Serum 25-Hydroxyvitamin D Response to Daily Oral Supplementation with 800 IU Cholecalciferol in Premenopausal Women Living in Maine

Monica Nelson

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SERUM 25-HYDROXYVITAMIN D RESPONSE TO DAILY ORAL SUPPLEMENTATION WITH 800 IU CHOLECALCIFEROL IN PREMENOPAUSAL WOMEN LIVING IN MAINE

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A THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (in Food and Nutrition Sciences)

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The purpose of this research was to measure the serum 25-hydroxyvitamin D \([25(\text{OH})\text{D}]\) response to daily supplementation with 800 IU vitamin D\(_3\) during winter in premenopausal women living in Maine, and to examine the effects of body composition and hormonal contraceptive use on baseline serum 25(OH)D levels and the response to supplementation.

One hundred twelve women (22.2±3.7 years old) received placebo from March 2005 until September 2005 when they were randomized to receive either placebo or 800 IU vitamin D\(_3\) through February 2006. Eighty-six women completed the study. Body composition was measured by dual-energy x-ray absorptiometry. Actual vitamin D\(_3\) content of the supplements averaged 885 IU per capsule.

In February 2005 the mean±SD serum 25(OH)D was 62.0±23.4 nmol/L in all subjects. Twenty-nine percent of subjects had optimal serum 25(OH)D levels (≥75 nmol/L). Serum 25(OH)D levels were significantly higher \((p < 0.0005)\) in the 58
hormonal contraceptive users (68.6±24.0 nmol/L) than in the 28 non-users (48.3±14.8 nmol/L). The 25(OH)D concentration increased with estrogen dose. Subjects in the highest tertile body fat (>33%) had significantly lower serum 25(OH)D levels (47.8±17.3 nmol/L) than subjects in the middle and lowest tertiles (69.4±23.8 and 69.0±22.2 nmol/L). Estrogen dose, percent body fat, and alcohol consumption were significant predictors of February 2005 serum 25(OH)D levels.

Serum 25(OH)D levels increased by 35.3±23.2 nmol/L from February 2005 to February 2006 in the treatment group, compared to 10.9±16.9 nmol/L in the placebo group. Treatment group, magnitude of summer increase in 25(OH)D, estrogen dose, and baseline serum 25(OH)D levels, but not body fat, were significant predictors of the one-year change in 25(OH)D levels.

Daily supplementation with 800 IU vitamin D3 during winter achieved optimal 25(OH)D levels (≥75 nmol/L) in 80% of subjects, indicating that this dose is too low to optimize vitamin D status in the population as a whole. Body fat does not appear to influence the serum 25(OH)D response to supplementation with vitamin D3, except through its influence on the baseline serum 25(OH)D level. Further research is needed to determine whether there is a health benefit to the higher serum 25(OH)D levels in oral contraceptive users.
DEDICATION

I dedicate this dissertation to my wonderfully supportive husband, Wassim Mazraany, and our two darling daughters, Ameera and Aayah, whose births marked the beginning and end of this endeavor and forced me to learn to balance school and family. Wassim’s support and encouragement gave me the strength and motivation to persevere on this rollercoaster ride. Thank you for believing in me.
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I am very grateful for my advisor, Dr. Susan Sullivan, for giving me the opportunity to conduct this research. Thank you for all of the time you invested in me – I have learned so much from you. Despite the hurdles we encountered over the past five years, overall, this has been a tremendous experience. I have truly enjoyed working with you. I would also like to thank my committee members, Dr. Adrienne White, Dr. Richard Cook, Dr. Dorothy Klimis-Zacas, and Dr. Jim Blum for their input, advice, and thoughtful review of this dissertation.

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I would like to thank Jamie Thompson, Misty Cram, and Jessica Charette who assisted with entering data in the computer – it wasn’t a fun task, but it was so important, and they did a great job. I am also extremely grateful for Jennifer, Joanna, Kristi, Cindy, and the other University of Maine friends who have helped me along the way.

Last, but not least, I would like to thank all of the women who participated in this study – it could not have happened without them.
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Chapter 1
INTRODUCTION

Serum 25-hydroxyvitamin D [25(OH)D] levels are used to measure the adequacy of vitamin D status. The consensus among researchers is that 25(OH)D concentrations of at least 75 nmol/L are optimal.\(^1,2\) Serum 25(OH)D levels reflect both dietary intake of vitamin D and cutaneous synthesis of vitamin D after exposure to solar ultraviolet-B radiation.\(^3\) An individual’s serum 25(OH)D concentration is usually less than optimal after winter in Maine (44°53’N)\(^4\) due to insufficient sunlight from November through February.\(^5\) Vitamin D insufficiency is widespread at northern latitudes such as Maine, even in populations with dietary vitamin D intake equal to, or greater than, the current Dietary Reference Intake of 200 IU daily for 19 to 50 year olds.\(^4,6-8\)

Insufficient vitamin D levels are of concern because vitamin D insufficiency is associated with suboptimal calcium absorption\(^9,10\) and increased parathyroid hormone levels,\(^11-15\) which lead to increased bone resorption, accelerated cortical bone loss, and increased risk of fracture.\(^1\) Currently, an estimated 10 million Americans have osteoporosis, 8 million of whom are women.\(^16\) In addition to the effect on bone, inadequate vitamin D may play a role in the development of type 1 diabetes,\(^17\) multiple sclerosis,\(^18\) hypertension,\(^19\) and in the outcome of certain cancers.\(^20\) Since vitamin D insufficiency is widespread, even in populations that meet the Dietary Reference Intake, further research is needed to determine the intake required to achieve optimal serum 25(OH)D concentrations, and, once this is determined, strategies must be developed to raise the population to optimal levels.
Research is needed to determine how much vitamin D₃ intake is needed to optimize serum 25(OH)D levels for different age groups. The purpose of this research project was to evaluate the serum 25(OH)D response to supplementation with 800 IU vitamin D₃ during winter. The primary objective was to achieve optimal serum 25-hydroxyvitamin D levels in premenopausal women living in Maine. The secondary objective was to examine the effects of body mass index (BMI), body composition, and oral contraceptive use on baseline serum 25-hydroxyvitamin D levels and the response to oral vitamin D supplementation.
Chapter 2

LITERATURE REVIEW

Metabolism of Vitamin D

Vitamin D can be synthesized in the skin upon exposure to ultraviolet-B radiation and is, therefore, not technically a vitamin, but rather a steroid hormone. There are two forms of vitamin D: vitamin D₂, ergocalciferol, which is found in plants, and vitamin D₃, cholecalciferol, which is synthesized in the skin of animals and humans upon exposure to sunlight. Vitamin D written without a subscript may refer to either vitamin D₂ or vitamin D₃.²¹

Skin synthesis is the primary source of vitamin D₃ and provides 90 to 100% of the vitamin D requirement for most people.³ In the skin of humans, 7-dehydrocholesterol (provitamin D₃), which is produced in relatively large quantities, absorbs ultraviolet-B radiation (290-315 nm wavelengths) upon exposure to sunlight. Absorption of the radiation causes transformation of 7-dehydrocholesterol to previtamin D₃. Previtamin D₃ is thermodynamically unstable and is isomerized to form the more stable vitamin D₃ in one to three days. Vitamin D₃ diffuses from the skin into the extracellular space and is picked up by vitamin D-binding protein (DBP) in the dermal capillary bed. DBP carries vitamin D₃ primarily to the liver. However, some (approximately 12%) vitamin D is deposited in adipose tissue for storage along the way.²²-²⁴

Vitamin D-binding protein is the primary transport protein for 25(OH)D, 1,25(OH)₂D, and vitamin D₃. Less than 5% of the binding sites on DBP are occupied by vitamin D sterols.²⁵,²⁶ DBP is produced in the liver and has a high affinity for 25(OH)D; 88% of 25(OH)D is bound to DBP; 12% is bound to albumin, and less than 1% is in the
free (unbound) form. Other vitamin D metabolites are bound to DBP with less affinity. The regulation of DBP is unclear. Lower levels are found in nephrotic syndrome (due to urinary losses) and severe liver disease (due to poor production), but this does not seem to affect the binding capacity of DBP because binding sites are largely unoccupied under normal conditions. Estrogen increases hepatic production of DBP, resulting in higher concentrations during pregnancy and oral contraceptive use. The concentration of DBP is not affected by vitamin D deficiency or excess.

When individuals are exposed to higher doses of ultraviolet radiation, the serum 25(OH)D response does not increase proportionately resulting in vitamin D intoxication. Instead, previtamin D₃ and vitamin D₃ absorb sunlight and are converted to other photoproducts, including lumisterol, tachysterol, suprasterols, and toxisterols, which are sloughed off with the skin rather than continuing on the pathway to 25(OH)D production. Therefore, the skin cannot generate excessive quantities of vitamin D₃ to cause vitamin D intoxication upon exposure to sunlight.

Vitamin D is slowly released from adipose tissue during times of vitamin D deprivation. The mechanism for the release of vitamin D from adipose tissue is unknown.

About 50% of the vitamin D consumed from the diet is incorporated into micelles and absorbed by passive diffusion into intestinal cells. Within the intestinal cell, vitamin D is incorporated into chylomicrons and excreted into the lymphatic system for transport to the blood. In the blood, some vitamin D is transferred from the chylomicrons to DBP for delivery to extrahepatic tissues for storage. After chylomicrons enter the
blood, they undergo conversion to chylomicron remnants that contain the vitamin D and are taken up by the liver via hepatic receptors.23

Vitamin D₃ synthesized in the skin after exposure to ultraviolet-B (UVB) radiation is transported mainly via DBP, whereas vitamin D ingested from the diet is transported primarily by chylomicrons in the bloodstream.²³,²⁵,³² Upon reaching the liver, delivered either by chylomicron remnants or DBP, vitamin D is hydroxylated by a 25-hydroxylase enzyme, forming 25(OH)D, the major circulating form of vitamin D. Although 25-hydroxylase is less efficient when vitamin D is in abundance, 25-hydroxylase is poorly regulated. Therefore, the serum 25(OH)D concentration reflects overall vitamin D status.²³ A membrane receptor on the hepatocyte quickly picks up chylomicron remnants, resulting in a quicker increase in serum 25(OH)D concentration after oral vitamin D consumption compared to cutaneous production.³¹,³² In contrast, vitamin D from the skin diffuses into the blood more slowly and enters the liver more gradually, allowing for prolonged production of 25(OH)D.³² Serum 25(OH)D levels have been found to be sustained for a longer period following cutaneous vitamin D synthesis than after oral consumption of vitamin D.³²,³³

From the liver, 25(OH)D is released back into the bloodstream where it is transported bound to DBP to the kidney and other tissues. In the kidney and other tissues, 25(OH)D is further hydroxylated by a 1-α-hydroxylase enzyme, producing the active form of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)₂D], which interacts with nuclear vitamin D receptors (VDR) in the cells to influence gene transcription.²³

The primary function of 1,25(OH)₂D is to maintain serum calcium levels within a normal range so as to support cellular activities and neuromuscular function.²⁹
decrease in serum calcium concentration stimulates the parathyroid gland to secrete parathyroid hormone (PTH), which goes to the kidney to stimulate production of 1,25(OH)₂D from 25(OH)D. DBP carries 1,25(OH)₂D to the intestines where it acts via the vitamin D receptor in the intestines to increase active calcium absorption. If there is inadequate dietary calcium to maintain serum calcium concentrations, 1,25(OH)₂D and PTH work together to stimulate reabsorption of calcium from the renal tubules and mobilize calcium from the skeleton to maintain normal serum calcium levels.29,34 The steroid hormone, 1,25(OH)₂D, is only made in the kidneys as needed, and production is tightly regulated by PTH and calcium.20

High concentrations of 1,25(OH)₂D inhibit 1-α-hydroxylase activity, and increase 24-hydroxylase enzyme activity, which acts to degrade 1,25(OH)₂D to calcitroic acid for excretion.35 The 24-hydroxylase enzyme produces 24,25-dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D, intermediates in the degradation of 1,25(OH)₂D to calcitroic acid.36 Calcitroic acid is excreted via bile in feces.37 Twenty-five-hydroxyvitamin D is also broken down via this pathway.36 High serum 1,25(OH)₂D levels decrease the half-life of 25(OH)D,38 most likely by increasing metabolic clearance as noted by increased fecal, urinary, and biliary excretion of catabolic products.39-41 An increase in 1,25(OH)₂D due to inadequate serum calcium and vitamin D can accelerate the depletion of 25(OH)D stores.42

Serum concentrations of 1,25(OH)₂D are only about 1/100 of serum concentrations of 25(OH)D and the half-life of 1,25(OH)₂D is only four to six hours, compared to three to four weeks for 25(OH)D.42
Recently, vitamin D receptors and 1-α-hydroxylase were found in many tissues outside the kidney; these tissues produce 1,25(OH)\(_2\)D, which acts locally to regulate cell growth. This 1,25(OH)\(_2\)D does not enter circulation and is therefore not measured as part of the serum concentration. Extrarenal production of 1,25(OH)\(_2\)D is dependent on an adequate supply of 25(OH)D reaching the tissues.\(^{20}\)

In summary, vitamin D, whether consumed orally or synthesized cutaneously upon exposure to UVB radiation, is transported to the liver where it is hydroxylated to 25(OH)D. Then, 25(OH)D is carried by DBP from the liver to the kidney and extrarenal tissues where it is further hydroxylated to the biologically active 1,25(OH)\(_2\)D.

**Functions of Vitamin D**

The primary function of vitamin D is to maintain serum calcium levels within a normal range so as to support cellular activities and neuromuscular function.\(^{29}\) Vitamin D is necessary for calcium absorption, for bone mineralization, and in the prevention of rickets, osteomalacia, and osteoporosis. In addition to the calcemic roles, vitamin D also appears to have important non-calcemic, non-skeletal-related functions including: reducing the risk of developing autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, and multiple sclerosis; improving immune function; and improving the outcome of certain cancers.

The functions of vitamin D are carried out through the actions of the active form, 1,25(OH)\(_2\)D, which interacts with nuclear vitamin D receptors (VDRs). VDRs act through vitamin D-responsive elements (VDREs) to either initiate or suppress gene transcription.\(^{34,43}\)
Calcemic Functions

Calcium Absorption

Vitamin D is necessary for active transport of calcium across the intestinal cell mucosa. When serum calcium levels start to decrease, the parathyroid gland is stimulated to secrete PTH, which then increases renal 1,25(OH)₂D production. The 1,25(OH)₂D interacts with nuclear vitamin D receptors (VDRs) in the intestinal cells activating genes for calcium binding protein (calbindin) and other proteins involved in calcium and phosphorus transport across the cell membranes, through the cells, and into circulation to maintain serum calcium levels.⁴³

If there is inadequate dietary calcium to maintain serum calcium concentrations, 1,25(OH)₂D and PTH work together to stimulate reabsorption of calcium from the renal tubules and mobilize calcium from the skeleton to maintain normal serum calcium levels.²⁹,³⁴

Without vitamin D the intestine only absorbs 10% to 15% of dietary calcium via passive diffusion. Calcium absorption increases to 30% with adequate vitamin D and up to 80% during growth, lactation, and pregnancy.⁴⁴ Postmenopausal women with mean serum 25(OH)D levels of 86.5 nmol/L absorbed 45% to 65% more calcium than did women with mean serum 25(OH)D levels of 50.1 nmol/L.⁹ In another study, after adjusting for age, calcium intake, and 1,25-dihydroxyvitamin D concentration, serum 25(OH)D level was the most significant determinant of calcium absorption in elderly women.⁴⁵
Bone Mineralization and Fracture Risk

Vitamin D is essential for the prevention of rickets (poor mineralization resulting in softening of the bones) in children and osteomalacia (defective bone mineralization) and osteoporosis (porous, thinned bones) in adults. Rickets causes deformity and increased risk of fracture in children. Osteomalacia results in pain, weakness, and fragility of bones in adults. Rickets, osteomalacia, and osteoporosis increase fracture risk.20

When there is inadequate 25(OH)D, calcium absorption decreases and serum calcium drops, which stimulates secretion of PTH by the parathyroid gland, resulting in secondary hyperparathyroidism. Parathyroid hormone alone, and in cooperation with 1,25(OH)2D, induces maturation of osteoclasts in the bone, which release hydrochloric acid and enzymes to destroy the bone matrix, releasing calcium and other minerals into the circulation.34,44 Over time, the withdrawal of mineral from the bone and destruction of the matrix results in osteoporosis.29 Secondary hyperparathyroidism also increases renal tubular absorption of calcium and loss of phosphorus in the urine resulting in an inadequate calcium-phosphate product to promote mineralization of the bone, which leads to osteomalacia and rickets.44

Accumulation of peak bone mass during childhood and adolescence and the rate of bone loss during aging are two of the main factors contributing to osteoporosis.46,47 Although accumulation of bone mass is most rapid during late childhood and early adolescence, bone mass can continue to accumulate until women are in their late twenties47 and bone loss typically begins after age 30. Vitamin D intake is positively
correlated with bone mineral density (BMD) in young adult women. Similarly, serum 25(OH)D levels and BMD are positively correlated in adolescent girls.

In the elderly, men and women receiving 700 IU vitamin D$_3$ and 500 mg calcium daily over three years experienced a significant reduction in bone loss and a 54% reduction in non-vertebral fractures. Researchers have found that supplementation with vitamin D significantly reduces bone loss in elderly subjects and fracture risk. The decrease in fracture risk is likely due to both the improvements in bone density and in muscle strength.

**Skeletalmuscular Comfort**

In osteomalacia, it is believed that the osteoblasts deposit collagen matrix on the endosteal and periosteal surfaces of the skeleton, but without adequate calcium phosphate product, the matrix is rubbery and is not strong enough to support the weight of the skeleton. Instead, the rubbery matrix expands under the periosteal covering, putting pressure on the covering, which is innervated with sensory pain fibers, resulting in the deep bone aching and pain associated with osteomalacia. Muscle pain and weakness probably precede bone disease and can occur in patients without signs of osteomalacia. However, the mechanism for this muscle pain is not yet known.

Osteomalacia, which causes generalized aching in the bones, and muscle pain and weakness, is common in vitamin D deficiency and can be misdiagnosed as fibromyalgia. But, unlike in fibromyalgia, correction of the vitamin D deficiency can provide nearly complete pain relief. In Minnesota, more than 90% of hospital inpatients with nonspecific muscle aches and bone pain had severe vitamin D deficiency. In Denmark, 88% of Arab women with muscle weakness and bone pain
were severely vitamin D deficient. In the Arab women, treatment with vitamin D, which increased serum 25(OH)D levels by over 400% (from 6.7 ± 0.6 to 34.4 ± 2.0 nmol/L) significantly reduced subjective complaints of muscle and bone discomfort, in most cases within one and a half months. Vitamin D supplementation also significantly improved objective measures of muscle power (maximal voluntary quadricep contraction, single twitch stimulation, maximal production rate, and maximal relaxation rate) in the same women. Middle-aged men and women experienced a 24.8 ± 8.0% improvement in overall muscle power when mean serum 25(OH)D levels increased from 7.0 ± 0.7 to 48.3 ± 8.3 nmol/L.

In the elderly, low serum 25(OH)D levels are associated with decreased muscle strength and increased risk of falling. Improvement in the function of type-II muscle fibers after supplementation with vitamin D may explain the reduced risk of falling seen in some supplementation studies.

**Non-Skeletal Functions**

In the United States and worldwide, people who live at higher latitudes, and therefore have limited access to sunlight, have increased risk of prostate, colon, breast, ovarian, and esophageal cancers and non-Hodgkins lymphoma, multiple sclerosis, type 1 diabetes, rheumatoid arthritis, and hypertension.

Adequate amounts of the active form of vitamin D, 1,25(OH)₂D, are necessary in cancer prevention and other diseases. In the kidneys, low calcium levels promote the production of 1,25-dihydroxyvitamin D; however, this is not the case in the extra-renal tissues where adequate supply of the substrate, 25(OH)D, is necessary for 1,25(OH)₂D production.
Most of the studies relating vitamin D status to disease rates are based on associations and are, therefore, not necessarily causal. Thus, the role of vitamin D in cancer and autoimmune disease is still somewhat controversial.

**Cancer**

The active form of vitamin D, 1,25(OH)₂D, plays an important role in the proliferation and differentiation of cells. Normal tissue and some cancer cells (including prostate, colon, breast, and lung cancer cells) have 1-α-hydroxylase and are able to convert 25(OH)D into 1,25(OH)₂D, which interacts with the vitamin D receptor (VDR) in these cells, decreasing their differentiation and promoting their maturation, to regulate cell growth and possibly prevent malignancy.²⁰,²⁹

Studies show that serum 25(OH)D levels of at least 50 nmol/L decrease the risk of developing and dying of colon, prostate, and breast cancer by 30% to 50%.³⁰,⁶⁷,⁶⁸,⁷⁰ In a review of 18 observational studies looking at 25(OH)D levels or oral vitamin D intake and colon cancer, Gorham et al⁷⁶ found that colorectal cancer risk can be decreased by 50% with serum 25(OH)D levels above 80 nmol/L compared with 29 nmol/L or with oral intake of at least 1000 IU vitamin D3 daily compared with 100 IU daily.

In a study designed to look at fracture risk, women in rural Nebraska receiving 1400 to 1500 mg calcium and 1100 IU vitamin D₃ daily for four years had a 0.402 relative risk (RR) for all cancers compared to women receiving placebo.⁷⁷ The RR dropped to 0.232 when only cancers diagnosed after the first year were included (cancers diagnosed during the first year might have been present, but undiagnosed, at the start of the study). Subjects receiving 1400 to 1500 mg calcium, but no vitamin D, daily for four years had a RR of 0.532 for all cancers compared to placebo. Their RR did not change
when only cancers diagnosed after the first year were included. Over the four-year period, out of 1180 women, there were 50 non-skin cancer diagnoses occurring in the breast (19), colon (3), lung (7), lymph/leukemia/myeloma (10), uterus (3), and other (8). Baseline serum 25(OH)D levels and treatment were significant predictors of cancer risk using linear regression analysis. The RR for the calcium-only group suggests a protective effect of calcium, or, the researchers suggest, it could be a chance occurrence since, with the exception of colon cancer, no other studies have ever shown an association between calcium and cancer. High calcium intake may reduce 1,25(OH)2D concentration, which would result in slower consumption and degradation of 25(OH)D.42 This would act like a higher dose of vitamin D and could explain the protective effect of calcium.

**Autoimmune Diseases**

Development of type 1 diabetes, multiple sclerosis, and rheumatoid arthritis has been prevented in mice prone to these diseases after treatment with 1,25(OH)2D at an early age.73,78-82 The likely mechanism of action is that 1,25(OH)2D interacts with T-helper lymphocytes, which suppresses the inflammatory responses of T-helper type 1 lymphocytes.34

The risk of developing type 1 diabetes was reduced by 80% in non-obese diabetic mice, prone to type 1 diabetes, who received 1,25(OH)2D throughout their lives.78,81

In humans, children in Finland who received the recommended dose of 2000 IU vitamin D daily during their first year of life in 1966 were 80% less likely to develop type 1 diabetes over the next 30 years.17 Additionally, children who had been diagnosed with rickets during childhood were three times more likely to develop type 1 diabetes.17
Experimental autoimmune encephalomyelitis (EAE) is the animal model for multiple sclerosis (MS). Administration of adequate 1,25(OH)2D to animals can eliminate or suppress EAE at any stage of development.79 However, this therapy caused hypercalcemia in the animals.

Munger and colleagues,18 analyzing serum samples of white military personnel, found an inverse relationship between serum 25(OH)D levels before age 20 and risk of developing multiple sclerosis. For the Hispanics and African Americans no significant relationship was seen, probably due to the smaller sample size and, in blacks, lower serum 25(OH)D levels. Among whites, the odds ratio of developing MS was 0.38 in the highest quintile of serum 25(OH)D (>99.1 nmol/L) compared to the lowest quintile of 25(OH)D (<63.3 nmol/L). Having serum 25(OH)D concentrations above 100 nmol/L reduced the risk of multiple sclerosis by 51% compared with serum concentrations less than 75 nmol/L.18

In mice, supplementation with 1,25(OH)2D decreased symptoms and arrested the progression of arthritis in animals inflicted with an animal version of rheumatoid arthritis (RA). Supplementation with 1,25(OH)2D prevented the development of arthritis in mice.73

Immunity

Researchers believe that 1,25(OH)2D stimulates the innate immune response. Vitamin D prevents macrophages from releasing too many cytokines and chemokines83 and enhances macrophage ability to function.84,85 The active form of vitamin D, 1,25(OH)2D, activates the gene for production of the antimicrobial peptide (AMP),
cathelicidin. Antimicrobial peptides act like antibiotics to destroy invading microorganisms, and accelerate wound healing.

In human studies, there is evidence that adequate vitamin D status benefits immunity. When serum from African Americans with low 25(OH)D levels was inoculated with *Mycobacterium tuberculosis*, macrophages produced 63% less cathelicidin than did macrophages in serum from white Americans with higher 25(OH)D levels. Addition of 25(OH)$_2$D to the African American serum increased cathelicidin production to that seen in the white serum.

When Wayse and colleagues compared 80 children with lower respiratory infections to healthy controls, 80% of the ill children had serum 25(OH)D levels less than 25 nmol/L, compared to only 31% of the controls. Some researchers suggest adequate vitamin D levels may protect against influenza. In a psychiatric hospital in California, none of the men receiving high doses of vitamin D for treatment of deficiency became ill during an influenza outbreak that affected over 100 men at the facility. In 756 young Finnish men serving in the military, Laaksi and colleagues found that men who had serum 25(OH)D levels less than 40 nmol/L in July missed significantly more days of military duty due to respiratory infection over the following six months. The men with vitamin D deficiency missed a median of four days compared to two days missed by the control group.

**Hypertension**

Adequate vitamin D may help prevent hypertension by preventing overstimulation of the renin angiotensin system (RAS). The proposed mechanism for the effect of vitamin D on hypertension, as demonstrated in mice, is that 1,25(OH)$_2$D down-
regulates renin gene transcription through a mechanism involving the VDR.\textsuperscript{93} VDR knock-out mice had increased renin expression and increased angiotensin II production resulting in hypertension, which was corrected with an angiotensin converting enzyme (ACE) inhibitor.\textsuperscript{93} Further research is needed to examine the molecular mechanism for vitamin D regulation of renin expression.

Hypertensive patients exposed to UVB radiation for three months experienced a 162\% mean increase in serum 25(OH)D levels and a 6 mmHg mean drop in diastolic and systolic blood pressure; there was no change in a group exposed to UVA radiation.\textsuperscript{19} This change in blood pressure upon exposure to UVB is similar to that obtained from medications.\textsuperscript{19} Data from the Health Professionals’ Follow Up Study and the Nurses’ Health Study revealed the pooled relative risk of incident hypertension was 3.18 for men and women with serum 25(OH)D levels less than 37.5 nmol/L compared with those with levels greater than 75 nmol/L.\textsuperscript{75}

**Inflammation**

Likely through its role in enhancing the immune system, decreasing cytokine production\textsuperscript{83} and enhancing macrophage function,\textsuperscript{84,85} vitamin D also plays a role in decreasing inflammation.\textsuperscript{94} Studies have reported an inverse relationship between C-reactive protein and serum 25(OH)D levels.\textsuperscript{94,95} Van den Berghe and colleagues\textsuperscript{94} saw a decrease in C-reactive protein and interleukin-6 (IL-6) concentrations in critically ill patients receiving 500 IU vitamin D\textsubscript{3} in parenteral nutrition daily. Timms and colleagues\textsuperscript{95} gave 47 vitamin D deficient adults (25(OH)D levels less than 27 nmol/L) either 500 or 50,000 IU vitamin D\textsubscript{3} intramuscularly every three months for one year. Serum 25(OH)D levels increased to 37.5 nmol/L in the high dose group, and 32.9 nmol/L
in the low dose group. The researchers reported difficulty in accurately measuring 500 IU vitamin D₃ to be given intramuscularly and speculated that subjects received injections containing more than 500 IU which might explain the lack of a difference in final serum 25(OH)D levels between the two dosage groups. Overall, C-reactive protein levels decreased by 23% in the subjects receiving vitamin D₃; there was no placebo group.⁹⁵

In men and women over 50 years old participating in NHANES III, serum 25(OH)D concentrations were inversely associated with periodontal attachment loss.⁹⁶ Periodontal attachment loss is the loss of periodontal ligament and alveolar bone, which is characteristic of periodontal disease.⁹⁶ Men in the lowest quintile lost 0.39 mm more attachment than those in the highest quintile of serum 25(OH)D concentration; women in the lowest quintile of serum 25(OH)D lost 0.26 mm more periodontal attachment. BMD and attachment loss were not significantly associated. The researchers credit the anti-inflammatory effects of vitamin D for the inverse association with periodontal disease.⁹⁶ Possibly, vitamin D inhibits the release of proinflammatory cytokines in response to bacteria in the dental plaque. Without sufficient vitamin D, the cytokines stimulate bone resorption in the affected teeth.⁹⁶ The same researchers looked at the association between gingivitis and serum 25(OH)D levels in NHANES III participants.⁹⁷ Gingivitis is a better tool for examining the effect of vitamin D on inflammation because, unlike periodontal disease, its development is completely unrelated to bone. The highest quintile of serum 25(OH)D (median 99.6 nmol/L) had 20% lower odds of bleeding than the lowest quintile (median 32.4 nmol/L). There was a significant inverse association between serum 25(OH)D levels and bleeding of the gums on probing (a measure of gingivitis). This
association did not reach a threshold at a point where gingival inflammation leveled off, suggesting that serum 25(OH)D concentrations higher than 90-100 nmol/L may be needed to reduce inflammation.

**Summary**

Vitamin D is essential for maintaining skeletal strength throughout the lifecycle and may also play important roles in the prevention and treatment of several cancers, hypertension, and autoimmune diseases. The actions of vitamin D are carried out by the active hormonal form, 1,25(OH)₂D, interacting with a vitamin D receptor in the cell nucleus to enhance or suppress gene transcription. Adequate serum 25(OH)D is critical to provide adequate substrate for hydroxylation to 1,25(OH)₂D. Because 1,25(OH)₂D produced outside the kidneys does not enter into circulation, measurement of the active form of vitamin D does not reflect adequacy of vitamin D status. Instead, assessment of serum 25(OH)D concentration is the appropriate indicator of vitamin D status.

**Optimal Levels of Serum 25(OH)D**

Serum 25-hydroxyvitamin D concentration is the best measure of vitamin D status because it reflects both cutaneous synthesis of vitamin D and absorption of vitamin D from the diet. The enzyme that converts vitamin D into 25(OH)D, 25-hydroxylase, is poorly regulated. Therefore, 25(OH)D concentration reflects overall vitamin D status. In the past, optimal levels of 25(OH)D were the levels that prevented osteomalacia and rickets.

Most laboratories use 37.5 to 50 nmol/L (15 to 20 ng/mL) as the lower limit of normal for 25(OH)D. To obtain the normal range, a diverse population of asymptomatic
subjects was sampled. However, this “normal” population could include individuals with inadequate sun exposure and suboptimal serum 25(OH)D levels. Hollis and colleagues suggest the “normal” population should be redefined as sun-replete individuals such as lifeguards, surfers, and farmers from locations where vitamin D is synthesized year-round. In 93 surfers and skateboarders in Honolulu, Hawaii (21°N) with self-reported sun exposure of three or more hours on five or more days per week for the previous three months, serum 25(OH)D levels ranged from 28 to 178 nmol/L. Similarly, the median serum 25(OH)D levels of 30 sun-replete healthy men who spent long hours outdoors during the summer in Nebraska (landscaping, construction, farming, or recreation) was 122 nmol/L (interquartile range: 100 to 154 nmol/L) in late summer.

The optimal level of 25(OH)D is the concentration associated with the maximum suppression of PTH, maximum calcium absorption, maximum bone mineral density and reduced rate of bone loss, reduced rate of falling and improved muscle strength, and reduced risk of fractures. During a round table discussion at the 5th International Symposium on the Nutritional Aspects of Osteoporosis, held in Lausanne, Switzerland in May 2003, five of the six leading vitamin D researchers agreed that, based on the evidence available, the optimal concentration of serum 25(OH)D is at least 70 to 80 nmol/L. Further research is needed to determine the optimal concentration of serum 25(OH)D required for the non-calcemic functions of vitamin D; however, preliminary evidence suggests levels of at least 75 nmol/L are desirable.

Maximum Suppression of PTH

Many studies have found an inverse relationship between serum 25(OH)D levels and PTH levels. Researchers have found that serum PTH levels increase when
serum 25(OH)D levels drop below 37 nmol/L,11,15 50 nmol/L,12 78 nmol/L,13 95 nmol/L,101 and 110 nmol/L.14 Possible explanations for the wide range of estimated threshold levels include variations in ethnicity, age, and calcium intake of the subjects, renal insufficiency, and the use of unstandardized assays for serum 25(OH)D levels.102,103

**Maximum Calcium Absorption**

In a study of postmenopausal women, calcium absorption increased with serum 25(OH)D levels up to 80 nmol/L, at which point fractional calcium absorption plateaued. Women with mean serum 25(OH)D levels of 86.5 nmol/L absorbed 45% to 65% more calcium than did women with mean serum 25(OH)D levels of 50.1 nmol/L.9 In healthy men, calcium absorption did not change significantly when serum 25(OH)D levels dropped from 122 nmol/L at the end of summer to 74 nmol/L at the end of winter.10 These studies suggest that the threshold for maximum calcium absorption is at serum 25(OH)D levels of at least 75 nmol/L.

**Reduced Rate of Falling / Improved Muscle Strength**

Low serum 25(OH)D levels are associated with decreased muscle strength and increased risk of falling in the elderly. Increasing serum 25(OH)D levels from 30 to 65 nmol/L significantly increased muscle strength (measured by the “timed up&go” test, knee flexor strength, knee extensor strength, and grip strength) and reduced the number of falls in older men and women.64 In another study, elderly men and women with serum 25(OH)D levels between 50 and 74 nmol/L were twice as likely to experience loss of grip strength (an indicator of muscle strength that is positively correlated with both lower- and upper-extremity strength) compared to subjects with serum 25(OH)D levels above 75 nmol/L.65
Reduced Risk of Fractures

A meta-analysis of vitamin D supplementation and fracture risk revealed that fractures were prevented at serum 25(OH)D levels of at least 74 nmol/L, and optimal fracture prevention was achieved when serum 25(OH)D levels reached at least 100 nmol/L. In one study, overall fracture risk was decreased by 22% in community-dwelling men and women with a mean serum 25(OH)D level of 74.3 nmol/L, compared to those with a mean of 53.4 nmol/L. Other vitamin D supplementation studies that failed to achieve serum 25(OH)D levels greater than 75 nmol/L did not find a significant reduction in fracture risk.

Maximum Bone Mineral Density and Reduced Rate of Bone Loss

In an analysis of NHANES III data, Bischoff-Ferrari and colleagues found a positive correlation between serum 25(OH)D levels and BMD, which continued as serum 25(OH)D levels increased above 100 nmol/L.

In another study, when serum 25(OH)D levels were maintained above 90 nmol/L in 100 postmenopausal women, wintertime bone loss was decreased compared to the control group, resulting in a slight gain in BMD of the spine over one year. Similarly, postmenopausal women with a mean serum 25(OH)D level of 100 nmol/L experienced significantly less femoral bone loss over one year than subjects with a mean serum 25(OH)D level of 66.3 nmol/L. These studies suggest that levels even greater than 75 nmol/L may be optimal to prevent bone loss in postmenopausal women.

Conclusion

The above evidence suggests that optimal serum 25(OH)D levels are at least 75 nmol/L, and possibly higher. Based on the recent editorial by Dawson-Hughes and
colleagues,¹ which reports an informal consensus among prominent vitamin D researchers to establish 75 nmol/L as the lower end of the optimal serum 25(OH)D range, this level was selected as the cut-off for determining optimal concentrations of serum 25(OH)D in this research study. Increasing serum 25(OH)D levels in the US to at least 75 nmol/L would result in an expected 4% to 5% increase in BMD; a 4% to 6% increase in lower extremity function in older adults; and a 25% reduction in hip or non-vertebral fractures.¹⁰³

Factors Influencing Serum 25(OH)D Levels

Dietary Vitamin D Intake

Recommended Dietary Intake Levels of Vitamin D

The recommended Adequate Intake set by the Food and Nutrition Board of the Institute of Medicine for Vitamin D, in the absence of sunlight, is 200 IU for children and adults up to 50 years old; 400 IU for adults 51 through 70 years old; and 600 IU for adults over 70 years old.²¹ However, the data used to set this intake recommendation in 1997 are now obsolete and most researchers agree that at least 800 to 1000 IU daily is needed for optimal vitamin D status in the absence of sunlight.¹

Sources of Vitamin D

Most people rely on food to meet their vitamin D requirement during times of inadequate sunlight. Few foods, primarily oily fish such as salmon, mackerel, and sardines; cod liver oil; and organ meats, are natural sources of vitamin D. Oily fish is not consumed daily by most individuals in the United States; therefore, the US population must rely on fortified foods for vitamin D.¹¹⁰ In fact, 65 to 87% of dietary vitamin D
intake comes from fortified foods in the US. A list of foods that contain vitamin D can be found in Appendix A.

In the early 1930’s the United States began fortifying milk with 100 IU vitamin D2 or D3 per eight ounce cup in an effort to prevent rickets in children. However, the actual amount of vitamin D in milk has been found to vary considerably. In addition to milk, some orange juice, some cereals and breads, some yogurts, and some margarines are fortified with vitamin D in the US (see Appendix A). Despite fortified food, women 19 to 50 years old only consumed an average of 168 IU vitamin D daily from fortified and naturally occurring food sources leading some researchers to argue for higher levels of fortification in a larger variety of foods. However, even with more fortification, supplemental vitamin D may still be necessary for individuals with inadequate sun exposure, including those living at high latitudes.

Sunlight, in the form of ultraviolet-B (UVB) radiation, is the primary source of vitamin D, providing 90 to 100% of the requirement for most people who are exposed to adequate sunlight. The amount of vitamin D1 produced by whole-body exposure to one minimal erythemal dose (MED) of sunlight (the amount needed to cause a light pinkness to the skin) is equivalent to the consumption of 10,000 to 25,000 IU oral vitamin D2. However, the exact amount of vitamin D produced in response to sunlight will vary by age and skin type, and is highly variable between individuals, with some individuals having low serum 25(OH)D levels despite abundant sun exposure.
Factors that Influence Cutaneous Synthesis of Vitamin D

Anything that reduces the number of UVB photons absorbed by the skin or decreases the amount of 7-dehydrocholesterol in the skin will decrease or eliminate vitamin D synthesis.

Latitude, Time of Day, and Season

The position of the sun in the sky varies daily and seasonally. When the sun is low on the horizon, UV radiation must travel farther, is more easily scattered, and is more absorbed by ozone than when the sun is directly overhead, resulting in fewer UVB photons reaching the earth’s surface. Because of the tilt of the earth on its axis, UV radiation must travel farther to reach areas closer to the north or south poles than to reach areas nearer the equator. Therefore, the ability of the skin to synthesize vitamin D is affected by latitude, season, and time of day.

Above 35°N latitude, no previtamin D₃ is produced in the skin for four to six months during winter. In Boston (42°N) human skin samples synthesized the greatest amount of previtamin D₃ in June and July and did not synthesize any previtamin D₃ on cloudless days from November through February. In Edmonton, Canada (52°N), no previtamin D₃ was synthesized from October through March. In Orono, Maine (44°N), no vitamin D₃ synthesis occurred in human skin samples on a sunny day at the end of February. Below 35° latitude (Los Angeles and Puerto Rico), however, previtamin D₃ was formed year-round. Similar results were found in the southern hemisphere; in Argentina previtamin D₃ synthesis was negligible in Ushuaia (55°S), but adequate in Buenos Aires (34°S) during winter. The period when there is insufficient UVB for vitamin D synthesis is known as “Vitamin D Winter”.
In North America (30°N to 43°N), serum 25(OH)D levels are reported to be at their lowest point at the end of the Vitamin D Winter in February, rapidly increase after March, reach their peak in August, and then slowly decline after September.\textsuperscript{6,121,122}

Time of day also affects the zenith angle of the UVB rays, and, therefore, affects vitamin D synthesis. In Boston in July, vitamin D synthesis can occur as early as 0700 EST and continue as late as 1700 EST, with peak synthesis between 1000 and 1400.\textsuperscript{123} In August in Orono, Maine, significant vitamin D\textsubscript{3} synthesis only occurred between 1000 and 1600.\textsuperscript{118} During spring and autumn in Boston, vitamin D synthesis only occurs between 1000 EST and 1500 EST.\textsuperscript{115}

In summary, season, latitude, and time of day affect the amount of UVB radiation reaching the earth’s surface and, therefore, affect cutaneous vitamin D synthesis. Maximum vitamin D synthesis occurs at the equator at midday during summer. No vitamin D is synthesized during winter at high latitudes at any time of day.

Pollution

Aerosols in the atmosphere deflect UVB photons, thus decreasing the amount of UVB radiation reaching the earth’s surface.\textsuperscript{120} High ozone levels in the troposphere absorb UVB photons and reduce cutaneous synthesis of vitamin D.\textsuperscript{115} Extremely high atmospheric ozone levels can extend the duration of “Vitamin D Winter” by two months and increase the latitude for “Vitamin D Winter” by approximately ten degrees.\textsuperscript{120} Individuals living in locations with high levels of pollutants in the air may have lower serum 25(OH)D levels than in areas with low pollution rates.
Cloud Cover

Ultraviolet-B radiation at the earth’s surface can be reduced by as much as 45% on a cloudy day. Sullivan and colleagues compared *in vitro* previtamin D₃ production on a cloudy summer day with a sunny summer day in Maine and found previtamin D₃ production was reduced by 50% when cloudy.

Sunscreen

Sunscreens, although beneficial in the prevention of skin cancer, prevent vitamin D synthesis in the skin. Sunscreen with a sun protection factor (SPF) of eight or greater reduces vitamin D production by more than 95% if applied and reapplied according to package instructions. In fact, twenty white individuals who used sunscreen on sun-exposed areas for more than one year had serum 25(OH)D levels 55% lower than matched controls (40.2 versus 91.3 nmol/L) during summer. Two (10%) of the sunscreen users had vitamin D deficiency (serum 25(OH)D levels less than 20 nmol/L).

On the other hand, Wolpowitz and Gilchrest argue that most individuals do not apply sufficient amounts of sunscreen, reapply it as directed, or cover all sun-exposed body surfaces and, therefore, are not receiving enough sun protection to prevent vitamin D synthesis. They also argue that, even if properly applied, sunscreens with SPF-15 permit 1/15 (6%) of UVB photons to penetrate the skin and, therefore, vitamin D deficiency due to sunscreen use is highly unlikely.

In Australia, Marks and colleagues gave 113 people either sunscreen with SPF-17 or a placebo cream. All subjects were instructed to apply the cream daily, and to reapply if they were likely to have sweated, washed, or rubbed it off during the day. All
subjects were advised to avoid sun exposure at midday, and to wear hats and clothing for sun protection since they could be in the placebo group and getting no other sun protection. The researchers found no significant difference in serum 25(OH)D levels between the sunscreen users and the control group. However, there were some weaknesses in this study, which may explain why Marks and colleagues failed to find any difference in serum 25(OH)D levels. Subjects were only instructed to apply cream to their head, neck, forearms and dorsum of each hand, which left their legs, and upper arms exposed to sunlight and could explain why sunscreen users synthesized vitamin D. All subjects were encouraged to protect themselves from sun by avoiding midday sun and wearing protective hats and clothing; therefore, subjects in the placebo group likely had decreased vitamin D synthesis. Overall, serum 25(OH)D levels increased only 12.3 nmol/L, which was less than the seasonal differences of 20.1 nmol/L\(^4\) and 31 nmol/L\(^{129}\) seen in other studies between winter and summer.

Thus, sunscreen, if applied to all exposed skin, and reapplied frequently, can prevent vitamin D synthesis. In reality, however, most individuals do not apply and reapply sunscreen to all sun-exposed areas regularly enough to cause vitamin D deficiency.

**Clothing**

Clothing absorbs UVB radiation, preventing vitamin D synthesis. When subjects wearing black or white clothes made from cotton, polyester, or wool fabric were exposed to six MEDs of ultraviolet radiation, they did not produce any vitamin D.\(^{130}\) Vitamin D deficiency is common in sunny countries such as Saudi Arabia, Jordan, and the United
Arab Emirates, where, due to cultural clothing customs, women rarely expose their skin to sunlight.\textsuperscript{131-133}

**Aging**

In general, compared to younger people, older people are more likely to stay inside during the peak period for vitamin D production, wear more clothing, and use more sunscreen than younger people, putting them at risk for lower serum 25(OH)D levels.\textsuperscript{134} In addition, the amount of 7-dehydrocholesterol in the skin may decrease by more than 50\% by age 70,\textsuperscript{135} possibly due to decreased skin thickness and skin mass.\textsuperscript{136} Despite the decrease in 7-dehydrocholesterol, the elderly are still able to make vitamin D\textsubscript{3}, it just takes longer to make the same amount as a younger person.\textsuperscript{137} Elderly stroke patients in Japan who were exposed to 15 minutes of sunlight on their hands and faces every pleasant weather day for one year increased their mean serum 25(OH)D levels from 18 to 52 nmol/L. In contrast, in a similar group of patients who were not regularly exposed to sunlight, mean serum 25(OH)D levels dropped from 17 to 13 nmol/L.\textsuperscript{137} Older individuals may have lower baseline serum 25(OH)D levels than younger people due to decreased sun exposure and a decreased ability to synthesize vitamin D in the skin.

**Melanin**

The skin pigment melanin acts as natural sunscreen.\textsuperscript{115} People with dark skin do not make vitamin D\textsubscript{3} as efficiently as people with white skin.\textsuperscript{5,138,139} Dark-skinned individuals with Fitzpatrick skin types V or VI (dark brown or black) require ten to fifty times more sun exposure to produce the same amount of vitamin D\textsubscript{3} as lighter skinned individuals with skin types II or III (fair or slightly beige skin).\textsuperscript{138} In fact, in Boston in June, a black skin sample (type V) had negligible conversion of 7-dehydrocholesterol to
vitamin D₃ after 20 to 30 minutes of UVB exposure, whereas a white skin sample (type II) exhibited significant vitamin D₃ production after just five to ten minutes.¹⁴⁰

Analysis of data from NHANES III found that mean serum 25(OH)D levels in non-Hispanic whites were 1.2 to 1.7 times higher than levels in Mexican Americans or non-Hispanic blacks.¹²¹ In Boston, 20 to 40 year old white women had serum 25(OH)D levels more than twice those of black women in summer and in winter.¹⁴¹

Tanning Beds

Tanning beds that provide an artificial source of UVB radiation promote vitamin D₃ synthesis. At the end of winter in Boston, the serum 25(OH)D levels of adults who had used a tanning bed in the previous six months were 90% higher than adults who did not use a tanning bed.¹⁴² The tanners, who had tanned an average of 6.2 years, had significantly lower PTH levels and higher total hip BMD than non-tanners.

Skin pigmentation also affects vitamin D production from artificial UVB radiation. After 12 weeks of artificial UVB radiation with 0.75 MED in each tanning session, serum 25(OH)D levels increased 210% in subjects with skin type II (fair), 187% in subjects with skin type III (medium), 125% in subjects with skin type IV (olive or light brown), and 40% in subjects with skin type V (brown).¹⁴⁰

Other Factors that Influence Serum 25(OH)D Levels

Obesity

There is an inverse relationship between serum 25(OH)D levels and body mass index (BMI) or body fat¹⁴,¹²⁹,¹³⁹,¹⁴³-¹⁴⁹ As DBP transports vitamin D to the liver for hydroxylation, some vitamin D is deposited in the adipose tissue for storage.²²,²³,²⁴,¹⁴⁶ Obese individuals with large amounts of adipose tissue pick up and store more vitamin D,
resulting in lower serum 25(OH)D levels.\textsuperscript{146} It is believed that once the adipose tissue has been saturated, the vitamin D remains in the serum and is delivered to the liver for hydroxylation, but there are no long term data on vitamin D supplementation in obese adults.\textsuperscript{34}

Wortsman and colleagues\textsuperscript{146} found that obese individuals had a 57\% lower serum 25(OH)D response to UV radiation compared to normal weight peers. However, the amount of 7-dehydrocholesterol converted to vitamin D\textsubscript{3} was not different between the two groups. Therefore, the researchers concluded that obesity does not affect the ability of the skin to make vitamin D\textsubscript{3}, but affects the release from skin into the circulation.\textsuperscript{146}

Arunabh and colleagues\textsuperscript{129} found a significant difference in serum 25(OH)D levels between women with the lowest and highest quartiles of total body fat (<31\% body fat and 56.6 nmol/L compared with >42\% body fat and 44.2 nmol/L). There was a significant inverse correlation between fat quartile and the likelihood of achieving serum 25(OH)D levels of at least 80 nmol/L. Because total body fat correlated with serum 25(OH)D levels more strongly than either BMI or weight, researchers concluded that adiposity, rather than body mass, impacts serum 25(OH)D levels.\textsuperscript{129,148} Similarly, Bolland and colleagues\textsuperscript{150} found lower serum 25(OH)D levels at the end of summer in post-menopausal women and older men in the highest quartile of fat mass compared to those in the lowest quartile; however, they did not find a significant difference in serum 25(OH)D levels at the end of winter.

NHANES III data demonstrated that white women with a healthy BMI (18.5 to 25) had higher 25(OH)D levels than white obese women (BMI ≥30).\textsuperscript{139} No relationship was seen in African American women possibly due to the higher proportion of lean body
mass or to the lower serum 25(OH)D levels in African Americans.\textsuperscript{139} Eighty-nine percent of morbidly obese adults undergoing gastric bypass surgery in Bangor, Maine had serum 25(OH)D levels less than 80 nmol/L before surgery.\textsuperscript{151}

Vitamin D stored in adipose tissue is slowly released during times of vitamin D deprivation.\textsuperscript{3,22,29,30} However, in the obese, vitamin D is presumed to be sequestered deep in the body fat, so it is less bioavailable than in normal weight individuals.\textsuperscript{43}

Bell and colleagues\textsuperscript{143} suggested an alternative hypothesis to explain the lower serum 25(OH)D levels associated with obesity. They suggested that the elevated 1,25(OH)\textsubscript{2}D levels seen in obese individuals exert negative feedback on 25(OH)D production in the liver.\textsuperscript{143} However, the findings of Wortsman and colleagues\textsuperscript{146} contradict this theory as they found no difference in 25(OH)D production in obese subjects receiving oral vitamin D.

In research, BMI or, preferably, body fat, should be accounted for when evaluating vitamin D status. Clinically, obese individuals should be assessed for vitamin D deficiency.

**Estrogen**

Oral contraceptives are prescribed most often for preventing pregnancy, but are sometimes used to control acne, or moderate menses. Oral contraceptive pills must be taken daily and may contain either progestin only, or a combination of estrogen (ethinyl estradiol) and progestin. Depending on the prescription, the active pills may provide 15, 20, 25, 30, or more than 35 μg estrogen per day. The active pills may be monophasic, biphasic, or triphasic and are taken for the first 21 days of the menstrual cycle followed by 7 days of inactive pills (placebo) or no pill. Monophasic oral contraceptives provide
the same amount of estrogen and progestin every day for the first 21 days. Biphasic pills provide the same amount of estrogen for the first 21 days, but the progestin dose is lower in the second half of the cycle. Triphasic pills have the same or varying doses of estrogen, and varying doses of progestin throughout the cycle. In addition to the oral contraceptive pill, hormonal contraception is available as a skin patch, vaginal ring, injection, or implant. The vaginal ring and skin patch provide a steady low dose of estrogen equivalent to less than 15 μg per day.

Serum 25(OH)D levels are 15 to 25 nmol/L higher in oral contraceptive users than in non-users. In rural Iowa during summer, mean serum 25(OH)D levels were 25% (15 nmol/L) higher in 20-34 year old white women on oral contraceptives compared to those not on oral contraceptives. In Boston during winter, Harris and Dawson-Hughes found that oral contraceptive users had serum 25(OH)D levels 41% (24.1 nmol/L) higher than non-users. Analysis of NHANES III data collected in summer and winter found mean serum 25(OH)D levels 24.3% (24.8 nmol/L) higher in white oral contraceptive users compared to non-users.

The mechanism for the increased serum 25(OH)D levels in oral contraceptive users is uncertain. In male rats, estrogen administration increases mitochondrial 25-hydroxylase activity in the liver, which increases serum 25(OH)D levels. In rabbits and cattle, vitamin D-binding protein stimulates 25-hydroxylase activity. Since estrogen stimulates hepatic production of DBP in humans, DBP levels are increased in oral contraceptive users. However, in humans, there is no evidence that the increase in serum 25(OH)D levels in oral contraceptive users is due to increased 25-hydroxylation in the liver.
Oral contraceptives are believed to increase serum 25(OH)D levels due to the increase in DBP causing increased circulating concentrations of serum vitamin D sterols. Estrogen delivered transcutaneously or via skin patch does not increase serum DBP concentrations. Approximately one month following pregnancy, or after oral contraceptives are discontinued, DBP concentrations return to normal.

Although the DBP and total 25(OH)D concentrations are increased, the percentage of sterol in the free form is decreased, and the concentration of free 25(OH)D remains unchanged. Likewise, studies have shown an increase in DBP and total 1,25(OH)₂D concentrations after three months of oral estrogen use, but there was no increase in the concentration of free 1,25(OH)₂D.

According to the free hormone hypothesis, the biologic activity of a hormone is affected by its free (unbound) concentration, not by its bound concentration. Therefore, since total serum 25(OH)D levels are increased in oral contraceptive users due to increased DBP concentration, but the concentration of free 25(OH)D remains similar to those not using oral contraceptives, one could presume there should not be a physiologic advantage to oral contraceptive use in terms of serum 25(OH)D status. However, evidence of the effect of elevated serum 25(OH)D in oral contraceptive users on health is lacking.

The change in endogenous estrogen levels during the menstrual cycle is not associated with any detectable change in 25(OH)D levels. The phases of change in estrogen levels may be too short to detect changes in 25(OH)D levels. Hormone replacement therapy with one to two mg estradiol daily in postmenopausal women
increased plasma levels of vitamin D-binding protein, but did not affect serum 25(OH)D levels.

Gender

Serum 25(OH)D concentrations were generally 20% higher in elderly men than in elderly women living in Boston in summer and fall, but not from February to May. The difference in serum 25(OH)D levels may have been due to a difference in sun exposure as the men reported spending more time outdoors. Dietary intake of vitamin D was similar between the men and women. Elderly women have decreased estrogen levels, which may also lower their serum 25(OH)D levels.

NHANES III data showed the rate of vitamin D insufficiency was two times higher in females than males of the same age. Another study of older women and men in the Netherlands found that women had lower 25(OH)D levels than men, but this difference disappeared after adjustment for body fat.

The differences in serum 25(OH)D concentration seen between men and women may be explained by differences in sun exposure and body fat. Therefore, it is not clear whether there is a gender difference in serum 25(OH)D levels.

Calcium Intake

In healthy men fed their normal diets (estimated to contain 800 mg calcium) plus 2000 mg calcium for six to seven weeks during winter, serum 25(OH)D levels increased significantly more than in a control group. Serum 1,25(OH)_{2}D levels decreased significantly in the group receiving calcium. The authors hypothesized the increase in serum 25(OH)D and decrease in serum 1,25(OH)_{2}D could be due to one of the following: a) increased calcium intake decreases PTH secretion, which decreases 1,25(OH)_{2}D and
causes an increase in 25(OH)D due to a released negative feedback inhibition of 25-
hydroxylase enzyme; or b) increased calcium intake stimulates 25-hydroxylation of
vitamin D; or c) increased calcium intake, or the resulting decrease in 1,25(OH)₂D, may
decrease the metabolism of 25(OH)D.¹⁶⁴

The findings of Bell and colleagues,¹⁶⁵ that 1,25(OH)₂D may inhibit the formation
of 25(OH)D from vitamin D₃ support the first hypothesis. In addition, in hypocalcemic,
vitamin D-deficient rats, Haddad and colleagues¹⁶⁶ found that dietary calcium stimulated
hepatic microsomal 25-hydroxylase activity. In support of the second hypothesis,
researchers found decreased 25-hydroxylase activity¹⁶⁶ and decreased 25(OH)D
production⁴⁰ in calcium deficient rats.

There is also much research supporting the third hypothesis that increased
calcium, or the resulting decrease in 1,25(OH)₂D, may decrease 25(OH)D catabolism.
High serum 1,25(OH)₂D levels decrease the half-life of 25(OH)D,³⁸ most likely by
increasing metabolic clearance.³⁹-⁴¹ In rats fed a calcium free diet, the resulting
secondary hyperparathyroidism caused an increase in 1,25(OH)₂D, which increased the
metabolic clearance rate of 25(OH)D.³⁹,⁴⁰ Bolt and colleagues⁴⁰ found the clearance rate
of 25(OH)D was similar to the increased production rate of 1,25(OH)₂D found in other
studies of rats deprived of calcium, leading the researchers to conclude that the increased
turnover of 25(OH)D was due to increased production of 1,25(OH)₂D.

Therefore, the decreased 25(OH)D levels in calcium deficient rats are likely due
to both increased metabolic clearance of 25(OH)D for the production of 1,25(OH)₂D, and
decreased production of 25(OH)D from vitamin D.⁴⁰ Similarly, in humans, infusion with
1,25(OH)₂D stimulated the excretion of 25(OH)D catabolic products, resulting in a shorter half-life of 25(OH)D.⁴²

Calcium intake may affect baseline serum 25(OH)D levels in individuals with very low calcium intakes by stimulating serum 1,25(OH)₂D production thereby lowering serum 25(OH)D levels, and decreasing 25-hydroxylation of vitamin D.

**Alcohol Consumption**

Moderate alcohol consumption increases estrogen levels in postmenopausal women,¹⁶⁷ which may reduce bone loss¹⁶⁸ and increase serum 25(OH)D levels. In elderly women, some researchers have found a positive association between moderate alcohol consumption and serum 25(OH)D levels,¹⁶⁹,¹⁷⁰ while others have found no association with serum 25(OH)D levels.¹⁶⁸ Lamberg-Allardt and colleagues¹⁷¹ found a positive association between alcohol and serum 25(OH)D levels in healthy adult men and women (30 to 42 years). Moderate alcohol consumption may influence serum 25(OH)D levels and, therefore, should be considered when evaluating vitamin D status.

**Smoking**

Some studies have shown a negative association between smoking and serum 25(OH)D levels in healthy adult men and/or women,⁹²,¹⁷¹-¹⁷³ elderly women,¹⁴⁷,¹⁷⁰ and adult patients with Crohn’s Disease.¹⁷⁴ Other studies, however, have failed to find any relationship between smoking and vitamin D status.¹²⁹,¹⁴⁹,¹⁶⁹ Another study found a relationship between smoking and 25(OH)D levels in adult women, but not in men.¹⁷³ Some of the studies¹²⁹,¹⁶⁹,¹⁷₅ may have failed to find a relationship between smoking and serum 25(OH)D levels due to a low percentage of participants who were smokers.
The mechanism for the relationship between smoking and serum 25(OH)D levels is uncertain, but may be due to changes in sex hormones.\textsuperscript{172,175,176} Some researchers have found that, in postmenopausal women receiving hormone replacement therapy, estrogen levels are lower in smokers than in non-smokers\textsuperscript{177,178} presumably due to increased hepatic clearance of estrogen.\textsuperscript{177} Lower estrogen levels could result in lower serum 25(OH)D levels. However, Ortego-Centeno and colleagues\textsuperscript{179} found higher levels of serum estradiol and serum hormone binding globulin (SHBG) in premenopausal smokers than in non-smokers. SHBG increases with estrogen and thyroid hormones.\textsuperscript{176} Other researchers found that SHBG levels were significantly reduced after smoking cessation in postmenopausal women\textsuperscript{176} and in men and women with a mean age of 32.2 years.\textsuperscript{175} Thus, serum 25(OH)D levels may be lower in smokers, but the mechanism for this possible decrease is uncertain.

**Assay Methods**

Because serum 25(OH)D is highly hydrophobic and exists in two forms, 25(OH)D\textsubscript{2} and 25(OH)D\textsubscript{3}, it is very difficult to assay.\textsuperscript{180} Furthermore, assay results vary by 33\% or more between labs.\textsuperscript{181,182} Therefore, it is difficult to compare results between supplementation studies.

High performance liquid chromatography (HPLC), DiaSorin Radioimmunoassay (RIA), Nichols Advantage Competitive Binding Protein Assay, and Immunodiagnostic Systems (IDS) RIA are the assays that have been most commonly used for determining 25(OH)D levels in research over the past few years.\textsuperscript{180} Recently, however, it was discovered that the very popular Nichols Advantage assay and the IDS RIA did not detect 25(OH)D\textsubscript{2} well and are, therefore, no longer used.\textsuperscript{180} The DiaSorin RIA, when used by
experienced personnel, was able to accurately measure both 25(OH)D$_2$ and 25(OH)D$_3$\textsuperscript{180}. Measuring 25(OH)D by HPLC is considered the gold standard. However, this slow and expensive method is only accurate if it is performed by a highly experienced individual\textsuperscript{180}. Until a method to standardize assays is developed, caution must be used when comparing results from different research studies\textsuperscript{183}.

**Diseases and Medications**

Diseases characterized by intestinal malabsorption, liver disease, and kidney disease as well as some medications such as corticosteroids, anticonvulsants, some weight loss medications, and medications for treatment of high cholesterol affect serum 25(OH)D levels. Higher rates of vitamin D insufficiency have been documented in patients with malabsorptive diseases such as cystic fibrosis\textsuperscript{184-186}, celiac disease\textsuperscript{187}, and inflammatory bowel disease (Crohn’s disease and ulcerative colitis)\textsuperscript{174} than in healthy controls, often despite oral vitamin D supplementation. Vitamin D insufficiency is common in liver disease due to fat malabsorption and also due to poor hydroxylation\textsuperscript{188}.

Patients with nephrotic syndrome have low serum 25(OH)D levels due to a combination of urinary excretion of vitamin D-binding protein, and long-term glucocorticoid use\textsuperscript{189}. Weng and colleagues\textsuperscript{189} found 90\% of children with nephrotic syndrome had serum 25(OH)D levels less than 75 nmol/L.

Corticosteroids decrease calcium absorption\textsuperscript{190}, which increases PTH, stimulating renal 1,25(OH)$_2$D, and leading to a decrease in serum 25(OH)D levels\textsuperscript{42}. Orlistat, a pancreas lipase inhibitor prescribed for weight loss, may cause malabsorption of vitamin D and/or calcium\textsuperscript{191}.
The impact of the hypolipidemic drug cholestyramine on serum 25(OH)D levels was mixed. Knodel and colleagues\textsuperscript{192} reported impairment of vitamin D absorption in people taking large doses of cholestyramine (more than 32 grams daily). Two other studies, however, showed no deleterious effects of cholestyramine therapy (24 gm daily) on serum 25(OH)D levels.\textsuperscript{193,194}

Serum 25(OH)D levels are lower in individuals taking anticonvulsant medications, including, but not limited to, phenobarbitol, phenytoin, carbamazapine, oxycarbazepine and valproate.\textsuperscript{195,196} Although research results are mixed, anticonvulsant medications are believed to induce 24-hydroxylase enzymes which promotes the degradation of 25(OH)D.\textsuperscript{195,196}

**Factors that Influence Response to Supplementation**

**Baseline Serum 25(OH)D Levels**

Studies have shown that subjects with lower serum 25(OH)D levels have a larger increase in serum 25(OH)D levels due to supplementation (hereafter referred to as response to supplementation) than individuals with higher serum 25(OH)D levels.\textsuperscript{28,145,197-199} Barger-Lux and colleagues\textsuperscript{145} believe that this difference in response to supplementation depending on baseline levels suggests that hepatic 25-hydroxylation is a saturable process.

When Trang and colleagues\textsuperscript{197} gave adults 4000 IU vitamin D\textsubscript{3} daily for 14 days, the serum 25(OH)D levels of subjects in the lowest tertile of baseline serum levels (10 to 34 nmol/L) increased 30.6 ± 16.2 nmol/L, compared to a 13.3 ± 13.9 nmol/L increase in the highest tertile (50 to 86 nmol/L).\textsuperscript{197} In another study of young and old men living in the Boston area, there was a significant inverse correlation between baseline serum
25(OH)D levels and response to supplementation with 800 IU vitamin D₃ daily during winter. Baseline serum 25(OH)D levels (but not BMI or vitamin D intake) were predictive of response to supplementation in the young men, but not in the old men.

In Ireland, DeLappe and colleagues supplemented elderly women with 800 IU vitamin D₃ and 1000 mg calcium for three months. The 36 women who had baseline serum 25(OH)D levels less than 50 nmol/L increased mean serum 25(OH)D concentrations significantly from 28.9 to 52.5 nmol/L; the 15 women who had baseline levels greater than 50 nmol/L increased their serum levels from 73.9 nmol/L to 76.1 nmol/L, an insignificant change. In nursing home residents in Brazil, serum response to 7000 IU vitamin D₃ per week was significantly influenced by baseline serum 25(OH)D levels, but not body fat. Residents with baseline levels less than 50 nmol/L increased serum levels by 25 nmol/L compared to a 13 nmol/L increase in residents with baseline serum 25(OH)D levels greater than 50 nmol/L. On the other hand, Orwoll and colleagues did not see any effect of baseline serum 25(OH)D levels on response to supplementation with 1000 IU vitamin D₃ in 30 to 82 year old men. However, with a mean of 60 nmol/L, there may not have been enough low serum 25(OH)D levels to see an effect.

One study also observed a stronger response to ultraviolet radiation in subjects with low baseline serum 25(OH)D levels. In 13 German men and women, 20 to 57 years old, when exposed to a high dose of ultraviolet radiation over 21 days (total of 36 J/cm²), serum 25(OH)D levels increased twice as much in subjects with baseline serum 25(OH)D levels less than 25 nmol/L compared to those with baseline serum levels greater than 50 nmol/L.
Obesity

The effect of body fat on response to supplementation is uncertain. Wortsman and colleagues\textsuperscript{146} found no difference between obese and normal weight subjects in their response to oral supplementation with a one-time dose of 50,000 IU vitamin D\textsubscript{2}. Likewise, elderly subjects in Brazil, given 7000 IU vitamin D\textsubscript{3} weekly, had a similar 25(OH)D response to supplementation, regardless of tertile of body fat.\textsuperscript{200} In another study, however, when given three different doses of vitamin D\textsubscript{3}, heavier men had less of a response to supplementation.\textsuperscript{145} The researchers calculated that men weighing 85 kg had approximately 65\% of the response to supplementation as did 55 kg men.\textsuperscript{145}

Calcium Intake

A high calcium diet may increase the serum 25(OH)D response to oral vitamin D supplementation by increasing serum calcium levels slightly, which will decrease PTH secretion, thereby decreasing production of 1,25(OH)\textsubscript{2}D. Decreasing 1,25(OH)\textsubscript{2}D will slow the depletion of 25(OH)D stores\textsuperscript{42} because less 25(OH)D will be used as substrate for 1,25(OH)\textsubscript{2}D production. In one study, serum 25(OH)D levels increased significantly less in eight normal subjects taking 100,000 IU vitamin D\textsubscript{2} with 2000 mg calcium for four days than when taking the same amount of vitamin D\textsubscript{2} alone.\textsuperscript{202} In another study however, Goussous and colleagues,\textsuperscript{203} using intakes of calcium and vitamin D within the range that are usually recommended, found that daily calcium intakes of 500 or 1500 mg did not affect the serum 25(OH)D response to supplementation with 800 IU vitamin D\textsubscript{3} for three months during winter.
Cholecalciferol (Vitamin D₃) versus Ergocalciferol (Vitamin D₂)

Until recently, vitamin D₂ and vitamin D₃ were believed to be equally effective at increasing serum 25(OH)D levels. However, recent studies show that D₃ is the more effective form of supplemental vitamin D.¹⁹⁷,²⁰⁴,²⁰⁵

Tjellesen and colleagues²⁰⁴ gave 19 premenopausal women either 4000 IU vitamin D₂ or vitamin D₃ for eight weeks. In the vitamin D₂ group, 25(OH)D₂ increased 68 nmol/L, but 25(OH)D₃ decreased by 51.5 nmol/L (more than would be expected due to season), so the total 25(OH)D remained unchanged. In the vitamin D₃ group, 25(OH)D₃ and total 25(OH)D increased 32 nmol/L. The researchers suggested the decrease in vitamin D₃ in the vitamin D₂ group may have been due to either competition for hydroxylase or up-regulated catabolism.²⁰⁴

A few years later, Trang and colleagues¹⁹⁷ gave volunteers either 4000 IU vitamin D₂ or vitamin D₃ or nothing for 14 days. Total 25(OH)D increased 23.3 nmol/L in the vitamin D₃ group versus only 13.7 nmol/L in the vitamin D₂ group, and 3.0 nmol/L in the untreated group.¹⁹⁷ The researchers attributed this difference to the findings of Holmberg and colleagues²⁰⁶ that, in hepatic mitochondria, the hydroxylation rate of vitamin D₃ is five times that of vitamin D₂. In the same study, no hydroxylation of vitamin D₂ and very little hydroxylation of vitamin D₃ was detected in the microsomes.

Armas and colleagues²⁰⁵ gave 30 men either 50,000 IU vitamin D₂ or vitamin D₃ or nothing daily for 28 days. Serum 25(OH)D₂ and 25(OH)D₃ increased the same amount in the first three days, suggesting they were absorbed and hydroxylated the same. However, by the end of the study, total 25(OH)D was 22 nmol/L higher in the vitamin D₃ group than in the vitamin D₂ group.²⁰⁵ Since DBP has a higher affinity for D₃ than D₂²⁵
the researchers concluded that vitamin D₃ is metabolized more slowly and vitamin D₂ is cleared more quickly, resulting in a lower response to supplementation.²⁰⁵ In work by Guo and colleagues,²⁰⁷ it appears that vitamin D₂ is preferentially 24-hydroxylated and is therefore degraded more quickly. Vitamin D₃, however, is preferentially 25-hydroxylated.

**Dose of Vitamin D₃**

Higher doses of vitamin D₃ increased serum 25(OH)D levels less per unit of vitamin D consumed than did lower doses of vitamin D₃.²⁰⁸,²⁰⁹ When Vieth and colleagues²¹⁰ gave subjects either 1000 or 4000 IU vitamin D₃ for three months during late winter, the increases in serum 25(OH)D levels per microgram of vitamin D₃ input were 1.02 and 0.59 nmol/L, respectively. In another study, subjects were given either 5000 or 10,000 IU vitamin D₃ daily and serum 25(OH)D levels increased 0.736 and 0.636 nmol/L per microgram of vitamin D₃ intake, respectively.²⁰⁹

**Aging**

Aging does not appear to affect response to supplementation with oral vitamin D₃.¹⁹⁸,²¹¹ Harris and colleagues¹⁹⁸ found no significant difference between 18 to 35 year old men and 62 to 79 year old men in their response to supplementation with 800 IU vitamin D₃. Vieth and colleagues²¹¹ found that adults less than 50 years old and adults more than 70 years old consuming similar amounts of vitamin D had no difference in serum 25(OH)D levels. However, the researchers did find that older adults needed a higher 25(OH)D concentration to maximally suppress PTH and, therefore, may need a higher recommended intake than younger adults.²¹¹
Absorption

Diseases characterized by intestinal malabsorption can affect serum response to oral supplementation with vitamin D3. Some medications, such as some weight loss medications, and some medications for the treatment of high cholesterol induce fat malabsorption and may, therefore, also affect serum 25(OH)D levels.

Despite vitamin D supplementation, higher rates of vitamin D insufficiency have been documented in patients with malabsorptive diseases such as cystic fibrosis, celiac disease, and inflammatory bowel disease (Crohn’s disease and ulcerative colitis) than in healthy controls, suggesting a reduced response to supplementation in these disorders.

Orlistat, a pancreas lipase inhibitor prescribed for weight loss, may cause malabsorption of vitamin D and/or calcium and would, therefore, reduce response to supplementation. Large doses of cholestyramine induce fat malabsorption and impairment of vitamin D absorption. Two other studies, however, showed no deleterious effect of cholestyramine therapy on serum 25(OH)D levels.

Gender

In Brazilian nursing home patients, Canto-Costa and colleagues saw no difference in response to supplementation between men and women given 7000 IU vitamin D3 weekly for twelve weeks.

Other

Estrogen, alcohol consumption, and smoking influence baseline serum 25(OH)D levels, but their impact on the response to supplementation has yet to be determined.
Summary

The typical American diet provides little vitamin D and, therefore, has a minimal effect on serum 25(OH)D levels. The majority of vitamin D for most people comes from cutaneous synthesis upon exposure to sunlight. Winter at high latitudes, pollution, and cloudy skies scatter UVB photons reducing cutaneous synthesis of vitamin D and lowering serum 25(OH)D levels. Sunscreen, clothing, and dark skin pigmentation block UVB rays from reaching the skin, thereby decreasing synthesis of vitamin D and serum 25(OH)D levels. Increased body fat, elevated 1,25(OH)₂D levels, smoking, and some medical conditions and medications cause decreased serum 25(OH)D levels. On the other hand, hormonal contraceptives and moderate alcohol consumption result in increased serum 25(OH)D levels. These factors must be considered when assessing serum 25(OH)D levels in individuals and populations. In addition, 25(OH)D assays are not standardized, which makes comparisons between studies difficult.

Baseline serum 25(OH)D levels are known to be inversely associated with response to oral vitamin D supplementation. Medical conditions characterized by malabsorption and medications inducing fat malabsorption are believed to hinder response to supplementation. However, the effects of obesity, calcium intake, estrogen, gender, alcohol, and smoking on serum 25(OH)D response to supplementation are uncertain at this time.

Prevalence of Vitamin D Insufficiency

Many studies have documented high rates of vitamin D deficiency in premenopausal women in North America, particularly at the end of winter and in northern latitudes (Table 1). When comparing these studies, it is important to remember
the many factors that influence serum 25(OH)D levels, including the type of assay used, the latitude and season in which serum 25(OH)D levels were drawn, and the age and race of the subjects. The cut-off values for 25(OH)D indicating insufficiency vary among the studies; they are all higher than the 27.5 nmol/L used to set the DRI in 1997, but most are lower than the optimal level of 75 nmol/L.

Analysis of NHANES III data for non-Hispanic white, non-Hispanic black, and Mexican American women between the ages of 20 and 39 years, living in the US at lower latitudes (median latitude 32ºN) in winter, found 40% of subjects with serum 25(OH)D levels less than 50 nmol/L and 55% with levels less than 62.5 nmol/L. Because of the unpredictable weather at higher latitudes during winter, the NHANES III data was collected at 25ºN to 47ºN (median 39ºN) during summer. Eighteen percent of 20 to 39 year old non-Hispanic white, non-Hispanic black, and Mexican American women living in the north were found to have serum 25(OH)D levels less than 50 nmol/L and 30% had levels less than 62.5 nmol/L during summer.

In Omaha (41ºN), Kinyamu and colleagues found a much lower incidence of vitamin D insufficiency than other researchers. They compared institutionalized and free-living elderly women with younger women and found only 6% of 25 to 35 year old women had serum 25(OH)D levels less than 30 nmol/L between November and May. Eight percent of the institutionalized women, and 1.6% of the free-living elderly women had serum 25(OH)D levels below 30 nmol/L. More recently in Omaha, Armas and colleagues found a much higher incidence of vitamin D deficiency with 58% of young men and women (19 to 49 years old) having levels below 80 nmol/L at the end of summer and 96% at the end of winter. The same researchers observed that although
Table 1. Prevalence of vitamin D insufficiency in North America.

<table>
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<tr>
<th>Study</th>
<th>Assay Used*</th>
<th>Latitude</th>
<th>Season</th>
<th>Gender</th>
<th>Age</th>
<th>Race</th>
<th>Mean 25(OH)D (nmol/L)</th>
<th>% Below 50 nmol/L</th>
<th>% Below 75 nmol/L</th>
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<td>Winter</td>
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<td>CPB</td>
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<td>F</td>
<td>25 – 35</td>
<td>n/a</td>
<td>70.6 ± 26.3</td>
<td>6% (30 nmol/L)</td>
<td></td>
</tr>
<tr>
<td>Rucker et al. 20027</td>
<td>RIA</td>
<td>51°N Calgary</td>
<td>Winter</td>
<td>M, F</td>
<td>27 – 89</td>
<td>98% White</td>
<td>57.3 ± 21.3</td>
<td>39%</td>
<td>86% (80 nmol/L)</td>
</tr>
<tr>
<td>Sullivan et al. 20054</td>
<td>CPB</td>
<td>44°N Bangor</td>
<td>Winter</td>
<td>F</td>
<td>9 – 11</td>
<td>White</td>
<td>55.9 ± 16.5</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Tangpricha et al. 20028</td>
<td>CPB</td>
<td>42°N Boston</td>
<td>March</td>
<td>M, F</td>
<td>18 – 29</td>
<td>60% White</td>
<td>70 ± 25</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>Vieth et al. 20016</td>
<td>RIA</td>
<td>43°N Toronto</td>
<td>Winter</td>
<td>F</td>
<td>18 – 35</td>
<td>White</td>
<td>58 ± 24</td>
<td>21% (40 nmol/L)</td>
<td></td>
</tr>
<tr>
<td>Weng et al. 2007213</td>
<td>RIA</td>
<td>40°N Philadelphia</td>
<td>Winter</td>
<td>M, F</td>
<td>6 – 21</td>
<td>White</td>
<td>~75</td>
<td>-</td>
<td>51%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Black</td>
<td>~45</td>
<td>-</td>
<td>94%</td>
</tr>
</tbody>
</table>

*RIA = Radioimmunoassay, CPB = Competitive Protein Binding
subjects who reported just casual summer sun exposure (i.e. walking to and from the car) had a seasonal fluctuation in serum 25(OH)D levels, their summer sun exposure was not sufficient to increase serum 25(OH)D levels above 80 nmol/L. In a study of healthy young men and women (18 to 29 years) in Boston (42°N), Tangpricha and colleagues found that 36% had serum 25(OH)D levels less than 50 nmol/L in March.

In 18 to 35 year old women living in Canada (43°N), where milk is fortified with vitamin D at the same level as in the US, 21% of white women, some of whom were taking multivitamin supplements, had 25(OH)D levels less than 40 nmol/L in winter. In Calgary, Canada (51°N), 20% of 27 to 89 year old men and women had serum 25(OH)D levels less than 40 nmol/L; 39% had levels less than 50 nmol/L; and 86% had levels less than 80 nmol/L during winter.

Similar rates of vitamin D insufficiency were seen in children and adolescents in the US. In Bangor, Maine (44°N), over a three-year period, 48% of healthy white adolescent girls (9 to 11 years) were found to have at least one serum 25(OH)D level less than 50 nmol/L at the end of winter and all subjects were found to have at least one serum 25(OH)D level less than 75 nmol/L at the end of winter. Likewise, 51% of white 6 to 21 year olds in the Philadelphia area (40°N) had serum 25(OH)D levels less than 75 nmol/L during winter. The researchers found a much higher prevalence of vitamin D insufficiency in black children with 94% presenting with serum levels less than 75 nmol/L during winter.

As mentioned previously, serum 25(OH)D levels fluctuate seasonally. Sullivan and colleagues saw a 28% (20.1 nmol/L) drop in mean serum 25(OH)D levels from summer to winter in adolescent girls. Arunabh and colleagues found a 37% drop (from
84 to 53 nmol/L) in mean serum 25(OH)D levels from summer to winter in white women. In 24 to 70 year old women, serum 25(OH)D levels increased 18.0 nmol/L (36%) from winter to summer in London. In white women in Toronto, Canada, serum 25(OH)D levels measured in summer were 31% higher than those measured in winter.

Although serum 25(OH)D levels are higher in summer than in winter, there are still cases of vitamin D deficiency at the end of summer. Seventeen percent of adolescent girls in Bangor, Maine had serum 25(OH)D levels less than 50 nmol/L at the end of summer. Nationally, NHANES III data revealed 18% of women living between 25 and 47°N latitude had serum 25(OH)D levels less than 50 nmol/L at the end of summer. NHANES III data also showed that the rate of vitamin D insufficiency was two times higher in females than males of the same age.

Although less common, vitamin D deficiency is present at the end of summer in some individuals, and vitamin D deficiency rates are much higher at the end of winter, affecting up to 94% of some populations. It is not known whether there is a cumulative negative effect of repeated cycles of insufficient wintertime serum 25(OH)D levels during adolescence and young adulthood on the ability to achieve peak bone mass. However, given the many important functions of vitamin D, optimizing levels of serum 25(OH)D year-round is a logical goal.

Supplementation Studies

Previous Supplementation Trials

The general consensus among vitamin D researchers is that the optimal concentration for serum 25(OH)D is at least 75 nmol/L. Many people do not reach these levels in winter. The amount of vitamin D needed to optimize levels for various groups
needs to be determined. Once this amount is ascertained, strategies must be developed to raise the entire population to optimal levels. A number of vitamin D supplementation trials have been completed. When comparing these studies, one must consider the many factors that may affect response to supplementation, including season and exposure to UVB radiation (whether through natural or artificial means), body fat, baseline serum 25(OH)D levels, the form of vitamin D used (vitamin D$_2$ or vitamin D$_3$), the type of assay used, and, in women, exogenous estrogen use. In addition, it is important to consider whether the supplementation period was long enough for the serum 25(OH)D to reach an equilibrium concentration. The half-life of serum 25(OH)D levels is approximately three to four weeks$^{42}$ and the concentration equilibrium is reached approximately four weeks after supplementation begins.$^{216}$ When comparing studies, one must also keep in mind that many studies do not measure the actual vitamin D content of the supplement. Some manufacturers may put more vitamin D in the supplement than the label states in order to compensate for storage losses,$^{216}$ so the response to supplementation may be exaggerated in some studies if the actual vitamin D content is not measured.

In the US, no supplementation studies have focused on the vitamin D needs of premenopausal women. In the few studies worldwide that have supplemented premenopausal women with vitamin D$_3$, they have either failed to consider hormonal contraceptive use, had a small sample size, included men in the sample, or did not account for changing serum 25(OH)D levels due to season. Rarely were the supplements evaluated for actual vitamin D$_3$ content.

Van der Klis and colleagues$^{217}$ gave 800 IU vitamin D$_3$ to six Dutch premenopausal women, five of whom were on oral contraceptives, for four weeks during
March. Serum 25(OH)D levels increased from 46.2 nmol/L to approximately 82 nmol/L, a 1.8 nmol/L increase per microgram of vitamin D3. There was no placebo group to control for the decreasing serum 25(OH)D levels that would be expected during March in the Netherlands (52°N).217

Barnes and colleagues218 administered a chewable supplement containing either 600 IU vitamin D3 and 1500 mg calcium or just 1500 mg calcium daily to 27 men and women aged 18 to 27 years for eight weeks, from January to March, in Northern Ireland (55°N). Use of oral contraceptives was not mentioned. Serum 25(OH)D levels increased 38.6 nmol/L from a baseline level of 47.9 nmol/L in the group receiving vitamin D, and decreased 7.2 nmol/L in the control group. After adjusting for the change in the control group, serum 25(OH)D levels increased 2.1 nmol/L per microgram of vitamin D3 in the treatment group.218

In another study, 14 men and women, aged 22 to 60 years, drank one cup of orange juice fortified with 1000 IU vitamin D3 and 350 mg calcium daily; another 12 subjects drank one cup of orange juice fortified with calcium only every day for 12 weeks, from March until May, in Boston (42°N).219 Serum 25(OH)D levels increased from 37.0 to 94.0 nmol/L in the group receiving vitamin D fortified orange juice. In the control group, serum 25(OH)D levels increased from 50.0 to 73.0 nmol/L due to the spring season. After accounting for the change in the control group, serum 25(OH)D levels increased 1.4 nmol/L per microgram of vitamin D3. Information on oral contraceptive use and menopausal stage was not provided.

In Denmark (56°N), 19 healthy premenopausal women, 5 of whom were taking oral contraceptives, were given either 4000 IU vitamin D2 or vitamin D3 and 500 mg
calcium for eight weeks from September to November. The capsules were analyzed for actual vitamin D content and were found to contain 4400 IU vitamin D3. There was no placebo group to describe the seasonal decrease in serum 25(OH)D levels that would be expected between September and November. In the vitamin D3 group, serum 25(OH)D levels increased from 77.5 nmol/L to 113.4 nmol/L, a change of 0.33 nmol/L per microgram of vitamin D3. After adjusting for a 51.5 nmol/L drop in the serum 25(OH)D concentration in the group supplemented with vitamin D2, the change per microgram of vitamin D3 increased to 0.79 nmol/L. The researchers suggested the drop in serum 25(OH)D levels in the vitamin D2 group was too large to be due to the seasonal change in vitamin D3 production alone and suggested competition between vitamin D2 and vitamin D3 for hydroxylation as the cause.

Vieth and colleagues gave 61 men, and pre-and postmenopausal women either 1000 or 4000 IU vitamin D3 for three months, starting in January and February in Toronto, Canada (43°N). In the group receiving 1000 IU, serum 25(OH)D levels increased from 43.3 nmol/L to 68.7 nmol/L, a 1.02 nmol/L increase per microgram of vitamin D3. In the group receiving 4000 IU, serum 25(OH)D levels increased from 37.9 to 96.4 nmol/L, an increase of 0.59 nmol/L per microgram of vitamin D3.

In a frequently cited attempt to help determine the dose-response relationship for vitamin D3 and serum 25(OH)D levels, Heaney and colleagues studied 67 healthy young men (age 38.7 ± 11.2 years, BMI 26.2 ± 2.4) with daily dietary vitamin D intakes less than 200 IU during winter in Omaha, Nebraska (41°N). Subjects consumed either placebo or 1000, 5000, or 10,000 IU vitamin D3 tablets daily for five months. Serum 25(OH)D levels decreased 11 nmol/L in the placebo group, and increased 12, 92, 159
nmol/L in the 1000, 5000, 10,000 IU groups, respectively. Using the dose-response curves from this data, the researchers determined that serum 25(OH)D levels increase by 0.7 nmol/L for every microgram (40 IU) of vitamin D3 input.209

In the supplementation studies involving young women described above, the increase in serum 25(OH)D levels per microgram of vitamin D3 intake ranged from 0.59 to 2.1 nmol/L, which bracket the value obtained by Heaney and colleagues.209 In the studies in which baseline serum 25(OH)D levels were lowest, the change in serum 25(OH)D levels in response to supplementation was the greatest.210,219

Theoretical Dose Required

Previous studies of 18 to 35 year old men and women in North American (41° to 43°N) have found serum 25(OH)D levels at the end of winter in the range of approximately 58 nmol/L6 to 70 nmol/L.8,212 Using the 0.7 nmol/L increase in 25(OH)D levels per microgram of vitamin D3 intake suggested by Heaney and colleagues,209 a dose of 285 to 970 IU vitamin D3 would be required to optimize the mean vitamin D status of these populations. Furthermore, in a recent editorial by Dawson-Hughes and colleagues,1 researchers suggested at least 800 to 1000 IU vitamin D3 would be necessary to achieve a mean serum 25(OH)D concentration of 75 nmol/L.

Risk and Safety Monitoring

Vitamin D toxicity is not subtle. It causes soft tissue calcification, hypercalcemia, and dehydration. Hypercalcemia is characterized by fatigue, muscle weakness, nausea and vomiting, thirst, and loss of appetite. Development of hypercalciuria (increased ratio of urinary calcium to urinary creatinine) precedes hypercalcemia in vitamin D intoxication220 and is, therefore, a useful and convenient monitor of vitamin D tolerance.
However, hypercalciuria can be caused by many factors unrelated to hypercalcemia and vitamin D intoxication, and should not be considered an adverse effect of vitamin D supplementation if serum calcium levels remain normal.216

Based on a serum 25(OH)D response to supplementation of approximately 1.0 nmol/L per microgram of vitamin D3 intake, the current Adequate Intakes for vitamin D, 200 IU for 1 to 50 year olds; 400 IU for 51 to 70 year olds; and 600 IU for those older than 70, would only increase serum 25(OH)D levels by 4, 8, and 12 nmol/L, respectively. The current tolerable upper limit (2000 IU) would increase serum 25(OH)D levels by 35 nmol/L. Vitamin D intoxication occurs when serum 25(OH)D levels exceed 220 nmol/L221 to 375 nmol/L,216,222,223 or more.224 Using NHANES data, Nesby-O’Dell and colleagues139 found the mean serum 25(OH)D levels of white women in the US to be 82.5 ± 1.5 nmol/L. In summer in Toronto (43°N), 95% of white women had serum 25(OH)D levels no higher than 90 nmol/L.6 The highest serum 25(OH)D levels of sun-replete individuals in the US were 154 to 178 nmol/L.10,99 The current tolerable upper limit, if consumed daily, would be unlikely to cause vitamin D intoxication, even in sun-replete individuals in the US.

Vitamin D toxicity has only occurred in individuals taking more than 10,000 IU daily. Mawer and colleagues221 reported vitamin D intoxication in eight patients who had been taking 50,000 to 200,000 IU vitamin D2 daily for six or more years. Koutkia and colleagues223 reported a patient who presented to the hospital with hypercalcemia after taking an over-the-counter vitamin D3 supplement for two years. When analyzed, the supplement contained 26 to 430 times the labeled amount of vitamin D3. The patient had been taking 156,000 to 2,604,000 IU vitamin D3 for an unknown length of time.223
Serum calcium levels usually normalize within one to four months of discontinuing vitamin D supplements, but it may take years for serum 25(OH)D levels to drop below 115 nmol/L after reaching toxic levels, even without exposure to UVB radiation.

Although the body protects itself from vitamin D intoxication upon exposure to excessive sunlight, in theory, adults receiving profuse amounts of sunshine may be more susceptible to vitamin D toxicity when taking supplements because of the cumulative effect of sun and supplements. In addition, patients with granulomatous diseases such as sarcoidosis and tuberculosis should avoid vitamin D supplements and sunshine because granulomas cause hypercalcemia due to unregulated production of calcitriol.

Summary

The documented cases of vitamin D intoxication have occurred at intake levels far exceeding the upper limit of 2000 IU. Although vitamin D intoxication is rare, and is highly unlikely to occur in individuals supplemented with 800 IU vitamin D₃, safety monitoring of serum calcium and urinary calcium to creatinine ratio may detect changes in calcium excretion related to vitamin D intoxication. At “safe” supplementation levels, the existing literature on supplementation in adult men and women indicates a response to supplementation of approximately 1.0 nmol/L per microgram of vitamin D₃ intake. However, most studies have included men in the sample, and failed to consider the effect of exogenous hormones, body composition, and season on the response to supplementation. A supplementation study of premenopausal women in the United States, that takes into consideration oral contraceptive use, seasonal fluctuations in serum 25(OH)D levels, and body composition, is needed to help determine how much vitamin D₃ is needed to optimize serum 25(OH)D levels in this population.
Rationale for Research Project

Vitamin D has many skeletal and non-skeletal purposes. There is a high prevalence of vitamin D insufficiency in Maine and other areas above 35°N latitude due to inadequate UVB radiation for synthesis of vitamin D during winter. Many factors influence an individual’s vitamin D status, including sun exposure, skin color, body composition, and oral contraceptive use. Researchers speculate that approximately 800 to 1000 IU vitamin D₃ is required to raise serum 25(OH)D levels to at least 75 nmol/L in the absence of sunlight. Further research is needed to determine how much vitamin D₃ is required to optimize serum 25(OH)D levels for different age groups, while taking into consideration sun exposure, skin color, body composition, and hormonal status. There have been no studies looking at the serum 25(OH)D response to supplementation with 800 IU vitamin D₃ in premenopausal women in the United States that takes into consideration the use of hormonal contraceptives and body composition.
Chapter 3

METHODS AND MATERIALS

Study Design

In February 2005, 112 women aged 19 to 35 years enrolled in the study to receive six months of placebo daily followed by five months of either an 800 IU oral vitamin D₃ supplement or a matching placebo daily. The goal was to achieve a serum 25(OH)D level of at least 75 nmol/L in the treatment group during winter.

The project took place over 16 months (Figure 1):

- November 2004 – February 2005: Four-month recruitment period
- February 2005: Measurement of baseline serum 25(OH)D levels
- March – September 2005: Six-month run-in period
- September 2005 – February 2006: Five-month placebo-controlled, double-blind vitamin D supplementation period

Blood samples were drawn in February 2005, September 2005, and February 2006 for analysis of serum 25(OH)D, PTH, and calcium levels. Urine samples were obtained in February 2005, September 2005, and February 2006 for analysis of urinary calcium and creatinine levels.

Each participant had a total body scan using dual energy x-ray absorptiometry (DXA) in March 2005 and again in March 2006 for determination of percent body fat and bone mineral density. Height was measured in March 2005 and March 2006; weight in light clothing without shoes was measured in March 2005, September 2005, and March 2006.
Figure 1. The Vitamin D Study timeline.
Upon enrollment, subjects completed a Health History Questionnaire (Appendix B), which was then reviewed with them to determine eligibility for the study. Every three months thereafter, participants completed a brief lifestyle questionnaire (Appendix B) to update their health history and provide information about sun exposure, oral contraceptive use, and so on. In addition, three-day food records were collected upon enrollment and during the following winter.

**Subjects and Recruitment**

One hundred twelve women, aged 19 to 35 years, living in the Bangor, ME area, were recruited from a pool of roughly 6000 female students at the University of Maine and 15,000 women in this age range in Penobscot County, Maine. Enrollment was open to any ethnic or racial group. Premenopausal women were chosen as a homogeneous group in which to assess vitamin D intake requirements.

During the fall semester of 2004, students were recruited at the University of Maine through mailings to all female freshman, sophomores, and juniors (see Appendix C), and through e-mail folders and bulletin boards. Women within the community were recruited through fliers on public bulletin boards (see Appendix D) and through a local television station.

To be eligible for participation in the study, subjects were required to have a body mass index between 18.5 and 40 kg/m², and be free of any disease or condition that influences calcium, or vitamin D metabolism, such as hyper- and hypothyroidism, primary hyperparathyroidism, diabetes, celiac disease, cystic fibrosis, liver disease, kidney disease, or inflammatory bowel disease. Women who were pregnant or planned to become pregnant during the study period were excluded, as were those taking any
medications that affect calcium or vitamin D metabolism, including corticosteroids and anti-convulsant medications. Subjects were asked not to travel to a southern latitude between September and March, and to refrain from using tanning booths. Subjects had not regularly consumed any calcium or vitamin D-containing supplement within the previous four months and agreed not to take any calcium, vitamin D, or multivitamin supplements other than those provided during the study period. Subjects with baseline serum 25(OH)D levels less than 22.5 or greater than 175 nmol/L were excluded.

Subjects were recruited in November and December 2004. In January 2005 informational meetings were held for women interested in participating in the study. Interested individuals completed a brief, confidential Health History Questionnaire (see Appendix B), which was then reviewed with them to screen for eligibility.

Written informed consent was obtained from each subject. This study was approved by the University of Maine Human Subjects Committee and St. Joseph Hospital’s Institutional Review Board. The consent form is found in Appendix E.

January 2005

Interested individuals attended informational meetings to learn more about the study and to be screened for eligibility. At this time, written informed consent was obtained from each subject. Appointments were scheduled to have blood drawn at the University of Maine Cutler Health Center in February and to have dual energy x-ray absorptiometry (DXA) scans for assessment of body composition at the Maine Center for Osteoporosis Research and Education (MECORE) in March. Subjects were given instructions for completing a three-day food record to include two weekdays and one weekend day.
Baseline - February / March 2005

In February 2005, prior to the University of Maine spring break, participants reported to Cutler Health Center to have their blood drawn for measurement of baseline serum 25(OH)D, PTH, and calcium levels. A random urine sample was obtained from each subject for measurement of urinary calcium and creatinine. Participants answered a brief lifestyle questionnaire to update their health history and provide information about nutritional supplement use, contraceptives, and sun exposure, including winter vacation locations and use of tanning booths. In March 2005, participants reported to the Maine Center for Osteoporosis Research and Education (MECORE) for DXA scan and measurement of height and weight.

At MECORE, subjects received their first three-month supply of placebo capsules, which were manufactured by Tishcon, Corporation (Westbury, NY). During the run-in period from March to September 2005, all subjects took a placebo for six months. Subjects with less than 75 percent compliance with placebo, as determined by pill counts every three months, were to be dismissed from the trial.

June 2005

In June 2005, subjects who were in the Bangor area for the summer reported to the Vitamin D Research Room (123 Hitchner Hall, University of Maine) to return unused capsules for assessment of compliance and to pick-up a new supply of placebo. At this time, subjects completed a lifestyle questionnaire to update their health history (see Appendix B). Because estrogen-containing medications affect serum 25(OH)D levels, the lifestyle questionnaire given every three months included questions to detect changes in use or non-use of prescription contraceptives.
A new supply of placebo was mailed to subjects who were not in the Bangor area during the summer. Subjects were instructed to finish the pills in their current pill bottle before starting the new bottle. All unused capsules were to be returned for counting in September. The lifestyle questionnaire was mailed to these subjects along with an addressed and stamped envelope for ease of return.

**September 2005**

Subjects were contacted via email in late August 2005 to schedule appointments for phlebotomy at Cutler Health Center in September. In September, subjects reported to Cutler Health Center again to provide blood and urine samples, and to have their weights measured. Participants returned unused placebo capsules.

In August, individuals were randomly assigned to either the treatment group or the control group using Research Randomizer (version 3.0) software. The random number generator assigned subjects to either the treatment group or the placebo group. One-third of the subjects (36/103) were randomized to the placebo group; two-thirds (67/103) to the treatment group. At the time of randomization, *t*-tests were conducted and no significant differences were seen between the two groups regarding baseline serum 25(OH)D levels, percent body fat, or age. An investigator not directly involved with the subjects transmitted the list of subjects in the treatment group and the placebo group to the pharmacist at Cutler Health Center to be packaged in child-resistant vials labeled with each subject’s name and identification number. The researchers and subjects remained blinded to the content of the capsule taken by the subjects. In September, subjects picked up a three-month supply of either placebo or 800 IU vitamin D₃ and completed another lifestyle questionnaire (see Appendix B), which included a
skin type questionnaire that was used to categorize them according to Fitzpatrick skin type (I-VI).227

December 2005

In December 2005 subjects reported to the Vitamin D Research Room to return unused capsules for assessment of compliance and to pick-up a new supply of capsules. Returned capsules were counted to measure percent compliance. Subjects completed another lifestyle questionnaire to update their health histories (see Appendix B). Instructions for recording three-day food records were reviewed with subjects and they were asked to again record intake on two weekdays and one weekend day.

February / March 2006

In February 2006, prior to the University of Maine spring break, participants reported to Cutler Health Center to provide blood and urine samples. Participants answered a final brief lifestyle questionnaire to update their health history (see Appendix B) and returned unused capsules, which were counted to measure percent compliance. The compliance rates were calculated for September to December and December to February, and the mean percent compliance was used in data analysis. In March 2006, participants reported to the Maine Center for Osteoporosis Research and Education (MECORE) for the final DXA scan and measurement of height and weight.

Vitamin D₃ Supplements

The supplements and matching placebo were manufactured by Tishcon Corporation (Westbury, NY). The capsules were analyzed for actual vitamin D₃ content using high performance liquid chromatography (HPLC) on three separate occasions. At the time of manufacture (February 2005), Tishcon Corporation measured 869 IU vitamin
D₃ per capsule. At the beginning and end of the supplementation period, an independent laboratory (Analytical Laboratories of Anaheim, Inc) measured 956 IU vitamin D₃ (September 2005) and 832 IU vitamin D₃ (April 2006). The average vitamin D₃ content of the capsules was 885 IU vitamin D₃. No vitamin D₃ was detected in the placebo capsules by either the manufacturer or the independent laboratory.

Qualified medical personnel at Cutler Health Center packaged the capsules in child-resistant vials labeled with each subject’s name and identification number. The placebo capsules contained maltrin and magnesium stearate; the vitamin D capsules contained maltrin, magnesium stearate, and vitamin D₃.

**Analysis of Serum and Urine Samples**

Non-fasting serum samples were obtained by skilled phlebotomists from Cutler Health Center in February 2005, September 2005, and February 2006. Samples from all subjects were drawn within a two-week time period. Ten milliliter blood specimens were centrifuged within two hours of being drawn, and the serum was removed and frozen in one milliliter aliquots at -70°C until analysis. Serum samples were shipped on dry ice to the Medical University of South Carolina, Charleston for analysis of 25(OH)D and PTH by Dr. Bruce Hollis. Serum 25(OH)D was measured by radioimmunoassay. The intraassay and interassay coefficients of variation were less than 10%. Intact PTH was measured by radioimmunoassay (DiaSorin Inc., Corp., Stillwater, MN). The intraassay and interassay coefficients of variation were less than 10%. Serum samples were assayed for baseline 25(OH)D levels in March 2005 to determine eligibility for the study and to assure that mean baseline 25(OH)D levels were not significantly different between groups at randomization. Serum samples from all three points in time were batch-
analyzed at the conclusion of the study to avoid inter-assay variation. Serum samples were protected from ultraviolet light during processing.

Serum 25(OH)D levels were measured in September and February when levels are at their highest and lowest points of the year, respectively.\textsuperscript{122} Five months elapsed from the start of vitamin D\textsubscript{3} supplementation to the next measurement of serum 25(OH)D to allow ample time for equilibration at the new level of intake before levels were reassessed.

Serum calcium was measured by a colorimetric assay using the clinical analyzer (Beckman-Coulter CX-4 PRO) in the Clinical Nutrition Laboratory at the University of Maine. A random urine sample was collected from each subject in February 2005, September 2005, and February 2006 for measurement of calcium and creatinine content. Urinary calcium and creatinine were measured by a colorimetric assay using the clinical analyzer (Beckman-Coulter CX-4 PRO) in the Clinical Nutrition Laboratory at the University of Maine, and the urinary calcium to creatinine ratio was calculated. The calcium to creatinine ratio was used to monitor tolerance to the supplementation.

**Anthropometric Measurements**

Height was measured in March 2005 and March 2006 using a calibrated stadiometer. Weight in light clothing, without shoes, was measured using an electronic standing scale in March and September 2005 and March 2006.

Body fat content was measured using dual energy x-ray absorptiometry (DXA) on a Hologic QDR 2000 (Hologic Inc., Waltham, MA, USA) by a single DXA technician in March 2005 and March 2006 at the Maine Center for Osteoporosis Research and Education. DXA is a valid measure of body composition and body fat.\textsuperscript{229} The DXA
machine was calibrated against a phantom daily. The coefficient of variation (CV) was 0.6%. Urine pregnancy tests were confirmed negative prior to DXA scans.

**Questionnaires**

Every three months between February 2005 and February 2006, participants answered a brief lifestyle questionnaire to update their health history and provide information about nutritional supplement use, intake of alcoholic beverages, hormonal contraceptive use, and sun exposure, including winter vacation locations and use of tanning booths. Specific brand names of hormonal contraceptives were obtained and a daily average intake of estradiol was calculated. Subjects also completed a skin type questionnaire to categorize them according to Fitzpatrick skin type (I-VI).

**Dietary Intake Assessment**

Subjects completed three-day food records in February 2005 and again in February 2006. Instructions for recording three-day food records (two weekdays and one weekend day) were reviewed with subjects prior to keeping each food record. Subjects were instructed to record intake immediately after consumption and to include as much information as possible including brand names, package sizes, and recipes when possible. Serving sizes were either measured with measuring cups when possible, or estimated using common household items (i.e. ¼ cup is approximately the size of a golf ball). Food records were analyzed using Nutritionist Pro software, version 2.5 (Axxya Systems, Stafford, TX) for calories, protein, calcium, sodium, and vitamin D intake.

**Sun Exposure Questionnaire**

The February 2005 and September 2005 Lifestyle Questionnaires (see Appendix B) included a series of questions used for calculating sun exposure. Subjects were
asked the approximate number of days worked per week, the number of days off per week, and the number of days spent on vacation. In addition, they were asked how many hours they spent outside between 1000 EST and 1500 EST while at work, on their days off, or on vacation. Based on a 16-week summer (May through August), the average number of hours spent outside per week was calculated. An example showing the calculation of the average weekly number of hours of sun exposure can be found in Appendix F.

**Statistical Analysis**

All data from subject records were entered into a Microsoft Excel spreadsheet. The statistical analysis was performed using SPSS, Student Version 13.0.

Vitamin D intake, sun exposure, body fat, BMI, and oral contraceptive use are known to affect serum 25(OH)D levels. Both multiple and logistic regression were used to measure the effects of these variables on serum 25(OH)D levels, the serum 25(OH)D response to supplementation with 800 IU vitamin D₃, and the seasonal increase in serum 25(OH)D levels. Additionally, age, years of oral contraceptive use, tanning during winter, BMI, sun exposure during the previous summer, skin type, the summer increase in serum 25(OH)D levels, smoking, day of the menstrual cycle on which blood was drawn, and dietary calcium and vitamin D intake were included as independent variables in the logistic regression analysis of baseline serum 25(OH)D levels. The cut-off for baseline serum 25(OH)D levels in the binary logistic regression was 75 nmol/L (the optimal serum 25(OH)D level). In the analysis of the one-year change in serum 25(OH)D levels, estrogen dose, the magnitude of the summer increase in serum 25(OH)D levels, baseline 25(OH)D levels, the randomization group, mean body fat, change in
serum calcium levels, smoking, one-year change in calcium and vitamin D intake, alcohol consumption, the one-year change in calcium to creatinine ratio, one-year change in sun exposure, tanning bed use during winter, and the number of days with a cold were included as independent variables. The cut-off for one-year change in serum 25(OH)D levels in the binary logistic regression was 40 nmol/L (0.25 standard deviation above the mean).

In the logistic regression analyses, the Wald statistic was used to test the significance of the regression coefficients for each independent variable.\textsuperscript{231} Nagelkerke’s $R^2$ was used to approximate the percent of variance explained by the model.\textsuperscript{232} The Hosmer and Lemeshow test, a chi-square test of goodness of fit, was used to determine whether the regression model fit the data at an acceptable level.\textsuperscript{233}

Student’s $t$-test was used to compare means between two variables, and one-way analysis of variance was used to compare means between multiple variables. Assumptions were tested by examining normal probability plots of residuals and scatter diagrams of residuals versus predicted residuals. No violations of normality, linearity, or homogeneity of variance were detected.

Statistical significance was set at 0.05.
Chapter 4

RESULTS

Subject Characteristics

In February 2005, 112 subjects enrolled in the study, 98 of whom completed the study in February 2006 (a retention rate of 88%). Ten subjects chose to withdraw from the study and four subjects were excluded from the study because their baseline (February 2005) serum 25(OH)D levels were outside the range (22.5 to 175 nmol/L) established for inclusion criteria (two were above and two were below the acceptable range). Reasons cited for withdrawing from the study included moving out of the area (5/10), desiring to take a multivitamin (2/10), and personal reasons (3/10). Twelve subjects who either started or quit using hormonal contraceptives during the study were excluded from analysis, leaving a total of 86 participants (Figure 2, flowchart).

The baseline (February 2005) characteristics are summarized in Tables 2a and 2b for the 103 subjects who were randomized to receive either placebo or 800 IU vitamin D₃. Independent samples t-tests revealed no significant differences between the groups.
Table 2a. Comparison of baseline characteristics between the treatment and placebo groups in the 103 subjects who were randomized.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>67 22.0 ± 2.9</td>
<td>36 22.3 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>67 25.8 ± 4.9</td>
<td>36 26.4 ± 5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>67 30.0 ± 7.4</td>
<td>36 30.7 ± 7.6</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>67 164.8 ± 6.2</td>
<td>36 163.3 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67 70.1 ± 14.6</td>
<td>36 70.0 ± 13.3</td>
<td>NS</td>
</tr>
<tr>
<td>2004 Summer sun exposure (h/wk)</td>
<td>67 16.0 ± 9.4</td>
<td>36 18.1 ± 8.6</td>
<td>NS</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>67 61.9 ± 25.1</td>
<td>36 60.3 ± 22.1</td>
<td>NS</td>
</tr>
<tr>
<td>Serum PTH (pg/mL)</td>
<td>67 30.7 ± 9.7</td>
<td>36 34.0 ± 14.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>67 9.7 ± 0.27</td>
<td>36 9.7 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Urine calcium:creatinine</td>
<td>67 0.14 ± 0.08</td>
<td>36 0.13 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Years of contraceptive use*</td>
<td>43 2.67 ± 2.1</td>
<td>25 3.2 ± 3.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Only includes subjects using hormonal contraceptives

Treatment and placebo groups were compared with use of independent samples \( t \)-tests.

Table 2b. Comparison of the daily nutrient intake at baseline (February 2005) between the treatment and placebo groups in the 103 subjects who were randomized.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal/d)</td>
<td>67 1759 ± 455</td>
<td>36 1749 ± 418</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>67 67.4 ± 19.8</td>
<td>36 67.1 ± 18.6</td>
<td>NS</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>67 2980 ± 1007</td>
<td>36 2905 ± 996</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>67 890 ± 403</td>
<td>36 903 ± 357</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin D (IU/d)</td>
<td>67 143 ± 120</td>
<td>36 139 ± 101</td>
<td>NS</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples \( t \)-tests.
116 Subjects Recruited

February 2005
4 No-shows for blood draw
(n = 112)

March – June 2005
4 excluded for baseline 25(OH)D levels outside range
3 w/d for personal reasons
2 w/d to take vitamins
(n = 103)

Randomization

June – Sept 2005
5 moved away
(n = 98)

12 excluded for changing oral contraceptive use
(n = 86)

Placebo Group
31 Subjects

Vitamin D Group
55 Subjects

Figure 2. Flowchart of participation.
Characteristics of Subjects Who Did Not Complete the Study

Serum PTH levels were significantly higher in the 26 individuals who did not complete the study compared to the 86 subjects who completed the study, but there were no other significant differences at baseline (Table 3).

Table 3. Comparison of baseline characteristics of subjects who completed the study and those who did not.

<table>
<thead>
<tr>
<th></th>
<th>Did Not Complete the Study</th>
<th>Completed the Study</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Age (y)</td>
<td>26</td>
<td>21.7 ± 2.1</td>
<td>86</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26</td>
<td>25.9 ± 4.9</td>
<td>86</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>26</td>
<td>58.2 ± 32.6</td>
<td>86</td>
</tr>
<tr>
<td>Serum PTH (pg/mL)</td>
<td>26</td>
<td>36.6 ± 13.5</td>
<td>86</td>
</tr>
<tr>
<td>2004 Summer sun exposure (h/wk)</td>
<td>26</td>
<td>17.0 ± 8.6</td>
<td>86</td>
</tr>
<tr>
<td>Calorie intake (kcal/d)</td>
<td>25</td>
<td>1745 ± 426</td>
<td>86</td>
</tr>
<tr>
<td>Protein intake (g/d)</td>
<td>25</td>
<td>69.5 ± 24.3</td>
<td>86</td>
</tr>
<tr>
<td>Sodium intake (mg/d)</td>
<td>25</td>
<td>3089 ± 1039</td>
<td>86</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>25</td>
<td>847 ± 453</td>
<td>86</td>
</tr>
<tr>
<td>Vitamin D intake (IU/d)</td>
<td>25</td>
<td>149 ± 86</td>
<td>86</td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

Baseline Characteristics of Subjects Who Completed the Study

The baseline (February 2005) characteristics are summarized in Tables 4a and 4b for the 86 subjects who completed the study. Independent samples t-tests revealed no significant differences between the treatment and placebo groups.
Table 4a. Comparison of treatment and placebo groups at baseline (February 2005) in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Age (y)</td>
<td>55</td>
<td>22.3 ± 3.1</td>
<td>31</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>55</td>
<td>25.5 ± 4.8</td>
<td>31</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>55</td>
<td>29.8 ± 7.3</td>
<td>31</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>55</td>
<td>164.7 ± 6.2</td>
<td>31</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55</td>
<td>69.3 ± 14.1</td>
<td>31</td>
</tr>
<tr>
<td>2004 Summer sun exposure (h/wk)</td>
<td>55</td>
<td>15.4 ± 9.3</td>
<td>31</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>55</td>
<td>62.1 ± 24.0</td>
<td>31</td>
</tr>
<tr>
<td>Serum PTH (pg/mL)</td>
<td>55</td>
<td>29.7 ± 9.1</td>
<td>31</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>55</td>
<td>9.8 ± 0.27</td>
<td>31</td>
</tr>
<tr>
<td>Urine calcium:creatinine</td>
<td>55</td>
<td>0.149 ± 0.80</td>
<td>31</td>
</tr>
<tr>
<td>Oral contraceptive use (y)</td>
<td>55</td>
<td>1.8 ± 2.0</td>
<td>31</td>
</tr>
<tr>
<td>Compliance (%)</td>
<td>55</td>
<td>97.5 ± 11.4</td>
<td>31</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 4b. Comparison of the daily intake from food at baseline (February 2005) between the treatment and placebo groups in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Calories (kcal/d)</td>
<td>55</td>
<td>1729 ± 451</td>
<td>31</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>55</td>
<td>67.0 ± 19.2</td>
<td>31</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>55</td>
<td>2899 ± 941</td>
<td>31</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>55</td>
<td>892 ± 385</td>
<td>31</td>
</tr>
<tr>
<td>Vitamin D (IU/d)</td>
<td>55</td>
<td>138 ± 125</td>
<td>31</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

**Baseline Serum 25(OH)D Levels**

Thirty-eight percent of the subjects were vitamin D deficient (serum 25(OH)D levels less than 50 nmol/L), 33% were vitamin D insufficient (serum 25(OH)D 50 to 74 nmol/L), and 29% had optimal vitamin D levels (serum 25(OH)D levels at least 75 nmol/L). Pearson’s chi-square revealed no significant differences in the percent of
subjects in the treatment and placebo groups with optimal, insufficient, or deficient serum 25(OH)D levels at baseline (Figure 3). Independent samples $t$-tests showed no significant differences in mean serum 25(OH)D levels between the groups (Table 5).

![Bar chart showing the frequency of vitamin D sufficiency, insufficiency, and deficiency at baseline (February 2005) in the 86 subjects who completed the study.]

**Figure 3.** Frequency of vitamin D sufficiency, insufficiency, and deficiency at baseline (February 2005) in the 86 subjects who completed the study.

**Table 5.** Comparison of mean serum 25(OH)D levels in the placebo and treatment groups by level of baseline serum 25(OH)D levels.

<table>
<thead>
<tr>
<th>Baseline Serum 25(OH)D Category</th>
<th>Placebo Group</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$n$</td>
</tr>
<tr>
<td>&lt;50 nmol/L</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Mean Serum 25(OH)D ± SD (nmol/L)</td>
<td>40.8 ± 5.0</td>
<td>37.7 ± 8.6</td>
</tr>
<tr>
<td>50 – 74 nmol/L</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Mean Serum 25(OH)D ± SD (nmol/L)</td>
<td>65.1 ± 7.1</td>
<td>62.3 ± 8.1</td>
</tr>
<tr>
<td>≥75 nmol/L</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Mean Serum 25(OH)D ± SD (nmol/L)</td>
<td>89.0 ± 11.0</td>
<td>92.7 ± 14.9</td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared with use of independent sample $t$-tests. Percent of subjects in treatment versus placebo groups were compared using Pearson’s chi-square test, with no significant differences.
**Hormonal Contraceptives**

Thirty-four subjects (62%) in the treatment group and 24 (77%) in the placebo group used hormonal contraceptives. Pearson chi-square analysis indicated there was no significant difference in the percent of subjects using hormonal contraceptives in the treatment group compared to the placebo group. Baseline serum 25(OH)D levels were not significantly different between the placebo and treatment groups for the subjects not on hormonal contraceptives, on the low or high estrogen doses, or for the hormonal contraceptive users overall (Table 6a). However, serum 25(OH)D levels were significantly higher in the subjects in the medium estrogen dose group who were in the treatment group compared to the placebo group (Table 6a). Serum 25(OH)D levels were significantly higher in hormonal contraceptive users than in non-users, and analysis of variance showed that serum 25(OH)D levels increased as exogenous estrogen dose increased (Table 6b). Serum 25(OH)D levels and exogenous estrogen dose were positively correlated ($r = 0.463$, $p < 0.0005$). Ninety-seven percent of non-hormonal contraceptive users compared to 51% of hormonal contraceptive users had serum 25(OH)D levels less than 75 nmol/L at baseline (February 2005).
Table 6a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among levels of exogenous estrogen exposure in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>Non-hormonal contraceptive users</td>
<td>21</td>
<td>49.0 ± 16.0</td>
<td>7</td>
</tr>
<tr>
<td>Hormonal contraceptive users</td>
<td>34</td>
<td>70.2 ± 24.8</td>
<td>24</td>
</tr>
<tr>
<td>Non-hormonal contraceptive users</td>
<td>21</td>
<td>49.0 ± 16.0</td>
<td>7</td>
</tr>
<tr>
<td>Low exogenous estrogen (15 μg/d)</td>
<td>6</td>
<td>46.5 ± 7.6</td>
<td>3</td>
</tr>
<tr>
<td>Medium exogenous estrogen (20-25 μg/d)</td>
<td>9</td>
<td>80.2 ± 22.6</td>
<td>6</td>
</tr>
<tr>
<td>High exogenous estrogen (&gt;25 μg/d)</td>
<td>19</td>
<td>72.9 ± 25.2</td>
<td>15</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 6b. Baseline (February 2005) serum 25(OH)D levels among levels of exogenous estrogen exposure in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-hormonal contraceptive users</td>
<td>28</td>
<td>48.3 ± 14.8</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Hormonal contraceptive users</td>
<td>58</td>
<td>68.6 ± 24.0</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>A  Non-hormonal contraceptive users</td>
<td>28</td>
<td>48.3 ± 14.8</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>B  Low exogenous estrogen (15 μg/d)</td>
<td>9</td>
<td>49.5 ± 11.6</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>C  Medium exogenous estrogen (20-25 μg/d)</td>
<td>15</td>
<td>68.9 ± 24.2</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>D  High exogenous estrogen (&gt;25 μg/d)</td>
<td>34</td>
<td>73.6 ± 24.2</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among estrogen doses with use of ANOVA. Tukey’s post-hoc analysis: A,C (p = 0.012); A,D (p < 0.0005); B,D (p = 0.013); B,C (p = 0.121)

Body Mass Index

Pearson chi-square analysis shows no significant differences in the percent of subjects in the treatment group compared to the placebo group who were of healthy
weight, overweight, or obese according to their BMI (Figure 4). Serum 25(OH)D levels did not differ between treatment and control groups among the three BMI categories (Table 7a). There was a significant inverse correlation between baseline serum 25(OH)D levels and BMI ($r = -0.383$, $p < 0.0005$), and serum 25(OH)D levels are significantly lower in the obese subjects compared to the healthy-weight subjects (Table 7b).

![Figure 4. Percent of subjects of healthy weight, overweight, and obese at baseline (February 2005) in the 86 subjects who completed the study.](image)

Table 7a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among BMI categories in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>BMI</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>$n$</td>
</tr>
<tr>
<td>$&lt;25$ kg/m$^2$</td>
<td>29</td>
<td>68.8 ± 24.6</td>
<td>17</td>
</tr>
<tr>
<td>$25 – 30$ kg/m$^2$</td>
<td>18</td>
<td>61.6 ± 21.2</td>
<td>7</td>
</tr>
<tr>
<td>$&gt;30$ kg/m$^2$</td>
<td>8</td>
<td>39.1 ± 11.9</td>
<td>7</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples $t$-tests.
Table 7b. Baseline (February 2005) serum 25(OH)D levels among BMI categories in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>BMI</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &lt;25 kg/m²</td>
<td>46</td>
<td>69.2 ± 23.2</td>
<td>0.001</td>
</tr>
<tr>
<td>B 25 – 30 kg/m²</td>
<td>25</td>
<td>59.7 ± 22.1</td>
<td></td>
</tr>
<tr>
<td>C &gt;30 kg/m²</td>
<td>15</td>
<td>43.8 ± 14.6</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among levels of BMI with use of ANOVA. Tukey’s post-hoc analysis: A,C (p < 0.0005); B,C (p = 0.069); A,B (p = 0.184).

Percent Body Fat

There was no significant difference in serum 25(OH)D levels (Table 8a) in the treatment group compared to the placebo group among tertiles of percent body fat. There was a significant inverse correlation between baseline serum 25(OH)D levels and percent body fat (r = -0.405, p < 0.0005) and serum 25(OH)D levels were significantly lower in subjects in the highest tertile of body fat compared to those in the lower tertiles (Table 8b).

Table 8a. Comparison of baseline (February 2005) serum 25(OH)D levels between treatment and placebo groups among tertiles of body fat in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Percent Body Fat Tertile</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>&lt;26%</td>
<td>19</td>
<td>69.8 ± 22.7</td>
<td>9</td>
</tr>
<tr>
<td>26 – 33%</td>
<td>17</td>
<td>68.3 ± 25.4</td>
<td>12</td>
</tr>
<tr>
<td>&gt;33%</td>
<td>19</td>
<td>48.9 ± 18.9</td>
<td>10</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Table 8b. Baseline (February 2005) serum 25(OH)D levels among tertiles of body fat in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Percent Body Fat Tertile</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &lt;26%</td>
<td>28</td>
<td>69.0 ± 22.2</td>
<td></td>
</tr>
<tr>
<td>B 26 – 33%</td>
<td>29</td>
<td>69.4 ± 23.8</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>C &gt;33%</td>
<td>29</td>
<td>47.8 ± 17.3</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of body fat with use of ANOVA. Significant differences (Tukey’s post-hoc analysis): A,C (p = 0.001); B,C (p = 0.001).

Weight

There was no significant difference in baseline serum 25(OH)D levels between the treatment and placebo group among the tertiles of body weight (Table 9a). Weight was inversely correlated with serum 25(OH)D levels (r = -0.302, p = 0.005). However, serum 25(OH)D levels did not differ among the tertiles of body weight (Table 9b).

Table 9a. Comparison of baseline (February 2005) serum 25(OH)D levels between treatment and placebo groups among tertiles of body weight in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Baseline Body Weight Tertile</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>&lt;62.5 kg</td>
<td>21</td>
<td>65.1 ± 22.0</td>
<td>8</td>
</tr>
<tr>
<td>62.5 – 71 kg</td>
<td>16</td>
<td>62.5 ± 27.3</td>
<td>14</td>
</tr>
<tr>
<td>&gt;71 kg</td>
<td>18</td>
<td>58.2 ± 24.0</td>
<td>9</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 9b. Baseline (February 2005) serum 25(OH)D levels among tertiles of body weight in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Baseline Body Weight Tertile</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;62.5 kg</td>
<td>29</td>
<td>66.3 ± 21.8</td>
<td></td>
</tr>
<tr>
<td>62.5 – 71 kg</td>
<td>30</td>
<td>64.2 ± 24.7</td>
<td></td>
</tr>
<tr>
<td>&gt;71 kg</td>
<td>27</td>
<td>55.0 ± 22.8</td>
<td>0.170</td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of weight with use of ANOVA.
Skin Type

Skin types II and III made up 76% of the overall group (Figure 5). There were no significant differences in the frequency of each skin type between the treatment and placebo groups. Within each skin type there was no difference in serum 25(OH)D level between the treatment and placebo groups (Table 10a). Skin type was not significantly correlated with serum 25(OH)D levels in February 2005 ($r = 0.181, p = 0.095$), nor was there a significant difference in serum 25(OH)D levels among skin types (Table 10b).

![Figure 5. Frequency of skin type at baseline (February 2005) in the 86 subjects who completed the study.](image)

### Table 10a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among skin types in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>Treatment Group</th>
<th></th>
<th>Placebo Group</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td></td>
</tr>
<tr>
<td>I Extremely fair</td>
<td>2</td>
<td>28.8 ± 16.6</td>
<td>2</td>
<td>65.6 ± 43.0</td>
<td>NS</td>
</tr>
<tr>
<td>II Fair</td>
<td>13</td>
<td>60.7 ± 23.5</td>
<td>10</td>
<td>58.2 ± 22.2</td>
<td>NS</td>
</tr>
<tr>
<td>III Medium</td>
<td>29</td>
<td>62.6 ± 24.2</td>
<td>13</td>
<td>59.1 ± 23.8</td>
<td>NS</td>
</tr>
<tr>
<td>IV Olive or Light brown</td>
<td>10</td>
<td>70.8 ± 22.7</td>
<td>6</td>
<td>72.8 ± 16.0</td>
<td>NS</td>
</tr>
<tr>
<td>V Brown</td>
<td>1</td>
<td>46.3</td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples $t$-tests.
Table 10b. Baseline (February 2005) serum 25(OH)D levels among skin types in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Extremely fair</td>
<td>4</td>
<td>47.2 ± 34.1</td>
<td></td>
</tr>
<tr>
<td>II Fair</td>
<td>23</td>
<td>59.6 ± 22.5</td>
<td></td>
</tr>
<tr>
<td>III Medium</td>
<td>42</td>
<td>61.5 ± 23.8</td>
<td>NS</td>
</tr>
<tr>
<td>IV Olive or Light brown</td>
<td>16</td>
<td>71.5 ± 19.9</td>
<td></td>
</tr>
<tr>
<td>V Brown</td>
<td>1</td>
<td>46.3</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among skin type with use of ANOVA.

Tanning Bed Use

Seven (13%) subjects in the treatment group and four (13%) subjects in the placebo group used a tanning salon prior to having their blood drawn in February 2005. There was no significant difference in mean 25(OH)D levels between the treatment and placebo groups (Table 11a). Although subjects who used a tanning salon had higher serum 25(OH)D levels than did those who did not use a tanning salon, independent samples t-tests revealed this difference was not significant (Table 11b).

Table 11a. Baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among users and non-users of tanning beds during winter 2004-2005 in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>No tanning bed use – Winter 2004-2005</td>
<td>48</td>
<td>59.4 ± 22.6</td>
<td>27</td>
</tr>
<tr>
<td>Tanning bed use – Winter 2004-2005</td>
<td>7</td>
<td>80.2 ± 27.3</td>
<td>4</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Table 11b. Baseline (February 2005) serum 25(OH)D levels among users and non-users of tanning beds during winter 2004-2005 in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tanning bed use –</td>
<td>75</td>
<td>60.2 ± 22.7</td>
<td>0.061</td>
</tr>
<tr>
<td>Winter 2004-2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanning bed use –</td>
<td>11</td>
<td>74.3 ± 25.6</td>
<td></td>
</tr>
<tr>
<td>Winter 2004-2005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

Summer Sun Exposure

There were no significant differences in serum 25(OH)D levels between the treatment and placebo groups in any tertile of summer sun exposure (Table 12a).

Summer sun exposure and serum 25(OH)D levels were positively correlated ($r = 0.229$, $p = 0.034$). However, analysis of variance showed that the increase in serum 25(OH)D levels among tertiles of sun exposure was not significant (Table 12b).

Table 12a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among tertiles of weekly sun exposure during summer 2004 in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Summer Sun Exposure</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>&lt;11 h/wk</td>
<td>22</td>
<td>57.9 ± 22.6</td>
<td>7</td>
</tr>
<tr>
<td>11 – 20 h/wk</td>
<td>17</td>
<td>60.6 ± 19.8</td>
<td>12</td>
</tr>
<tr>
<td>&gt;20 h/wk</td>
<td>16</td>
<td>69.5 ± 29.2</td>
<td>12</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Table 12b. Baseline (February 2005) serum 25(OH)D levels among tertiles of weekly sun exposure during summer 2004 in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Summer Sun Exposure</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11 h/wk</td>
<td>29</td>
<td>58.11 ± 22.7</td>
<td>NS</td>
</tr>
<tr>
<td>11 – 20 h/wk</td>
<td>29</td>
<td>62.0 ± 21.1</td>
<td></td>
</tr>
<tr>
<td>&gt;20 h/wk</td>
<td>28</td>
<td>66.1 ± 26.4</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of sun exposure with use of ANOVA.

Dietary Vitamin D Intake

There was no significant difference in serum 25(OH)D levels between the treatment and placebo groups in any tertile of dietary vitamin D intake (Table 13a).

There was no correlation between serum 25(OH)D and vitamin D intake, nor did serum 25(OH)D levels differ significantly among tertiles of vitamin D intake (Table 13b).

Table 13a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among tertiles of dietary vitamin D intake in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Dietary Vitamin D Tertile</th>
<th>n</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
</tr>
<tr>
<td>&lt;73 IU/d</td>
<td>20</td>
<td>60.7 ± 28.0</td>
<td>8</td>
<td>56.3 ± 26.2</td>
</tr>
<tr>
<td>73 – 159 IU/d</td>
<td>17</td>
<td>65.7 ± 26.7</td>
<td>13</td>
<td>60.6 ± 22.9</td>
</tr>
<tr>
<td>&gt;159 IU/d</td>
<td>18</td>
<td>60.3 ± 16.4</td>
<td>10</td>
<td>68.0 ± 20.0</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 13b. Baseline (February 2005) serum 25(OH)D levels among tertiles of dietary vitamin D intake in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Dietary Vitamin D Tertile</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;73 IU/d</td>
<td>28</td>
<td>59.4 ± 27.1</td>
<td>NS</td>
</tr>
<tr>
<td>73 – 159 IU/d</td>
<td>30</td>
<td>63.5 ± 24.8</td>
<td></td>
</tr>
<tr>
<td>&gt;159 IU/d</td>
<td>28</td>
<td>63.0 ± 17.8</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of vitamin D intake with use of ANOVA.
Dietary Calcium Intake

There was no significant difference in serum 25(OH)D levels between the treatment and placebo groups in any tertile of dietary calcium intake (Table 14a). There was no correlation between serum 25(OH)D and calcium intake, nor did serum 25(OH)D levels differ significantly among tertiles of calcium intake (Table 14b).

Table 14a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among tertiles of dietary calcium intake in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Dietary Calcium Intake</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>&lt;703 mg/d</td>
<td>20</td>
<td>63.3 ± 28.5</td>
<td>9</td>
</tr>
<tr>
<td>703 – 983 mg/d</td>
<td>19</td>
<td>65.1 ± 23.8</td>
<td>11</td>
</tr>
<tr>
<td>&gt;983 mg/d</td>
<td>16</td>
<td>57.1 ± 18.0</td>
<td>11</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 14b. Baseline (February 2005) serum 25(OH)D levels among tertiles of dietary calcium intake in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Dietary Calcium Intake</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;703 mg/d</td>
<td>29</td>
<td>62.1 ± 28.0</td>
<td>NS</td>
</tr>
<tr>
<td>703 – 983 mg/d</td>
<td>30</td>
<td>62.2 ± 22.5</td>
<td></td>
</tr>
<tr>
<td>&gt;983 mg/d</td>
<td>27</td>
<td>61.7 ± 19.4</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of calcium intake with use of ANOVA.

Alcohol Consumption

Alcohol consumption was positively associated with serum 25(OH)D levels \( (r = 0.221, p = 0.040) \). Among tertiles of alcohol consumption, serum 25(OH)D levels did not differ significantly between the treatment and placebo groups (Table 15a). Analysis of variance revealed significant differences in serum 25(OH)D levels between the lowest and highest tertiles of alcohol consumption (Table 15b).
Table 15a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among tertiles of alcohol consumption in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Alcohol Intake</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>0 drinks/wk</td>
<td>18</td>
<td>51.1 ± 20.0</td>
<td>12</td>
</tr>
<tr>
<td>0.5 - 3 servings/wk</td>
<td>20</td>
<td>59.1 ± 18.5</td>
<td>11</td>
</tr>
<tr>
<td>&gt;3 servings/wk</td>
<td>17</td>
<td>77.3 ± 26.9</td>
<td>8</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 15b. Baseline (February 2005) serum 25(OH)D levels among tertiles of alcohol consumption in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Alcohol Intake</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 drinks/wk</td>
<td>30</td>
<td>53.9 ± 20.9*</td>
<td>0.011</td>
</tr>
<tr>
<td>0.5 - 3 servings/wk</td>
<td>31</td>
<td>61.3 ± 19.2</td>
<td></td>
</tr>
<tr>
<td>&gt;3 servings/wk</td>
<td>25</td>
<td>72.6 ± 27.3*</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of alcohol consumption with use of ANOVA.
*Tukey’s post-hoc analysis: p = 0.008

Smoking

Ninety-three percent (51/55) of the treatment group and 97% (30/31) of the placebo group did not smoke cigarettes. Four subjects (3 in the treatment group and 1 in the placebo group) smoked less than half a pack of cigarettes per day. One subject in the treatment group smoked more than one pack of cigarettes per day. Serum 25(OH)D levels were not significantly different between the placebo and treatment groups among smokers and non-smokers (Table 16a). There was a trend toward an inverse relationship between smoking and serum 25(OH)D levels ($r = -0.180, p = 0.097$). Serum 25(OH)D levels, however, were not significantly different between smokers and non-smokers (Table 16b).
Table 16a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among smokers and non-smokers in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>51</td>
<td>63.0 ± 24.3</td>
<td>30</td>
</tr>
<tr>
<td>Smokers</td>
<td>4</td>
<td>50.0 ± 17.4</td>
<td>1</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.

Table 16b. Baseline (February 2005) serum 25(OH)D levels among smokers and non-smokers in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>81</td>
<td>63.0 ± 23.5</td>
<td>0.125</td>
</tr>
<tr>
<td>Smokers</td>
<td>5</td>
<td>46.4 ± 17.1</td>
<td></td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

**Serum PTH**

There were no significant differences in serum PTH levels between the treatment and placebo groups among tertiles of serum 25(OH)D levels (Table 17a). There was no significant correlation between baseline serum PTH levels and serum 25(OH)D levels, nor were there any significant differences in serum PTH levels among tertiles of serum 25(OH)D at baseline (Table 17b).

Table 17a. Comparison of baseline (February 2005) serum PTH levels between the treatment and placebo groups among tertiles of serum 25(OH)D levels in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Baseline Serum 25(OH)D Tertiles</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean PTH ± SD (pg/mL)</td>
<td>n</td>
</tr>
<tr>
<td>Lowest tertile (&lt;47 nmol/L)</td>
<td>16</td>
<td>32.6 ± 7.2</td>
<td>12</td>
</tr>
<tr>
<td>Middle tertile (47 – 72.4 nmol/L)</td>
<td>20</td>
<td>27.6 ± 10.2</td>
<td>9</td>
</tr>
<tr>
<td>Highest tertile (≥72.5 nmol/L)</td>
<td>19</td>
<td>29.5 ± 9.1</td>
<td>10</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Table 17b. Baseline (February 2005) serum PTH levels among tertiles of serum 25(OH)D in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Baseline Serum 25(OH)D Tertiles</th>
<th>n</th>
<th>Mean PTH ± SD (pg/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lowest tertile (&lt;47 nmol/L)</td>
<td>28</td>
<td>33.0 ± 11.6</td>
<td></td>
</tr>
<tr>
<td>B Middle tertile (47 – 72.4 nmol/L)</td>
<td>29</td>
<td>29.0 ± 12.2</td>
<td>NS</td>
</tr>
<tr>
<td>C Highest tertile (≥72.5 nmol/L)</td>
<td>29</td>
<td>30.7 ± 10.1</td>
<td></td>
</tr>
</tbody>
</table>

Mean PTH levels were compared among tertiles of baseline 25(OH)D levels with use of ANOVA.

Menstrual Cycle

Serum 25(OH)D levels did not differ between treatment and placebo groups during any phase of the menstrual cycle (Table 18a), nor did serum 25(OH)D levels differ among menstrual cycle phases (Table 18b). However, there was a positive correlation between day of menstrual cycle and serum 25(OH)D levels ($r = 0.319$, $p = 0.003$).

Table 18a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among three menstrual cycle phases in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
</tr>
<tr>
<td>Days* 0 – 10</td>
<td>19</td>
<td>59.2 ± 22.2</td>
<td>7</td>
</tr>
<tr>
<td>Days* 11 – 20</td>
<td>20</td>
<td>64.1 ± 23.6</td>
<td>12</td>
</tr>
<tr>
<td>Days* 21 - 35</td>
<td>11</td>
<td>63.7 ± 21.7</td>
<td>9</td>
</tr>
</tbody>
</table>

*Day of menstrual cycle on which blood was drawn for 25(OH)D analysis. Treatment and placebo groups were compared with use of independent samples $t$-tests.
Table 18b. Baseline (February 2005) serum 25(OH)D levels among three menstrual cycle phases in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Days*</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>26</td>
<td>63.1 ± 23.0</td>
<td></td>
</tr>
<tr>
<td>11 – 20</td>
<td>32</td>
<td>64.2 ± 24.2</td>
<td>NS</td>
</tr>
<tr>
<td>21 – 35</td>
<td>20</td>
<td>57.9 ± 19.5</td>
<td></td>
</tr>
</tbody>
</table>

*Day of menstrual cycle on which blood was drawn for 25(OH)D analysis. Mean 25(OH)D levels were compared among menstrual cycle phase with use of ANOVA.

Number of Days with a Cold

The number of days with a cold is being used as a rough estimate of subjects’ immune status. Eighty-five out of 86 subjects completed this question on the questionnaire. Serum 25(OH)D levels differed significantly between treatment and placebo groups in the highest tertile of the number of days with a cold, but were not significantly different in the lower two tertiles (Table 19a). Serum 25(OH)D levels did not differ among tertiles of the number of cold days (Table 19b). There was no correlation between the number of cold days and serum 25(OH)D levels.

Table 19a. Comparison of baseline (February 2005) serum 25(OH)D levels between the treatment and placebo groups among tertiles of number of days with a cold in 85 of the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean 25(OH)D ± SD (nmol/L)</td>
<td>n</td>
</tr>
<tr>
<td>0 to 0.66 days</td>
<td>18</td>
<td>67.2 ± 23.9</td>
<td>10</td>
</tr>
<tr>
<td>0.67 to 7 days</td>
<td>19</td>
<td>64.3 ± 28.7</td>
<td>11</td>
</tr>
<tr>
<td>&gt; 7 days</td>
<td>17</td>
<td>53.7 ± 17.2</td>
<td>10</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Table 19b. Baseline (February 2005) serum 25(OH)D levels among tertiles of number of days with a cold in 85 of the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Days with a Cold (d)</th>
<th>n</th>
<th>Mean 25(OH)D ± SD (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 0.66</td>
<td>28</td>
<td>64.1 ± 22.9</td>
<td></td>
</tr>
<tr>
<td>0.67 to 7</td>
<td>30</td>
<td>60.8 ± 26.9</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;7</td>
<td>27</td>
<td>60.8 ± 20.6</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among tertiles of days with a cold with use of ANOVA.

Variables Affecting Baseline Serum 25(OH)D Levels

Linear and logistic regression analyses were performed to determine which factors influenced baseline serum 25(OH)D levels. Both methods identified estrogen dose, percent body fat, and alcohol consumption to be significant predictors of baseline serum 25(OH)D levels. In addition, logistic regression found the number of days with a cold during winter 2004-2005 to be significant.

The advantage of using logistic regression is that the dependent variable, serum 25(OH)D level, can be categorized into optimal (≥75 nmol/L) and suboptimal (<75 nmol/L) levels, and one can determine which parameters predict optimal serum 25(OH)D levels, which is especially useful to a clinician. The logistic regression results are described below.

Logistic regression analysis was used to predict the probability that the baseline serum 25(OH)D levels would be greater than 75 nmol/L. A backward stepwise approach was used for this analysis. In the backward stepwise model, all of the variables start in the model and, in a stepwise fashion, one variable is eliminated at each step. Estrogen dose, percent body fat, number of days with a cold, and alcohol consumption were the predictors included in the final model.
A test of the full model versus a model with constant only was statistically significant, $\chi^2 (df = 4) = 42.661, p < 0.0005$. Table 20a shows the logistic regression coefficient, Wald test, and odds ratio for estrogen dose, percent body fat, number of days with a cold, and alcohol consumption. Table 20b shows the logistic regression coefficient and Wald test for the variables eliminated from the final model.

The model was able to correctly classify 86% of the subjects with serum 25(OH)D levels less than 75 nmol/L and 72% of those with levels greater than 75 nmol/L, with an overall success rate of 81% (Table 20c). Nagelkerke $R^2$ was 0.577 and $-2 \text{Log likelihood}$ was 57.456. Fifty-eight percent of the variation in baseline serum 25(OH)D levels can be explained by the logistic regression model.

A Hosmer and Lemeshow Test was performed, $\chi^2 (df = 8) = 2.429, p < 0.965$. Therefore we did not reject the null hypothesis that the observed and predicted values are the same and concluded that the model fits the data reasonably well.

Table 20a. Logistic regression predicting baseline serum 25(OH)D levels from estrogen dose, percent body fat, number of days with a cold, and alcohol consumption.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>S.E.</th>
<th>Wald</th>
<th>$p$</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen dose</td>
<td>0.164</td>
<td>0.043</td>
<td>14.503</td>
<td>&lt;0.0005</td>
<td>1.179</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>-0.103</td>
<td>0.057</td>
<td>3.304</td>
<td>0.069</td>
<td>0.902</td>
</tr>
<tr>
<td>Number of days with a cold</td>
<td>-0.141</td>
<td>0.060</td>
<td>5.460</td>
<td>0.019</td>
<td>0.869</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.240</td>
<td>0.109</td>
<td>4.866</td>
<td>0.027</td>
<td>1.272</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.519</td>
<td>1.806</td>
<td>0.707</td>
<td>0.400</td>
<td>0.219</td>
</tr>
</tbody>
</table>
Table 20b. Variables eliminated from the backward stepwise logistic regression model predicting baseline serum 25(OH)D levels.

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable Eliminated</th>
<th>Beta coefficient</th>
<th>Wald Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Smoking</td>
<td>-59.815</td>
<td>0.000</td>
<td>0.999</td>
</tr>
<tr>
<td>2</td>
<td>Years of oral contraceptive use</td>
<td>-0.005</td>
<td>0.000</td>
<td>0.984</td>
</tr>
<tr>
<td>3</td>
<td>Sun exposure summer 2004</td>
<td>-0.003</td>
<td>0.007</td>
<td>0.933</td>
</tr>
<tr>
<td>4</td>
<td>Tanning bed use</td>
<td>-0.107</td>
<td>0.010</td>
<td>0.920</td>
</tr>
<tr>
<td>5</td>
<td>BMI</td>
<td>-0.015</td>
<td>0.008</td>
<td>0.929</td>
</tr>
<tr>
<td>6</td>
<td>Summer 25(OH)D increase</td>
<td>0.004</td>
<td>0.095</td>
<td>0.758</td>
</tr>
<tr>
<td>7</td>
<td>Age</td>
<td>0.040</td>
<td>0.103</td>
<td>0.748</td>
</tr>
<tr>
<td>8</td>
<td>Vitamin D from diet</td>
<td>-0.001</td>
<td>0.406</td>
<td>0.524</td>
</tr>
<tr>
<td>9</td>
<td>Calcium from diet</td>
<td>-0.001</td>
<td>0.406</td>
<td>0.524</td>
</tr>
<tr>
<td>10</td>
<td>Skin type</td>
<td>0.352</td>
<td>0.630</td>
<td>0.428</td>
</tr>
<tr>
<td>11</td>
<td>Day of menstrual cycle</td>
<td>0.035</td>
<td>0.962</td>
<td>0.327</td>
</tr>
</tbody>
</table>

Table 20c. Classification table.

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th>&lt;75 nmol/L</th>
<th>≥75 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;75 nmol/L</td>
<td>48</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>≥75 nmol/L</td>
<td>7</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 48/56 = 86%
Specificity = 18/25 = 72%
Positive Predictive Value = 48/55 = 87%
Negative Predictive Value = 18/26 = 69%
Total Correct = 66/81 = 81%

September 2005 Serum 25(OH)D Levels and the Seasonal Increase in Levels

In September 2005, the mean serum 25(OH)D level was 103.0 ± 33.2 nmol/L. The mean seasonal summer increase was 41.0 ± 26.1 nmol/L. Seventy-seven percent of subjects had optimal serum 25(OH)D levels (≥75 nmol/L); 22% had insufficient levels (50 – 74 nmol/L); and no subjects had deficient levels (<50 nmol/L) at the end of the summer.

There was no significant difference in September serum 25(OH)D levels or the seasonal increase in serum 25(OH)D levels between the treatment and placebo groups.
There was also no significant difference between treatment and placebo groups regarding September serum 25(OH)D levels and the seasonal change when these groups were further divided into hormonal contraceptive users (OCP+) and non-users (OCP-; Table 21a). Hormonal contraceptive users had significantly higher September serum 25(OH)D levels in the treatment group ($p = 0.025$) and in the placebo group ($p = 0.007$) compared with non-users. The seasonal summer increase did not differ significantly between hormonal contraceptive users in the treatment group ($p = 0.362$) or in the placebo group ($p = 0.090$) (Table 21a), or in the overall group (Table 21b). Nor did the seasonal change differ significantly among doses of estrogen (Table 21b).

### Table 21a. Comparison of the September serum 25(OH)D levels and the seasonal summer increase in serum 25(OH)D levels between treatment and placebo groups in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>(nmol/L)</td>
<td>$n$</td>
</tr>
<tr>
<td>September 25(OH)D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>104.7 ± 33.1</td>
<td>31</td>
</tr>
<tr>
<td>OCP-</td>
<td>27</td>
<td>94.6 ± 25.0</td>
<td>10</td>
</tr>
<tr>
<td>OCP+</td>
<td>28</td>
<td>114.4 ± 37.3</td>
<td>21</td>
</tr>
<tr>
<td>Seasonal summer increase in 25(OH)D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>42.6 ± 27.9</td>
<td>31</td>
</tr>
<tr>
<td>OCP-</td>
<td>27</td>
<td>46.1 ± 25.6</td>
<td>10</td>
</tr>
<tr>
<td>OCP+</td>
<td>28</td>
<td>39.2 ± 30.1</td>
<td>21</td>
</tr>
</tbody>
</table>

*OCP- group includes individuals with $\leq 15$ μg daily estrogen (contraceptive patch and ring users)

Treatment and placebo groups were compared with use of independent samples $t$-tests.
Table 21b. Seasonal increase in serum 25(OH)D levels among levels of estrogen dose in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Estrogen Dose</th>
<th>n</th>
<th>Summer Increase in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-oral contraceptive users*</td>
<td>37</td>
<td>41.3 ± 26.3</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive users</td>
<td>49</td>
<td>40.9 ± 26.3</td>
<td>NS</td>
</tr>
<tr>
<td>Non-oral contraceptive users*</td>
<td>28</td>
<td>40.9 ± 25.5</td>
<td></td>
</tr>
<tr>
<td>Low-exogenous estrogen (15 μg/d)</td>
<td>9</td>
<td>42.6 ± 30.1</td>
<td>NS</td>
</tr>
<tr>
<td>Medium exogenous estrogen (20-25 μg/d)</td>
<td>15</td>
<td>48.8 ± 29.4</td>
<td></td>
</tr>
<tr>
<td>High exogenous estrogen (&gt;25 μg/d)</td>
<td>34</td>
<td>37.3 ± 24.4</td>
<td></td>
</tr>
</tbody>
</table>

*Non-oral contraceptive users include individuals with ≤15 μg daily estrogen (contraceptive patch and ring users).
Subject groups were compared with use of independent samples t-tests.
Mean 25(OH)D levels were compared among levels of estrogen dose with use of ANOVA.

Pearson correlations were performed between the magnitude of seasonal change in serum 25(OH)D levels and estrogen dose, percent body fat, baseline serum 25(OH)D levels, skin type, and summer sun exposure. There was a significant inverse relationship between skin type and seasonal change (r = -0.282, p = 0.009). Individuals with darker skin pigmentation experienced less seasonal increase in serum 25(OH)D levels than did those with lighter skin. One-way analysis of variance with Tukey’s post-hoc analysis identified significant differences between September serum 25(OH)D levels in subjects with extremely fair skin and those with light brown skin (Table 22). There were no significant correlations between the seasonal change in serum 25(OH)D levels and estrogen dose, percent body fat, baseline serum 25(OH)D levels, or summer sun exposure.
Table 22. Seasonal change in serum 25(OH)D levels among skin types in the 86 subjects who completed the study.

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>n</th>
<th>Summer Increase in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Extremely fair</td>
<td>4</td>
<td>73.9 ± 7.7*</td>
<td>0.028</td>
</tr>
<tr>
<td>II Fair</td>
<td>23</td>
<td>42.5 ± 32.7</td>
<td></td>
</tr>
<tr>
<td>III Medium</td>
<td>42</td>
<td>41.7 ± 21.6</td>
<td></td>
</tr>
<tr>
<td>IV Olive / Light brown</td>
<td>16</td>
<td>30.7 ± 23.6*</td>
<td></td>
</tr>
<tr>
<td>V Brown</td>
<td>1</td>
<td>15.3</td>
<td></td>
</tr>
</tbody>
</table>

Mean 25(OH)D levels were compared among skin type with use of ANOVA. *Tukey’s post-hoc analysis: mean difference is significant ($p = 0.015$).

Logistic regression analysis was performed to determine which factors influenced the summer seasonal increase in serum 25(OH)D levels. Logistic regression analysis was used to predict the probability that the summer increase in serum 25(OH)D levels would be at least 60 nmol/L. A backward stepwise approach was used for this analysis. Skin type was the only predictor included in the final model.

A test of the full model versus a model with constant only was statistically significant, $X^2 (df = 1) = 15.160$, $p < 0.0005$. Table 23a shows the logistic regression coefficient, Wald test, and odds ratio for skin type. Table 23b shows the logistic regression coefficient and Wald test for the variables eliminated from the final model.

The model was able to correctly classify 100% of the subjects with a seasonal change less than 60 nmol/L and 20% of those with a change greater than 60 nmol/L, with an overall success rate of 95% (Table 23c). Nagelkerke $R^2$ was 0.244 and -2 Log likelihood was 78.124. Twenty-four percent of the variation in the seasonal change in serum 25(OH)D levels was explained by the logistic regression model.

A Hosmer and Lemeshow Test was performed, $X^2 (df = 1) = 0.237$, $p < 0.627$. Therefore we did not reject the null hypothesis that the observed and predicted values were the same and concluded that the model fits the data reasonably well.
### Table 23a. Logistic regression predicting the seasonal increase in serum 25(OH)D levels from skin type.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>S.E.</th>
<th>Wald</th>
<th>p</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin type</td>
<td>-1.385</td>
<td>0.406</td>
<td>11.656</td>
<td>0.001</td>
<td>0.250</td>
</tr>
<tr>
<td>Constant</td>
<td>2.465</td>
<td>1.048</td>
<td>5.529</td>
<td>0.019</td>
<td>11.761</td>
</tr>
</tbody>
</table>

### Table 23b. Variables eliminated from the backward stepwise logistic regression model predicting the seasonal increase in serum 25(OH)D levels.

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable Eliminated</th>
<th>Beta coefficient</th>
<th>Wald Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Body fat</td>
<td>0.001</td>
<td>0.001</td>
<td>0.977</td>
</tr>
<tr>
<td>2</td>
<td>Sun exposure summer 2005</td>
<td>-0.005</td>
<td>0.032</td>
<td>0.859</td>
</tr>
<tr>
<td>3</td>
<td>Baseline serum 25(OH)D</td>
<td>0.012</td>
<td>0.705</td>
<td>0.401</td>
</tr>
<tr>
<td>4</td>
<td>Estrogen dose</td>
<td>-0.011</td>
<td>0.287</td>
<td>0.592</td>
</tr>
<tr>
<td>5</td>
<td>Sunscreen use</td>
<td>0.254</td>
<td>1.222</td>
<td>0.269</td>
</tr>
</tbody>
</table>

### Table 23c. Classification table.

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th>&lt;75 nmol/L</th>
<th>≥75 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;75 nmol/L</td>
<td>66</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥75 nmol/L</td>
<td>16</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 66/66 = 100%
Specificity = 4/20 = 20%
Positive Predictive Value = 66/82 = 80%
Negative Predictive Value = 4/4 = 100%
Total Correct = 70/74 = 95%
One-Year Change

Body mass index, percent body fat, weight, vitamin D intake, and urinary calcium:creatinine ratio changed significantly between February 2005 and February 2006 (Table 24a). However, these changes were not significantly different between the placebo and treatment groups (Table 24b).

Table 24a. Comparison of parameters in February 2005 and February 2006 in the overall group (n=86).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>February 2005 Mean ± SD</th>
<th>February 2006 Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>85</td>
<td>25.8 ± 5.0</td>
<td>26.5 ± 5.5</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>85</td>
<td>30.0 ± 7.5</td>
<td>31.3 ± 7.7</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85</td>
<td>69.7 ± 14.0</td>
<td>71.8 ± 15.1</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Vitamin D from diet (IU/d)</td>
<td>83</td>
<td>137 ± 118</td>
<td>103 ± 96</td>
<td>0.008</td>
</tr>
<tr>
<td>Summer sun exposure, 2004 and 2005 (h/wk)</td>
<td>86</td>
<td>16.3 ± 9.2</td>
<td>15.4 ± 9.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>86</td>
<td>9.7 ± 0.26</td>
<td>9.8 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Urine calcium:creatinine</td>
<td>86</td>
<td>0.141 ± 0.083</td>
<td>0.168 ± 0.113</td>
<td>0.046</td>
</tr>
<tr>
<td>Number of cold days</td>
<td>85</td>
<td>6.47 ± 7.45</td>
<td>5.48 ± 6.19</td>
<td>NS</td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

Table 24b. Comparison of one-year change in parameters between treatment and placebo groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Treatment Group</td>
<td>Placebo Group</td>
<td>p</td>
</tr>
<tr>
<td>Change in BMI (kg/m²)</td>
<td>55</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Change in body fat (%)</td>
<td>55</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Change in weight (kg)</td>
<td>55</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Change in vitamin D intake (IU/d)</td>
<td>53</td>
<td>30</td>
<td>0.211</td>
</tr>
<tr>
<td>Change in sun exposure (h/wk)</td>
<td>55</td>
<td>31</td>
<td>NS</td>
</tr>
<tr>
<td>Change in serum calcium (mg/dL)</td>
<td>55</td>
<td>31</td>
<td>0.150</td>
</tr>
<tr>
<td>Change in urine calcium:creatinine</td>
<td>55</td>
<td>31</td>
<td>0.095</td>
</tr>
<tr>
<td>Change in number of cold days</td>
<td>54</td>
<td>31</td>
<td>NS</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Tolerance to Vitamin D Supplementation

Serum calcium and urinary calcium and creatinine levels were measured to monitor tolerance to the supplementation. Mean serum calcium levels and the urinary calcium to creatinine ratios were not significantly different between the placebo group and the treatment group after five months of supplementation with 800 IU vitamin D₃ (Table 25a). There was no significant difference in serum calcium levels or the calcium to creatinine ratio in the treatment group between February 2005 and February 2006 (Table 25b). In the placebo group, however, the urinary calcium to creatinine ratio increased significantly from February 2005 (0.13 ± 0.09) to February 2006 (0.18 ± 0.14); serum calcium levels did not change in the placebo group (data not shown). The one-year change in serum calcium and in the urine calcium to creatinine ratio was not significantly different between the treatment and placebo groups (Table 24b). After the supplementation period, three subjects in the treatment group and four subjects in the placebo group had urinary calcium to creatinine ratios above 0.40, indicating hypercalciuria. However, all seven subjects had serum calcium levels within normal limits.

Table 25a. Comparison of serum calcium levels and urinary calcium to creatinine ratios in treatment and placebo groups after five month supplementation period.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>55</td>
<td>9.7 ± 0.33</td>
<td>31</td>
</tr>
<tr>
<td>Urinary calcium to creatinine ratio</td>
<td>55</td>
<td>0.16 ± 0.10</td>
<td>31</td>
</tr>
</tbody>
</table>

Treatment and placebo groups were compared with use of independent samples t-tests.
Table 25b. Comparison of serum calcium and urinary calcium to creatinine ratios in the treatment group in February 2005 (before supplementation) and in February 2006, after five months of supplementation.

<table>
<thead>
<tr>
<th></th>
<th>February 2005</th>
<th>February 2006</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 55</td>
<td>n = 55</td>
<td></td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.8 ± 0.27</td>
<td>9.7 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary calcium to creatinine ratio</td>
<td>0.15 ± 0.08</td>
<td>0.16 ± 0.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

Response to Supplementation

As seen in Table 26, serum 25(OH)D levels increased by 35.3 ± 23.2 nmol/L in the treatment group between February 2005 and February 2006. In the placebo group, serum 25(OH)D levels increased 10.9 ± 16.9 nmol/L. The net treatment effect was a 24.4 nmol/L increase in serum 25(OH)D levels.

Table 26. One-year change in serum 25(OH)D levels.

<table>
<thead>
<tr>
<th></th>
<th>February 2005</th>
<th>February 2006</th>
<th>One-Year Change in Serum 25(OH)D Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 25(OH)D (nmol/L)</td>
<td>n 25(OH)D (nmol/L)</td>
<td>(nmol/L)</td>
</tr>
<tr>
<td>Treatment</td>
<td>55 62.1 ± 24.0†</td>
<td>97.4 ± 31.3*†</td>
<td>35.3 ± 23.2*</td>
</tr>
<tr>
<td>Placebo</td>
<td>31 61.9 ± 22.6†</td>
<td>72.7 ± 27.8*†</td>
<td>10.9 ± 16.9*</td>
</tr>
</tbody>
</table>

*Treatment and placebo groups compared using independent samples t-tests, p < 0.0005. †February 2005 and February 2006 serum 25(OH)D levels compared using paired samples t-tests, p < 0.0005.

The actual vitamin D₃ content of the vitamin D capsules was measured by the manufacturer at the time they were made (February 2005, 869 IU), and by an independent laboratory at the beginning (September 2005, 956 IU) and end (April 2006, 832 IU) of the supplementation period. The mean vitamin D₃ content of the capsules was 885 IU (22 μg). The net treatment effect of 24.4 nmol/L in those receiving 885 IU vitamin D₃ daily corresponds to an increase of 1.1 nmol/L for every μg of supplemental vitamin D₃ intake.
Examination of Predictor Variables for Response to Supplementation in the Treatment Group

Baseline Serum 25(OH)D Levels

There was no correlation between baseline (February 2005) serum 25(OH)D levels and the one-year change in serum 25(OH)D levels, nor was there any significant difference in the one-year change in serum 25(OH)D levels among categories of baseline serum 25(OH)D levels in the treatment group (Table 27a). In the placebo group, there was no significant difference in the one-year change in serum 25(OH)D levels among categories of baseline serum 25(OH)D levels (Table 27b).

Table 27a. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among categories of baseline serum 25(OH)D levels.

<table>
<thead>
<tr>
<th>Baseline serum 25(OH)D level (nmol/L)</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 nmol/L</td>
<td>19</td>
<td>34.6 ± 22.0</td>
<td>NS</td>
</tr>
<tr>
<td>50 – 75 nmol/L</td>
<td>21</td>
<td>40.0 ± 22.7</td>
<td></td>
</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>15</td>
<td>29.6 ± 25.4</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among categories of baseline serum 25(OH)D levels with use of ANOVA.

Table 27b. One-year change in serum 25(OH)D levels in the placebo group (n = 31) among categories of baseline serum 25(OH)D levels.

<table>
<thead>
<tr>
<th>Baseline serum 25(OH)D level (nmol/L)</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 nmol/L</td>
<td>14</td>
<td>14.0 ± 17.6</td>
<td>NS</td>
</tr>
<tr>
<td>50 – 75 nmol/L</td>
<td>7</td>
<td>5.8 ± 16.3</td>
<td></td>
</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>10</td>
<td>10.0 ± 16.9</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among categories of baseline serum 25(OH)D levels with use of ANOVA.

Hormonal Contraceptive Use

There was no correlation between exogenous estrogen dose and the one-year change in serum 25(OH)D levels in the treatment group. Analysis of variance revealed
no difference in one-year change in serum 25(OH)D levels among levels of exogenous estrogen dose in the treatment group, or in the placebo group (Table 28).

**Table 28. One-year change in serum 25(OH)D levels in the treatment and placebo groups among levels of exogenous estrogen exposure.**

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>One-Year Change in Serum 25(OH)D (nmol/L)</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>Non-hormonal contraceptive users</td>
<td>21</td>
<td>31.3 ± 21.2</td>
<td>NS</td>
<td>7</td>
</tr>
<tr>
<td>Hormonal contraceptive users</td>
<td>34</td>
<td>37.8 ± 24.4</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Non-hormonal contraceptive users</td>
<td>21</td>
<td>31.3 ± 21.2</td>
<td>NS</td>
<td>7</td>
</tr>
<tr>
<td>Low exogenous estrogen (15 µg/d)</td>
<td>6</td>
<td>34.6 ± 19.3</td>
<td>NS</td>
<td>3</td>
</tr>
<tr>
<td>Medium exogenous estrogen (20-25 µg/d)</td>
<td>9</td>
<td>32.9 ± 23.8</td>
<td>NS</td>
<td>6</td>
</tr>
<tr>
<td>High exogenous estrogen (&gt;25 µg/d)</td>
<td>19</td>
<td>41.0 ± 26.6</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

Hormonal contraceptive users and non-users were compared with use of independent samples $t$-tests.

Mean changes in 25(OH)D levels were compared among levels of estrogen use with use of ANOVA.

**Mean Body Mass Index**

There was no correlation between mean BMI and the one-year change in serum 25(OH)D levels in the treatment group. There was no significant difference in the one-year change in serum 25(OH)D levels among BMI categories in the treatment group (Table 29), or in the placebo group (data not shown).
Table 29. One-year change in serum 25(OH)D levels in the treatment group \((n = 55)\) among mean BMI categories.

<table>
<thead>
<tr>
<th>Mean BMI</th>
<th>(n)</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25 kg/m(^2)</td>
<td>26</td>
<td>37.7 ± 21.0</td>
<td>NS</td>
</tr>
<tr>
<td>25 – 30 kg/m(^2)</td>
<td>20</td>
<td>34.3 ± 27.6</td>
<td></td>
</tr>
<tr>
<td>&gt;30 kg/m(^2)</td>
<td>9</td>
<td>30.5 ± 20.1</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among levels of BMI with use of ANOVA.

Mean Percent Body Fat

There was no correlation between mean percent body fat and the one-year change in serum 25(OH)D levels in the treatment group. There was not a significant difference in the one-year change in serum 25(OH)D levels among tertiles of mean percent body fat in the treatment group (Table 30a), or in the placebo group (Table 30b).

Table 30a. One-year change in serum 25(OH)D levels in the treatment group among tertiles of mean body fat.

<table>
<thead>
<tr>
<th>Tertile of Mean Body Fat</th>
<th>(n)</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;26%</td>
<td>19</td>
<td>39.3 ± 26.8</td>
<td>NS</td>
</tr>
<tr>
<td>26 – 33%</td>
<td>18</td>
<td>32.9 ± 22.9</td>
<td></td>
</tr>
<tr>
<td>&gt;33%</td>
<td>18</td>
<td>33.4 ± 20.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among tertiles of body fat with use of ANOVA.

Table 30b. One-year change in serum 25(OH)D levels in the placebo group \((n = 31)\) among tertiles of mean body fat.

<table>
<thead>
<tr>
<th>Tertile of Mean Body Fat</th>
<th>(n)</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;27%</td>
<td>10</td>
<td>9.3 ± 19.2</td>
<td>NS</td>
</tr>
<tr>
<td>27 – 34.9%</td>
<td>11</td>
<td>15.3 ± 17.2</td>
<td></td>
</tr>
<tr>
<td>≥35%</td>
<td>10</td>
<td>7.6 ± 14.7</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among tertiles of body fat with use of ANOVA.
Mean Body Weight

There was no correlation between mean weight and the one-year change in serum 25(OH)D levels in the treatment group. There was no significant difference in the one-year change in serum 25(OH)D levels among tertiles of mean weight in the treatment group (Table 31), or in the placebo group (data not shown).

Table 31. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among tertiles of mean body weight.

<table>
<thead>
<tr>
<th>Mean Body Weight Tertile</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;62 kg</td>
<td>18</td>
<td>36.0 ± 23.9</td>
<td>0.205</td>
</tr>
<tr>
<td>62 – 74 kg</td>
<td>19</td>
<td>41.5 ± 21.1</td>
<td></td>
</tr>
<tr>
<td>&gt;74 kg</td>
<td>18</td>
<td>27.9 ± 23.8</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among tertiles of weight with use of ANOVA.

Skin Type

There was no correlation between skin type and the one-year change in serum 25(OH)D levels in the treatment group. There was no significant difference in the one-year change in serum 25(OH)D levels among skin type classifications in the treatment group (Table 32), or in the placebo group (data not shown).

Table 32. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among skin types.

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Extremely fair</td>
<td>2</td>
<td>35.1 ± 17.9</td>
<td></td>
</tr>
<tr>
<td>II Fair</td>
<td>13</td>
<td>36.8 ± 22.0</td>
<td></td>
</tr>
<tr>
<td>III Medium</td>
<td>29</td>
<td>37.8 ± 25.8</td>
<td>NS</td>
</tr>
<tr>
<td>IV Olive or Light brown</td>
<td>10</td>
<td>26.4 ± 19.0</td>
<td></td>
</tr>
<tr>
<td>V Brown</td>
<td>1</td>
<td>32.5</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among skin type with use of ANOVA.
Tanning Bed Use

As seen in Table 33, in the treatment group, subjects who only tanned during winter 2005-2006 had a significantly higher one-year change in serum 25(OH)D levels than did subjects who only tanned during winter 2004-2005. In the placebo group, there was no significant difference in the one-year change in serum 25(OH)D levels according to tanning bed use (data not shown).

Table 33. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among users and non-users of tanning beds.

<table>
<thead>
<tr>
<th>Tanning Bed Use</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tanning bed use</td>
<td>45</td>
<td>34.9 ± 22.0</td>
<td></td>
</tr>
<tr>
<td>Tanning bed use both winters</td>
<td>3</td>
<td>39.2 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Tanning bed use – winter 2004-2005 only</td>
<td>3</td>
<td>6.6 ± 19.0*</td>
<td>0.031</td>
</tr>
<tr>
<td>Tanning bed use – winter 2005-2006 only</td>
<td>4</td>
<td>58.0 ± 28.9*</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among levels of tanning bed use with use of ANOVA.
*Tukey’s post-hoc analysis: p = 0.017

Dietary Vitamin D Intake

There was no correlation between mean vitamin D intake and the one-year change in serum 25(OH)D levels in the treatment group. There was no significant difference in the one-year change in serum 25(OH)D levels among tertiles of mean vitamin D intake in the treatment group (Table 34), or in the placebo group (data not shown).
Table 34. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among tertiles of mean dietary vitamin D intake.

<table>
<thead>
<tr>
<th>Mean Vitamin D Intake</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;56 IU</td>
<td>18</td>
<td>33.6 ± 16.8</td>
<td></td>
</tr>
<tr>
<td>56 – 125 IU</td>
<td>19</td>
<td>29.5 ± 24.8*</td>
<td>0.199</td>
</tr>
<tr>
<td>&gt;125 IU</td>
<td>18</td>
<td>42.9 ± 26.0*</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among levels of vitamin D intake with use of ANOVA.

*Tukey’s post-hoc analysis: p = 0.184

Dietary Calcium Intake

There was no correlation between mean calcium intake and the one-year change in serum 25(OH)D levels in the treatment group. There was no significant difference in the one-year change in serum 25(OH)D levels among tertiles of mean calcium intake in the treatment group (Table 35), or in the placebo group (data not shown).

Table 35. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among tertiles of mean dietary calcium intake.

<table>
<thead>
<tr>
<th>Mean Calcium Intake</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;725 mg/d</td>
<td>18</td>
<td>31.7 ± 13.9</td>
<td>NS</td>
</tr>
<tr>
<td>726 to 981 mg/d</td>
<td>19</td>
<td>35.8 ± 29.6</td>
<td></td>
</tr>
<tr>
<td>&gt;981 mg/d</td>
<td>18</td>
<td>38.3 ± 23.7</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among levels of calcium intake with use of ANOVA.

Alcohol Consumption

There was no significant difference in the one-year change in serum 25(OH)D levels among tertiles of alcohol consumption in the treatment group (Table 36), or in the placebo group (data not shown).
Table 36. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among tertiles of alcohol consumption.

<table>
<thead>
<tr>
<th>Tertile of Alcohol Consumption</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 drinks/wk</td>
<td>18</td>
<td>40.2 ± 18.8</td>
<td>NS</td>
</tr>
<tr>
<td>≤3 servings/wk</td>
<td>20</td>
<td>31.7 ± 26.6</td>
<td></td>
</tr>
<tr>
<td>&gt;3 servings/wk</td>
<td>17</td>
<td>34.3 ± 23.7</td>
<td></td>
</tr>
</tbody>
</table>

Mean change in 25(OH)D levels were compared among tertiles of alcohol consumption with use of ANOVA.

Smoking

There was no significant difference in the one-year change in serum 25(OH)D levels between smokers and non-smokers in the treatment group (Table 37), or in the placebo group (data not shown).

Table 37. One-year change in serum 25(OH)D levels in the treatment group (n = 55) among smokers and non-smokers.

<table>
<thead>
<tr>
<th>Tobacco Use</th>
<th>n</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>51</td>
<td>34.8 ± 22.8</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers</td>
<td>4</td>
<td>41.0 ± 31.7</td>
<td></td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

Magnitude of Summer Increase in Serum 25(OH)D Levels

The seasonal summer increase in serum 25(OH)D levels was positively correlated with the one-year change in serum 25(OH)D levels in the treatment group (r = 0.402, p = 0.002). Analysis of variance revealed significant increases in the one-year change in serum 25(OH)D levels among tertiles of the summer increase in serum levels in the treatment group (Table 38a), but not in the placebo group (Table 38b).
Table 38a. One-year change in serum 25(OH)D levels in the treatment group \((n = 55)\) among tertiles of magnitude of seasonal change.

<table>
<thead>
<tr>
<th>Magnitude of Seasonal Change</th>
<th>(n)</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;29 nmol/L</td>
<td>18</td>
<td>25.4 ± 21.0*</td>
<td>0.051</td>
</tr>
<tr>
<td>29 – 52 nmol/L</td>
<td>19</td>
<td>36.3 ± 21.1</td>
<td></td>
</tr>
<tr>
<td>&gt;52 nmol/L</td>
<td>18</td>
<td>44.0 ± 24.7*</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among tertiles of seasonal change with use of ANOVA.
*Tukey’s post-hoc analysis: \(p = 0.041\)

Table 38b. One-year change in serum 25(OH)D levels in the placebo group \((n = 31)\) among tertiles of magnitude of seasonal change.

<table>
<thead>
<tr>
<th>Magnitude of Seasonal Change</th>
<th>(n)</th>
<th>One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;27 nmol/L</td>
<td>10</td>
<td>4.0 ± 11.6</td>
<td>NS</td>
</tr>
<tr>
<td>27 – 42 nmol/L</td>
<td>11</td>
<td>16.0 ± 11.4</td>
<td></td>
</tr>
<tr>
<td>&gt;42 nmol/L</td>
<td>10</td>
<td>12.1 ± 16.9</td>
<td></td>
</tr>
</tbody>
</table>

Mean changes in 25(OH)D levels were compared among tertiles of seasonal change with use of ANOVA.

Serum PTH Concentration

There was no correlation between February 2006 serum PTH levels and the one-year change in serum 25(OH)D levels. As seen in Table 39, February 2006 PTH levels in the treatment group were significantly higher in subjects who started out in the lowest tertile of baseline serum 25(OH)D levels compared to those in the highest tertile. Furthermore, in the subjects who started out in the highest tertile of serum 25(OH)D levels, serum PTH levels were significantly lower in February 2006, after the supplementation period, than in February 2005. In the placebo group, the February 2005 and February 2006 PTH levels were not significantly different among tertiles of baseline serum 25(OH)D levels, nor was the one-year change in PTH significantly different among tertiles of baseline serum 25(OH)D levels (data not shown).
Table 39. February 2006 serum PTH levels and the one-year change in serum PTH levels in the treatment group (n = 55) among tertiles of baseline serum 25(OH)D levels from the overall group.

<table>
<thead>
<tr>
<th>Baseline serum 25(OH)D tertiles (nmol/L)</th>
<th>n</th>
<th>February 2005 Mean Serum 25(OH)D Levels (nmol/L)</th>
<th>February 2006 Mean Serum 25(OH)D Levels (nmol/L)</th>
<th>February 2006 mean serum PTH levels (pg/mL)</th>
<th>p</th>
<th>February 2005 mean serum PTH levels (pg/mL)</th>
<th>p</th>
<th>One-year change in PTH levels p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lowest tertile (&lt; 47 nmol/L)</td>
<td>16</td>
<td>37.4 ± 6.9</td>
<td>71.7 ± 25.7</td>
<td>36.2 ± 10.4*</td>
<td>0.183</td>
<td>32.6 ± 7.2</td>
<td>NS</td>
<td>0.183</td>
</tr>
<tr>
<td>B Middle tertile (47 – 72.4 nmol/L)</td>
<td>20</td>
<td>58.9 ± 8.3</td>
<td>97.6 ± 25.7</td>
<td>30.4 ± 11.3</td>
<td>0.004</td>
<td>27.6 ± 10.2</td>
<td>NS</td>
<td>0.346</td>
</tr>
<tr>
<td>C Highest tertile (≥ 72.5 nmol/L)</td>
<td>19</td>
<td>88.8 ± 13.9</td>
<td>118.7 ± 25.1</td>
<td>23.5 ± 10.3*</td>
<td>0.038</td>
<td>29.5 ± 9.1</td>
<td>NS</td>
<td>0.038</td>
</tr>
</tbody>
</table>

*Tukey’s post-hoc analysis: February 2006 PTH A,C (p = 0.003)
Variables Affecting One-year Change in Serum 25(OH)D Levels

Linear and logistic regression techniques were used to determine which factors predict the one-year change in serum 25(OH)D levels. Both methods identified the treatment group, magnitude of the seasonal summer increase in 25(OH)D levels, estrogen dose, and baseline serum 25(OH)D levels to be significant predictors of one-year change. The logistic regression results are described below (Table 40a).

Logistic regression analysis was used to predict the probability that the one-year increase in serum 25(OH)D levels would be greater than 40 nmol/L. A backward stepwise approach was used for this analysis. In the backward stepwise model, all of the variables start in the model and, in a stepwise fashion, one variable is eliminated at each step. Estrogen dose, summer increase in 25(OH)D levels, baseline 25(OH)D levels, and treatment group were the predictors included in the final model.

A test of the full model versus a model with constant only was statistically significant, $\chi^2 (df = 4) = 37.351, p < 0.0005$. Table 40a shows the logistic regression coefficient, Wald test, and odds ratio for estrogen dose, summer increase in 25(OH)D levels, baseline 25(OH)D levels, and treatment. Table 40b shows the logistic regression coefficient and Wald test for the variables eliminated from the final model.

The model was able to correctly classify 91% of the subjects with a change in 25(OH)D less than 40 nmol/L and 65% of those with a change in levels greater than 40 nmol/L, with an overall success rate of 83% (Table 40c). Nagelkerke $R^2$ was 0.509 and $-2 \log \text{likelihood}$ was 65.847. Fifty-one percent of the variation in one-year change can be explained by the logistic regression model.
A Hosmer and Lemeshow Test was performed, $X^2 (df = 8) = 9.572, p < 0.296$. Therefore we did not reject the null hypothesis that the observed and predicted values are the same and concluded that the model fits the data reasonably well.

**Table 40a. Logistic regression predicting the one-year change in serum 25(OH)D levels from estrogen dose, summer serum 25(OH)D increase, baseline 25(OH)D levels, and treatment category.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>S.E.</th>
<th>Wald</th>
<th>$p$</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen dose</td>
<td>0.073</td>
<td>0.028</td>
<td>6.581</td>
<td>0.010</td>
<td>1.075</td>
</tr>
<tr>
<td>Summer increase</td>
<td>0.045</td>
<td>0.014</td>
<td>10.227</td>
<td>0.001</td>
<td>1.046</td>
</tr>
<tr>
<td>Baseline 25(OH)D</td>
<td>-0.028</td>
<td>0.015</td>
<td>3.346</td>
<td>0.067</td>
<td>0.973</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.338</td>
<td>0.928</td>
<td>12.948</td>
<td>&lt;0.0005</td>
<td>28.170</td>
</tr>
<tr>
<td>Constant</td>
<td>-8.394</td>
<td>2.185</td>
<td>14.755</td>
<td>&lt;0.0005</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Table 40b. Variables eliminated from the backward stepwise logistic regression model predicting one-year change in serum 25(OH)D levels.**

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable Eliminated</th>
<th>Beta coefficient</th>
<th>Wald Coefficient</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean calcium intake</td>
<td>0.000</td>
<td>0.024</td>
<td>0.877</td>
</tr>
<tr>
<td>2</td>
<td>Skin type</td>
<td>-0.087</td>
<td>0.037</td>
<td>0.848</td>
</tr>
<tr>
<td>3</td>
<td>Mean body fat</td>
<td>-0.016</td>
<td>0.071</td>
<td>0.789</td>
</tr>
<tr>
<td>4</td>
<td>Change in serum calcium</td>
<td>0.374</td>
<td>0.136</td>
<td>0.713</td>
</tr>
<tr>
<td>5</td>
<td>Smoking</td>
<td>1.350</td>
<td>0.202</td>
<td>0.653</td>
</tr>
<tr>
<td>6</td>
<td>Change in vitamin D intake</td>
<td>0.002</td>
<td>0.224</td>
<td>0.636</td>
</tr>
<tr>
<td>7</td>
<td>Alcohol</td>
<td>-0.093</td>
<td>0.431</td>
<td>0.511</td>
</tr>
<tr>
<td>8</td>
<td>Change in calcium:creatinine</td>
<td>2.224</td>
<td>0.693</td>
<td>0.405</td>
</tr>
<tr>
<td>9</td>
<td>Days with a cold in 2006</td>
<td>0.051</td>
<td>0.859</td>
<td>0.354</td>
</tr>
<tr>
<td>10</td>
<td>Change in sun exposure</td>
<td>0.034</td>
<td>1.139</td>
<td>0.286</td>
</tr>
<tr>
<td>11</td>
<td>Tanning bed use 2005-2006</td>
<td>1.450</td>
<td>2.374</td>
<td>0.123</td>
</tr>
</tbody>
</table>

**Table 40c. Classification table.**

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th>&lt;40 nmol/L</th>
<th>≥40 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 nmol/L</td>
<td>52</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>≥40 nmol/L</td>
<td>9</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 52/57 = 91%
Specificity = 17/26 = 65%
Positive Predictive Value = 52/61 = 85%
Negative Predictive Value = 17/22 = 77%
Total Correct = 69/83 = 83%
One-Year Change in Serum PTH Levels

Serum PTH followed a seasonal pattern, increasing during winter and decreasing during summer. There were no significant differences in serum PTH levels between the treatment and placebo groups in February 2005, September 2005, or February 2006. Mean serum PTH levels did not change significantly from February 2005 to February 2006 in the placebo group or in the group receiving 800 IU vitamin D3 (Table 41); however, they did change significantly in the treatment group who were in the highest tertile of baseline serum 25(OH)D levels (Table 39). The one-year change in serum PTH levels was not significantly different among tertiles of one-year change in serum 25(OH)D levels (Table 42).

Table 41. Change in serum PTH levels from February 2005 to February 2006.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>February 2005 PTH (pg/mL)</th>
<th>September 2005 PTH (pg/mL)</th>
<th>February 2006 PTH (pg/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>55</td>
<td>29.7 ± 9.1</td>
<td>28.4 ± 14.5</td>
<td>29.7 ± 11.7</td>
<td>NS</td>
</tr>
<tr>
<td>Placebo group</td>
<td>31</td>
<td>32.9 ± 14.4</td>
<td>30.3 ± 17.4</td>
<td>32.3 ± 15.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Independent samples t-tests revealed no significant differences between treatment and placebo groups (p > 0.05).
Independent samples t-tests revealed no significant differences between PTH levels in February 2005 and February 2006 (p > 0.05).

Table 42. One-year change in serum PTH levels in the whole group (n = 86) among tertiles of one-year change in serum 25(OH)D.

<table>
<thead>
<tr>
<th>Mean One-Year Change in Serum 25(OH)D (nmol/L)</th>
<th>n</th>
<th>Mean One-Year Change in Serum PTH (pg/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (26.5 ± 24.1)</td>
<td>86</td>
<td>-0.22 ± 13.0</td>
<td>-</td>
</tr>
<tr>
<td>Lowest tertile (&lt;15 nmol/L)</td>
<td>28</td>
<td>3.0 ± 11.7</td>
<td></td>
</tr>
<tr>
<td>Middle tertile (15 – 38.74 nmol/L)</td>
<td>29</td>
<td>-2.4 ± 13.7</td>
<td>NS</td>
</tr>
<tr>
<td>Highest tertile (≥38.75 nmol/L)</td>
<td>29</td>
<td>-1.1 ± 13.2</td>
<td></td>
</tr>
</tbody>
</table>

Mean change in PTH levels were compared among tertiles of change in 25(OH)D levels with use of ANOVA.
Summary of Serum 25(OH)D Levels Over 12 Months

Serum 25(OH)D levels fluctuated seasonally in the placebo group and the winter decrease was attenuated in the treatment group (Figure 6).

![Figure 6. One-year fluctuation in serum 25(OH)D levels in the treatment and placebo groups.](image)

Of the 55 subjects receiving daily 800 IU vitamin D₃ supplementation, 80% had optimal serum 25(OH)D levels at the end of winter (Figure 7). As seen in Table 42, subjects who achieved optimal serum 25(OH)D levels at the end of winter had significantly higher baseline serum 25(OH)D levels and lower percent body fat. In addition, the subjects who used oral contraceptives appeared to be more likely to have optimal serum 25(OH)D levels (Table 43). However, a Pearson Chi-Square revealed this difference was not significant.
Table 43. Characteristics of subjects in the treatment group who achieved optimal serum 25(OH)D levels (≥75 nmol/L) in February 2006.

<table>
<thead>
<tr>
<th></th>
<th>February 2006 Serum 25(OH)D &lt;75 nmol/L (Suboptimal)</th>
<th>February 2006 Serum 25(OH)D ≥75 nmol/L (Optimal)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 25(OH)D (nmol/L)</td>
<td>40.9 ± 16.4</td>
<td>67.4 ± 22.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Percent Body Fat</td>
<td>35.4 ± 7.4</td>
<td>29.9 ± 7.1</td>
<td>0.028</td>
</tr>
<tr>
<td>Oral Contraceptive Use (%)</td>
<td>27</td>
<td>57</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Subject groups were compared with use of independent samples t-tests.

Figure 7. Changes in the prevalence of vitamin D deficiency and insufficiency over one-year, and in response to 800 IU vitamin D₃ supplementation.

Summary of Association Between Serum 25(OH)D Levels and BMI or Body Composition

There was a significant inverse correlation between serum 25(OH)D levels and BMI and percent body fat in February 2005, September 2005, and February 2006 (in both treatment and placebo groups). However, there was no significant correlation between response to supplementation and BMI or body fat, nor was there a correlation between the seasonal increase in 25(OH)D levels during summer and BMI or body fat. Percent

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body fat was a significant predictor of baseline serum 25(OH)D levels, but was not a significant predictor of the one-year change in serum 25(OH)D levels after treatment with 800 IU vitamin D₃.

**Summary of Association Between Serum 25(OH)D Levels and Hormonal Contraceptives**

There was a significant positive association between serum 25(OH)D levels and exogenous estrogen dose at all time points. Hormonal contraceptive use was a strong predictor of both baseline serum 25(OH)D levels and of the one-year change in serum 25(OH)D levels.
Chapter 5

DISCUSSION

The purpose of this research was to evaluate the serum 25(OH)D response to supplementation with 800 IU vitamin D₃ during winter in premenopausal women living in Maine. The goals were to achieve optimal serum 25(OH)D levels in the supplemented group at the end of winter, and to examine the effects of body composition and oral contraceptive use on baseline serum 25(OH)D levels and response to supplementation.

One hundred twelve women aged 19 to 35 years were recruited to participate in the study. All subjects received placebo from March 2005 until September 2005, at which time they were randomized to receive either 800 IU vitamin D₃, or matching placebo until March 2006. Blood samples were collected from each subject for the analysis of serum 25(OH)D, parathyroid hormone, and calcium levels in March 2005, September 2005, and March 2006. Every three months from March 2005 until March 2006, the subjects picked up a new supply of supplements and completed a brief questionnaire about other factors that affect serum 25(OH)D levels, such as sun exposure, medical conditions, medications, and skin type. In addition, they completed a three-day food record during the winter to assess dietary vitamin D intake. Body fat was measured at the beginning and end of the study using a dual energy x-ray absorptiometry (DXA) scan.

There were high rates of vitamin D insufficiency and deficiency among the subjects in February 2005. Serum 25(OH)D levels increased greatly over the summer and there were no cases of vitamin D deficiency at the end of summer. Eighty percent of
the treatment group had optimal serum 25(OH)D levels at the end of February 2006, after receiving 800 IU vitamin D₃ daily during winter.

**Vitamin D Status in Maine**

Vitamin D insufficiency is common in Maine. At the end of winter, 29% of subjects in this study of white premenopausal women had optimal serum 25(OH)D levels (≥75 nmol/L). In Maine, due to the tilt of the earth, there is insufficient ultraviolet-B radiation from November until March for cutaneous vitamin D synthesis resulting in high levels of vitamin D insufficiency at the end of winter. The vitamin D synthesized during the summer, and the vitamin D consumed from the diet are not able to sustain optimal serum 25(OH)D levels through winter. If vitamin D insufficiency is common in this group of active young women, it is likely indicative of vitamin D insufficiency in all age groups in Maine.

The rates of deficiency in the current study were similar to those seen in other studies in the United States. Analysis of NHANES III data found 40% of premenopausal women (20 to 39 years) had levels less than 50 nmol/L during winter in the southern US (latitude range 25° to 41°N; median 32°N). In the southern US, at latitudes below 35°N, there is sufficient sun for cutaneous synthesis of vitamin D year-round, and, therefore, a lower prevalence of vitamin D deficiency would be expected at the end of the winter. However, the higher prevalence of vitamin D deficiency seen in the NHANES III data is most likely due to the lower serum 25(OH)D levels present in the non-Hispanic Black and Mexican American women who made up 38% and 47% of the sample, respectively. In the 12 to 29 year old female age group in the south, 15% of non-Hispanic white females had serum 25(OH)D levels less than 50 nmol/L during
winter compared to 70% of non-Hispanic black, and 41% of Mexican American females.\textsuperscript{121} Thirty-six percent of 18 to 29 year old healthy men and women in Boston (42°N) had serum 25(OH)D levels less than 50 nmol/L in March.\textsuperscript{8}

Few other studies have examined optimal serum 25(OH)D levels at the end of summer. In the current study, there was a large increase in serum 25(OH)D levels during the summer due to increased cutaneous synthesis of vitamin D. Rates of vitamin D insufficiency were much lower at the end of the summer, and there was no occurrence of vitamin D deficiency (25(OH)D <50 nmol/L). Other studies, on the other hand, showed higher rates of vitamin D deficiency during summer in the US than the current study.\textsuperscript{4,121} NHANES III data showed that, in the northern US, 18% of 20 to 39 year old women had serum 25(OH)D levels less than 50 nmol/L, and only 70% of women in the north had serum 25(OH)D levels greater than 62.5 nmol/L during summer.\textsuperscript{121} The higher prevalence of vitamin D deficiency seen in the NHANES data is again most likely due to the lower levels of serum 25(OH)D seen in non-Hispanic blacks and Mexican Americans. People with dark skin experience a smaller seasonal summer increase in serum 25(OH)D levels than do people with light skin.\textsuperscript{129,141} In addition, the NHANES data includes all serum 25(OH)D levels measured during summer (between April and October) rather than at the end of the summer. Therefore, it includes many serum levels that had not yet peaked, as well as some that had peaked and started to decrease due to declining sun exposure. However, 17% of white adolescent girls in Maine had serum 25(OH)D levels below 50 nmol/L at the end of summer.\textsuperscript{4} Unlike the NHANES data, this higher incidence of deficiency cannot be explained by race or time of measurement, but may be due to the use of a different assay.
In areas far from the equator, such as Maine, serum 25(OH)D levels increase during summer and decrease during winter when there is insufficient sunlight for cutaneous vitamin D synthesis. Over time, the suboptimal serum 25(OH)D levels seen at the end of winter in many people, and even year-round in some people, may result in lower bone mineral densities, increased risk of osteoporosis and fractures, increased risk of autoimmune disease, increased risk of certain cancers, and decreased immune function.\textsuperscript{30} During winter, individuals living in Maine and other sun-deprived areas need supplemental vitamin D to maintain optimal serum 25(OH)D levels.

**Estrogen**

Serum 25(OH)D levels are significantly higher in women who use hormonal contraceptives.\textsuperscript{139,152,153} In addition, although there was no correlation between exogenous estrogen dose and response to supplementation, logistic regression revealed that exogenous estrogen appeared to be one of the forces causing a better response to oral vitamin D supplementation in premenopausal women, independent of estrogen’s effects on baseline serum 25(OH)D levels.

The elevation in serum 25(OH)D levels due to oral contraceptive use in the current study was similar to that seen in other studies.\textsuperscript{139,152,153} At the end of winter, serum 25(OH)D levels were 20.3 nmol/L higher in hormonal contraceptive users. Both Nesby-O’Dell and colleagues,\textsuperscript{139} and Harris and Dawson-Hughes\textsuperscript{153} saw similar differences of 24.8 nmol/L and 24.0 nmol/L, respectively, in white women at the end of winter. Sowers and colleagues\textsuperscript{152} saw a 15.0 nmol/L difference in summertime serum 25(OH)D levels between oral contraceptive users and non-users, which is similar to the 21 nmol/L difference in the current study.
The current study was the only study to look at the effect of the dose of estrogen on serum 25(OH)D levels. Serum 25(OH)D levels increased as the estrogen dose within the contraceptives increased above 15 μg. The lack of an effect at doses of 15 μg could be because 15 μg estradiol was too low to cause an increase in 25(OH)D levels, or it could be because the 15 μg contraceptives were not taken orally, but are in the form of a skin patch, or vaginal ring.

No other studies have examined the effect of estrogen on response to supplementation. The two theories offering explanations for the increased levels of serum 25(OH)D levels in subjects receiving estrogen can also be used as possible explanations for the improved response to supplementation. If estrogen increases hydroxylation activity in the liver, a higher percentage of vitamin D intake would be hydroxylated,\textsuperscript{154,155} increasing serum 25(OH)D levels more in women receiving exogenous estrogen. On the other hand, if estrogen increases serum 25(OH)D levels by increasing vitamin D-binding protein concentration, then more DBP is present in circulation, and more 25(OH)D could be bound to DBP in women receiving estrogen, resulting in more serum 25(OH)D being measured.\textsuperscript{25} Similarly, increased DBP could result in more vitamin D\textsubscript{3} being picked up in circulation and transported to the liver for hydroxylation, causing an increase in serum 25(OH)D levels.

It is not known whether there is a physiological benefit to the increased serum 25(OH)D levels in oral contraceptive users, or if oral contraceptive use simply raises the measurable 25(OH)D. PTH is the only measure available in this study to evaluate the functional effect of serum 25(OH)D levels, and there was no significant difference in mean PTH levels between oral contraceptive users and non-users at baseline (Table 17c).
despite significantly higher serum 25(OH) levels. However, other researchers suggest that oral contraceptives may impact PTH levels independently of vitamin D,\textsuperscript{234} so PTH is of limited use in examining the effect of oral contraceptive use on vitamin D status. Furthermore, due to the large amount of individual variation in serum PTH levels, a larger number of subjects would be needed to use PTH to evaluate the functional effect of serum 25(OH)D levels. Further research is needed to determine whether oral contraceptive use provides a beneficial effect on vitamin D status.

Oral contraceptive use must be considered in any research involving premenopausal women and adolescents looking at a change in serum 25(OH)D levels, and all contraceptives do not affect vitamin D status equally.

**Body Composition**

Increased body fat, BMI, and weight were associated with lower serum 25(OH)D levels in the current study. However, as also reported by Arunabh,\textsuperscript{129} body fat percentage had the strongest correlation with serum 25(OH)D levels, indicating that it is adiposity, and not simply body mass, that affects serum 25(OH)D levels. Therefore, body fat was the variable included in the regression analyses and was found to be a significant predictor of baseline serum 25(OH)D levels, while controlling for other variables known to affect serum 25(OH)D levels such as, season, vitamin D intake, exogenous estrogen, and age. The change in serum 25(OH)D levels due to supplementation, however, was not affected by body fat content.

The impact on baseline serum 25(OH)D levels was greatest in those with the highest tertile of body fat (>33% body fat). This difference in serum 25(OH)D levels based on body fat is consistent with the findings of Arunabh and colleagues,\textsuperscript{129} who
found significantly lower serum 25(OH)D levels in the highest quartile (>44% body fat) compared with the lowest quartile (<31% body fat) of body fat in women, and with Nesby-O'Dell and colleagues who found significantly lower serum 25(OH)D levels in white women with BMI greater than 30 compared to those with normal BMI (18.5 to 24.9).

As vitamin D is transported by DBP to the liver for hydroxylation, some is deposited in adipose tissue for storage along the way. Obese people have larger amounts of adipose tissue, and therefore pick up more vitamin D from circulation, resulting in lower serum 25(OH)D levels.

The change in serum 25(OH)D levels due to supplementation was not affected by body fat content. This lack of relationship is consistent with the findings of Wortsman and colleagues who found that obese individuals experienced an attenuated increase in vitamin D3 upon exposure to sunlight compared to non-obese peers, but the response to supplementation with oral vitamin D2 was not different between the two groups. Likewise, Brazilian nursing home residents responded similarly to a weekly dose of 7000 IU vitamin D3 regardless of body fat category. In contrast, Barger-Lux and colleagues saw BMI, but not body weight, contribute significantly to the variance in 25(OH)D response to supplementation with vitamin D3. It is not clear why BMI influenced response to supplementation in the study by Barger-Lux and colleagues, but not in others. Barger-Lux and colleagues used much larger doses of vitamin D3 (1000, 10,000, 50,000 IU), so more of it may have been transferred from the chylomicrons to vitamin D-binding protein in circulation, some of which is then deposited in adipose tissue for storage.
Body composition affects baseline serum 25(OH)D levels and, therefore, indirectly affects response to supplementation. Because obese women have lower baseline serum 25(OH)D levels than non-obese women, obese women require a higher dose of vitamin D₃ to optimize their vitamin D status. However, overweight and obese individuals do not need a higher dose of vitamin D₃ to produce the same incremental serum 25(OH)D response to supplementation as normal weight individuals.

**Sun Exposure**

Cutaneous synthesis of vitamin D following exposure to UVB radiation provides 80% to 90% of the vitamin D input for people who spend time in sunlight.³ The increase in serum 25(OH)D levels provided by one MED (minimal erythemal dose) of sunlight (the amount that causes a slight pinkness to the skin) while wearing a bathing suit is equivalent to the consumption of 10,000 to 25,000 IU oral vitamin D₂.¹¹⁵ However, the exact amount of vitamin D produced in response to sunlight varies by age and skin type.¹¹⁶ Comparatively, consumption of eight ounces of fortified milk provides only about 100 IU vitamin D, and three ounces of salmon provides 245 to 988 IU vitamin D₃.²³⁵

Summer sun exposure, as measured in this study, was not a significant predictor of serum 25(OH)D levels, or of the summer increase in serum 25(OH)D levels. Sun exposure not being predictive of serum 25(OH)D levels is not likely due to a lack of effect, but is more likely a result of the difficulty in estimating sun exposure. Sun exposure and, therefore, cutaneous synthesis of vitamin D, vary on a daily basis depending on the weather and cloud cover, the type of clothing worn, the amount of sunscreen used and frequency of application of sunscreen, and the length of time spent
outside, which makes cutaneous vitamin D synthesis a very difficult variable to quantify with validity. To truly ascertain the impact of sun exposure on serum 25(OH)D levels, a valid and reliable measurement tool must be developed. To further complicate matters, however, cutaneous vitamin D production is highly variable between individuals, with some individuals having low serum 25(OH)D levels despite abundant sun exposure.\textsuperscript{117}

**Alcohol Consumption**

The current study, like others\textsuperscript{169-171} showed higher levels of serum 25(OH)D levels with consumption of moderate amounts of alcohol, compared to no alcohol consumption. The likely mechanism for alcohol’s effect on serum 25(OH)D levels is due to an increase in estrogen levels with alcohol consumption.\textsuperscript{167} It is not clear whether this increase in serum 25(OH)D levels due to estrogen provides any physiological benefit, or simply increases the amount of measurable 25(OH)D.

**Number of Days with a Cold**

The number of days with a cold was used as a rough indicator of immune status. The subjects were simply asked how many times they had a cold in the previous three months, and the approximate number of days the cold lasted (see Lifestyle Questionnaires in Appendix B). Surprisingly, this simple retrospective measure was a significant variable in the logistic regression looking at determinants of baseline serum 25(OH)D levels. Women with higher serum 25(OH)D levels may have improved immunity over women with lower 25(OH)D levels, which may be associated with fewer days with a cold. Researchers believe 1,25(OH)\textsubscript{2}D stimulates the innate immune response.\textsuperscript{83-85} It is possible that raising serum 25(OH)D levels during winter provides more substrate for production of 1,25(OH)\textsubscript{2}D, which may protect against colds and the
Further research in this area would be beneficial, especially to children, the elderly, and the immunosuppressed during cold and flu season.

**Tanning Bed Use**

Although serum 25(OH)D levels were much higher in the 11 subjects who used a tanning bed during winter than in subjects who did not tan, this difference was not significant due to the small number of tanners in this study. At the end of winter in Boston, Tangpricha and colleagues\textsuperscript{142} saw serum 25(OH)D levels 90\% higher in subjects who used tanning beds in the previous six months compared with adults who did not tan. Artificial UVB radiation promotes cutaneous vitamin D synthesis and may provide an alternative to oral supplementation for increasing serum 25(OH)D levels during winter. However, due to the increased risk of melanoma and squamous cell carcinoma in tanning bed users,\textsuperscript{236} artificial UVB radiation for tanning is not an acceptable public health approach to improving vitamin D status.

**Seasonal Increase in Serum 25(OH)D Levels**

The 66\% summer increase in serum 25(OH)D levels was much greater than that seen in other studies.\textsuperscript{4,6,215} Serum levels in adolescents living in the Bangor area (44°N) increased 14 nmol/L or 28\% during the summer.\textsuperscript{4} In 24 to 70 year old women living in London (51°N), serum 25(OH)D levels were 36\% higher in summer than in winter.\textsuperscript{215} In white 18 to 35 year old women living in Toronto (43°N), serum 25(OH)D levels measured in summer were 31\% higher than in winter.\textsuperscript{6}

There are many factors that affect cutaneous synthesis of vitamin D, and therefore, affect the seasonal summer increase in serum 25(OH)D levels. London has a reputation for cloudiness and more air pollution than Maine, which could explain the
lower seasonal increase in serum 25(OH)D levels seen in London. In all of the studies, cloud cover and temperature could affect the amount of clothing worn, which affects vitamin D synthesis. The blood samples in the current study were specifically drawn when serum 25(OH)D levels are at their lowest (end of February) and highest (September), whereas in the Toronto and London studies, blood samples were measured during winter and during summer, so not all levels had reached their nadir and peak. It is also possible that the subjects in the current study exhibited more sun-seeking behavior than those in the other studies.

Skin type was the only variable determined to predict the seasonal change in serum 25(OH)D levels. As expected, subjects with darker skin experienced a smaller rise in serum 25(OH)D levels than did subjects with lighter skin, which is consistent with the findings of other researchers. Melanin acts as a natural sunscreen, so individuals with darker skin require more ultraviolet exposure to effect the same increase in serum 25(OH)D levels as individuals with lighter skin. Using artificial UVB radiation, Armas and colleagues found that together, skin type and the UVB dose explained 80% of the variation in the serum 25(OH)D response to UVB radiation. Age, gender, BMI, body surface area, and baseline 25(OH)D levels did not significantly contribute to the variation in serum 25(OH)D response.

The seasonal summer 2005 increase in serum 25(OH)D levels was unexpectedly a significant predictor of the one-year change (February 2005 to February 2006) in serum 25(OH)D levels. Since serum 25(OH)D levels increase every summer, and individual sun exposure behavior is presumed to be consistent from year to year, the affect on February serum 25(OH)D levels was expected to be similar from year to year, and,
therefore, was not expected to affect the overall one-year change in serum 25(OH)D levels. Indeed, in a three-year study by Sullivan and colleagues, the summer serum 25(OH)D levels increased to approximately the same levels every September and decreased to approximately the same levels every March. The difference found in the current study suggests greater cutaneous synthesis in summer 2005 than in summer 2004, resulting in higher serum 25(OH)D values in February 2006. The one-year increase in serum 25(OH)D levels in the placebo group also suggests a bigger influence of summer on February levels during the second February. Analysis of climatological data from the National Oceanic and Atmospheric Administration revealed that summer 2004 was cooler than summer 2005, so the subjects may have spent less time outside, or may have worn more clothing while outside in 2004 than in summer 2005, both of which would decrease vitamin D production. Temperatures in summer 2004 (May through August) were 6.4°F below normal compared to 1.1°F below normal in summer 2005. During May through August 2004, Bangor had 236 cooling degree days compared to 405 cooling degree days during the same period in summer 2005. One cooling degree day is accumulated for each whole degree that the daily mean temperature is above 65°F.

Researchers cannot assume that the summer increase in serum 25(OH)D levels will have a consistent impact on serum 25(OH)D levels the following February from year to year. Therefore, it is important to control for this change with a placebo group when looking at the one-year change in serum 25(OH)D levels.
Baseline Serum 25(OH)D Levels

Although there was no correlation between baseline serum 25(OH)D levels and response to supplementation, logistic regression analysis identified baseline serum 25(OH)D levels as having an independent effect on response to supplementation. Subjects with lower baseline serum 25(OH)D levels had a stronger serum 25(OH)D response to supplementation, which is consistent with other research studies.\textsuperscript{197-200} The hydroxylation of vitamin D\textsubscript{3} to 25(OH)D is likely a saturable process,\textsuperscript{145} causing a weaker response to supplementation in individuals with higher baseline serum 25(OH)D levels.

Rather than adjusting vitamin D supplementation dosages depending on baseline serum 25(OH)D, a single dose could be used for everyone since people with lower baseline serum 25(OH)D levels have a greater response to supplementation, and people with higher baseline levels have a weaker response. Because vitamin D toxicity has only occurred after exceptionally high intakes of vitamin D, one dose can be used safely for everyone.

Magnitude of Response to Supplementation

On average, serum 25(OH)D levels increased 1.1 nmol/L for every microgram of supplemental vitamin D\textsubscript{3} input, which is within the range (0.59 to 2.1 nmol/L) found by other researchers.\textsuperscript{145,198,209,210,219} In fact, Barger-Lux and colleagues\textsuperscript{145} estimated that in a 70 kg person (the mean weight of subjects in the current study), serum 25(OH)D levels would increase by 22 nmol/L on 800 IU supplemental vitamin D\textsubscript{3} daily, which would be a 1.1 nmol/L increase for each mcg of input.
The current study confirms the findings of other researchers that serum 25(OH)D levels increase approximately 1.0 nmol/L for every microgram of oral vitamin D₃ input. However, these numbers (0.7, 1.0, 1.1, and so on) are just average responses to one microgram of vitamin D₃. Some individuals may have a stronger or lesser response depending on baseline vitamin D status, and estrogen use.

**Adequacy of Dose**

Eighty percent of the premenopausal women receiving 800 IU vitamin D₃ daily achieved optimal vitamin D status at the end of the second winter, compared to 29% of the subjects at baseline. The women for whom 800 IU was inadequate had lower mean baseline serum 25(OH)D levels, had a higher mean body fat content, and were less likely to be taking oral contraceptives; 73% of women with suboptimal serum 25(OH)D levels after supplementation were not on oral contraceptives. Thirty percent of the women receiving 800 IU vitamin D₃ daily who were not using oral contraceptives had suboptimal serum 25(OH)D levels after five months of supplementation.

An adequate dose of vitamin D₃ would increase serum 25(OH)D levels sufficiently to suppress PTH secretion during winter. Other researchers have seen a suppression of PTH secretion with serum 25(OH)D levels of at least 75 nmol/L.¹³ As seen in Table 39, in the treatment group, the one-year change (decrease) in serum PTH levels was significant in the highest tertile of baseline serum 25(OH)D levels. The February 2006 serum PTH levels were also significantly lower in those with the highest baseline serum 25(OH)D levels compared to those in the lowest serum 25(OH)D tertile. It appears that 800 IU vitamin D₃ increased serum 25(OH)D levels in the highest tertile of baseline serum 25(OH)D levels enough to result in significant suppression of PTH.
The women in the treatment group were consuming approximately 1000 IU vitamin D daily from supplementation and diet. The current Adequate Intake (200 IU) set for this age group by the Food and Nutrition Board\textsuperscript{21} is only one-fifth of the amount consumed in this study. An AI is set when the Board feels there is not enough evidence available to establish a Recommended Daily Allowance (RDA). By definition, the RDA is the average daily intake required to meet the needs of 97% to 98% of healthy individuals in a specific age and gender group.\textsuperscript{21}

In the current study, 97.5% of subjects had baseline serum 25(OH)D levels greater than 28.0 nmol/L. Therefore, in order to ensure that 97% to 98% of subjects have optimal serum 25(OH)D levels of at least 75 nmol/L, the individuals with baseline serum 25(OH)D levels as low as 28.0 nmol/L would need to be optimized. Evidence suggests a linear relationship between vitamin D\textsubscript{3} intake and the increase in serum 25(OH)D levels.\textsuperscript{209} Therefore, based on the 1.1 nmol/L increase in serum 25(OH)D levels per microgram of vitamin D\textsubscript{3} input seen in the current study, approximately 1700 IU vitamin D\textsubscript{3} would be required daily to optimize the vitamin D status of 97.5% of the population. Doing the same calculation, but using a 0.7 nmol/L increase per microgram as determined by Heaney and colleagues,\textsuperscript{209} 2700 IU would be required for 97.5% of the population to achieve serum 25(OH)D levels above 75 nmol/L. Similarly, using NHANES III data and a 0.7 nmol/L increase per microgram of vitamin D\textsubscript{3} input, Heaney\textsuperscript{134} determined that approximately 2600 IU vitamin D\textsubscript{3} would be needed to meet the needs of 97.5% of a population of 60 to 79 year old white women. There are many factors that affect baseline serum 25(OH)D levels and response to oral supplementation, therefore 0.7 and 1.1 nmol/L per microgram of vitamin D\textsubscript{3} intake are just estimates.
Nevertheless, many studies\textsuperscript{145,198,209,210,219} have found values in the same range and Heaney believes these estimations are very close to the amount needed.\textsuperscript{134}
Chapter 6

CONCLUSIONS

Researchers speculate that approximately 800 to 1000 IU vitamin D3 is required to achieve optimal serum 25(OH)D levels of at least 75 nmol/L in the absence of sunlight. However, further research is needed to determine how much vitamin D3 is required to optimize serum 25(OH)D levels for different age groups.

The purpose of this study was to measure the serum 25(OH)D response to daily supplementation with 800 IU vitamin D3 during winter in premenopausal women living in Maine, and to examine the effects of body composition and hormonal contraceptive use on baseline serum 25(OH)D levels and on the response to supplementation. From the sample of white premenopausal women in the current study, many conclusions can be extrapolated into the entire population.

There is a high rate of vitamin D insufficiency in the northeastern United States and serum 25(OH)D levels fluctuate seasonally. In February 2005, less than one-third of young women in Maine had optimal serum 25(OH)D levels (≥75 nmol/L). Serum 25(OH)D concentrations increased 66% during the summer, and 77% of young women had optimal levels at the end of the summer. For 80% of this relatively homogeneous sample, 800 IU supplemental vitamin D3 daily was sufficient to achieve serum 25(OH)D levels of at least 75 nmol/L at the end of winter.

Exogenous estrogen from hormonal contraceptive use increases serum 25(OH)D levels, and, in the current study, also increased the serum 25(OH)D response to supplementation. Further research is needed to determine whether or not these higher serum 25(OH)D levels are merely a result of an increase in the amount of 25(OH)D that
is measured or actually represent a functional improvement in vitamin D status. Percent body fat, on the other hand, is negatively associated with serum 25(OH)D levels. Women with higher percent body fat, have lower serum 25(OH)D levels. Body fat did not, however, affect serum 25(OH)D response to supplementation in the current study. Women who started with lower serum 25(OH)D levels had a greater increase in serum 25(OH)D levels during supplementation than women who started with higher serum 25(OH)D levels.

Because of the large variation in vitamin D requirements due to factors such as body composition, oral contraceptive use, and initial serum 25(OH)D levels, as well as the many factors affecting vitamin D synthesis, including skin color, sun exposure, and geographic location, it is very difficult to develop a strategy to optimize vitamin D status for everyone. For the health of the nation, the Food and Nutrition Board must review the abundant research that has been conducted since the current Adequate Intake for vitamin D was established in 1997 and revise their recommendations. Ideally, everyone should have their serum 25(OH)D levels measured at the end of winter to determine supplementation needs. However, screening everyone for vitamin D deficiency would be expensive. Therefore, people who do not receive adequate sunlight, which includes everyone who resides at latitudes above 35°N, may need 800 IU vitamin D₃ or more daily during winter. Given the low risk of toxicity, vitamin D supplementation recommendations should be high enough to optimize serum 25(OH)D levels in the population regardless of body composition, baseline serum 25(OH)D levels, or skin color.
In the current study, 800 IU vitamin D$_3$ was only adequate to optimize vitamin D status in 80% of this sample of white, young adult women. Therefore, supplementation with 800 IU vitamin D$_3$ is not likely to optimize vitamin D status in the population as a whole. Further research is needed to determine the dose of vitamin D$_3$ required to optimize vitamin D status in the US population as a whole.
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APPENDICES
Appendix A

Vitamin D Content of Foods
## VITAMIN D CONTENT OF FOODS

### Table A1. Vitamin D content of fish.

<table>
<thead>
<tr>
<th>Food</th>
<th>International Units (IU) Per Serving (mean ± SEM when available)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod liver oil, 1 Tablespoon</td>
<td>1,360(^a)</td>
</tr>
<tr>
<td>Salmon, wild-caught, 3½ ounces</td>
<td>988 ± 524(^b)</td>
</tr>
<tr>
<td>Salmon, farm raised, <em>raw</em>, 3½ ounces</td>
<td>240 ± 108(^b)</td>
</tr>
<tr>
<td>Salmon, farm raised, <em>baked</em>, 3½ ounces</td>
<td>240(^b)</td>
</tr>
<tr>
<td>Salmon, farm raised, <em>fried in vegetable oil</em>, 3½ ounces</td>
<td>123(^b)</td>
</tr>
<tr>
<td>Blue fish, 3½ ounces</td>
<td>280 ± 68(^b)</td>
</tr>
<tr>
<td>Cod, 3½ ounces</td>
<td>104 ± 24(^b)</td>
</tr>
<tr>
<td>Trout, farm raised, 3½ ounces</td>
<td>388 ± 212(^b)</td>
</tr>
<tr>
<td>Tuna, Ahi-yt, 3½ ounces</td>
<td>404 ± 440(^b)</td>
</tr>
<tr>
<td>Mackerel, cooked, 3½ ounces</td>
<td>24(^b) – 345(^a)</td>
</tr>
<tr>
<td>Tuna fish, canned in oil, 3 ounces</td>
<td>200(^a)</td>
</tr>
<tr>
<td>Sardines, canned in oil, drained, 1¼ ounces</td>
<td>250(^a)</td>
</tr>
</tbody>
</table>
Table A2. Vitamin D content of foods fortified with vitamin D.

<table>
<thead>
<tr>
<th>Food</th>
<th>International Units (IU) Per Serving</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MILK</strong></td>
<td></td>
</tr>
<tr>
<td>Milk, nonfat, reduced fat, and whole fat, vitamin D fortified, 1 cup</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soy Milk Silk&lt;sup&gt;®&lt;/sup&gt; regular, lowfat and fat free, 8 oz</td>
<td>120&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soy Milk, 8&lt;sup&gt;th&lt;/sup&gt; Continent&lt;sup&gt;®&lt;/sup&gt;, regular, lowfat, and fat free, 8 oz</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>YOGURT</strong></td>
<td></td>
</tr>
<tr>
<td>Colombo&lt;sup&gt;®&lt;/sup&gt; Classic&lt;sup&gt;®&lt;/sup&gt; and Light Yogurt, 6 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colombo&lt;sup&gt;®&lt;/sup&gt; Lowfat Yogurt, 8 oz</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dannon&lt;sup&gt;®&lt;/sup&gt; Light &amp; Fit&lt;sup&gt;®&lt;/sup&gt;, 6 oz</td>
<td>60 – 80&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dannon&lt;sup&gt;®&lt;/sup&gt; Activia&lt;sup&gt;®&lt;/sup&gt;, 4 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dannon&lt;sup&gt;®&lt;/sup&gt;, Fruit on the Bottom and Fruit Blends, 6 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dannon&lt;sup&gt;®&lt;/sup&gt;, Danimals&lt;sup&gt;®&lt;/sup&gt;, 4 oz</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stonyfield Farm&lt;sup&gt;®&lt;/sup&gt;, Organic Whole Milk and Lowfat, and All Natural Fat Free, 6 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stonyfield Farm&lt;sup&gt;®&lt;/sup&gt;, 2-a-Day Yogurt, 6 oz</td>
<td>80</td>
</tr>
<tr>
<td>Yoplait&lt;sup&gt;®&lt;/sup&gt; Original Fruit Flavors, Custard Style, and Light Flavors, 6 oz</td>
<td>80&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yoplait&lt;sup&gt;®&lt;/sup&gt; Trix&lt;sup&gt;®&lt;/sup&gt; Yogurt, 4 oz</td>
<td>40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yoplait&lt;sup&gt;®&lt;/sup&gt; Whips&lt;sup&gt;®&lt;/sup&gt;, Fruit Flavors 4 oz</td>
<td>40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yoplait&lt;sup&gt;®&lt;/sup&gt; Go-Gurt&lt;sup&gt;®&lt;/sup&gt;, 2.25 oz tube</td>
<td>24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CEREAL</strong></td>
<td></td>
</tr>
<tr>
<td>General Mills: Cheerios&lt;sup&gt;®&lt;/sup&gt;, Chex&lt;sup&gt;®&lt;/sup&gt;, Wheaties&lt;sup&gt;®&lt;/sup&gt;, Total&lt;sup&gt;®&lt;/sup&gt;, Trix&lt;sup&gt;®&lt;/sup&gt;, Lucky Charms&lt;sup&gt;®&lt;/sup&gt;, Kix&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kellogg’s Special K&lt;sup&gt;®&lt;/sup&gt;, Frosted Mini Wheats&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kellogg’s Rice Krispies&lt;sup&gt;®&lt;/sup&gt;, All Bran&lt;sup&gt;®&lt;/sup&gt;, Corn Flakes&lt;sup&gt;®&lt;/sup&gt;, Froot Loops&lt;sup&gt;®&lt;/sup&gt;, Corn Pops&lt;sup&gt;®&lt;/sup&gt;, Frosted Flakes&lt;sup&gt;®&lt;/sup&gt;, Smart Start&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quaker Cap’n Crunch&lt;sup&gt;®&lt;/sup&gt;, Life&lt;sup&gt;®&lt;/sup&gt;, Oatmeal Squares&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post Honey Bunches of Oats&lt;sup&gt;®&lt;/sup&gt;, Golden Crisp&lt;sup&gt;®&lt;/sup&gt;, Grape Nuts&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post Shredded Wheat&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kashi Heart to Heart&lt;sup&gt;®&lt;/sup&gt;, Go Lean&lt;sup&gt;®&lt;/sup&gt;, Go Lean Crunch&lt;sup&gt;®&lt;/sup&gt;, 1 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Food</td>
<td>International Units (IU) Per Serving (mean ± SEM when available)</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>MARGARINE</td>
<td></td>
</tr>
<tr>
<td>Margarine, fortified, 1 tablespoon</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Land O’Lakes®, 1 Tbsp</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fleischman’s® Original (stick), 1 Tbsp</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fleischman’s® made with olive oil, 1 Tbsp</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Promise® Regular, Light, 1 Tbsp</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smart Balance®, 1 Tbsp</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smart Balance®, Light, 1 Tbsp</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shedd’s Spread® Country Crock® with calcium and vitamins, 1 Tbsp</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ORANGE JUICE</td>
<td></td>
</tr>
<tr>
<td>Orange Juice, fortified, 8 oz (such as, Tropicana Pure Premium® Healthy Kids, Tropicana Pure Premium® Calcium + Vitamin D, Minute Maid Kids+®, Minute Maid® Calcium and Vitamin D)</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Orange Juice, not fortified with vitamin D, 8 oz</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>OTHER</td>
<td></td>
</tr>
<tr>
<td>Egg yolk, 1 whole</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver, beef, cooked, 3½ ounces</td>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cheese, Swiss, 1 ounce</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>


<sup>c</sup>According to package label 2007 Sep 18
Appendix B

Health History Questionnaire

And

Lifestyle Questionnaires
Health History Screening Form
(January 2005)

We are asking these health history questions because certain conditions and medications affect vitamin D metabolism. A positive response may or may not disqualify you from participation in the study. Please answer as accurately as possible.

Date of Birth ______________________

Do you have, or have you ever had:

Diabetes   Y   N  Celiac Disease / Sprue   Y   N
Cystic Fibrosis   Y   N  Gluten Intolerance   Y   N
Crohn’s Disease   Y   N  Liver Disease   Y   N
Ulcerative Colitis   Y   N  Kidney Disease   Y   N
Chronic Diarrhea   Y   N  Pacemaker   Y   N
Whipple’s Disease   Y   N

Do you have any other medical conditions? If so, please explain:  

What is your current height? _______  Weight? _______

BMI = _______

Please do not write in this box.

Are you pregnant?   Yes   No   Maybe

Are you planning to become pregnant in the next 12 months?   Yes   No

Do you have a regular menstrual cycle (occurring every 23-35 days)? If not, how often do you menstruate?
Health History Screening Questionnaire, continued
(January 2005)

Are you currently taking:
Steroids, including inhalers (i.e. prednisone)
Anti-seizure medication (i.e. phenytoin, valproic acid, and others)
Thyroid medication (i.e. synthroid)
Cholesterol-lowering medication (i.e. Questran, Colestid)
Depo-Provera
Mineral Oil

Do you take any vitamins or minerals? If so, which ones, how much, and how often?

What other medications or herbal supplements do you take, and how often?

Will you be able to be available on a weekday between 8:00 am and 5:00 pm to have your blood drawn in March 2005, September 2005, and March 2006, and to have your body composition measured in March 2005 and 2006?

Y  N

Due to the nature of this study, we ask that all participants refrain from taking supplemental multivitamins, calcium, or vitamin D other than those on an approved list. You must also have no plans to travel south of Southern California, Southern Texas, Louisiana, New Mexico, Georgia, Alabama, or Florida over winter 2004/2005 and 2005/2006. (Spring Break will be okay)
Please help us estimate the amount of time you typically spent outdoors last summer between the hours of 10:00 am and 3:00 pm between the beginning of May and the end of August 2004.

**Work Day:** On a typical workday last summer, how much time did you spend outdoors between the hours of 10 am and 3 pm?

On average, how many days per week did you work last summer?

**Day Off:** On a typical day off last summer, how much time did you spend outdoors between the hours of 10 am and 3 pm?

On average, how many days off per week did you have last summer?

**Vacation Time:** While you were on vacation last summer, how many hours per day did you spend outdoors between the hours of 10 am and 3 pm?

How many days were you on vacation last summer?

When you were outside, did you wear sunscreen on your face?

Never Rarely Sometimes Often Always

When you were outside did you wear sunscreen on the rest of your body?

Never Rarely Sometimes Often Always

To which part(s) of your body did you typically apply sunscreen?

- [ ] Arms
- [ ] Back and Shoulders
- [ ] Hands
- [ ] Legs
- [ ] All Bare Skin

What number SPF sunscreen did you typically wear? ____________________
Lifestyle Questionnaire, continued

(March 2005)

Did you use a tanning bed in the past 5 months? If so, how many times? _______

Have you traveled to a southern climate since October? If so, where and for what dates?

Are you allergic to any of the following ingredients that are in the vitamin D or placebo capsules? Placebo: Gelatin, maltrin, magnesium stearate. Vitamin D₃: gelatin and synthetic vitamin D₃. Yes No

When was the first day of your last period? __________________________

Are you pregnant? Yes No Maybe

Do you use a birth control pill, patch, shot, or ring? If so, which brand?

If yes, have you been on this birth control for at least 4 months? Y N

Please list any new medications that you took during the past 2 months:

Did you take any vitamins, minerals, or herbal supplements during the past 2 months? If so, which ones, how much, and how often did you take them?
Lifestyle Questionnaire, continued

(March 2005)

Do you have any chronic medical condition? If so, please explain.

How many times did you have a cold or the flu in the past 3 months?
How many days did it usually last?

Do you smoke? Y N If yes, how many packs/day? ______________

Which of the following best describes how often you take an antacid such as Tums or Rolaids?

At least once a day At least once a week Never
At least once a month Less than once a month

Which antacid do you take?

Which of the following best describes how often you eat fat-free snacks that contain the fat substitute, olestra (i.e. WOW Chips)?

At least once a day At least once a week Never
At least once a month Less than once a month
Contact Information

Do you have a new address or phone number since we last saw you? If so, please provide below:


Your next visit for the Vitamin D Study is in June. You will need to pick up a new supply of capsules and return the unused ones. Please provide your summer address and phone number so that we can get in contact with you.

June Address: ________________________________

June Phone Number: __________________________

If you are not going to be near Orono this summer, are you planning to be on campus or in the Bangor area anytime during June? If so, when could we connect?
Lifestyle Questionnaire
(June 2005)

Please let us know if your contact information will change this fall.

Over the past 3 months:

Did you experience any side effects that you think are related to the capsules given to you in this study? If yes, please describe

Approximately how many capsules did you forget to take during the past 3 months?

Did you use a tanning bed? If so, how many times?

Have you traveled to a southern climate since March? If so, where and for what dates?

Are you pregnant? Yes No Maybe

Do you use a birth control pill, patch, shot, or ring? If so, has your prescription changed in the past 3 months (what brand do you use now)? What month did this change occur?

Have you quit using a birth control pill, patch, shot, or ring in the past 3 months? If so, what month did you stop taking it?
Lifestyle Questionnaire, continued
(June 2005)

Please list any new medications that you took during the past 3 months:

Did you take any vitamins, minerals, or herbal supplements during the past 3 months? If so, which ones, how much, and how often did you take them? (Not including the capsule for this study)

How often do you take an antacid such as Tums or Rolaids?
- At least once a day
- At least once a week
- Never
- At least once a month
- Less than once a month

Which antacid did you typically take?

Do you smoke? Y N
If yes, how many packs/day? __________

Were you diagnosed with any chronic medical condition in the past 3 months?

How many times did you have a cold or the flu in the past 3 months?
How many days did it usually last?

Which of the following best describes how often you eat snacks that contain the fat substitute Olestra (i.e. WOW Chips or Light Chips)?
- At least once a day
- At least once a week
- Never
- At least once a month
- Less than once a month

How do you remember to take your daily capsule? I would like to compile these responses (without names attached) to give the other participants some suggestions.
Please change the above address label to reflect your current address. This is the address to which your October check will be sent.

Do you receive a paycheck from the University of Maine? Y N (When I submit the information for your check, payroll needs to know this.)

Please help us estimate the amount of time you typically spent outdoors between the hours of 10:00 am and 3:00 pm between the beginning of May and the end of August 2005.

**Work Day:** If you worked or went to school, on a typical work or school day, how much time did you spend outdoors between the hours of 10 am and 3 pm?

On average, how many days per week did you work or go to school?

**Day Off:** On a typical day off, how much time did you spend outdoors between the hours of 10 am and 3 pm?

On average, how many days off did you have per week?

**Vacation Time:** If you went on vacation, while you were on vacation, how many hours per day did you spend outdoors between the hours of 10 am and 3 pm?

How many days were you on vacation?

**Over the past 3 months:**

When you were outside, did you wear sunscreen on your face?

Never Rarely Sometimes Often Always
Lifestyle Questionnaire, continued
(September 2005)

When you were outside, did you wear sunscreen on the rest of your body?

Never  Rarely  Sometimes  Often  Always

To which part(s) of your body did you typically apply sunscreen?

□ Arms  □ Back and Shoulders  □ Hands  □ Legs  □ All Bare Skin

What number SPF sunscreen did you typically wear? ______________________

Did you use a tanning bed? If so, how many times? ______________________

When was the first day of your last period? ______________________________

Are you pregnant?  Yes  No  Maybe

Did you experience any side effects from the capsules given to you in this study?

If yes, please describe: ________________________________________________

Approximately how many capsules did you forget to take in the past 3 months?

Do you use a birth control pill, patch, shot, or ring? If so, has your prescription changed in the past 3 months (what brand do you use now)? What month did this change occur?

Have you quit using a birth control pill, patch, shot, or ring in the past 3 months? If so, what month did you stop taking it?
Lifestyle Questionnaire, continued
(September 2005)

Please list any medications that you took over the past 3 months:

Did you take any vitamins, minerals, or herbal supplements over the past 3 months? If so, which ones, how much, and how often did you take them? Please include brand names if possible. (Do not include the capsule for this study)

How often do you take an antacid such as Tums or Rolaids?
- At least once a day
- At least once a week
- At least once a month
- Less than once a month

Which antacid do you typically take? ________________________________

Do you smoke? Y N If yes, how many packs/day? ________________

Were you diagnosed with any chronic medical condition in the past 3 months?

How many times did you have a cold or the flu in the past 3 months?
How many days did it usually last?

How often do you eat fat-free snacks that contain the fat substitute, olestra (i.e. WOW Chips or Light Potato Chips)?
- At least once a day
- At least once a week
- Never
- At least once a month
- Less than once a month
Skin Type Questionnaire  
(September 2005)

Skin color affects the amount of vitamin D your skin is able to make. Skin type is often categorized by the Fitzpatrick skin type scale which ranges from very fair (skin type I) to very dark (skin type VI). This classification is based on a person’s complexion and response to sun exposure. Please check the description which best fits your skin type:

- **Skin Type I:** Persons with this skin type have blond or red hair and very fair skin or ivory white skin. They often have freckles and blue eyes. They easily burn, never tan, and are extremely sensitive to sunlight.

- **Skin Type II:** Persons with this skin type usually have blue or hazel eyes, and red or blonde hair. Their skin color is white or fair and they may have freckles. They typically burn easily and tan slightly or slowly.

- **Skin Type III:** Persons with this skin type have fair skin and are blond or brunette. Their skin is white to slightly beige or olive. They tan slowly and moderately, gradually turning to a light brown color. They sometimes burn.

- **Skin Type IV:** Persons with this skin type usually have beige, light brown, or olive-colored skin, dark eyes, and dark hair. They tan easily and moderately, and rarely burn.

- **Skin Type V:** Persons with this skin type are similar to those with skin type IV, but they never burn. They tan profusely to a deep brown or black color.

- **Skin Type VI:** Persons with this skin type have dark eyes and hair and dark brown or black skin. They never burn.

To further help us understand the ability of your skin to make vitamin D, how would you describe (the largest portion of) your ethnicity?

- White
- Asian
- Black or African American
- Indian or Alaska Native
- Hispanic or Latino
- Native Hawaiian or Other Pacific Islander
- Some other race
The Vitamin D Study

Lifestyle Questionnaire
(December 2005)

Phone Number:

If you are a student, in what semester did you start (year and month) at UM or UCB?

**Over the past 3 months:**

Did you experience any side effects from the capsules given to you in this study?

If yes, please describe:

Approximately how many capsules did you forget to take in the past 3 months?

Are you pregnant? Yes No Maybe

Do you use a birth control pill, patch, shot, or ring? (circle one) Yes No

If so, as your prescription changed in the past 3 months? (circle one) Yes No

In what month did this change occur?

Which brand do you use now?

Have you quit using a birth control pill, patch, shot, or ring in the past 3 months? If so, what month did you stop taking it?

How often do you take an antacid such as Tums or Rolaids?

- At least once a day
- At least once a week
- Never
- At least once a month
- Less than once a month

Which antacid do you typically take?
Lifestyle Questionnaire, continued
(December 2005)

Please list any medications that you took during the past 3 months:

Did you take any vitamins, minerals, or herbal supplements during the past 3 months? If so, which ones, how much, and how often did you take them? (Not including the capsule for this study)

Did you use a tanning bed in the past 3 months? If so, how many times?

Did you travel to a southern climate in the past 3 months? If so, where, and for what dates?

Did you ski in the past 3 months? If so, how many hours do you spend skiing each week during the day (don’t count night skiing)?

Do you smoke? Y N If yes, how many packs/day? ____________

Have you been diagnosed with any chronic medical condition in the past 3 months?

How many times did you have a cold or the flu in the past 3 months?
How many days did it usually last?

How often do you eat fat-free snacks that contain the fat substitute, olestra (i.e. WOW Chips)?
   At least once a day   At least once a week   Never
   At least once a month   Less than once a month
The Vitamin D Study

Lifestyle Questionnaire
(March 2006)

Please change the above address label to reflect your current address.

Are you currently a UM student (I need to know this for billing purposes)?  Y  N

**Over the past 3 months:**

Did you experience any side effects from the capsules given to you in this study?  Y  N

If yes, please describe ____________________________________________________________

Approximately how many capsules did you forget to take in the past 3 months? _______

Did you use a tanning bed in the past three months? _____________________________
If so, where and how many times? _____________________________

Did you travel to a southern climate in the past three months? If so, where, and for what dates?

Did you ski? If so, how many hours do you spend skiing each week during the day (don’t count night skiing)?

Please list any medications that you took over the past 3 months:

Did you take any vitamins, minerals, or herbal supplements over the past 3 months? If so, which ones, how much, and how often did you take them? (Not including the capsule for this study)

When was the first day of your last period? _________________________________
Do you use a birth control pill, patch, shot, or ring?  
Y  N
If so, has your prescription changed in the past 3 months (what brand do you use now)?
What month did this change occur?

Have you quit using a birth control pill, patch, shot, or ring in the past 3 months?  If so, what month did you stop taking it?

If you have ever used a birth control pill:
Approximately how many years did you /have you used it?
Which years did you use a birth control pill?

Do you smoke?  Y  N
If yes, how many packs/day?  

On average, how many days a week do you consume alcohol?

Using the serving information below, on average, how many servings of alcohol do you drink in a week?

1 serving of alcohol:
1 can or bottle (12 oz) or ½ of a “big red” or Solo cup of beer / 1 bottle (12 oz) of wine coolers / 5 oz wine / 1 shot of liquor (1 ½ oz) / 1 Dixie cup of jello shots

Have you been diagnosed with any chronic medical condition in the past 3 months?

How many times did you have a cold or the flu in the past 3 months?
How many days did it usually last?

How often do you eat fat-free snacks that contain the fat substitute, olestra (i.e. WOW Chips)?
At least once a day  At least once a week  Never
At least once a month  Less than once a month

How often do you take an antacid such as Tums or Rolaids?
At least once a day  At least once a week  Never
At least once a month  Less than once a month

What brand of antacid do you most often use?  

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Appendix C

Recruitment Letter
November 16, 2004

Dear Student:

Did you know that almost half of the women living in Maine have low levels of vitamin D in their blood, putting them at increased risk of developing weak bones or other health problems? These low levels occur because the sun is not strong enough in winter in Maine for the skin to make vitamin D. However, researchers are not sure how much extra vitamin D women need during winter.

We plan to do a study to find out how much vitamin D is needed to maintain normal blood levels in winter in Maine. We are looking for women between the ages of 19 and 35 to participate in “The Vitamin D Study”, a 12-month supplementation study. For a relatively small effort on your part, you can help make a difference in vitamin D research.

What the study involves:

- Filling out short questionnaires every 3 months
- Taking a vitamin D or placebo capsule daily (A placebo is a capsule that looks like the vitamin D capsule, but contains an inactive, harmless substance instead)
- Body composition measurement at the beginning and end of the study

We are looking for women to sign up for the study this fall. The first testing will take place in March 2005. Compensation will be $200 for completion of the study.

Please consider participating in our study and encourage your friends to do the same. If you are interested in participating or would like more information, please call 581-1622.

Thank you,

Monica Nelson, MPH, RD
Graduate Student
Food Science and Human Nutrition
University of Maine

Susan Sullivan, DSc, RD
Director Didactic Program
Food Science and Human Nutrition
University of Maine
Appendix D

Recruitment Flyer
DO YOU GET ENOUGH SUN?

Skin makes vitamin D when it is exposed to sunlight. During winter in Maine, the sun is not strong enough for skin to make vitamin D. Researchers at the University of Maine are looking for **women between the ages of 19 and 35** to participate in a 12-month study to find out how much extra vitamin D young women need to keep blood levels high enough during winter in Maine.

What the study involves:

- Blood tests every 6 months (3 tests - less than two tablespoons of blood will be drawn each time.)
- Filling out short questionnaires every 3 months
- Taking a vitamin D capsule or a sugar pill daily
- Body composition measurement in Bangor in March 2005 & 2006

We are looking for women to sign up for the study this fall. The first testing will take place in March 2005. Compensation will be $200 for completion of the study.

For more information, please call Susan Sullivan, D.Sc., R.D. of the Department of Food Science and Human Nutrition at the University of Maine or Monica Nelson, graduate student, at 581-1622.
Appendix E

Consent Form
PATIENT INFORMATION AND INFORMED CONSENT STATEMENT

TITLE: Serum 25-Hydroxyvitamin D Response to Customized Doses of Vitamin D₃ in Premenopausal Women

DATE: June 1, 2005

You have been asked to take part in a research study being conducted by Dr. Susan Sullivan, faculty member, and Monica Nelson, graduate student, in the Department of Food Science and Human Nutrition at the University of Maine. Funding for this study is being provided by the United States Department of Agriculture (USDA).

You have initially qualified to take part in this study because you are a 19-35 year old healthy woman. You cannot have any serious medical conditions such as diabetes, celiac disease or Crohn’s disease or take any prescription medications such as steroids that affect vitamin D and calcium metabolism. If you are pregnant or become pregnant, you will not be allowed to continue in this research study. Your body mass index (BMI) must be between 18.5 and 40 to participate. BMI is the product of 705 times your weight in pounds divided by your height in inches, squared (BMI = lbs/inches² x 705). These conditions and medications will be discussed with you in detail prior to your enrolling in the study. In addition, you must agree to avoid taking most vitamin, mineral, and herbal supplements, other than those on an approved list. You must avoid tanning booths during the study period, and you must try to avoid traveling south of 35° north latitude (southern California, southern Texas, New Mexico, Louisiana, Alabama, Georgia, and Florida) from October through February (see attached map).

WHY IS THIS STUDY BEING DONE?

Vitamin D is important for building and maintaining healthy bones. Vitamin D may also reduce a person’s risk for some cancers, immune disorders, high blood pressure and type I diabetes. In Maine, it is common to have low blood levels of vitamin D because there is not enough sunlight in the winter for the skin to make vitamin D. Exposure to strong sunlight during winter can ruin the results of this study, therefore you must try to avoid traveling south from October through February. If southern travel becomes necessary please wear sunscreen (minimum SPF 8) and avoid the sunlight as much as possible.

The main purpose of this study is to help identify how much extra vitamin D young women in Maine need to keep blood levels normal during winter. This study will also look at how body fatness affects vitamin D levels in the blood. There will be about 120 women in this study.

Initial here to confirm page has been read ________
WHAT IS INVOLVED IN THE STUDY?

In this study you will either be in the treatment group where you will receive a vitamin D capsule containing 800 International Units (IU) of vitamin D, or in the control group where you will receive a placebo (we call it a sugar pill even though it does not contain any sugar). The sugar pill looks exactly like the vitamin D capsule, but does not contain any vitamin D. A computer will randomly decide if you will be in the treatment or control group. You will not be told which group you are in. You will have a 2 in 3 chance of receiving vitamin D and a 1 in 3 chance of receiving a sugar pill.

If you meet the requirements for the study and agree to take part in it, you will need to take one vitamin D capsule or sugar pill every day. Taking the capsules every day is very important for this study to be successful. If you forget to take a capsule one day, you can take two pills the following day.

You will need to report to Cutler Health Center at the University of Maine three times over the next 12 months for testing (March 2005, September 2005, and March 2006). You will also report to the research office at the University of Maine to pick up a new supply of capsules every three months. In March 2005 and March 2006 you will visit the Maine Center for Osteoporosis Research and Education (MECORE) in Bangor for body composition measurement.

In January 2005 there will be an informational meeting at the University of Maine. The estimated time for this meeting is 1-2 hours. During this time you will:

• Be given time to ask questions about the study
• Be asked to read and sign this informed consent
• Be asked questions about your health and medications to make sure you qualify for the study. You will be asked questions such as:
  o Do you have, or have you ever had diabetes, celiac disease, cystic fibrosis, gluten intolerance, Crohn’s disease, ulcerative colitis…

The first visit to Cutler Health Center will be in March 2005. The estimated time for this visit is 30-45 minutes. During this time you will:

• Have your weight and height measured
• Have less than 2 tablespoons of blood drawn
• Provide a urine sample

Blood will be drawn to measure calcium, parathyroid hormone, and vitamin D. Urine will be collected to measure calcium.

After the first blood draw, if your blood vitamin D is very low (<22.5 nmol/L) or high (>175 nmol/L), you will not be allowed to continue in the study, and you will be advised to speak with your doctor for evaluation at your own expense.

Initial here to confirm page has been read ________
If you meet the requirements and want to be in the study, the computer will choose which one of the treatments you will receive (vitamin D or sugar pill). During the 12 months of the study, you will be on sugar pill for six of the months and either vitamin D or sugar pill for the other six months. Neither you nor the researchers will know whether you are taking vitamin D or the sugar pill. No one at the clinic will be able to tell you which pill you are taking. This information will be available at Cutler Health Center in case of emergency.

You will return to Cutler Health Center for two more visits over the next 12 months. These visits will be in September 2005, and March 2006. At each of these visits, the testing will be the same as described above. The estimated time for each of these visits is 30 minutes.

Starting in March 2005 you will visit the research office at the University of Maine every three months to return any unused capsules and receive a new supply. You will be asked to stop taking all other multivitamins, vitamin D, or calcium supplements during the study. You will be given a list of vitamins such as vitamin C and folate that you will be allowed to take. The estimated time for this visit is 15 minutes. At this time you will be asked brief questions about your health, such as:

- Did you take any vitamins, minerals, or herbal supplements over the past 3 months? If so, which ones, how much, and how often did you take them?

Between January and March 2005 and 2006 you will be asked to keep a three-day record of what you eat and drink. The day after you finish this record, the researcher will call you to find out what you ate and drank. This information will be used to estimate how much vitamin D, calcium, phosphorus, protein, and calories you ate. The estimated time for this phone call is 30 minutes. In addition, during your visits to the research office, you will be asked some questions about the foods that you typically eat and drink.

In March 2005 and 2006 you will visit the Maine Center for Osteoporosis Research and Education (MECORE) in Bangor to have your body fat and bone density measured by dual energy x-ray absorptiometry (DXA). The DXA scanner is similar to an x-ray. During the exam you will lie on a padded table and the arm of the machine will pass over your body. You will not be enclosed in any way during this exam.

The estimated time for this MECORE visit is ½ hour. During this time you will:

- Have your weight and height measured
- Take a urine pregnancy test before your body fat is measured because an embryo should not be exposed to the small amount of radiation used to measure body fat.
- Have a total body DXA scan to measure your body fat and bone density

Initial here to confirm page has been read ________
POSSIBLE BENEFITS OF THE STUDY:

This study is being done to find out more about vitamin D needs during winter. It may not provide any direct benefit to you. If you would like, at the end of the study, you and your doctor will be given your body fat and vitamin D results along with information on how to optimize vitamin D levels and body composition. Based on this information, decisions can be made to help decrease your future risk of osteoporosis and other health concerns.

If you complete all of the requirements you will be compensated a total of $200 for your mileage, time, and effort. You will be paid in April 2005 ($25), October 2005 ($50), and March 2006 ($125). If you choose to discontinue the study before March 2006, you may keep any money you have received.

POSSIBLE RISKS OR DISCOMFORTS OF THE STUDY:

You will have blood drawn three times during the study. When you have blood drawn you may have some pain, bleeding, or a black and blue spot at the site of the blood draw. The total blood volume drawn will be less than two tablespoons.

During the bone density scan (DXA) you will be exposed to less radiation than you would get during a chest x-ray or a long flight in an airplane. The radiation may be harmful to an embryo, therefore you will be withdrawn from the study if you become pregnant.

There are no known side effects of 800 IU of vitamin D which is in the safe dose range set by the Food and Nutrition Board. In comparison, a light-skinned person wearing a swimsuit in the summer will absorb about 20,000 IU of vitamin D in the amount of time it takes her skin to get lightly pink. If you have any concerns about this vitamin D supplementation, please contact the researchers at 581-1622.

CONFIDENTIALITY:

The results of all testing and questionnaires will be recorded on special forms. These forms will have no name on them, but will use a code number to protect your privacy. All records will be kept for no more than five years following completion of the study. Your name will not be used in any publication describing this research. Medical information from the study will be made available to your physician upon your request after you have signed a written release.

Initial here to confirm page has been read ________
VOLUNTARY PARTICIPATION:

Taking part in this study is voluntary. You can choose not to answer any questions on the forms. You are free to leave the study at any time. If you fail to meet the requirements of the study, the researchers can remove you from the study at any time. There will be no charge to you or to your insurance company for tests or services that are required by this study. You will receive compensation for your time, travel and general inconvenience. St. Joseph Hospital and the University of Maine do not provide financial payment for any injury resulting from this study.

ADDITIONAL INFORMATION:

To become a part of this study, you must sign this consent. By signing this consent, you are confirming the following:

1) You have had time to consider if you want to take part in this study. You have read or had read to you this consent and had it explained to you in language you are able to understand.

2) You have had a chance to ask questions, and you have received answers that fully satisfy your questions.

3) You understand the information in this consent form. You willingly agree to take part in this study.

4) You understand the study has been reviewed and approved by an ethical research review committee to protect your legal rights.

5) You have been given a copy of this informed consent.

6) If you have any further questions about this study, you may contact Susan Sullivan at (207) 581-3130 or Monica Nelson at (207) 581-1622. If you have any questions about your rights as a research subject you may contact Gayle Anderson, Assistant to the University of Maine’s Protection of Human Subjects Review Board, at (207) 581-1498.

______________________________
Subject Signature / Date
Appendix F

Calculation of Sun Exposure
**CALCULATION FOR DETERMINING SUN EXPOSURE**

Example:

Work days – 6 days per week; 1 hour sun per day

Days off – 1 day per week; 3 hours sun per day

Vacation days – 2 weeks; 5 hours sun per day

**Table F1. Calculation used for determining sun exposure.**

<table>
<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multiply days worked per week x 16 weeks = Work Days</td>
<td>6x16=96 work days</td>
</tr>
<tr>
<td>2</td>
<td>Multiply days off per week x 16 weeks = Days Off.</td>
<td>1x16=16 days off</td>
</tr>
<tr>
<td>3</td>
<td>Determine how many days of vacation would have been days worked versus days off based on typical week from Steps 1 and 2.</td>
<td>2 weeks vacation = would have been 12 work days, 2 days off</td>
</tr>
<tr>
<td>4</td>
<td>Subtract vacation days from Work Days or Days Off as appropriate</td>
<td>96 - 12 = 84 days worked; 16 - 2 = 14 days off</td>
</tr>
<tr>
<td>5</td>
<td>Multiply new Work Days (from Step 4) by number of hours in sun per work day = Total Work Sun</td>
<td>84 days worked x 1 hour sun = 84 hours work sun</td>
</tr>
<tr>
<td>6</td>
<td>Multiply new Days Off (from Step 4) by number of hours in sun per day off = Total Day-Off Sun</td>
<td>14 days off x 3 hours sun = 42 hours day-off sun</td>
</tr>
<tr>
<td>7</td>
<td>Multiply vacation days x hours of sun per vacation day = Total Vacation Sun</td>
<td>14 vacation days x 5 hours sun = 70 hours vacation sun</td>
</tr>
<tr>
<td>8</td>
<td>Add up Total Work Sun + Total Day-Off Sun + Total Vacation Sun = Total Sun</td>
<td>84 + 42 + 70 = 196 hours sun</td>
</tr>
<tr>
<td>9</td>
<td>Divide Total Sun by 16 to determine number of hours of sun per week.</td>
<td>196 / 16 = 12.25 hours sun / week</td>
</tr>
</tbody>
</table>
BIOGRAPHY OF THE AUTHOR

Monica Nelson was born in Malone, New York on November 17, 1970. She was raised in Hamilton, New York and graduated from Hamilton Central School in 1988. She attended Cornell University and graduated in 1992 with a Bachelor’s degree in Nutritional Sciences.

While working as a clinical dietitian in Staten Island, New York, Monica attended New York University and graduated in 1997 with a Master of Public Health degree in nutrition. Monica worked as a pediatric dietitian at Loma Linda University Children’s Hospital in Loma Linda, California prior to moving to Maine in 2002 at which time she entered the Food and Nutrition Sciences graduate program at the University of Maine.

Monica is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from the University of Maine in December, 2007.