Vitamin D status and bone health in Inuit women 40 years of age and older

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2. ABSTRACT

Low serum 25-hydroxyvitamin D (25(OH)D) concentrations and calcium intakes and a high risk of fracture have been separately reported in Inuit women. We examined vitamin D status, nutrient intake, and forearm bone mineral density (fBMD) in Inuit women (*n*=419, aged 40-90 y) from Nunavut. Fasting serum was assayed for 25(OH)D, osteocalcin (OC) and parathyroid hormone (PTH). Vitamin D status was below optimal (≤ 75 nmol/L) in 69.4% of pre- and 37.6% of postmenopausal women. Vitamin D and calcium intakes (per 1000 kcal) tended to increase with age. Low fBMD was observed in 33% of post- (T-score < -1.5) and 2% of premenopausal women (Z-score < -2). Predictors of fBMD included body mass index and OC, and age in the post- and PTH in the premenopausal group. Lower vitamin D status and dietary nutrient density among premenopausal women suggests a greater risk for osteoporosis with aging compared to the previous generation.

3. RÉSUMÉ

Des bas niveaux sériques de vitamine D (25(OH)D), un apport alimentaire insuffisant en calcium, ainsi qu'un risque élevé de fracture ont été rapportés séparément chez les femmes Inuit. Nous avons évalué le statut de vitamine D, l'apport alimentaire de nutriments, et la densité minérale osseuse à l'avant-bras (aDMO) chez les femmes Inuit (*n*=419, âgées de 40 à 90 ans) du Nunavut participant dans l'Enquête de Santé Inuit 2007/2008. Les taux sériques de 25(OH)D, d'ostéocalcine (OC) et de parathormone (PTH) ont été mesurés. Le statut de vitamine D était sous-optimal (≤ 75 nmol/L) dans chez 69.4% des femmes pré-ménopausées et 37.6% des femmes ménopausées. Les apports alimentaires de vitamine D et de calcium (par 1000 kcal) avaient tendance à augmenter avec l'âge. La aDMO était basse chez 33% des femmes ménopausées (T-score < -1.5) et 2% des femmes pré-ménopausées (Z-score < -2). Les prédicteurs de la aDMO comprenaient l'indice de masse corporelle et l'OC, ainsi que l'âge dans les femmes ménopausées et la PTH dans les femmes pré ménopausées. L'alimentation et le statut de vitamine D des femmes pré ménopausées suggèrent que, avec l'âge, leur risque pour l'ostéoporose sera plus élevé que la génération précédente.

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6. LIST OF ABBREVIATIONS

%BF – percent body fat

%CV – percent coefficient of variation

$1,25(OH)_2D$ – 1,25-dihydroxyvitamin D, or calcitriol

$24,25(OH)_2D$ – 24,25-dihydroxyvitamin D

25(OH)D – 25-hydroxyvitamin D, or calcidiol

AI – adequate intake

ALP – alkaline phosphatase

bALP – bone alkaline phosphatase

BMD – bone mineral density

BMI – body mass index

BRU – bone remodeling unit

CINE – Centre for Indigenous People's Nutrition and Environment

COX – cyclooxygenase

CTX – C-telopeptide

DBP – vitamin D binding protein

DPD – deoxypyridinoline

DRI – dietary reference intake

DXA – dual-energy x-ray absorptiometry

EAR – estimated average requirement

EDTA – ethylenediaminetetraacetic acid

EER – estimated energy requirement

ELISA – enzyme-linked immunosorbent assay

fBMD – forearm bone mineral density

FFQ – food frequency questionnaire

FM – fat mass

FNBHS – First Nations Bone Health Study

GHYL – galactosyl hydroxylysine

Glc.GHYL – glucosyl galactosyl hydroxylysine

HOB – human osteoblast-like cells

HPLC – high pressure liquid chromatography

HRT – hormone replacement therapy

HUVEC – human umbilical venous endothelial cells

IGF – insulin-like growth factor

IGFBP – insulin-like growth factor binding protein

IL – interleukin

IPY – International Polar Year

IRMA – immunoradiometric assay

LBM – lean body mass

M-CSF – macrophage-colony stimulating factor

mRNA – messenger ribonucleic acid

MSC – mesenchymal stem cell

OC – osteocalcin

OHPr – hydroxyproline

OPG – osteoprotegerin

PA – physical activity

pDXA – peripheral dual-energy x-ray absorptiometry

PGE – prostaglandin E

PTH – parathyroid hormone

PYD – pyridinoline

QCT – quantitative computed tomography

QUS – quantitative ultrasound

RANKL – receptor activator of NF-κB ligand

RDA – recommended dietary allowance

RIA – radioimmunoassay

RXR – retinoic acid receptor

TGF – transforming growth factor

TNF – tumour necrosis factor

UL – upper limit

UV – ultraviolet

VDR – vitamin D receptor

VEGF – vascular endolthelial growth factor

WHO – World Health Organization

7. CONTRIBUTION OF AUTHORS

The first author of the both manuscripts contained in this thesis participated in the 2008 IHS as an interviewer and dietary quality control manager, performed the statistical analysis and subsequent interpretation of the data, reviewed relevant literature, and drafted the manuscripts. Biochemical measurements of serum 25(OH)D, PTH and OC were also performed by the first author. The entry and cleaning of dietary data was performed by a number of students, including the first author. All co-authors reviewed drafted manuscripts and provided suggestions for improvement, highlighting areas deserving further development and suggesting helpful references.

8. INTRODUCTION

Disparities between the health status of Aboriginal populations and the national population are known to exist in Canada and globally (1). Though the absolute burden of mortality and morbidity in Canadian Aboriginal populations has decreased substantially in the past 50 years or so, in large part due to the massive decline in infectious disease, mortality and morbidity rates remain higher, and infectious disease rates have plateaued at a higher level than the general Canadian population (1, 2). For Inuit, chronic diseases such as diabetes and cardiovascular disease are on the rise, and accidents, suicides, violence and substance abuse are responsible for much of the ill health and premature mortality in many Inuit communities (2). Research is needed to inform the prioritization of health needs and determine solutions for eliminating health disparities. Compared to other Canadian Aboriginal populations, Inuit have been the subject of more published research, which is surprising based on their smaller numbers (3). Despite this, many areas of health remain relatively unexplored amongst Inuit populations. A health survey conducted in 2004 in Nunavik (Northern Québec) named "Qanuippitaa" (how are we?) prompted a desire in Nunavumiut populations for similar health research that could practically inform policies and procedures to minimize the consequences of the rapid transitions occurring in their communities. "Qanipitali" (how are we too?), the Nunavut Health Survey was thus undertaken in 2007, and quickly evolved into the Inuit Health Survey due to the desire of Inuit in Nunatsiavut (Northern Labrador) and the Inuivialuit Settlement Region (Western Arctic) to be included in the project. Using data derived from this wide-spanning research endeavor, this study's aim was thus to add to the knowledge on Inuit health with respect to vitamin D status and bone health.

9. LITERATURE REVIEW

9.1 Inuit Health and Vitamin D Status

Arctic Indigenous peoples have thrived for thousands of years in a harsh environment due to their ability to adapt to the climate and live off the land, with knowledge of hundreds of species of plants and animals (4). In recent times, however, an upward trend in obesity has been observed globally in Indigenous populations, presumably due to potential genetic predisposition, as well as a decreased level of physical activity combined with an energy-dense diet containing a higher proportion of market than country food (5). Prior to colonial contact in the Americas, the Inuit derived 100% of their dietary energy from country food (defined as animal and plant species culturally identified as food and harvested from the local environment) (5). At the turn of the 20th century, upon the establishment of the Hudson's Bay stores, the intake of market foods (defined as foods shipped from the South and purchased in stores) began to increase. A century later, the percent of energy derived from country food had significantly decreased, accounting for only 14.1-36.0% of dietary energy amongst Inuit. Variance in this percentage was accounted for by age, sex, and body mass index (BMI), with older age, male sex, and lower BMI being associated with deriving a higher proportion of dietary energy from country food (5). This phenomenon, commonly referred to as nutrition transition, combined with other social, societal and environmental factors, has resulted in the presence of large health disparities between Canadian Aboriginal and non-Aboriginal peoples with respect to shorter life expectancy and increased rates of chronic disease (2, 6).

Recent research implicates vitamin D in many chronic diseases, including osteoporosis, diabetes, cancer and immune diseases (see **Section 9.5**) (7), however, the data on vitamin D intake and status is sparse for the Aboriginal population. A high prevalence of vitamin D concentrations below the normal range was reported in mothers and children in two large Manitoba Cree communities, with 76% of mothers having serum concentrations of 25-hydroxyvitamin D (25(OH)D) less than 25 nmol/L (8). Vitamin D intake was significantly lower and the risk of deficiency (based on the recommended intake

of 10 μg/d for pregnant women) was higher in native (Inuit and First Nations) versus non-native mothers in the Northwest Territories (9). It should be noted, however, that the risk of deficiency for the group as a whole with and without supplementation remained high at 39.0% and 80.8%, respectively. Older studies from the mid-80's to early 90's examining nutrient intake and status among native Canadians in Alberta also found low intakes and plasma concentrations of several micronutrients, including vitamin D (9). An analysis of the contribution of a selection of market and country foods to the diets of Nunavik women revealed that 40% of the intake of a number of nutrients, including vitamin D, came from country foods, though the major source of energy was market food; low calcium intakes were also reported in this population (10). More recently, Kuhnlein et al (5) described the displacement of a large proportion of the consumption of country foods by market foods in Dene/Métis, Inuit and First Nations populations, and thus a decline in the intake of a number of nutrients, including vitamin D. Vitamin D and calcium intake was investigated in Aboriginal (rural and urban) and White (urban) women in Manitoba during the 2002 First Nations Bone Health Study (FNBHS). There were no ethnic differences in overall vitamin D intake, however, rural Aboriginal women obtained all of their vitamin D from food sources (as opposed to supplements), and had significantly higher intakes than both the urban Aboriginal and White women. Calcium intake was low in all women, but especially urban Aboriginal women (11).

Overall, the Aboriginal population seems to be at increased risk for vitamin D deficiency, and low calcium intakes have been observed in Aboriginal compared to White women. The following sections will examine the features, formation and regulation of vitamin D as well as its importance for health (and particularly bone health), current requirements, and major sources for Inuit; a review of bone metabolism and ethnic differences therein is also provided.

9.2 Vitamin D: Features, Formation, and Regulation

Vitamin D can be endogenously synthesized from a precursor, 7-dehydrocholesterol (or provitamin D_3), which in adults is concentrated in the epidermis and, to a lesser extent, the dermis (12). This precursor is transformed by

a phytolytic process to previtamin D_3 upon exposure to ultraviolet B (UVB) radiation (290-315nm), followed by a slow, heat-dependent isomerization to vitamin D_3 (or cholecalciferol, see **Figure 1**). The conversion of provitamin D_3 to previtamin D_3 in the skin is affected by skin pigmentation, age, use of sunscreen, time of day, season and latitude (12, 13). Melanin is a skin pigment that absorbs UVB and thus acts as a natural sunscreen, therefore in those with darker skin tones, less UVB is able to pass into the epidermis and dermis where 7-dehydrocholesterol is located. An inverse relationship between the concentration of 7-dehydrocholesterol present in the epidermis and age has been observed, leading to a decreasing ability to synthesize vitamin D_3 with age (7, 12). Depending on the solar zenith angle of the sun, the ozone layer can become more or less capable of absorbing UVB radiation from the sun, thus depending on the location, season, and time of day, the amount of UVB radiation reaching the skin will differ (7, 14). During the winter months, individuals living at latitudes north of 40°N or south of 40°S produce no vitamin D (15). UVB radiation usually peaks around midday, though the exact time of the peak as well as its magnitude varies depending on one's location (12). As an additional consideration, human behavior and social customs can affect how much any given individual's skin is exposed to the sun. In the warmer months, the use of sunscreen and protective clothing is promoted to prevent skin cancer. A study of adolescent girls wearing concealing clothing because of religious dress codes revealed high levels of vitamin D deficiency (13). Finally, obesity can influence the release of vitamin D_3 from the skin into the bloodstream, as increased subcutaneous fat will cause a sequestering of the fat-soluble vitamin. Wortsman et al found serum concentrations of 25(OH)D after sun exposure to be 57% lower in obese (BMI > 30 kg/m^2) versus non-obese (BMI $\leq$ 25) subjects of the same skin color (white; skin types II and III) (16).

Once formed, vitamin D_3 is carried out of the skin into the bloodstream attached to vitamin D binding protein (DBP), after which it can be stored in adipose tissue or further transformed by mitochondrial and microsomal vitamin D-25-hydroxylase enzymes (also called P450C25 or CYP27A1) in the liver to

25(OH)D, the major circulating form of vitamin D (see **Figure 1**) (12, 13, 17-19). P450C25 can also be found in the skin, kidney and intestine, however the major determinant of serum concentrations of 25(OH)D is the liver (20). Like vitamin D_3, however, 25(OH)D is metabolically inert and must undergo a final hydroxylation in the mitochondria of cells in the renal proximal convoluted tubule by 25(OH)D-1α-hyrdoxylase (also called P450C1 or CYP27B1) to $1,25(OH)_2D$, or calcitriol, the active hormone (12, 13, 17, 18). Though enzyme expression is highest in the kidney, P450C1 is also present in the skin, intestine, macrophages and osteoblasts, but just as the liver determines the serum concentration of 25(OH)D, the kidney determines the serum concentration of $1,25(OH)_2D$ (20). Another form of vitamin D, called vitamin D_2, is produced naturally by plankton from ergosterol (or provitamin D_2) and artificially by irradiation of the mold ergot, which can contain up to 2% ergosterol (17). The use of the term "vitamin D" encompasses both the D_2 and D_3 forms.

Vitamin D can thus be obtained from the diet, though not in substantial amounts (see **Section 9.7**). As a fat-soluble vitamin, vitamin D is taken up in the small intestine with the help of bile salts and free fatty acids and transported into the blood via the intestinal-lymphatic system, circulating in the form of a chylomicron/lipoprotein complex (21). As is the case with endogenously produced vitamin D, dietary vitamin D is bound in the circulation to DBP (60%) or lipoproteins (40%), whereupon some is deposited in body fat reserves while the rest is shuttled to the liver (12, 21, 22). It should be noted, however, that the lower proportion of vitamin D bound to DBP decreases the half-life of dietary compared to endogenously produced vitamin D (22). Additionally, vitamin D_3 is said to be 2 to 3 times more effective than vitamin D_2 in raising serum concentrations of 25(OH)D (22).

The synthesis of vitamin D is under both photochemical and physiologic regulation. In the skin, previtamin D_3 can either undergo thermal isomerization to vitamin D_3 or, if it absorbs more UVB radiation, isomerize to biologically inert isomers lumisterol and tachysterol (12, 23). Vitamin D_3 itself can also undergo isomerization to inactive 5, 6-transvitamin D_3 suprasterols upon continued

exposure to UVB radiation, thus preventing prolonged sun exposure from increasing serum 25(OH)D to toxic concentrations (12, 23). Once vitamin D_3 enters circulation, the products of its transformations to 25(OH)D and calcitriol negatively inhibit the respective enzymes by which they were hydroxylated (20). This negative feedback is not so tightly regulated, however, that an increase in dietary vitamin D or exposure to sunlight would be prevented from increasing serum concentrations of 25(OH)D, it would just occur at a progressively slower rate. In the kidney, the production of calcitriol by P450C1 is stimulated by parathyroid hormone (PTH), and thus indirectly by a decrease in serum calcium (see **Sections 9.3.2, 9.3.3 and 9.4**), or a decrease in serum phosphorous concentrations (12, 20). 24-hyrdoxylase (also called P450C24 or CYP24) is an enzyme that can transform calcidiol into inactive 24, 25-dihydroxyvitamin D (24, $25(OH)_2D$) that is quickly excreted in bile. P450C24 is thus responsible for the maintenance of normal concentrations of circulating $1,25(OH)_2D$ and therefore intracellular calcium concentrations (see **Section 9.4**); its activity is enhanced by calcitriol and inhibited by a decrease in serum phosphorous (20). Though its activity is highest in the proximal tubule of the kidney, P450C24 can also be found in more distal kidney tubule segments and nearly all cells in the body; however its induction by calcitriol is unique to the kidney (20).

To exert its various actions (see **Sections 9.4 and 9.5**), vitamin D must bind to vitamin D receptors (VDR) in the nucleus of cells and can thus influence the transcription of messenger RNA (mRNA). VDR are members of the steroid hormone zinc-finger superfamily of receptors, selectively binding calcitriol to the retinoic acid receptor (RXR) to form a heterodimer that interacts with vitamin D-responsive elements encoded in genes (12). Though the VDR has 500-1000 times higher affinity for $1,25(OH)_2D$, serum concentrations of 25(OH)D are approximately 1000 times higher than $1,25(OH)_2D$ (24), therefore the biological activity of 25(OH)D cannot be completely ruled out (25, 26). Though somewhat controversial, the existence of a membrane-bound form of VDR which would allow vitamin D to exert non-genomic actions involving the generation of second messengers or the phosphorylation of intracellular proteins has also been

proposed (27). Elucidation of these aspects is important in order to understand the relationships between vitamin D status and chronic diseases, including osteoporosis.

9.3 Bone Metabolism

9.3.1 Bone Structure

Bone is composed of an organic matrix (osteoid) within which hydroxyapatite crystals (precipitated calcium phosphate salts) are deposited (18, 24). Collagen type I comprises 90-95% of the organic matrix that determines the shape and three-dimensional structure of bone, with the remaining percentage made up of proteoglycans, glycoproteins, and lipids (28). Cortical (or lamellar in adults and woven in infants) bone forms the hard outer layer of bone - the shaft (or diaphyses) and protective caps - whereas trabecular (also called spongy or cancellous) bone forms the lacy interior of cuboidal bones, flat bones and the ends (or epiphyses) of long bones (29, 30). Cortical bone comprises 80% of the skeleton, concentrated in the appendicular skeleton, and the remaining 20% of the skeleton is made up of trabecular bone, mostly in the axial skeleton and metaphyses of long bones (29). Due to differences in the architecture of the structural units from which they are composed, trabecular bone is more porous than cortical bone and has a much greater surface area despite its lesser abundance in the skeleton (31).

Bone contains 99% of the body's calcium (including skeleton and teeth), making it critical to calcium homeostasis (maintained within the narrow range of 2.2-2.6 mmol calcium/L) (18, 28). The control of calcium metabolism encompasses two aspects: calcium balance and calcium homeostasis. The former ensures that calcium intake equals excretion over the long term and the latter, that normal serum calcium concentrations are constantly maintained. Control of calcium balance is controlled by adjusting the intestinal absorption and urinary excretion of calcium (18). Control of calcium homeostasis, on the other hand, is on a minute-to-minute basis and involves calcium exchanges between the bone and the extracellular fluid and, to a lesser extent, urinary calcium excretion (18). The first resource of calcium in the bone is the labile pool of calcium contained in

bone fluid and partially mineralized bone. Under conditions of chronic hypocalcemia, the body will draw on the stable pool of calcium in mineralized bone, which can decrease bone mineral density (BMD) over the long term (18, 24). Each of the two types of bone described above lose calcium at a different rate, with the loss of cortical bone beginning around the 4th decade of life and proceeding at a slow and steady pace, and the loss of trabecular bone subject to the day-to-day regulation of hormones but becoming particularly significant in the 3rd decade of life and losing bone mineral content at a much faster rate (32). While most people think of bone as a relatively inert tissue, it is actually extremely dynamic, with bone remodeling constantly taking place at sites termed bone remodeling units (BRU) or bone multicellular units (33, 34); the entire skeleton is remodeled every 10 years (35). Trabecular bone remodeling takes place mainly to maintain calcium homeostasis as described above, whereas cortical bone remodeling occurs primarily in response to loading, to repair microdamage, and to maintain cell viability (29), all of which are essential to maintain bone's adaptability to changes in use and to remove damaged areas while maintaining metabolic balance for the rest of the organism (36, 37). An additional difference between the two types of bone is that the remodeling (or turnover) rate is about eight times faster in trabecular than in cortical bone, thus the greatest turnover is noted in trabecular-rich regions of the thoraco-lumbar spine and certain areas of the femur (38).

9.3.2 Bone Cells

The two major types of bone cells involved in the process of bone remodeling are osteoclasts (which break down bone) and osteoblasts (which build bone). Osteoclasts arise from hematopoietic monocyte-macrophage precursor cells or B-220-positive pre-B-cells and are found on the outer surfaces of bone, anchored to the bone matrix by integrins in their sealing zones (34). This sealing zone forms the barrier around a bone-resorbing compartment between the osteoclast's characteristically ruffled border and the bone surface in which there is an extremely low pH; this acidic environment, combined with the action of acid proteases that are synthesized and secreted by osteoclasts, allows for the

dissolution of mineralized bone (28, 34, 39). This resorptive phase takes about 2 to 3 weeks in adults, and is subsequently followed by bone formation by osteoblasts (24).

Osteoblasts arise from mesenchymal stem cells (MSC) (40) and are found in both the outer (periosteum) and inner (endosteum) layers of bone (18). Osteoblasts are responsible for the bone formation phase, producing the organic matrix of bone, which then undergoes primary mineralization (60-70%) within 6-12 hours, followed by secondary mineralization (remaining 30-40%) in 1-2 months (28). Some osteoblasts then become entombed in the bone around them, and are referred to as osteocytes; these cells remain metabolically active and connect to osteoblasts via extensions of their cell membranes, forming the osteocytic-osteoblastic bone membrane (18). It is across this membrane that calcium from the labile pool is rapidly transferred to the blood when serum PTH, which rises in response to a decrease in serum calcium, activates calcium pumps in the membrane, resulting in calcium from bone fluid and newly mineralized bone being pumped into osteocytes and osteoblasts then transferred to the plasma (18). Any remaining osteoblasts on the surface of bone become quiescent and flatten out into lining cells (24).

Many other types of cells can also be found within bone. Osteoprogenitor cells, also called stromal cells (24, 33) or MSC (40), are present within the bone marrow. It is from these cells that osteoblasts arise and from which osteocytes receive nutritional support (28). The hematologic precursors of osteoclasts can also be found within the bone marrow (33). Other cells within the bone marrow include adipocytes, which have a reciprocal relationship with osteoblasts, T-cells, which are a source of RANKL (receptor activator of NF-κB ligand, see **Section 9.3.3**) and mast cells. The relationship between mast cells and bone health is ill-defined, as they have been implicated in osteoporosis via either a mere increase in their number or an increase in number and a subsequent increase in interleukin (IL)-6 production (see **Section 9.3.3**) (24). Lining cells are flat, fibrocyte-like cells that are derived from (or closely related to) the osteoblast cell line and cover all free bone surfaces. It is to these cells that osteocytes communicate information

about the amount of mechanical strain that is occurring in their domain, presumably causing the lining cells to retract and initiate local bone remodeling projects, though much remains to be elucidated on this topic (24). Since bone is a vascularized tissue, endothelial cells should also be mentioned, releasing such substances as endothelial growth factor, cytokines, and other regulatory factors such as nitric oxide and prostaglandins into the local environment, all of which can have an influence on bone metabolism (34).

9.3.3 Bone Remodeling

As mentioned above, bones are constantly in a state of turnover. Children and adolescents are in positive calcium balance and modeling can take place to strengthen the structure of bone (28, 33). When peak bone mass is achieved early in the third decade of life, bone resorption by osteoclasts and bone formation by osteoblasts are coupled and take place at approximately equal rates (28). The uncoupling of bone turnover takes place during somatic growth, ageing, metabolic bone disease, increased or decreased mobility, therapeutic interventions, and other conditions, usually leading to changes in bone strength, structure, and mass (41). The rate at which this bone turnover occurs has also been linked with a higher risk of fracture in postmenopausal women (36, 41). The balance of bone resorption over bone formation is influenced by local, hormonal, nutritional and mechanical factors (37). There are four stages in bone remodeling that occur at each BRU: activation, resorption, reversal, and formation (34). Though they perform opposite tasks, osteoclasts and osteoblasts interact in a unique way during the bone remodeling process and a number of local factors produced by osteoblasts, osteocytes, and stromal cells are involved. Pre-osteoblasts are the target cells for the initiation of the first stage of the bone remodeling cycle (activation), and their differentiation is activated by the transcription factor core binding factor I (or RUNX2), the expression of which is enhanced by a variety of local and systemic factors (34). Osteoblasts can then begin to secrete macrophage-colony stimulating factor (M-CSF) and, under the influence of bone resorption regulators that rely on one of three different signal pathways, express a cytokine called RANKL on their cell membranes. RANKL is a soluble membrane-bound ligand that binds to

RANK on the membranes of hematopoietic monocyte-macrophage precursor cells and, in the presence of M-CSF and its receptor on osteoclasts, leads to their differentiation into active osteoclasts (34, 40). The three aforementioned signal pathways, each of which is initiated by a different set of hormones, cytokines, or prostaglandins, are: (i) the vitamin D nuclear receptor-mediated pathways (e.g. for calcitriol), (ii) the protein kinase A-mediated pathways (e.g. for PTH, prostaglandin E_2 (PGE_2), and interleukin 1 (IL-1)), and (iii) the gp130-mediated pathways (e.g. for leukemia inhibitory factor, IL-6, IL-11, and oncostatin M). All of these pathways exert indirect effects on bone resorption by stimulating osteoblasts to produce RANKL, which then exerts the direct effects on bone resorption described above (40). The active osteoclasts proceed to resorb bone during the resorption phase of remodeling until reversal, the most poorly understood step of the cycle, during which osteoclasts undergo apoptosis and osteoblasts are recruited to the remodeling site for the final formation phase (34).

As mentioned previously, PTH rises in response to a decrease in serum calcium – it is the most important hormone regulating calcium homeostasis and the principal determinant of how much bone remodeling occurs at any given moment (24, 37). PTH is produced by the parathyroid glands (28). Intermittent administration of PTH has been known to have an anabolic effect on bone since the 1930's (42). However, excess bone loss has often been associated with the elevated concentrations of PTH that accompany aging and cause an increase in bone resorption (36), with PTH preferentially increasing the loss of cortical rather than trabecular bone (43). PTH exerts its effects by acting on both the kidney and bone (see **Figure 1**). In the kidney, it increases the activation of 25(OH)D to $1,25(OH)_2D$ (see **Section 9.2**) and the reabsorption and excretion of calcium and phosphorous, respectively, in the distal tubule (18). In bone, it stimulates the production of osteoclast-activating factors by osteoblasts (28) and enhances the activity of newly formed osteoclasts so that they become active osteoclasts (20). In women 55 years of age and older, PTH had a negative correlation with BMD and a positive correlation with serum C-telopeptide (CTX), a marker of bone resorption (see **Section 9.3.4**), with CTX lagging behind PTH by 1-2 months (44).

PTH also indirectly promotes the absorption of calcium by increasing serum concentrations of $1,25(OH)_2D$, which increases the absorption of calcium in the intestine (see **Section 9.2**) (18). Overall, an increase in serum calcium that exceeds the needs for bone formation established by the previous cycle of bone resorption is obtained, and the surplus is used to re-establish normal serum calcium concentrations (24).

The presence of estrogen receptors in bone cells, especially osteoblasts, has been well established (33). The decrease in estrogen concentrations that accompanies menopause leads to deleterious consequences in bone (18, 33, 40), as estrogen is the primary hormone responsible for maintaining bone mass in adult women (33). More specifically, there is a decrease in bone formation during the years preceding menopause that, when associated with an acceleration in bone resorption at menopause, leads to significant bone loss during these years (39). Through a variety of cellular mechanisms (see **Figure 1** for some examples), a 50 to 100% increase in the biomarkers of bone metabolism may be observed in postmenopausal women.

Mechanical factors are another determinant of bone turnover, and whereas PTH is the main determinant of *how much* bone remodeling occurs, the pattern of strain is the main determinant of *where* bone remodeling occurs (24). An increase in mechanical stress is a significant stimulus to modeling in adults (33), and new bone will be formed at those sites where mechanical strains are detected most frequently (18, 24, 33, 37). The removal of mechanical stress on bones, such as that observed during prolonged bed rest or space travel, results in a decrease in BMD (18, 24).

9.3.4 Biomarkers of Bone Turnover

Several biomarkers of bone turnover, arising due to their production by osteoblasts (markers of bone formation) or from the breakdown of osteoid (markers of bone resorption), can be measured in serum and urine. Several caveats must be kept in mind, however, when interpreting the concentrations of these biomarkers. Firstly, the distinction between markers of bone formation and resorption is not always clear. Osteocalcin (OC), for example, is produced by

osteoblasts and correlates well with the bone formation rate, however, it is also released during bone resorption (39, 45). Secondly, most of the markers are also produced by tissues other than bone, therefore a proportion of their concentration derives from non-skeletal origins. Thirdly, any changes in the concentrations of these biomarkers reflect the skeleton as a whole, and therefore cannot be used to assess fracture risk at a specific site. Finally, elevated concentrations of these markers may reflect a number of other diseases apart from postmenopausal osteoporosis, making it important to identify any secondary sources of bone loss (39, 45).

Alkaline phosphatase (ALP) is a membrane-bound enzyme produced by osteoblasts; it is thought to play a role in the initiation of mineralization via the hydrolysis of any surrounding organic phosphate compounds that would otherwise inhibit mineralization, the increase of local concentrations or transportation of inorganic phosphate, and/or the binding of calcium (24, 46). ALP concentrations in serum rise in vitamin D deficiency (see **Section 9.4**), and this is thought to occur as a result of disrupted bone formation (36). There are several isoforms of ALP arising from the liver, bone, intestine, spleen, kidney, placenta, and it can sometimes be expressed by certain tumours. Serum concentrations of total ALP show good correlation with bone ALP (bALP) in healthy adults and, if liver disease is ruled out, are a good indicator of osteoblast activity and the extent of new bone formation. The measurement of bALP by electrophoresis, precipitation, immunoradiometric assay (IRMA), or radioimmunoassay (RIA) is preferred for clinical use due to its higher specificity, however some assays can still show up to 20% cross-reactivity with liver ALP (39).

Osteoblasts also produce OC, a small glycoprotein containing residues of calcium-binding γ-carboxyglutamic acid, which, though its exact role is unknown, has the ability to bind hydroxyapatite. It is thought to play a key role in the organization of the extracellular matrix, limiting bone mineralization or regulating bone turnover (39). It can be measured in serum by immunoassay (RIA, IRMA, enzyme-linked immunosorbent assay (ELISA)) as an indicator of osteoblast

function and thus a marker of bone formation in particular and bone turnover in general (39, 46).

Collagen, which makes up the majority of osteoid, is also broken down into fragments during bone resorption, resulting in the release of varying amounts of hydroxyproline (OHPr) (the major breakdown product of collagen), and the pyridinium crosslinks pyridinoline (PYD) and deoxypyridinoline (DPD), which can be subsequently measured in urine (36). Hydroxyproline can be measured by colorimetric or high pressure liquid chromatography (HPLC) methods, but is considered to be a less specific indicator of collagen turnover, as significant amounts of it are derived from newly synthesized collagen, taken up from foods, liberated during the metabolism of elastin and C1q, and can be found in tissues other than bone (39). PYD and DPD, on the other hand, are only present in mature collagens, not taken up from food and, with the exception of PYD which is also present in cartilage, ligaments, and vessels, are uniquely present in bone and dentin. The pyridinium crosslinks can be quantified in urine by HPLC or in serum by ELISA (39). N-telopeptide (NTX) and CTX, which are protein fragments derived from the terminal ends of collagen I whose highest contribution is from bone, can be measured in serum or urine by ELISA (39). These telopeptides are more specific for bone than PYD and DPD, NTX even more so than CTX (46). Other collagen breakdown products include galactosyl hydroxylysine (GHYL) and glucosyl galactosyl hydroxylysine (Glc.GHYL), the former being more abundant in bone than in other human tissues. GHYL can be measured in urine, but analytical methods are time-consuming and Glc.GHYL has not been widely studied or validated as a marker of bone resorption (46).

9.4 Vitamin D and Bone Metabolism

The principal physiologic function of vitamin D is in the maintenance of intra- and extracellular calcium concentrations. In order to accomplish this function, vitamin D regulates the absorption of calcium and phosphorous in the intestine, their release from bone, and their excretion in the distal tubule of the kidney (12).

In the intestine, calcitriol binds to nuclear VDRs within enterocytes, promoting the transcription and translation of mRNA for the proteins that make up the epithelial calcium channels and the calcium-binding protein calbindin. These proteins enhance cellular uptake of calcium from the small intestine. In the jejunum and ileum, calcitriol enhances uptake of phosphorous, though the mechanism responsible for this remains unidentified (12).

If a further increase in serum calcium is required, calcitriol will bind to VDRs within osteoblasts, enhancing the production of RANKL on their surface and indirectly increasing the number of osteoclasts as RANKL interacts with RANK on the membranes of osteoclast precursor cells (see **Section 9.3.3**) (12). As the number of active osteoclasts increases, serum calcium and phosphorous subsequently rise due to the promotion of bone resorption (12).

Calcitriol can also bind to VDRs in the cells of the distal tubule of the kidney, increasing the reabsorption of the last 1% of the filtered load of calcium while decreasing the reabsorption of phosphorous (17, 18). This inverse action results from the solubility characteristics of calcium phosphate salts in the blood such that when serum calcium increases, serum phosphate decreases, and vice versa. It should be noted, however, that serum phosphorous is not as tightly regulated as serum calcium (18).

There is also evidence that calcitriol enhances bone formation as well as resorption and influences the balance of calcium between blood and bone in ways that are not yet fully understood (47). In vitro studies in human and rat cell cultures suggest that it may play a role in coupling bone resorption and formation during the bone remodeling cycle (27) and have anabolic effects on osteoblasts such as via the stimulation of osteoblast production of type I collagen, ALP (48), OC (12, 24, 48) and osteopontin (12, 24). A study examining the short-term (3-day) treatment of rats with high-dose calcitriol revealed that although an initial increase in bone resorption (as evidenced by increased serum and urinary calcium concentrations) was observed, histomorphometry revealed that the treatment was able to increase osteoblast and osteoid perimeter, osteoid area, and decrease osteoclast perimeter, the latter being presumably an indirect action mediated by

calcitriol's ability to decrease serum PTH. The enlargement of osteoblast and osteoid perimeter and osteoid area was hypothesized to have been caused by an increase in osteoblast number and/or the stimulation of exisiting osteoblasts (49). Finally, additional indirect effects of calcitriol on osteoblasts may relate to its role in the interdependent relationship between osteoblasts and endothelial cells. An in vitro study by Wang et al (50) revealed that when human osteoblast-like cells (HOB) were cultured with human umbilical venous endothelial cells (HUVEC), osteoblast production of vascular endothelial growth factor (VEGF) stimulated the proliferation of HUVEC, which subsequently increased HUVEC production of the osteoblast growth factors insulin-like growth factor (IGF)-1 and endothelin-1 and thus stimulated the proliferation and differentiation of osteoblast cells as evidenced by an increase in ALP production. Especially interesting was that calcitriol was able to enhance these effects, presumably by increasing the expression of VEGF by osteoblasts, VEGF receptors on HUVEC, and IGF-1 receptors on and the release of IGF-binding proteins by HOB. In addition to calcitriol, steady-state concentrations of VEGF can also be increased by PTH, IGF-1, and PGE, all of which have been shown to stimulate bone formation in vivo (50). In summary, it is clear that calcitriol can exert anabolic effects on osteoblasts via both direct and indirect pathways, and though there may be limited evidence that it plays a direct role in bone mineralization at physiological concentrations, it does play a role in maintaining normal concentrations of serum calcium and phosphorous which are supersaturating to bone and essential for its proper mineralization (12, 17, 51).

Another role of vitamin D that has implications for bone health is in the parathyroid gland. Calcitriol is responsible for maintaining normal parathyroid status by keeping the expression of the gene for preproparathyroid hormone reasonably suppressed, as well as preventing the proliferation of parathyroid gland cells (17). In vitamin D deficiency, the proliferation of the parathyroid gland and production of PTH leads to secondary hyperparathyroidism and its detrimental effects on bone health (17). Not only does vitamin D play a direct role in the parathyroid gland, but the actions of calcitriol in the kidney and bone

require the participation of PTH; vitamin D and PTH can thus be said to have a close, interdependent relationship (17, 18). An inverse relationship between serum 25(OH)D and PTH has been well-documented in a number of population groups, including postmenopausal women (43, 52-58), elderly men and women (59-61), healthy adults (14), and hospital inpatients (62). In terms of their relationship to BMD, serum 25(OH)D positively and PTH negatively associates with BMD at a number of sites. A positive relationship between serum 25(OH)D and BMD has been observed for the hip (total (63, 64), trochanter (58), and femoral neck (63, 65)), lumbar spine (60, 63, 65), and proximal and ultradistal forearm (63). In addition, serum concentrations of 25(OH)D below 30 nmol/L (12 ng/mL) and above 60 nmol/L have been associated with an increased and decreased risk of osteoporotic fractures, respectively, in older adults (66, 67). The negative association of serum PTH with BMD has been reported at the total hip (43, 56, 61), mid- and ultradistal forearm (44), and the spine (60). The inverse relationship between these two biomarkers is also seen when the biomarkers of bone metabolism are examined. A negative correlation between serum 25(OH)D and the urinary markers of bone resorption PYD (53), DPD (61) and OHPr (53, 61) has been observed in postmenopausal, elderly, and both postmenopausal and elderly women, respectively. A similar negative correlation has been reported between serum 25(OH)D and the serum markers of bone formation OC and bALP (61) in elderly women, and total ALP (53, 58, 62) in postmenopausal women and medical inpatients. Taken together, these results suggests that increased serum 25(OH)D is indicative of lower bone turnover. Elevated serum PTH concentrations are associated with greater bone turnover and thus higher concentrations of the urinary markers of bone resorption CTX (44, 65) and DPD (43) in older women, and markers of bone formation such as serum OC (43, 60) in older men and women, and bALP (43) in postmenopausal women. In terms of the relatively novel markers of bone metabolism RANKL and osteoprotegerin (OPG), though PTH and pharmacologic doses of $1,25(OH)_2D$ both have the ability to stimulate the production of RANKL and inhibit the production of OPG by osteoblasts (40), physiologic doses of $1,25(OH)_2D$ are thought to inhibit the

increase in RANKL caused by PTH (68). It seems, therefore, that 1,25$(OH)_2$D can be thought of as a less potent form of PTH that maintains bone turnover within an acceptable range but also has the ability to provide additional benefits to bone, both indirect, via its ability to increase calcium absorption and direct, via the proposed anabolic effects within bone tissue itself discussed previously.

9.5 Other Functions of Vitamin D

VDRs have been found in a variety of cells aside from osteoblasts, enterocytes, and the cells of the distal tubule of the kidney, leading researchers to speculate an even broader role for vitamin D in the human body. To date, over 20 cell types have been found to contain VDRs, including skin keratinocytes, colon cells, ovarian cells, pituitary gland cells, lymphocytes and parathyroid gland cells (7, 13, 17). In addition to this, as previously mentioned (see **Section 9.2**), the enzymatic machinery required to activate 25(OH)D to calcitriol is also found outside the kidney in a variety of tissue types, allowing calcitriol to exert local actions at a number of locations throughout the body (7, 12, 17, 69). P450C1 exists in prostate, breast, colon, and esophagus tissue (12). Cross-sectional and case-control analyses of epidemiologic data show an association between latitude and UVB exposure (and thus risk of developing vitamin D deficiency) and the prevalence of certain diseases including hypertension, prostate, breast and colon cancers, multiple sclerosis, diabetes, and even chronic fatigue and metabolic syndrome (25).

9.6 Current Recommendations for Vitamin D Intake

There has never been an objective way to assess the requirements of vitamin D (69), thus no estimated average requirement (EAR) or recommended dietary allowance (RDA) has been put forward by the Food and Nutrition Board of the Institute of Medicine. Instead, the 1997 dietary reference intake (DRI) value is an adequate intake (AI) based on the average observed or experimentally determined intake of a group (or groups) of healthy people. The AI for vitamin D was set at 5 μg/d (200 IU/d) for those aged 0-50, 10 μg/d (400 IU) for those aged 51-70 (and for pregnant women) and 15 μg/d (600 IU/d) for those over 70 years of age (70). These recommendations were made with the assumption that there

would be no significant contribution of vitamin D from exposure to sunlight. The upper limit (UL) for vitamin D was set at 50 μg (2000 IU)/d for individuals over 1 year of age, and 25 μg (1000 IU)/d for infants (70). Currently, these levels are considered to be quite outdated and in dire need of revision, with most experts today recommending a daily intake of 25 μg (1000 IU) vitamin D for adults (15, 71). Additionally, it is suggested that the upper limit should be set at 50 μg/d (2000 IU/d) for those ≤1 year old and 100 μg/d (4000 IU/d) for those over 1 year of age (12), as toxicity is only likely to be observed at vitamin D intakes greater than 250 μg/d (10000 IU/d) (12, 15, 23, 72). The daily use of vitamin D by the body is thought to be ~100 μg (4000 IU), therefore most of this must come from reserves stored in the body during the summer months (17). It must therefore be taken into account that certain segments of the population for whom cutaneous vitamin D synthesis is decreased or the release of vitamin D from adipose tissue stores is impeded (see **Section 9.2**) may have higher vitamin D requirements than others.

There has been much debate over how the recommendations for vitamin D should be set. Criteria of serum concentrations of 25(OH)D that produce minimum concentrations of PTH, maximum absorption of calcium, or minimum incidence of negative bone-related health outcomes (e.g. fractures, bone resorption, bone gain or loss) have been put forward (15, 71). Issues arise when considering the concentrations of serum 25(OH)D required to minimize serum concentrations of PTH, as concentrations ranging from 75-110 nmol/L (30-44 ng/mL) have proven adequate; additionally, calcium intake can influence the minimum concentration of 25(OH)D required for this function (73). Furthermore, the optimal concentrations of both 25(OH)D and PTH required for bone health remain unclear (15, 74). The normal range of 25(OH)D is wide at 25-137.5 nmol/L (10-55 ng/mL) and the lower limit can shift up or down depending on the population; for example, it is thought to be greater than 50 nmol/L in the elderly (≥ 65 years of age) (15, 70, 74). As defined by the Food and Nutrition Board of

the Institute of Medecine (70), if serum 25(OH)D is:

- < 37.5 nmol/L (15 ng/mL), this is classified as vitamin D deficiency
- < 50 nmol/L (20 ng/mL), this is classified as vitamin D insufficiency
- 400-1250 nmol/L (160-500 ng/mL), this is classified as vitamin D toxicity, or hypervitaminosis D

According to more recent research using maximal calcium absorption as an indicator of optimal vitamin D status, concentrations of ~80 nmol/L (32 ng/mL) 25(OH)D have also been suggested (15, 71, 74). Interestingly, this was the concentration at which a 33% decrease in osteoporotics fracture risk was observed during a randomized, placebo-controlled trial administering ~20 μg/d (~800 IU/d) of vitamin D_3 or a placebo to healthy British men and women between 65 and 85 years of age (74). In terms of benefits to bone mass, these tend to be maximized when 25(OH)D concentrations are 90-100 nmol/L (75).

General recommendations for vitamin D intake via sun exposure have also been put forward by the scientific community. It has been determined that exposure of 6% of the body's surface to 1 minimum erythemal dose of sunlight is the equivalent of ingesting 600-1,000 IU (15-25 μg) of vitamin D, making it reasonable to conclude that the exposure of one's arms and legs, or face, hands and arms to suberythemal doses of sunlight (approximately 5-15 minutes, just before the skin turns pink) 2 to 3 times per week is enough to satisfy one's vitamin D requirement. Optimally, this should be on a clear day between the hours of 10h00 and 15h00, as this is when UVB availability is at its highest. (12, 22, 23, 51). Evidently, there may be dermatologic concerns regarding such sun exposure; however, studies have shown that, though painful sunburns before the age of 20 have been associated with all types of skin cancer, lifetime sun exposure was associated with a reduced risk of malignant melanoma, the most deadly form of skin cancer (22, 76). To minimize the risk of skin cancer, and particularly malignant melanoma, sun protection should be applied to the face and neck before sunlight exposure, as the small amount of vitamin D they provide does not justify the exposure of these vulnerable areas. Following the 5-15 minute exposure,

sunscreen with a SPF of at least 15 (or any other form of sun protection) should be applied if remaining outside (22).

9.7 Vitamin D in the Food Supply of the Canadian Arctic

Vitamin D is naturally present in very few foods, including fatty fish, liver and other organ meats, egg yolks and mushrooms, though the vitamin D content of the latter two foods is variable (7, 77). In the Canadian Arctic, Aboriginal peoples have access to many natural sources of vitamin D in fish such as arctic char, lake trout and whitefish, sea mammals such as seal, and beluga and narwhal whales, and land animals such as caribou and muskox (see **Table 2**) (78, 79).

In addition to these natural sources, fortified market foods are also available in the North. Though fortification practices vary from country to country, in Canada, fluid milk and substitutes of plant origin (4.4 µg/250 mL) as well as margarines (13.25 µg/100 g) are fortified with vitamin D. Other milk products such as evaporated milk, goat's milk, and powdered milk, as well as meal replacements, nutritional supplements, formulated liquid diets, and some egg products can also be fortified with vitamin D (77). On the whole, however, consumption of fish and sea mammals is the major determinant of vitamin D intake for Arctic Indigenous peoples, as market foods containing vitamin D are not consumed in significant quantities (79). Important to note is that nutrient values for animal foods reflect their cholecalciferol (vitamin D_3) content, however these values may be underestimates as meat and liver contain significant quantities of 25(OH)D_3, which is 5 times more biologically active than vitamin D_3 (80). This may be particularly relevant for Inuit, and especially older Inuit adults, who derive a large portion of their energy from animal-source country foods (78).

9.8 Prevalence and Potential Consequences for Bone Health of Vitamin D Deficiency and Insufficiency

9.8.1 Prevalence of Vitamin D Deficiency and Insufficiency in Canada

Deficient and insufficient concentrations of serum 25(OH)D have been a common finding around the globe, raising much concern as adequate vitamin D is associated with the prevention of a number of chronic diseases causing significant morbidity and mortality (81). Ward (82) describes vitamin D deficiency in

Canadian infant-mother pairs as a persistent problem despite access to fortified food and supplements. The prevalence of vitamin D insufficiency (defined as serum 25(OH)D < 40 nmol/L (16 ng/mL)) and deficiency (25(OH)D < 25 nmol/L (10 ng/mL)) at the end of winter in children and adolescents 2-16 years of age in Edmonton, Alberta was 34% and 6%, respectively (83). The significant predictors of insufficiency were vitamin D intake (per kilogram of body weight), age, and male sex, and it was thought that older children were at higher risk because vitamin D intake did not increase in proportion to body mass. 20-28% of young (18-35 y) women from Toronto, Ontario had insufficient concentrations of serum 25(OH)D (< 40 nmol/L (16 ng/mL)) from December to April. Over the whole year, the prevalence of vitamin D insufficiency was higher in Asian and Indo-Asian (including two Native North American women, grouped together because of skin color) versus white women (25.6% versus 14.8%, respectively) (84). In a sample of healthy adults (27-89 y, ~70% women) from Calgary, Alberta, 34% experienced vitamin D insufficiency (25(OH)D < 40 nmol/L (16 ng/mL)) at least once during the year (85). Age, BMI and travel to lower latitudes were significant predictors, the first two inversely and the latter one positively related to serum 25(OH)D (85). Significant seasonal variation in concentrations of serum 25(OH)D (with lower concentrations in early spring compared to late summer) has translated into seasonal differences in the prevalence of vitamin D insufficiency (25(OH)D < 50 nmol/L (20 ng/mL)) and deficiency (25(OH)D < 25 nmol/L (10 ng/mL)) in both ambulatory (86) and institutionalized (87) older adults in Canada.

In summary, three significant findings are confirmed by these and other studies reporting a high prevalence of vitamin D insufficiency in North America (88). Firstly, seasonality and latitude are important determinants of serum 25(OH)D in healthy young as well as in older individuals, and thus the issue of vitamin D insufficiency is not exclusively relevant to home-bound or institutionalized elderly populations and hospital inpatients. Secondly, race/ethnicity has a significant effect on serum 25(OH)D concentrations in adults over a range of latitudes and in different seasons. Finally, current food

fortification practices in Canada and the United States are ineffective in reducing the prevalence of vitamin D insufficiency, with the consumption of fortified milk resulting in modest to no increases in serum concentrations of 25(OH)D (88), and average serum concentrations of 25(OH)D in Canada being similar to those observed in Europe, where food is not fortified with vitamin D (84). It is thus clear that inadequate vitamin D intake is one of the many factors responsible for the high prevalence of vitamin D deficiency and insufficiency observed in the Canadian population, with average intakes reported to be around 5µg from food alone, and only a few micrograms higher with supplementation (81).

9.8.2 Osteoporosis

Osteoporosis is a disease that is known to affect both the quantity (i.e. density) and quality of bone (89, 90). It is thus characterized by low bone mass and a deterioration of the microarchitectural structure of bone, leading to enhanced bone fragility and an increased risk of disabling fractures that can increase dependency and the risk of death (90-92). It can be generalized or localized, and when generalized, can arise as a primary or secondary disorder. Primary osteoporosis includes postmenopausal and senile osteoporosis, while secondary osteoporosis arises due to endocrine or gastrointestinal disorders, certain types of drug therapy, neoplasia or miscellaneous causes (93). The achievement of a high peak bone mass, as well as having a larger bone size, can potentially lower the risk of osteoporosis and related fractures later in life (90, 94). Peak bone mass is higher in men than in women, and in blacks than in whites, therefore white females are at the highest risk for low bone mass (93). An individual's bone mass and size are primarily (60-80%) accounted for by genetic factors, however individual potential may not be achieved if lifestyle factors or the onset of chronic diseases do not permit it (94). These lifestyle factors include nutritional determinants, body weight and composition, smoking, physical activity and the use of certain medications (see **Table 3** and **Section 9.11**) (90, 92, 95-97). In addition to the achieved level of peak BMD, other factors influencing osteoporosis risk include age and hormonal status (93). The progression of

osteoporosis is thought to begin when serum 25(OH)D concentrations fall below ~80 nmol/L (32 ng/mL) (74).

In 1997, a conservative estimate of the direct and indirect medical costs of hip fractures alone in the United States was $131.5 billion (98). Though they are most frequently observed in postmenopausal white women, the number of osteoporotic fractures in men and nonwhite persons is projected to increase over the next several decades (90, 92). Furthermore, the elderly currently represent the fastest growing age group, thus even if age-adjusted incidence rates for fracture remain stable, the total number of fractures will rise substantially over the next several decades. For example, it is estimated that the number of hip fractures will rise from 1.7 million in 1990 to 6.3 million in 2050 (98). Hip fractures are considered the most devastating consequence of osteoporosis, causing serious disability and excess mortality (98). Irrespective of the site, however, the occurrence of a fracture predicts a substantially greater risk of sustaining another fracture at a different site (99).

9.8.3 Osteomalacia/Rickets

In contrast to osteoporosis, osteomalacia (in adults) or rickets (in children) is caused by a defect in the mineralization of bone rather than a simple decrease in bone mass. These diseases are usually caused by vitamin D deficiency, which leads to impaired calcium absorption, a decrease in serum calcium concentrations, and secondary hyperparathyroidism (24, 100). No more than 10-15% of dietary calcium is absorbed in vitamin D deficiency; in vitamin D sufficiency, approximately 30% of dietary calcium can be absorbed, and this percentage is even higher in pregnancy, lactation and during periods of rapid growth (51). Since vitamin D also plays a role in phosphorus absorption, serum phosphorus is also decreased with vitamin D deficiency and since PTH does not correct for this and in fact aggravates the situation by increasing phosphorus excretion in the kidney, bone mineralization is impaired. In osteomalacia, newly formed bone matrix fails to mineralize properly during the process of bone remodeling, leading to a loss of stiffness and potential deformities in bone (e.g. bowing of the legs, misshapen pelvis) (24). Bone matrix formation is also impaired, though not to the same

extent as bone mineralization (24, 100). Osteomalacia is thought to occur when serum concentrations of 25(OH)D fall below 20 nmol/L (8 ng/mL) (74).

9.9 Measuring Vitamin D Status

The body's two sources of vitamin D are cutaneous synthesis and dietary intake, therefore these two factors, along with anything that may interfere with the absorption or synthesis of the fat soluble vitamin, should be taken into account when assessing vitamin D status (70). Secondly, the serum concentration of 25(OH)D should be quantified, as it is a reflection of both skin synthesis and dietary intake of vitamin D, as well as vitamin D stores. Finally, since vitamin D's ultimate effect on the body is the maintenance of bone health, an evaluation of skeletal health in terms of bone turnover and bone density is also warranted and is described in the next section (70).

9.9.1 Dietary Intake and Sun Exposure

Evaluating dietary intake in relation to bone health requires the use of tools that can assess food intake over the long term. Food frequency questionnaires (FFQ) that are culturally appropriate and include foods that contain substantial amounts of the nutrient(s) of interest, but whose intakes are not uniform across the population are used quite frequently for this purpose. The results obtained correlate fairly well with results obtained from 28-day dietary records (101, 102). A FFQ can be qualitative (e.g. Willett) or quantitative/semi-quantitative (e.g. Block), and though an exact estimation of the intake of any one nutrient cannot be directly determined, relative intakes of specific nutrients of individuals are placed in rank order for comparison between or among groups (102, 103). Twenty-four-hour dietary recalls are used to quantitatively assess recent food intake. Though many repeat recalls are required to accurately assess an individual's nutrient intake and this tool is less useful on its own for the evaluation of dietary intake as it relates to bone health, 24-hour recalls can be used to validate FFQs and/or help with the correct estimation of portion sizes (101, 102). Food records or diaries are also considered inappropriate for evaluating long-term intake as recent food intake is recorded and, in addition, there tends to be low compliance and modification of eating patterns during the

recording period. Finally, food or diet histories are tools that combine the 24-hour recall, history of usual food intake, and data on food preparation during an interview with a trained dietetics professional; although this method is considered the "gold standard" for assessing the validity of other methods, it is not commonly practiced due to its time-consuming nature (101, 102). Methodological issues that must be dealt with regardless of the method chosen are the correlation of variables (e.g. energy and iron intake), day-to-day variability in eating patterns, and incomplete information on the nutrient content of certain foods (102, 103).

Both the amount of sun exposure that an individual receives and the amount of vitamin D produced during each exposure are extremely difficult to quantify (70). Questions regarding the amount of time spent in the sun and the use of sunscreen may be useful for categorizing individuals according to their level of sun exposure and determining its contribution to total vitamin D intake.

9.9.2 Serum 25-Hydroxyvitamin D

Though there is much debate over a number of the topics surrounding vitamin D, one area of consensus is that serum 25(OH)D is the appropriate functional indicator of vitamin D status (104). Cholecalciferol has a short half-life of approximately 24 hours and the assay for this molecule is difficult (12). Calcitriol's concentration in serum is 1000 times less than 25(OH)D, it has an even shorter half life than cholecalciferol (4-6 hours), and since its production is tightly regulated, its measurement does not paint an accurate picture of vitamin D status, with concentrations observed to be normal or even elevated in vitamin D insufficiency (12). 25(OH)D has a half life of approximately 3 weeks and better reflects substrate availability from cutaneous production and dietary intake due to its relatively unregulated production (12).

The measurement of 25(OH)D can be challenging, however, as several problems have been reported with currently available 25(OH)D assays. Though it is more abundant than calcitriol, 25(OH)D's concentration in serum remains low, and only 0.03% of 25(OH)D is found free in serum (105). An extraction step is usually required to release 25(OH)D from its binding proteins, with 85% and 15% of 25(OH)D being bound to DBP and albumin, respectively. When extraction is

performed, there is risk of co-precipitation, but if this is not performed, the lipophilic nature of 25(OH)D will render assays for this molecule susceptible to matrix effects (105). Assays must also have the ability to equally detect $25(OH)D_2$ and $25(OH)D_3$. Underestimation of $25(OH)D_2$ has been frequently observed, especially with competitive binding protein assays such as Nichols Advantage, but also with the IDS Gamma-B RIA and enzyme immunoassay for 25(OH)D. While it is reported that the DiaSorin RIA employing an ^{125}I-labelled tracer detects both molecules equally, some have reported under-recognition of the D_2 isoform (105). The under-detection of $25(OH)D_2$ is not suspected to be of any major consequence, however, unless there is supplementation with this form of the vitamin, as the major animal form of vitamin D is vitamin D_3 and the main source for most people is cutaneous synthesis (74).

HPLC with UV detection is reported as the "gold standard" method for the measurement of 25(OH)D (105) and liquid chromatography-tandem mass spectrometry is reported to perform even better (106), however, until very recently, they were far too costly and complex for routine clinical and laboratory use (105, 107). When validated and performed by experienced laboratory technicians, the DiaSorin RIA has shown good correlation with HPLC methods, and immunoassays remain the more popular methods due to their relative ease of use, speed and cost (105). More recently, automated chemiluminescence-based assays have been developed, utilizing either the DBP or a specific antibody for 25(OH)D detection, and seem promising due to their precision and speed (107). In a study validating the performance of the DiaSorin LIAISON® 25 OH Vitamin D automated assay against both the DiaSorin RIA and HPLC, there were no significant differences between the total recovery of 25(OH)D in 329 clinical samples using RIA or LIAISON® assays, or the total recovery in a subset of 10 samples that were analyzed first by HPLC and then by LIAISON® (107). The former test was also used to assess the cross-reactivity of the LIAISON® assay for both $25(OH)D_2$ and $25(OH)D_3$, as these subjects also received supplementation with ergocalciferol; cross-reactivity was thus established as 100% (107). In addition to these results, the functional sensitivity of the

LIAISON® assay (defined as the concentration below which the imprecision exceeded 20%) was reported as 17.5 nmol/L (7 ng/mL) (107), a value which is far below the currently defined threshold for vitamin D deficiency (37.5 nmol/L (15 ng/mL)) (70).

Vitamin D metabolites have been shown to be quite stable, capable of being stored at 30°C for up to 2 weeks and unaffected by up to 4 freeze-thaw cycles; even after a year of frozen (-20°C) storage, no loss in vitamin D metabolites was reported. It is preferable to perform vitamin D assays on serum rather than plasma, though ethylenediaminetetraacetic acid (EDTA) and lithium-heparin treated plasma have been reported to be satisfactory (105).

9.10 Evaluating Bone Health

Bone health can be evaluated on the basis of bone strength, and the two components that influence bone strength are bone density and bone quality (29). As of yet, it is impossible to measure total bone density because there is no way of detecting the organic matrix component of bone (24). Bone mineral density, however, can be measured in a number of ways, and accounts for approximately 70% of bone strength (29) and 60-90% of the variability in elastic modulus (108). Volumetrically, the proportion of mineral and organic matrix in bone is about 50:50 in both health and disease, therefore BMD can be considered an appropriate surrogate measure of total bone density (24). Currently available methods for measuring BMD and bone quality are discussed below.

9.10.1 Dual-Energy X-Ray Absorptiometry (DXA)

Its wide use in research studies, low cost, and broad availability have allowed DXA to be considered as the "gold standard" for measuring BMD (108) and for the non-invasive diagnosis of osteoporosis (109). The basic principle behind DXA is that X-ray photons of two different energy densities are differentially attenuated based in part on their energy levels and on the density of the tissue through which they must pass (29, 108). The DXA is equipped with an X-ray source, collimators and detectors and the highest dose of X-ray that is used is 0.01 to 0.04 millirem (for a whole body scan), an extremely low dose compared to a conventional chest X-ray which uses approximately 40 millirem (29).

One of the major advantages of DXA is that it can be used clinically for the diagnosis of osteoporosis and the monitoring of therapy. Diagnoses are made according to T-scores that represent the number of standard deviations that the subject's hip (total proximal femur, femoral neck, or trochanter), lumbar spine (posterior-anterior L1-L4), or forearm (33% or 1/3 radius) BMD deviate from a young-normal sex-specific adult reference population (109). According to the World Health Organization (WHO), T-scores less than or equal to -2.5 are in the osteoporotic range, those that are between -1.0 and -2.5 are in the osteopenic range, and those greater than -1.0 can be considered normal. Most of the research used to establish these cut-offs was done in postmenopausal women, so certain guidelines must be followed if they are to be applied in other populations. For premenopausal women (20 years of age to menopause), Z-scores that use an age-matched reference population should be used rather than T-scores, and the diagnosis for osteoporosis should not be made unless there is low BMD with secondary causes (e.g. glucocorticoid therapy) or risk factors for fracture (e.g. personal history of fracture as an adult). For non-Caucasians, though the current recommendation is to use Caucasian reference data, further research is encouraged to determine if fractures occur at the same BMD in Caucasians as non-Caucasians. Guidelines regarding the application of WHO cut-offs in men and children are also available, along with instructions regarding which patients should receive BMD testing, which sites should be measured, and a list of the risk factors for osteoporosis and related fractures (109). The reference population and/or the statistical methods used to calculate the young-normal mean and standard deviation used to generate T-scores may vary depending on the manufacturer of the device. Until one universal normative database is created, T-scores may need to be recalculated based on a young normal mean and standard deviation calculated from, for example, epidemiological data from the National Health and Nutrition Examination Survey (110).

Peripheral DXA (pDXA) devices that measure the phalanges, forearm, and/or calcaneus are also available (29, 108). For the purposes of diagnosing osteoporosis, aside from devices that measure the forearm (and specifically, the

distal 33% radius), cut-points for each specific pDXA device are not yet available, thus they may only be used for screening purposes (109). The utility of forearm compared to central (hip and spine) BMD in identifying individuals with low bone mass and thus at potential risk for osteoporosis and related fractures has been highlighted in a number of studies. For example, Patel et al (111) reported that the presence of one or more key clinical risk factors for low BMD was associated with decreases in Z-scores of similar magnitude in both distal forearm (radius and ulna, measured by pDXA) and central (measured by DXA) BMD in a large (n=1009) group of women in the United Kingdom. Additionally, the proportion of women classified into normal, osteopenic and osteoporotic groups was similar using either central or forearm BMD measurements (111). Other studies have reported similarly strong associations between forearm BMD and the risk of non-spine fractures (112).

There are certain limitations of DXA, however. With DXA, the BMD that is measured is areal BMD (in g/cm^2), a measure that is influenced by body and bone size and does not take bone thickness, an important determinant of bone quality and thus bone strength, into account. Also, because of its two dimensional nature, it integrates both cortical and trabecular bone into one measurement (108).

9.10.2 Quantitative Computed Tomography (QCT)

As with DXA, central and peripheral QCT devices are available, with measurements being obtained using a standard CT scanner equipped with the appropriate software and calibration phantom(s). Central QCT (cQCT) allows for the measurement of the spine and proximal femur, while peripheral QCT devices measure the forearm or tibia (108). Both types allow for the differentiation of cortical from more metabolically active trabecular bone, and measurements are of volumetric rather than areal BMD (in g/cm^3) (108). There is limited clinical utility of QCT, however, as it has not been used in many studies examining fracture risk and no cut-off points (such as the WHO's cut-points for DXA) are available for making the diagnosis of osteoporosis. Though this technology was previously characterized by high radiation doses and low precision, significant improvements have been made and its use is encouraged in future studies (108).

9.10.3 Quantitative Ultrasound (QUS)

A unique measure of bone quality rather than bone density, QUS uses high-frequency sound waves to measure both changes in the intensity of sound waves (termed broadband ultrasound attenuation) as well as the velocity of transmitted sound (the speed of sound) as they pass through bone (29). "Wet" and "dry" QUS systems are available, the former, older system utilizing a temperature-stabilized water bath, and the latter, newer system using silicon pads and ultrasound gel to couple the transducers to the site of interest. The tibia, phalanges, and, most commonly, the calcaneus, are measured with QUS, though exactly what QUS measures is still unclear. The measurements obtained give an idea of the mechanical properties of cortical and trabecular bone such as bone connectivity, spacing, thickness, and number of trabeculae, which in turn determine bone stiffness, failure load and fracture risk (29). Studies have shown that QUS is useful in assessing many determinants of fracture risk other than BMD, and have advantages over DXA in terms of portability and the lack of exposure to ionizing radiation (29). Validated heel QUS devices are known to predict fragility fractures at various sites in postmenopausal women and men over 65 years of age, independently of central DXA (109). Further studies are needed on this device, however, especially the newer "dry" systems (29).

9.10.4 Standard X-Rays

The use of standard x-rays for assessing bone mineral density has proved to be a subjective technique characterized by poor inter- and intra-observer reproducibility and a low correlation with quantitatively measured BMD. As its use in assessing bone health only extends to an ability to detect bone fractures, the use of the quantitative techniques for measuring BMD discussed above are much more widespread (108).

9.10.5 Bone Turnover Markers

Bone turnover has also been associated with bone health, with higher rates of bone turnover being independently associated with the risk of fracture in a number of studies (41, 113, 114). Bone turnover's association with bone loss has not been as concretely established, and may depend on the skeletal site, gender,

and menopausal age, with a greater association seen in older postmenopausal women and being less predictive of bone loss at the hip and spine. Also, bone resorption markers tend to be better predictors of bone loss than bone formation markers (41). Both preanalytical and analytical variation can interfere with the proper measurement of the biomarkers of bone turnover, and there are a limited number of variables over which control can be exerted (39, 115). Pre-analytical variability is a combination of uncontrollable and controllable biological and technical factors; these are first described in general for all bone turnover markers, and further details are provided for the bone turnover markers of particular interest.

Uncontrollable variables include age, with bone turnover markers being higher in infants and children than in adults, gender, with them being higher in men than in women in the second and third decades, decreasing to their lowest concentrations in men between the ages of 50 and 60, and increasing thereafter (115). For women, the main changes in bone turnover occur at menopause, as previously described. Other uncontrollable factors include ethnicity and geography, with differences in bone turnover markers being observed both between and within ethnic groups at different geographical locations. Fractures will also result in an increase in bone turnover (particularly bone formation) markers, with the pattern of increase in markers depending on the fracture site. Fracture effects must be taken into account for at least 6 months after a fracture when interpreting bone turnover markers (115). Pregnancy increases the concentrations of most bone turnover markers beginning in the second trimester and concentrations may remain elevated up to 1 year postpartum, particularly if breastfeeding is practiced (115). As with BMD, certain drugs can also affect bone turnover, including antiresorptive drugs (HRT, bisphosphonates, selective estrogen receptor modulators), which can significantly affect all markers of bone turnover, long-term corticosteroid and short-term heparin treatment, which tend to decrease bone formation markers, and long-term anticonvulsant and gonadotrophin releasing hormone agonist treatment, which increase bone turnover. Oral contraceptives can also affect bone turnover, though effects are

thought to be age dependent, having little significance in younger women and slightly decreasing bone turnover in older (>35 years of age) women (115). Diseases such as primary hyperparathyroidism, diabetes, and thyroid disease increase bone turnover, and other diseases where the mechanisms of clearance and/or metabolism are impaired or there is an increase in the contribution of non-bone sources can also distort the interpretation of concentrations of bone turnover markers. For example, moderate impairments in renal function (glomerular filtration rate 50 ml/min) can have significant effects on serum concentrations of OC, CTX and NTX, whose main clearance mechanisms are via the kidney (39), and increased degradation of collagen has been observed in cases of rheumatoid and osteoarthritis (115). Finally, bed-rest/immobility is the final uncontrollable factor influencing bone turnover, with an increase in bone resoption observed in as little as 2 to 4 days of immobility, and the decrease in bone resorption with remobilization progressing at a much less rapid rate (115).

Controllable sources of preanalytical variability include circadian rhythm, which has the largest impact on bone turnover concentrations and is described in more detail for the markers of interest below. Small changes in bone turnover markers have also been observed during the luteal phase of the menstrual cycle, and while some report this as insignificant (115), others suggest taking samples between the third and seventh day of the menstrual cycle (39). The season at which the sample is taken may account for up to 12% of the variability of bone turnover markers, with an approximate increase of 20% from summer to winter. This and the geographical effects are thought, at least in part, to be due to the seasonal and geographical variations observed in serum 25(OH)D, with less significance of these effects at latitudes between 40°N and 40°S (115). Diet, and collagen intake in particular, only affects the measurement of urinary OHPr, though calcium supplementation may depress concentrations of bone turnover markers, depending on the time of day when the supplement is taken (115). The effects of exercise can be difficult to quantify, as they depend on age as well as the type and intensity of the exercise performed. Nevertheless, it is recommended

that subjects be asked to refrain from exercise at least 24 hours before phlebotomy (115).

The measurement of OC can be complicated by the fact that it can rapidly degrade into fragments in serum, with significant losses occurring after 1-2 hours at room temperature, and that the breakdown of osteoid can also lead to the release of OC fragments, high concentrations of which are indicative of increased bone resorption rather than formation (39). Its concentrations in serum can be measured by immunoassay, though much care must be taken in the handling of samples due to its potential for rapid degradation. Hemolysis, repeated freeze-thawing cycles, and/or lipema will confound assay results, as the release of proteases by erythrocytes or the action of serum proteases can degrade OC resulting in reduced values, and lipids may bind to OC rendering it nonimmunoreactive (46). The use of an assay for 1-43 (N-terminal/mid-molecule) OC fragment can eliminate some, but not all, of the problems related to intact (1-49) OC's instability, as 1-43 OC is one of the larger degradation products of OC and may also be directly generated by osteoblasts; it represents one third of the circulating OC pool (39). A circadian rhythm is observed for OC, with a slow decline during the morning leading to a nadir between 1200h and 1600h followed by a gradual rise that peaks between 0200h and 0400h; the amplitude of variation ranges from 5-20% of the 24-hour mean concentration (115). Because vitamin K is required for carboxylation of glutamic acid to γ-carboxyglutamic acid, vitamin K deficiency or vitamin K antagonists such as coumarin and warfarin can interfere with the synthesis of functional OC, though not the total amount. It is most likely, however, that OC is not the sole factor involved in the potential adverse effects of vitamin K deficiency on bone, and it is thought that vitamin K deficiency may point to overall poor nutrition and/or that other vitamin K-dependent proteins involved in bone and calcium homeostasis may play a role in the observed bone pathology (46).

PTH is an extremely labile molecule, with a half-life of approximately 5 minutes (28). Seasonal fluctuations as well as circadian and ultradian rhythms have been described for PTH. Serum concentrations of PTH fluctuate 20% above

and below the annual average in the winter and summer, respectively, varying directly with bone turnover and inversely with serum concentrations of 25(OH)D and bone density (116). A phase shift delay of 1 month has been observed between PTH and 25(OH)D (with 25(OH)D decreasing before PTH begins increasing), and serum CTX has been reported to rise 1-2 months after an increase in serum PTH (44). The circadian rhythm of PTH is characterized by a nadir in late morning followed by two peaks: one in late afternoon and a more pronounced one in early morning. The magnitude of the early morning peak can be altered by the intake of calcium and phosphate, and in patients with renal hyperparathyroidism, a nightly dose of calcitriol has been shown to decrease morning blood calcium and phosphorous concentrations and may help to improve bone density (116). Fasting has also been shown to eliminate the circadian rhythm of PTH, as well as decrease mean concentrations of the intact hormone (117). Induced wakefulness with hourly snacks can reduce the amplitude of the PTH peaks but does not alter its circadian rhythm (118). An ultradian rhythm in serum PTH has also been reported, with seven pulses per hour accounting for approximately 30% of PTH secretion. A study by Joseph et al (119) revealed a single sustained peak beginning in the late afternoon in postmenopausal compared to premenopausal women and elderly men. The preservation of the oscillatory pattern of PTH secretion is thought to be of primordial importance in maintaining normal bone metabolism; it is increasingly being taken into consideration during the development of pharmacologic treatments for both renal and non-renal bone disorders (116). In terms of stability, intact PTH is best measured in EDTA plasma rather than serum, and though it can be refrigerated (at 2-8°C) for up to 8 hours, for prolonged storage, it can be frozen at -20°C or lower for up to 4 months (120).

9.11 Nutrition and Bone Health

Many nutrients including protein, calcium, magnesium, manganese, phosphorus, zinc, and vitamins A, D, K, and C all have known biological roles in bone cell metabolism and/or matrix formation (see **Table 3**). Calcium and vitamin D have been shown to be effective in preventing and treating osteoporosis when

used in adjunct to other therapies, and adequate protein is also recommended for optimal bone health (121). Evidence for other micronutrients comes mostly from epidemiological or cross-sectional studies, and thus, additional intakes of these nutrients cannot be recommended in the prevention or treatment of osteoporosis (121). Minimizing one's intake of sodium and caffeine, two nutrients that are suspected to increase the urinary excretion of calcium, is also recommended, with caffeine intake only thought to play a significant role in older persons with low calcium intakes (92, 121). Ethanol has a direct suppressive effect on bone formation whether consumption is acute or chronic, and chronic alcoholism can result in decreased concentrations of 25(OH)D and $1,25(OH)_2D$ (122).

9.12 Vitamin D and Bone Metabolism in Northern Aboriginal Populations

Ethnic variation is known to have a strong impact on bone health and vitamin D status. Differences in bone health are thought to be due to a combination of genetic differences in accretion of peak bone mass (123), timing or rate of adult bone loss, body size, bone size (123, 124) and hip geometry (124). One similarity between ethnic groups, however, is that decreasing peripheral BMD, regardless of measurement site or use of a white reference population, has been shown to increase the risk of fracture in white, black, Asian, Hispanic, and Native American ethnic groups (124). In terms of vitamin D metabolism, ethnic differences are thought to be attributable to genetic differences in the production of and response to PTH (125-128) and $1,25(OH)_2D$ (127-129) or the way calcium is metabolized (125, 128). It is therefore important to take ethnicity into account when measuring bone health, as it can be considered a proxy measure for all of the above factors (130). Current knowledge of vitamin D and bone metabolism specific to Aboriginal populations is discussed in this section.

Renjmark et al (129) compared concentrations of calciotropic hormones and biomarkers of bone metabolism in 4 groups: Inuit Greenlanders living in Nuuk on traditional (group A) or Western (group B) fare, Inuit Greenlanders living in Denmark on Western fare (group C), and Danes from Denmark on Western fare (group D). Nuuk is located at 65°N, while the Denmark location was at 55°N, therefore summers are longer in Denmark compared to Nuuk, and

traditional as compared to Western fare was reported to contain much more vitamin D from food sources such as fatty fish and sea mammals. Additionally, skin pigmentation is greater in Greenlanders than Danes and Greenlanders tend to wear more protective clothing and spend less time in the sun than their Danish counterparts (129). The prevalence of vitamin D insufficiency (<40 nmol/L (16 ng/mL)) was common in all groups but highest in group B, and seasonal variation in 25(OH)D was observed in all groups. Despite similar concentrations of 25(OH)D which were higher than those in groups B and C, groups A and D had very different PTH concentrations (A<D), and this was thought to be due to differences in $1,25(OH)_2D$ concentrations (A>D), or a lower "set-point" for calcium-regulated PTH release in Greenlanders. 25(OH)D concentrations were higher in older individuals (presumably because of a higher vitamin D intake), lower in the winter, lower in those on Western fare, and higher in Danes compared to Greenlanders. That latitude did not have a significant effect on concentrations of 25(OH)D was surprising, and points to the fact that the existence of a period with sun exposure may be more important than the actual length of the summer (129). PTH concentrations were higher in Danes, inversely related to 25(OH)D, and increased with age, while $1,25(OH)_2D$ concentrations were higher in Greenlanders. Evolution may thus have selected for Greenlanders to have higher P450C1 activity due to their low intake of calcium and high prevalence of lactose intolerance. Ethnic differences were found in the biomarkers of bone metabolism, with serum bALP and OC being higher and lower, respectively, in Greenlanders than Danes; seasonal variation in both biomarkers was only observed in subjects living in Denmark, with higher concentrations observed during the winter than the summer (129).

A number of older studies employing suboptimal methods for measuring bone mass suggest differences in bone mass between Inuit and Caucasians (131). In a recent study, however, lower BMD (as measured by pDXA) was found at the calcaneus but not at the forearm in Greenland Inuit compared to Caucasian individuals, with this difference disappearing upon adjustment for weight. For all

participants, male gender and increased body size (BMI and height) were associated with increased BMD (131).

Research regarding the bone health of Canadian Aboriginals (and Inuit in particular) is lacking. An early study by Evers et al (132) examined bone density in First Nations compared to Caucasian postmenopausal females in Southwestern Ontario, but the first major initiative was the previously described 2002 FNBHS. BMD was measured at the lumbar spine, hip and total body for urban participants by DXA, and at the distal forearm and calcaneus for all participants by pDXA. After adjustments for age and weight or BMI, the latter two variables being higher in Aboriginal versus White women due to a greater amount of adipose tissue, site-specific BMD was found to be lower for the calcaneus, forearm and total body in Aboriginal compared to White women (133, 134). There was a significant effect of ethnicity on bone area after adjustment for age, height and weight, and as there were no ethnic differences in BMC after the same adjustments, it was determined that the lower BMD in Aboriginal compared to White women could be attributed to their larger bone area (130). However, after adjustment for body composition, namely the ratio of lean to fat mass, which was lower in Aborginal versus White women, ethnic differences in BMD were eliminated (134). For both Aboriginal and White women, a smaller increase in BMD was observed with an increase in fat mass versus lean mass, and predictors of BMD included age and weight at all sites. PTH predicted BMD at the radius, lumbar spine, femoral neck, total hip and whole body, ALP at the calcaneus and whole body, OPG at the lumbar spine and total hip, and OC at the calcaneus. Surprisingly, 25(OH)D along with phosphate and CTX, did not significantly contribute to the variation in BMD at any site (135).

Comparisons were also made between Aboriginal and White women in terms of vitamin D status and various markers of bone metabolism. The prevalence of vitamin D deficiency (defined as serum 25(OH)D < 37.5 nmol/L) was greater in rural (32%) and urban (30.4%) Aboriginal women compared to urban White women (18.6%) (11). Serum 25(OH)D was negatively correlated with BMI, insulin, C-peptide and hemoglobin A1c, and positively correlated with

insulin-like growth factor binding protein (IGFBP)-1 and IGFBP-3 (136). Even after adjustment for BMI and the markers of glucose metabolism however, ethnic differences in 25(OH)D remained, showing reduced concentrations in Canadian Aboriginal women (136). Surprisingly, OPG was significantly greater in Aboriginals and correlated negatively with BMD at 4 out of 5 sites measured (heel, spine, hip and total body). It is thought that this may point to a counter-regulatory response similar to that observed with aging and inflammatory bowel disease, which both have a negative impact on bone metabolism. Thus, had RANKL also been measured, an unusual elevation in this parameter in Aboriginals compared to Whites may also have been observed. There was no difference between serum concentrations of homocysteine, but concentrations of both vitamin B_{12} and folate were significantly lower in Aboriginals compared to Whites. The concentrations of certain novel markers of bone metabolism may thus be important to the explanation of ethnic differences in bone metabolism (137). There were no differences between concentrations of serum CTX, OC or phosphate (135, 136), but serum calcium and 25(OH)D were lower and ALP, PTH, and OPG were higher in Aboriginal compared to White women (135).

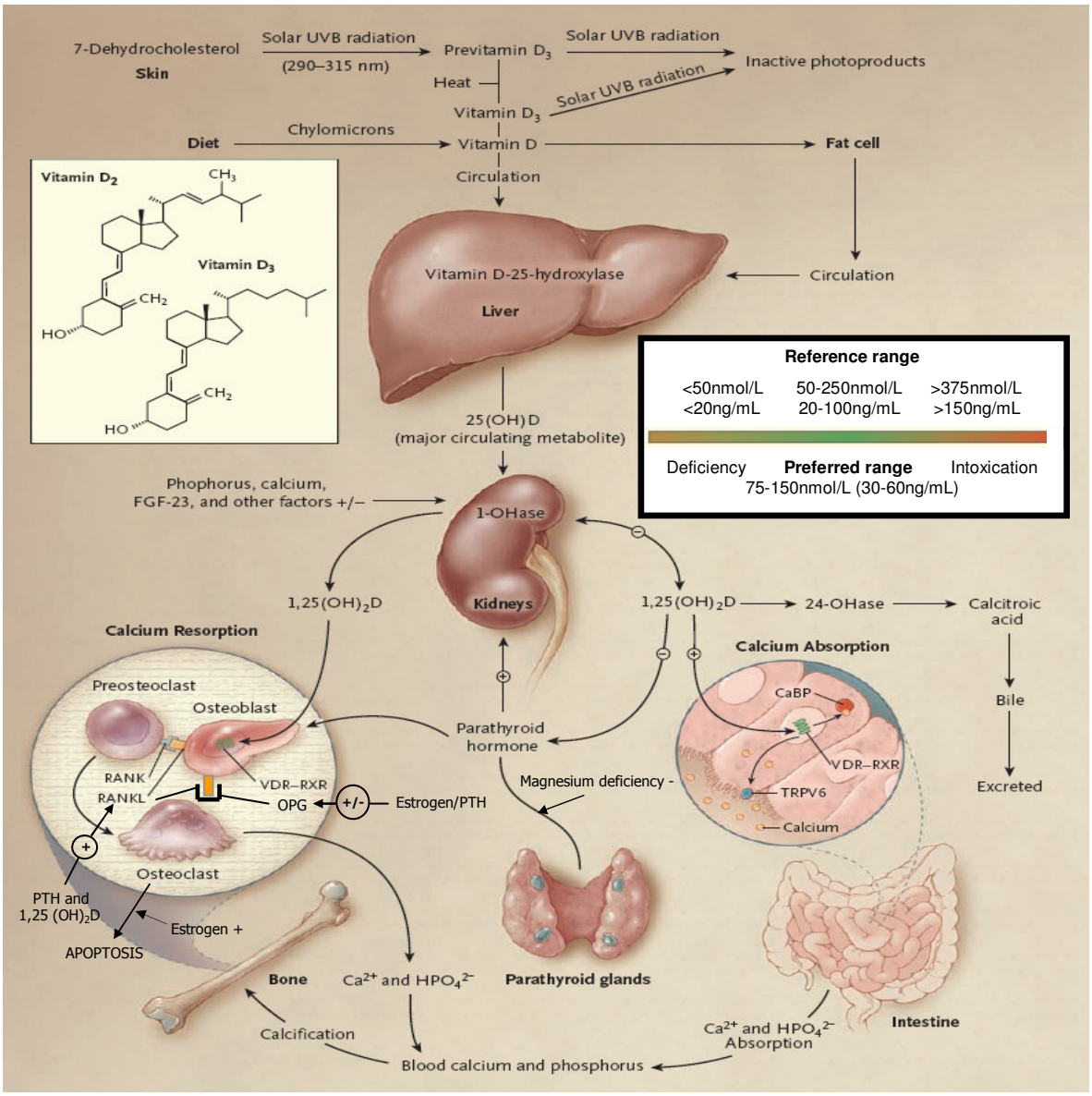
Amongst Inuit in Alaska, hip fracture incidence in women over 64 years of age was higher than that of white women in the rest of the United States from 1979-89 and increased further from 1996-99 (138). In men, hip fracture incidence was also higher than white US counterparts from 1996-99 (138). A retrospective matched cohort study examining fracture risk among First Nations people revealed that the risk of any fracture was considerably higher in First Nations compared to age and sex-matched non-First Nations people (139). Wrist, spine and hip fractures occurred predominantly in older people and women, while craniofacial fractures occurred mostly in men and younger adults of either sex (139). Income quintile, geographic area of residence, and diabetes were shown to contribute to, though not fully account for, the excess risk of fracture associated with First Nations ethnicity (140).

Evers et al (132) found that bone density (calculated as a mean cortical area ratio) at the radius and second metacarpal was significantly lower in First

Nations versus Caucasian women at both sites. In terms of the determinants of bone mass, age at menopause was less significant than either chronologic age or years post menopause in both groups. Amongst First Nations women, the primary determinants of bone density in the radius were obesity and the number of years post menopause, and in the metacarpal, BMI and smoking were the most significant predictors, with smoking showing an unusual positive correlation with BMD. Amongst Caucasian women, the primary determinants of BMD at either site were the number of years since menopause and cigarette smoking (this time with the more commonly observed negative correlation). Surprisingly, BMI was not a significant predictor of bone density at either site in Caucasian women. The relatively small sample size (n=77) and the suboptimal method of measuring BMD employed in this study should be taken into account, however, when gauging the value of these results.

In summary, the Canadian Aboriginal population is at increased risk for fracture, and this may not be accounted for by a lower BMD, as differences in BMD between Aboriginal and White women disappear after adjustment for body composition in some studies. Significant predictors of bone mass at various sites in Aboriginal women included weight/BMI, ratio of lean to fat mass, number of years post menopause, smoking, and serum OC, ALP, PTH and OPG. Ethnic differences were observed with respect to serum calcium and 25(OH)D, and serum OPG, ALP and PTH, which were lower and higher, respectively, in Aboriginal compared to White women. These differences, combined with low vitamin D and calcium intakes, suggest that the Aboriginal population is at increased risk for vitamin D deficiency and low bone mass, and that continued monitoring and evaluation of their vitamin D status and bone health is warranted.

Figure 1: Synthesis, metabolism and actions of vitamin D on bone metabolism, and selected other factors influencing bone turnover



Adapted from Holick, 2007 (23)

Figure 1. Once vitamin D is activated to $1,25(OH)_2D$, (or calcitriol), it exerts a variety of actions related to bone. A decrease in serum calcium causes an increase in serum PTH which stimulates the formation of calcitriol. Calcitriol then acts to increase serum calcium and phosphorus via enhanced absorption from the gastrointestinal tract, stimulation of bone resorption, and an increased reabsorption of calcium in the kidney. Bone resorption is stimulated when calcitriol and PTH upregulate the synthesis of RANKL on the membranes of osteoblasts, which then binds with RANK on the surface of osteoclast precursors, transforming them into active osteoclasts. PTH also inhibits the synthesis of OPG, a decoy receptor for RANKL, by osteoblasts, further stimulating bone resorption. Calcitriol inhibits PTH synthesis by the parathyroid glands; magnesium deficiency can also produce this effect. Estrogen's positive effects on bone turnover stem in part from its stimulation of OPG synthesis by osteoblasts and its stimulation of active osteoclast apoptosis.

Table 1: Vitamin D (as cholecalciferol) in Canadian arctic country foods

Food	N	Vitamin D *(μg/100g)*	SD
Sea Mammals			
Seal, ringed, brain, raw	2	1.5	1.04
Seal, ringed, blubber, raw	1	1.6	N/A
Seal, bearded, meat, boiled	1	1.8	N/A
Beluga, muktuk, raw	1	1.8	N/A
Seal, ringed, eyes, raw	2	2.6	1.68
Narwhal, oil, aged	1	4	N/A
Narwhal, blubber, raw	1	10	N/A
Seal, ringed, liver, raw	3	10.7	4.47
Beluga, blubber, boiled	1	13.3	N/A
Beluga, oil, aged	1	26.7	N/A
Land Animals			
Caribou, kidney, raw	3	1.1	0.89
Caribou, liver, raw	4	1.4	1.2
Muskox, fat, raw	2	3.2	3.38
Fish and Shellfish			
Sculpin, flesh and bones, boiled	1	2	N/A
Whitefish, flesh, raw	2	4.4	0.85
Cisco, eggs, raw	2	6.5	1.84
Sculpin, flesh and bones, raw	1	14.1	N/A
Loche, eggs, raw	2	15.2	3.13
Lake trout, flesh, raw	3	19.7	16.74
Arctic char, flesh, raw	4	25.8	24.39
Loche, liver, raw	2	317.9	10.89

Adapted from Kuhnlein et al, 2006 (79)

Table 2(A): Roles and potential mechanisms of lifestyle factors known to affect bone mass - *Nutritional determinants*

Factor	Role(s) and Potential Mechanism(s)	Source
Nutritional Determinants		
Calcium	-Required for bone mineralization	(90, 92)
Protein	-Adequate intake required for bone formation and maintenance -Excess proposed to be calciuric, though calcium intake and overall acid-base balance of diet affect its impact	(92)
Copper, zinc, manganese, vitamin C	-Cofactors for the synthesis of collagen cross-links that are essential to the structure of bone	(24)
Vitamin K	- Required for the γ-carboxylation of the glutamic acid residues in three bone glycoproteins	(24, 90)
Phosphorus	-Required for bone mineralization -An excess in serum decreases serum calcium, however this is thought to be counteracted by ↑ calcium reabsorption in distal tubule	(18, 24, 92)
Sodium, caffeine	-High consumption thought to be calciuric, with caffeine only thought to be of significant risk to older individuals with low calcium intakes	(92)
Ethanol	-Acute and chronic doses can have (-) effects on bone formation -Moderate consumption ↑ conversion of androstenedione to estrone, an estrogenic compound which can have beneficial effects on bone	(92, 122)
Fluoride	-Can (+) osteoblasts and replace hydroxyl ions in hydroxyapatite crystals in bone -Has potential to ↑ crystalline size but ↓ elasticity and quality of bone, ↑ its compression while ↓ its tensile strength	(92)
Vitamin A	-Required for differentiation of bone cells -Bone has nuclear receptors that can bind both vitamins A and D - excess of vitamin A thought to compete with vitamin D for binding sites leading to the consequences observed during vitamin A excess or toxicity such as ↑ bone resorption, hypercalcemia, and fractures	(92)
Vitamin D	-Enhances absorption of calcium and phosphorus in the intestine, their release from bone, and their excretion in the distal tubule of the kidney -May directly enhance bone formation via direct and indirect anabolic effects on osteoblasts	(12, 17, 51)

Table 2(B): Roles and potential mechanisms of lifestyle factors known to affect bone mass – *Body weight and composition, and physical activity*

Factor	Role(s) and Potential Mechanism(s)	Source
Body Weight and Composition, Physical Activity		
Body weight	-Positive correlation of BMD with BMI or weight reported in several studies on Caucasian participants, though ethnic differences known to exist -Mechanism likely involoves mechanical loading on weight-bearing bones -Low BMI (< 20 kg/m^2 or possibly higher) ↑, while BMI > 25 kg/m^2 ↓ the risk of fracture -BMI > 30 → immobility, osteoarthritis and ↑ tendency to fall (costs > benefits of ↑ weight)	(24, 92, 94, 141-145)
Lean body mass (LBM)	-Acquisition associated with many of the factors that (+) bone formation such as insulin, growth hormones, androgens and PA -↑ LBM associated with ↑ muscle strength, which has been correlated with BMD at various sites in perimenopausal women; contributes significantly to skeletal loading - Positive correlations of LBM with bone mass regardless of body weight have been observed	(92, 134, 144, 145)
Fat mass (FM)	-Important source of estrogen in postmenopausal women -Evidence suggests that BMD ↓ and fracture risk ↑ in lean versus obese subjects, however negative correlations between FM and bone mass have been observed -↑ FM associated with chronic disease and hormone profiles may differ depending on whether bulk of FM is stored viscerally or subcutaneously	(92, 134, 143, 144, 146, 147)
Physical Activity (PA)	-Associated with ↑ BMD (magnitude of ↑ depends on type of physical activity (magnitude, frequency, rate and gradient of mechanical stimuli involved)) -Benefits may also include ↑ muscle strength and bone volume,↓ fall rates, and changes in trabecular morphology -Mechanism may include bone fluid flow and shear stress effects leading to deformation of cytoskeletal structure and activation of associated signaling pathways; intracellular membrane channels and bone cell production of autocrine and paracrine factors such as NO, prostaglandins and glutamate may also be involved	(18, 30, 92, 94, 95)

BMD – bone mineral density, BMI – body mass index, LBM – lean body mass, FM – fat mass, PA – physical activity, NO – nitric oxide

Table 2(C): Roles and potential mechanisms of lifestyle factors known to affect bone mass – *Smoking and the use of certain medications*

Factor	Role(s) and Potential Mechanism(s)	Source
Smoking and the Use of Certain Medications		
Smoking	-Associated with ↓ BMD, ↑ risk of fracture and ↑ rate of bone loss -Effects presumably due to negative effects on estrogen metabolism and characteristics commonly possessed by smokers including ↓ body weight, ↑ caffeine and alcohol consumption, and earlier menopause in women	(92)
Glucocorticoid medications	-Accelerate bone loss and ↑ risk of fracture -↑ excretion and ↓ absorption of calcium, reduce gonadal hormone concentrations, (-) osteoblast function and ↑ bone resorption	(14, 37)
Anti-epileptic drugs	-Disturb calcium endocrine system that produce effects similar to those observed in vitamin D insufficiency and in some cases, deficiency	(96)
Oral anticoagulants	-Vitamin K antagonists	(97)
Oral contraceptives	-Associated with ↓ bone turnover and ↓ BMD in young women -May modify skeletal response to PA and effects may be worsened by inadequate calcium intakes	(95)

BMD – bone mineral density, PA – physical activity

10. RATIONALE AND OBJECTIVES

Vitamin D plays an essential role in bone, overall health and disease prevention. Aging Inuit women are at increased risk for vitamin D deficiency due to habitation at higher latitudes, darker skin pigmentation, advancing age, and ongoing "nutrition transition" which has led to decreased intakes of vitamin D and an increased prevalence of obesity. Lower BMD at various sites, a higher risk of fracture, and lower calcium intakes and serum 25(OH)D concentrations have been observed in First Nations compared to White counterparts, of particular relevance to postmenopausal women, who are already at increased risk for osteoporosis.

The objectives of this research were to 1) evaluate vitamin D status by the prevalence of concentrations indicating severe deficiency (25(OH)D ≤ 25 nmol/L), mild deficiency (25.1-50 nmol/L), suboptimal (50.1-75 nmol/L), optimal (75.1-125 nmol/L), supra optimal (> 125 nmol/L) and pharmacologic (> 225 nmol/L) status, 2) evaluate bone health by the prevalence of low forearm BMD T-scores < -1.5 (for women > 50 years) or Z-scores < -2 (for women 40-50 years), and 3) identify factors contributing to variability in forearm BMD in the target population, and 4) evaluate intakes of bone-related nutrients.

Results of this research will contribute to the growing body of knowledge concerning the relationship between vitamin D and bone metabolism, and shed some light on the dynamics of this relationship in a unique population. Useful information will also be obtained regarding the need and potential targets for nutrition interventions amongst this population.

11. MANUSCRIPT 1[1]

11.1 Abstract

Vitamin D status and predictors of forearm bone mineral density as measured by peripheral dual-energy X-ray absorptiometry in Inuit women 40 years of age and older

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Aging Inuit women are at increased risk for vitamin D deficiency due to habitation at higher latitudes, darker skin pigmentation, advancing age, and ongoing "nutrition transition" which has caused decreased intakes of vitamin D and an increased prevalence of obesity. Lower serum 25-hydroxyvitamin D (25(OH)D) concentrations and higher risk of fracture have been separately reported in Inuit women, of particular relevance to postmenopausal women, who are already at increased risk for osteoporosis. This study aimed to evaluate vitamin D status and bone health in 419 Inuit women (40-90 years of age) from 2 regions of Nunavut during August and September 2007/2008. Fasting serum 25(OH)D as measured by a Liaison autoanalyzer was ≤ 75 nmol/L in 49.2% of women (n=374), with a higher prevalence in the pre- (69.4%) versus the postmenopausal (37.6%) group ($\chi^2 = 48.23$, $P<0.001$). Age and 25(OH)D were positively correlated in the post- (r=0.44, $P<0.001$) but not the premenopausal group. Forearm BMD (fBMD) measured by peripheral dual-energy X-ray absorptiometry was low in 60 (31.8%) of the postmenopausal group (T-score < -1.5) and in 2 (1.8%) of the premenopausal women (Z-score < -2). Multiple regression analyses revealed that osteocalcin and either weight, body mass index or percent body fat significantly predicted variability in fBMD, along with parathyroid hormone in most models and age in all models for the pre- and postmenopausal groups, respectively. Lower vitamin D status among premenopausal women suggests a greater risk for osteoporosis with aging compared to the previous generation.

Key words: Forearm bone mineral density; vitamin D; dual-energy X-ray absorptiometry; Inuit; women.

[1] Manuscript prepared for submission.

11.2 Introduction

Information regarding the bone health and vitamin D status of Canadian Inuit is extremely limited. Vitamin D is an important nutrient for bone health, and unlike most populations, Inuit have many rich sources of this vitamin in their country food supply (79). Ongoing nutrition transition, however, has led to a decrease in the intake of such country foods in favor of market foods, and this, combined with the decreased practice of traditional activities, has led to a decrease in the intake of many nutrients, including vitamin D, and an increased prevalence of obesity amongst this population (5). Habitation at latitudes well above 40°N and darker skin pigmentation are additional risk factors for vitamin D deficiency among Inuit. Recently, a higher prevalence of vitamin D deficiency despite similar vitamin D intakes was reported in First Nations in Manitoba compared to White women (11). This is of particular relevance in aging women, who are at increased risk for osteoporosis following menopause, and in whom this risk is further increased when vitamin D deficiency subsequently leads to secondary hyperparathyroidism (39).

Ethnic differences in bone health are known to exist and thought to be due to a combination of genetic differences in the accretion of peak bone mass (123), timing or rate of adult bone loss, body size, bone size (123, 124) and hip geometry (124). In terms of vitamin D metabolism, ethnic differences are thought to be attributable to genetic differences in the production of and response to parathyroid hormone (PTH) (125-128) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (127-129) or altered calcium metabolism (125, 128). An investigation of ethnic differences in the vitamin D status and biomarkers of bone metabolism between Greenland Inuit living in Nuuk or Denmark consuming traditional or westernized fare, and Danes living in Denmark consuming westernized fare revealed that serum bone alkaline phosphatase (bALP) and $1,25(OH)_2D$ were higher, while PTH and osteocalcin (OC) were lower, in Inuit versus Danes. The presence of vitamin D insufficiency (25-hydroxyvitamin D (25(OH)D) < 40 nmol/L) was highest in Inuit Greenlanders consuming westernized fare (129). Differences in calcaneal bone mineral density (BMD) between Greenland Inuit and Caucasians were explained

by differences in body size (BMD and weight higher in Caucasians) (131). Ethnic differences in weight- and body mass index (BMI)-adjusted BMD at the calcaneus, total body and distal forearm were accounted for by a lower ratio of lean to fat mass in First Nations versus White women, combined with a smaller increment in BMD from gains in fat versus lean mass in both populations (134). First Nations women also had lower serum 25(OH)D and PTH, and higher osteoprotegerin (OPG), which correlated negatively with BMD at the calcaneus, spine, hip and total body, than White counterparts (135). Lower bone density at the radius and second metacarpal has been reported in First Nations versus Caucasian females in Southwestern Ontario (132). An increased fracture risk has also been observed in First Nations, with wrist, spine and hip fractures occurring predominantly in older people and women (139). Higher fracture rates were also observed in Alaskan Inuit men and women compared to white US counterparts (138).

Risk factors for poor vitamin D status and bone health may be similar amongst Canadian Aboriginal populations. The aim of this study was thus to investigate the bone health and vitamin D status, as well as potentially ethnic-specific predictors of BMD in aging Inuit women, a unique population in whom such valuable information is lacking.

11.3 Methods

Study Population

Data was collected during the International Polar Year (IPY) Inuit Health Survey between August and September, 2007 and 2008; the overall participation rate for the survey was 71.0%. For this report, all participants were Nunavut women 40 years of age and older from 20 communities (19 coastal, 1 inland) in the Qikiqtaaluk (Baffin) and Kivalliq (Keewatin) regions (57-76°N, *mean*=67°N) whose households were randomly selected to participate in the survey. Entrance criteria were that the women not be pregnant and be physically and mentally capable of boarding the research facility (Canadian Coast Guard Ship Amundsen) and completing the required tests and questionnaires.

Ethics

Ethics approval was obtained from McGill University's Institutional Review Board, and a research license was also granted from the Nunavut Research Institute. Research agreements were signed by each participating hamlet and all participants gave informed written consent.

Anthropometry

Standing height was measured to the nearest 0.1 centimeter using a portable stadiometer (Seca Road Rod #214, Stadiometer.com, Snoqualmie, WA, USA) and weight (measured to the nearest 0.1 kilogram) and percent body fat were obtained using a TBF-300A Body Composition Analyzer/Scale (Tanita Corporation of America, Arlington Heights, IL, USA). Body mass index was then calculated using weight and height measurements (weight in kilograms divided by height in meters squared).

Biochemical Tests and Classification of Vitamin D Status

Chemiluminescent immunoassays (CLIA) were used to measure serum 25(OH)D (#310600), PTH (#310910) and OC (#310950) on a LIAISON® automated analyzer (DiaSorin, Stillwater, MN). All assays were conducted on samples obtained from participants in the fasted state at a standardized time of day (between 0800h and 1200h). Samples were analyzed in multiple assays in singlet, but controls were measured in duplicate in order to obtain an average percent coefficient of variation (%CV) for each assay. The average interassay %CV for the 25(OH)D, PTH and OC assays were 4.5, 2.6, and 2.2, respectively. Our laboratory participates in the Vitamin D External Quality Assessment Scheme (DEQAS; Charing Cross Hospital, London, UK), and our results have been in range with other reported Liaison results.

The assay for 25(OH)D is a direct competitive CLIA designed to measure total 25(OH)D (D_2 and D_3 isoforms), and has shown minimal (<1%) cross reactivity with vitamin D_2 and D_3, and 3-epi-25(OH)D_3, 40% cross-reactivity with1,25(OH)$_2D_2$ and 17% cross reactivity with 1,25(OH)$_2D_3$. The assays for PTH and OC are both direct, two site, sandwich type CLIAs. For PTH, the concentration of intact (1-84) PTH is measured and the assay has shown minimal

cross-reactivity with the various PTH fragments, with the exception of PTH 7-84, which had a cross-reactivity of 52%. For OC, the assay is designed to measure the intact (1-49) molecule, but was shown to have 87% cross-reactivity with the 1-43 fragment.

In terms of vitamin D status, individuals were classified based on 25(OH)D concentrations as severely deficient (25(OH)D ≤ 25 nmol/L), mildly deficient (25.1-50 nmol/L), suboptimal (50.1-75 nmol/L), optimal (75.1-125 nmol/L), supra optimal (> 125 nmol/L) and pharmacologic (> 225 nmol/L).

Bone Mineral Density

Areal BMD at the distal forearm was measured using peripheral dual-energy X-ray absorptiometry (pDXA; GE/Lunar PIXI, Fort Myers, FL). BMD values from the PIXI are expressed in g/cm^2 and also as age standardized Z-score and T-score values; all measures were explored in data analysis. The non-dominant arm was used for the measurement unless affected by previous fracture or severe arthritic changes. Previous history of fracture(s) was obtained via self-report. The reference population against which BMD scores were compared to obtain Z- and T-scores was composed of ambulatory white subjects aged 20-45 years with no history of chronic disease or medications affecting bone, and no history of symptomatic, atraumatic fractures. All reference data was collected in the United States using PIXI systems. Quality control using the forearm phantom provided with the unit was performed daily before any measurements were taken, with a minimum of three times per week; long-term precision error was <0.5%. In vivo variability was assessed through the calculation of a %CV using three repeated measurements with repositioning on two randomly selected patients per day, one at the beginning and the other at the end of the day; the average %CV was 1.68.

Low bone mass was classified as forearm BMD (fBMD) Z-score less than -2 (for premenopausal women) and T-score less than -1.5 (for postmenopausal women). Our pDXA device measured both bones at the distal forearm rather than the 1/3 radius, therefore cutoffs were intentionally set more conservatively than those of the WHO (T-score between -1.0 and -2.5 for osteopenia in

postmenopausal women (148)). When returning results to participants, women in this category were referred to their health care centers for further evaluation.

Other Variables

Interviewer-administered questionnaires allowed us to obtain information on menopausal status. This was defined as no menstrual periods for the past 3 months due to menopause or hysterectomy, or age > 50 y if no other information (which was the case for n=20 women (5.2%)). Questionnaires also allowed us to obtain information regarding diabetes status (classified as having diabetes if fasting plasma glucose ≥ 7 mmol/L or oral glucose tolerance test ≥ 11.1 mmol/L, or if self-reported status confirmed by medication(s)) and smoking status (current, past or never smoker). Nurses recorded the use of medications, including those that could influence BMD including hormone replacement therapy, oral corticosteroids, bisphosphonates, thyroid replacement hormone, and contraceptives. Covariates were included in regression models if their frequency overall or within a particular group was ≥ 10.

Statistical Analysis

Due to highly probable differences in bone metabolism, pre- and postmenopausal women were analyzed separately in multivariate analyses. Comparisons of two means between and within groups were performed using either t-tests or Mann-Whitney U-tests for normally or non-normally distributed variables, respectively, with normality being tested using the Kolmogorov-Smirnov method. Similarly, for more than two means, oneway ANOVA or Kruskal-Wallis oneway ANOVA was used for normally or non-normally distributed variables, respectively. The Bonferroni adjustment was used in cases of multiple comparisons with α=0.05. Pearson's Chi-square tests were used to compare proportions of categorical variables between and within groups. Prior to multivariate analyses, pairwise Pearson and Spearman correlations were calculated between all continuous and categorical variables, respectively, to establish collinearity. Various models were constructed using either fBMD or Z-score (for premenopausal group) or T-score (for postmenopausal group) as the dependent variable, centered continuous variables as the regressors to facilitate

model interpretation, and $P<0.05$ accepted as significant. Normality of residuals was ensured using the Kolmogorov-Smirnov test along with visual examination of residual-normal quantile plot, and heteroskedasticity was evaluated using the Breusch-Pagan/Cook-Weisburg test. Variance inflation factors were examined post-regression to ensure the absence of multicollinearity. Outliers in multivariate analyses were identified using leverage versus residual plots; those with high leverage were either corrected if due to errors in data entry or tested for their influence on regression parameters by successive exclusion alone and in combination with other outliers (see **Appendix**). Once highly influential outliers were removed, non-significant regressors were eliminated from the final model until the R^2 between the full model's fitted values and the reduced model's fitted values fell below 0.95; no regressors with P values exceeding 0.2 were included in final models. All statistical analyses were performed using Stata/SE 9.2 (StataCorp LP, College Station, Texas).

11.4 Results

Overall Population Characteristics

Pre- and postmenopausal groups differed in nearly every characteristic except mean serum PTH concentration and the frequency of use of thyroid replacement hormone. The postmenopausal group had significantly higher age, weight, BMI, percent body fat (%BF), 25(OH)D, OC, proportion of past smokers and persons with diabetes, and lower height, fBMD and proportion of current smokers than the premenopausal group (**Table 1**). Out of the 414 women from the Baffin and Kivalliq regions 40 years of age and older who participated in the Inuit Health Survey, 31 (7.5%) accepted to be in the survey but, for whatever reason, could not board the ship and complete the survey (partial participants), 296 (71.5%) had complete datasets for multiple regression and 5 (1.2%) of these were excluded as outliers. Three of these outliers had unusual fBMD (three high and one low) for their weight and age, and one had inexplicably high OC concentrations. Final regression analyses therefore included 291 (70.3%) women (101 premenopausal and 190 postmenopausal).

Vitamin D Status

Overall, 49.2% of the 374 women in whom serum 25(OH)D was measured had severely deficient, mildly deficient or suboptimal (i.e. 25(OH)D ≤ 75 nmol/L) vitamin D status, with 69.4% of the premenopausal (*n*=137) and 37.6% of postmenopausal (*n*=237) women in these categories (**Figure 1**). None of the premenopausal women were in the supra-optimal category and none of the women in either group were in the pharmacologic category. Age was significantly positively correlated with 25(OH)D in the postmenopausal (r=0.44, P<0.001), but not the premenopausal (r=0.16, P=1.000) group (**Figure 2**). The proportion of women below and above 75 nmol/L was significantly different in the pre- versus the postmenopausal group (χ^2=48.23, P<0.001).

Evaluation of Bone Health

Forearm BMD was measured in 310 women (111 premenopausal and 199 postmenopausal) and was low in 64 (32.2%) postmenopausal women. Women in that category had significantly higher mean age, OC and 25(OH)D concentrations, and lower mean weight, height, BMI, and %BF (**Table 2**). The postmenopausal women (*n*=44) in whom fBMD was not measured were similar to the postmenopausal women with normal fBMD, differing only with respect to a lower mean serum 25(OH)D (**Table 2**). Only 2 (1.8%) premenopausal women were classified as having low fBMD.

Predictors of Forearm BMD

In both groups, results were almost identical whether fBMD or T- or Z-Score was used as the dependent variable. Results are thus reported using fBMD as the dependent variable in order to facilitate comparisons between groups.

Because of a high correlation between weight, BMI and %BF, a different model was constructed for each of these anthropometric predictors within each group (**Table 3**). For the premenopausal women, full models included age, height, current and past smoking status, 25(OH)D, PTH and OC, along with either weight (model A), BMI (B) or %BF (C). Models explained 23.7-32.8% of the variability in fBMD. In all models, OC and either weight, BMI or %BF were significant predictors, with OC explaining 4.2-5.2%, weight 27.0%, BMI 27.8%, and %BF

14.8% of the variability in fBMD. For the postmenopausal women, full models included the same variables as for the premenopausal women, along with whether or not the non-dominant arm was used for the fBMD measurement, presence or absence of diabetes, and whether or not the participant was taking thyroid replacement hormone. Models explained 60.8-62.0% of the variability in fBMD. This time, in all three models, age, OC and either weight, BMI or %BF were all highly significant predictors of fBMD ($P<0.001$), with age accounting for the majority (41.9-48.9%), weight 8.9%, BMI 8.1%, %BF 8.2%, and OC 2.7-3.2% of the variability in fBMD.

Table 1: Population characteristics for overall and each menopausal group[1]

	Overall *(n=414)*[2]	**Premenopausal** *(n=140)*[3]	**Postmenopausal** *(n=243)*[4]	***P***[5]
Age *(years)*	54 ± 11 (40 to 90)	45 ± 7 (40 to 82)	59 ± 10 (41 to 90)	<0.001
Weight *(kg)*	66.3 ± 16.0 (36.4 to 129.6)	64.3 ± 16.5 (36.4 to 129.6)	67.4 ± 15.7 (37.0 to 121.0)	0.0270
Height *(cm)*	152.0 ± 5.5 (126.0 to 168.0)	153.2 ± 5.0 (140.0 to 168.0)	151.2 ± 5.7 (126.0 to 168.0)	0.0035
BMI *(kg/m^2)*	28.7 ± 6.7 (16.2 to 53.9)	27.4 ± 6.9 (16.2 to 53.9)	29.4 ± 6.5 (18.3 to 49.2)	0.0010
Body Fat *(%)*	35.0 ± 9.5 (7.5 to 53.4)	32.6 ± 9.8 (7.5 to 53.4)	36.4 ± 9.0 (10.5 to 53.2)	0.0002
25(OH)D *(nmol/L)*	76.5 ± 34.3 (10.0 to 207.7)	60.5 ± 24.8 (10 to 122.6)	85.7 ± 35.3 (15.6 to 207.7)	<0.001
PTH *(pmol/L)*	4.2 ± 1.8 (1.0 to 15.1)	4.3 ± 1.8 (1.4 to 12.8)	4.2 ± 1.9 (1.0 to 15.1)	0.4122
OC *(nmol/L)*	2.1 ± 0.9 (0.9 to 7.3)	1.7 ± 0.6 (0.9 to 4.6)	2.3 ± 1.0 (0.9 to 7.3)	<0.001
fBMD *(g/cm^2)*	0.453 ± 0.085 (0.176 to 0.665)	0.495 ± 0.056 (0.342 to 0.650)	0.430 ± 0.090 (0.176 to 0.665)	<0.001
Current Smokers *(n (%))*	235 (62.7)	105 (77.2)	130 (54.2)	<0.001
Past Smokers *(n (%))*	113 (30.1)	24 (17.6)	89 (37.5)	<0.001
With Diabetes *(n (%))*	28 (7.5)	4 (2.9)	24 (10.0)	0.011
Taking TH *(n (%))*	11 (3.1)	3 (2.2)	8 (3.5)	0.523

BMI: body mass index, 25(OH)D: serum 25-hydroxyvitamin D, PTH: serum parathyroid hormone, OC: serum osteocalcin, fBMD: forearm bone mineral density, TH: thyroid hormone

[1]Values are mean±SD (range) unless otherwise noted

[2]Overall sample size, with missing data for: weight, BMI and PTH - 11.8%, of overall height - 11.6%, %BF – 12.3%, 25(OH)D – 9.7%, OC – 10.1%, fBMD – 25.1%, smoking status – 9.4%, diabetes status – 7.0%, medications (including TH) – 10.6%

[3]Overall premenopausal sample size, with missing data for: weight, height and BMI - 3.6% of overall , %BF – 5%, 25(OH)D and OC – 2.1%, PTH – 5.7%, fBMD – 20.7%, smoking status - 2.9%, medications (including TH) – 4.3%

[4]Overall postmenopausal sample size, with missing data for: weight, BMI and %BF – 5.3% of overall, height and medications (including TH) – 4.9%, 25(OH)D – 2.5%, PTH – 4.1%, OC – 3.3%, fBMD – 18.1%, smoking and diabetes status – 1.2%

[5]*P*-values between pre- and postmenopausal groups

Table 2: Differences between postmenopausal women with low, normal and unknown fBMD[1]

	Low BMD *(n=64)*[2]	**Normal BMD** *(n=135)*[3]	**Unknown BMD** *(n=44)*[4]	***P***
Age *(years)*	67 ± 9 (50 to 90)[a]	56 ± 7 (41 to 78)[b]	60 ± 11 (41 to 83)[c]	<0.001
Weight *(kg)*	58.9 ± 14.9 (38.6 to 95.8)[a]	70.6 ± 15.1 (37 to 121)[b]	71.0 ± 13.6 (48.2 to 97)[b]	0.0001
Height *(cm)*	149.8 ± 6.4 (126.0 to 168)[a]	152.2 ± 5.2 (135.8 to 164.3)[b]	149.7 ± 5.6 (142 to 165)[a]	0.0022
BMI *(kg/m^2)*	26.2 ± 6.1 (18.3 to 42.9)[a]	30.4 ± 6.3 (18.5 to 49.2)[b]	31.6 ± 5.7 (22.2 to 45.5)[b]	<0.001
Body Fat (%)	32.3 ± 9.8 (14.2 to 49.4)[a]	37.6 ± 8.6 (10.5 to 53.2)[b]	39.3 ± 6.5 (27.8 to 49.5)[b]	0.0003
25(OH)D *(nmol/L)*	98.2 ± 34.3 (23.6 to 195.2)[a]	85.6 ± 32.3 (22.4 to 207.7)[b]	67.2 ± 38.7 (15.6 to 148.0)[c]	0.0001
PTH *(pmol/L)*	4.3 ± 2.2 (1.3 to 15.1)	4.1 ± 1.8 (1.0 to 10.5)	4.3 ± 1.7 (1.7 to 9.8)	0.9047
OC *(nmol/L)*	2.7 ± 1.3 (1.2 to 7.3)[a]	2.1 ± 0.8 (0.9 to 4.9)[b]	2.3 ± 1.0 (1 to 4.9)[ab]	0.0102
fBMD *(g/cm^2)*	0.327 ± 0.053 (0.176 to 0.396)[a]	0.479 ± 0.056 (0.397 to 0.665)[b]	-	<0.001

BMI: body mass index, PTH: serum parathyroid hormone, OC: serum osteocalcin, fBMD: forearm bone mineral density

[1]Values are means ± SD (range); means in a row with superscripts without a common letter differ, Bonferonni-adjusted for multiple comparisons (α=0.05)

[2]Overall sample size for normal BMD, with missing data for: 25(OH)D, PTH and OC – 1.6% of overall

[3]Overall sample size for low BMD, with missing data for: weight, BMI and %BF – 0.7% of overall, 25(OH)D – 3.0%, PTH – 5.2%, OC – 4.4%

[4]Overall sample size for unknown BMD, with missing data for: weight, height, BMI and %BF – 27.3%, 25(OH)D and OC – 2.3%, PTH - 4.5%

Table 3: Results from reduced multiple linear regression models for predictors of forearm bone mineral density (g/cm^2) in pre- and postmenopausal groups

	β coefficient (±SE)	*P*	R^2%	Model	
				R^2	Root MSE
Premenopausal Group (n=101)					
Model A					
Constant	0.487 ± 0.007	0.000	-	0.311	0.046
Weight (kg)	0.002 ± 0.0003	0.000	0.270		
OC (nmol/L)	-0.025 ± 0.009	0.005	0.052		
PTH (pmol/L)	-0.007 ± 0.003	0.024	0.031		
Height (cm)	-0.002 ± 0.001	0.079	-		
Model B					
Constant	0.487± 0.006	0.000	-	0.328	0.046
BMI (kg/m^2)	0.004 ± 0.001	0.000	0.278		
OC (nmol/L)	-0.025 ± 0.009	0.004	0.052		
PTH (pmol/L)	-0.007 ± 0.003	0.020	0.032		
Model C					
Constant	0.523 ± 0.023	0.000	-	0.237	0.048
Body Fat (%)	0.002 ± 0.001	0.000	0.148		
OC (nmol/L)	-0.023 ± 0.009	0.014	0.042		
PTH (pmol/L)	-0.003 ± 0.003	0.174	-		
Current Smoker (1=yes, 0=no)	-0.037 ± 0.023	0.105	-		
Past Smoker (1=yes, 0=no)	-0.040 ± 0.024	0.108	-		
Postmenopausal Group (n=190)					
Model A					
Constant	0.465 ± 0.005	0.000	-	0.620	0.056
Weight (kg)	0.002 ± 0.0003	0.000	0.089		
OC (nmol/L)	-0.017 ± 0.004	0.000	0.031		
Age (y)	-0.006 ± 0.0004	0.000	0.419		
Model B					
Constant	0.465 ± 0.005	0.000	-	0.611	0.057
BMI (kg/m^2)	0.004 ± 0.001	0.000	0.081		
OC (nmol/L)	-0.017 ± 0.004	0.000	0.032		
Age (y)	-0.006 ± 0.0004	0.000	0.450		
Model C					
Constant	0.466 ± 0.005	0.000	-	0.608	0.058
Body Fat (%)	0.003 ± 0.001	0.000	0.082		
OC (nmol/L)	-0.016 ± 0.004	0.000	0.027		
Age (y)	-0.007 ± 0.0004	0.000	0.489		

OC: serum osteocalcin, PTH: serum parathyroid hormone, BMI: body mass index

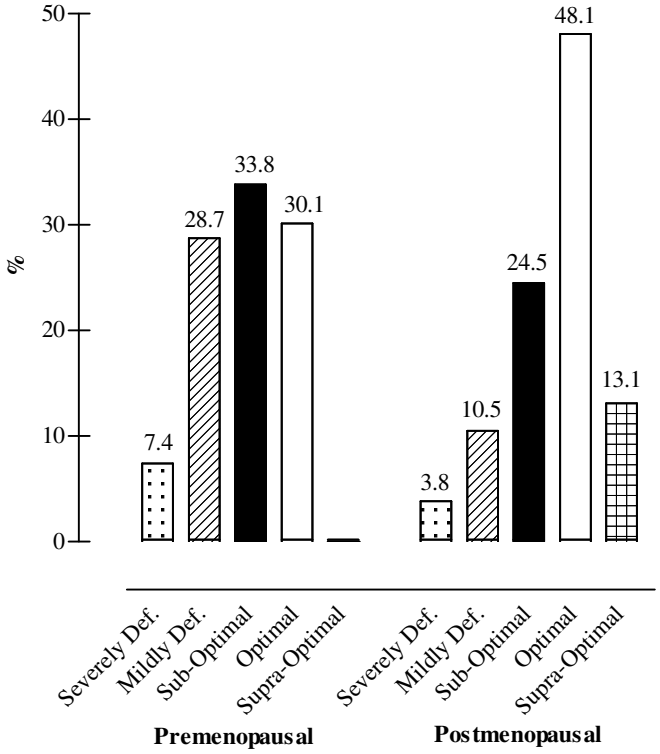

Figure 1. Proportion of Inuit women 40-90 years of age (*n*=374) in each vitamin D status group divided by menopausal status. Bars represent severely deficient (⊡; < 25 nmol/L), mildly deficient (▨ ; 25.1-50 nmol/L), sub-optimal (■ ; 50.1-75 nmol/L), optimal (□ ; 75.1-125 nmol/L) and supra-optimal (⊞ ; >125 nmol/L) vitamin D status.

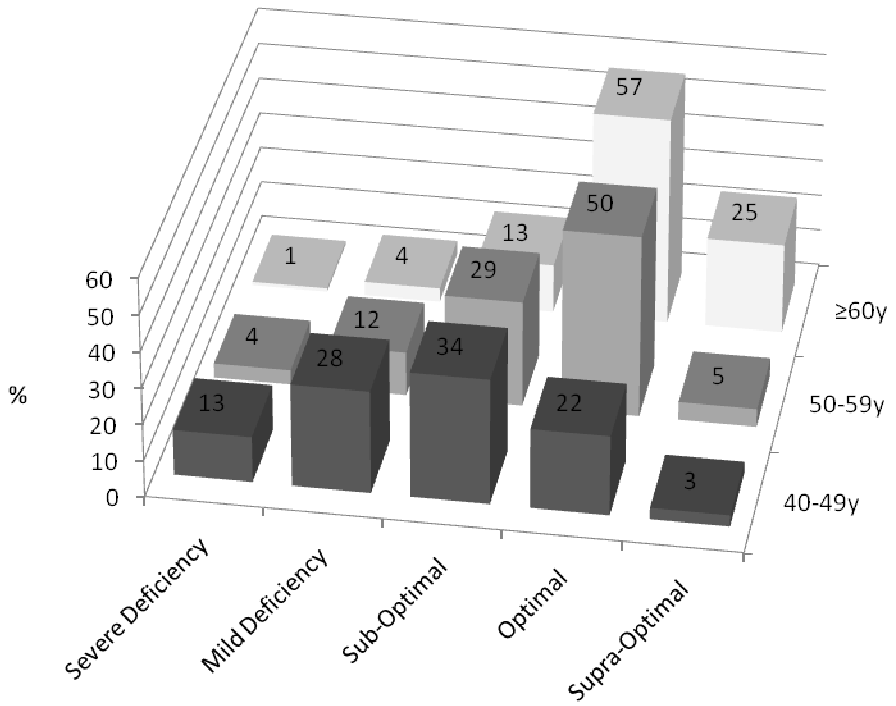

Figure 2. Proportion of postmenopausal Inuit women (*n*=237) in each vitamin D status category, divided by age group. Vitamin D status categories represent severely deficient (25(OH)D< 25 nmol/L), mildly deficient (25.1-50 nmol/L), sub-optimal (50.1-75 nmol/L), optimal (75.1-125 nmol/L) and supra-optimal (>125 nmol/L) 25(OH)D concentrations.

11.5 Discussion

At the point in the year when 25(OH)D should be at its highest, a large proportion of Inuit women had below optimal concentrations, particularly the younger, premenopausal women. 25(OH)D was not a significant predictor of fBMD, and in fact, its concentrations were highest in the postmenopausal women with low bone density. Women in this category also had a higher mean age and lower mean weight, BMI and %BF, suggesting that the effects of these negative predictors of fBMD may have outweighed any potential positive effects of 25(OH)D on bone. Other possibilities include that these older women exhibited resistance to the effects of calcitriol (the active form of vitamin D), such as that previously reported in the elderly (149), or that 25(OH)D was not maintained at these high concentrations throughout the year, allowing for the seasonal occurrence of accelerated bone loss.

A study in Nuuk (64°N), Greenland revealed that 23% of Inuit consuming traditional compared to 74% consuming westernized fare had 25(OH)D concentrations below 40 nmol/L during the period from May to September. Overall, 22.5% of our target population (14.5% of the postmenopausal and 35.1% of the premenopausal women) had 25(OH)D concentrations that were in the mild or severely deficient categories (i.e. 25(OH)D ≤ 50 nmol/L), comparable to Greenland Inuit consuming traditional fare. In a sample of healthy adults in Calgary (51°N), a city that receives more hours of sunshine per year than any other Canadian city, the proportion of those with 25(OH)D concentrations less than 50 and 80 nmol/L was highest in the fall (November-December, 43% and 94%, respectively) and lowest in the summer (August-September, 14% and 68%). During a similar summer time period, the proportion of women in our study population with serum 25(OH)D at or below 50 and 75 nmol/L was 35.1% and 69.4%, respectively, in the premenopausal group, and 14.5% and 37.6% in the postmenopausal group. Age has been previously correlated with total daily intake of country food amongst Inuit (5). A higher consumption of vitamin D-rich country foods, including fish and sea mammals (79), is thus a likely explanation

for the higher 25(OH)D concentrations observed in the older postmenopausal versus the younger post- and premenopausal Inuit women.

In a group of 30-49 year old Inuit women in Greenland, mean BMD at the right and left forearms was 0.484 and 0.474 g/cm^2 (131), lower than our mean value for the premenopausal women (0.495 g/cm^2), and higher than that for the postmenopausal women (0.430 g/cm^2), likely due to the Greenlanders younger mean age. In postmenopausal white women 55-70 years of age in the UK who met the conventional criteria for BMD examination, 39% of those in whom fBMD was measured with a similar pDXA device (Osteometer DTX-200) had T-scores in the intermediate range (-1.4 to -2.6, defining 90% sensitivity and specificity for that particular device) (150). With a similar cutoff in our postmenopausal women (T-score < -1.5), 31.8% were classified as having low fBMD. This seems comparable to the UK group, with their percentage likely being higher due to a higher risk study population. It is not convention to apply the WHO classification methods to premenopausal women, and osteoporosis can only be diagnosed if there is low BMD with secondary causes or risk factors for fracture (148).

Age was a significant predictor of fBMD in the post- but not the premenopausal group, consistent with reports that BMD at this site only begins to decline in women around age 50 (151). Weight, BMI, and %BF were equivalent predictors of fBMD in the postmenopausal group, while in the premenopausal group, %BF accounted for approximately half the variability in fBMD than weight or BMI. In postmenopausal women, body fat's contribution to BMD may be explained by both its influence on overall body weight, estrogenic effects and/or the effects of adipocyte-derived hormones such as leptin and adiponectin, which have been shown to influence bone by a variety of different mechanisms (147, 152). Age and weight have been similarly identified as predictors of fBMD in women 65 years and older in Norway (151), along with serum OC and PTH in First Nations women (aged 25-75 y) (135). Low body weight has also been associated with low BMD in women aged 40-60 years of age (153), along with low BMI in women aged 40-59 years of age (154). Amongst younger Inuit in

Greenland (aged 30-50 y), BMI but not age, height, weight or smoking status was a significant predictor of fBMD (131).

Though seasonal variability in BMD closely paralleling that of 25(OH)D has been observed at a number of sites, including the mid-radius (155), and 25(OH)D has shown positive relationships with BMD at a number of sites (63-65, 67, 156), it was not a significant predictor of fBMD in our study. This relationship was also absent at the radius and five other sites in a recent study of First Nations women 25-75 years of age in Manitoba (135). It is thought that once a certain concentration of 25(OH)D (~80 nmol/L (71)) is reached, a plateau in PTH concentrations occurs approximately 1 month later, with a leveling out of the concentrations of bone turnover markers (specifically, CTx) in the following 1-2 months (44). It can therefore be postulated that once this plateau is reached, BMD change occurs at a much slower pace, remaining relatively constant until the concentration of 25(OH)D, and subsequently PTH, once again reaches that threshold concentration. The fact that 25(OH)D was only measured at a single summer time point in our study may have concealed its' relationship with fBMD, as concentrations of 25(OH)D exceeding the proposed threshold that would not necessarily have led to further improvements in fBMD were present in many individuals. Concentrations of PTH, and potentially the PTH-influenced biomarkers of bone turnover, may better reflect BMD when measured at a single time point, explaining the appearance of PTH and OC as predictors of BMD at various sites in the First Nations study, which also employed single measurements of 25(OH)D and BMD (135). In our study, OC was a predictor of fBMD in all models, along with PTH in the premenopausal group. The absence of PTH as a predictor in the postmenopausal group may be due to the confounding influence of calcium and phosphorus intakes (116), or to the fact that menopause and aging, rather than poor vitamin D status, may be the major influence on PTH concentrations in these women (36, 157). The appearance of PTH and OC as predictors provides indirect evidence of vitamin D's role in bone health, as 25(OH)D has been shown to inhibit PTH synthesis and thus PTH-driven increases in bone turnover, for which OC is a marker (23, 39).

One limitation of our study is that the WHO cutoffs for osteoporosis and osteopenia are not applicable to the site we were able to measure (148), and device-specific cutoffs have not yet been developed for the measurement of fBMD using PIXI technology. Despite these limitations, peripheral BMD has been shown to be highly predictive of overall fracture risk (158), and has been recommended as a safe, comfortable and moderate cost screening tool for the detection of osteoporotic bone loss (112, 159). Additionally, the use of pDXA, and specifically the GE Lunar PIXI, has been validated for Arctic field studies by a group in Greenland (160). Access to a number of important and well-measured bone-related covariates allowed our regression models to explain a considerable amount of the variability in fBMD. Genetic factors may account for a large portion of remaining unexplained variability, along with modifiable lifestyle factors such as exercise and alcohol consumption, which have been reported as predictors of BMD at various sites (161-163). Unfortunately, these variables were not available to us at the time of our analyses. Additionally, the contribution of variables such as diabetes status and the use of thyroid replacement hormone may not have reached significance due to their limited representation in the population, while a low percentage of never smokers may not have allowed for a significant distinction between the fBMD of current, past and never smokers.

In summary, vitamin D concentrations among premenopausal Inuit women fell far below those of more Southern dwelling Canadian adults. The results for the postmenopausal women are more encouraging, with concentrations exceeding those of Southern dwelling Canadians providing evidence that, despite habitation at extreme Northern latitudes, the achievement of optimal 25(OH)D concentrations is highly possible in aging Inuit women. An investigation into the predictors of serum 25(OH)D would be beneficial to inform efforts to increase intakes of vitamin D and promote a safe amount of sun exposure amongst this population. Higher weight, BMI and %BF had protective effects on fBMD, while increased concentrations of OC in all women, and PTH in premenopausal women, had negative effects on fBMD. Approximately one third of postmenopausal women were classified as having low fBMD, a proportion which seems

comparable to other populations. Lower vitamin D status among premenopausal women, however, suggests a greater risk for osteoporosis with aging compared to the previous generation. Proper access of aging Inuit women to screening tools such as pDXA, along with effective therapy for low bone mass, should thus be ensured in the Canadian arctic.

12. INTRODUCTION TO MANUSCRIPT 2

Based on the large discrepancy between pre- and postmenopausal groups with respect to vitamin D status, we decided to explore whether the differences between these groups extended to nutrient intakes. Suspecting that the higher prevalence of deficient, insufficient, and suboptimal 25(OH)D concentrations in the pre- versus the postmenopausal group was caused by a greater impact of nutrition transition amongst this younger group, we divided our sample of women into three age-based sub-groups (40-49 y, 50-59 y, and ≥ 60 y). Subsequently, our main objectives were to look for trends in the intakes of selected bone related nutrients, and re-examine vitamin D status by age group.

13. MANUSCRIPT 2[2]

13.1 Abstract

Nutrient intakes and vitamin D status in Inuit women 40 years of age and older

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Objectives: To determine nutrient intakes and vitamin D status in Inuit women ≥ 40 y.

Study Design: Participants were women ≥ 40 years of age (*n*=416) who were randomly selected to participate in the 2007/2008 International Polar Year Inuit Health Survey from two regions in Nunavut.

Methods: Nutrient values were derived from single 24-hour recalls using CANDAT. The concentration of 25-hydroxyvitamin D (25(OH)D) was measured on fasting serum samples using a LIAISON® automated analyzer. Nutrient intakes were compared between three sub-groups (40-49 y, 50-59 y, ≥ 60 y) using nonparametric K-sample tests on equality of medians. The proportion of women in each vitamin D status group (severely deficient (25(OH)D ≤ 25 nmol/L), mildly deficient (25.1-50 nmol/L), suboptimal (50.1-75 nmol/L), optimal (75.1-125 nmol/L), supra optimal (> 125 nmol/L)) was compared between sub-groups using a Pearson Chi-square test.

Results: Complete nutrient data and 25(OH)D measurements were obtained for 333 and 374 women, respectively. Trends towards increasing intakes of protein, manganese, phosphorus, zinc, polyunsaturated fatty acids, vitamin D, calcium and magnesium per 1000 kilocalories with age was observed. Mean intakes were below the estimated average requirement for magnesium and below the adequate intake for vitamin K and calcium. The proportion of women with serum 25(OH)D ≤ 75 nmol/L significantly decreased with age (40-49 y, 72.2%; 50-59 y, 45.9%; ≥ 60 y, 20.0%).

Conclusions: Women 50 years of age and older tend to have higher vitamin D concentrations and more nutrient-dense diets than women 40-49 years of age. The adequacy of vitamin K, calcium and magnesium warrants further investigation over multiple days.

Key Words: Vitamin D; nutrition; Inuit; women.

[2] Manuscript prepared for submission.

13.2 Introduction

Traditional food use is decreasing around the globe amongst Indigenous populations for a variety of social and economic reasons (164). Inuit in the Canadian Arctic are not exempt from this nutrition transition. An increasing prevalence of obesity and a decreasing intake of many nutrients has been reported in this population, especially in younger age groups, as the consumption of market foods increases at the expense of traditional (or country) foods (5). Inuit country foods are rich sources of many vitamins and minerals, including vitamin D, which is abundantly present in fish and sea mammals (79). In adult Inuit women from Nunavik, though market food was the major contributor to energy intake, 40% of vitamin D intake was derived from traditional food (10). Amongst Yukon First Nations, Dene/Métis, and Inuit communities in Canada, days when traditional food was consumed were significantly higher in vitamin D and other nutrients than days when it was not consumed (5). In terms of nutrient adequacy, the percent of Inuit men and women over 40 years of age meeting or exceeding the adequate intake (AI) for vitamin D was 91.0 and 100, respectively (4). This was greater than the percent for men (59.0) and women (76.0) under 40 years of age, likely due to a higher intake of traditional sea mammal meats and fats amongst older age groups (4).

With cutaneous synthesis of vitamin D being limited at extreme Northern latitudes, a decreased intake of nutrient-rich traditional food and an increased prevalence of obesity, a risk factor for vitamin D deficiency (16), is likely to put the adequacy of Inuit vitamin D intake in jeopardy. For example, a study in Nuuk (64°N), Greenland revealed that 23% of Inuit consuming traditional compared to 74% consuming westernized fare had 25(OH)D concentrations below 40 nmol/L during the period from May to September (129). Along with other nutrients such as protein, vitamin C, calcium, and magnesium, many of which are abundant in country foods, vitamin D is essential for bone health, particularly in postmenopausal women in whom the risk for osteoporosis is increased. Interrelations exist between vitamin D and other nutrients such as vitamin A, an excess of which may produce a relative deficiency of vitamin D, and calcium,

whose absorption across the gastrointestinal tract is enhanced by vitamin D (92). Rarely have studies assessed vitamin D status in Inuit as determined by serum 25-hydroxyvitamin D (25(OH)D) (9), and data for the reports on nutrient intake amongst Inuit date back a decade or more (4, 5, 10). The aim of this study was therefore to assess the vitamin D status and intake of bone-related nutrients in older Inuit women. Three age groups (40-49 years, 50-59 years, and 60 years or older) were examined to represent premenopausal, early post-menopausal, and post-menopausal women, and to attempt to approximate the age at which nutrition transition has the greatest impact on vitamin D status and nutrient intakes in this population.

13.3 Methods

Study Population

Data was collected during the International Polar Year (IPY) Inuit Health Survey between August and September, 2007 and 2008; the overall participation rate for the survey was 71.0%. For this report, all participants were Nunavut women 40 years of age and older from 20 communities (19 coastal, 1 inland) in the Qikiqtaaluk (Baffin) and Kivalliq (Keewatin) regions (57-76°N, *mean*=67°N) whose households were randomly selected to participate in the survey. Entrance criteria were that the women not be pregnant and be physically and mentally capable of boarding the research facility (Canadian Coast Guard Ship Amundsen) and completing the required tests and questionnaires.

Ethics

Ethics approval was obtained from McGill University's Institutional Review Board, and a research license was also granted from the Nunavut Research Institute. Research agreements were signed by each participating hamlet and all participants gave informed written consent.

Anthropometry

Standing height was measured to the nearest 0.1 centimeter using a portable stadiometer (Seca Road Rod #214, Stadiometer.com, Snoqualmie, WA, USA) and weight (measured to the nearest 0.1 kilogram) and percent body fat were obtained using a TBF-300A Body Composition Analyzer/Scale (Tanita

Corporation of America, Arlington Heights, IL, USA). Body mass index was then calculated using weight and height measurements (weight in kilograms divided by height in meters squared).

25(OH)D Assay and Classification of Vitamin D Status

A chemiluminescent immunoassay (CLIA) was used to measure serum 25(OH)D (#310600) on a LIAISON® automated analyzer (DiaSorin, Stillwater, MN). Assays were conducted on samples obtained from participants in the fasted state at a standardized time of day (between 0800h and 1200h). Samples were analyzed in multiple assays in singlet, but controls were measured in duplicate in order to obtain an average percent coefficient of variation (%CV); the average interassay %CV was 4.5. Our laboratory participates in the Vitamin D External Quality Assessment Scheme (DEQAS; Charing Cross Hospital, London, UK), and our results have been in range with other reported Liaison results.

The assay for 25(OH)D is a direct competitive chemiluminescent immunoassay (CLIA) designed to measure total 25(OH)D (D_2 and D_3 isoforms), and has shown minimal (<1%) cross reactivity with vitamin D_2 and D_3, and 3-epi-25(OH)D_3, 40% cross-reactivity with1,25$(OH)_2D_2$ and 17% cross reactivity with 1,25$(OH)_2D_3$.

In terms of vitamin D status, individuals were classified based on 25(OH)D concentrations as severely deficient (25(OH)D ≤ 25 nmol/L), mildly deficient (25.1-50 nmol/L), suboptimal (50.1-75 nmol/L), optimal (75.1-125 nmol/L), supra optimal (> 125 nmol/L) and pharmacologic (> 225 nmol/L).

Dietary Intake

Dietary intake of selected macro- and micronutrients in the target population was assessed using a single 24-hour recall; supplement intakes were also assessed on a separate questionnaire. Twenty-four-hour recalls were administered by trained interviewers which included dietitians, bilingual interviewers and graduate students of the School of Dietetics and Human Nutrition at McGill University. Interviewers received both initial and ongoing training throughout the survey from registered dietitians. Nutrient values were obtained using the CANDAT program (Godin London Inc., London, ON), and the

food database used was derived from the Canadian Nutrient File (Health Canada, Ottawa, ON) and McGill University's institute file, a complementary nutrient file containing data obtained from food labels and field research from its' Centre for Indigenous Peoples Nutrition and Environment. Nutrients intakes were evaluated both as total amounts and per 1000 kilocalories ((total nutrient intake/total kcal intake) x 1000), with statements of nutrient adequacy made with reference to current Dietary Reference Intakes (DRI) (165).

Statistical Analysis

After testing for normality using the Kolmogorov-Smirnov method, comparisons of two means between and within groups were performed using Mann-Whitney U-tests for non-normally distributed variables. Similarly, for more than two means, Kruskal-Wallis nonparametric ANOVA was used. Since most nutrients were non-normally distributed, nonparametric K-sample tests on the equality of medians were used to compare intakes between age groups. The Bonferroni adjustment was used to adjust for multiple comparisons with α=0.05. Pearson's Chi-square tests were used to compare proportions of categorical variables between groups. All statistical analyses were performed using Stata/SE 9.2 (StataCorp LP, College Station, Texas).

13.4 Results

Overall Population Characteristics

Characteristics of the study population overall and by age group are presented in **Table 1**. Women in the oldest age group were significantly shorter than those in the youngest age group (P=0.0015), and women in the two oldest age groups had significantly higher body fat than those in the youngest age group (P=0.0014). Mean BMI for each age group was in the overweight category.

Vitamin D Status

Overall, 184 of the 374 women (49.2%) in whom serum 25(OH)D was measured had severely deficient, mildly deficient or suboptimal (i.e. 25(OH)D ≤ 75 nmol/L) vitamin D status (**Figure 1**). Significant differences in mean 25(OH)D were found between age groups (P<0.001) (**Table 1**). Significant differences were also observed between age groups in the proportion of women in each vitamin D

status category (χ^2=104.60, P<0.001), and above and below 75 nmol/L (χ^2=75.54, P<0.001). Women 40-49 years of age had the highest (72.2%), and women ≥ 60 years of age had the lowest (20.0%) proportion of participants in the less than optimal (≤ 75 nmol/L) categories; women in the 50-59 year age group fell somewhere in between, with 45.9% of participants having 25(OH)D concentrations at or below 75 nmol/L (**Figure 2**).

Nutrient Intakes

Completed 24-hour recalls were obtained and analyzed from 333 women, and intakes are reported by age group in **Table 2**. A significant (P=0.002) difference between age groups was found for kilocalories, with those over 60 years of age consuming fewer kilocalories than those in the two younger age groups. Similar significant differences and trends were found between these groups in overall intake of vitamin K (P=0.004), saturated fatty acids (SFA, P=0.006), monounsaturated fatty acids (MUFA, P=0.246) and polyunsaturated fatty acids (PUFA, P=0.441). These differences no longer existed, however, when nutrient data were expressed per 1000 kilocalories. A significant negative trend between age group and nutrient intake was observed for both overall and kilocalorie-adjusted intake of carbohydrate (P<0.001 overall and per 1000 kcals) and vitamin C (P=0.041 overall, P=0.035 per 1000 kcals). Though there was no trend in overall protein intake, a trend for increasing protein intake with age was seen per 1000 kilocalories and was significant between those in the two oldest versus the youngest age group(s) (P=0.001). A trend towards increasing intake with age was also observed for manganese, with a significant difference between those in the youngest versus the oldest age group for overall intake (P=0.007), and a difference between the two youngest and the oldest age groups for intake per 1000 kilocalories (P<0.001). There was a similar trend for overall phosphorus intake (P=0.540), with that trend becoming significant between all groups when adjusted for kilocalorie intake (P<0.001). Another significant trend was seen for zinc intake per 1000 kilocalories (P=0.023), with a significantly higher intake in the oldest versus the youngest age group. Positive, but non-significant, trends between nutrient intake per 1000 kilocalories and age were also seen for vitamin

D (P=0.076), calcium (P=0.068), and magnesium (P=0.465). No trends or significant differences between age groups were found for vitamin A, whether overall or per 1000 kilocalories.

In all groups, mean intakes were below the estimated average requirement (EAR) for magnesium, and below the AI for vitamin K and calcium. Mean intakes were above the EAR for vitamin A, vitamin C, phosphorus, zinc, and above the AI for manganese. Vitamin D intake was below the AI in 79.6% of women (76.8% in those 40-49y, 88.7% in those 50-59y, and 74.4% in those ≥60y). Proportion of energy from fat and protein was within the acceptable macronutrient distribution range (AMDR) for all age groups, and carbohydrate was within range for the youngest, but below range for the other two groups.

Table 1: Population characteristics overall and by age group[1]

	Overall *(n=419)*[2]	**40-49y** *(n=178)*[3]	**50-59y** *(n=119)*[4]	**≥60y** *(n=122)*[5]	***P***[6]
Age *(years)*	54 ± 11 (40 to 90)	44 ± 3 (40 to 49)[a]	54 ± 3 (50 to 59)[b]	69 ± 6 (60 to 90)[c]	0.0001
Weight *(kg)*	66.3 ± 16.3 (36.4 to 129.6)	65.2 ± 17.1 (36.4 to 129.6)	68.0 ± 15.7 (37 to 104.8)	66.1 ± 15.4 (38.6 to 121.0)	0.1755
Height *(cm)*	152.0 ± 5.5 (126.0 to 168.0)	153.1 ± 5.0 (140.0 to 168.0)[a]	151.8 ± 5.5 (135.8 to 165.2)[ab]	150.6 ± 6.0 (126.0 to 168.0)[b]	0.0015
BMI *(kg/m^2)*	28.7 ± 6.8 (16.2 to 53.9)	27.8 ± 7.2 (16.2 to 53.9)	29.5 ± 6.8 (18.9 to 49.2)	29.0 ± 6.0 (18.3 to 49.1)	0.0329
% Body Fat	35.0 ± 9.6 (7.5 to 54.5)	32.9 ± 9.9 (7.5 to 54.5)[a]	35.8 ± 9.9 (10.4 to 51.4)[b]	37.0 ± 8.1 (15.2 to 53.2)[b]	0.0014
25(OH)D *(nmol/L)*	76.6 ± 34.4 (10.0 to 207.7)	58.6 ± 25.8 (10.0 to 152.5)[a]	78.6 ± 30.3 (20.6 to 178.2)[b]	99.9 ± 34.4 (23.2 to 207.7)[c]	0.0001

BMI – body mass index; 25(OH)D – 25-hydroxyvitamin D

[1]Values are mean ± standard deviation (range)

[2]Overall sample size, with missing values for: weight and BMI – 11.7%, height – 11.5%, %BF – 12.2%, 25(OH)D – 9.5%

[3]Overall sample size for 40-49y women, with missing values for: weight, height and BMI – 12.4%, %BF – 13.5%, 25(OH)D – 11.8%

[4]Overall sample size for 50-59y women, with missing values for: weight, height, BMI and %BF – 7.6%, 25(OH)D – 6.7%

[5]Overall sample size for ≥60y women, with missing values for: weight, BMI and %BF – 14.8%, height – 13.9%, 25(OH)D – 10.7 %

[6]*P* for comparison between age groups by Kruskal-Wallis nonparametric ANOVA; means in a row with superscripts without a common letter differ by Mann-Whitney U-test, Bonferonni-adjusted for multiple comparisons (α=0.05)

Table 2(A): Mean and median macronutrient intakes of Inuit women 40-90 years of age (*n*=333) and comparisons between age groups

	Daily Intakes by Age Group[1]			
Macronutrients	40-49 y *(n=150)*	50-59 y *(n=97)*	≥60 y *(n=86)*	P^2
Energy (Kcal)	1778 (1268-2626)[a] [1998±973]	1784 (1328-2484)[a] [1938±874]	1420 (985-2004)[b] [1509 ±835]	0.002
EER[3]	1787	1719	1589	
Protein (g)	83.0 (49.9-119.2) [97.1±70.7]	94.3 (58.6-134.5) [104.4±62.6]	87.2 (51.5-128.4) [102.2±72.9]	0.299
PER 1000 Kcal	42.1 (32.1-61.9)[a]	51.1 (34.8-73.1)[b]	59.0 (43.1-86.7)[b]	0.001
% Energy *(AMDR – 10-35%)*	19.4	21.5	27.1	
Carbohydrate (g) *(EAR – 100)*	211.4 (140.4-306.3)[a] [230.4±126.4]	175.0 (114.3-277.3)[a] [204.5±125.3]	128.5 (84.1-174.4)[b] [138.2±87.7]	<0.001
PER 1000 Kcal	128.1 (95.9-149.7)[a]	105.1 (75.4-135.4)[b]	94.1 (69.8-122.8)[b]	<0.001
% Energy *(AMDR – 45-65%)*	46.1	42.2	36.6	
Fat (g)	61.4 (35.8-90.9)[a] [74.4±56.6]	61.5 (38.4-91.8)[ab] [72.5±49.6]	46.1 (29.2-76.4)[b] [58.5±45.9]	0.022
PER 1000 Kcal	33.7 (25.0-41.8)	36.3 (25.5-42.5)	34.6 (25.5-44.6)	0.504
%Energy *(AMDR – 20-35%)*	33.5	33.7	34.9	
SFA (g)	19.6 (12.7-29.2)[a]	17.6 (10.4-29.1)[a]	15.1 (7.9-22.2)[b]	0.006
PER 1000 Kcal	10.4 (8.4-13.0)	10.8 (8.2-13.6)	10.1 (7.4-12.9)	0.181
MUFA (g)	23.1 (13.7-37.7)	21.5 (12.4-36.5)	18.0 (10.1-33.0)	0.246
PER 1000 Kcal	12.5 (9.5-17.3)	12.7 (8.7-17.5)	14 (10.1-20.3)	0.288
PUFA (g)	9.2 (4.9-16.4)	7.7 (4.8-13.8)	7.4 (4.3-14.6)	0.441
PER 1000 Kcal	5.1 (3.8-6.7)	5.2 (3.3-6.7)	5.6 (4.5-7.3)	0.271

Kcal – kilocalories; EER – estimated energy requirement; AMDR – acceptable macronutrient distribution range; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; RAE – retinol activity equivalents; EAR – estimated average requirement; AI – adequate intake

[1]Values are median (Q1-Q3) and [mean ± standard deviation]

[2]From nonparametric K-sample tests on the equality of medians; medians in a row with superscripts without a common letter differ by Mann-Whitney U-test, Bonferonni-adjusted for multiple comparisons (α=0.05)

[3]Calculated using DRI EER equations using mean age, weight, and height for each age group and assuming a sedentary activity level

Table 2(B): Mean and median micronutrient intakes of Inuit women 40-90 years of age (*n*=333) and comparisons between age groups

	Daily Intakes by Age Group[1]			
Micronutrients	40-49 y *(n=150)*	50-59 y *(n=97)*	≥60 y *(n=86)*	*P*[2]
Vitamin A (µg RAE)	340 (151-966)	414 (181-808)	349 (71-783)	0.513
(EAR - 500)	[864±1217]	[768±1069]	[849±1447]	
PER 1000 Kcal	215 (82-451)	208 (98-556)	231 (59-530)	0.930
Vitamin C (mg)	65 (16-136)[a]	61 (13-143)[a]	21 (5-92)[b]	0.041
(EAR – 60)	[114±175]	[101±117]	[66±94]	
PER 1000 Kcal	29 (9-92)[ab]	30 (8-78)[a]	14 (5-64)[b]	0.035
Vitamin D (µg)	2 (1-5)	2 (1-5)	2 (0-10)	0.310
(AI - 40-50 y :5; >50 y :10)	[7±22]	[10±38]	[18±37]	
PER 1000 Kcal	1 (0-3)	1 (0-2)	2 (0-6)	0.076
Vitamin K(µg)	14 (6-28)[a]	11 (4-20)[ab]	8 (2-22)[b]	0.004
(AI – 90)	[23±27]	[17±21]	[17±22]	
PER 1000 Kcal	8 (3.3-16)	6 (2-12)	6 (2-19)	0.146
Calcium (mg) *(AI - 40-50 y :1,000; >50y :1,200)*	359 (235-555) [461±385]	366 (228-587) [483±392]	301 (155-518) [417±418]	0.534
PER 1000 Kcal	203 (149-297)	215 (139-341)	237 (144-365)	0.068
Magnesium (mg)	197 (129-267)	204 (138-268)	171 (120-233)	0.194
(EAR – 265)	[217±115]	[222±103]	[222±103]	
PER 1000 Kcal	110 (87-134)	112 (93-139)	124 (90-171)	0.465
Manganese (mg)	2.2 (1.3-3.4)[a]	2.7 (1.9-3.9)[ab]	2.9 (1.9-4.1)[b]	0.007
(AI – 1.8)	[2.7±2.0]	[3.4±2.5]	[4.2±4.8]	
PER 1000 Kcal	1.2 (0.7-2.0)[a]	1.4 (1.0-2.3)[a]	2.2 (1.3-3.6)[b]	<0.001
Phosphorus (mg)	1055 (711-1514)	1092 (777-1576)	1109 (679-1594)	0.540
(EAR – 580)	[1214±793]	[1278±767]	[1273±909]	
PER 1000 Kcal	571 (453-723)[a]	682 (489-803)[b]	810 (651-1066)[c]	<0.001
Zinc (mg)	11.4 (5.7-18.3)	12.7 (8.1-16.2)	11.0 (5.4-16.9)	0.552
(EAR – 6.8)	[15.4±15.5]	[15.4±12.4]	[15.1±13.3]	
PER 1000 Kcal	6.1 (3.6-10.1)[a]	6.7 (4.5-9.9)[ab]	7.6 (5.2-13.0)[b]	0.023

Kcal – kilocalories; RAE – retinol activity equivalents; EAR – estimated average requirement; AI – adequate intake

[1]Values are median (Q1-Q3) and [mean ± standard deviation]

[2]From nonparametric K-sample tests on the equality of medians; medians in a row with superscripts without a common letter differ by Mann-Whitney U-test, Bonferonni-adjusted for multiple comparisons (α=0.05)

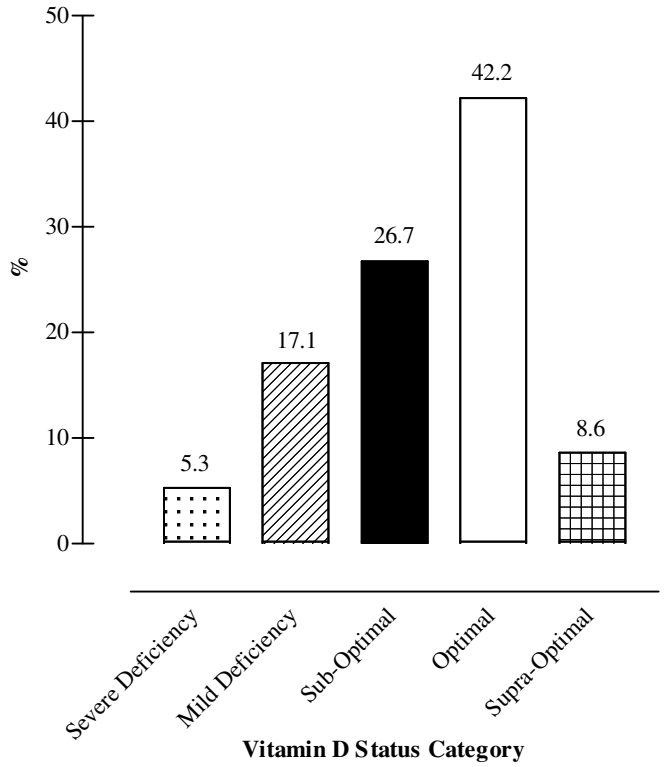

Figure 1. Proportion of Inuit women 40-90 years of age in each vitamin D status category (n=374). Bars reflect proportion of women categorized as having severely deficient (⬚; <25 nmol/L), mildly deficient (▨; 25.1-50 nmol/L), suboptimal (■; 50.1-75 nmol/L), optimal (□; 75.1-125 nmol/L), and supra-optimal (▦; >125 nmol/L) concentrations of serum 25(OH)D.

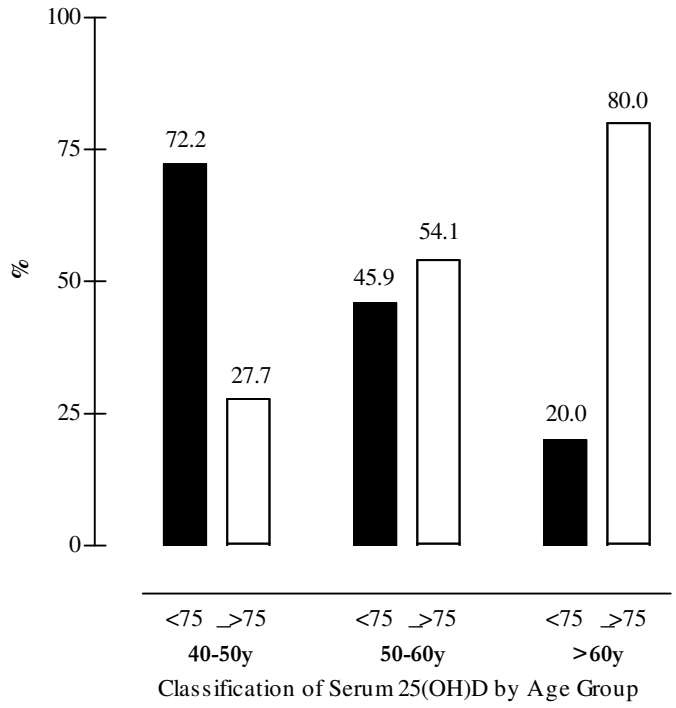

Figure 2. Proportion of Inuit women with 25(OH)D above and at or below 75 nmol/L in each age group (*n*=374). Women with 25(OH)D concentrations above (clear bars) and at or below (solid bars) 75 nmol/L reflect the proportion of women with optimal or above optimal and less than optimal vitamin D status, respectively.

13.5 Discussion

Valuable information regarding the extent of nutrition transition amongst women in this population segment can be gleaned from this study. At the point in the year when 25(OH)D should be at its highest, a large proportion of Inuit women had below optimal concentrations, with this proportion being highest in the youngest and lowest in the oldest age group. The dietary nutrient density of most nutrients, including vitamin D, tended to increase with age.

A study from Nuuk, Greenland revealed that 23% of Inuit consuming traditional compared to 74% consuming westernized fare had 25(OH)D concentrations below 40 nmol/L during the period from May to September (129). Overall, 22.5% of our target population had 25(OH)D concentrations that were at or below 50 nmol/L, comparable to those Greenland Inuit consuming traditional fare, with a clear improvement in vitamin D status with age as 38.7% of those 40-49 years, 15.6% of those 50-59 years, and only 6.4% of those 60 years of age or older were classified in the mild or severely deficient (i.e. 25(OH)D ≤ 50 nmol/L) status categories. In a sample of healthy adults in Calgary (51°N), a city that receives more hours of sunshine per year than any other Canadian city, the proportion of those with 25(OH)D concentrations less than 50 and 80 nmol/L was highest in the fall (November-December, 43% and 94%, respectively) and lowest in the summer (August-September, 14% and 68%) (85). During a similar summer time period, the proportion of women in our youngest age group with serum 25(OH)D at or below 50 and 75 nmol/L (38.7% and 72.2%, respectively) was comparable to that seen at the worst time for those in Calgary, while vitamin D status in the 50-59 y (15.6% and 45.9%) and at or over 60 y (6.4% and 22%) age groups appears to be comparable to or better than Canadian counterparts at the best time.

Trends towards higher intakes of carbohydrate and vitamin C in the younger women provide evidence for higher market food consumption in this age group. Intakes of these nutrients are higher on days when traditional food is not consumed (5), and are likely derived from popular market foods such as sugar, white bread, biscuits, crystal powdered drinks, and soft drinks (78, 166). Trends

towards higher intakes of protein, PUFA, vitamin D, calcium, magnesium, manganese, phosphorus and zinc with increasing age group suggest a higher consumption of traditional food in older women. With the exception of PUFA and calcium, all of these nutrients are typically higher on days when traditional food is consumed (5). Traditional food supplies 40% of the vitamin D, phosphorus and zinc intakes of Nunavik women despite market food accounting for the majority of energy intake (10). Overall, in agreement with previous studies, these trends seem to point to a more nutrient-dense diet in older Inuit women, likely attributable to a higher consumption of country foods.

The role of vitamin D and calcium in bone health has been firmly established, however, the benefits of increasing intakes of other bone-related nutrients is less well accepted despite their well-known biological functions in such processes as collagen formation, bone mineralization, and as enzyme cofactors (24, 90, 92, 121). Intakes of vitamin C, phosphorus and zinc are likely adequate, as mean intakes were above the EAR for these nutrients. Mean intakes of magnesium were below the EAR, suggesting that a multiple day assessment should be undertaken to more accurately assess the extent of inadequacy. For nutrients with an AI, when mean intakes are at or above the AI, as is the case for manganese, it can be generally assumed that there is a low prevalence of inadequacy in the population. Though the trend towards increasing calcium per 1000 kilocalories with age approached significance, mean overall intakes in all three age groups were far below the AI. Calcium likely remains a nutrient of concern in this population, possibly due a low intake of dairy products (10, 166). As for vitamin D, serum 25(OH)D indicated a higher prevalence of adequacy with increasing age, reflected in mean overall intakes but not in the proportion below the AI. Mean intakes of vitamin K were below the AI in all groups, suggesting that intakes of this nutrient may also need improvement. It seems safe to say that macronutrient intake was adequate in all age groups, as even though the proportion of energy from carbohydrate was below the AMDR in the two older age groups, mean intakes were still above the EAR.

Energy intake was lowest in the oldest age group, however, no differences in weight or BMI existed between the three groups, and the fact that body fat was significantly higher in the two older age groups may simply be a consequence of normal metabolic changes and/or decreased levels of physical activity that tend to accompany aging. Mean energy intakes were above the average estimated energy requirement for a sedentary activity level in all age groups, and particularly the two younger age groups. This suggests an imbalance between energy intake and expenditure and is a likely explanation of why the average BMI in each age group was in the overweight category.

A major limitation of this study is that the proportion of the population with "at risk" intakes of most of the nutrients examined cannot be firmly established with only a single 24-hour recall; adjustment for within-person variability would be required to make more definitive statements about adequacy (167). Despite this, previous studies support the observed concern for the adequacy of calcium and magnesium in Inuit populations (4, 10). Another issue is that the nutrient database from which average intakes were calculated is incomplete with respect to vitamin D values, and particularly its content in the variously consumed forms of country foods (e.g. dried, aged, raw). Furthermore, while cholecalciferol is the only form of vitamin D currently reported in nutrient analyses, $25(OH)D_3$ is present in many animal foods and is suspected to have 5 times the biological activity of the former, traditionally measured form (80). This may explain why, despite having a proportion below the AI for vitamin D that was similar to those 40-49 years of age, women 60 years of age and older had significantly higher mean 25(OH)D concentrations than their younger counterparts. Thankfully, access to serum concentrations of 25(OH)D allowed for the accurate evaluation of vitamin D status.

Vitamin D status is in agreement with trends in intake of the nutrient amongst these three age groups. Improved vitamin D status along with trends in the intake of other nutrients suggests a more nutrient-dense diet in older women, likely due to a higher intake of country foods. Intakes of vitamin A, vitamin C, phosphorus, zinc and manganese are likely adequate. The adequacy of intakes of

vitamin K, calcium and magnesium warrants further investigation. That the 25(OH)D concentrations of older Inuit women living at extreme Northern latitudes far exceeds those of adults living in the sunniest city in Canada provides strong evidence that Inuit nutrition has the potential to sustain good health. An increased consumption of country foods and nutrient-dense market foods should be promoted, particularly amongst younger age groups. Individual intervention must be accompanied by efforts to minimize the environmental and social factors negatively impacting on the supply of Inuit country food, and maximize the availability and affordability of high-quality market food amongst Northern communities.

14. STRENGTHS, LIMITATIONS AND CONCLUSIONS

Research amongst Indigenous groups can only be successful when it is based on a participatory research process (168). The generous assistance with the planning and execution of the survey supplied by the Nunavut IHS Steering Committee and community research assistants certainly qualifies as a strength in that respect, and without it, the Inuit Health Survey would have been largely, if not completely, unsuccessful. With this in mind, researchers had to be flexible, as research and community needs did not always overlap to the desired extent. In addition, because of the logistic complexity of such a large-scale survey, the needs of each individual researcher could not always be catered to, even despite best efforts to do so.

In bone regression models, the inclusion of additional predictors such as alcohol consumption, exercise habits, personal history of fracture as an adult, and history of fragility fracture in a first degree relative, would have allowed for a more complete picture of variability in forearm bone density (121, 161, 163). Alcohol is known to decrease OC concentrations, and acute consumption leads to a transient decrease followed by an increase in PTH concentrations, explaining the higher average PTH concentrations present in chronic alcohol consumers. Moderate alcohol consumption, however, has been shown to have a beneficial effect on bone in postmenopausal women, likely due to its estrogenic effects (122). Therefore, in addition to explaining additional variability in forearm BMD, the inclusion of alcohol consumption in regression models may have helped to explain outliers, allowing for a more complete and inclusive model. The examination of medical records for history of personal fracture and family history of fracture was not possible during the survey, though participants receiving bone densitometry were questioned as to their previous history of fracture, with such self-reported data known to be generally reliable (169). The data that was collected pertaining to alcohol consumption had limited applicability to our analyses. Exercise habits are also suspected to have been negatively affected by nutrition transition (164), and though self-reported data on exercise habits was collected during the survey, it was not available for use at the time of our

analyses. Even the effects of covariates for which data was collected were not able to be detected due to their low frequency of appearance in the population. Increasing the sample size by extending future analyses to all three regions may help to resolve this issue.

The current "gold standard" for the diagnosis of osteoporosis is DXA, specifically central measurements at the spine and hip, and/or peripheral measurements at the 33% radius if one or both central measures cannot be obtained (148). We were not able to perform cDXA measurements on participants during the survey due to limited space on board the research facility, the CCGS Amundsen. The speed and portability of pDXA made for a high quality alternative, and its appeal was further enhanced by its previous validation for use in Arctic field studies (160). Though the pDXA device chosen was not able to measure the 33% radius, pDXA measurements at various sites, including the distal radius, have been shown to accurately predict future fracture (158). Additionally, certain inaccuracies inherent in DXA measurements are minimized at the forearm due to minimal surrounding tissues (112), though there is a higher proportion of yellow marrow at this site, particularly in older individuals, which is thought to cause an underestimation of true BMD (170). In terms of safety, pDXA measurements reduce the radiation dose to the gonads that is present with cDXA (112).

Vitamin D status as assessed by serum 25(OH)D was below optimal (≤ 75 nmol/L) in a significant proportion of Inuit women in the younger age groups. In contrast, women in the oldest age group had high mean concentrations of 25(OH)D, with 80% classified as having optimal or above optimal vitamin D status (25(OH)D > 75 nmol/L). This provides evidence that despite older age, darker skin pigmentation, and habitation at extreme latitudes, the achievement of high 25(OH)D concentrations is possible in this population. Regression analysis of the predictors of serum 25(OH)D including latitude and/or UVB exposure data, frequency of consumption of vitamin D-rich traditional foods (e.g. arctic char, beluga oil, seal liver) and %BF, would likely have allowed us to pinpoint the causes of vitamin D deficiency, providing potential targets for future interventions

to improve vitamin D status in this population. Also, in order to provide a more complete evaluation of vitamin D status in this population, seasonal (and especially winter) assessments of serum 25(OH)D would be required. Finally, further analyses should be conducted on the vitamin D content of country foods in each of the various forms consumed (e.g. dried, aged, raw), and should include the measurement of both cholecalciferol and $25(OH)D_3$, the latter of which is suspected to have 5 times the biological activity of the former, traditionally measured form (80).

Apart from vitamin D, for which biochemical data was available, the adequacy of other bone-related nutrients could have been more accurately assessed had replicate 24-hour recalls been available to adjust for intra-individual variability in intakes. For logistic reasons, however, only single 24-hour recalls were obtained in the adult survey. Coefficients of intra-individual variability from previous studies in Inuit populations may become available for future analyses, and would provide a means of more accurately estimating the prevalence of nutrient inadequacies amongst this population.

Finally, the generalizability of these results to other age and gender groups is limited. Women approaching or experiencing the menopausal transition were targeted for bone health evaluation because of their increased risk for osteoporosis, with a higher risk of hip, wrist and spine fracture being reported in First Nations women and older people compared to white counterparts in Manitoba (139). Identification of the predictors of bone density in younger women is also important, however, as age was shown to have a greater impact on forearm bone density than all other factors examined for the postmenopausal women in our study, highlighting the need for the maximization of peak bone mass before the menopausal transition. Further research is also warranted in men, as despite the fact that they begin to experience accelerated bone loss at a later age than women, 20% of men over 50 years of age will experience a fragility fracture in their remaining lifetime (98). Vitamin D status should also be evaluated in other population groups since it may vary considerably by community and age group depending on the extent of nutrition transition and the

availability of vitamin-D rich traditional fish and sea mammals. There is also a need to evaluate vitamin D status amongst other Aboriginal populations, who may be at higher risk for vitamin D deficiency than Inuit and in whom research is lacking despite a larger representation in the population (3). In a study of mothers in a Manitoba Cree community, 76% were found to have 25(OH)D concentrations below 25 nmol/L (8), and in a report by Kuhnlein et al, a much higher proportion of Dene/Métis and Yukon First Nations compared to Inuit men and women had vitamin D intakes below the AI (79).

Final conclusions from this study are that vitamin D status in postmenopausal (approximately 50 years of age and older) Inuit women in the Baffin and Kivalliq regions was better, while that of premenopausal women was worse, than that of Canadians living at more Southern latitudes. Forearm bone health in all women seemed standard for the age groups examined, with 33% of the postmenopausal women and virtually none (2%) of the premenopausal women being classified as having low forearm BMD. In the premenopausal group, a higher body weight, BMI or, to a lesser extent, percent body fat, were predictive of higher, while higher OC and PTH were predictive of lower forearm bone mineral density. In the postmenopausal group, a higher body weight, BMI or percent body fat were equally predictive of higher, while increasing age and OC were predictive of lower forearm BMD. Though 25(OH)D was not a predictor in any of the models examined, the appearance of PTH and OC as predictors provided indirect evidence of its role in bone health. Dietary nutrient density seemed to increase with increasing age, and this, combined with a high prevalence of below optimal vitamin D concentrations in premenopausal women, suggest that bone health may be a concern in future generations of postmenopausal Inuit women. Mean intakes of vitamin K, magnesium and calcium were below the AI or EAR, and warrant further investigation with multiple 24-hour recalls. With respect to bone health, the availability of the proper screening tools and treatment for low bone mass should be ensured for postmenopausal women in this population, and education on the maximization of peak bone mass provided to younger age groups. In terms of improved nutrient intake and vitamin D status,

which will also have an impact on bone health, interventions should emphasize a safe amount of sun exposure and promote an increased intake of country foods and high quality market foods. The long-term success of these interventions will largely depend on the resolution of the social, economic and environmental factors that are currently fueling the progression of nutrition transition in the Canadian arctic.

15. LITERATURE CITED

1. MacMillan HL, MacMillan AB, Offord DR, Dingle JL. Aboriginal health. CMAJ 1996;155:1569-78.
2. Bjerregaard P, Young TK, Dewailly E, Ebbesson SO. Indigenous health in the Arctic: an overview of the circumpolar Inuit population. Scand J Public Health 2004;32:390-5.
3. Young TK. Review of research on aboriginal populations in Canada: relevance to their health needs. BMJ 2003;327:419-22.
4. Kuhnlein HV, Receveur O, Soueida R, Berti PR. Unique patterns of dietary adequacy in three cultures of Canadian Arctic indigeous peoples. Public Health Nutr 2007;11:349-60.
5. Kuhnlein HV, Receveur O, Soueida R, Egeland GM. Arctic Indigenous Peoples Experience the Nutrition Transition with Changing Dietary Patterns and Obesity. J. Nutr. 2004;124:1447-1453.
6. Health Canada. First Nations and Inuit Health. 2007.
7. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008;87(Suppl):1080S-6S.
8. Lebrun JB, Moffatt MEK, Mundy RJT, et al. Vitamin D Deficiency in a Manitoba Community. Canadian Journal of Public Health 1993;84:394-396.
9. Waiters B, Godel JC, Basu TK. Perinatal Vitamin D and Calcium Status of Northern Canadian Mothers and Their Newborn Infants. Journal of the American College of Nutrition 1998;18:122-126.
10. Blanchet C, Dewailly E, Ayotte P, Bruneau S, Receveur O, Holub BJ. Contribution of Selected Traditional and Market Foods to the Diet of Nunavik Inuit Women. Journal of Dietetic Practice and Research 2000;61:50-59.
11. Weiler HA, Leslie WD, Krahn J, Steiman PW, Metge CJ. Canadian Aboriginal Women Have a Higher Prevalence of Vitamin D Deficiency than Non-Aboriginal Women Despite Similar Dietary Vitamin D Intakes. J. Nutr. 2007;137:461-465.
12. Holick MF. Vitamin D. In: Shils ME, ed. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams & Wilkins, 2006:376-395.
13. Wardlaw GM, Hampl JS. Vitamin D. In: Meyers LM, ed. Perspectives in Nutrition. New York: McGraw-Hill, 2007:309-316.
14. Chapuy M-C, Preziosi P, Maamer M, et al. Prevalence of Vitamin D Insufficiency in an Adult Normal Population. Osteoporosis Int 1997;7:439-443.
15. Weaver CM, Fleet JC. Vitamin D requirements: current and future. Am J Clin Nutr 2004;80(suppl):1735S-9S.
16. Wortsman J, Matsuoka L, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690-3.
17. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80(suppl):1689S-96S.

18. Sherwood L. Endocrine Control of Calcium Metabolism. In: Julet M, ed. Human Physiology: From Cells to Systems. 5th ed. Belmont: Brooks/Cole - Thomson Learning, 2004:733-742.
19. Blum M, Dolnikowski G, Seyoum E, et al. Vitamin D_3 in fat tissue. Endocr 2008;33:90-4.
20. Omdahl JL, Morris HA, May BK. Hydroxylase Enzymes of the Vitamin D Pathway: Expression, Function, and Regulation. Annu. Rev. Nutr. 2002;22:139-66.
21. Holick MF. Vitamin D. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:403-456.
22. Holick MF. Sunlight, UV-Radiation, Vitamin D and Skin Cancer: How Much Sunlight Do We Need? Adv Exp Med Biol 2008;624:1-15.
23. Holick MF. Vitamin D Deficiency. N Engl J Med 2007;357:266-81.
24. Heaney RP. Bone Biology in Health and Disease. In: Shils ME, ed. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams & Wilkins, 2007:1314-1325.
25. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? British Journal of Nutrition 2003;89:552-72.
26. Pfeifer M, Begerow B, Minne HW. Vitamin D and Muscle Function. Osteoporosis Int 2002;13:187-194.
27. Farach-Carson MC. Bioactive analogs that stimulate subsets of biological activities of $1\alpha,25(OH)_2D_3$ in osteoblasts. Steroids 2001;66:357-361.
28. Brook CGD, Marshall NJ. Calcium Regulation. Essentials of Endocrinology. 4th ed: Blackwell Science, 2001:119-136.
29. Lee RD, Nieman DC. Nutritional Assessment in Disease Prevention. In: Meyers LM, ed. Nutritional Assessment. 4th ed. New York: McGraw-Hill, 2007:297-303.
30. Zernicke R, MacKay C, Lorincz C. Mechanisms of bone remodeling during weight-bearing exercise. Appl Physiol Nutr Metab 2006;31:655-660.
31. Jee WSS. The skeletal tissues. In: Weiss L, ed. Histology: Cell and tissue biology. 5th ed, 1983:206-254.
32. Whitney EN, Rolfes SR. Osteoporosis and Calcium. Understanding Nutrition. 9th ed. Belmont: Wadsworth/Thompson Learning, 2002:420-427.
33. Spelsberg TC, Subramaniam M, Riggs BL, Khosla S. The Actions and Interactions of Sex Steroids and Growth Factors/Cytokines on the Skeleton. Molecular Endocrinology 1999;13:819-828.
34. Raisz LG. Bone Physiology: Bone Cells, Modeling, and Remodeling. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:43-62.
35. Rosen CJ. Nutrition and Bone Health in the Elderly. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:211-226.
36. Need AG. Bone resorption markers in vitamin D insufficiency. Clinica Chimica Acta 2006;In Press.

37. Lemaire V, Tobin FL, Greller LD, Cho CR, Suva LJ. Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. Journal of Theoretical Biology 2004;229:293-309.
38. Rosen CJ, Tenenhouse A. Biochemical markers of bone turnover. Postgraduate Medecine 1998;104:1-11.
39. Seibel MJ. Biochemical Markers of Bone Turnover Part I: Biochemistry and Variability. Clin Biochem Rev 2005;26:97-122.
40. Aubin JE, Bonnelye E. Osteoprotegerin and its Ligand: A New Paradigm for Regulation of Osteoclastogenesis and Bone Resorption. Osteoporosis Int 2000;11:905-913.
41. Seibel MJ. Biochemical Markers of Bone Turnover Part II: Clinical Applications in the Management of Osteoporosis. Clin Biochem Rev 2006;27:123-138.
42. Kostenuik PJ, Capparelli C, Morony S, et al. OPG and PTH-(1-34) Have Additive Effects on Bone Density and Mechanical Strength in Osteopenic Ovariectomized Rats. Endocrinology 2001;142:4295-4304.
43. Sahota O, Mundey MK, San P, Godber IM, Lawson N, Hosking DJ. The relationship between vitamin D and parathyroid hormone: calcium homeostasis, bone turnover, and bone mineral density in postmenopausal women with established osteoporosis. Bone 2004;35:312-319.
44. Pasco JA, Henry MJ, Kotowicz MA, et al. Seasonal Periodicity of Serum Vitamin D and Parathyroid Hormone, Bone Resorption, and Fractures: The Geelong Osteoporosis Study. J Bone Miner Res 2004;19:752-758.
45. Brown JP, Albert C, Nassar BA, et al. Bone turnover markers in the management of postmenopausal osteoporosis. Clinical Biochemistry 2009;42:929-42.
46. Calvo MS, Eyre DR, Gundberg CM. Molecular Basis and Clinical Application of Biological Markers of Bone Turnover. Endocrine Review 1996;17:333-358.
47. Need AG, Nordin BEC. Misconceptions - Vitamin D insufficiency causes malabsorption of calcium. Bone 2008;42:1021-4.
48. Reichel H, Koeffler H, Norman A. The role of vitamin D endocrine system in health and disease. N Engl J Med 1989;320:980-91.
49. Erben RG, Scutt AM, Miao D, Kollenkirchen U, Haberey M. Short-Term Treatment of Rats with High Dose 1,25-Dihydroxyviatmin D_3 Stimulates Bone Formation and Increases the Number of Osteoblast Precursor Cells in Bone Marrow. Endocrinology 1997;138:4629-4635.
50. Wang DS, Miura M, Demura H, Sato K. Anabolic Effects of 1,25-Dihydroxyvitamin D_3 on Osteoblasts Are Enhanced by Vascular Endothelial Growth Factor Produced by Osteoblasts and by Growth Factors Produced by Endothelial Cells. Endocrinology 1997;138:2953-2962.
51. Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. Curr Opin Endocrinol Diabetes 2002;9:87-98.
52. Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B. Effect of Vitamin D Intake on Seasonal Variations in Parathyroid

Hormone Secretion in Postmenopausal Women. N Engl J Med 1989;321:1777-83.
53. Jesudason D, Need AG, Horowitz M, O'Loughlin PD, Morris HA, Nordin BEC. Relationship between Serum 25-Hydroxyvitamin D and Bone Resorption Markers in Vitamin D Insufficiency. Bone 2002;31:626-630.
54. Need A, Horowitz M, O'Loughlin P, Coates P, Nordin B. What is the cause of the hyperparathyroidism in vitamin D deficiency? J Bone Miner Res 2005;20(suppl. 1):S390.
55. Need AG, Horowitz M, Morris HA, Nordin BEC. Vitamin D status: effects on parathyroid hormone and 1,25-dihydroxyvitamin D in postmenopausal women. Am J Clin Nutr 2000;71:1577-81.
56. vonMülen DG, Greendale GA, Garland CF, Wan L, Barrett-Connor E. Vitamin D, parathyroid hormone levels and bone mineral density in community-dwelling older women: The Rancho Bernardo Study. Osteoporosis Int 2005;16:1721-26.
57. Need AG, O'Loughlin PD, Morris HA, Horowitz M, Nordin BEC. The Effects of Age and Other Variables on Serum Parathyroid Hormone in Postmenopausal Women Attending an Osteoporosis Center. The Journal of Clinical Endocrinology & Metabolism 2004;89:1646-1649.
58. Lips P, Duong T, Oleksik A, et al. A Global Study of Vitamin D Status and Parathyroid Function in Postmenopausal Women with Osteoporosis: Baseline Data from the Multiple Outcomes of Raloxifene Evaluation Clinical Trial. The Journal of Clinical Endocrinology & Metabolism 2001;86:1212-1221.
59. Kinyamu HK, Gallagher JC, Rafferty KA, Balhorn KE. Dietary calcium and vitamin D intake in elderly women: effect on serum parathyroid hormone and vitamin D metabolites. Am J Clin Nutr 1998;67:342-8.
60. Gallagher JC, Kinyamu HK, Fowler SE, Dawson-Hughes B, Dalsky GP, Sherman SS. Calciotropic Hormones and Bone Markers in the Elderly. Journal of Bone and Mineral Research 1998;13:475-482.
61. Sahota O, Masud T, San P, Hosking DJ. Vitamin D insufficiency increases bone turnover markers and enhances bone loss at the hip in patients with established vertebral osteoporosis. Clinical Endocrinology 1999;51:217-221.
62. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in Medical Inpatients. N Engl J Med 1998;338:777-83.
63. Stilgren LS, Hegedüs LM, Beck-Nielsen H, Abrahamsen B. Osteoprotegerin Levels in Primary Hyperparathyroidism: Effect of Parathyroidectomy and Association with Bone Metabolism. Calcif Tissue Int 2003;73:210-216.
64. Harwood RH, Sahota O, Gaynor K, Masud T, Hosking DJ. A randomised, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: The Nottingham Neck of Femur (NoNOF) Study. Age and Ageing 2004;33:45-51.
65. Bhattoa HP, Bettembuk P, Ganacharya S, Balogh A. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone

metabolism in community dwelling postmenopausal Hungarian women. Osteoporosis Int 2004;15:447-51.
66. Looker AC, Mussolino ME. Serum 25-hydroxyvitamin D and Hip Fracture Risk in Older U.S. White Adults. Journal of Bone and Mineral Research 2008;23:143-150.
67. Macdonald HM, Mavroeidi A, Barr RJ, Black AJ, Fraser WD, Reid DM. Vitamin D status in postmenopausal women living at higher latitudes in the UK in relation to bone health, overweight, sunlight exposure and dietary vitamin D. Bone 2008;42:966-1003.
68. Ueno Y, Shinki T, Nagai Y, Murayama H, Fujii K, Suda T. In vivo administration of 1,25-dihydroxyvitamin D3 suppresses the expression of RANKL mRNA in bone of thyroparathyroidectomized rats constantly infused with PTH [Abstract]. J Cell Biochem 2003;90:267-77.
69. Vieth R. Why the optimal requirement for vitamin D_3 is probably much higher than what is officially recommended for adults. Journal of Steroid Biochemistry & Molecular Biology 2004;89-90:575-579.
70. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes FaNB, Institute of Medecine,. Vitamin D. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington: National Academy Press, 1997:250-287.
71. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporosis Int 2005;16:713-6.
72. Vieth R, Bischoff-Ferrari HA, Boucher BJ, et al. The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr 2007;85:649-50.
73. Adami S, Viapiana O, Gatti D, Idolazzi L, Rossini M. Relationship between serum parathyroid hormone, vitamin D sufficiency, age, and calcium intake. Bone 2008;42:267-270.
74. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. Am J Clin Nutr 2004;80(suppl):1706S-9S.
75. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimates of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.
76. Kennedy C, Bajdik CD, Willemze R, de Gruijl FR, Bouwes Bavnick JN. The influence of painful sunburns and lifetime of sun exposure on the risk of actinic keratoses, seborrheic warts, melanocytic nevi, atypical nevi and skin cancer. J Invest Dermatol 2003;120:1087-93.
77. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr 2004;80(suppl):1710S-6S.
78. Kuhnlein HV, Receveur O. Local Cultural Animal Food Contributes High Levels of Nutrients for Arctic Canadian Indigenous Adults and Children. The Journal of Nutrition 2007;137:1110-4.
79. Kuhnlein HV, Barthet V, Farren A, et al. Vitamins A, D, and E in Canadian Arctic traditional food and adult diets. Journal of Food Composition and Analysis 2006;19:495-506.

80. Gibson SA, Ashwell M. New vitamin D values for meat and their implication for vitamin D intake in adults. Proc Nutr Soc 1997;56:116A.
81. Whiting SJ, Green TJ, Calvo MS. Vitamin D intakes in North America and Asia-Pacific countries are not sufficient to prevent vitamin D insufficiency. Journal of Steroid Biochemistry & Molecular Biology 2007;103:626-630.
82. Ward LM. Vitamin D deficiency in the 21st century: a persistent problem among Canadian infants and mothers. CMAJ 2005;172:769-770.
83. Roth DE, Martz P, Yeo R, Prosser C, Bell M, Jones AB. Are National Vitamin D Guidelines Sufficient to Maintain Adequate Blood Levels in Children? Canadian Journal of Public Health 2005;96:443-449.
84. Vieth R, Cole D, Hawker G, Trang H, Rubin L. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. European Journal of Clinical Nutrition 2001;55:1091-1097.
85. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. CMAJ 2002;166:1517-24.
86. Vecino-Vecino C, Gratton M, Kremer R, Rodriguez-Mañas L, Duque G. Seasonal Variance in Serum Levels of Vitamin D Determines a Compensatory Response by Parathyroid Hormone: Study in an Ambulatory Elderly Population in Quebec. Gerontology 2006;52:33-39.
87. Liu BA, Gordon M, Labranche JM, Murray TM, Vieth R, Shear NH. Seasonal Prevalence of Vitamin D Deficiency in Institutionalized Older Adults. Journal of the American Geriatrics Society 1997;45:598-603.
88. Calvo MS. Prevalence of Vitamin D Insufficiency in Canada and the United States: Importance to Health Status and Efficacy of Current Food Fortification and Dietary Supplement Use. Nutrition Reviews 2003;61:107-113.
89. Papaioannou A, Giangregorio L, Kvern B, Boulos P, Ioannidis G, Adachi J. The osteoporosis care gap in Canada. BMC Musculoskeletal Disorders 2004;5:11-16.
90. Cashman KD. Diet, Nutrition, and Bone Health. The Journal of Nutrition 2007;137:2507S-12S.
91. Tenenhouse A, Joseph L, Kreiger N, et al. Estimation of the Prevalence of Low Bone Density in Canadian Women and Men Using a Population-Specific DXA Reference Standard: The Canadian Multicentre Osteoporosis Study (CaMos). Osteoporosis Int 2000;11:897-904.
92. Dawson-Hughes B. Osteoporosis. In: Shils ME, ed. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams & Wilkins, 2007:1339-1352.
93. Burns DK, Kumar V. The Musculoskeletal System. In: Kumar V, Cotran RS, Robbins SL, eds. Basic Pathology. 6th ed. Philadelphia: W.B. Saunders Company, 1997:669-671.
94. Ruffing J, Cosman F, Zion M, et al. Determinants of bone mass and bone size in a large cohort of physically active young adult men. Nutrition & Metabolism 2006;13.

95. Fiatarone Singh MA. Exercise and Bone Health. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:515-548.
96. Gascon-Barré M. Antiepileptic Drugs and Bone Health. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:647-665.
97. Booth SL, Charette AM. Vitamin K, Oral Anticoagulants, and Bone Health. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:457-478.
98. Sambrook P, Cooper C. Ostoporosis. Lancet 2006;367:2010-8.
99. Kanis JA, Johnell O, Laet CD, et al. A meta-analysis of previous fracture and subsequent fracture risk. Bone 2004;35:375-82.
100. Kumar V, Cotran RS, Robbins SL. Environmental Diseases. In: Kumar V, Cotran RS, Robbins SL, eds. Basic Pathology. 6th ed. Philadelphia: W.B. Saunders, 1997:248-252.
101. Kakar S, Einhorn TA. Nutritional Assessment of Nutrients for Bone Health. In: Dawson-Hughes B, Holick MF, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:105-112.
102. Willett W. 24-Hour Dietary Recall and Food Record Methods. Nutritional Epidemiology. 2nd ed. New York: Oxford University Press, 1998:50-73.
103. Anderson JJB, Switzer BR, Stewart P, Symons M. Nutritional Assessment: Analysis of Relations Between Nutrient Factors and Bone Health. In: Dawson-Hughes B, Holick MF, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:113-128.
104. Heaney RP. Vitamin D: How Much Do We Need, and How Much is Too Much? Osteoporosis Int 2000;11:553-555.
105. Wootton AM. Improving the Measurement of 25-hydroxyvitamin D. Clin Biochem Rev 2005;26:33-36.
106. Lensmeyer GL, Wiebe DA, Binkley N, Drezner MK. HPLC Method for 25-Hydroxyvitamin D Measurement: Comparison with Contemporary Assays. Clinical Chemistry 2006;52:1120-1126.
107. Ersfeld DL, Rao DS, Body J-J, et al. Analytical and clinical validation of th 25 OH vitamin D assay for the LIAISON® automated analyzer. Clinical Biochemistry 2004;37:867-874.
108. Lenchik L, Vatti S, Register TC. Interpretation of Bone Mineral Density As It Relates to Bone Health and Fracture Risk. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:63-84.
109. International Society for Clinical Densitometry. Official Positions of the International Society for Clinical Densitometry. West Hartford, 2007:1-14.
110. Faulkner KG, Roberts LA, McClung MR. Discrepancies in Normative Data between Lunar and Hologic DXA Systems. Osteoporosis Int 1996;6:432-436.
111. Patel R, Blake GM, Fogelman I. Peripheral and Central Measurements of Bone Mineral Density Are Equally Strongly Associated with Clinical Risk Factors for Osteoporosis. Calcif Tissue Int 2007;80:89-96.

112. Augat P, Fuerst T, Genant HK. Quantitative Bone Mineral Assessment at the Forearm: A Review. Osteoporosis Int 1998;8:299-310.
113. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical Markers of Bone Turnover, Endogenous Hormones and the Risk of Fractures in Postmenopausal Women: The OFELY Study. Journal of Bone and Mineral Research 2000;15:1526-36.
114. Sherman SS, Tobin JD, Hollis BW, Gundberg CM, Roy TA, Plato CC. Biochemical Parameters Associated with Low Bone Density in Healthy Men and Women. Journal of Bone and Mineral Research 1992;7:1123-30.
115. Hannon R, Eastell R. Preanalytical Variability of Biochemical Markers of Bone Turnover. Osteoporosis Int 2000;6 (Suppl):S30-44.
116. Schmitt CP, Homme M, Schaefer F. Structural organization and biological relevance of oscillatory parathyroid hormone secretion. Pediatric Nephrology 2005;20:346-351.
117. Schlemmer A, Hassager C. Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption. European Journal Of Endocrinology 1999;140:332-337.
118. Fuleihan GE-H, Klerman EB, Brown EN, Choe Y, Brown EM, Czeisler CA. The parathyroid hormone circadian rhythm is truly endogenous - a general clinical research center study. Journal of Clinical Endocrinology & Metabolism 1997;82:281-6.
119. Joseph F, Chan BY, Durham BH, et al. The Circadian Rhythm of Osteoprotegerin and Its Association with Parathyroid Hormone Secretion. The Journal of Clinical Endocrinology & Metabolism 2007;92:3230-3238.
120. ALPCO Diagnostics. Intact PTH (1-84) EIA. Salem: ALPCO Diagnostics, 2007.
121. Brown JP, Josse RG. 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada. CMAJ 2002;167:S1-34.
122. Laitinen K, Välimäki M. Alcohol and Bone. Calcif Tissue Int 1991;49 (Suppl):S70-3.
123. Finkelstein JS, Lee M-LT, Sowers M, et al. Ethnic Variation in Bone Density in Premenopausal and Early Perimenopausal Women: Effects of Anthropometric and Lifestyle Factors. Journal of Clinical Endocrinology & Metabolism 2002;87:3057-3067.
124. Barrett-Connor E, Siris ES, Wehren LE, et al. Osteoporosis and Fracture Risk in Women of Different Ethnic Groups. Journal of Bone and Mineral Research 2005;20:185-194.
125. Harris SS, Soteriades E, Coolidge JAS, Mudgal S, Dawson-Hughes B. Vitamin D Insufficiency and Hyperparathyroidism in a Low Income, Multiracial, Elderly Population. Journal of Clinical Endocrinology & Metabolism 2000;85:4125-4130.
126. Fuleihan GE-H, Gundberg CM, Gleason R, et al. Racial Differences in Parathyroid Hormone Dynamics. Journal of Clinical Endocrinology & Metabolism 1994;79:1642-1647.
127. Dawson-Hughes B. Racial/ethnic considerations in making recommendations for vitamin D for adult and elderly men and women. American Journal of Clinical Nutrition 2004;80(suppl):1763S-6S.

128. Harris SS. Nutrition and Skeletal Health in Blacks. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press, 2004:227-232.
129. Renjmark L, Jorgensen ME, Pedersen MB, et al. Vitamin D Insufficiency in Greenlanders on a Westernized Fare: Ethnic Differences in Calciotropic Hormones Between Greenlanders and Danes. Calcif Tissue Int 2004;74:255-263.
130. Leslie WD, Metge CJ, Weiler HA, Doupe M, Wood Steiman P, O'Neil JD. Bone density and bone area in Canadian Aboriginal women: the First Nations Bone Health Study. Osteoporosis Int 2006;17:1755-1762.
131. Andersen S, Boeskov E, Laurberg P. Ethnic Differences in Bone Mineral Density Between Inuits and Caucasians in North Greenland Are Caused by Differences in Body Size. Journal of Clinical Densitometry 2005;8:409-14.
132. Evers SE, Orchard JW, Haddad RG. Bone Density in Postmenopausal North American Indian and Caucasian Females. Human Biology 1985;57:719-726.
133. Leslie WD, Metge CJ, Weiler HA, et al. Site-Specific Reductions in Bone Density in Canadian Aboriginal Women: The First Nations Bone Health Study. J Bone Miner Res 2004:S288.
134. Leslie WD, Weiler HA, Lix LM, Nyomba BLG. Body composition and bone density in Canadian White and Aboriginal women: The First Nations Bone Health Study. Bone 2008;42:990-5.
135. Weiler HA, Leslie WD, Bernstein CN. Parathyroid hormone is predictive of low bone mass in Canadian Aboriginal and White women. Bone 2008;42:498-504.
136. Leslie WD, Krahn J, Weiler HA, et al. Vitamin D Insufficiency in Canadian Aboriginal Women: The First Nations Bone Health Study. J Bone Miner Res 2004:S423.
137. Leslie WD, Krahn J, Bernstein CN, et al. Ethnic Differences in Novel Markers of Bone Metabolism and Fracture Risk: The First Nations Bone Health Study. J Bone Miner Res 2004;20(Suppl 1):S275.
138. Pratt WB, Holloway JM. Incidence of hip fracture in Alaska Inuit people: 1979-89 & 1996-99. Alaska Med 2001;43:2-5.
139. Leslie WD, Derksen S, Metge C, et al. Fracture risk among First Nations people: a retrospective matched cohort study. CMAJ 2004;171:869-73.
140. Leslie WD, Derksen S, Metge CJ, et al. Demographic Risk Factors for Fracture in First Nations People. Canadian Journal of Public Health 2005;96:S45-S50.
141. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive Association between 25-Hydroxy Vitamin D Levels and Bone Mineral Density: A Population-Based Study of Younger and Older Adults. The American Journal of Medecine 2004;116:634-639.
142. Hampson G, Martin FC, Moffat K, et al. Effects of dietary improvement on bone metabolism in elderly underweight women with osteoporosis: a randomised controlled trial. Osteoporosis Int 2003;14:750-756.

143. Shapses SA, Cifuentes M. Body Weight/Composition and Weight Change. In: Holick MF, Dawson-Hughes B, eds. Nutrition and Bone Health. Totowa: Humana Press Inc., 2004:549-573.
144. Wardlaw GM. Putting body weight and osteoporosis into perspective. American Journal of Clinical Nutrition 1996;63(suppl):433S-436S.
145. Zhao L-J, Liu Y-J, Liu P-Y, Hamilton J, Recker RR, Deng H-W. Relationship of Obesity with Osteoporosis. The Journal of Clinical Endocrinology & Metabolism 2007;92:1640-6.
146. Castro JP, Joseph LA, Shin JJ, et al. Differential effect of obesity on bone mineral density in White, Hispanic and African American women: a cross sectional study. Nutrition & Metabolism 2005;2:9-15.
147. Ribot C, Tremollieres F, Pouilles J-M, Bonneau M, Germain F, Louvet J-P. Obesity and Postmenopausal Bone Loss: The Influence of Obesity on Vertebral Density and Bone Turnover in Postmenopausal Women. Bone 1988;8:327-331.
148. Lewiecki EM, Watts NB, McClung MR, et al. Official Position of the International Society for Clinical Densitometry. The Journal of Clinical Endocrinology & Metabolism 2004;89:3651-3655.
149. Pattanaungkul S, Riggs BL, Yergey AL, Vieira NE, O'Fallon WM, Khosla S. Relationship of intestinal calcium absorption to 1, 25-dihydroxyvitamin D [1, 25$(OH)_2$D] levels in young versus elderly women: evidence for age-related intestinal resistance to 1,25$(OH)_2$D action. J Clin Endocrinol Metab 2000;85:4023-7.
150. Blake GM, Chinn DJ, Steel SA, et al. A list of device-specific thresholds for the clinical interpretation of peripheral x-ray absorptiometry examinations. Osteoporosis Int 2005;16:2149-56.
151. Forsmo S, Langhammer A, Forsen L, Schei B. Forearm bone mineral density in an unselected population of 2,779 men and women - The HUNT Study, Norway. Osteoporosis Int 2005;16:562-7.
152. Zhao L-J, Jiang H, Papasian CJ, et al. Correlation of Obesity and Osteoporosis: Effect of Fat Mass on the Determination of Osteoporosis. Journal of Bone and Mineral Research 2008;23:17-24.
153. Waugh EJ, Lam M-A, Hawker GA, et al. Risk factors for low bone mass in healthy 40-60 year old women: A systematic review of the literature. Osteoporosis Int 2009;20:1-21.
154. Morin S, Tsang JF. Weight and body mass index predict bone mineral density and fractures in women aged 40-59 years. Osteoporosis Int 2009;20:363-70.
155. Rapuri PB, Kinyamu HK, Gallagher JC, Haynatzka V. Seasonal Changes in Calciotropic Hormones, Bone Markers, and Bone Mineral Density in Elderly Women. J Clin Endocrinol Metab 2002;87:2024-32.
156. Mezquita-Raya P, Munoz-Torres M, Luna JDD, et al. Relation Between Vitamin D Insufficiency, Bone Density, and Bone Metabolism in Healthy Postmenopausal Women. Journal of Bone and Mineral Research 2001;16:1408-15.

157. Gabaroi DC, Peris P, Monegal A, et al. Search for hidden secondary causes in postmenopausal women with osteoporosis. Menopause 2009;17:1-5.
158. Siris ES, Miller PD, Barrett-Connor E, et al. Identification and Fracture Outcomes of Undiagnosed Low Bone Mineral Density in Postmenopausal Women: Results from the National Osteoporosis Risk Assessment. JAMA 2001;286:2815-22.
159. Picard D, Brown JP, Rosenthall L, et al. Ability of Peripheral DXA Measurements to Diagnose Osteoporosis As Assessed By Central DXA Measurement. Journal of Clinical Densitometry 2004;7:111-8.
160. Andersen S, Boeskov E, Holm J, Laurberg P. Feasibility of Dual-Energy X-ray Absorptiometry in Arctic field studies. Circumpolar Health 2003:280-283.
161. Seeman E. Pathogenesis of bone fragility in women and men. Lancet 2002;359:1841-50.
162. Gerdhem P, Obrant KJ. Effects of Cigarette Smoking on Bone Mass as Assessed by Dual-Energy X-ray Absorptiometry and Ultrasound. Osteoporosis Int 2002;13:932-6.
163. Beitz R, Dören M. Physical activity and postmenopausal health. J Br Menopause Soc 2004;10:70-4.
164. Popkin BM. An overview on the nutrition transition and its health implications: the Bellagio meeting. Public Health Nutr 2002;5:93-103.
165. Otten JJ, Hellwig JP, Meyers LD. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Washington: National Academies Press, 2006.
166. Moffat MEK, O'Neil J, Young TK. Nutritional Patterns of Inuit in the Keewatin Region of Canada. Arctic Medical Research 1994;53 (Suppl. 2):298-300.
167. Gibson RS. Measuring food consumption of individuals. Principles of Nutritional Assessment. New York: Oxford, 2005:55-59.
168. Sims J, Kuhnlein HV. Indigenous Peoples Participatory Health Research. Planning and Management. Preparing Research Agreements. Geneva: WHO, 2003.
169. Honkanen K, Honkanen R, Heikkinen L, Kroger H, Saarikoski S. Validity of self-reports of fractures in perimenopausal women. Am J Epidemiol 1999;150:511-6.
170. Bolotin HH, Sievänen H. Inaccuracies Inherent in Dual-Energy X-Ray Absorptiometry In Vivo Bone Mineral Density Can Seriously Mislead Diagnostic/Prognostic Interpretations of Patient-Specific Bone Fragility. Journal of Bone and Mineral Research 2001;16:799-804.

16. APPENDIX - *Regression Models With and Without Outliers: Percent Change in Regression Coefficients and Selection of "Best" Model*

1) POSTMENOPAUSAL WOMEN: Model A (Dependent variable: **T-Score**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)							Final Regression Coefficient for "Best" Model (VII; n=166)
		345 (I)[1]	813 (II)	1271 (III)	345 & 813 (IV)	345 & 1271 (V)	813 & 1271 (VI)	345, 813 & 1271 (VII)	
Age	-0.1101143*	1.32*	-1.00*	-1.73*	0.33*	-0.98*	-2.70*	-1.93*	-0.1079937*
Weight	0.0216562*	18.28*	3.90*	-10.89*	22.04*	4.44*	-6.87*	8.30*	0.0234526*
Height	0.0056738	-137.29	-61.54	28.07	-197.16	-107.30	-33.32	-166.69	-0.0037839
Non-Dominant Arm Used for Measurement?	0.4334786	-16.04	-8.59	-4.18	-24.41	-22.81	-12.64	-31.00	0.2991164
Diabetes Status	-0.072928	1.33	-193.07	-60.58	-188.12	-82.00	-251.18	-268.05	0.1225578
Taking Thyroid Hormone?	-0.2234264	-47.58	288.14	-24.84	235.33	-84.78	261.16	194.78	-0.6586088
Current Smoker	-0.5951707	-1.57	0.18	2.86	-1.39	2.28	3.00	2.41**	-0.6095217**
Past Smoker	-0.2796232	53.13	-19.73	-3.95	33.57	51.04	-23.46	31.76	-0.3684258
25(OH)D	0.0018001	-46.81	-48.27	-59.20	-93.99	-131.27	-106.26	-176.93	-0.0013848
PTH	-0.0365162	28.83	-48.20	-27.53	-18.56	-7.25	-74.94	-53.64	-0.0169307
OC	-0.0743242	65.56**	-12.49	191.77**	53.06**	333.72*	176.80**	318.53*	-0.3110688*
Constant	-0.248996	-33.11	-1.29	-15.06	-34.26	-55.93	-16.14	-56.82	-0.107521
R^2_{adj}	0.4691	0.5737	0.4852	0.4865	0.5929	0.6106	0.5024	0.6300	0.6300
Root MSE	1.1392	0.97664	1.1113	1.1227	0.944	0.93523	1.0947	0.90159	0.90159

25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; non-dominant arm used for measurement? – 0=no, 1=yes; taking thyroid hormone? – 0=no, 1=yes; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

2) POSTMENOPAUSAL WOMEN: Model B (Dependent variable: **T-Score**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)							Final Regression Coefficient for "Best" Model (V; n=167)
		345 (I)[1]	813 (II)	1271 (III)	345 & 813 (IV)	345 & 1271 (V)	813 & 1271 (VI)	345, 813 & 1271 (VII)	
Age	-0.1098341*	1.35*	-1.00*	-1.69*	0.36*	-0.90*	-2.66*	-1.85*	-0.1088426*
BMI	0.0511747*	16.83*	3.66*	-10.72*	20.35*	3.07*	-6.95*	6.70*	0.0527482*
Height	0.0238016	-18.78	-11.69	-1.59	-30.17	-22.15	-13.16	-33.35	0.0185297
Non-Dominant Arm Used for Measurement?	0.4394139	-15.53	-8.41	-4.24	-23.72	-22.36	-12.52	-30.37	0.3411536
Diabetes Status	-0.0782971	-2.61	-180.33	-56.42	-179.42	-80.69	-234.47	-254.35	-0.0151167
Taking Thyroid Hormone?	-0.2218924	-47.57	290.02	-24.73	236.99	-84.72	263.17	196.50	-0.033901
Current Smoker	-0.5806357	-1.46	0.18	3.15	-1.27	2.80	3.29	2.94	-0.596884
Past Smoker	-0.2738741	53.79	-20.21	-3.78	33.76	51.96	-23.77	32.22	-0.4161786
25(OH)D	0.0017598	-47.65	-49.35	-59.80	-95.85	-133.24	-107.93	-179.89	-0.0005849
PTH	-0.0364018	28.17	-48.44	-27.30	-19.44	-7.77	-74.97	-54.37	-0.0335737
OC	-0.0722954	68.09**	-12.72	195.64**	55.36**	342.57**	180.39**	327.07**	-0.3199542**
Constant	-0.2659746	-30.75	-1.13	-14.61	-31.73	-52.86	-15.53	-53.60	-0.1253734
R^2_{adj}	0.4710	0.5753	0.4871	0.4880	0.5945	0.6117	0.5040	0.6311	0.6117
Root MSE	1.1371	0.97481	1.1092	1.121	0.94218	0.93388	1.093	0.90026	0.93388

25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; non-dominant arm used for measurement? – 0=no, 1=yes; taking thyroid hormone? – 0=no, 1=yes; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

3) POSTMENOPAUSAL WOMEN: Model C (Dependent variable: **T-Score**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)							Final Regression Coefficient for "Best" Model (V; n=167)
		345 (I)[1]	813 (II)	1271 (III)	345 & 813 (IV)	345 & 1271 (V)	813 & 1271 (VI)	345, 813 & 1271 (VII)	
Age	-0.1170472*	2.46*	-0.70*	-2.41*	1.77*	-0.65*	-3.08*	-1.31*	-0.116292*
% Body Fat	0.0319093*	28.76*	5.12*	-8.08**	33.73*	19.75*	-2.91*	24.73*	0.0382114*
Height	0.012868	-59.70	-26.00	3.68	-85.15	-58.76	-22.16	-83.92	0.0053073
Non-Dominant Arm Used for Measurement?	0.4321866	-16.66	-8.66	-4.60	-25.16	-24.03	-13.15	-32.37	0.3283167
Diabetes Status	0.0200514	-12.37	709.64	149.70	687.09	190.03	852.42	879.80	0.0581555
Taking Thyroid Hormone?	-0.2847033	-33.72	227.13	-23.51	190.24	-67.93	202.13	153.73	-0.091311
Current Smoker	-0.6677009	-1.84**	0.29	0.60	-1.55**	-1.15**	0.88	-0.87**	-0.6600259**
Past Smoker	-0.2914519	52.89	-18.74	-4.42	34.27	50.46	-22.97	32.08	-0.4385319
25(OH)D	0.0019273	-45.90	-45.19	-61.46	-90.31	-132.46	-105.68	-175.65	-0.0006256
PTH	-0.0384927	33.67	-44.96	-27.01	-10.74	-0.71	-71.35	-44.33	-0.0382194
OC	-0.0620063	63.11	-17.30	245.81**	45.88	401.12*	226.16**	381.34*	-0.3107231*
Constant	-0.1919244	-43.32	-2.15	-15.51	-45.32	-67.30	-17.49	-69.09	-0.0627513
R^2_{adj}	0.4612	0.5686	0.4770	0.4818	0.5879	0.6111	0.4978	0.6309	0.6111
Root MSE	1.1477	0.98254	1.12	1.1278	0.94979	0.93469	1.0998	0.90053	0.93469

25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; non-dominant arm used for measurement? – 0=no, 1=yes; taking thyroid hormone? – 0=no, 1=yes; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

4) POSTMENOPAUSAL WOMEN: Model A (Dependent variable: **fBMD**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)							Final Regression Coefficient for "Best" Model (VII, n=166)
		345 (I)[1]	813 (II)	1271 (III)	345 & 813 (IV)	345 & 1271 (V)	813 & 1271 (VI)	345, 813 & 1271 (VII)	
Age	-0.0066389*	1.30*	-1.00*	-1.71*	0.31*	-0.97*	-2.68*	-1.92*	-0.0065115*
Weight	0.0012939*	18.21*	3.95*	-10.86*	22.01*	4.41*	-6.80*	8.31*	0.0014014*
Height	0.0003567	-130.00	-59.13	26.60	-187.52	-101.56	-32.38	-158.65	-0.0002092
Non-Dominant Arm Used for Measurement?	0.0264073	-15.67	-8.52	-4.09	-23.97	-22.30	-12.48	-30.42	0.0183755
Diabetes Status	-0.0050868	1.13	-167.20	-51.78	-162.98	-70.06	-216.86	-231.26	0.0066768
Taking Thyroid Hormone?	-0.0136211	-46.45	285.50	-24.29	233.94	-82.81	259.13	194.31	-0.0400884
Current Smoker	-0.0365699	-1.52	0.17	2.78	-1.34	2.21	2.91	2.34**	-0.0374264**
Past Smoker	-0.0171686	51.51	-19.41	-3.84	32.26	49.47	-23.04	30.50	-0.0224045
25(OH)D	0.0001046	-47.90	-50.19	-60.71	-97.01	-134.51	-109.66	-182.03	-0.0000858
PTH	-0.0021172	29.60	-50.21	-28.30	-19.80	-7.49	-77.71	-55.83	-0.0009351
OC	-0.0044065	65.82**	-12.72	192.82**	53.08**	335.35*	177.57**	319.86*	-0.018501*
Constant	0.4747218*	1.03*	0.04*	0.47*	1.07*	1.75*	0.51*	1.78*	0.4831483*
R^2_{adj}	0.4746	0.5782	0.4911	0.4917	0.5978	0.6146	0.5082	0.6345	0.6345
Root MSE	0.06793	0.05828	0.06622	0.06695	0.05627	0.05581	0.06523	0.05375	0.05375

25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; non-dominant arm used for measurement? – 0=no, 1=yes; taking thyroid hormone? – 0=no, 1=yes; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*P<0.01

**P<0.05

5) POSTMENOPAUSAL WOMEN: Model B (Dependent variable: **fBMD**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)							Final Regression Coefficient for "Best" Model (VII, n=166)
		345 (I)[1]	813 (II)	1271 (III)	345 & 813 (IV)	345 & 1271 (V)	813 & 1271 (VI)	345, 813 & 1271 (VII)	
Age	-0.006622*	1.33*	-1.00*	-1.67*	0.35*	-0.89*	-2.64*	-1.84*	-0.0064999*
BMI	0.0030585*	16.76*	3.70*	-10.69*	20.32*	3.05*	-6.88*	6.72*	0.0032639*
Height	0.0014398	-18.47	-11.68	-1.56	-29.85	-21.80	-13.12	-32.99	0.0009648
Non-Dominant Arm Used for Measurement?	0.0267618	-15.18	-8.34	-4.15	-23.30	-21.86	-12.37	-29.81	0.0187846
Diabetes Status	-0.0054122	-2.25	-157.59	-48.65	-156.80	-69.56	-204.27	-221.38	0.0065695
Taking Thyroid Hormone?	-0.0135285	-46.44	287.34	-24.18	235.58	-82.75	261.11	196.01	-0.0400459
Current Smoker	-0.0356974	-1.41	0.18	3.05	-1.23	2.71	3.19	2.85**	-0.0367145**
Past Smoker	-0.016825	52.12	-19.87	-3.66	32.42	50.34	-23.32	30.92	-0.0220267
25(OH)D	0.0001022	-48.83	-51.37	-61.35	-99.00	-136.69	-111.45	-185.23	-0.0000871
PTH	-0.0021106	28.92	-50.47	-28.07	-20.70	-8.02	-77.74	-56.59	-0.0009162
OC	-0.0042842	68.40**	-12.96	196.79**	55.41**	344.37*	181.25**	328.57*	-0.0183607*
Constant	0.4737043*	1.03*	0.04*	0.49*	1.06*	1.77*	0.52*	1.79*	0.4821951*
R^2_{adj}	0.4765	0.5798	0.4930	0.4933	0.5994	0.6157	0.5097	0.6356	0.6356
Root MSE	0.06781	0.05816	0.06609	0.06685	0.05616	0.05573	0.06512	0.05367	0.05367

BMI – body mass index; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; non-dominant arm used for measurement? – 0=no, 1=yes; taking thyroid hormone? – 0=no, 1=yes; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

6) POSTMENOPAUSAL WOMEN: Model C (Dependent variable: **fBMD**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)							Final Regression Coefficient for "Best" Model (V, n=167)
		345 (I)[1]	813 (II)	1271 (III)	345 & 813 (IV)	345 & 1271 (V)	813 & 1271 (VI)	345, 813 & 1271 (VII)	
Age	-0.0070531*	2.43*	-0.70*	-2.39*	1.74*	-0.64*	-3.06*	-1.30*	-0.0070078*
% Body Fat	0.0019057*	28.67*	5.18*	-8.06**	33.69*	19.67*	-2.84*	24.72*	0.0022806*
Height	0.0007868	-58.11	-25.69	3.58	-83.26	-57.19	-21.94	-82.07	0.0003368
Non-Dominant Arm Used for Measurement?	0.0263304	-16.28	-8.59	-4.50	-24.70	-23.49	-12.98	-31.76	0.0201454
Diabetes Status	0.0004736	-31.17	1814.86	377.93	1758.04	479.56	2175.25	2244.26	0.0027448
Taking Thyroid Hormone?	-0.0172823	-33.06	226.02	-23.10	189.84	-66.65	201.46	154.00	-0.0057636
Current Smoker	-0.0409078	-1.79**	0.29	0.59	-1.50**	-1.12**	0.86	-0.84**	-0.0404513**
Past Smoker	-0.017875	51.33	-18.45	-4.30	32.99	48.97	-22.57	30.86	-0.0266288
25(OH)D	0.0001122	-46.88	-46.88	-62.92	-93.00	-135.56	-108.82	-180.39	-0.0000399
PTH	-0.0022348	34.52	-46.78	-27.74	-11.69	-0.78	-73.88	-46.17	-0.0022174
OC	-0.0036726	63.42	-17.64	247.47**	45.85	403.55*	227.42**	383.37*	-0.0184933*
Constant	0.4781347*	1.03*	0.05*	0.37*	1.08*	1.61*	0.42*	1.65*	0.4858269*
R^2_{adj}	0.4666	0.5730	0.4830	0.4871	0.5928	0.6149	0.5035	0.6353	0.6149
Root MSE	0.06844	0.05863	0.06674	0.06725	0.05662	0.05578	0.06554	0.05369	0.05578

25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; non-dominant arm used for measurement? – 0=no, 1=yes; taking thyroid hormone? – 0=no, 1=yes; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

7) PREMENOPAUSAL WOMEN: Model A (Dependent variable: **Z-Score**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)			Final Regression Coefficients for "Best" Model (III, n=101)
		174 (I)[1]	450 (II)	174 & 450 (III)	
Age	0.0082676	22.63	42.02	66.38	0.0137559
Weight	0.0227763*	2.15*	21.82*	24.60*	0.0283802*
Height	-0.0140235	6.98	30.79	38.77	-0.019461
Current Smoker	-0.6206172	-2.06	-19.31	-21.93	-0.484501
Past Smoker	-0.5427713	0.74	-5.56	-4.95	-0.5159289
25(OH)D	-0.0008006	-255.68	104.76	-155.21	0.000442
PTH	-0.0935499	-15.59	17.36**	1.79	-0.0952256
OC	-0.2053574	89.51**	14.59	106.95*	-0.4249961*
Constant	0.7016074	-9.02	-8.59	-18.09	0.5746674
R^2_{adj}	0.2022	0.2446	0.2550	0.3015	0.3015
Root MSE	0.82071	0.79705	0.79571	0.76908	0.76908

Current & past smoker – 0=no, 1=yes; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

8) PREMENOPAUSAL WOMEN: Model B (Dependent variable: **Z-Score**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)			Final Regression Coefficients for "Best" Model (III, n=101)
		174 (I)[1]	450 (II)	174 & 450 (III)	
Age	0.0091101	20.71	40.75	63.10	0.0148582
BMI	0.0542248*	2.09*	22.01*	24.73*	0.0676336*
Height	0.0045382	-12.84	-6.19	-19.55	0.0036512
Current Smoker	-0.5879392	-2.25	-21.85	-24.73	-0.4425392
Past Smoker	-0.5212743	0.69	-6.78	-6.24	-0.4887506
25(OH)D	-0.0007337	-279.11	114.95	-168.97	0.000506
PTH	-0.0939659	-15.55	17.56**	2.02	-0.0958664
OC	-0.2089711	88.03**	14.99	105.87*	-0.4302098*
Constant	0.6817458	-9.34	-9.60	-19.44	0.5491881
R^2_{adj}	0.2078	0.2504	0.2626	0.3093	0.3093
Root MSE	0.81779	0.79399	0.79164	0.76478	0.76478

BMI – body mass index; current & past smoker – 0=no, 1=yes; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC - osteocalcin; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

9) PREMENOPAUSAL WOMEN: Model C (Dependent variable: **Z-Score**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)			Final Regression Coefficients for "Best" Model (I, n=102)
		174 (I)[1]	1169 (I)	174 & 1169 (III)	
Age	-0.0055281	-29.09	-17.56	-43.62	-0.0031166
% Body Fat	0.0390271*	1.09*	-6.57*	-4.91*	0.037112*
Height	-0.0108715	7.06	-10.92	-3.14	-0.0105299
Current Smoker	-0.6097538	-1.47	6.27	4.27	-0.6357993
Past Smoker	-0.5938832	0.85	-5.91	-4.54	-0.5669441
25(OH)D	-0.0018307	-105.88	35.58	-68.77	-0.0005718
PTH	-0.0764982	-19.36	66.15**	41.40	-0.1081681
OC	-0.1986612	89.15**	-10.94	75.11**	-0.3478729**
Constant	0.5973511	-10.14	1.38	-8.43	0.5470191
R^2_{adj}	0.1893	0.2277	0.1962	0.2305	0.2305
Root MSE	0.82728	0.8059	0.81854	0.79915	0.79915

Current & past smoker – 0=no, 1=yes; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

10) PREMENOPAUSAL WOMEN: Model A (Dependent variable: **fBMD**)

Independent Variables	**Regression Coefficients with Outliers Included**	**% Change in Regression Coefficient with Removal of Outlier(s)**			**Final Regression Coefficients for "Best" Model (III, n=101)**
		174 (I)[1]	**450 (II)**	**174 & 450 (III)**	
Age	0.0001517	87.61	134.81	228.74	0.0004987
Weight	0.0013874*	2.51*	21.09*	24.33*	0.0017249*
Height	-0.0010758	6.46	23.63	30.99	-0.0014092
Current Smoker	-0.0350512	-2.59	-20.13	-23.42	-0.0268437
Past Smoker	-0.0324539	0.88	-5.48	-4.75	-0.0309127
25(OH)D	-0.0000805	-180.62	61.37	-121.49	0.0000173
PTH	-0.0061572	-16.83	15.53**	-1.20**	-0.0060836**
OC	-0.0096798	134.88**	18.23	156.78*	-0.0248562*
Constant	0.5265254*	-0.85*	-0.67*	-1.57*	0.5182624*
R^2_{adj}	0.1884	0.2400	0.2382	0.2950	0.2950
Root MSE	0.05035	0.0483	0.04896	0.0467	0.0467

Current & past smoker – 0=no, 1=yes; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

11) PREMENOPAUSAL WOMEN: Model B (Dependent variable: **fBMD**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)			Final Regression Coefficients for "Best" Model (III, n=101)
		174 (I)[1]	450 (II)	174 & 450 (III)	
Age	0.0002036	65.82	107.51	178.24	0.0005665
BMI	0.003309*	2.44*	21.27*	24.43*	0.0041173*
Height	0.0000553	-74.68	-29.84	-107.38	-4.08E-06
Current Smoker	-0.0330183	-2.85	-22.95	-26.58	-0.0242424
Past Smoker	-0.031135	0.82	-6.69	-6.06	-0.0292492
25(OH)D	-0.0000762	-190.94	65.22	-128.08	0.0000214
PTH	-0.006189	-16.77	15.72**	-0.95**	-0.00613**
OC	-0.0098987	132.01**	18.67	154.34*	-0.0251760*
Constant	0.5252963*	-0.86*	-0.73*	-1.64*	0.5166896*
R^2_{adj}	0.1947	0.2466	0.2465	0.3037	0.3037
Root MSE	0.05016	0.04809	0.0487	0.04641	0.04641

Current & past smoker – 0=no, 1=yes; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$

12) PREMENOPAUSAL WOMEN: Model C (Dependent variable: **fBMD**)

Independent Variables	Regression Coefficients with Outliers Included	% Change in Regression Coefficient with Removal of Outlier(s)			Final Regression Coefficients for "Best" Model (III, n=101)
		174 (I)[1]	450 (II)	174 & 450 (III)	
Age	-0.0006895	-16.65	-8.93	-23.94	-0.0005244
% Body Fat	0.0023806*	1.28*	-6.84*	-4.88*	0.0022645*
Height	-0.0008847	6.21	-8.51	-1.65	-0.0008701
Current Smoker	-0.0343584	-1.86	7.06	4.52	-0.0359102
Past Smoker	-0.0355662	1.02	-6.26	-4.62	-0.0339242
25(OH)D	-0.0001431	-96.69	28.93	-66.95	-0.0000473
PTH	-0.005122	-20.65	62.72**	36.16	-0.0069743
OC	-0.0092701	136.42**	-14.88	117.59**	-0.0201704**
Constant	0.5201525*	-0.83*	0.10*	-0.71*	0.5164682*
R^2_{adj}	0.1759	0.2226	0.1847	0.2263	0.2263
Root MSE	0.05074	0.04885	0.05014	0.0484	0.0484

Current & past smoker – 0=no, 1=yes; 25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; OC – osteocalcin; MSE – mean square error

[1]Values in this row are outlier ID number(s) (MODEL NUMBER)

*$P<0.01$

**$P<0.05$