organ that is constantly being remodeled in order to maintain its own structural integrity as well as to balance blood electrolyte concentrations.⁴ During the process of remodeling, aged or damaged bone is removed and replaced with new bone.

Removal and renewal of the extracellular matrix not only changes the physical structure of bone but also releases calcium and phosphate ions into the blood stream. Since calcium and phosphate ions are crucial for many cell and tissue processes, it is important they remain within normal physiological ranges. Generally accepted physiological levels of total serum calcium and phosphate in healthy adults can range from 8.5 to 10.5 mg/dL and 2.5 to 4.5 mg/dL, respectively.¹⁷ However, these normal ranges tend to decrease after birth and have been reported to fluctuate with age, sex, and pregnancy.^{18,19}

Under normal physiological conditions, bone deposition by the osteoblasts is typically in balance with bone resorption by the osteoclasts.¹¹ This helps to maintain a sufficient bone mineral density (BMD) while altering the substructure so that its strength is directly correlated to the forces being applied to it. Bone turnover and remodeling is

dependent on tissue type. It is estimated that only 2-3% of cortical bone is remodeled and replaced per year in healthy adults.⁴ This is significantly lower than cancellous bone, which can reach turnover rates of 25% per year.²⁰

The process is subdivided into four successive phases: activation, resorption, reversal, and formation.⁴ Activation begins with osteoblasts secreting RANKL, which then binds to the RANK receptor on the osteoclasts.¹¹ This causes the movement of the mononuclear osteoclast precursor cells from the vasculature to the remodeling site, where they fuse to become activated multinuclear cells.⁴ In the resorption phase, these activated osteoclasts secrete hydrogen and chloride ions to acidify and mobilize the mineralized portions of the matrix. The osteoblasts also help with digestion of the organic matrix by releasing a series of enzymes from intracellular lysosomes.⁴

During the reversal stage, the digestive activity of the osteoclasts is reduced and inhibited by several other ligands, including transforming growth factor beta (TGF- β) and osteoprotegerin.⁴ These molecules act as antagonists to the RANK receptor, thereby shutting down the resorption mechanism.¹¹ Osteoblasts are then attracted to the released TGF- β and subsequently undergo chemotaxis to the resorption site where they begin to proliferate.²¹ A host of other growth factors act on these osteoblasts to fully activate them. The final phase of remodeling is the formation phase, where the matured osteoblasts begin to fill in the digested areas with type I collagen to form a new organic network.²¹ Mineralization follows as calcium and phosphate ions bind to the matrix, forming a hydroxyapatite crystalline structure. Following mineralization, the majority of osteoblasts undergo a programmed death, thought to be due to TGF- β and other growth factors in a negative feedback mechanism.²¹

Bone remodeling is a continuous process that is partially dependent on biomechanical loading of the skeleton. Therefore, in order to respond to stress, bones must experience stress. Similar to how targeted bone formation occurs at locations of high stress, a lack of loading leads to inhibition of bone formation and reduced turnover.²² Extreme examples of this can be seen in astronauts who are subject to long-duration space flight, in which they experience zero gravitational forces and are almost completely non-weight bearing. It has been reported that astronauts lose 1.0% to 1.5% of their bone density for every month spent in flight.²³ The implication is that skeletal loading is a requirement for proper bone formation and turnover.

Of the major functions of the skeletal system, one of bone's more critical functions is that of a repository for calcium and phosphate. The mineralized portion of the boney extracellular matrix is composed of crystalized hydroxyapatite. Many physiological processes, such as muscular contraction, membrane potential regulation and the release of neurotransmitters, require a net flux of calcium ions. Two important regulatory hormones that help to maintain appropriate serum calcium levels are parathyroid hormone and calcitonin. Parathyroid hormone is produced and secreted from chief cells in the parathyroid glands when there is a detected drop in blood calcium concentration. The released parathyroid hormone then acts on osteoblasts to promote RANKL expression, thus inducing subsequent changes in osteoclasts to increase bone resorption.²¹ This process releases calcium and phosphate into the surrounding capillaries.

Calcitonin, another calcium regulatory hormone, is produced in parafollicular cells in the thyroid glands. In contrast to parathyroid hormone, calcitonin release is

stimulated by an increase in plasma calcium concentrations. It acts as an antagonist to the RANKL/RANK system and subsequently inhibits bone resorption.²¹ Finally, another important regulatory hormone that greatly affects bone metabolism is vitamin D. Vitamin D is the main topic of this thesis and will be discussed in detail in the following section.

CHAPTER 3

VITAMIN D

Vitamins constitute a group of organic molecules that are necessary for proper cellular function and must be obtained almost exclusively through the diet. There are officially 13 essential human vitamins, each playing an integral role in normal cellular activity.²⁴ Vitamins A, E, D and K belong to a subcategory known as fat-soluble vitamins. These vitamins are typically absorbed in conjunction with dietary lipids and are easily stored within cells. Alternatively, the eight B vitamins and vitamin C are water-soluble. This property makes them poor candidates for storage and thus need to be acquired through the diet on a daily basis. Although some vitamins have overlapping roles, each differs from the next in both structure and function. Most assist with the enzymatic reactions of carbohydrate, lipid and protein metabolisms, but some function as antioxidants and signaling hormones.²⁴ One particular vitamin that is important for bone cell physiology and overall skeletal health is vitamin D.

Beginning in the late 19th and early 20th century, British and American researchers found that certain foods irradiated with ultraviolet light acquired preventative properties that could be used to treat rickets, a disease in which bones are inadequately mineralized and misshapen.²⁵ Investigation into these foods led to the discovery of a compound that would later be termed vitamin D. With additional work, researchers were able to isolate several subclasses of vitamin D metabolites that differed slightly in their molecular organization. Two of the most predominantly studied metabolites at the time were

vitamin D2 and vitamin D3. These metabolites became the clinically and nutritionally relevant forms for humans.²⁵ Since its initial isolation in the 1930s, numerous functions throughout the body have been attributed to vitamin D, including immune response, cancer cell inhibition, and cellular growth, proliferation and differentiation.²⁶ Because vitamin D plays such a critical part in numerous physiological processes, the current research in this field is expansive and covers many metabolic pathways and disorders.

3.1 VITAMIN D METABOLISM

Unlike other vitamins, healthy individuals have the ability to produce vitamin D in substantial amounts, so long as certain conditions are met. Endogenous production of vitamin D in humans is in the form of vitamin D3, otherwise known as cholecalciferol. The process begins with the precursor steroid molecule 7-dehydrocholesterol. As the skin is exposed to ultraviolet B light, specifically between wavelengths of 290-315 nm, 7-dehydrocholesterol in the epidermis is irradiated and converted to a compound known as pre-vitamin D3 by breaking the bond between carbons 9 and 10 and rearranging the surrounding double-bonds within the ring.²⁷ Pre-vitamin D3 then undergoes a thermal isomerization to form cholecalciferol, the inactive and non-hydroxylated form of vitamin D3.²⁵ Two additional products formed during the irradiation of 7-dehydrocholesterol are lumisterol and tachysterol.²⁵ These compounds are thought to act in a regulatory manner and are converted either forward to cholecalciferol or back to pre-vitamin D3, depending on the metabolic needs of the body.

In order to exert its physiological effects on specific target cells, cholecalciferol must first be modified and activated via two separate hydroxylations. Due to their fat-soluble property, the majority of vitamin D metabolites are dissolved and transported

through the bloodstream bound to carrier proteins. Following its formation, cholecalciferol is transported from the skin to the liver using a specific carrier protein called vitamin D binding protein (DBP).²⁶ Human DBP is made up of 458 amino acids and is similar in structure to serum albumin.²⁸ DBP is responsible for binding and transporting roughly 85% of the vitamin D metabolites, while serum albumin carries the remaining 15%.²⁹ Because of its lipophilic structure, only a miniscule amount of vitamin D is physically dissolved in the serum.

After arriving at the liver, vitamin D₃ is transported into hepatocytes where the first hydroxylation step occurs.³⁰ Cytochrome P450 enzymes are responsible for the hydroxylation of vitamin D metabolites, but there is still debate on which cytochrome(s) is/are responsible for hydroxylating cholecalciferol specifically. There are two categories of cytochrome P450 enzymes based on their functional location: microsomal and mitochondrial. Both types carry out reactions using energy from the electron transport chain.³¹ In the case of microsomal cytochrome enzymes, two reduced nicotinamide adenine dinucleotide phosphate (NADPH) electron carriers are oxidized by the enzyme NADPH P450 reductase, which then transfers two electrons to the cytochrome P450 enzyme.³¹ Similarly, mitochondrial cytochrome enzymes also use NADPH. However, ferredoxin reductase replaces NADPH P450 reductase as the oxidizing agent in this process.³¹ Ferredoxin reductase catalyzes the oxidation of the NADPH, and the reduction and subsequent activation of the cytochrome P450 enzyme occurs using a reduced ferredoxin intermediate.³¹ While multiple microsomal and mitochondrial cytochrome P450 enzymes are used during vitamin D metabolite activation, current research suggests that the microsomal CYP2R1 enzyme found in hepatocytes is the primary enzyme

responsible for the first hydroxylation of cholecalciferol.³² The product 25-OH hydroxycholecalciferol, also referred to as calcifediol or 25(OH)D, is the major inactive form of vitamin D found in circulation and serves as a functional reservoir for the body. Once in circulation, 25(OH)D has a half-life of roughly 15 days.³³

The active form of vitamin D is associated with serum calcium ion regulation. When there is a need for increased amounts of vitamin D, as in with hypocalcemia, 25(OH)D undergoes an additional activating hydroxylation. The production of active vitamin D is under close regulation and varies depending on the influences of other hormones, namely parathyroid hormone (PTH) and calcitonin. Should serum calcium levels begin to fall, calcium-sensing receptors on chief cells in the parathyroid glands cease to inhibit PTH synthesis, resulting in a subsequent increase in PTH production and secretion.³⁴

One site of PTH function is in the proximal convoluted tubule of the kidney, where there is a resultant increase in the synthesis of the enzyme 25-hydroxyvitamin D-1 α -hydroxylase.³⁴ Unlike the liver, in which there are suspected to be several functional cytochrome P450 hydroxylases, thorough research has concluded that there is but a single cytochrome P450 enzyme functioning in renal vitamin D activation.³¹ CYP27B1 is a mitochondrial cytochrome enzyme responsible for the hydroxylation of 25(OH)D at the alpha carbon of the parent chain, converting it to 1,25-OH₂ dihydroxyvitamin D, also called calcitriol or 1,25(OH)₂D.²⁵ From here, 1,25(OH)₂D enters circulation through renal capillaries, binds to DBP, and is transported to target cells. With a relatively short half-life, remaining in circulation between 10 and 24 hours after its formation, 1,25(OH)₂D is the functional vitamin D metabolite but is rarely used to determine vitamin D status.³⁵ It

is worth noting CYP27B1 is also found in a variety of extrarenal locations. However, the conversion and activation of 25(OH)D in these sites is under local control of numerous other factors and is not directly linked to renal production of 1,25(OH)₂D.³⁶

The regulation of active 1,25(OH)₂D is important due to its expansive list of target cells and tissues. Aside from its normal biological function, 1,25(OH)₂D has regulatory effects on its own production. When production and activation exceed the body's demand, it acts via a negative feedback mechanism to inhibit cellular transcription of both PTH in the parathyroid and CYP27B1.²⁶ Additionally, it signals the production of CYP24A1, a third important mitochondrial cytochrome P450 enzyme.²⁵ CYP24A1 is functionally similar to the activating enzyme CYP27B1 and is found in the renal tubules as well as many target tissues.²⁵ However, CYP24A1 hydroxylates both 25(OH)D and 1,25(OH)₂D at the 24th carbon, leading to a series of catabolic reactions that eventually degrade and cleave them into calcitriol acid.²⁵ In doing so, excess vitamin D metabolites can be discarded and mineral homeostasis is protected.

3.2 VITAMIN D INTESTINAL ABSORPTION

When endogenous production of vitamin D3 fails to meet the metabolic demands of the body, there are additional exogenous sources from which vitamin D can be acquired. This typically occurs in individuals that lack sufficient exposure to the sun's radiation. Under these conditions, the majority of vitamin D is obtained through the diet. Certain species of fish are rich in vitamin D, and food products such as milk, yogurt and orange juice are commonly fortified with vitamin D.³⁷

In the 1930s, when the study of vitamin D was just beginning, a team of British researchers lead by F.A. Askew were the first to isolate and describe the other major

vitamin D metabolite.³⁸ Vitamin D2, also referred to as ergocalciferol, is produced in various plants and fungi by irradiation of the compound ergosterol in a process very similar to that of vitamin D3 in animals.²⁵ Ergocalciferol differs from cholecalciferol by the presence a methyl side group and a double bond between carbons 22 and 23. Because of the overall similarity, vitamin D2 has been widely used as a supplement in humans and is the common metabolite used in vitamin D prescriptions. However, while there are many common features between ergocalciferol and cholecalciferol, researchers have learned that the potencies between the two forms are not equivalent and that ergocalciferol tends to be less effective in humans.³⁹

The lipophilic nature of the vitamin D metabolites leads to the notion that intestinal absorption should mimic that of cholesterol and other lipids. The primary site of dietary vitamin D absorption is in the small intestines, with roughly 75% of absorption occurring in the jejunum and ileum.⁴⁰ Research has shown that the mechanism of vitamin D uptake by enterocytes is largely based on concentration and may occur using both passive diffusion and membrane transporter proteins.⁴¹ Absorption of vitamin D metabolites at low dietary concentrations primarily occurs using membrane-bound proteins similar to cholesterol transporters.⁴¹ Alternatively, at higher pharmacological concentrations, most vitamin D transporters are saturated and simple diffusion is the primary mode of absorption.⁴¹ After movement into surrounding enterocytes, vitamin D is packaged into chylomicrons along with other lipids, proteins and carbohydrate.⁴² From here, the majority of vitamin D-containing chylomicrons enter into the lymphatic system, while a small amount is transferred directly to the hepatic portal system. In either case,

the final destination is the liver, where the vitamin D is released, hydroxylated to form 25(OH)D, bound to DBP and reintroduced to circulation.

3.3 MECHANISM OF ACTION

It is widely known that hormonal vitamin D affects numerous physiological processes in every organ system in the body.⁴³ The primary function is regulation of calcium ion absorption and resorption in the small intestine and kidneys to maintain mineral homeostasis. Vitamin D can also work in conjunction with PTH to mobilize calcium from the reserves found in bone. Additionally, vitamin D plays a vital role in skeletal health, including bone development and the mineralization and bone remodeling processes.

As previously described in the bone physiology section, bone contains three prominent cell types: osteocytes, osteoblasts, and osteoclasts. Osteocytes are, by far, the most numerous of the three cell types and work to monitor the condition of the bone microenvironment. Osteoblasts respond to various signals and are responsible for mineral deposition and bone growth. Osteoclasts are cells that resorb the bone matrix. Working together, osteoblasts and osteoclasts help to maintain calcium and phosphate homeostasis as well as the integrity of the bone's microstructure. Each cell type displays their own unique characteristics, and they respond to extracellular signals, like vitamin D, in different manners.

Hormonally active vitamin D, 1,25(OH)₂D, generally works via genomic pathways to induce protein synthesis and secretion.²⁵ The initial step in these signaling pathways begins with simple diffusion of 1,25(OH)₂D across the target cell's membrane. Once within the cell, there is a subsequent interaction with an intracellular nuclear

hormone receptor known as vitamin D receptor (VDR).³⁶ After binding 1,25(OH)₂D, VDR undergoes a conformational change that allows it to heterodimerize with an additional protein receptor known as retinoid X receptor (RXR).⁴⁴ Only then can the vitamin D/VDR/RXR complex translocate to the nucleus. Coactivators and corepressors are recruited, and the transcription complex binds to DNA at short, specific gene promoter regions upstream of target genes called vitamin D response elements (VDRE).³⁶ Ultimately, this pathway increases or decreases the production of proteins, thereby altering downstream cellular mechanisms.

The major effect 1,25(OH)₂D has on bone is promoting the release of calcium into circulation when there are inadequate amounts of dietary and renal calcium absorption and reabsorption. By binding to VDRs on osteoblasts, 1,25(OH)₂D induces the production of a protein called receptor activator of NK-κB ligand (RANKL).⁴⁵ Receptor activator of NK-κB ligand subsequently binds to the RANK receptor found on adjacent precursor osteoclasts and initiates osteoclastogenesis, a process in which precursor osteoclasts fully mature and become activated.⁴⁵ In this specific pathway, the activated osteoclasts begin to resorb the mineralized matrix and release calcium back into circulation. In addition to the production of RANKL, calcitriol hinders osteoblastic production of osteoprotegerin (OPG), a glycoprotein that inhibits osteoclastogenesis, thereby allowing the effects of RANKL to be maximized.⁴⁶

Secondary to serum calcium homeostasis, 1,25(OH)₂D exhibits regulatory effects on skeletal health and maintenance. In early stages of bone growth, 1,25(OH)₂D has been shown to decrease the amount of type I collagen produced by osteoblasts, thus limiting the amount of extracellular matrix produced.⁴⁷ However, in later stages of bone

development there is an increase in type I collagen production. Thus, the effects of vitamin D on osteoblast function are based on the specific cell stage and extracellular matrix requirements. This concept, along with indirectly promoting osteoclastogenesis, proves important in that 1,25(OH)₂D can not only regulate the primary stages of bone growth but can also modulate the preexisting microstructure of bone. Without this modulation, bone's ability to adapt is lessened and the skeletal infrastructure may become jeopardized.

1,25(OH)₂D also affects the production of several other proteins in bone cells. First, it upregulates the synthesis of osteopontin, a protein known to increase the growth, migration and survival of osteoblasts.⁴⁸ This increases the functionality of osteoblasts and promotes mineral deposition. Osteocalcin, another protein secreted by osteoblasts, is thought to improve and maintain the overall structural integrity of the bone.⁴⁸ In order to regulate its own synthesis, 1,25(OH)₂D acts on osteocytes and osteoblasts to increase the production of a signaling molecule called fibroblast growth factor 23 (FGF23).⁴⁶ Fibroblast growth factor 23 inhibits the upstream production of CYP27B1 and increases the production of CYP24A1, thus functioning to prevent further activation and decrease overall levels of vitamin D metabolites.

Other prominent 1,25(OH)₂D signaling pathways found in bone cells are the mitogen-activated protein kinase (MAPK) and Wnt pathways. During bone development, the MAPK pathway inhibits the formation of cartilage and causes the selective differentiation and maturation of osteoblast precursor cells into osteoblasts in order begin the mineralization process.⁴⁵ Alternatively, the Wnt pathway is responsible for causing stromal stem cells found in bone marrow to differentiate into osteoblasts rather than

adipocytes. This prevents the accumulation of fat within the bone marrow and also allows for progression of bone development.⁴⁵

Hormonal vitamin D is a nutrient required for the development and maintenance of the human skeleton. Importantly, it helps to regulate many of the cellular processes associated with growth and mineralization. Without sufficient levels, these signaling pathways will be reduced and the overall health of the bone may become compromised.

3.4 CLINICAL IMPLICATIONS OF VITAMIN D DEFICIENCY

Vitamin D deficiency is a global issue and is brought on by many different causes. The metabolite 25(OH)D is the primary metabolite measured to determine an individual's vitamin D status due to its extended half-life, inactivity, and relatively loose regulation compared to the hormonally active form 1,25(OH)₂D.⁴⁹ Laboratory 25(OH)D tests range in price from \$50 to \$220, depending on insurance coverage and the specificity and test type.⁵⁰ Because of the wide-spread effects of vitamin D and the large variation among individuals' metabolic needs, there are several accepted values used in determining appropriate vitamin D status.

The most commonly used recommendations for vitamin D status are those described by the Endocrine Society.⁵¹ The sufficient threshold is listed as circulating levels of 25(OH)D presenting above 30 ng/ml. Levels between 20 and 29 ng/ml are listed as insufficient, while levels below 20 ng/ml are listed as deficient. There are several biochemical techniques that are used to measure serum 25(OH)D levels. Common techniques include radioimmunoassays, high performance liquid chromatography, and liquid chromatography combined with mass spectrometry.⁵¹ In rare cases, the accumulation of 25(OH)D to levels above 100 ng/ml, either from exogenous acquisition

or due to certain pathological states and diseases, can cause vitamin D toxicity, termed hypervitaminosis D.⁵²

One of the main causes of vitamin D deficiency worldwide is the lack of sun exposure. In healthy individuals, vitamin D production is directly correlated to the exposure to the sun's radiation. Research suggests that being exposed to the sun's radiation twice a week for approximately 30 minutes is enough to produce vitamin D in appropriate amounts, up to the equivalent of 20,000 international units (IU) (one microgram equals 40 IU).⁵³ This timeframe may be extended several hours based on geographical residence and season, as well as skin color, the amount of clothing worn, and the use of protective sunscreens.⁵³ When individuals do not meet this requirement, or if they live in geographical locations with reduced sunlight, they may be at a higher risk of developing vitamin D deficiency. This most often occurs in individuals living at higher latitudes (above and below the 33° north and south latitudes) or in areas of continuous cloud coverage.⁵¹

Several categories of vitamin D dietary reference intakes have been laid out by The Endocrine Society. In general, it is recommended that individuals acquire 600 IU of dietary vitamin D per day.⁴⁹ Individuals at risk of vitamin D deficiency should acquire more, with recommendations up to 1,000 IU per day for children and up to 2,000 IU per day for adults. In cases of diagnosed deficiencies, vitamin D supplements may be provided in tablets or capsules of up to 50,000 IU once a week. Supplements are relatively inexpensive, ranging in price based on dosages. Vitamin D3 1,000 IU cost around \$0.07 per capsule, while dosages of 50,000 IU cost up to \$0.30 per capsule.⁵⁴

Vitamin D deficiency can affect multiple organ systems and physiological processes. With regards to bone health, the mineralization and remodeling processes that are required to maintain the structural integrity of the bone are hindered, resulting in weaker, more fragile bones with lower BMD. Certain populations, aside from their geographical location, may be at an increased risk for vitamin D deficiency. One such risk factor is an individual's age. In adolescents, most bones are still in the developmental stage and have increased metabolic activities. Therefore, the vitamin D requirements in this group are greater so that the demands of bone growth are met. Alternatively, elderly individuals have bones that are generally less metabolically active. Vitamin D requirements are elevated to ensure maintenance of bone strength and BMD. A lack of vitamin D can lead to several diseases, such as rickets in children and osteoporosis or osteomalacia in the elderly.⁴²

Other populations that may be severely affected by vitamin D deficiency are those with highly active lifestyles. Two groups at the center of current vitamin D research are military recruits and elite athletes.^{2,3} Military recruits are generally beginning a new daily regimen of running and physical training. Because of this rapid transition, their bones have not experienced the continuous loading needed to initiate the remodeling process, and they become at risk for developing skeletal injuries. Conditioned athletes are also at risk for skeletal injuries, albeit by a different mechanism. Athletes may have elevated bone density that can match the physical forces being applied to them, but without sufficient vitamin D, the turnover and recovery processes fall short. In addition, both of these populations are subject to dietary nutrient deficiencies, resulting in reduced musculoskeletal strength and a higher risk for injury.^{55,56}

Due to vitamin D's extensive list of responsibilities, it is important that both endogenous production and nutritional absorption meet the metabolic demands of the body. Discrepancies in vitamin D levels may affect a wide array of physiological processes such as mineral absorption in the intestines, cell cycles, cancer suppression, immune responses and skeletal health. Likewise, there are many diseases and disordered states that are either caused directly by or associated with an imbalance in vitamin D metabolism. Thus, the clinical implications of vitamin D have prompted a wide range of research areas focusing on adverse effects of vitamin D deficiencies and supplemental treatments.

CHAPTER 4

STRESS FRACTURES: AN OVERUSE INJURY

Among the many roles of the human skeletal system, two primary functions are to support the weight of the body and to adapt to changing forces it experiences. Bones consistently experience cyclic loading during everyday activities like walking. Their resilience to these changing forces is due in part to dynamic turnover process, helping to ensure the bone's microstructure is mechanically sound.⁴ It is important to balance the forces experienced by a bone with that bone's overall strength. However, when demands on a bone exceed its biomechanical strength, homeostasis is lost and the bone begins to fail, leading to skeletal injuries.⁵⁷

One of the most commonly occurring skeletal fractures is the stress fracture. The simplistic definition of a stress fracture is a small, hairline crack that is created following overuse and overloading of a bone.⁵⁸ Unlike other fractures, which typically arise from a single traumatic event, stress fractures are unique in that they develop as a result of repetitive stresses applied to the bone over an extended period of time.⁵⁹ The term "stress fracture" encompasses two further divided subcategories: fatigue fractures and insufficiency fractures.⁶⁰ In healthy individuals, the development of a fracture in a physiologically normal bone when it is exposed to abnormally large or repetitive stresses is known as a fatigue fracture. This type of stress fracture is most commonly seen in athletes and military personnel who have dramatically changed their training regimens.^{57,61} Alternatively, individuals with decreased bone mineral density and more

fragile bones may develop a fracture with exposure to normal physiological loading. This type of stress fracture is known as an insufficiency fracture and is typically found in elderly patients with osteoporosis and patients with disorders that cause excessively fragile bones.⁶¹

As a sports-related injury, stress fractures are most commonly seen in the pelvis and weight-bearing bones of the lower extremity.⁶² The pelvic girdle and lower extremities support the entire weight of the body as well as function directly in locomotion. Thus, it is reasonable to conclude that bones in the lower extremity experience much higher loads compared to those of the upper extremity. Supporting research shows the action of running can increase the forces placed on the lower extremities by up to three times that from static normal body weight.⁶³ The bones in the lower extremity that most commonly develop stress fractures are the tibia, fibula, tarsals, metatarsals, and femur. However, in individuals with high tensile forces, such as baseball pitchers, rowers and golfers, it is not uncommon to find stress fractures in the ribs and upper extremities.⁶⁴

Stress fracture injuries can be extremely debilitating if they are not caught and treated early. Current research in the fields of orthopedics and sports medicine aims to further understand the causes of stress fractures. By understanding how they form, treatment and preventative measures can be implicated to reduce their occurrence and keep individuals active and healthy.

4.1 ETIOLOGY AND PATHOPHYSIOLOGY

The key principle behind the formation of stress fractures is the repetitive application or cycling of a load that exceeds the bone's overall strength. Stress fractures

develop over an extended period of time because of the bone's insufficient repair mechanism. In this sense, it is generally challenging to pinpoint an exact moment in which the bone's substructure becomes compromised. As individuals increase their activity levels, the bone remodeling and repair processes that normally maintain structural homeostasis fail to respond adequately and the integrity of the bone is reduced.³ Based on the notion that stress fractures develop longitudinally rather than acutely, stress fractures can be placed within a much broader stress injury continuum.⁶⁴ Additionally, numerous intrinsic and extrinsic risk factors affect the likelihood and severity of this injury.

When a load is placed on a physiologically healthy bone, such as when walking or running, the bone's material composition keeps it rigid while simultaneously offering slight flexibility. The organic material allows it to change its shape and act as a spring to absorb energy, while the mineralized matrix functions to maintain its stiffness.⁶⁵ Compressional, tensile and torsional forces cause a bone to bend and twist. The overall change in shape compared to its original shape is known as strain.⁶⁴ Strain is a materialistic function and is closely associated with elasticity, which is determined by the structural composition of the bone, i.e. the relative amounts of mineralized to organic material. The overall range in which a bone can change shape and rebound is known as its elastic range of motion.¹²

As the bone is exposed to cyclic loading and unloading that exceeds its elasticity, it enters into what is known as its plastic range. Once this threshold has been reached, the bone's microstructure has been sufficiently damaged and the bone can no longer return to its original shape.⁶⁴ The compressive or tensile forces acting on the bone essentially break

the conjoining bonds between the hydroxyapatite crystals surrounding the collagen fibers, resulting in microscopic cracks.⁶⁶ However, this microdamage is normal and even necessary to invoke the repair process which allows for adaptation to the outside stressors.⁶⁴ Bone's structural homeostasis relies on the balance between the amount of normal microdamage sustained and the efficiency in which the damage can be repaired.¹¹

Accumulation of the strain-induced damage initiates the bone's remodeling process to rebuild and adapt its microstructure.⁶⁴ This process, described in the chapter on bone physiology, begins with osteoclastic resorption of both mineralized and organic materials. When the accrual of damage and speed of repair are kept in balance, the remodeling cycle can last anywhere between 1 of 5 months.¹¹ With repeated loading, however, the equilibrium between damage and repair is interrupted and the bone becomes compromised. Due to the degenerative nature of the remodeling process' initial step, there comes a time when the bone is more structurally impaired than what was caused solely from the microdamage.⁶⁵ During this vulnerable timeframe, continued application of loads without a sufficient recovery period can potentially lead to the first stage of the stress injury continuum known as a stress reaction.⁶⁴

Stress reactions differ from stress fractures in that there is no visible fracture line. Stress reactions still occur from significant amounts of microdamage, but the overall macroscopic continuity of the bone is kept intact.⁶⁷ This initial stage of the injury continuum is generally when individuals notice the first signs of pain or discomfort. Over time as more damage is generated without sufficient repair, the compromised bone will develop a partial hairline fracture. Diagnostically, the injury progresses to a stress fracture when the bone's continuity is disrupted and medical imaging shows a visible

break.⁶⁷ As the injury reaches the more severe end of the spectrum, it is possible for the stress fracture to develop into a complete fracture, which may lead to additional complications and can require surgical fixation.

The progression of stress fracture formation is in part determined by the rate of load cycling and the force exerted on the bone.⁶⁴ In this way, higher intensity loads applied over a shorter period of time inflict relatively more damage than lower intensity loads over longer periods of time. This relationship is also the basis behind stress fracture recovery, which will be discussed in a subsequent section.

4.2 RISK FACTORS

If not for bone's remarkable turnover and maintenance strategies, the forces that individuals experience as a part of their daily routine would jeopardize their bone's durability and longevity. Aside from the mechanical physiology of bone, there are a number of other factors that play a role in determining overall skeletal health. A discrepancy in any one of these elements could potentially heighten the risk for developing a stress fracture or stress injury. Therefore, it is imperative that all aspects of this injury be studied and accounted for.

Most of the risk factors that contribute to stress fracture formation can be assigned to one of two categories. Extrinsic factors are those found in the environment that have an outside influence on the body.⁶⁴ These include things like activity type, training and recovery timeframes, and equipment used. In contrast, intrinsic factors are those that are directly associated with the body's ability to adapt and maintain homeostasis.⁶⁴ Elements such as the biomechanical alignment and anatomy of the musculoskeletal system, hormone production, tissue metabolism and personal physique play a role in how the

body responds to stresses. All of these risk factors affect how likely an individual is to develop a stress fracture, but many of them are also interconnected and affect one another.

In active populations, certain activities place individuals at a higher risk for developing a stress fracture solely due to the nature of that activity. Athletes partaking in sports that commonly place high loads on the lower extremities, such as endurance runners or track and field athletes, are more prone to injury than athletes of low-impact sports, like cyclists.⁶⁰ Likewise, newly recruited military personnel who may have moved too quickly from a sedentary lifestyle to one full of marching and physical training are also at much higher risk. Although bones have the extraordinary ability to adapt, the process does not occur overnight; it takes several months for bone to repair and reorganized to fully compensate for newly added stresses. Novice runners are of particular concern for developing stress fractures because their rapid increase in training and mileage overwhelms the bone's ability to repair the excessive damage.⁶⁷

Using the proper equipment and footwear may help remove the added stress of activities like running and jumping. Many conditioned runners opt for lightweight shoes that provide minimal support while allowing for a more natural foot strike. Alternatively, novice runners and military recruits that are not as conditioned to the extreme forces of running should be fitted for shoes with added cushion and support in order to prevent excessive damage. Proper footwear also helps with biomechanical alignment of the foot and ankle, thus reducing the strain placed on those bones and joints.

A rapid change in the intensity of an activity, or the using the wrong equipment or footwear, can greatly affect the amount of stress added to the lower extremities.

However, the intrinsic properties of the musculoskeletal system play an even greater role in biomechanical stability. Bone characteristics such as mineral density, microstructure, shape and length all affect the amount of force it can withstand without significant damage.⁶⁷ Bones with lower mineral density, or those that are misshapen or misaligned, become structurally compromised and may not sufficiently dissipate energy. When considering gait and stride, individuals with abnormal foot arches may be subject to over-pronation or supination during walking or running, therefore reducing the natural absorptive abilities of the foot and ankle. Muscle strength and flexibility also help reduce forces of impacts during running and other movements.

Other intrinsic factors include hormonal regulation and tissue metabolism. Many systemic hormones are directly linked to skeletal health. Parathyroid hormone, thyroid hormone, calcitonin, vitamin D and the reproductive hormones all affect the mineralization and cellular metabolism of bone in some way.¹¹ Diseases and conditions that inhibit hormone production may also greatly reduce bone's turnover, leading to impaired adaptation. Adequate nutrient intake is also vital to bone health. Without the proper building blocks, like calcium and amino acids, the physical composition of bone will be altered. Physiological processes work to maintain a level of homeostasis, but diseased states and malnutrition greatly decrease the efficiency of these processes.

Stress fractures are complicated injuries and are affected by numerous factors. They may arise in individuals because of one or many interconnected issues. Understanding the role these factors play in the overall development of this injury is critical in both prevention and treatment.

4.3 CLINICAL PRESENTATION

Stress fractures are a common injury in many physically active individuals, as well as the elderly and patients with irregular bone mass. Studies have shown that stress fractures can account for up to 14% of physician visits in general,⁶⁸ with even higher percentages in runners and military recruits.⁶⁴ Among patients that are diagnosed, the injury is most often found in bones of the lower extremity. Tibial stress fractures are the most prominent and represent almost half of all diagnosed stress fractures.⁶¹ They are also found repeatedly in the fibula, tarsals, metatarsals, and femur.⁶¹

Regardless of background and site of injury, many patients complain of the common symptoms. One complaint that patients present with is bone pain during weight-bearing activities, usually linked to a recent change in activity level. They may also note that the pain intensity increases with exercise and is reduced with rest, although this may not always be true.⁶¹ In cases where the injury has progressed patients may have difficulty with normal gait and standing. One simple diagnostic tool many physicians use is called the “hop-test”.⁶⁹ During this test, patients hop on one leg to reproduce the stress associated with increased exercise. Although not conclusive, this test provides clinicians with information to differentiate between bone pain and muscular, tendinous, or ligamentous issues.

There are many other secondary observations that can be made in conjunction with these primary symptoms. Patients often describe the site of injury as being tender with palpation.⁶¹ Depending on the severity of the fracture, some may be able to pinpoint an exact location where the tenderness is most intense. This is more noticeable in tibial and tarsal stress fractures and less in femur fractures due to the relative amounts of

musculature and soft tissue covering the bone itself. There may also be slight edema around the injury site, but again this is not always present.

Like many other pathologies and disorders, there are a number of differential diagnoses that share the same symptoms as stress fractures. Medial tibial stress syndrome, or shin splints, is a disorder involving pain and inflammation of the distal third of the tibia and the associated muscles.⁶¹ Patients with medial tibial stress syndrome commonly present with the same symptoms and sites of pain as those with tibial stress fractures. Other disorders like compartment syndromes, tendinopathies and ligamentous injuries are also confused with stress fractures. Many of these injuries are still stress-related but do not primarily affect the bone. Positive identification of a stress fracture with imaging techniques ultimately excludes many of these differential diagnoses.

Modern advancements in medical imaging have provided physicians with an accurate way to diagnosis stress fractures from other potential issues. Among the most commonly used techniques are radiographs, computed tomography (CT), ultrasonography, radioactive bone scans, and magnetic resonance imaging (MRI).⁶⁸ Each of these technologies offers different advantages and disadvantages for imaging bone.

Historically, bone injuries were imaged using x-rays to create a radiograph. Radiographic imaging shows relative densities of tissues and, therefore, allow for clear visualization of bone over soft tissues. During bone damage, the repair process generally takes several weeks to lay down new bone. This delay causes radiographic imaging to be less reliable for acute bone injuries and early stages of stress fractures, only positively diagnosing roughly 10% of early stage injuries.⁶⁰ However, the speed and ease of radiographic imaging become useful for viewing bone that has progressed to the callous-

forming phases. A second choice for imaging stress fractures is the use of computed tomography. CT scans use a series of x-ray images from various planes and compute them into a single image. This imaging technique is beneficial over standard radiographs because of the added detail and specificity.⁶⁰

Ultrasonography is also a reliable and inexpensive way to image structural changes in bone. This technique can be used during early phases of bone change, but is limited in the depth that it can image.⁶⁸ Ultrasound imaging is generally used on areas with thin overlying soft tissue, such as the medial tibia and bones of the foot.

Bone scintigraphy is a technique used to evaluate bone metabolism. This technique collects images of an injected radioactive tracer that has accumulated in bone with elevated metabolic activity, such as with repair and remodeling of damaged bone.⁷⁰ This accumulation appears on the recorded image as a “hot spot”. While bone scintigraphy can be used for a variety of purposes, it is beneficial in distinguishing between bone abnormalities and non-skeletal conditions.⁶⁸

The most useful technique in diagnosing bone and other tissue alterations is the MRI scan. MRIs create a powerful magnetic field around the body, and in doing so it alters the alignment of protons of hydrogen atoms. A computer then detects the signal and displays it on a monitor. MRI scans have recently become more popular for diagnosing stress fractures for several reasons. Primarily, the fact that they distinguish between tissue types and changing tissue structures allows physicians to positively identify a stress reaction prior to it developing into a fracture.⁷¹ Additionally, because it shows all tissue types, there is the potential to find added changes in soft tissue that may be associated with bone injuries. Overall, this technique allows for earlier diagnosis,

which may result in quicker recovery time and less surgical intervention.⁷¹ The major downfall of MRIs compared to those covered above is that MRI scans are relatively expensive and are commonly used as a secondary measure.

All of the above radiology techniques offer benefits and disadvantages, but regardless they are vital to the diagnosis and outcome of stress fractures. Symptoms and history only guide the physician to draw an educated initial diagnosis. A visual confirmation can ensure the patient receives more specific and personalized treatment.

4.4 REHABILITATION

Making the correct diagnosis of a stress fracture at the onset of symptoms is crucial for the recovery process of a patient. Bone healing and remodeling averages around eight weeks in time,⁷² but may last anywhere from one to four months depending on the specific site of injury.⁶⁸ The initial stage of recovery consists primarily of rest in order to reduce pain and allow bone healing and remodeling to occur without further damage. Clinicians typically advise against weight-bearing activities that place too much load on the injured bone. However, it is important to only reduce the amount of loading to a minimal level because bone remodeling is partially dependent on skeletal loading. Depending on the severity of the fracture, it may be necessary to cast and immobilize the affected bone to facilitate recovery.⁶⁷ In the most extreme cases where the injury has progressed to a complete fracture, surgical fixation of the bones may be required.

During the initial phases of rehabilitation, clinicians work with patients to maintain their physical strength and cardiovascular endurance by incorporating alternative, low-impact activities.⁷² After two or three pain-free weeks, the patient may begin working on developing core strength, flexibility and proper ambulatory

biomechanics if the injury is in the lower extremity. High-impact activities such as running and jumping are generally excluded until all pain and tenderness have subsided. On average, patients may return to full participation between two and four months after beginning rehabilitation. This timeline is extremely variable based on the patient's fitness level and history. As an example, young professional endurance athletes may recover and return quicker than novice middle-aged runners.^{73,74}

Many individuals that develop stress fractures do so because of one or more risk factors previously mentioned. Most of these risk factors are avoidable. An additional part of the recovery process involves removing these risk factors to further prevent injuries from reoccurring.⁷⁵ Maintaining a balanced diet, seeking professional training plans, allowing sufficient recovery periods following training sessions and utilizing proper footwear and running mechanics are easy adjustments that can limit stress and damage added to the skeletal system.⁷⁶ Based on bone's reorganization timeline, it is much more efficient to prevent injuries like stress fractures from developing than it is to treat and recover from them.

CHAPTER 5

CURRENT CONCEPT: VITAMIN D AND STRESS FRACTURES IN COLLEGIATE AND PROFESSIONAL ATHLETES

5.1 VITAMIN D IN COLLEGIATE AND PROFESSIONAL ATHLETES

The benefits of vitamin D in skeletal health have been known for a long time, but only recently has there been an increase in interest regarding its benefits specifically in athletic populations. Aside from insufficient bone metabolism, there are many other issues that may develop as a result of poor vitamin D status in athletes. These include reduced muscle strength, compromised immune responses, and decreased overall athletic performance.^{77,78} The wide-ranging physiological actions of vitamin D make it a crucial nutrient to monitor in athletes. Current research strives to fully understand its benefits in order to prevent injuries and keep athletes healthy and performing.

Overall Vitamin D Status

Data from several recent publications indicate a high prevalence of vitamin D deficiency among athletes, particularly in elite athletes belonging to collegiate teams and professional organizations. In a 2012 study examining vitamin D statuses in a group of male and female NCAA Division I athletes from a variety of sports at a single university, researchers found 75 of 223 examined athletes (33.6%) had suboptimal 25(OH)D levels.⁷⁹ Maroon et al. and Shindle et al. found an alarming 68.7% and 80.9% of NFL players on two separate teams had 25(OH)D levels below the sufficient range,

respectively.^{80,81} In addition, two studies on European and Asian professional soccer players found surprisingly high percentages of athletes with low vitamin D status, reporting 84% of 342 athletes and 91.4% of 93 athletes, respectively.^{82,83} The results of these studies show very clearly that vitamin D deficiency is prevalent even among highly conditioned athletes worldwide.

Intrinsic Factors

Vitamin D metabolism in athletes can be influenced by several intrinsic factors. Absorbance of UVB radiation can account for up to 90% of vitamin D production.⁸⁴ Therefore, cutaneous synthesis of vitamin D is greatly impacted by the lightness or darkness of an athlete's skin. Individuals with darker skin tend to absorb relatively less UVB radiation when compared to those with lighter skin. Thus, it is expected that vitamin D production should be slightly lower in athletes with darker skin. Results from numerous studies support this notion. The previously mentioned NCAA and NFL studies found significantly lower levels of serum 25(OH)D in athletes with darker skin complexions.^{79,80} The NCAA study also reported dark-skinned athletes were 15.2 times more likely to have poor vitamin D status compared to light-skinned athletes.⁷⁹ Results from Bescos Garcia and colleagues show that 25(OH)D concentrations in dark-skinned basketball players were, on average, half of those measured in light-skinned players.⁸⁵ These studies demonstrate a noticeable and expected trend that skin tone can affect vitamin D status and should be accounted for when evaluating athletes during physicals.

Another intrinsic variable that potentially affects circulating 25(OH)D levels is the relative amount of adipose tissue present in an athlete. The fat-soluble nature of vitamin D allows it to be taken up and stored in adipocytes. This suggests that increasing

adiposity may elevate the risk of vitamin D deficiency. The effects of obesity on vitamin D status in non-athletic populations has been previously researched.^{86,87} However, very few studies have examined this concept in-depth with athletic populations, and the results have been mixed. Fitzgerald and colleagues found a slight inverse correlation between circulating 25(OH)D and the amount of fat mass in male ice hockey players.⁸⁸

Researchers noted that each 1-kg increase in fat mass led to a reduction of circulating 25(OH)D by 1.1%. Additional studies by Lewis et al. and Larson-Meyer and Willis also reported an inverse trend in fat mass and vitamin D levels among several collegiate athlete subgroups, although none of their results were statistically significant.^{89,90} A more recent study by Heller and colleagues demonstrated total body mass and fat mass both significantly predicted a decrease in vitamin D availability among collegiate athletes.⁹¹

Conversely, results from Kopec and colleagues show no significant fluctuations in 25(OH)D levels based on fat mass.⁹² Wilson et al. also present differing fat mass values among professional jockeys did not significantly affect vitamin D status.⁹³ These results are further supported by Hamilton and colleagues, who did not find any significant relationship between vitamin D and adiposity.⁸³ The general conception that vitamin D status should be related to fat mass is decidedly inconclusive for athletic populations. However, with athletes' tendencies to have lower levels of body fat, more research is needed to determine adiposity's relation to vitamin D status in this population.⁹⁴

Vitamin D status has also been shown to vary between the sexes, yet it remains unclear if there is a true correlation. In the study by Villacis et al. on NCAA athletes of varying sports, the researchers found male athletes had a significantly higher risk of poor vitamin D status when compared to female athletes.⁷⁹ However, Halliday and colleagues

reported no difference in 25(OH)D concentration between male and female athletes of numerous specialties at three different measurement points in their study.⁹⁵ This is further supported by several additional studies, none of which found differences between sexes.^{96,97}

Environmental Factors

In addition to athletes' intrinsic characteristics, environmental conditions also play role in vitamin D metabolism. The specific setting in which activities are held vary by discipline and can impact the amount of UVB radiation an athlete receives. Indoor teams, such as swimming and diving, basketball, volleyball, and gymnastics, are constantly shielded from the sun due to the locations of their sports. Vitamin D metabolism of indoor athletes may be depressed, which can put them at greater risk for developing skeletal injuries. Participation in outdoor sports (i.e. soccer, track and field, or endurance running) may lead to increased production of vitamin D by giving athletes more exposure to the sun. Several studies have examined the variations in vitamin D status based on a sport's environment.

Results from Peeling et al. show 25(OH)D levels of indoor athletes averaged roughly 36 ng/ml.⁹⁶ While this is still above the suggested minimum for sufficient status, it is significantly lower than the average found in both outdoor and mixed environment athletes, whose values were 52.4 and 53.2 ng/ml, respectively. A study out of Spain analyzed vitamin D levels of 408 elite athletes of differing specialties, of which 82% were below sufficient status.⁹⁸ These researchers detected a significant decrease in serum 25(OH)D levels among athletes training indoors when compared to those training

outdoors. Allison and colleagues also found significant changes in 25(OH)D based on the athlete's sun exposure.⁹⁴

Furthermore, Halliday and colleagues tested vitamin D levels in collegiate athletes on a variety of indoor and outdoor teams over the course of a college school year. They found that athletes participating outdoors had 25(OH)D levels significantly higher than athletes of indoor sports during tests following the summer, and moderate but insignificantly elevated levels during the winter and spring.⁹⁵ This discrepancy during winter and spring measurements may be caused by the overall drop in enrollment throughout the course of their study, leading to a reduction in statistical power. The previously mentioned study from Villacis and colleagues also noted larger but insignificant percentages of vitamin D insufficiency in indoor athletes.⁷⁹ However, after analysis they determined these results were likely due to skin complexion rather than environment.

Contrasting these results, another group of researchers lead by John Fitzgerald tested indoor junior and collegiate ice-hockey players and found only 37.7% of their cohort was vitamin D insufficient, with none of the athletes showing deficiency.^{88,99} Hockey is almost exclusively played indoors, making these results somewhat shocking. While the literature tends to support differences between vitamin D statuses of indoor and outdoor training athletes, it is clear that there are other factors involved.

Different seasons may also have a profound effect on vitamin D metabolism. Fewer hours of daylight and less radiation reaching the earth's surface during winter months means even athletes participating outdoors should produce relatively less vitamin D than they do during summer months. Morton et al. discovered that average summer

25(OH)D concentrations were roughly twice that of winter months.¹⁰⁰ On a professional soccer team in Poland, Kopec et al. noted the prevalence of low vitamin D status rose from 50% in the summer to 83% during winter and early spring.⁹² Additionally, indoor ballet dancers were also reported to be significantly lower in vitamin D status during winter months compared to summer months.⁹⁷

Knowing vitamin D status can fluctuate with the time of year, it is reasonable that higher vitamin D levels acquired over the summer may help protect against vitamin D deficiency throughout the winter and into spring. A study by Galan et al. sought to find a baseline summer 25(OH)D serum concentration that could potentially maintain a sufficient status through the winter months. Their analysis of professional soccer players from Spain found that levels greater than 48.8 ng/ml were able to maintain vitamin D sufficiency through the winter and into February.¹⁰¹ This study had a relatively small cohort, but it provides a basic concept to which other research can be added. Understanding the seasonal changes in vitamin D production can help athletes and clinicians prepare for and treat these expected drops.

Similar to the change in radiation levels from summer to winter months, certain geographical locations experience more direct sunlight than others. The solar zenith angle is the angle at which sun rays enter the atmosphere, with steeper angles allowing more photons to pass through the ozone and reach the surface.¹⁰² Researchers found that cutaneous vitamin D production is almost completely halted during winter months at latitudes greater than 35 degrees north or south.^{90,103,104} However, very few publications list and evaluate the residential latitude of their athlete cohort. More studies that examine

geography, including latitude and climate, are needed to determine their effects on vitamin D status in athletes.

Nutrition and Parathyroid Hormone

When an athlete's exposure to sun does not suffice, vitamin D can also be obtained from the diet. Major sources of dietary vitamin D include fish, eggs, and dairy products.³⁷ Some elite athletes are meticulous about their nutrition, but many lack sufficient vitamin D intake and put themselves at risk for inadequacy issues. One way of examining dietary intake, and nutrient intake in general, is to incorporate self-reported, multi-day nutritional questionnaires into study protocols to discover exactly how much dietary vitamin D athletes are receiving.

In 21 professional basketball players, average dietary vitamin D intake was a mere 139 IU per day,⁸⁵ staggeringly low in relation to the Endocrine Society's recommended 600 IU per day.¹⁰³ Halliday's study reported average dietary intakes of 242, 282, and 204 IU per day for fall, winter, and spring seasons, respectively.⁹⁵ An analysis of 31 male professional horse jockeys found that average vitamin D intake was less than 100 IU per day.⁹³ These reported numbers are shockingly lower than the recommended daily minimum. Training or performing indoors places a heavier reliance on the diet to provide adequate vitamin D, and it seems this may be a concern for athletes.

Endogenous production and dietary intake of vitamin D may not always meet metabolic demands. In these instances, supplemental treatment can help to offset these discrepancies. The benefits of supplemental vitamin D has been studied in the general population for a variety of conditions and diseases. However, data on supplementation

specifically in athletes is only beginning to emerge. A randomized, double-blind study out of the University of Kentucky tested whether long-term vitamin D supplementation affected overall performance in swimming and diving athletes.⁸⁹ Over a period of 6-months, a supplemental group provided with 4,000 IU daily was able to maintain a relatively constant status, while the placebo group dropped an average of 20 ng/ml. A second more recent study found comparable results over 8 weeks of either supplementation or placebo. The treatment group receiving 5,000 IU per day had elevated vitamin D levels averaging 23.1 ng/ml, whereas the levels in the placebo group decreased an average of 1.6 ng/ml.¹⁰⁵

Magee and colleagues tested several supplemental dosages and their effects on raising vitamin D levels in elite male and female Irish athletes consisting of boxers, paralympians, Gaelic hurlers and soccer players. Each of the supplemental groups had statistically significant increases in serum vitamin D levels compared to a non-treated control group, whose mean 25(OH)D levels actually decreased.¹⁰⁶ In study by Close et al., no participant in the supplemented group was at an optimal level at initial testing (in this case researchers used 40 ng/ml). After 8 weeks of daily supplementation of 5,000 IU, 60% of the treatment group showed elevated levels above 40 ng/ml.¹⁰⁷ Findings from these studies support the effectiveness of supplemental treatment for vitamin D deficiency in athletes. With so many additional health benefits, supplementation is an efficient and inexpensive way to keep athletes injury free.

With one of its primary functions being a regulator of skeletal health, it is understandable that changes in vitamin D should be related to factors such as calcium levels, parathyroid hormone (PTH) production, and bone mineral density (BMD). Studies

have evaluated fluctuations of PTH with regards to 25(OH)D levels. Both Kopec et al. and Galan et al. reported insignificant but inverse correlations of PTH and 25(OH)D levels.^{92,101} Contrasting these findings, studies by Lewis and colleagues and Halliday and colleagues failed to find a correlation in PTH and vitamin D changes.^{89,95} However, Lombardi et al. found several blood bone turnover markers as well as PTH were inversely correlated to 25(OH)D levels.¹⁰⁸ With regards to BMD, inadequate 25(OH)D levels would lead to decreased calcium absorption and falling calcium levels, prompting a release of PTH that would, in turn, act to initiate bone resorption and elevate calcium levels. Thus, while evidence is not completely clear in athlete populations, poor vitamin D status may be linked to a drop in bone density, which will hinder the integrity of their skeletal system.

Association with the Female Athlete Triad

Certain groups of female athletes are at risk for developing one or multiple conditions associated with the Female Athlete Triad. The Triad is a syndrome that occurs due to inadequate energy intake, menstrual irregularity or dysfunction, and low bone mineral density.¹⁰⁹ Particularly prevalent in younger females, athletes seeking to maintain slender profiles or meet weight restrictions tend to follow abnormal dieting habits.¹⁰⁹ It is estimated that roughly 78% of the female athlete population experiences at least one of the three conditions.¹¹⁰ Abnormal dieting can lead to insufficient nutrient intake, which can cause decreased levels of estrogen and leptin, two hormones that play a role in bone mineralization and menstruation, respectively.¹⁰⁹ Bone mineral density, which tends to peak during pubescent years, also becomes hindered, further jeopardizing skeletal integrity.¹⁰⁹

Studies have examined the prevalence of the Triad among different female competitive sports groups. In a study of 80 female varsity high school athletes, Hoch et al. reported 36% of athletes had low energy intakes, 54% had amenorrhea, and 16% had low BMD.¹¹¹ Similar results were found by Schtscherbyna and colleagues in a cohort of 78 elite female swimmers.¹¹² Cobb et al. noticed a correlation between low BMD and abnormal menses, as well as disordered eating being directly associated with low BMD.¹¹³

Athletes that experience any one of the three components making up the Female Athlete Triad may be at an elevated risk for sustaining skeletal injuries caused by normal physical activities. Reducing weight and maintaining body image by disordered eating can prevent female athletes from acquiring essential elements of bone health like calcium and vitamin D.

5.2 STRESS FRACTURES IN COLLEGIATE AND PROFESSIONAL ATHLETES

Overall, poor vitamin D status is highly prevalent in collegiate and professional athlete populations of a variety of backgrounds. Current research interests in the fields of orthopedic and sports medicine focuses on the effects of vitamin D on skeletal turnover and remodeling in high-performance athletes. The previous chapters described how the bone remodeling process works to adapt to stresses, and how vitamin D plays a central role in the cellular regulation of osteoblast and osteoclast function during mineralization and resorption. When bone turnover does not match physical demands placed on the body, overuse injuries like stress fractures can develop. Thus, the question is raised: is there a correlation between vitamin D status and the risk of stress fracture among elite athlete populations?

Incidence Rates in Athletes

Stress fractures can be a debilitating injury and can hinder athletic performance. Keeping athletes healthy and active by preventing stress fracture injuries is a major goal in the field of sports medicine. With such a wide variety of sports and athlete demographics, the total occurrence rate of stress fractures is not completely defined. Stress fractures are estimated to account for between 10% to 20% of total sports related injuries diagnosed in clinic.^{114,115} Because of the nature of stress fracture development, sports involving repeated skeletal loading put athletes at a potentially higher risk of injury. However, incidence rates do not present consistently between differing sports.

Several studies have examined the occurrence of stress fractures across specialties. In general, research shows that running athletes tend to be at a relatively higher risk. Of individuals seeking medical attention for running-related injuries, Brubaker et al. reported a stress fracture incidence of 15.6%.¹¹⁶ Studies by Nattiv et al. and Bennell et al. found that stress fracture occurrences specifically in track and field athletes were 8.7% and 21.1% of total injuries over a period of a year, respectively.^{114,117} Hame and colleagues discovered the number of stress fractures sustained by cross-country or track and field athletes equaled roughly that of every other sport combined.¹¹⁸ Multiple other studies found the highest percentages of stress fractures were reported in cross-country runners.¹¹⁹⁻¹²¹ However, other high-impact sports, such as soccer^{110,122} and basketball^{123,124} also show high incidence rates.

Several other differences may influence the incidence or risk of stress fractures. Variations in hormone levels and biomechanical alignment of the skeleton between the sexes may have an impact, although very few studies have examined this in depth.¹²⁵ In

the multi-year study by Arendt and colleagues, stress injuries in females were roughly twice as prominent as in males.¹¹⁹ This was also the case with Hame et al., who found more female athletes developed stress fractures than did male athletes.¹¹⁸ In contrast, Nattiv et al. and Iwamoto et al. did not find any significant differences between sexes.^{117,123} If there is any association between sexes and stress fracture occurrence, the correlation is small. Further research is needed to properly identify sex as a risk factor.

Nutritional Aspects and Vitamin D

Although the overall mechanism and pathophysiology of stress fracture development has been examined, there is a relative lack of supporting information on the nutritional variances that can affect the progression of the injury. Much of the existing data relating to vitamin D status as a predictor for stress fracture development has been in military recruits. Because of the excessive running and physical training of both recruits and elite athlete populations, many of the results can be implied for both groups. Two case-control studies found slight inverse correlations between vitamin D status and stress fracture incidences, although neither were statistically significant.^{126,127} In addition, several prospective studies examined baseline 25(OH)D concentrations following initial enrollment of recruits and monitored them for stress fractures.^{55,128-131} Four of the 5 studies saw lower mean 25(OH)D levels in recruits that developed stress fractures compared to control groups that did not sustain stress fractures.^{55,128,129,131}

Vitamin D directly regulates mineralization and calcium homeostasis, and therefore has a profound effect on bone mineral density. Many researchers have examined the correlation between calcium, BMD, and the prevalence of stress fractures due to inadequacies in each. Results from Wentz et al. indicate that calcium intake has a

direct relation to risk of developing a stress fracture.¹³² In a study evaluating 25 athletes with diagnosed stress fractures to 25 matched controls, calcium intake was the only significant nutritional factor that varied between the two groups.¹³³ This drastically contrasts findings by Bennell et al. that athletes that sustained stress fractures consumed more calcium than athletes without injury.¹³⁴ More prospective studies are needed to evaluate dietary vitamin D and calcium intake in reference to stress fracture occurrence.

Female Athlete Triad

Female athletes experiencing any one of the three conditions that make up the Female Athlete Triad are at a potential risk for sustaining skeletal injuries. Decreased bone density is prevalent with inadequate vitamin D status and low calcium and other nutrient intake. In addition, hormonal imbalances can affect the bone's turnover process, thus limiting its ability to repair and adapt.

A study by Nieves et al. sought to identify nutritional factors that could affect stress fracture rates in female runners. After following 125 female runners for several years, a total of 17 athletes were diagnosed with a stress fracture.¹³⁵ In their report, it was found that consumption of dairy products and vitamin D had multiple benefits on skeletal health: 1) high dairy and low fat diets proved helpful in reducing stress fracture incidence, 2) there was between 40% and 62% overall decrease in stress fracture risk with each additional dairy serving per day, and 3) both dairy intake and vitamin D intake improved BMD at several different anatomical locations.¹³⁵ Schnackenburg and colleagues tested BMD at the lower femoral neck and hip in 19 female runners diagnosed with stress fractures and 19 matched controls. They found overall BMD was significantly lower in the group of females with stress fractures.¹³⁶

Furthermore, several studies describe additional risk factors. An analysis by Barrack et al. on stress fracture incidence among 259 female athletes showed that low BMD and inadequate nutritional intake brought the highest risk.¹³⁷ In a study on female military recruits, Rauh and colleagues report secondary amenorrhea being linked to an increased risk of stress fracture by almost three times that of eumenorrheic females.¹³⁸ By monitoring diets, allowing for adequate nutrient intake and consuming dairy products, at-risk female athletes can properly maintain calcium and vitamin D statuses. This, in turn, can increase overall bone mineralization and density, which can increase bone strength and reduce stress fracture incidences.

Preventive Supplementation

Ultimately, the aim for research on vitamin D's role in bone health and the incidence rate of stress fracture development is to find a strong correlation between the two. By understanding the connection, steps can be taken to promote expedited recovery from the injury and prevention of subsequent stress fractures. Testing 25(OH)D levels and further supplementing athletes with below optimal status is an efficient and inexpensive way to promote bone health.

An important study by Lappe and colleagues tested whether calcium and vitamin D supplementation could reduce the stress fracture incidence rate among U.S. female navy recruits. Of the 3,700 subjects that completed the study, results indicate that vitamin D and calcium supplementation reduced the occurrence of stress fractures in the treatment group by 21% when compared to the placebo group.¹³⁹ This study provides strong evidence that supplemental intervention can prevent stress fractures. These findings are supported by the previous study from Nieves, who also found increased

calcium intake being linked to increased bone density, thus reducing stress fracture risk.¹³⁵

A study by Gaffney-Stromburg and colleagues found that calcium and vitamin D supplementation was able to increase calcium levels and regulate PTH and other turnover markers.¹⁴⁰ These results indicate that supplemental treatment can help normalize bone turnover in response to increased stress during training, thus protecting bone from injury. Furthermore, a retrospective study by Simon et al. reported the effects of vitamin D and bisphosphonate supplementation in elite athletes with diagnosed bone marrow edema. Bone marrow edema is the swelling and increase of interstitial fluid in an injured bone.¹⁴¹ The study found that supplementation had several positive outcomes: 1) normalization of vitamin D status in all 25 cases, 2) reduction in pain in 64% of athletes, and 3) normalized bone turnover markers.¹⁴² Bone marrow edema and stress fractures frequently develop simultaneously.¹⁴³ Together, these studies show promise that vitamin D supplementation has the potential to treat and prevent skeletal injuries like stress fractures.

5.3 CONCLUDING REMARKS

There is a high prevalence of both vitamin D inadequacy and stress fracture occurrence among elite athletic populations. Overuse skeletal injuries not only cause pain during activities but can also prevent athletes from participating. This can be detrimental to athletes who strive to be stronger, faster, and to outmatch the competition. Research suggests that correcting vitamin D deficits can have an enormous impact on skeletal health, as well as many other functions in the body. The primary goal of sports medicine clinicians and researchers is the prevention of injury and maintenance of athlete health.

Testing serum 25(OH)D levels and providing supplemental treatment are easy and inexpensive steps towards reaching this goal. Continued research on this topic may trend towards incorporating a standardized testing and treatment protocol for inadequate vitamin D status in athletes in order to prevent injuries.

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