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# Effects of Season on Vitamin D and Bone Metabolism in a Healthy Population of Western Canadians

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA

October, 2001

## ABSTRACT

Individuals living in countries at higher latitudes are more prone to seasonal vitamin D insufficiency, which can lead to secondary hyperparathyroidism and subsequent bone loss. The primary objective of this thesis was to evaluate seasonal variations in bone and calcium metabolism and prevalence of vitamin D insufficiency. A total of 188 randomly selected subjects from the Calgary cohort of the Canadian Multicentre Osteoporosis Study participated and were tested at quarterly intervals over a one-year period. This study documented the predicted rise of 25-hydroxy vitamin D during the spring and summer months and a subsequent decrease during fall and winter months. Vitamin D insufficiency (defined as <50 nmol/L of 25-hydroxy vitamin D) was found in 61% of all participants at least once during this 12-month study. Parathyroid hormone declined in the summer, but also in the fall, so that the anticipated reciprocal relationship between 25-hydroxy vitamin D and parathyroid hormone was not documented. There was no relationship between 25-hydroxy vitamin D and bone mineral density. While these findings support a general recommendation for dietary vitamin D supplementation in Canada, the implications of seasonal vitamin D insufficiency on bone health need further investigation.

ACKNOWLEGEMENTS

I would like to express my deepest appreciation to my supervisor Dr. David Hanley, who has been an exemplary role model as a scientist, physician and human being. His "open-door" policy allowed me to stay highly motivated throughout this thesis project and his invaluable clinical expertise often brought meaning to the statistics. I would also like to thank members of my supervisory and defence committees - Dr. Tish Doyle-Baker, Dr. Steve Edworthy, Dr. Otto Rorstad and Dr. Ron Zernicke - for their time and effort.

I am indebted to Jane Allan not only for her irreplaceable organizational skills that made this study possible, but also her for patience and friendship. Special thanks also go to Howard Cheng for the technical analysis of all the assays, Teresa Williams for her invaluable secretarial aid and Jacob Jaremko for his expert editorial assistance.

Finally, I would like to thank all the graduate students, technicians and professors of the McCaig Centre, whose diverse background and research expertise was truly inspirational. I will always look back on the friendships formed as some of the best times of my life.

The financial support of the Alberta Heritage Foundation for Medical Research and the Alexa W. Church Medical Research Scholarship is recognized.

*To my family for all their support and encouragements*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ~ | approximately |
| <, ≤ | less than, less than or equal to |
| >, ≥ | greater than, greater than or equal to |
| $1,25(OH)_2D$ | 1,25-dihydroxy vitamin D |
| 25(OH)D | 25-hydroxy vitamin D |
| BMD | bone mineral density |
| BMI | body mass index |
| BUA | broadband ultrasound attenuation |
| CaMos | Canadian Multicentre Osteoporosis Study |
| CDA | computed digital absorptiometry |
| Cm | centimetre |
| CPBA | competitive protein binding assays |
| CV | coefficient of variation |
| dB/MHz | decibel per megahertz |
| DBP | vitamin D binding protein |
| DEXA | dual energy x-ray absorptiometry |
| $g/m^2$ | gram per metre squared |
| GEE | generalized estimating equations |
| IRMA | immunoradiometric assay |
| IU | international units |
| kg | kilogram |
| $kg/m^2$ | kilogram per metre squared |
| L1, L4 | lumbar vertebrae 1, lumbar vertebrae 4 |
| m | metre |
| m/s | metres per second |
| mg | milligram |

| | |
|---|---|
| mm | millimetre |
| mmol/L | millimole per litre |
| mo | month |
| n | number |
| ng/L | nanogram per litre |
| nmol/L | nanomole per litre |
| pg/mL | picogram per millilitre |
| PTH | parathyroid hormone |
| QUS | quantitative ultrasound |
| SCV | standarized coefficient of variation |
| SD | standard deviation |
| SOS | speed of sound |
| U/L | units per litre |
| UV | ultraviolet |
| yr | year |

# CHAPTER 1

## REVIEW OF THE LITERATURE

## 1.1 INTRODUCTION

### *1.1.1 History*

Despite its name, vitamin D is not a vitamin but a prohormone produced in the skin after exposure to ultraviolet (UV) B sunlight.[1] The discovery of vitamin D, and the recognition that it is a hormone and not a vitamin, has only occurred within the last 80 years. Yet the substance appears to be evolutionarily very ancient, produced by phytoplankton exposed to sunlight as early as 750 million years ago.[2] The Polish physician Sniadecki first noted in 1822 that the dreaded "English disease" (or rickets) was caused by inadequate exposure to sunlight.[3] In the first epidemiological study on rickets in 1890, Palm documented that children who were well-nourished but lived in the sunless environments of the British Isles were severely afflicted by rickets, whereas children from China, Japan and India, who were poorly nourished yet exposed to sunlight were free of rickets.[4] Despite these findings, however, doctors and scientists were reluctant to believe that such a crippling bone disease could be cured with a remedy as simple as sunlight. It took another 30 years before Huldschinski (1919) provided unequivocal evidence for the first time showing that the radiation exposure from a mercury-vapor quartz lamp alone was sufficient to cure rickets.[5] At approximately the same time, other investigators showed that rickets could be cured with administration of cod-liver oil.[6] The confusion over whether rickets was cured by solar exposure or by ingestion of a dietary factor was resolved

when Powers showed that the healing effects in rachitic rats was the same after treatment with either cod liver oil or radiation exposure.[7]

### *1.1.2 Chemistry and Structure*

When written without a subscript, vitamin D represents both vitamin $D_3$ (from animal sources) and vitamin $D_2$ (from plant sources). The molecular structure of vitamin D is similar to other steroid hormones such as estradiol, cortisol or aldosterone because of the root ring structure (Figure 1.1). The only structural difference between $D_2$ and $D_3$ is the presence of a methyl group at carbon 24 and a double bond between carbons 22 and 23 on the side chain of vitamin $D_2$ (Figure 1.1). Vitamin $D_2$ and $D_3$ are handled identically in the body though the latter is about 10-20 times more potent, presumably because vitamin $D_2$ binds less efficiently to the protein that carries it throughout the circulation.[8]

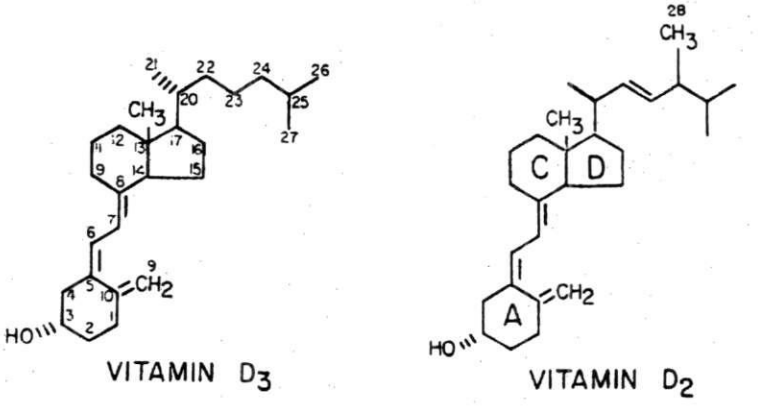


**Figure 1.1** Structure of vitamin $D_2$ (from plant sources) and $D_3$ (from animal sources). The only structural differences between vitamin $D_2$ and $D_3$ is their side chains: Vitamin $D_2$ contains double bond between carbon 22 and 23 and a methyl group on carbon 24. Adapted from Holick.[8]

### *1.1.3 Sources of Vitamin D*

Vitamin D can be obtained either from skin synthesis after sun exposure or through the diet. Nutritional vitamin D also originates from photosynthesis in living organisms. Among the few natural sources of vitamin D are egg yolks, liver and fish liver oils. The primary dietary source of vitamin D is in foods artificially fortified with vitamin D, such as milk, margarine, cereal and breads. However, food supplemented with vitamin D does not always contain the stated concentrations, as gross errors of either under or over fortification have been detected in several milk products.[8-10] As a result, skin synthesis of vitamin D is considered the most common source of vitamin D in man and vertebrates.[9] Holick has estimated that exposure of hands, face and arms for 10-15 min between 9 AM and 3 PM in the summer two or three times a week is equivalent to vitamin D supplement of 200 IU/day.[10] The current dietary recommendation of vitamin D in Canada is 200 IU/day for adults (600 IU/day for those over the age of 70)[11] although Vieth has argued that the requirement should be at least 800 IU/ day or even as high as 4000 IU/ day.[12]

## 1.2 PHOTOBIOLOGY OF VITAMIN $D_3$

The epidermis and dermis layers of the skin contain stores of 7-hydrocholesterol (provitamin $D_3$), although the epidermis accounts for greater than 80% of the total vitamin $D_3$ produced in the skin.[13] During exposure to sunlight, UVB photons between 290-315 nm in wavelength break the carbon 9-10 bond of

provitamin $D_3$, yielding previtamin $D_3$ (Figure 1.2). Previtamin $D_3$ is thermodynamically unstable and immediately isomerizes to vitamin $D_3$ by a temperature dependent process that takes ~10 hrs in humans at body temperatures (37°).[1, 14] The change in conformation of previtamin $D_3$ to vitamin $D_3$ allows it to move into the extracellular space, where it is drawn to the dermal capillary bed by vitamin D binding protein (DBP) and carried into the circulation.[15] Several regulatory mechanisms within the photochemical pathway for vitamin $D_3$ prevent possible vitamin D intoxication resulting from excessive sunlight exposure. Previtamin $D_3$ also absorbs UVB photons, thereby converting to the biologically inert photoproducts lumisterol and tachysterol, so that the amount of previtamin $D_3$ is maintained at no more than ~10-15% of the initial provitamin $D_3$ concentration.[16] Furthermore, vitamin $D_3$ produced in the skin is sensitive to both UVB and UVA radiation (~up to 345 nm) and is photosynthesized to suprasterol 1, suprasterol 2, and 5,6-*trans*-vitamin $D_3$ if it does escape into the circulation.[17]

### *1.2.1 Factors that Influence Cutaneous Vitamin D Production*

The cutaneous production of previtamin $D_3$ is essentially dependent on the concentration of provitamin $D_3$ and the amount of UVB photons that are absorbed by the skin. Several factors, including the latitude, time of year, skin pigmentation, use of sunscreens and age of the individual have important consequences on the cutaneous production of Vitamin $D_3$.

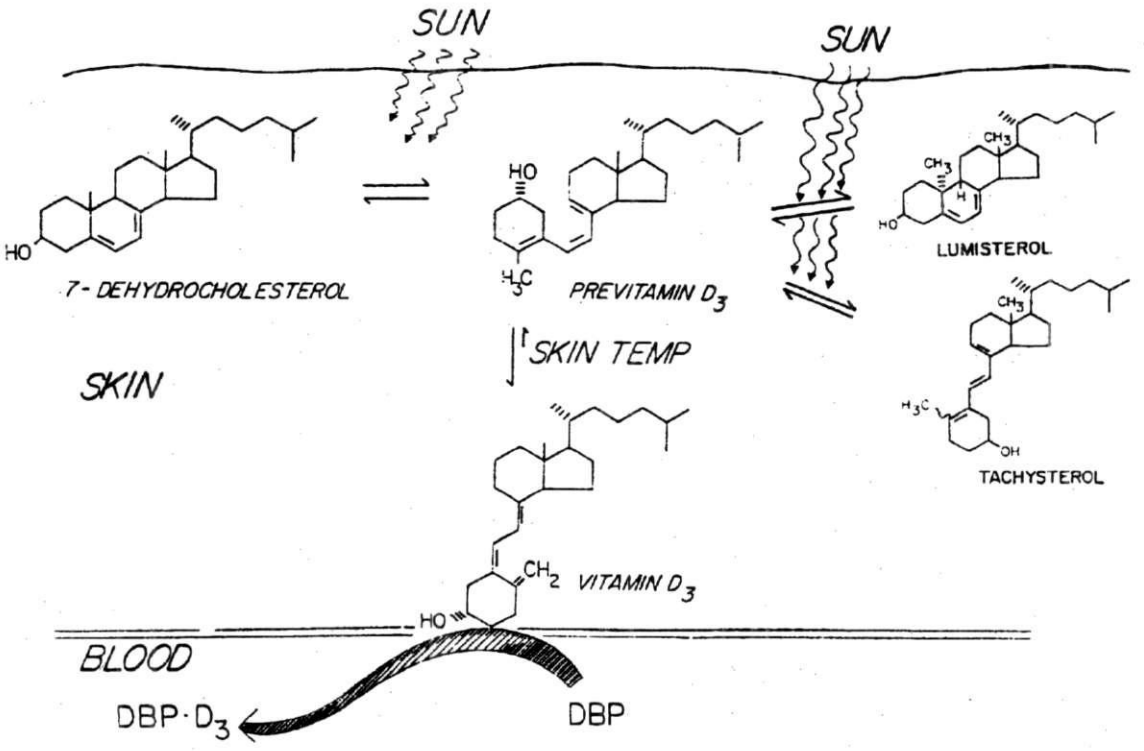


**Figure 1.2** Photochemical events that lead to the production of vitamin $D_3$. From Holick.[1]

*1.2.1.1 Season and Latitude.* The amount of UVB photons that reach the earth's surface is dependent on the distance the photons must travel in the atmosphere, as a function of the solar zenith angle. The solar zenith angle is measured as the angle between the Sun's rays and the point in the sky directly overhead, and changes with rotation of the earth about the sun (season) and its own axis (time of day), so that the angle is larger in the winter and when the sun is low. The zenith angle also increases at latitudes further away from the equator. As the zenith angle increases, sunlight is filtered at a more oblique angle through the stratospheric ozone layer thus decreasing the amount of UVB radiation available to

synthesize previtamin $D_3$.[16] Human skin samples *in vitro* exposed to sunlight produced no previtamin $D_3$ from November through February in Boston (42.2° North) and October through March in Edmonton (52° North), whereas further south in Los Angeles (24° North) and Puerto Rico (18° North) previtamin $D_3$ was effectively converted year round (Figure 1.3).[18] The time of day in which vitamin $D_3$ is produced in the skin is also affected by the season. During the summer months in Boston, UVB radiation from 700 to 1700 hours of Eastern Daylight Savings Time (EDT) was sufficient to produce vitamin $D_3$, and in the spring and autumn these hours were reduced to ~900 to 1500 EDT.[19]

*1.2.1.2 Age.* Aging significantly reduces the concentration of provitamin $D_3$ in the epidermis,[13] reducing the amount of vitamin $D_3$ produced by the skin. In comparison to a young healthy adult, people over the age of 70 yrs produce less than 30% of vitamin $D_3$ when exposed to equal amounts of sunlight.[20]

*1.2.1.3 Sunscreen and Clothing.* Topical sunscreens, although extremely valuable for the prevention of skin cancer and damaging effects of sun exposure, also prevent the photosynthesis of previtamin D. Topical sunscreen with a sun protection factor of 8 was completely effective in preventing vitamin D synthesis, and thus may result in vitamin D deficiency amongst regular sunscreen users.[21, 22] Most fabrics used in clothing absorb UVB radiation, and therefore also prevent skin synthesis of vitamin $D_3$.[23]

*1.2.1.4 Melanin.* Melanin acts as a natural sunscreen by competing with previtamin $D_3$ for UVB photons. As a result, people with increased melanin skin pigmentation have less circulating vitamin $D_3$ compared to individuals with a lighter skin tone, and require longer sun exposure to produce the same amount of vitamin $D_3$.[24, 25]

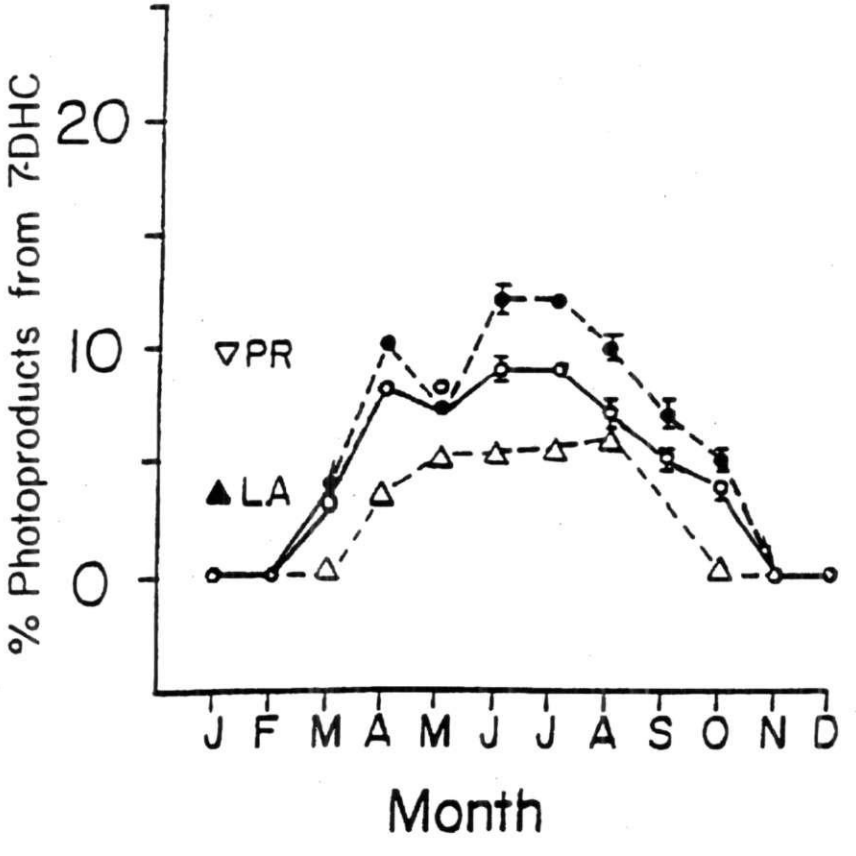


**Figure 1.3** Photosynthesis of previtamin D3 after exposure of 7-dehydrocholesterol (7-DHC) to sunlight in Boston (42° N) for 1 h (○) and 3 h (●); in Edmonton (52° N) for one hour each month for 1 y (Δ); and in Los Angeles (34° N) (▲) and Puerto Rico (18° N) (▽) in January. No previtamin $D_3$ was produced from November until February in Boston and in Edmonton this period was extended from October until March. Adapted from Webb *et al.*[18]

## 1.3 METABOLISM OF VITAMIN D

Once vitamin D enters the circulation, it is bound to DBP and carried to the liver, where cytochrome P450-vitamin D-hydroxylase adds an hydroxyl group to the 25-carbon forming 25-hydroxyvitamin D (25(OH)D) (Figure 1.4). The hepatic hydroxylase is not tightly regulated so that all the vitamin D produced in the skin or ingested from the diet is essentially converted to 25(OH)D. 25(OH)D is subsequently transported to the kidney, where cytochrome P450-monooxygenase, 25(OH)D 1α-hydroxylase adds another hydroxyl group to convert 25(OH)D to $1,25(OH)_2D$.[19] Unlike the first hydroxylation in the liver, the second hydroxylation in the kidneys is tightly regulated by negative feedback inhibition of $1,25(OH)_2D$. Renal conversion of 25(OH)D to $1,25(OH)_2D$ is stimulated by an increase in parathyroid hormone (PTH) and phosphorus or a decrease in extracellular ionized calcium.[19]

Although the kidney is the major source of circulating $1,25(OH)_2D$, studies have shown that other tissue cells, such as keratinocytes and placental cells, are capable of producing $1,25(OH)_2D$.[26, 27] However, the extra-renal sites of $1,25(OH)_2D$ production do not appear to have an important role in calcium homeostasis.[19]

## 1.4 PHYSIOLOGICAL ACTIONS OF 1,25-DIHYDROXYVITAMIN D

### *1.4.1 Cellular Mechanisms of Action*

Although the dihydroxylation increases the affinity of vitamin D for water, $1,25(OH)_2D$ is still very lipid soluble, therefore able to easily move through cell membranes. All target tissues for vitamin D contain a nuclear vitamin D receptor. The mechanism of action of $1,25(OH)_2D$ is similar to other steroid hormones. Once $1,25(OH)_2D$ has entered the cell, it binds with its nuclear receptor and forms a heterodimer with the retinoid-X receptor, that, along with other transcription factors, activates the transcription of specific genes for the production of messenger ribonucleic acids that code for calcium-transporting or growth-regulating proteins.[28]

Not all biological actions of vitamin D occur at the level of gene transcripton. The most characterized non-genomic action of $1,25(OH)_2D$ is the rapid transmembrane transport of calcium ("transcaltachia") by the opening of voltage-gated calcium channels that occurs when vitamin D interacts with a plasma membrane receptor protein.[29, 30] Transcaltachia initiated by vitamin D has been documented in the plasma membranes of intestinal epithelial cells, osteoblasts, hepatocytes, muscle, and parathyroid cells.[31]

### *1.4.2 Physiological Actions of $1,25(OH)_2D$ in the Small Intestine*

The major physiologic function of vitamin D is to maintain calcium homeostasis through absorption of dietary calcium in the small intestine.[19, 31-33]

$1,25(OH)_2D$ directly facilitates the transport of calcium into and across the intestinal plasma membrane and transfers the calcium across the basolateral membrane into the circulation.

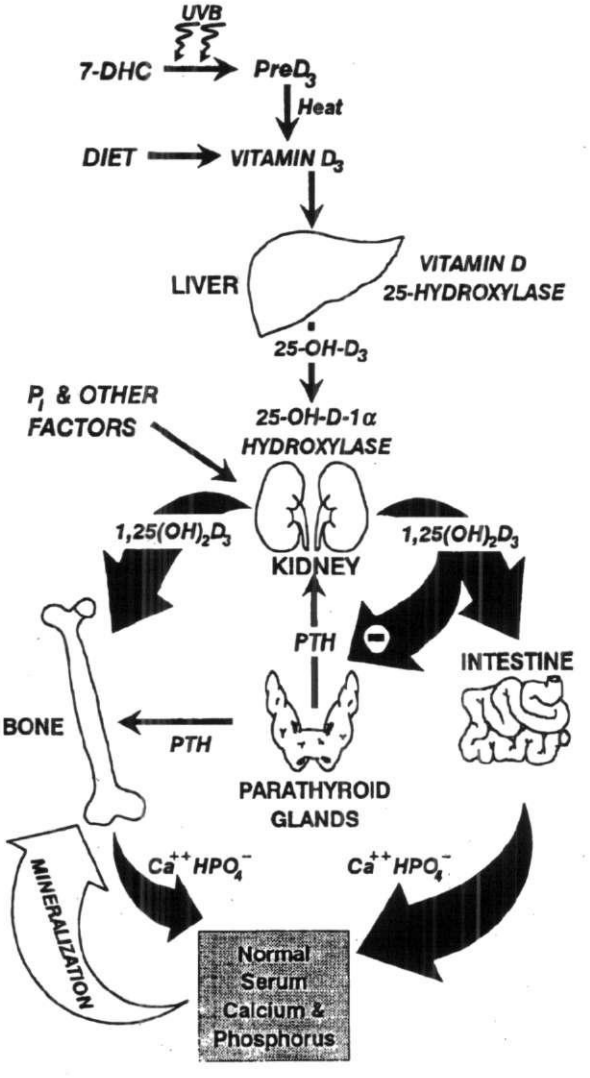


**Figure 1.4** Metabolism of vitamin $D_3$ from cutaneous synthesis and the diet to 25(OH)D and $1,25(OH)_2D$. Once formed, $1,25(OH)_2D$ carries out biologic functions of vitamin D on the intestine and bone. Parathyroid hormone (PTH) promotes the synthesis of $1,25(OH)_2D$, which, in turn, stimulates intestinal calcium transport and bone calcium mobilization, and regulates the synthesis of PTH by negative feedback. Adapted from Holick.[19]

### *1.4.3 Physiological Actions of 1,25(OH)$_2$D in Bone*

1,25(OH)$_2$D acts both indirectly and directly on bone. Through the previously mentioned ability to stimulate intestinal absorption of mineral ions, 1,25(OH)$_2$D indirectly regulates the mineralization of osteoid by maintaining the normal ranges of extracellular calcium and phosphorus necessary for hydroxyapatite formation.[8] 1,25(OH)$_2$D also directly modulates osteoblastic production of several matrix proteins, including Type 1 collagen,[34] alkaline phosphatase,[35] osteocalcin,[36] osteopontin,[37] and matrix-Gla protein.[38] 1,25(OH)$_2$D also stimulates the differentiation of osteoclast progenitors into mature osteoclast-like cells,[39] likely mediated through the osteoblast as expression of the vitamin D receptor gene has only been demonstrated in the osteoblast.[40] It appears that 1,25(OH)$_2$D causes osteoblasts to release osteoclast-activating factor,[41] which stimulates the osteoclasts to resorb bone. Finally, 1,25(OH)$_2$D increases $\alpha_v\beta_3$ integrin expression in the osteoclast's bone resorbing ruffled border[42] (integrin molecules are necessary to localize and anchor osteoclasts to the resorbing surface of bone).

### *1.4.4 Physiological Actions of 1,25(OH)$_2$D in Other Tissues*

1,25(OH)$_2$D acts directly on parathyroid hormone (PTH) secretion by suppressing transcription of the gene responsible for PTH synthesis.[43] Although the vitamin D receptor as well as vitamin D responsive calcium binding proteins (calbindins) are found on the distal nephron segments, there is little evidence of

vitamin D playing an important role in regulating calcium transport in the kidney.[44] In addition to the well established effects of vitamin D on the intestine, bone and parathyroid gland, new and previously unappreciated functions of the vitamin D hormone are being found in other tissues and systems, including the skin, immune system, embryonic cells, and islet cells of the pancreas (Table 1.1). While a detailed study of these non-classical target tissues is beyond the scope of this review, it should be pointed out that many of these non-classical target tissues and proposed "alternative" functions of $1,25(OH)_2D$ were initially discovered *in vitro*, so that the physiological significance remains to be elucidated *in vivo* humans.[8]

**Table 1.1** Vitamin D Receptor Distribution Among Mammalian Tissue. From Holick.[8]

| | | | |
|---|---|---|---|
| Intestine | Pituitary | Thymus | Uterus |
| Kidney | Parotid | Lymphocytes | Placenta |
| Bone | Pancreas | Monocytes/Macrophages | Breast |
| Parathyroid | Stomach | Testes | Embryonic liver |
| Brain | Skin | Ovaries | Embryonic muscle |

## 1.5 CLINICAL SIGNIFICANCE OF VITAMIN D INSUFFICIENCY

### *1.5.1 Epidemiology of Osteoporosis*

Osteoporosis, a systemic disease characterized by low bone mass, is a major health problem that leads to a significant increase in fractures.[45, 46] Osteoporotic fractures have a catastropic impact upon the quality of life in the elderly and the cost

of health care. Following a hip fracture there is a 10-20% chance of mortality over the next six months, 50% of sufferers will be unable to walk without assistance, and 25% will require long-term care.[45] Statistics Canada reported ~25,000 hip fractures in 1990 with an annual cost > $400 million.[47] With the demographic shift toward an increased life expectancy, it is projected that one in four women (one in eight men) over the age of 50 will suffer from osteoporosis.[47]

### *1.5.2 Role of Vitamin D in the Etiology and Treatment of Osteoporosis*

The cause of osteoporosis is multi-factorial, but if body weight is held constant, the three most important factors to influence bone density are: (1) physical activity, (2) gonadal hormones, and (3) nutrition.[48] For adults of industrialized countries, the nutrients most important for bone health are calcium and vitamin D.[48]

Vitamin D deficiency and insufficiency are commonly used terms to describe reduced supplies of an essential nutrient that leads to impaired function. The concept of vitamin D "deficiency" which results in overt clinical disease, such as rickets in children or osteomalacia in adults, needs to be distinguished from the concept of vitamin D "insufficiency" (or subclinical vitamin D deficiency) which has a biological effect on calcium homeostasis and skeletal metabolism, mainly through compensatory hyperparathyroidism without apparent clinical symptoms. As the body becomes vitamin D insufficient, the efficiency of calcium absorption from the intestine decreases from 30-50% to no more than 15%.[10] PTH not only acts to conserve calcium by increasing renal tubular reabsorption of calcium, but also

mobilizes stem cells to become active resorbing osteoclasts. Secondary hyperparathyroidism occurring with vitamin D insufficiency has been well documented[49-53] and may contribute to osteoporotic fractures in the elderly.[54-58] Studies have shown a direct relationship between low serum 25(OH)D with bone mineral density (BMD)[57, 59] with coinciding increases in PTH.[60] Dawson-Hughes *et al.* have also shown that vitamin D supplementation (without high calcium intake) can prevent seasonal bone loss.[61] Only a few studies have also examined the effect on fracture of vitamin D supplementation without high calcium supplementation, with conflicting results. Lips *et al.*[62] found that daily supplementation of 400 IU of vitamin $D_3$ per day did not decrease the incidence of hip fractures and other peripheral fractures in elderly Dutch population over a 4 year period, while Heikinheimo *et al.*[63] demonstrated that a single injection of 150,000-300,000 IU vitamin $D_2$ in the fall (equivalent to physiological doses of 410-820 IU of vitamin $D_3$/day) significantly decreased all fractures in an elderly Finish population.

### *1.5.3 Vitamin D Insufficiency and Age*

Elderly individuals are at a particular risk for vitamin D insufficiency because of reduced mobility and activity (leading to reduced sunlight exposure),[64] a decrease in the capacity of the skin to produce vitamin D,[13, 20] a decline in renal function,[65] and a decrease in renal 1α-hydroxylase activity with age.[66]

### *1.5.4 Vitamin D Insufficiency and Season*

Vitamin D insufficiency is more prevalent in countries located at higher latitude due to a restricted UVB exposure during the winter months.[18] In 1992 McKenna[67] published a review on all vitamin D studies reported from 1971 to 1990. A total of 117 studies were grouped into regions of North America, Scandinavia and Europe. This meta-analysis revealed that vitamin D status varied in all regions with the season in both the elderly and young adults.

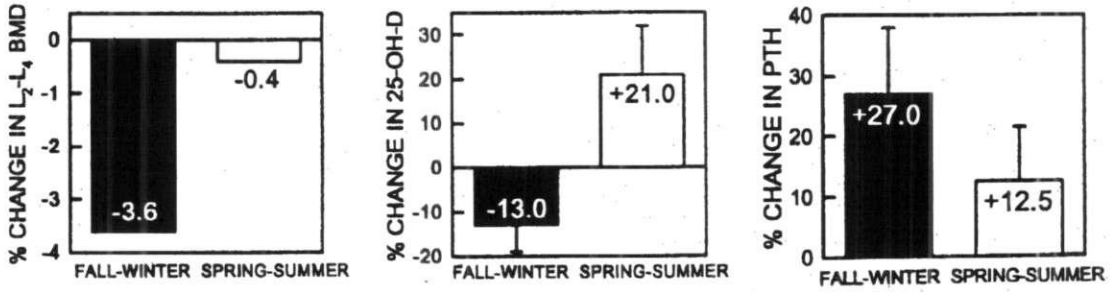


**Figure 1.5** Percent change in the L2-L4 BMD (left); 25(OH)D concentration (middle); and circulating PTH (right) in menopausal women during the winter and summer in rural Maine. Adapted from Rosen.[60]

### *1.5.5 Genetic and Age-Related Alteration of the Vitamin D Receptor*

Another important pathological mechanism of vitamin D in the development of osteoporosis is the genetic polymorphism of the vitamin D receptor.[68] Despite the initial excitement this hypothesis brought into the field of bone research, it is currently not known to what extent genetic variations of the vitamin D receptor affect the development of osteoporosis in subjects with different ethnic backgrounds.[69]

There is also evidence for an age-related decline in intestinal vitamin D receptor expression, leading to decreased sensitivity to $1,25(OH)_2D$ and impairing intestinal calcium absorption.[70]

### *1.5.6 Assessment of Vitamin D Insufficiency*

Although 25(OH)D itself has been shown to contribute up to one-eighth of calcium absorption in healthy adults,[71] 25(OH)D is thought mainly to act as the precursor pool for $1,25(OH)_2D$ in humans.[8] However, it is now recognized and accepted that 25(OH)D is the best measurable indicator of vitamin D production or nutrition.[72] The main reason for this is because circulating levels of 25(OH)D are about three orders of magnitude higher than $1,25(OH)_2D$, so that even low levels of 25(OH)D provide enough substrate for the formation of $1,25(OH)_2D$. Furthermore, in the event of vitamin insufficiency or deficiency, 25(OH)D is efficiently converted to $1,25(OH)_2D$ so that even if a small amount of food containing vitamin D was consumed, it would be completely converted to $1,25(OH)_2D$. For example, individuals with osteomalacia have shown to have elevated levels of $1,25(OH)_2D$, while having low levels of 25(OH)D.[73]

Currently there is some debate over defining the threshold for vitamin D insufficiency, which is based on the relationship between circulating PTH and 25(OH)D levels. An important consideration when interpreting the results of such studies is the type of assay used for measuring serum 25(OH)D.[74] In general, competitive protein binding assays (CPBA), which use rat serum as the binding agent,

cost less and require less work than radioimmunoassays (RIA), which bind with an antibody specific for the 25-hydroxyl-containing side chain. However, CPBAs have been shown to produce spuriously high 25(OH)D levels compared with either RIA or methods using chromatographic purification before doing the CPBA.[75] After adjusting for differences in assay techniques,[74] most studies suggest that serum 25(OH)D <40 nmol/L are indicative of vitamin D insufficiency,[49,61,76,77] while 25(OH)D levels <25 nmol/L should be used to define vitamin D deficiency.[78]

### *1.5.7 Assessment of Osteoporosis*

The definition of osteoporosis is a decrease in bone mass and deterioration of bone microstructure with an increase in bone fragility and risk of fracture.[79] In the last decade considerable progress has been made in developing diagnostic tools to non-invasively assess skeletal mass, and some of these may also address skeletal microarchitecture. These technologies can be grouped into 2 categories: those that use ionizing radiation such as radiographic absorptiometry, quantitative computed tomography, dual-energy x-ray absorptiometry (DEXA); and those that are not radiation based, such as qualitative ultrasound (QUS) and magnetic resonance imaging (MRI). The following section will focus only on DEXA and QUS, as these techniques are used to assess bone in the proposed study. In addition, the measurement of skin thickness as a potential screening tool for osteoporosis will be discussed.

*1.5.7.1 Dual-Energy X-Ray Absorptiometry.* DEXA provides an areal bone mineral density (BMD, g/cm$^2$) and is currently considered the gold standard of bone densitometry. BMD is thought to be the single best predictor of fracture: a decrease of ~10% in BMD increases the fracture risk two fold.[80] The precision of most DEXA machines (1-2% or lower for the spine) is ideal for monitoring small changes in BMD over time. Although the ionizing radiation generated by DEXA machines is relatively low, they are expensive to operate, require a lot of space and are difficult to transport. These drawbacks limit the widespread use of DEXA as a screening tool for osteoporosis. In response to these limitations, a smaller and less expensive densitometer -AccuDEXA$^{TM}$- that measures BMD of the phalanges has recently been developed.[81] Dual-energy computed digital absorptiometry (CDA, Schick Technologies, Long Island City, NY) measures areal density of the phalanges with a precision and accuracy similar to DEXA measurements of the hip and spine. Additional features that make CDA an attractive screening tool include the relatively low cost and space requirement of the device, and ease of portability when compared to current DEXA scanners. Although BMD at a specific skeletal site best predicts fracture risk at that site,[82] studies have shown that several peripheral BMD measurements, including the phalanges, can predict total fracture risk.[80, 83, 84]

*1.5.7.2 Quantitative Ultrasound.* The use of QUS for the assessment of bone is of particular interest due to its low cost, portability, and freedom from ionizing radiation. In addition, it is thought that QUS-measured parameters not only represent bone density, but bone architecture as well.[85] It is generally accepted that fragility

fractures resulting from osteoporosis are not due solely to reduction in bone mass, but also changes in the microstructure of bone.[86] QUS machines measure two parameters: the speed of sound (SOS) and broadband ultrasound attenuation (BUA) through the bone. The most common site of measurement is the heel bone (os calcis) because of the high cancellous bone content, lack of overlying tissue, and ease of measurement. Bauer *et al.*[87] found that BUA and SOS are significant predictors of fracture risk, independent of BMD as measured by DEXA. Some authors have suggested that perhaps the combination of QUS and DEXA provides a better fracture prediction than either one alone.[85, 88] Currently, the poor short-term precision (~1.3-6%) of QUS limits the ability of QUS for monitoring bone changes over time.

*1.5.7.3 Skin Thickness.* Skin, like bone, is a specialized connective tissue whose matrix is primarily made up of type I collagen (~70%).[89] It is well established that after the age of 20, skin thickness decreases linearly with age.[90] Since bone mass also declines with age, various studies have investigated the relationship between skin thickness and bone, in the interest of determining if skin thickness measurements can replace established, but expensive, BMD measurements for screening purposes in the prevention of osteoporosis.[91] Some investigators,[92, 93] but not others,[94, 95] have advocated screening for osteoporosis by measuring the skin thickness.

# CHAPTER 2

## RATIONALE AND HYPOTHESIS

## 2.1 SUMMARY

Vitamin D deficiency has long been recognized as a cause for rickets in children and osteomalacia in adults. More recent is the awareness of a preclinical phase of vitamin D deficiency, termed "vitamin D insufficiency" that increases the risk for osteoporotic fractures.[96-98] Vitamin D can be obtained through the diet or from the skin after sun exposure. However, because few foods are a natural source of vitamin D[99] and fortification of foods with vitamin D is often unreliable,[100, 101] skin synthesis is thought to constitute the major source of vitamin D. Individuals living in countries at higher latitudes are more prone to seasonal vitamin D insufficiency because wintertime sunlight does not promote skin conversion of the vitamin D precursor. In what is now one of the most well known studies on the combined effects of season and latitude, Webb and her coworkers demonstrated that in Edmonton (latitude 52° North) cutaneous vitamin D production does not occur from October through March.[18]

Despite these findings, seasonal variations of vitamin D in a Canadian population and its relation to bone health have been poorly documented. Several studies have examined the vitamin D status of institutionalized elderly.[102-104] Despite the modest sample sizes of these studies ($n < 90$), they all noted a decline of 25(OH)D during the winter with a corresponding rise in PTH. However, low levels of 25(OH)D in these populations is often exacerbated by a limited mobility and activity

characteristic of elderly living in care homes.[64] Delvin *et al.*[105] examined a larger population (n=186) of low income, yet autonomous elderly (mean age ~73±5 yr), and though this study observed the typical decline of serum 25(OH)D during the winter, this study did not examine the corresponding PTH levels.

## 2.2 OBJECTIVES AND HYPOTHESIS

### *2.2.1 Seasonal Variations in Calciotropic Hormones*

The primary objectives of this thesis were to document the prevalence of vitamin D insufficiency (as reflected by serum 25(OH)D) and the contribution of seasonal variation in vitamin D physiology to this problem in a healthy population of Western Canadian men and women. Included in this objective was the examination of associations between 25(OH)D and blood concentrations of $1,25(OH)_2D$, parathyroid hormone (PTH) and other related biochemical markers, such as total serum calcium, inorganic phosphate, alkaline phosphatase and osteocalcin. Alkaline phosphatase and osteocalcin are both markers of bone formation and therefore an indicator of metabolic bone turnover.[106]

Together, the vitamin D hormone system and PTH are the principal regulators of plasma calcium concentrations and therefore influence skeletal calcium reserves. It was hypothesized that 25(OH)D would rise in the spring and summer

months and decrease in the fall and winter months, with a corresponding reciprocal relationship in PTH. We also hypothesized that seasonal variations in $1,25(OH)_2D$ would not accurately reflect 25(OH)D or PTH levels.

### *2.2.2 Cross-Sectional Relation between Calciotropic Hormones and Bone Mineral Density of the Hip and Spine*

A secondary objective of this thesis was to determine the relation between the two principal calciotropic hormones (Vitamin D and PTH) and BMD of the hip and spine as determined by DEXA. We hypothesized that people with a low 25(OHD) (or conversely high PTH) would have a lower BMD of the hip and spine.

### *2.2.3 Relations of Phalangeal Bone Mineral Density and Calcaneal Quantitative Ultrasound with Hip and Spine Bone Mineral Density*

Since BMD measurements of the hip and spine are currently considered the "gold standard" we also wanted to compare more affordable yet less established techniques, such as QUS, phalangeal DEXA and skin thickness with the BMD of the hip and spine. We hypothesized that phalangeal DEXA and skin thickness would be positively correlated with hip and spine BMD, while QUS parameters BUA and SOS would be negatively correlated with hip and spine BMD.

### *2.2.4 Seasonal Variations in Phalangeal Bone Mineral Density and Calcaneal Quantitative Ultrasound*

The seasonal variations of phalangeal BMD and QUS parameters were also examined. Given the lack of precision in measurements typical of QUS, we hypothesized that we would not be able to detect seasonal variations in SOS or BUA (QUS parameters) and we would be able to detect seasonal changes in phalangeal BMD as measured by CDA if there were significant seasonal variations in calcium metabolism and bone mass.

# CHAPTER 3

# METHODS

## 3.1 STUDY DESIGN AND PARTICIPANTS

An overview of the study design and recruitment is presented in Figure 3.1.

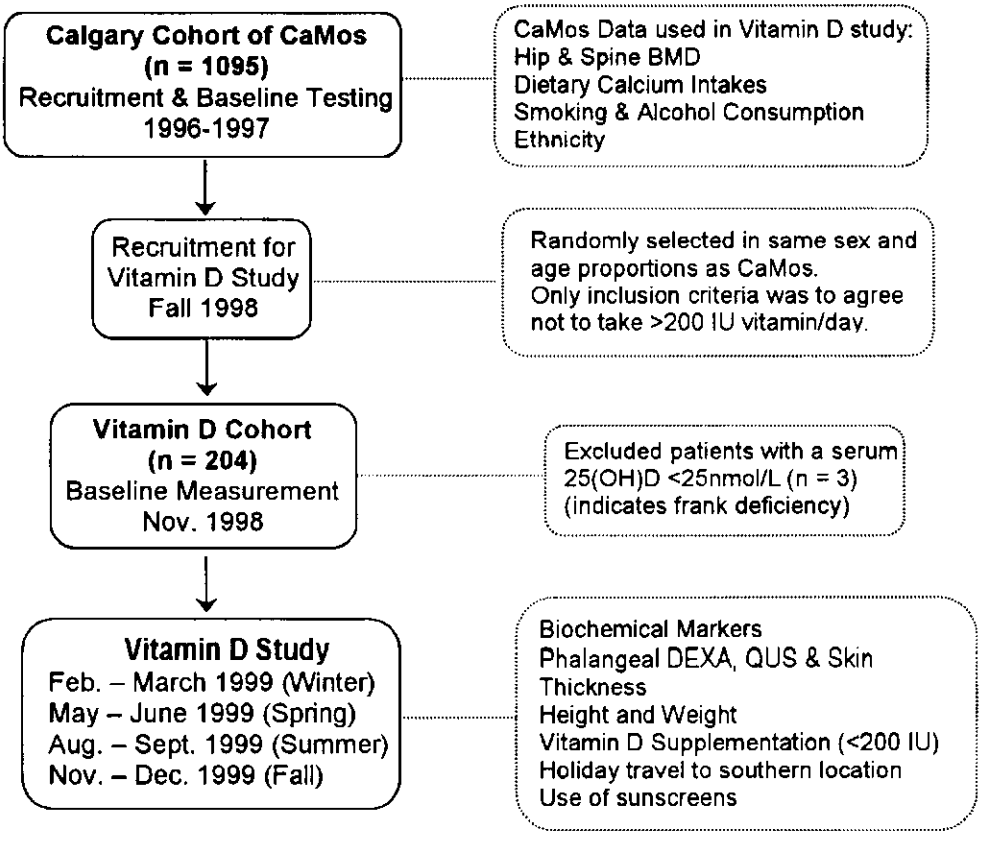


**Figure 3.1** An overview of the study design and recruitment of the Vitamin D study

All vitamin D participants were part of the Calgary cohort of the Canadian Multicenter Osteoporosis Study (CaMos). CaMos is an epidemiological study of osteoporosis in Canada which started in 1996 and is presently ongoing. It is a population-based sample of approximately 9500 Canadians aged 25 and older,

distributed across nine study centres in similar proportions of numbers, sex (approximately 30% men) and age. CaMos participants were recruited by telephone contact after an introductory letter was sent to households randomly selected from telephone listings. Details of the CaMos study-design, including the response rates and analysis of the characteristics on non-responders appear elsewhere.[107] The age strata of CaMos favor the recruitment of postmenopausal females, which is entirely appropriate given the increased incidence of osteoporosis in this group. Participants in the vitamin D study were randomly selected from the Calgary cohort of CaMos (n = 1095) in similar age and sex proportions (Table 3.1) and invited over the telephone to participate in the vitamin D study.

**Table 3.1** Age Distribution in CaMos, Calgary Cohort of CaMos and Vitamin D Study Participants by Sex

| Age (yr) | CaMos | (%) | Calgary Cohort | (%) | Vitamin D Sub-study | (%) |
|---|---|---|---|---|---|---|
| Women | | | | | | |
| 25-50 | 1040 | (16) | 110 | (14) | 20 | (14) |
| 50-60 | 1343 | (21) | 162 | (22) | 28 | (20) |
| 60-70 | 2053 | (32) | 242 | (31) | 43 | (31) |
| 70+ | 1883 | (31) | 257 | (33) | 23 | (34) |
| Total Women | 6319 | | 771 | | 140 | |
| Total% of Women | (67) | | (70) | | (69) | |
| | | | | | | |
| Men | | | | | | |
| 25-50 | 806 | (26) | 87 | (27) | 5 | (8) |
| 50-60 | 653 | (21) | 66 | (20) | 15 | (23) |
| 60-70 | 830 | (27) | 88 | (27) | 21 | (33) |
| 70+ | 815 | (26) | 83 | (26) | 23 | (36) |
| Total Men | 3104 | | 324 | | 64 | |
| Total% of Men | (33) | | (30) | | (31) | |
| Total Number | 9423 | | 1095 | | 204 | |

The only exclusion criteria for the vitamin D sub-study were: (1) vitamin D deficiency (serum 25(OH)D below 25 nmol/L) on pre-study screening, and (2) consumption of vitamin D supplements providing more than 200 IU/day. The frequency of visits and blood collection was the main reason for refusal. All potential and willing participants were asked not to exceed this intake for the duration of the study. Out of 463 people contacted by telephone, 204 agreed to participate. All participants were healthy, ambulatory and community dwelling. The University of Calgary Conjoint Medical Research Ethics Board approved the study, and all participants provided informed written consent (Appendix A).

Each participant came to the center four times at intervals of approximately 3 months. Blood collection was done in 4-6 week time periods starting February 1, May 1, August 1 and November 1. The choice of these sampling time points was dictated by our desire to obtain serum samples between August 1 and mid-September. This was thought to be the time when serum 25-OH Vitamin D levels would reflect the peak body stores of vitamin D. The highest cutaneous synthesis of vitamin D occurs during the months of June and July.[18] In addition, we wanted to ensure participation of subjects was not compromised by summer holiday travel, which, for Calgarians, typically occurs from mid-June through most of August. At each visit blood samples were collected between 7:30 and 10:30 AM after an overnight fast. Skin thickness, QUS and phalangeal DEXA were taken immediately following blood sampling, and interview-administered questionnaires were used to document holiday travel of ≥

1/day at a latitude below 40° N, vitamin D supplementation ≤ 200 IU and use of sunscreen (Appendix B). Height and weight were measured on the first and last visit using a stadiometer and balance scale after participants had removed shoes. Body mass index was calculated as mass/height$^2$ (kg/m$^2$). The average BMI of the first and last visit were calculated for the second and third visit. BMD of the hip and spine, dietary calcium intake, smoking status, alcohol intake, ethnicity and menopausal status in women were obtained from CaMos baseline questionnaires administered two years previously (Appendix C). Dietary calcium intake was estimated by an abbreviated food frequency questionnaire that was administered by a skilled technician using food models to help study participants estimate portion sizes. Calcium intake was expressed as intake of calcium in mg/day. Smoking status was graded as nonsmokers, current smokers, and ex-smokers; where current smokers and ex-smokers were defined as using cigarettes, pipes, cigars or chewing tobacco on daily basis for at least six months within the past year or ever, respectively. Alcohol consumption was categorized as less than or more than 1 beverage/day.

## 3.2 MEASUREMENTS

### *3.2.1 Biochemical Markers*

Serum phosphorus, alkaline phosphatase, total calcium and creatinine were measured by Roche Hitachi autoanalyzer (Roche Diagnostics, Laval, PQ) on the same

day of sampling. Blood samples for Osteocalcin, PTH, 25(OH)D, $1,25(OH)_2D$ were frozen at -80°C and measured in batches within the next month. Intact serum PTH and osteocalcin were measured by a one-step commercial immunoradiometric assay (IRMA) from DiaSorin Inc. (Stillwater, MN). The intra- and inter-assay coefficients of variation (CV, expressed as %) for PTH are 2.4-3.6% and 3.4-4.9%; and 4.5-6.3% and 7.1-9.5% for osteocalcin, respectively. Both 25(OH)D and $1,25(OH)_2D$ were measured in two-step procedures, also using a commercial IRMA kit from DiaSorin Inc. The assays involved a preliminary extraction and purification, followed by addition of antibody and tracer specific for 25(OH)D and $1,25(OH)_2D$. The intra- and inter-assay CVs for 25(OH)D were 11.7-12.5% and 9.4-11%; and 10.5-13.1% and 9.2-10.8% for $1,25(OH)_2D$ respectively.

### *3.2.2 Bone Mineral Density*

BMD of the spine and left hip were measured by DEXA using a QDR 4500c bone densitometer (Hologic, Waltham, MA). Lumbar spine BMD was determined as the average of measurements obtained from L1 to L4. Lumbar segments with aortic calcifications (as determined from standard x-rays by a radiologist) were omitted. The long-term %CV of BMD measurement with this scanner is 0.79% at the lumbar spine and 0.89% at the hip.

BMD of the middle phalanx of the middle finger (non-dominant side) was assessed by dual-energy computed digital absorptiometry using AccuDEXA$^{TM}$ (CDA,

Schick Technologies, Long Island City, NY). The long-term precision of CDA is 1.8%.[81] Quality control was performed by daily calibration using a phantom provided by the manufacturer.

### *3.2.3 Quantitative Ultrasound*

Quantitative ultrasound was measured at the calcaneus using Paris Ultrasound™ (Norland Medical Systems, Inc.). The ultrasound scanner is a dry system with pads coupled to the patient's heel using a standard ultrasound gel. The pads contain a pressurized reservoir of water, which form a coupling medium between the transducers and the heel. The device measures the slope of the frequency-dependent attenuation, broadband ultrasound attenuation (BUA, dB/MHz) and the speed of sound (SOS, m/s). Measurements were taken on the right calcaneus using a standardized positioning protocol. To improve accuracy for detecting small changes, scanning was repeated eight times, with repositioning of the foot after the first four scans. After each scan was completed, the data were automatically analyzed by the software and entered into a database. The BUA and SOS values of the eight scans were later averaged for statistical analysis. The average %CV of the eight measurements within subjects ranged both BUA and SOS was less than 1% during each season (calculated from logarithmic transformation of CV). Due to the unfavorable bias on instruments with a large mean value and relatively small changes that are observed with aging, disease or treatment (such as QUS parameters) in comparison with instruments that offer a small mean absolute values and large

changes (such as DEXA parameters), investigators have proposed the calculation of a standardized CV (SCV) whereby the SD is divided by the clinical range rather the mean.[108-110] The %SCV for all BUA and SOS measurements were 4.1% and 2.1%, respectively.

### *3.2.4 Skin Thickness*

Skin thickness was measured with digital micrometer calipers (precision 0.001 mm). With the subject's hand lying horizontal on a table, careful measurement of the skin fold was made by picking skin on the dorsum of the hand in an area situated over the third metacarpal. Measurements were read by computer software and automatically recorded into a database. Three consecutive measurements were made after repositioning and the mean of all three measurements was used in the analysis. The %CV of the three consecutive measurements from each individual ranged from 4.1-15.3%.

## 3.3 STATISTICAL ANALYSIS

Magnitude, distribution and symmetry of the data were examined with "box and whisker" plots. Outliers (values more than 1 standard deviation above or below the next closest value) were checked to ensure that data were correctly entered.

However, outliers were not omitted from any analysis. Data are presented as mean ± standard deviation (SD) unless otherwise noted. Student's *t*-tests were used to assess differences in baseline demographic data between genders. All data analysis was performed with Stata version 6.0 (Stata Inc., College Station, TX). A $P$-value $< 0.05$ (two-sided test) was considered statistically significant.

### *3.3.1 Longitudinal (Seasonal) Changes in Calciotropic Hormones, Phalangeal Bone Mineral Density and Calcaneal Quantitative Ultrasound*

To determine changes over time within each individual, longitudinal regression models using generalized estimating equations (GEE) were employed. GEEs were developed using the "quasi-likelihood approach."[111] This type of analysis allows for the assessment of repeated observations of a continuous outcome variable and a set of (time-dependent) covariates. Because repeated observations are made on each subject, correlation is anticipated and must be accounted for in the statistical analysis. Separate models were built for all biochemical markers, QUS and phalangeal DEXA. Log transformations of 25(OH)D, PTH, osteocalcin and BMI were used in the regression analysis to correct for right-skewed distributions, but presented as mean ± standard deviation for ease of interpretation. However, $P$ values are based on log-transformation data. Interaction variables were created to assess possible effect modification between age and BMI, as well as covariates that changed with each season (i.e., season and holiday travel). Non-significant predictor variables

were removed from the final model. Regression assumptions of normality, linearity and constant variance were checked with residual plots. All residual values (observed-expected values) were within a band of 2 standard deviations of zero and followed a random pattern.

### *3.3.2 Cross-sectional Relation Among Seasonal Measurements and Bone Mineral Density of the Hip and Spine*

Average values for 25(OH)D, PTH, QUS, phalangeal DEXA and skin thickness were calculated from seasonal measurements. Pearson's partial correlation coefficients were calculated to assess the strength of association between average values of seasonal measurements and BMD of the hip and spine.

Multivariate regression models were used to assess the ability of 25(OHD) PTH, QUS, phalangeal DEXA and skin thickness to predict BMD of the hip and spine after adjusting for other potential confounding variables. Age, BMI and sex were considered *a priori* confounding variables based on established and theoretical relationships between these variables and BMD, and therefore kept in the final model regardless of their significance. Due to the possible threshold effect of both PTH and 25(OH)D on BMD, both hormones were also categorized into quartiles and tested in multivariate regression models as categorical variables. Interaction terms were built to test for potential interaction between PTH and 25(OH)D as well as age and BMI, and non-significant predictor variables were removed from the final model. To facilitate comparison of coefficients within and across each model, normalized *beta*

coefficients were calculated and presented in summary tables. *Beta* coefficients are regression coefficients normalized by the ratio of the SD of the regressor (predictor variable) to the SD of the dependant variable (outcome variable).[112]

The precision error was calculated as the coefficient of variation (CV), or SD divided by the mean for each individual. The standardized coefficient of variation (SCV) was the SD of an individual's measurement divided by the range (the maximum measured value measured minus the minimum measured value). The lowest precision indicates the best precision.

# CHAPTER 4

RESULTS

## 4.1 SEASONAL VARIATIONS IN CALCIOTROPIC HORMONES

Ninety-two percent of subjects (188/204) that were recruited completed the study. Three women were excluded prior to the study for having serum 25(OH)D < 25nmol/L. An additional 13 participants dropped out during the study for various reasons: three women began taking vitamin D supplements ≥ 400 IUs; four women and one man could not make the time commitment, three men and one woman failed to show on test days, and one woman died. Of the 188 subjects that completed the study one man and one woman were Chinese and one man was South Asian; all others (n=185) were of white race.

**Table 4.1** Baseline Characteristics of the Study Population by Sex

| | Men (n=60) | Women (n=128) |
|---|---|---|
| Age (yr) | 63.8±11.9 | 64.3±12.7 |
| Weight (kg) | 82.1±14.3 | 71.1±16.5 |
| Height (cm) | 175.3±7.2 | 161.6±6.5 |
| Body mass index (kg/m$^2$) | 26.7±3.9 | 27.3±6.5 |
| Dietary calcium intake (mg/day) | 807±544 | 882±602 |
| History of smoking > 6 mo (%) | 53.9 | 31 |
| Current smoker > 6 mo (%) | 6.3 | 10 |
| Consumption of > 7 alcoholic beverages/ wk (%) | 15 | 13 |
| Menopausal (surgical or natural, %) | NA | 80 |

Note: data presented as mean plus minus standard deviation unless otherwise noted

Table 4.1 shows the demographic characteristics of the 188 men and women who finished the study. There were no significant sex differences in age and BMI ($P$ > 0.862). Seasonal demographics in vitamin D supplementation, use of sunscreen

and holiday travel are depicted in Table 4.2. The average duration of holidays taken was 24 days.

**Table 4.2** Vitamin D Supplementation, Sunscreen Use and Holiday Travel in Study Group

| | Winter (%) | Spring (%) | Summer (%) | Fall (%) |
|---|---|---|---|---|
| Vitamin D supplementation <200 IU | 25 (13) | 19 (10) | 27 (14) | 32 (17) |
| Regular use of Sunscreen | 21 (11) | 18 (10) | 52 (28) | 53 (28) |
| Holiday Travel >1 day < 40° N | 22 (12) | 16 (9) | 1 (0.5) | 12 (6) |

The seasonal changes in calciotropic hormones and related biochemical markers are summarized in Table 4.3 and shown graphically in Figure 4.1 for 25(OH)D, PTH and $1,25(OH)_2D$. As anticipated, there was a significant rise ($P < 0.01$) in serum 25(OH)D during spring (May-June) and summer (August-September) when compared to the winter (February-March). In the fall (November-December), 25(OH)D levels not only declined, but these levels were also significantly lower ($P = 0.008$) than those during the winter months. There were also several other independent predictors of 25(OH)D: both increasing age ($P = 0.007$) and BMI ($P < 0.0001$) were associated with significant decreases in 25(OH)D, whereas travel to lower latitudes (< 42°) for ≥ 1 day ($P = 0.002$) was associated with a significant increase in 25(OH)D. There was no significant interaction ($P > 0.263$) between holiday travel and season. Also worth noting is that sex ($P = 0.611$), low level vitamin D supplementation ($P = 0.282$) or use of sunscreen ($P = 0.123$) were not significant predictors of 25(OH)D.

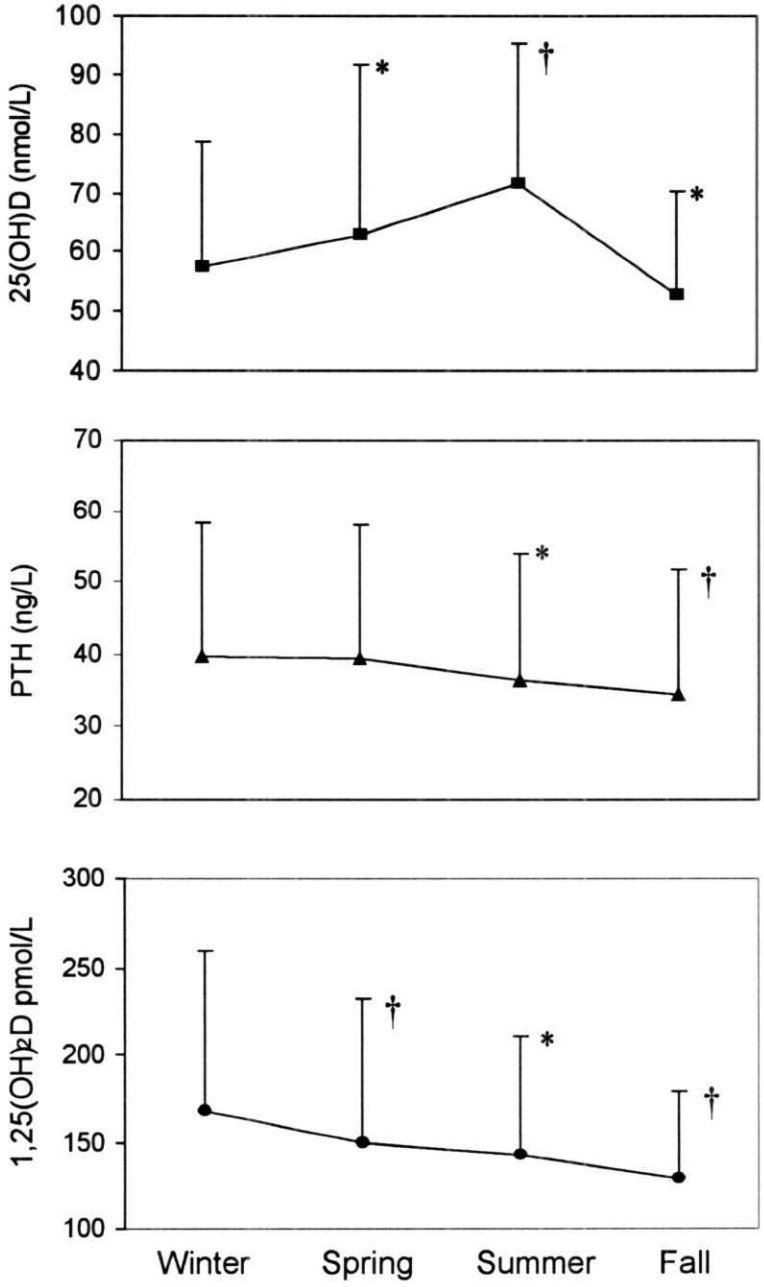


**Figure 4.1** Mean seasonal values for 25(OH)D (■), parathyroid hormone (▲) and 1,25-$OH_2D$ (•). Vertical lines represents standard deviation of the mean. *P* values compare seasonal within-subject variability to baseline winter season (*$P < 0.01$; †$P < 0.0001$) as determined by longitudinal regression models adjusted for other significant predictors (see legend of Table 4.3).

**Table 4.3** Seasonal Changes in Calciotropic Hormones and Related Biochemical Indices

| | Winter | Spring | Summer | Fall | Normal Range |
|---|---|---|---|---|---|
| Plasma 25(OH)D (nmol/L)* | 57.3±21.3 | 62.9±28.8<br>P = 0.001† | 71.6±23.6<br>P < 0.0001† | 52.9±17.2<br>P = 0.008† | 40-130 |
| Plasma 1,25-$OH_2D$ (pmol/L)* | 168.1±91.5 | 148.9±83.4<br>P < 0.0001‡ | 142.0±68.9<br>P = 0.001‡ | 128.9±50.8<br>P < 0.0001‡ | 45.3-145.4 |
| Serum PTH (ng/L)* | 39.5±18.8 | 39.3±18.7<br>P = 0.809§ | 36.3±17.8<br>P = 0.001§ | 34.5±17.3<br>P < 0.0001§ | 13-54 |
| Serum calcium (mmol/L) | 2.30±0.09 | 2.29±0.11<br>P = 0.075‖ | 2.28±0.09<br>P = 0.004‖ | 2.27±0.09<br>P = 0.001‖ | 2.10-2.55 |
| Serum inorganic phosphate (mmol/L) | 1.05±0.15 | 1.03±0.14<br>P = 0.288¶ | 1.09±0.14<br>P < 0.0001¶ | 1.10±0.15<br>P < 0.0001¶ | 0.8-1.6 |
| Serum alkaline phosphatase (U/L) | 80±24 | 80±24<br>P = 0.726** | 83±23<br>P < 0.0001** | 86±24<br>P < 0.0001** | 39-117 |
| Osteocalcin (nmol/L)* | 1.08±0.54 | 1.09±0.54<br>P = 0.463†† | 1.12±0.56<br>P = 0.008†† | 1.18±0.59<br>P < 0.0001†† | 0.46-2.07 |

Note: seasonal values presented as mean plus minus standard deviation; *log-transformations used in regression analysis to correct right-skewed data

*P* values compare seasonal within-subject variability to baseline reference season (winter)

†*P* value adjusted for age, BMI and holiday travel to lower latitudes

‡*P* value adjusted for age, BMI, PTH, 25(OH)D and inorganic phosphate

§*P* value adjusted for age, sex, BMI and serum calcium

‖*P* value adjusted for age and serum PTH

¶*P* value adjusted for sex, PTH and serum calcium

***P* value adjusted for age, BMI and serum calcium

††*P* value adjusted for serum calcium

Levels of $1,25(OH)_2D$ decreased significantly within the normal range in the spring ($P < 0.0001$), summer ($P = 0.001$) and fall ($P < 0.0001$) when compared to the winter months. In addition to season, lower $1,25(OH)_2D$ levels were associated with increasing age and BMI ($P < 0.0001$). Finally, the known complex regulation of $1,25(OH)_2D$ was supported by the biochemical parameters significant in the model: both increasing 25(OH)D ($P = 0.002$) and PTH ($P < 0.0001$) were positively associated with $1,25(OH)_2D$, whereas increased inorganic phosphate was negatively associated ($P = 0.001$) with $1,25(OH)_2D$ and postitively associated ($P < 0.0001$) with PTH.

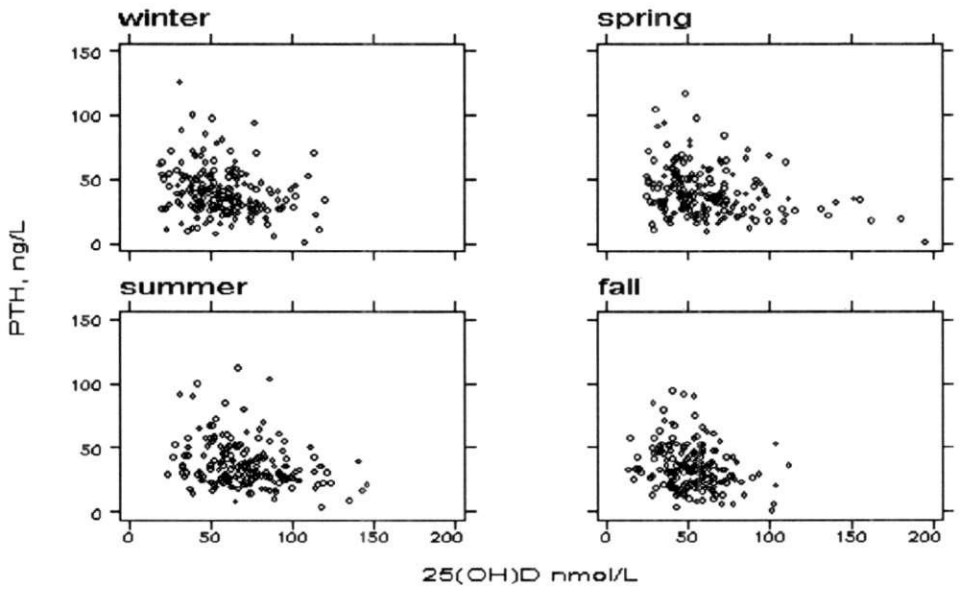


**Figure 4.2** Relation between 25(OH)D and PTH for each season

In comparison to 25(OH)D, serum PTH levels decreased significantly in the summer ($P = 0.001$) as well as in the fall ($P < 0.0001$), so that the anticipated inverse relation between 25(OH)D and PTH was not consistently observed. In addition to season, age ($P < 0.0001$), BMI ($P = 0.006$), sex ($P = 0.022$) and serum calcium ($P <$

0.0001) were independent significant predictors for serum PTH levels. 25(OH)D was not significant ($P = 0.206$) in the prediction of PTH (Figure 4.2).

In accord with the seasonal declines of PTH in the summer and fall serum inorganic phosphate increased during the summer and fall. Serum alkaline phosphatase and serum osteocalcin, on the other hand (markers that are expected to decrease with decreases in PTH) also increased in the summer and fall, which was an unusual and unexpected finding. However, although the seasonal variations in inorganic phosphate, alkaline phosphatase and osteocalcin during the summer and fall were significant ($P < 0.009$), mean values were within the normal reference range (Table 4.2) and therefore these seasonal changes may not be of clinical significance.

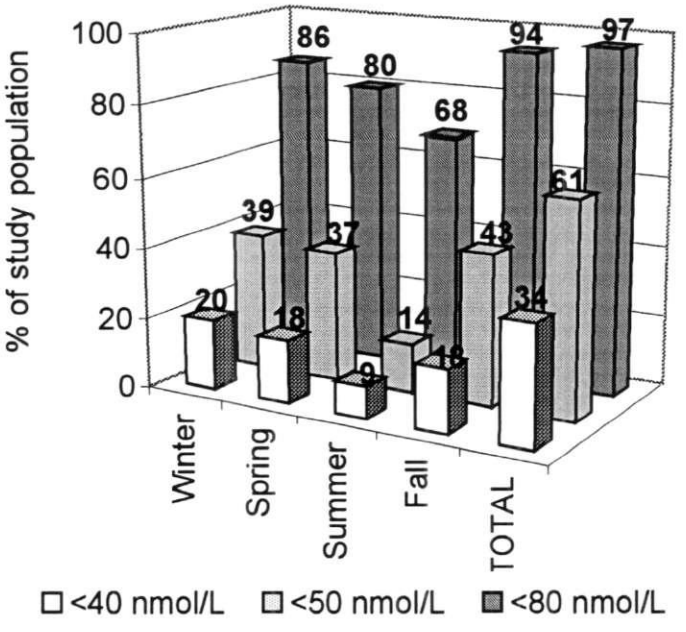


**Figure 4.3** Prevalence of vitamin D insufficiency defined as 25(OH)D levels <40 nmol/L, <50 nmol/L and 80 nmol/L during each season. Total bar represents percentage of individuals having vitamin D insufficiency at least once during the year. Exact percentages of each bar are noted by numbers.

The prevalence of vitamin D insufficiency using several proposed thresholds (less than 40, 50 or 80 nmol/L) per season is shown in Figure 4.3. The prevalence of vitamin D insufficiencies was lowest in the summer, and highest in the fall for all threshold levels. A total of 34% of all our participants had 25(OH)D levels < 40 nmol/L at one point during the year; 61% of participants has 25(OH)D levels < 50 nmol at one point during the year; and 97% had 25(OH)D levels < 80 nmol/L at one point during the year (based on threshhold levels commonly used in literature).

## 4.2 RELATION OF CALCIOTROPIC HORMONES, PHALANGEAL BONE MINERAL DENSITY, CALCANEAL QUANTITATIVE ULTRASOUND AND SKIN THICKNESS WITH HIP AND SPINE BONE MINERAL DENSITY

**Table 4.4** Mean values Densitometric, Quantitative Ultrasound, Skin Thickness and Biochemical Markers by Sex

| | Women (n=128) | Men (n=60) |
|---|---|---|
| Hip BMD ($g/cm^2$) | 0.863 ± 0.135 | 0.983 ± 0.137 |
| Spine BMD ($g/cm^2$) | 0.930 ± 0.149 | 0.986 ± 0.155 |
| PHALANGEAL DEXA[a] ($g/cm^2$) | 0.485 ± 0.087 | 0.614 ± 0.079 |
| BUA[a] (dB/MHz) | 112 ± 7 | 117 ± 5 |
| SOS[a] (m/s) | 1476 ± 49 | 1505 ± 47 |
| Skin thickness[a] (mm) | 0.077 ± 0.013 | 0.091 ± 0.013 |
| 25(OH)D[a] (nmol/L) | 61.1 ±19.6 | 61.3 ± 17.5 |
| PTH[a] (ng/L) | 38.9 ± 16.8 | 34.2 ± 14.9 |

Values represent mean ± SD
[a]Calculated average of 4 seasonal values

Average values for densitometric, QUS, skin thickness, and calciotropic hormones are given in Table 4.4. Men had a significantly higher BUA, SOS and skin thickness and BMD at all sites than women ($P < 0.0001$). There was no significant sex differences in serum 25(OH)D and PTH ($P > 0.069$)

The correlation coefficients of phalangeal DEXA, QUS, skin thickness, 25(OH)D and PTH are shown in Table 4.5. The strongest relation with hip and spine BMD ($r = 0.698$ and $r = 0.603$) was found with phalangeal DEXA. The correlation of QUS parameters and skin measurements was weaker with coefficients ranging from 0.188 to 0.576. In general, the correlation coefficients of all measurement devices were more strongly related at the hip than at the spine BMD. Of the calciotropic hormones, PTH was negatively related with BMD at both sites. There was no significant correlation between 25(OH)D and BMD at either hip and spine ($P > 0.8$).

**Table 4.5** Correlation of Phalangeal Bone Mineral Density, Calcaneal Quantitative Ultrasound, Skin Thickness and Calciotropic Hormones

| | Phalangeal DEXA ($g/cm^2$) | BUA (dB/MHz) | SOS (m/s) | Skin Thickness (mm) | 25(OH)D (nmol/L) | PTH (ng/L) |
|---|---|---|---|---|---|---|
| Hip BMD ($g/cm^2$) | 0.698<br>$P < 0.0001$ | 0.576<br>$P < 0.0001$ | 0.375<br>$P < 0.0001$ | 0.429<br>$P < 0.0001$ | -0.004<br>$P = 0.958$ | -0.258<br>$P = 0.0004$ |
| Spine BMD ($g/cm^2$) | 0.603<br>$P < 0.0001$ | 0.431<br>$P < 0.0001$ | 0.251<br>$P = 0.0005$ | 0.188<br>$P < 0.0001$ | -0.0131<br>$P = 0.859$ | -0.163<br>$P = 0.026$ |

Table 4.6 shows the ability of phalangeal DEXA, BUA, SOS and skin thickness to predict hip and spine BMD. In the prediction of hip and spine BMD all measurement devices remained significant ($P < 0.05$) except for skin thickness ($P > 0.265$). Phalangeal DEXA was the strongest predictor (*beta* = 0.72 and 0.59 for the spine and hip respectively) followed by BUA (*beta* = 0.33 and 0.36 for the spine and hip respectively) and then SOS (*beta* = 0.18 and 0.23 for the spine and hip respectively). The total variance in BMD that could be explained from the multivariate models of phalangeal DEXA and QUS ranged from 18-59%.

After adjusting for age, BMI and sex, higher levels of PTH were significantly correlated with decreased of hip ($P = 0.001$) but not with spine ($P = 0.066$) BMD. Analysis of PTH categorized as quartiles shows a significantly lower hip BMD in the first (lowest PTH values) and second quartile, but not the third quartile when compared to the fourth quartile (highest PTH values). In the multivariate regression model the average of seasonal 25(OH)D was not related to hip or spine BMD ($P > 0.75$), and there were no significant differences in hip and spine BMD within quartiles of 25(OH)D ( $P > 0.27$).

**Table 4.6** Multiple-Adjusted Regression Model of Calciotropic Hormones, Phalangeal Bone Mineral Density, Calcaneal Quantitative Ultrasound and Skin Thickness in the Prediction of Hip and Spine Bone Mineral Density

| | Hip BMD($g/cm^2$) | | Spine BMD ($g/cm^2$) | |
|---|---|---|---|---|
| | *Beta* | *P* | *Beta* | *P* |
| *Calciotropic Hormones* | | | | |
| 25(OH)D (nmol/L) | 0.017 | 0.778 | 0.003 | 0.968 |
| Age | -0.366 | < 0.0001 | -0.265 | < 0.0001 |
| BMI | 0.387 | < 0.0001 | 0.279 | < 0.0001 |
| Sex | -0.410 | < 0.0001 | -0.193 | 0.005 |
| | [a]$R^2$ = 39% | | $R^2$ = 0.15% | |
| PTH (ng/L) | -0.201 | 0.001 | -0.135 | 0.066 |
| Age | -0.309 | < 0.0001 | -0.225 | 0.002 |
| BMI | 0.420 | < 0.0001 | 0.309 | < 0.0001 |
| Sex | -0.383 | < 0.0001 | -0.175 | 0.011 |
| | $R^2$ = 43% | | $R^2$ = 16% | |
| Phalangeal BMD ($g/cm^2$) | 0.590 | < 0.0001 | 0.719 | < 0.0001 |
| Age | -0.173 | 0.001 | -0.026 | 0.608 |
| BMI | 0.312 | 0.316 | 0.193 | 0.001 |
| Sex | -0.060 | < 0.0001 | 0.234 | 0.001 |
| | $R^2$ = 59% | | $R^2$ = 44% | |
| *QUS* | | | | |
| BUA (dB/MHz) | 0.360 | < 0.0001 | 0.329 | < 0.0001 |
| Age | -0.233 | < 0.0001 | -0.141 | 0.039 |
| BMI | 0.325 | < 0.0001 | 0.226 | 0.281 |
| Sex | -0.286 | < 0.0001 | -0.079 | 0.001 |
| | $R^2$ = 49% | | $R^2$ = 23% | |
| SOS (m/s) | 0.236 | < 0.0001 | 0.179 | 0.013 |
| Age | -0.371 | < 0.0001 | -0.267 | < 0.0001 |
| BMI | 0.341 | < 0.0001 | 0.247 | 0.050 |
| Sex | -0.343 | < 0.0001 | -0.142 | < 0.0001 |
| | $R^2$ = 44% | | $R^2$ = 18% | |
| Skin thickness (mm) | 0.053 | 0.560 | -0.092 | 0.265 |
| Age | -0.347 | < 0.0001 | -0.305 | < 0.0001 |
| BMI | 0.369 | < 0.0001 | 0.301 | < 0.0001 |
| Sex | -0.385 | < 0.0001 | -0.236 | 0.004 |
| | $R^2$ = 39% | | $R^2$ = 15% | |

[a]$R^2$ is the proportion of variance of hip or spine BMD explained by regression model

## 4.3 SEASONAL VARIATIONS IN PHALANGEAL BONE MINERAL DENSITY AND CALCANEAL QUANTITATIVE ULTRASOUND

Seasonal changes in phalangeal DEXA and QUS parameters are summarized in Table 4.7. After adjusting for age and sex there were no significant seasonal variations in phalangeal DEXA. BUA decreased significantly in the spring while increasing significantly in the fall ($P < 0.0001$) when compared to the baseline winter measurements, though the biological relevance of these changes was minimal. In accordance with BUA, SOS increased significantly in both in the spring and in the fall. Seasonal fluctuations in both BUA and SOS did not follow the seasonal trends of 25(OH)D or PTH (see Table 3.3).

**Table 4.7** Seasonal Changes in Phalangeal Bone Mineral Density and Calcaneal Quantitative Ultrasound Parameters

| | Phalageal DEXA ($g/cm^2$) | BUA (dB/MHz) | SOS (m/s) |
|---|---|---|---|
| Winter | $0.525 \pm 0.104$ | $114 \pm 7$ | $1480 \pm 62$ |
| Spring | $0.528 \pm 0.103$<br>$P = 0.155$[a]<br>(0.6%)[d] | $113 \pm 8$<br>$P < 0.0001$[b]<br>(-0.68%) | $1488 \pm 54$<br>$P = 0.011$[c]<br>(0.64%) |
| Summer | $0.522 \pm 0.103$<br>$P = 0.109$[a]<br>(-0.93) | $114 \pm 8$<br>$P = 0.701$[b]<br>(0.14%) | $1474 \pm 54$<br>$P = 0.093$[c]<br>(-0.33%) |
| **Fall** | $0.529 \pm 0.104$<br>$P = 0.080$[a]<br>(0.4%) | $115 \pm 7$<br>$P < 0.0001$[b]<br>(1.4%) | $1497 \pm 64$<br>$P < 0.0001$[c]<br>(1.26%) |

Note: seasonal values presented as mean ± SD; $P$ values compare seasons to baseline reference (Winter)

[a] $P$ value adjusted for age and sex

[b] $P$ value adjusted for age, sex and BMI

[c] $P$ value adjusted for sex and BMI

[d] Values in brackets represent average percent change from reference value

# CHAPTER 5

DISCUSSION

## 5.1 SEASONAL VARIATIONS IN CALCIOTROPIC HORMONES

This thesis documents seasonal variations of the major circulating vitamin D metabolite, 25(OH)D, and a high prevalence of vitamin D insufficiencies in a healthy, community-dwelling population of Western Canadians situated at 51.4° N. Vitamin D and 25(OH)D are under the predominant influence of UVB radiation (between wavelengths 290-315 nm) from the sun. Webb and her colleagues[18] have shown that in Boston (located at 42° N), sun irradiation is unable to generate previtamin D *in vivo* from November through February, and in Edmonton (52° N), this period is extended from October through March. These data suggest that Canadians who are not taking vitamin D supplements may be at an increased risk for vitamin D insufficiency during winter months. The relation between seasonal vitamin D variations and geographic locations was supported by the general positive effect of travel to southern latitudes on the level of serum 25(OH)D documented in this study as well as in others.[50, 61] The inability to find significant interactions between holiday travel and season (which one would expect given that holiday travel as a covariate was not consistent within individuals across each season) may in part be influenced by the relatively low number of individuals traveling to lower climates during each season (Table 4.2). While seasonal variations in serum 25(OH)D amongst healthy populations have been frequently noted in several European countries and the United

States,[67] the effect of seasons on vitamin D status in Canadians has only been documented in the institutionalized elderly[102-104] and low-income elderly.[105]

This study also confirms the importance of age as a major determinant of vitamin D status. Regardless of season, increased age was associated with lower levels of $1,25(OH)_2D$ and 25(OH)D. The cause for the age-related decline in $1,25-(OH)_2D$ is multifactorial, including a decrease in renal 1α-hydroxylase activity with age,[66] compounded further by decreased renal function,[65] and the decreased availability of 25(OH)D as a substrate.[113] Declining levels of 25(OH)D with age have been attributed to impaired vitamin D absorption from the intestine[114] as well as a decline in the concentration of vitamin D precursors that are normally stored in the skin.[13]

After adjusting for season, age and travel to lower latitudes, we found that body mass index was inversely related to serum 25(OH)D. Such observations have also been noted in postmenopausal women,[115] elderly population samples[96, 116] and younger obese subjects.[117] Need *et al.*[115] have suggested that the inverse relationship between 25(OH)D and BMI is due to a larger body pool size and/or slower saturation and mobilization of vitamin D and 25(OH)D from adipose tissue.

Vitamin D "insufficiency" which contributes to negative calcium balance and secondary hyperparathyroidism should be distinguished from vitamin D "deficiency" which typically results in rickets in children or osteomalacia in adults. Chronic or seasonal vitamin D insufficiency, without ever leading to overt deficiency, has been

implicated in age-related bone loss that may result in osteoporotic fractures.[96, 97, 118, 119] In order to prevent the consequences of vitamin D insufficiency, studies have attempted to define a threshold level for vitamin D insufficiency based on baseline 25(OH)D and PTH levels in response to vitamin D therapy. After adjusting for differences in assay techniques,[74] most studies suggest that the threshold for serum 25(OH)D should be somewhere between 50-80 nmol/L.[49, 61, 76, 77] Using the most conservative estimate (< 40 nmol/L), 34% of our subjects were vitamin D insufficient at one point during the year. Using a threshold of < 50 nmol/L, approximately 61% of our study participants had insufficient vitamin D levels at one point, whereas virtually all participants (97%) had 25(OH)D levels below 80 nmol/L at least once during the year. Despite the high prevalence of vitamin D insufficiency in this population, we found no relation between PTH values and serum 25(OH)D at any time. Only a small proportion (10-17%) of participants had PTH levels above the "normal range" (provided by the manufacturer) of the DiaSorin assay (> 54 pg/mL) at any time point. Similar to other studies,[76, 120] we noted an increase in PTH with age and in women relative to the men. In addition, we found PTH to increase with BMI. The seasonal declines of PTH, which lagged behind seasonal increases in 25(OH)D by one season, may be the result of previously established vitamin D stores in body fat.[121]

Another possible reason that for the lack of association between PTH and 25(OH)D is that other factors, particularly calcium consumption, may serve to keep serum PTH levels low even during times of 25(OH)D insufficiency. Both Riggs *et*

*al.*[118] and Storm *et al.*[119] reported wintertime suppression of PTH with calcium supplementation without dietary vitamin D supplementation in postmenopausal women. In both of these studies, average calcium intake per day was well over 1500 mg/ day in each the intervention groups (placebo groups averaged between 700-800 mg/day). In addition, Storm *et al.* found a significant inverse relationship between PTH and 25(OH)D only when calcium consumption was < 800 mg/day, suggesting a threshold dose for calcium in order to suppress PTH.[119] An important limitation of this study was that dietary calcium assessments were not obtained during the vitamin D study period. Our subjects did report a relatively low calcium intake on entry into CaMos 2 years earlier, but are likely to have increased their calcium intake as a result of participation in an "osteoporosis study."

It is difficult to estimate the differential effect of vitamin D versus calcium supplementation on PTH status. Sorva *et al.*[122] failed to show an independent effect of calcium supplementation on PTH in patients with a greater degree of vitamin D insufficiency. McKenna has conceptualized different states of vitamin D insufficiency (Figure 5.1): (1) during normovitaminosis the effect of calcium intake can be judged without concern about vitamin D status; (2) during mild or short-term vitamin D insufficiency calcium can ameliorate the effect of vitamin D status; (3) during severe or long-term vitamin D insufficiency calcium intake cannot compensate for vitamin D deficiency.[78] The duration of vitamin D insufficiency is most certainly an important, though poorly studied, factor that may influence the renal hydroxylation

to maintain adequate levels of $1,25(OH)_2D$ in the face of diminishing 25(OH)D substrate concentrations.[78]

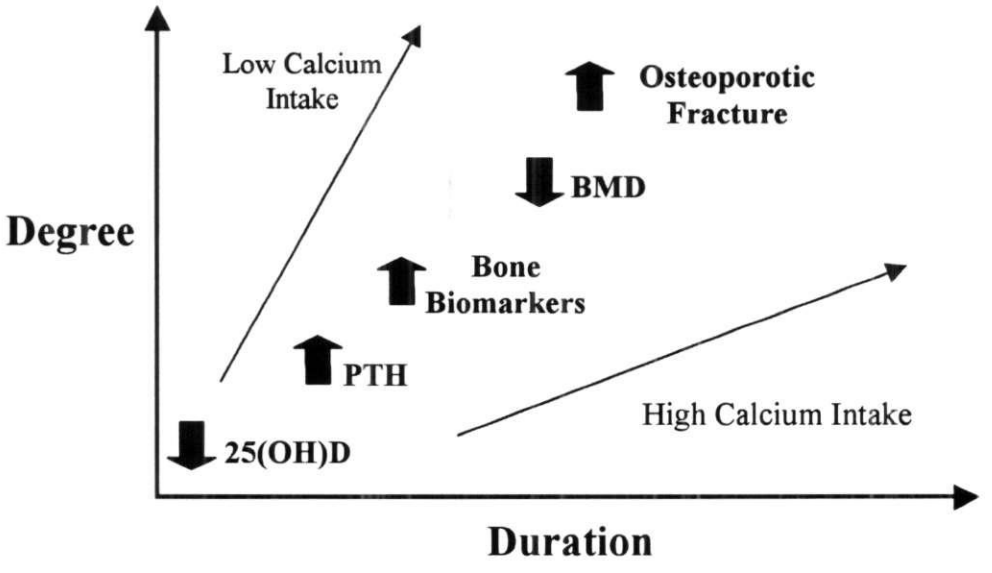


**Figure 5.1** Paradigm for the progression of vitamin D insufficiency through various stages to osteoporotic fractures that take into account the degree and duration of hypovitaminosis and the influence of calcium intake. Modified from McKenna *et al.*[78]

This is a large study of vitamin D metabolism in a Canadian population. However, several important factors must be considered when considering the results of this study to other Canadian populations. Firstly, the majority of our study participants (64%) were over the age of 60, thereby limiting the applicability of the results to a younger population. However, when we limited our analysis to only those in our study population below the age of 60 (n = 68), similar significant results were obtained. Our study sample was predominately Caucasian (except for 3 individuals), which does not accurately reflect more diverse ethnic communities such as

Vancouver and Toronto. However, given that people with increased skin pigmentation have less circulating vitamin $D_3$ compared to individuals with a lighter skin tone,[24, 25] it is likely that these study findings only overestimate serum 25(OH)D levels throughout the year in dark-skinned individuals. The recruitment of individuals for CaMos and the vitamin D study over the telephone may have introduced an ascertainment bias towards a more health-conscious population aware of nutritional factors that affected this study, such as vitamin D and calcium intakes. Finally, because of the importance of latitude on cutaneous vitamin D production, these results cannot be directly applied to other Canadian communities, particularly those with a more southern location. However, virtually all Canadian cities are located above 42° N and Webb and her co-workers[18] provided strong *in vitro* evidence of no cutaneous vitamin D production from November to February at a latitude of 41° N. It is therefore plausible that seasonal vitamin D insufficiencies in the rest of Canada are in fact similar to our population. Furthermore, there are also several factors unique to a Calgary population that may have biased our findings to underestimate the seasonal variations of 25(OH)D in a Canadian population. As shown in Table 5.1, Calgary receives the highest number of sunshine hours per year, particularly in the fall and winter, when compared to other Canadian cities. Though sunshine hours are not an accurate estimate of how much time people spend in the sun *per se*, several studies have noted a strong relation between the number of sunshine hours and levels of 25(OH)D.[54, 115] In addition, it is worth noting that at an

elevation of 1077 m, Calgary residents are situated at a considerably higher altitude than most other Canadians (Table 5.1). Though the effect of altitude on cutaneous 25(OH)D production has not been investigated, Rigel *et al.*[123] demonstrated approximately 8-10% increases of UVB radiation with every 300 m in elevation. As a result, given that participants in this study may have been exposed to a greater number of sunshine hours and resided at higher elevation than most Canadians, it is likely that the seasonal decreases observed in the fall and winter are less than those of other Canadian populations. This also suggests that the observed incidence of seasonal vitamin D insufficiency in this study may be a conservative estimate of the true incidence of vitamin D insufficiency in Canadians.

**Table 5.1** Normal[a] Hours of Sunshine[b] in Canada[124]

| | Latitude (° North) | Elevation (m) | Winter[c] | Spring[d] | Summer[e] | Fall[f] | Total |
|---|---|---|---|---|---|---|---|
| Calgary | 51.7 | 1077 | 425 | 756 | 807 | 407 | 2395 |
| Edmonton | 53.3 | 668 | 382 | 801 | 770 | 344 | 2297 |
| Victoria | 48.3 | 20 | 297 | 694 | 821 | 272 | 2084 |
| Toronto | 43.4 | 112 | 359 | 673 | 707 | 300 | 2039 |
| Quebec City | 46.5 | 102 | 373 | 627 | 635 | 283 | 1918 |
| Vancouver | 49.2 | 86 | 267 | 628 | 717 | 242 | 1854 |
| Whitehorse | 60.4 | 703 | 305 | 760 | 627 | 160 | 1852 |
| St.John's | 47.3 | 114 | 270 | 462 | 560 | 237 | 1529 |

[a]Normal Hours are calculated average of ~1960 to 1990
[b]Sunshine observations are made using the Stokes-Campbell sunshine recorder, which measures only "bright" sunshine rather than "visible" sunshine. For example, sunshine immediately after sunrise and just before sunset would not be bright enough to register.[124]
[c]Total Hours for January, February and March
[d]Total Hours for April, May and June
[e]Total Hours for July, Aug, September
[f]Total Hours for October, November and December

## 5.2 RELATION OF CALCIOTROPIC HORMONES, PHALANGEAL BONE MINERAL DENSITY, CALCANEAL QUANTITATIVE ULTRASOUND AND SKIN THICKNESS WITH HIP AND SPINE BONE MINERAL DENSITY

### *5.2.1 Calciotropic Hormones*

A cross-sectional examination of correlations between calciotropic hormones and BMD of the hip and spine showed that PTH was a significant predictor of the hip, but not spine BMD. This has been documented in other studies,[59, 125] and may be related to the difference in bone type: lumbar BMD measures primarily cancellous bone, whereas hip BMD is composed primarily of cortical bone. In hyperparathyroidism, cortical bone is generally affected more severely than cancellous bone.[126]

There was no relation between average seasonal 25(OH)D values and BMD of the hip or spine. As in our study, the significant association between PTH, but not 25(OH)D with BMD at the hip (or whole body) has been documented in elderly women.[125, 127] Conversely, another study examining elderly women and men[128] found that only 25(OH)D, and not PTH, was related to BMD. The different results in these studies cannot be explained and demonstrate the complexity of the vitamin D/ PTH endocrine regulation on bone.

This study had several limitations. Our findings, like that of any other that is cross-sectional study, cannot be taken as definitive evidence of a causal relationship. BMD measurements were obtained 2 years prior to 25(OH)D measurements and

small decreases of BMD may have occurred prior to the 25(OH)D measurement. This seems unlikely, however, given the general stability of BMD. Even though serum 25(OH)D levels are recognized as the best available indicator of vitamin D status, their significance in bone health is not fully elucidated.[129] Furthermore, given the seasonal variations of 25(OH)D, average values of four seasons may not adequately represent states of vitamin D insufficiency that had profound effects on bone. Finally, it is important to realize that bone tissue, relative to other tissue such as muscle, is slow to respond to systemic stresses imposed by hormones and nutrition, and even slower to evoke a change in measurable bone mass.[48] A gain or loss of bone amounting to 1-2% per year is typically all that can be produced in adults. Moreover, while the gain or loss of bone due to hormonal and systemic factors is real, its detection tends to be dwarfed by other factors that influence bone.[48] For example, in a recent 4-year longitudinal study of the contributions of calciotropic hormones, anthropometric and lifestyle factors to age-related bone loss in a random sample population of men and women, Dennison *et al.*[130] found that serum PTH, 25(OH)D and other biochemical markers of bone turnover did not predict bone loss after adjustment for adiposity.

On the other hand, the lack of association between 25(OH)D and other variables in these data reinforce the notion that the relative contribution of various pathogenic mechanisms of vitamin D in the development of osteoporosis (discussed in Chapter 1; i.e., altered vitamin metabolism and sensitivity with age, genetic and age-related variations in vitamin D receptor) are currently not well understood.

Given the diversity of factors that influence bone health in the human race, such knowledge may remain elusive.

### *5.2.2 Phalangeal Bone Mineral Density, Calcaneal Quantitative Ultrasound and Skin Thickness*

Owing to the high capital cost, space requirements and specialized operator training that currently limits the use of axial DEXA machines in the diagnosis and evaluation of osteoporosis, peripheral measurement devices assessing bone health have been developed as alternative screening tools for the diagnosis of osteoporosis. Several studies have found that peripheral bone sites - measured by either densitometry or ultrasound - can be used to predict both vertebral and hip fracture risk.[80, 84, 131, 132]

The findings of this study support previous data that peripheral measurements by phalangeal DEXA and QUS parameters BUA and SOS are adequate estimates of BMD of the hip and spine. Phalangeal DEXA was the strongest predictor of hip and spine BMD, followed by BUA and then SOS. It is important that the interpretation of these findings remain limited to the original purpose of this analysis, which was to determine the general relationship between densitometric and ultrasound parameters. The results of this thesis do not provide any information on the ability of the phalangeal DEXA and QUS to discriminate patients with osteoporosis from young normal controls. Calculating the sensitivity and specificity usually assesses the accuracy of a screening tool to correctly distinguish between those who have and

those who do not have osteoporosis. This type of analysis was not attempted because only a small proportion of participants (6% using the hip BMD and 11% using the spine BMD) in this population sample actually had osteoporosis. Future validation of the AccuDEXA™ and the Paris™ ultrasound in osteoporotic patients is warranted to determine the clinical utility of these devices.

Skin thickness has also been proposed as a cost-effective screening tool to identify those individuals at a higher risk for osteoporosis[91] mainly because type I collagen is a principal component of both skin and bone. Both Brincat *et al.*[92] and Chappard *et al.*[93] describe a simultaneous decrease in skin thickness and various measures of BMD. However, similar to the findings of other studies[94, 95] that have evaluated the actual relation between skin thickness and BMD measurements, we found only a weak correlation between skin thickness and BMD that was not significant after adjusting for age and BMI. These findings cast further doubt on the clinical utility of skin thickness as a screening tool for osteoporosis.

## 5.3 SEASONAL VARIATIONS IN PHALANGEAL BONE MINERAL DENSITY AND CALCANEAL QUANTITATIVE ULTRASOUND

The clinical utility of a diagnostic tool depends not only the ability to discriminate individuals with osteoporosis, but also on being able to monitor changes in bone with age, disease or treatment. The ideal monitoring tool is that which

measures a meaningful clinical parameter with a large rate of change and a high precision.

There were no significant seasonal variations in phalangeal BMD as measured by CDA observed in this healthy population sample of Canadian men and women. Though we observed significant seasonal variations in BUA and QUS, these changes did not follow the expected seasonal trend of calciotropic hormones. The seasonal changes (generally less than 1.5% for both BUA and SOS for all seasons) were well below the standardized precision error of 4.5%. Given the lack of patients with elevated levels of PTH, the ability of these devices to detect seasonal variations was expected to be poor in this study population. While phalangeal DEXA has not been tested in a longitudinal study, at least one study in a Swiss population of elderly institutionalized women showed that QUS parameters (especially BUA) were correlated with the hyperparathyroidism resulting from vitamin D insufficiency.[133] While this study was limited in the ability to detect seasonal changes of calciotropic hormones, the relative high %SCV for this particular QUS device suggests that better precision is needed before small changes in bone status can measured accurately.

# CHAPTER 6

## SUMMARY AND CONCLUSION

## 6.1 SUMMARY

The role of vitamin D in preventing osteoporosis, mainly through its role in maintaining a positive calcium balance, is well established. An in vivo study has shown that individuals living in countries at higher latitudes are prone to seasonal vitamin D insufficiency because of the inability of skin to synthesize the precursors for vitamin D. Despite this, the effect of season on vitamin D metabolism and bone health has not been documented in a healthy population of Canadians. The findings of this thesis are summarized with respect to: (1) Effect of season on vitamin D metabolism and prevalence of vitamin D insufficiency; (2) Relation of calciotropic hormones with bone; (3) Relation of quantitative ultrasound, phalangeal BMD and skin thickness with hip and spine BMD; (4) The ability of QUS and phalangeal DEXA to monitor seasonal variations of vitamin D. Recommendations for future research are addressed within each summary.

### *6.1.1 Seasonal Effects on Calciotropic Hormones and Prevalence of Vitamin D Insufficiency*

This study documented decreased levels of 25(OH)D during the fall and winter months. Other important risk factors for low 25(OH)D levels were an increased age and BMI. Using a threshold of <50 nmol/L of 25(OH)D, ~65% of the study participants suffered from vitamin D insufficiency during the year. However, given that the seasonal variations in PTH and other markers of bone turnover did not

follow the seasonal trends of 25(OH)D, and were generally within the accepted clinical values. These study results therefore suggest further questioning as to what an appropriate threshold level constitutes vitamin insufficiency. The study results may have been affected by variation of an unmeasured parameter, calcium intake, and careful documentation of this is vital in future studies of this nature in order to determine the independent effects of calcium and vitamin D on PTH. In addition, duration of vitamin D status (particularly when insufficient) and precise composition of lean and fat body tissue (as assessed by some DEXA machines) may be important factors that contribute to the endocrine regulation of vitamin D and PTH.

### *6.1.2 Relation between Calciotropic Hormones and Bone*

No relation was found between 25(OH)D and BMD of the hip and spine. A negative relation was found between PTH at the hip but not the spine. The inability of this study to show any significant relation between 25(OH)D and bone is likely due to the cross-sectional nature of this study, small sample size, measurement inadequacies of true 25(OH)D levels and to the difficulty in ascertaining the effects of this hormone in bone. Definitive studies to answer these questions will require long-term longitudinal studies, in a larger population of individuals with meticulous attention to other confounding variables, such as exercise and nutrition.

### *6.1.3 Relation of Calcaneal Quantitative Ultrasound, Phalangeal Bone Mineral Density and Skin Thickness with Hip and Spine Bone Mineral Density*

QUS parameters BUA and SOS and phalangeal BMD were adequate predictors of hip and spine BMD. Future studies should be conducted in osteoporotic patients to determine the diagnostic ability of these machines. Skin thickness of the hand was not related to hip or spine BMD, and is not likely to offer any more convenience of measurement compared to the other devices tested in this study.

### *6.1.4 Seasonal Variations in Phalangeal Bone Mineral Density and Calcaneal Quantitative Ultrasound*

Given the lack of variation in PTH throughout the year it was not possible to determine the clinical utility of these devices in monitoring the seasonal variations or progression of osteoporosis.

## 6.2 CONCLUSION

Vitamin D levels in a community-dwelling population of Canadians varied by season, and frequently below the recommended level of 25(OH)D. Despite these findings, the expected reciprocal elevation was not found in PTH levels. Although these results suggest a recommendation for dietary vitamin D supplementation may

be warranted in a Canadian population, the effect of vitamin D on bone health requires further, long-term studies.

This study also documented a strong relation between phalangeal BMD and heel QUS with BMD of the hip and spine, but more data is required to determine the clinical utility of these devices as a screening tool for osteoporosis and their ability to monitor changes over time.

# CHAPTER 7

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# APPENDIX A

CaMos
Canadian Multicentre Osteoporosis Study
*Étude Canadienne multicentrique sur l'ostéoporose*

THE UNIVERSITY OF CALGARY
David A. Hanley, M.D., FRCPC
Professor and Head, Division of Endocrinology and Metabolism
Faculty of Medicine, Department of Medicine

**Research Project Title:** **25- Hydroxy -Vitamin D Substudy of the Canadian Multicenter Osteoporosis Study**

**Investigator:** **Dr. David Hanley**

**Funding Agency:** **The Alberta Heritage Foundation for Medical Research**

**This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what participation involves. If you would like more details about something mentioned here, please feel free to ask. By reading this carefully it will help you understand the purpose of the study.**

**Purpose of the Study**

**As a participant of CaMos ( the Canadian Multicenter Osteoporosis Study) we are asking if you would be willing to participate in a second study within CaMos. The aim of this study is to determine whether seasonal changes in your body's Vitamin D production are also associated with changes in bone ultrasound and DEXA measurements. Vitamin D production by your body, may be reduced in the winter due to decreased exposure to sunlight. We can partially assess vitamin D production by measuring your blood level of 25- Hydroxy- Vitamin D. If you agree to participate in the substudy, you will be requested not to alter your intake of Vitamin D ( diet or vitamin pills) for one year, unless advised to do so by your own physician.**

**Procedure**

**The study involves quarterly blood and urine collection , calcaneus (heel bone) ultrasound measurement and dexa of the finger using the Accudexa. The Accudexa measures BMD by using Dual Energy Absorptiometry (DEXA). Using x-ray exposure that is trivial or about 1/100 of a dental x-ray, a bone density assessment is made within seconds. By simply placing your hand into the unit and positioning the middle finger over an ultra sensitive sensor, a measurement is made with no potential discomfort to you.**
**Every three months for one year, 15 ml (approximately the amount contained in three teaspoons) of blood will be drawn and you will be asked not to eat breakfast prior to the blood being taken. The blood and bone measurements will be done during the first two months of each season (Jan-Feb; April-May; July- Aug; Oct.- Nov). The relationship of blood levels of Vitamin D over one year, your previously measured bone density by DEXA (on admission to CaMos) , ultrasound measurement and BMD using Accudexa will be determined. The results of blood tests will be made available to you unless you specifically indicate that you do not wish to have them.**
**You are welcome to take the results to your family doctor and discuss them with him/ her.**

# APPENDIX B

## Vitamin D Questionnaire (#4)

Date:_____
Name:_____
ID:_____

Height:_____ *(visit #1 and #4 only)*

Weight:_____ *(visit #1 and #4 only)*

1. Have you taken any vitamin D or multi-vitamins? Yes ⇓ No
   If yes, how many IU of Vitamin D are you taking?_____

2. Have you spent any time outside of Canada in the last 3 months? Yes ⇓ No
   If yes, please indicate dates, where you went, and estimate how much time was spent in the sun per day:

3. Did you wear sunscreen on a regular basis? Yes ⇓ No
   If yes, what factor?_____

4. In the last year, have you had any of the following conditions: *(visit #4 only)*

| | | |
|---|---|---|
| Liver Disease | Yes | No |
| Kidney Disease | Yes | No |
| Crohn's Disease | Yes | No |
| Cancer | Yes | No |
| Diabetes | Yes | No |
| Convulsions or Seizures | Yes | No |
| Heart Disease | Yes | No |
| High Blood Pressure | Yes | No |

5. In the last year have you taken any of the following medications:

| | | |
|---|---|---|
| Estrogen, Glucocorticosteroids or Bisphosphonates? | Yes | No |
| Thyroid Medications? | Yes | No |

6. Did you have any major surgery in the last year? Yes No
   If you answered yes, please describe:

# APPENDIX C

Respondent I.D. # _____

CaMos

Canadian Multicentre Osteoporosis Study

*Étude canadienne multicentrique sur l'ostéoporose*

# QUESTIONNAIRE

1.7 * How would you best describe your race or colour?
(*Do not read list. Mark all that apply*)

- ☐ White
- ☐ Chinese
- ☐ South Asian (e.g. East Indian, Pakistani, Punjabi, Sri Lankan)
- ☐ Black (e.g. African, Haitian, Jamaican, Somali)
- ☐ Native/Aboriginal Peoples of North America (North American Indian, Métis, Inuit/Eskimo)
- ☐ Arab/West Asian (e.g. Armenian, Egyptian, Iranian, Lebanese, Moroccan)
- ☐ Filipino
- ☐ South East Asian (e.g. Cambodian, Indonesian, Laotian, Vietnamese)
- ☐ Latin American
- ☐ Japanese
- ☐ Korean
- ☐ Other (Specify _____)

1.8 How many years of school have you finished? (*Mark the highest grade completed*)

- ☐ less than grade 9
- ☐ grades 9-13, without certificate or diploma
- ☐ high school certificate or diploma
- ☐ trades or professional certificate or diploma (CEGEP in Quebec)
- ☐ some university without certificate or diploma
- ☐ university certificate or diploma
- ☐ university degree

1.9 * What is your current employment status?

- ☐ employed full time
- ☐ homemaker (full time)
- ☐ employed part time
- ☐ unemployed
- ☐ disability
- ☐ retired ⟶ How old were you? ____ years
- ☐ other (specify _____)

1.10 Do you live alone ? ☐ Yes ☐ No

↳ Do you live with another adult?
☐ Yes ☐ No

1.11 Do you have a particular doctor or clinic that you would call your regular doctor or clinic? ☐ Yes ☐ No

* See notes in manual

*In this section I would like to ask you questions that will help us understand how women's hormones relate to bone structure. We ask everyone these questions.*

## 5. REPRODUCTIVE HISTORY (FEMALES)

5.1 * Before menopause, have you ever gone 3 months or more without a menstrual period?
*(not including pregnancy or during breastfeeding)*

☐ Yes ☐ No
→ Go to 5.2

What was the longest single period of time without a menstrual flow? _____ months

If you count all the periods you have missed throughout your menstruating years, how many months would that be? _____ months
*(this question asks for the cumulative time)*

5.2 * Have your menstrual periods stopped for more than one year?
*(No period one year or more after last menstruation)*

☐ Yes ☐ No
→ At what age? _____ years

5.3 Have you had your uterus removed (*hysterectomy*)?

☐ Yes ☐ No
→ At what age? _____ years

5.4* Have you ever had one or both ovaries removed?

☐ Yes, one ovary removed at what age? _____
☐ Yes, both ovaries removed at what age? _____
*(if ovaries were removed on separate occasions, write the age at which the second ovary was removed)*
☐ Yes, do not know how many at what age? _____
☐ No

* See notes in manual

*I'm going to ask you a few questions on your eating habits.*

8.7 a) I am going to read two sentences for you. Please answer True (T) or False (F) for each statement as it pertains to you.

| | | |
|---|---|---|
| I enjoy eating too much to spoil it by counting calories or watching my weight. | T ☐ | F ☐ |
| I consciously hold back at meals in order not to gain weight. | T ☐ | F ☐ |

b) Which of these best describes you?

On a scale of 0 to 5, where 0 means no restraint in eating (*eating whatever you want, whenever you want it*) and 5 means total restraint (*constantly limiting food intake and never "giving in"*), what number would you give yourself?

0 Eat whatever you want, whenever you want it
1 Usually eat whatever you want, whenever you want it
2 Often eat whatever you want, whenever you want it
3 Often limit food intake, but often "give in"
4 Usually limit food intake, rarely "give in"
5 Constantly limiting food intake, never "giving in"

*Now the questions I will ask will relate to the use of tobacco.*

## 9. TOBACCO

9.1 Have you ever used any of the following tobacco products daily for at least 6 months?

| | | | |
|---|---|---|---|
| Cigarettes | ☐ Yes | ☐ No | |
| Pipes | ☐ Yes | ☐ No | |
| Cigars | ☐ Yes | ☐ No | |
| Chewing tobacco | ☐ Yes | ☐ No | → If NO to all: go to 9.3 |

9.2 Complete the following table for each product used.

→ At what age did you begin to ..... daily? (*for at least 6 months*)
→ Are you currently smoking?
→ At what age did you stop?
→ Approximately how many every day? (*number of cigarettes, bowls of pipe tobacco, number of cigars, number of chews*)
→ Have you temporarely stopped ..... and started again? (*total up all periods and covert to years*)

| | AGE STARTED | CURRENTLY SMOKING | | AGE STOPPED | AMOUNT PER DAY | TEMPORARELY STOPPED (YEARS) |
|---|---|---|---|---|---|---|
| | | YES | NO | | | |
| Cigarettes | | | | | | |
| Pipe | | | | | | |
| Cigar | | | | | | |
| Chewing tobacco | | | | | | |

9.3 a) On average, over the last month, have you been exposed to the tobacco smoke of others (*i.e. environmental tobacco smoke (ETS)*)?

☐ Not at all
☐ < 3 hours/day
☐ 3-8 hours/day
☐ 9 or more hours per day

b) Have you ever been exposed to ETS for more than 6 months?

☐ Yes ☐ No

↳ ☐ < 3 hours/day
☐ 3-8 hours/day
☐ 9 or more hours per day

Number of years _____

* See notes in manual

*Now I will ask you in detail about the foods you eat*

## 10. FOOD INTAKE

10.1 * How often (on the average) have you eaten the following items?

| | During the last 12 months? | | | | | In your 30's (If subject 40 years or over) | | | | In your teens? | | | | As a child? | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fold | Never | servings per month | servings per week | servings per day | Serving Size | Never | Less | Same | More | Never | Less | Same | More | Never | Less | Same | More |
| Milk to drink incl. choc. milk & hot cocoa w/milk | | | | | ☐ 125 ml (0.5 cup)<br>☐ 250 ml (1.0 cup)<br>☐ 375 ml (1.5 cup) | | | | | | | | | | | | |
| Milk on cereal | | | | | ☐ 60 ml (.25 cup)<br>☐ 125 ml (0.5 cup)<br>☐ 250 ml (1.0 cup) | | | | | | | | | | | | |
| Milk/cream in tea/coffee | | | | | ☐ 15 ml (1 tbsp)<br>☐ 30 ml (2 tbsp)<br>☐ 60 ml (4 tbsp) | | | | | | | | | | | | |
| Milk desserts (tapioca, rice pudding) | | | | | ☐ 125 ml (0.5 cup)<br>☐ 250 ml (1.0 cup) | | | | | | | | | | | | |
| Hard cheese (to eat, in sandwich or mixed dish) | | | | | ☐ 15 g (0.5 oz)<br>☐ 30 g (1 oz)<br>☐ 60 g (2 oz) | | | | | | | | | | | | |
| Yogurt | | | | | ☐ 125 ml (0.5 cup)<br>☐ 175 ml (single)<br>☐ 250 ml (1 cup) | | | | | | | | | | | | |
| Ice-cream, ice milk or frozen yogurt | | | | | ☐ 125 ml (0.5 cup)<br>☐ 250 ml (1.0 cup)<br>☐ 375 ml (1.5 cup) | | | | | | | | | | | | |
| Cream soups made with milk | | | | | ☐ 125 ml (0.5 cup)<br>☐ 160 ml (.67 cup)<br>☐ 250 ml (1.0 cup) | | | | | | | | | | | | |

* See notes in manual

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| | During the last 12 months? | | | | | In your 30's (If subject 40 years or over) | | | | In your teens? | | | | As a child? | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Food | Never | servings per month | servings per week | servings per day | Serving Size | Never | Less | Same | More | Never | Less | Same | More | Never | Less | Same | More |
| Canned salmon or sardines with bones | | | | | ☐ 30 g (1 oz)<br>☐ 60 g (2 oz)<br>☐ 90 g (3 oz) | | | | | | | | | | | | |
| Broccoli | | | | | ☐ 60 ml (.25 cup)<br>☐ 125 ml (0.5 cup)<br>☐ 250 ml (1 cup) | | | | | | | | | | | | |
| Dark leafy greens (bok choy, kale, gailan (Chinese broccoli), collards, dandelion greens) | | | | | ☐ 60 ml (.25 cup)<br>☐ 125 ml (0.5 cup)<br>☐ 250 ml (1 cup) | | | | | | | | | | | | |
| Dried peas or beans (navy, pinto, kidney) | | | | | ☐ 60 ml (.25 cup)<br>☐ 125 ml (0.5 cup)<br>☐ 250 ml (1 cup) | | | | | | | | | | | | |
| Whole wheat buns, bread, rolls, bagels | | | | | ☐ 1 serving = 1 slice<br>½ bagel<br>½ pita | | | | | | | | | | | | |
| White bread, buns, rolls, bagels, etc. | | | | | ☐ 1 serving = 1 slice<br>½ bagel<br>½ pita | | | | | | | | | | | | |
| Tofu | | | | | ☐ 60 ml (.25 cup)<br>☐ 125 ml (0.5 cup)<br>☐ 250 ml (1 cup) | | | | | | | | | | | | |
| Multivitamin, Vit. D or cod liver oil | | | | | ☐ 1 supplement | | | | | | | | | | | | |
| Calcium suppl. or "TUMS" | | | | | ☐ 200 mg<br>☐ 300 mg<br>☐ 500 mg | | | | | | | | | | | | |

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*Now some questions about the liquids/fluids you might choose to drink.*

## BEVERAGES *

10.2 How many of the following drinks did you consume?

*In these questions, one serving of alcoholic beverage is:*
- *1 bottle or can of beer or a glass of draft (12 oz):*
- *1 glass of wine or a wine cooler (4-5 oz)*
- *1 straight or mixed drink with (1-1½ oz) hard liquor*
- *1 serving of tea or coffee is 6 oz*
- *1 serving of cola is 12 oz - 1 can (355 ml)*

| Beverages | | During the past 12 months? | | | | In your 30's (If subject is 40 years or over) | | | | When in your teens? | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | None | Serving /month | Serving /week | Serving /day | None | Less | Same | More | None | Less | Same | More |
| Coffee | caffeinated | | | | | | | | | | | | |
| | decaffeinated | | | | | | | | | | | | |
| Tea | caffeinated | | | | | | | | | | | | |
| | decaffeinated | | | | | | | | | | | | |
| Colas | caffeinated | | | | | | | | | | | | |
| | decaffeinated | | | | | | | | | | | | |
| Alcoholic beverages | | | | | | | | | | | | | |

* See notes in manual

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