UNIVERSITY OF CALGARY

Optimizing Vitamin D Levels in Patients with Multiple Sclerosis

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Optimizing Vitamin D Levels in Patients with Multiple Sclerosis" submitted by Pavanjit Singh Ahluwalia in partial fulfilment of the requirements of the degree of Master of Science.

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2008 June 25
Date
Abstract

Multiple Sclerosis (MS) clinic physicians in Calgary, Alberta believe that MS patients should have at least minimally sufficient serum vitamin D levels (>80nmol/L) to maintain adequate bone health. Involving patients in the assessment and management of their own vitamin D needs may be effective and more efficient than having clinicians track levels. The objectives of this study were to determine the prevalence and selected correlates of vitamin D insufficiency in MS patients and to explore the process to optimize serum vitamin D levels by following a dose-adjustment algorithm for oral supplementation of vitamin. Two-hundred and thirteen patients who attended the Calgary MS Clinic between September 2006 and January 2007 participated. Each patient agreed to have serum 25(OH)D levels measured, complete questionnaires, and to adjust their oral vitamin D₃ dose over a six month period. There was a 62.4% prevalence of vitamin D insufficiency in participants at baseline. At 6-months, 47.6% of participants that had insufficient 25(OH)D levels at baseline and completed the study had their levels optimized to a serum level greater than 80 nmol/L.
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<th>Definition</th>
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<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen Presenting Cell</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CHR</td>
<td>Calgary Health Region</td>
</tr>
<tr>
<td>CHREB</td>
<td>Conjoint Health Research Ethics Board</td>
</tr>
<tr>
<td>CIMS</td>
<td>Canadian Impact of Multiple Sclerosis</td>
</tr>
<tr>
<td>CIS</td>
<td>Clinically Isolated Syndrome</td>
</tr>
<tr>
<td>CLS</td>
<td>Calgary Laboratory Services</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CYP27A1</td>
<td>Vitamin D 25-hydroxylase</td>
</tr>
<tr>
<td>CYP27B1</td>
<td>25-Hydroxyvitamin D 1α-hydroxylase</td>
</tr>
<tr>
<td>CYP24A1</td>
<td>25-Hydroxyvitamin D 24-hydroxylase</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic Cell</td>
</tr>
<tr>
<td>EAE</td>
<td>Experimental Autoimmune Encephalomyelitis</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-Pressure Liquid Chromatography</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon-Gamma</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin 2</td>
</tr>
<tr>
<td>IL-4</td>
<td>Interleukin 4</td>
</tr>
<tr>
<td>IL-5</td>
<td>Interleukin 5</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible Nitric Oxide Synthase</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>LC/MS/MS</td>
<td>Liquid Chromatography and Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin Basic Protein</td>
</tr>
<tr>
<td>M-DC</td>
<td>Myeloid Dendritic Cell</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>MOG</td>
<td>Myelin-Oligodendrocyte Glycoprotein</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix Metalloproteinases</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear Factor-Kappa Beta</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve Growth Factor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No-Observed-Adverse-Effect Level</td>
</tr>
<tr>
<td>NT-3</td>
<td>Neurotrophin 3</td>
</tr>
<tr>
<td>NT-4</td>
<td>Neurotrophin 4</td>
</tr>
<tr>
<td>p75&lt;sup&gt;NTR&lt;/sup&gt;</td>
<td>Low-Affinity Neurotrophin Receptor</td>
</tr>
<tr>
<td>P-DC</td>
<td>Plasmacytoid Dendritic Cell</td>
</tr>
<tr>
<td>PLP</td>
<td>Proteolipid Protein</td>
</tr>
<tr>
<td>PMCA</td>
<td>Plasma Membrane Ca&lt;sup&gt;2+&lt;/sup&gt; ATPase</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>Rag-1</td>
<td>Recombination Activating Protein</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor Activated NF-κB</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor Activated NF-κB Ligand</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>RRMS</td>
<td>Relapsing Remitting Multiple Sclerosis</td>
</tr>
<tr>
<td>RXR</td>
<td>Retinoid X Receptor</td>
</tr>
<tr>
<td>SPF</td>
<td>Sun Protection Factor</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming Growth Factor Beta</td>
</tr>
<tr>
<td>Th1</td>
<td>T-helper 1</td>
</tr>
<tr>
<td>Th2</td>
<td>T-helper 2</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T-cells</td>
</tr>
<tr>
<td>TRPV5</td>
<td>Epithelial Calcium Channel</td>
</tr>
<tr>
<td>TRPV6</td>
<td>Epithelial Calcium Channel</td>
</tr>
<tr>
<td>UL</td>
<td>Upper intake Level</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
</tr>
<tr>
<td>VDRE</td>
<td>Vitamin D Response Element</td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>Ergocalciferol</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Cholecalciferol</td>
</tr>
</tbody>
</table>
Chapter One: Introduction

Vitamin D is an essential molecule for the absorption of calcium and phosphorous in humans. Its deficiency is recognized as the cause of rickets in children and osteomalacia in adults. Diet is one source of vitamin D, but production in the skin after exposure to ultraviolet radiation from the sun remains the major source for most humans, hence being termed the ‘sunshine vitamin’. Emerging research suggests that vitamin D plays an important role beyond bone health, particularly in immune function and may be important in people with Multiple Sclerosis (MS). In Alberta, Canada, MS rates are amongst the highest in the world. A great proportion of the population of Calgary, Alberta is known to have insufficient levels of vitamin D. Evidence from research supports a role for vitamin D in reducing MS risk, but not necessarily a role impacting MS severity. MS clinic physicians in Calgary, Alberta believe that MS patients should have at least minimally sufficient serum vitamin D levels to maintain adequate bone health.

This thesis is comprised of two components. This first component reviews the current understanding of the emerging role of vitamin D in nervous system function and immune system functions.

The second component is a pilot study conducted with MS patients. The major aims of the pilot study were to determine the prevalence of vitamin D insufficiency in an MS cohort and to explore the process to optimize serum vitamin D levels by following a dose-adjustment algorithm for oral supplementation of vitamin D. Also, to assess the feasibility and acceptance of four different methods of managing patient vitamin D levels.
This study involved consenting MS clinic patients who had not yet had their vitamin D level optimized. Patients were stratified by Internet access and then randomized into self or directed management groups. This resulted in four study groups: 1) Self-Management with Internet access, 2) Directed-Management with Internet access, 3) Self-Management without Internet access and 4) Directed-Management without Internet access. Patients in the self-management groups were provided with their lab results by either given access to a secure website to check lab results or sent a letter in the mail and instructions to adjust vitamin D dose on their own. Whereas participants in the directed-management groups were provided with their 25(OH)D lab result and recommended a dose-adjustment when necessary sent via email or regular mail. The intention of this study was not to determine the influence of vitamin D on MS outcome, as the amount of vitamin D required for modulation of immune function is currently unknown.

The data generated by this study will improve our understanding of the magnitude of vitamin D insufficiency in patients with MS in Southern Alberta, most of whom have previously been advised to take a supplement. It will also provide preliminary data to determine how vitamin D levels can be efficiently optimized in people with MS.

1.1 Study Objectives

Objective 1: To determine the prevalence and selected correlates of vitamin D insufficiency among MS patients.

Objective 2: To examine participant adherence to the study protocol with respect to getting all the required blood tests and correctly adjusting their vitamin D dose.

Objective 3: To determine the proportion of participants in the four different study management groups who have their vitamin D levels optimized at 6-months.
**Objective 4:** Within 6 months, to correct the vitamin D insufficiency in 75% of the insufficient participants at baseline.

**Objective 5:** To examine the feasibility of using the four different study management methods to optimize vitamin D levels in terms of time to manage each group.

**Objective 6:** To assess overall patient satisfaction with participating in the optimization process.

**Objective 7:** To assess changes in participant behaviours that may increase vitamin D levels as a result of participating in the overall optimization process.

**Objective 8:** To assess the dose-response relationship of vitamin D supplementation and 25(OH)D levels over the study duration.
Chapter Two: Review of Literature

2.1 Vitamin D Physiology

Vitamin D was classified as a vitamin in the course of its discovery during the height of nutritional science in the early 20th century but later studies affirmed its classification appropriately as a prehormone (1). It is naturally produced in the plasma membrane of skin cells in the epidermis through a photochemical process. Under the exposure of ultraviolet B (UVB) rays (wavelength: 290-320 nm) a precursor, 7-dehydrocholesterol (provitamin D₃) is photolyzed resulting in a thermodynamically unstable product, previtamin-D₃, which undergoes a heat dependent isomerization to produce vitamin D₃ (2). Vitamin D₃ is very hydrophobic and the half-life ranges from 20 days to months (3). An internal system within the skin regulates the production of previtamin D₃; once previtamin D₃ formation reaches a maximum, further sun exposure only increases the concentration of biologically inactive photoisomers, lumisterol and tachysterol (2). These compounds rarely enter the circulation and are eventually eliminated during the natural turnover of the skin. Thus, prolonged sun exposure does not result in an excessive production of vitamin D₃. It is important to differentiate between vitamin D₂ and D₃. Vitamin D₃ is the natural form of vitamin D produced in skin, and vitamin D₂ is derived from irradiation of ergosterol in plant or fungal sources and is not produced by the human body (1). From this point forward all references to vitamin D are to vitamin D₃.

The vitamin D molecule itself does not hold any biological activity and modification by two hydroxylation reactions results in the active hormone molecule. The enzymes responsible for the metabolism of vitamin D are members of the cytochrome
P450 gene superfamily of mixed-function monooxygenases. This family includes a large number of related but distinct oxidative enzymes important in animal, plant and microorganism physiology (4). These enzymes are membrane-associated with the mitochondria or endoplasmic reticulum where they metabolize thousands of endogenous and exogenous compounds.

The first step in bioactivation occurs in the liver where vitamin D undergoes a hydroxylation at carbon 25 under 25-hydroxylase (CYP27A1) resulting in 25-hydroxyvitamin D [25(OH)D], which has a half-life ranging from 10 to 30 days (5, 6). 25(OH)D then travels to the kidneys where, primarily in the proximal convoluted tubule, there is a final hydroxylation at the carbon 1 position under 1-α-hydroxylase (CYP27B1) resulting in 1,25-dihydroxyvitamin D [1,25(OH)2D] (7). This metabolite is the least hydrophobic and has the shortest half-life, estimated at approximately 5-8 hours (6). Inactivation of vitamin D metabolites takes place through series of oxidative reactions at different side chain carbons by 24-hydroxylase (CYP24A1) (8). 24-hydroxylase metabolizes both 25-hydroxylated vitamin D metabolites to calcitroic acid which is then excreted in bile. 24-hydroxylase has broad tissue distribution, expressed in nearly all cells but the major site of expression appears to be the kidney (8). An overview of vitamin D metabolism is represented in Figure 1.1.
Although 25-hydroxylase is not exclusive to the liver, it is predominantly functionally active in this organ (9). 25(OH)D is the major circulating metabolite of vitamin D and is a marker of vitamin D availability to tissues; its levels are sensitive to both vitamin D intake and sun exposure (10). Serum 25(OH)D levels have been established to be the best indicator of nutritional status (11). Also, the kidneys are not the only site of the 1α-hydroxylase reaction, numerous extrarenal sites have been identified, including: bone, brain, liver, placenta, macrophages, prostate and skin (12). The contribution of these extrarenal sites to the systemic concentration of 1,25(OH)_{2}D is unknown and the kidneys remain the major site of production for circulating 1,25(OH)_{2}D.
Although 1,25(OH)\(_2\)D is responsible for the biological activity of vitamin D, its serum levels are not routinely measured as it provides essentially no information with respect to an individual’s nutritional vitamin D status (13). Serum levels of 1,25(OH)\(_2\)D are primarily used in the evaluation of several conditions including hereditary rickets and hypercalcemia associated with the following conditions of: sarcoidosis, tuberculosis, Hodgkin’s disease and lymphoma (13).

The systemic transport of vitamin D metabolites is mediated by a protein carrier, the vitamin D binding protein (DBP) and to a lesser degree, albumin. High binding affinity ensures DBP binds between 85% and 98% of each of the circulating vitamin D sterols, yet only 5% of DBP molecules are occupied by a vitamin D metabolite. The binding affinity varies for different vitamin D metabolites: 25(OH)D/24,25(OH)\(_2\)D>1,25(OH)\(_2\)D/vitamin D (14). The DBP having the least affinity for 1,25(OH)\(_2\)D ensures its small quantities are readily available when it is required but also requires strict control of its production.

Vitamin D exerts its biological action by the binding of 1,25(OH)\(_2\)D to a vitamin D receptor (VDR). The VDR is a member of the nuclear super family of steroid/thyroid hormone receptors and is a direct regulator of gene transcription (15). The VDR has variable affinity for vitamin D metabolites: 1,25(OH)\(_2\)D>25(OH)D/24,25(OH)\(_2\)D/vitamin D, ensuring preferential binding of the active molecule (16). VDR is the product of a single gene in humans and other organisms, although polymorphisms have been identified within the human VDR gene (17-19). To date, no functional phenotype has been associated with the different VDR polymorphisms. However, VDR isoforms have been reported to be associated with the susceptibility of certain diseases (20).
Tissue distribution of the VDR is extensive, surpassing 50 target tissues including cells of both tumorigenic and nontumorigenic origin (16). Levels of VDR vary greatly between tissues and even between different cell types within a given tissue. At the molecular level, 1,25(OH)₂D binds to cytoplasmic VDR inducing conformational changes. The VDR then traffics into the nucleus where it forms a heterodimer complex with a nuclear accessory factor, retinoid X receptor (RXR). RXR, a nuclear receptor for 9-cis retinoic acid, has been shown to be an compulsory partner of VDR in mediating high-affinity DNA-binding of 1,25(OH)₂D (15). 1,25(OH)₂D bound to the heterodimer complex subsequently modulates gene expression with the recruitment of additional co-activator or co-repressor complexes in specific vitamin D response elements (VDREs) within the promoter regions of responsive genes. The VDR can modulate the expression of vitamin D-responsive genes by different methods. It can positively regulate the expression of certain genes by binding to specific VDREs present in their promoter regions, or negatively regulate the expression of other genes by binding to negative VDREs, or inhibit the expression of some genes by antagonizing the action of certain transcription factors leading to indirect action (21). Vitamin D has been proposed to target cell surface receptors which might determine a variety of non-classical activities of 1,25(OH)₂D, however, the physiologic significance of a non-genomic mechanism remains to be established (22).

Molecular studies have reported that the VDR may be involved in the modulation of over 200 genes (23). The most established and studied role of vitamin D relates to its central function in the absorption of calcium and phosphorus, and in bone mineralization. Calcium is the fifth most abundant element in the human body and is crucial for many
physiological functions including neuronal excitability, muscle contraction and bone formation. Phosphorous is central to cellular metabolism, and in vertebrates it is essential to skeletal mineralization. The majority of the calcium and phosphorous in the human body is stored in the skeleton complexed together as a salt in an organic matrix (osteoid). Mineralization of the osteoid laid down by osteoblasts (bone forming cells) occurs only when the calcium x phosphate product is in the supersaturated state. The only way that calcium and phosphorous gain access to the body to serve their function is by their ingestion and absorption from dietary sources.

The main regulator of calcium levels is the parathyroid hormone (PTH); when serum calcium levels are low, calcium-sensing receptors trigger the release of PTH from the parathyroid glands. On its own, PTH acts directly on the bones and kidneys to increase the concentration of calcium but the inclusion of 1,25(OH)₂D substantially improves this process by taking advantage of a major source of calcium from the diet via the intestine. PTH stimulates the expression of 1 α-hydroxylase in the kidneys, thereby increasing the circulating level of 1,25(OH)₂D to aid in the normalization of serum calcium levels.

Calcium absorption occurs by two routes: active transcellular and passive paracellular transport. 1,25(OH)₂D functions to increase serum calcium concentrations through three separate mechanisms. First, it is involved in the induction of calcium channels involved in active intestinal calcium absorption. Active transcellular calcium transport involves a chain of calcium transport proteins (involved in apical influx, transport to the basolateral membrane and extrusion into the bloodstream). 1,25(OH)₂D modulates the expression of proteins including the epithelial calcium channels (TRPV5
and TRPV6), calbindin D 9K and plasma membrane calcium ATPase (PMCA) pump; all are involved at different steps of the transcellular movement of calcium in the intestine (24, 25). Furthermore, \(1,25(\text{OH})_2\text{D}\) stimulates active intestinal absorption of phosphate by directly regulating the expression of its transport protein in the intestine (26).

Vitamin D has a pronounced effect on calcium and phosphorous absorption in the intestine. In the absence of vitamin D, no more than 10-15\% of dietary calcium is absorbed, however, with adequate levels, this can increase by 30\% and during pregnancy, lactation and the growth spurt when \(1,25(\text{OH})_2\text{D}\) levels are attenuated, the efficiency of absorption can increase by 50-80\% (27). Approximately 60\% of dietary phosphorous is passively absorbed in the small intestine and \(1,25(\text{OH})_2\text{D}\) actively increases absorption by an additional 15-20\% (27).

If the plasma calcium concentration does not increase (typically because ingested calcium is unavailable) then PTH continues to be secreted which will continue to increase the production of \(1,25(\text{OH})_2\text{D}\). In such a state, the body will resort to mobilizing calcium from the only other source it has, the internal bone stores. This is a second mechanism by which vitamin D increases serum calcium levels. \(1,25(\text{OH})_2\text{D}\) does not directly act on the osteoclasts (bone resorbing cells) because they do not express the VDR (28). Instead \(1,25(\text{OH})_2\text{D}\) stimulates osteoblasts via the VDR to produce receptor activated nuclear factor NF-\(\kappa\)B (RANK) ligand (RANKL) on their surface (29). RANKL then interacts with RANK expressed by immature osteoclasts and this interaction results in a RANKL signal transduction to induce proosteoclasts to become mature osteoclasts. The mature osteoclasts then engage in bone resorption releasing calcium and phosphorous (29). Both vitamin D and PTH are required for this mobilization event (1).
The third mechanism by which vitamin D increases serum calcium concentrations involves the conservation of calcium at the kidneys. The distal renal tubule is responsible for reabsorption of the last 1% of the filtered load of calcium, and the 2 hormones (1,25(OH)\textsubscript{2}D and PTH) interact to stimulate the reabsorption of this filtered load (1).

A chronic state of vitamin D deficiency interferes with the normal functioning of the skeletal system and this has drastic negative effects if left untreated. In children the result is a serious condition called rickets, which is characterized by bone deformities of the long bones because of the accumulation of non-mineralization hypertrophic cartilage at epiphyseal zones with a lack of calcium and phosphate (30). In adults the result is osteomalacia, where new bone, osteoid, produced during the normal process of remodelling is not mineralized (30). Osteomalacia is not only associated with a mineralization defect of the skeleton but is also associated with isolated or global bone pain, muscle weakness and muscle pain which are symptoms that often go undiagnosed or misdiagnosed (31). Both rickets and osteomalacia can be corrected by exogenous vitamin D supplementation and serve to stress the central role vitamin D plays in skeletal health.

The extensive expression of the VDR suggests a role for vitamin D in many organ systems and syndromes beyond the scope of bone metabolism.
2.2 Vitamin D and the Central Nervous System

The central nervous system (CNS) is made of two main types of cells: neurons and glial cells. Neurons are the highly specialized functional units of the nervous system, which process and transmit electrical and cellular signals. Glia are non-neuronal cells that are in greater abundance than neurons and provide support and nutrition, maintain homeostasis, form myelin, and also participate in signal transmission in the nervous system. The main types of glial cells in the CNS are: astrocytes, oligodendrocytes, and ependymal cells. The most abundant glial cell in the CNS is the astrocyte. In addition to the cells of the nervous system there are also immune cells. Microglia are the resident macrophages that provide the first and main form of active immune defense in the CNS.

It is now appreciated that the CNS is a target organ of vitamin D. The VDR was first shown to be extensively expressed throughout the developing rat brain in 1998 and subsequently in the adult rat brain (32, 33). A recent study showed a similar pattern of widespread distribution throughout the human brain including expression in both neurons and glial cells (34). The presence of 1,25(OH)_{2}D and other metabolites of vitamin D in the cerebrospinal fluid (CSF) suggested that these molecules could cross the blood brain barrier (BBB) or be generated within the brain (35, 36). Studies have since confirmed the presence of the 1-α-hydroxylase enzyme in human brain neurons, supporting that the CNS can locally produce 1,25(OH)_{2}D from its substrate 25(OH)D (12, 34). The expression of 24-hydroxylase in astrocytes supports that these cells can also degrade vitamin D in the CNS (37).

The presence of vitamin D metabolites, along with the VDR and enzymes of bioactivation/metabolism in brain neurons and glial cells suggests that vitamin D plays a
role in the brain. There is still limited research describing the relationship between vitamin D and the nervous system, however, recent reviews have summarized the functions of vitamin D within the brain and evidence supports a possible role in neuroprotection and neurodevelopment.

2.2.1 Neuroprotection

Several investigators report neuroprotective effects of vitamin D in rodent models of excitotoxicity (38), encephalomyelitis (39), inflammation (40) and exposure to neurotoxins (41, 42). The processes underscoring this potential vitamin D mediated neuroprotection are not fully understood, however, several mechanisms have been put forth, including stimulation of neurotrophin production, reduction of calcium toxicity, and modulation of glutathione metabolism.

Neurotrophins are a sub-class of trophic factors which have a physiological role in neuronal survival, process outgrowth and regulation of plasticity (43). A number of different neurotrophins have been identified and include: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin-4 (NT-4) (44). The major trophic actions of neurotrophins are mediated by specific high-affinity transmembrane tyrosine receptors and are enhanced when a low-affinity co-receptor (p75NTR) is also present (44). Neurotrophins are important for the protection of neurons from excitotoxic, hypoxic and hypoglycemic damage emphasizing the survival-promoting role, hence they have been implicated in diseases such as Alzheimer’s, Parkinson’s, seizures and epileptogensis (43, 44).

Several investigators show that 1,25(OH)2D can regulate the production of specific neurotrophins in different cells of the CNS. In tissue culture, 1,25(OH)2D
increases the expression of NGF by neurons (45, 46). The action of 1,25(OH)\textsubscript{2}D on the production of NGF has also been shown in vivo. A single bolus of 1,25(OH)\textsubscript{2}D administered directly into the lateral ventricle induces NGF mRNA and protein in the adult rat brain (46). Neurotrophin expression has been shown in astrocytes and oligodendrocytes. 1,25(OH)\textsubscript{2}D treatment increases NGF and NT-3, but there is no impact on BDNF in astrocytes (47, 48). In oligodendrocytes, 1,25(OH)\textsubscript{2}D increases the production of NGF (49).

Another mechanism of neuroprotection may involve calcium metabolism. Because of its prominent role in regulating peripheral calcium, it was postulated that 1,25(OH)\textsubscript{2}D could also regulate calcium mediated processes in the brain. Calcium homeostasis, namely high intracellular concentrations of calcium, is recognized as having a critical role in the modulation of neuronal death related to excitotoxicity (50, 51). Mechanisms involved in reducing the intracellular concentration of calcium may therefore provide a therapeutic benefit in neuronal cell death. A vitamin D induced decrease in brain calcium may involve two distinct mechanisms. The first being that 1,25(OH)\textsubscript{2}D stimulates the expression of calcium binding proteins-parvalbumin and calbindins D9k and D28k (52-55); the second being that it also inhibits the expression of L-type calcium channels in the hippocampus (56). Both effects have been reported to protect neurons against toxic damage by reducing intracellular concentrations of calcium and suggest a role for vitamin D in the prevention of degenerative processes.

Oxidative stress is implicated in a variety of neurodegenerative disorders and may be a common mechanism underlying various forms of cell death including necrosis, apoptosis, and excitotoxicity. Regulation of detoxification, specifically glutathione
(GSH) metabolism may be another mechanism of vitamin D neuroprotection (57). GSH is an antioxidant molecule that plays multiple roles in the nervous system, including being a free radical scavenger and possible neurotransmitter. GSH depletion can enhance oxidative stress and may also increase the levels of excitotoxic molecules (58). The enzyme γ–glutamyl transpeptidase is crucial to the metabolism of glutathione (59). 1,25(OH)₂D upregulates γ–glutamyl transpeptidase activity and the expression of the corresponding gene in the rat brain (59, 60). 1,25(OH)₂D decreases brain hydrogen peroxide (a molecule that is toxic in high concentrations) and exerts a neuroprotective effect during brain damage caused by iron and zinc ions by increasing antioxidant defense by increasing brain glutathione (61, 62). As such, 1,25(OH)₂D may control brain detoxification processes via GSH regulation (57).

If the neuroprotective effects of vitamin D are fully realized, it may have potential as a therapeutic neuroprotector in a number of disorders including Multiple Sclerosis, Alzheimer’s disease and Parkinson’s disease.

2.2.2 Neurodevelopment

The VDR is expressed in the developing rat brain. An increase in VDR expression during embryonic brain development correlates with an increase in apoptosis and decrease in mitosis in brain tissue (33, 63). This correlation is consistent with the known properties of 1,25(OH)₂D as an proapoptotic and prodifferentiating agent in non-CNS tissues and as a regulator of neurotrophins in the brain (64). Collectively, this suggests that 1,25(OH)₂D, acting through the VDR, could play a role in brain development.
More direct evidence for the role of vitamin D in brain development stems from studies investigating a link between prenatal and early life hypovitaminosis D and brain development. It revolves around the ‘Neurodevelopmental Hypothesis’, which suggests there is an interaction between genetic and environmental factors during critical periods of development that affect later brain function (65).

The relevant studies have employed a vitamin D deficiency animal model where rat pups are born to mothers deprived of 1,25(OH)
3
2
D. This model has revealed a number of interesting findings. Maternal vitamin D deficiency markedly alters brain morphology, cellular differentiation, neurotrophin systems and even behavior of offspring. Alterations in gross brain morphology of the rat pups are characterized by an increased brain size (heavier), altered brain shape (cerebral hemispheres larger, longer not wider) and enlarged lateral ventricles compared to controls (66). By adulthood, external brain size and shape normalizes but the ventricles remain enlarged; this is an indicator of decreased brain tissue volume. Cellular proliferation is also increased in the rat pups; there is an increased proportion of cells in mitosis in vitamin D depleted offspring without a balancing increase in the proportion of cells in apoptosis in the four brain areas examined (the dentate gyrus of the hippocampus, the hypothalamus, the basal ganglia/amygdala and the cingulate gyrus of the cortex) (66). The specific cell types in the cellular proliferation experiments are not identified. However, vitamin D depletion does not affect the ratio of neurons to glia. The cell proliferation findings were replicated and vitamin D deficiency is found to correlate with a down-regulation of pro-apoptotic genes, and an up-regulation of mitotic genes, during pre- and perinatal stages but not postnally (67). The normalization of regulation post-natally suggests some unknown compensatory signals
become active. Nevertheless, these findings support the hypothesis that lack of vitamin D disrupts the normal sequence of apoptotic and mitotic activity during brain development.

Growth factor synthesis is also affected in the rat pups in the aforementioned studies. There is a significantly reduced expression of nerve growth factor (NGF) protein levels which correlates with a reduced expression of low affinity p75<sup>NTR</sup> receptor mRNA (66). Interestingly, binding of the p75<sup>NTR</sup> receptor by neurotrophins on its own triggers an opposing signal promoting cell apoptosis, instead of cell survival which occurs via binding in conjunction with the high-affinity neurotrophin receptors (44). Further a vitamin D response element has been identified in the promoter region of the p75<sup>NTR</sup> gene, suggesting the loss of expression of this receptor could decrease cell death processes that normally occur during brain development (68).

Therefore, even transient vitamin D deficiency has been shown to affect brain development. A study that assessed deficiency either until birth or until weaning reports restoration of the alterations when animals are examined at 10 weeks of age (69). However, some residual abnormalities, including enlarged lateral ventricles and reduced level of NGF expression persist, suggesting a long-term impact.

In relation to long-term impact, preliminary studies report behavioral consequences of vitamin D deficiency. Prenatal vitamin D deficiency in rats causes subtle behavioral consequences including hyperlocomotion, to alterations in learning and memory (70, 71). Interestingly, alterations in adult rat behavior in this model are not associated with impaired function of the hypothalamic pituitary axis (72). This suggests developmental vitamin D deficiency causes long-term impairment through an adverse event in brain development rather than via an alteration of the stress response. Moreover,
researchers report that a critical window may exist, as they are only able to observe the altered behavioral phenotype when hypovitaminosis D occurs during late gestation rather than during early gestation (73).

A dose effect also needs to be explored, as the degree of maternal hypovitaminosis D experienced by the animals in these experiments is severe and probably rare in clinical settings (66). The implication of hypovitaminosis D on the neurodevelopment of humans is unknown. The knowledge that vitamin D deficiency is widespread in pregnant women, together with the proposed associations with a range of persistent structural and cellular changes in the animal model, serve to stress the potential importance of identifying and ensuring adequate vitamin D levels in humans particularly during pregnancy (74).

Further studies are needed to clarify the role of vitamin D (and its deficiency) on the brain, as well as to understand its interplay with other mediators, as there is profound redundancy and plasticity within the brain. Established data indicate vitamin D may have a fundamental role in the CNS and may have a potentially far-reaching impact on neurodevelopmental and neurodegenerative disorders. These associations suggest that increasing rates of disorders such as autism may be related to increasing rates of vitamin D deficiency (75).
2.3 Vitamin D and the Immune System

The human immune system can be divided into two general branches: the innate and adaptive immune system. The innate system largely functions to provide the first line of defense against many common microorganisms and is essential for the control of common bacterial infections. The adaptive immune system employs T and B lymphocytes that use a large repertoire of receptors to recognize an extensive variety of antigens to generate effector cells for the elimination of pathogen. The cells of the adaptive immune system form the basis of immunological memory which ensures a more rapid and effective response on a second encounter with a pathogen, leading to the establishment of protective immunity. The adaptive immune system is also responsible for maintaining tolerance against 'self' antigens, and when this system fails it can lead to the development of destructive autoimmune conditions. Both divisions work together to provide a remarkably effective defense system for the human body.

The same VDR found on classical targets of vitamin D is widely expressed in the immune system. Most cells such as the antigen-presenting cells (APC) (including monocytes/macrophages, dendritic cells) and natural killer cells constitutively express the VDR (76-79). Resting T-cells express the VDR at very low levels, but upon activation the expression increases substantially. The CD8 subset has greater expression than the CD4 subset (77, 78). Studies to determine the expression of the VDR on B lymphocytes yield conflicting observations. The VDR is not expressed in normal resting B-cells; some studies report expression upon activation while others do not (78).

Some immune cells, in particular activated macrophages and dendritic cells, also have the capacity to synthesize 1,25(OH)2D, as they express the 1-α-hydroxylase enzyme.
(80). The 1-α-hydroxylase present in immune cells is identical to the renal enzyme, but regulation of its expression and activity is quite different. The renal enzyme is principally under the control of calcemic and bone signals, whereas the macrophage enzyme is primarily regulated by immune signals such as IFN-γ (81). This facilitates the generation of very high levels of the active vitamin D molecule within the local microenvironment.

The presence of the VDR on immune cells, and their ability to synthesize 1,25(OH)₂D, has triggered further investigation of the immune system as a target of vitamin D.

### 2.3.1 Target Cells within the Immune System

Antigen presenting cells (APC) are crucial for the generation and regulation of adaptive immune responses and are ultimately responsible for the activity of T-cells. Although any somatic cell can act as an APC, dendritic cells (DC) have come to be known as ‘professional’ APC. DCs derive from the same bone marrow precursor as macrophages and migrate to tissues where they become resident, in an immature state, to survey the local environment for pathogens (82). In a normal immune response, once a pathogen is taken up, the DC becomes activated and migrates toward a draining lymph node to present antigen to naïve T-cells. Upon activation, the DCs mature into highly effective APCs by halting antigen uptake and acquiring co-stimulatory molecules such as CD80 and CD86 that enable the DCs to fully activate antigen-specific T-cells. Mature and fully activated DCs induce a potent T-cell response by releasing important cytokines critical for T-cell differentiation into effector cells to eliminate the pathogen.

T-cells are specialized to recognize foreign antigens on infected immune cells only when peptide fragments are bound to glycoproteins of the major histocompatibility
complex (MHC) and brought to the cell surface. The MHC glycoproteins are encoded in a large cluster of genes that were first identified by their potent effects on the immune response to transplanted tissues (82). The 2 mains classes of MHC, I and II, deliver peptides from different cellular compartments to the surface of an infected cell for presentation. The different MHC classes are recognized by different T-cell subsets and result in the production of different armed effector T-cells. The MHC I molecule collects peptides derived from antigen protein in the cytosol and is recognized by T cells expressing the CD8 co-receptor. This interaction activates these CD8 T-cells to differentiate into cytotoxic T-cells. The MHC II molecule binds peptides derived from antigen proteins in intracellular vesicles and is recognized by T-cells expressing the CD4 co-receptor. This interaction activates these CD4 T-cells to differentiate into T-helper (Th1 or Th2) effector cells.

1,25(OH)_{2}D is one of the most powerful inhibitors of DC differentiation. Collectively, studies performed either on monocyte-derived DCs from human peripheral blood or on bone-marrow derived mouse DCs demonstrate that in vitro treatment with 1,25(OH)_{2}D leads to the continued expression of the monocytic marker CD14 and the failure to up-regulate the DC marker CD1a (83-86). Even when added to already immature DCs, 1,25(OH)_{2}D is able to cause the cells to revert to a monocyte phenotype (83).

Vitamin D also interferes with the maturation of DCs. When immature DCs are cultured with 1,25(OH)_{2}D and the maturation inducing stimuli lipopolysaccharide (LPS) or TNF-α, they have a reduced capability to express the full complement of MHC and co-stimulatory molecules (such as CD40, CD80 and CD86) (83, 85, 86). Also, an induction
of CD14 expression is observed. In contrast, mature DCs are resistant to the effects of vitamin D. This may be related to a reduced expression of VDR as mature monocytic DCs have a reduced expression of VDR as compared with monocyte precursors (84). 1,25(OH)$_2$D treatment does not reverse the DC phenotype and only slightly decreases the expression of the co-stimulatory molecules: CD80, CD86, HLA-DR (human MHC II equivalent) and of CD83 (marker for mature DCs) in mature DCs (83). These tissue culture studies suggest that DCs exposed to 1,25(OH)$_2$D early in their life cycle are maintained in a persistent immature state, which are able to take up antigen but are unable to present antigen to T-cells.

Both a specific antigen presented along with MHC peptides and a co-stimulatory signal must be provided by an APC to stimulate the clonal expansion of naïve T-cells. Activated T-cells synthesize the T-cell growth factor IL-2 and its receptor (IL-2R). The production of IL-2 indicates that a T-cell will proliferate and become an armed effector cell. The co-stimulatory signal provided by the interaction of CD80, CD86 on APCs with CD28 on T-cells is necessary for the synthesis and secretion of IL-2 (82). Antigen recognition, in the absence of co-stimulation, leads to T-cell tolerance which inactivates naïve T-cells by inducing a state known as 'anergy' with the inability to produce IL-2. This state of anergy prevents the naïve T-cells from proliferating and differentiating into effector cells when they encounter antigen, even if the antigen is subsequently presented by APCs. In a normal immune response against a pathogen this could have a detrimental impact as it would inhibit the T-cell response from eliminating the pathogen. On the other hand it also ensures tolerance of T-cells when a self-tissue antigen is encountered. This potentially limits an autoimmune response from developing into an autoimmune disease.
1,25(OH)\(_2\)D treatment alters the phenotype of DCs and also influences their functional capacity. When added to cultures of DCs generated from monocytes or already immature DCs, these conditioned DCs have a reduced ability to stimulate T-cells to proliferate (83, 85). The interaction not only reduces T-cell proliferation but also induces a state of hyporesponsivity in the exposed T-cells. T-cells first co-cultured with 1,25(OH)\(_2\)D-conditioned DCs are unable to respond to a second stimulation with control DC; IFN-\(\gamma\) secretion is inhibited in treated T-cells (86). IFN-\(\gamma\) is an important cytokine released by activated T-cells to combat pathogens. 1,25(OH)\(_2\)D also interferes with the secretion of the key cytokine IL-12 which is responsible for the differentiation of effector CD4 T-cells. 1,25(OH)\(_2\)D treatment decreases IL-12 secretion in tissue culture (85, 86). Overall, 1,25(OH)\(_2\)D profoundly affects all stages of the DC life cycle, inhibiting its differentiation, maturation and activation.

One concern that is raised is that 1,25(OH)\(_2\)D treatment could potentially result in a compromised immune system unable to resist infectious agents due to a profound suppression of DCs. However, there are two distinct subsets of DCs, defined as myeloid (M-DC) and plasmacytoid (P-DC) that have been identified in human and mouse blood (87). The two subsets have different properties. M-DCs are the most efficient APCs directly able to prime naïve T-cells and, depending on the circumstance, can become either immunogenic or tolerogenic. P-DCs however, play a key role in maintaining peripheral immune tolerance and are considered naturally occurring tolerogenic DCs. A very recent study reveals that modulation of DC tolerogenic properties by 1,25(OH)\(_2\)D as described previously is observed only in M-DCs and not P-DCs (88). By 1,25(OH)\(_2\)D not affecting P-DCs, their tolerogenic potential would remain unaltered. This may explain the
intact host resistance against infectious agents following 1,25(OH)₂D administration in a transplantation model (89).

Vitamin D also directly targets T-cells even without using APCs as an intermediary. 1,25(OH)₂D induces antigen specific inhibition of T-cell proliferation (90). The mechanism of inhibition likely involves IL-2, as 1,25(OH)₂D treatment reduces secretion of IL-2 from T-cells (90, 91). The VDR acts directly on the IL-2 promoter to repress transcriptional activation by 1,25(OH)₂D (92). This explains the earlier finding that 1,25(OH)₂D blocked the entry of activated T-cells into the S-phase in the cell cycle as the interaction between IL-2 and its receptor on the surface of activated T-cells is required for progression through the cell cycle (transition from G1 to S phase) (93).

During a normal immune response, different types of activated T-cells undergo different differentiation pathways where they acquire specialized features and effector functions. CD4 T-cells are thought to traditionally differentiate into T helper-1 (Th1) or T helper-2 (Th2) cell subsets. Cytokines play a dominant role in T-helper cell differentiation at the initial activation stage. The presence of IL-12 or IL-23 can preferentially generate Th1 cells that secrete cytokines like IFN-γ, IL-2 and TNF-α and promote cell-mediated immunity able to eliminate intracellular pathogens (94). In contrast, Th2 cells develop in the absence of IL-12 but in the presence of IL-4, producing cytokines like IL-4, IL-5, IL-10, TGF-β1, which are essential for the removal of extracellular pathogens. Alterations in the balance between Th1 and Th2 responses is associated with a variety of disease states. Excessive Th-2 response is associated with asthma whereas an excessive Th-1 response is associated with autoimmune disease. It is
generally accepted that Th1 and Th2 cells have opposing properties due to the cytokines they secrete: Th-1 being pro-inflammatory and Th2 anti-inflammatory.

Vitamin D not only influences T-cell proliferation but also cytokine secretion and the subsequent development of a Th1 or Th2 response. 1,25(OH)\(_2\)D modulates the expression of immune cytokines in T-cells at the level of transcription and gene expression. Several Th1-type cytokines are suppressed including IL-2, IL-12 and, IFN-\(\gamma\). Activation of the IFN-\(\gamma\) promoter is down-regulated by 1,25(OH)\(_2\)D. Binding of the VDR is detected at the IFN-\(\gamma\) promoter suggesting a possible mechanism of interference by 1,25(OH)\(_2\)D with the normal transcription of the IFN-\(\gamma\) gene (95). Inhibition of IL-12 by 1,25(OH)\(_2\)D is achieved through the direct targeting of an IL-12 gene subunit (96). The promoter region of this gene subunit contains a site for NF-\(\kappa\) \(\beta\) (a regulator of gene expression during immune and inflammatory responses), suggesting this transcription factor could be involved (97).

The role of 1,25(OH)\(_2\)D in regulating the differentiation of Th2 cells is unclear. Some studies have shown that 1,25(OH)\(_2\)D enhances the development of Th2 cells via a direct effect on naïve CD4\(^+\) T-cells. Naïve purified murine CD4\(^+\) T-cells stimulated in the absence of APC and cultured with 1,25(OH)\(_2\)D develop into a more Th2 than Th1 population characterized by enhanced proportions of IL-4, IL-5, and IL-10 secreting cells (98). The increased production of Th2 cytokines is associated with augmented expression of the Th2-specific transcription factors, GATA-3 and c-maf (98, 99). However, in other studies, differentiation into a Th2 phenotype is not clearly affected by 1,25(OH)\(_2\)D as IL-4 production is either down-regulated (100) or unchanged (101, 102). Therefore, it is unclear if 1,25(OH)\(_2\)D truly influences Th2 response.
The immunomodulatory effects of 1,25(OH)$_2$D however go beyond the Th1/Th2 cell balance and also involve another subset of T-cells called ‘regulatory T-cells’ (Tregs). There has been a recent resurgence of interest in Tregs, once called ‘suppressor T-cells’. Tregs were first identified as a subset of CD4$^+$ T-cells that possessed the capacity to maintain immune homeostasis and self-tolerance (103). A variety of different Tregs have been identified including CD4$^+$CD25$^+$Foxp3$^+$, IL-10-secreting T regulatory 1 (Tr1) cells, TGF-β-secreting Th3 cells, a repertoire of CD8$^+$ T-cells, and CD4$^+$CD8$^-$ double negative Treg cells (104). Tregs characterized by the co-expression of CD4$^+$, CD25$^{hi}$ and the Foxp3 transcription factor are one of the most studied Tregs in humans and rodents. Some Treg populations are naturally produced in the thymus, while others can be induced in peripheral lymphoid tissues from naïve T-cells as a consequence of antigen exposure (104). Numerous potential mechanisms of suppression by Tregs have been proposed and include direct T-cell-T-cell interaction involving TGF-β, IL-10 mediated suppression, and modification of the function of dendritic cells (103).

1,25(OH)$_2$D treatment induces CD4$^+$CD25$^+$ Treg production in animal models of disease. In a mouse model of allograft islet transplantation, mice are treated with both 1,25(OH)$_2$D and Mycophenolate mofetil (an inhibitor of T and B cell proliferation that also modulates APCs). The result is an increased percentage of CD4$^+$CD25$^+$ regulatory cells in draining lymph nodes and spleen (105). When these cells are transferred into a naïve mouse rendered diabetic by streptozotocin injection, the Tregs are able to prevent subsequent islet allograft rejection indicating an active mechanism of tolerance induction. Further, in a non-obese diabetes (NOD) mouse model, treatment with an analog of 1,25-(OH)$_2$D (Ro26-2198) significantly expands the number of CD4$^+$CD25$^+$ T-cells in
pancreatic lymph nodes (106). These CD4^+CD25^+ Tregs have suppressive activity as they inhibit autoreactive T-cells, inhibit LPS-induced production of IL-12, and delay the capacity of pathogenic T-cells to transfer disease into other recipients.

Induction of Tregs by tolerogenic DCs may provide an indirect explanation for the mechanism of Treg induction by 1,25(OH)_{2}D. Immature DCs have tolerogenic properties and can induce T-cells with suppressor activity (107). As previously described, in vitro treatment with 1,25(OH)_{2}D maintains DCs in an immature state therefore leading to DCs with a tolerogenic phenotype and function. 1,25(OH)_{2}D also induces DCs with tolerogenic properties in vivo; mice treated with vitamin D analog-conditioned DCs have prolonged skin graft survival compared with animals treated with unconditioned DCs (108). The increased proportion of Tregs in mice following vitamin D treatment is accompanied by DCs that express a tolerogenic phenotype (105). Based on this data, it has been suggested that tolerogenic DCs induced by 1,25(OH)_{2}D treatment may favour the induction of CD4^+CD25^+ Tregs rather than effector T-cells with the ability to modulate transplantation tolerance and autoimmune disease (107).

1,25(OH)_{2}D has multiple effects on the immune system. The APCs and T-cells are direct targets leading to the inhibition of effector T-cells and the enhanced frequency of regulatory T-cells. Further exploration into the immune properties of 1,25(OH)_{2}D could lead to 1,25(OH)_{2}D becoming an immunomodulatory agent perhaps suitable for the treatment of various human inflammatory diseases.
2.4 Overview of Multiple Sclerosis

2.4.1 Clinical Features

Multiple Sclerosis (MS) is a disease characterized by autoimmune demyelination and axon injury in the brain and spinal cord. The early thorough description of the pathology and clinical features of MS was by French neurologist Dr. Jean-Martin Charcot in 1868 (109). MS symptoms typically emerge between 20 and 40 years of age in approximately 70% of patients (110). There is a pattern of geographic distribution of MS with increased prevalence occurring farther from the equator; indeed, Canada has one of the highest rates of MS (240 per 100,000) in the world (111). The MS Society of Canada estimates that 55,000-75,000 people in Canada are affected by the disease and it is the most common neurological disease affecting young adults in Canada. Within a given population, the overall prevalence of MS is approximately twice as high in women as in men.

MS is a highly variable condition as it can be mild or can lead to death. The most common features of MS include fatigue, sensory disturbances, muscle weakness, lack of coordination, visual impairments, cognitive impairment and bowel and bladder impairment (110). The disease course varies but three common clinical patterns are recognized. The most common course is the Relapse-Remitting (RRMS) type. This is characterized by clearly defined acute relapses (attacks). A relapse is an episode of neurologic symptoms lasting one day to several months. Relapses are followed by partial or full recovery to the pre-existing level of disability (110). The other types of disease course are progressive, where patients have a gradual accumulation of disability with or without superimposed relapses (110). Progressive course can be divided into primary
progressive, with progression from disease onset and secondary progressive, where the patient starts with RRMS disease before progressing.

2.4.2 Pathogenesis

The pathogenesis of MS is immune-mediated CNS injury; it is considered an autoimmune disease. Several antigens are important including myelin basic protein (MBP), proteolipid protein (PLP), and myelin-oligodendrocyte glycoprotein (MOG) (112). Immunizing mice with spinal cord homogenate containing myelin proteins (MBP, MOG or PLP) results in a paralytic autoimmune disease with similarities to the inflammatory phase of MS (113). This animal model, referred to as experimental autoimmune encephalomyelitis (EAE), has contributed to the understanding of the physiological and pathological immune response related to MS (114).

The current working model of MS is that of molecular mimicry. In this model, it is proposed that peripheral immune cells specific for myelin proteins are activated as a result of interaction with a virus, another infectious agent, or other environmental stimulus (115). These T-cells would then recognize antigens presented by APCs and be activated to secrete proinflammatory cytokines, IL-1, IFN-γ and TNF-α (115). The activated T-cells would then migrate across the blood-brain barrier (BBB), mediated by an array of molecules including selectins, integrins and matrix metalloproteinases (MMPs) to enter the CNS (114). The breakdown of the BBB allows the entry of T-cells that cause active foci of inflammation which is generally perivenular (115).

In the CNS the immunoreactivity is amplified when antigens on CNS resident APCs (microglia) activate T-cells. It is suggested that in MS and EAE, autoreactive lymphocytes produce chemokines (chemical signals) and attract inflammatory cells into
the perivascular space. These recruited and activated inflammatory cells then produce cytokines like IL-1 and TNF-α and neurotoxic chemicals like nitric oxide (NO) which can damage oligodendrocytes and neurons (116).

Early research proposed that MS is a disease primarily mediated by MBP-specific CD4+ T-cells. This is supported by the involvement of MBP-specific CD4+ T-cells being a striking feature in MS patients versus healthy controls and EAE could be induced passively with sensitized CD4+-myelin-specific T-cells in an adoptive transfer model (112). This research is further supported by the inflammatory states in MS and EAE being attributed to Th1 cell types, wherein proinflammatory cytokines, TNF-α, IFN-γ and IL-12 prevailed (112). This led to the hypothesis that autoreactive CD4+ T-cells particularly of the Th1-type are at the center of the pathogenic process in MS (115).

CD4+ Th1 cells appear to play a predominant role in this model of MS pathogenesis; recent research supports the notion that a variety of immune cells including CD8+ T-cells, and B-cells must also be involved in MS development. It is difficult to explain the death of oligodendrocytes (myelin producing cells) and neurons by CD4+ Th1 cells because these cells only have MHC class I molecules which cannot present antigen to CD4+ T-cells (114); CD4+ T-cells only have the ability to recognize antigen associated with MHC class II molecules. A more appealing premise is that CD8+ cytotoxic T-cells, which recognize antigens associated with MHC class I molecules, account for the destruction of oligodendrocytes and subsequent neurons (112). In supporting this premise, is the development of progressive and destructive EAE when encephalitogenic myelin-specific CD8+ T-cells are transferred into a mouse (passive transfer model of EAE) (117, 118). Also, CD8+ cytotoxic T-cell expansion occurs in the cerebrospinal fluid
(CSF) of MS patients (119, 120). The CD8$^+$ cytotoxic T-cells also outnumber T-cells of the CD4$^+$ subtype in MS lesions (121). Further, in tissue culture, this T-cell subset kills oligodendrocytes and neurons in an antigen-specific manner leading to axonal damage similar to that seen in MS brain lesions (114). Therefore, the role of CD8$^+$ T-cells cannot be denied and adds another dimension to disease pathogenesis.

The occurrence of elevated antibody levels and oligoclonal immunoglobulin G (IgG) bands in the CSF of MS patients has suggested a role for both B-cells and plasma cells in MS pathogenesis (122). Various roles for B cells and IgGs have been proposed in MS. B-cells can function as APCs for T-cells and thus participate in CNS-specific restimulation of autoreactive T-cells, provide costimulatory signals to T-cells and recruit T-cells to the CNS. (112). The identification of antibodies reacting with distinct myelin antigens especially MOG in MS lesions, suggests B-cells may cause myelin destruction (123, 124). The full role of B cells and IgGs remains to be established.

The investigation of CD4$^+$CD25$^+$ Tregs in human autoimmunity has attracted considerable attention in recent years. Collective experiments in both animals and humans outlines their potential to modulate a variety of autoimmune conditions (109). Accordingly, Tregs are of interest in MS research because of their potential to inhibit ongoing pathogenic autoimmunity and restore self-tolerance. Studies reveal that Tregs are equally distributed in the blood and CSF of MS patients and their number and distribution does not differ from healthy controls (125, 126). However, with either polyclonal or myelin-specific stimulation, the suppressive function of patient-derived CD4$^+$CD25$^+$ Tregs is impaired. These studies provide insight into the pathogenic role of
Tregs; that they seem to be impaired in MS and this results in a decreased ability to control the immune response.

Overall, a number of immune cells have been implicated to have a role in the pathogenesis of MS. It is currently thought that CD4$^+$ T-cells are important at onset and the CD8$^+$ T-cells and impaired Tregs sustain the disease.
2.4.3 Etiology

Although the cause of MS remains unknown, it is thought to be the result of an interaction between genes and the environment. At present, the most widely accepted hypothesis for the etiology of MS is that susceptibility to the disease is genetically determined and onset is triggered by unknown environmental factors. Genetic factors play a role as the disease is reported to predominantly affect Caucasians whereas certain racial groups such as Black Africans, East Asians, and Native Americans consistently have lower rates of MS (110). Further, family studies have identified a clear familial aggregation with a higher age-adjusted risk for siblings than for second and third-degree relatives (127). The concordance rate between monozygotic twins for MS is around 35% and is higher than in children with both parents affected (20%) as opposed to offspring’s of single-affected couples (2%) (127). Genetic studies suggest that susceptibility to MS may be contributed by the effect of polymorphisms in a number of genes. Studies involving the entire human genome have indicated that there is no single gene which has a significant influence on MS; it appears there is a combined impact of a variety of genes (110). Genes related to certain Human Leukocyte Antigen (MHC class equivalent antigen-presentation genes in humans) class II molecules represent a risk factor for MS (110, 112).

There are a number of observations that support environmental factors having a role in the susceptibility of MS. These factors include the lack of complete concurrence for MS in identical twins, the prevalence of MS increasing with latitude even in countries that are relatively racially homogeneous, and the changes in risk found in migration studies (128). A wide variety of environmental factors have been suggested to have a
role; vitamin D, infection with a virus or pathogen (particularly the Epstein-Barr virus), and cigarette smoking have emerged as consistent predictors of MS risk (128, 129). Although, none of these proposed triggers have actually been proven to have an effect, indirect evidence supports compelling links.
2.5 Vitamin D and Its Link with Multiple Sclerosis

2.5.1 Epidemiology

The prevalence of MS shows a striking geographical pattern; it rises with increasing latitude in both hemispheres, from a low of 1–2 cases per 100,000 people near the equator, to greater than 200 cases per 100,000 people at latitudes higher than 50° N (113). Duration and intensity of sunlight has a strong correlation with latitude (129). It was almost 40 years ago that E.D. Acheson proposed that exposure to sunlight might reduce MS risk, since this striking inverse correlation with MS prevalence was shown (130). Further, cumulative research supports this hypothesis that reduced sunlight exposure increases MS risk. In a case-control study based on death certificates in the United States from 1984-1995, individuals with the highest levels of residential and occupational sunlight exposure have the lowest odds ratio (0.24) of mortality from MS (131). Moreover, an Australian case-control study reports that high sun exposure during childhood and early adolescence, and greater actinic damage (used as an objective marker of cumulative lifetime sun exposure), are associated with a decreased risk of MS (132). Unusual geographic patterns can also be explained, for example in Switzerland, districts at low altitudes (≤1000m) have higher MS rates, whereas districts at high altitudes (≥1000m) have lower MS rates despite genetically similar populations (113). UV light intensity is higher at high altitudes, thereby accounting for low MS rates at higher altitudes. Thus, the inverse correlation between high sunlight exposure and lower MS risk suggests that sunlight might be a protective environmental factor in MS.

The vitamin D endocrine system is highly responsive to sunlight and for most humans, exposure to sunlight is their major source of vitamin D (10). In 1974, Goldberg
suggested that the amount of vitamin D available in the environment, either from sunlight exposure or from the diet, might affect the prevalence of MS (133). The prevailing hypothesis is that sunlight, working through the vitamin D endocrine system, may be protective in MS due to the immunoregulatory functions of 1,25(OH)₂D₃ (113). The evidence supporting this hypothesis is indirect but is supported by research from a number of different areas, namely epidemiology and experimental animal and clinical studies.

Nutritional studies provide evidence that links vitamin D intake to lower MS risk. Lower MS prevalence occurs in coastal villages with greater fish consumption than in inland agricultural communities in Norway (fish is known to be an excellent source of vitamin D) (129). A longitudinal study also highlights the influence of vitamin D intake and MS risk. Pooled data from two prospective large cohorts of American women (the Nurses’ Health Study (NHS I; 92,253 women followed for twenty years) and Nurses’ Health Study II (NHS II; 95,310 women followed for ten years)) shows that even minimal vitamin D supplementation (as little as the 400 IU which is typically included in multivitamins) is associated with a 40% reduction in risk of developing MS (134). Further, in a case control study of US military personnel, MS cases were identified over a 12 year period and were matched (age, sex, ethnicity and dates of blood collection) to controls recruited from the same military population. Preclinical vitamin D status measured in serum collected from the time of military recruitment was estimated by averaging 25(OH)D levels of 2 or more serum samples collected before the date of initial MS symptoms. The risk of MS among Caucasians decreases with increasing serum levels of 25(OH)D, and levels above 100 nmol/L show the greatest risk reduction at 51% (135).
There is no significant association between vitamin D and MS risk among blacks and Hispanics (109 cases, 218 controls), who had lower 25(OH)D levels than Caucasians. The smaller sample size and substantially lower 25(OH)D levels in the blacks may have reduced the power to detect an association in this group. Also, there were no black cases or controls with 25(OH)D levels 100nmol/L or higher, therefore, the assessment of the association between high serum levels and MS risk was not possible. As childhood and adolescence appear to be important periods of exposure for the relationship between sun exposure and reduced MS risk, 25(OH)D levels greater than 100nmol/L before the age of 20 (16-19 years) compared to ages 20 or older, show a considerably stronger reduction in risk (91%) for MS development.

Evidence that month of birth is associated with rate of MS has also been intriguing. A study using data pooled from Canada, Denmark, Great Britain and Sweden found that people born in May have an increased MS risk and people born in November have a lower risk (136). This research suggests that climate, including its influence on vitamin D from sunlight, impacts the risk of MS during gestation or shortly after birth.

2.5.2 Vitamin D in the Autoimmune Model: Experimental Autoimmune Encephalomyelitis

Early in vitro evidence that T-cells express the VDR, and that 1,25(OH)₂D treatment directly inhibits T-cell proliferation and cytokine secretion, led to the hypothesis that vitamin D may have an influence in T-cell-mediated autoimmune diseases. A number of experimental autoimmune models have contributed to the expanding knowledge of the role of vitamin D on immune function. EAE was one of the first autoimmune animal models to establish a role for 1,25(OH)₂D. The biologically
active form of vitamin D 1,25(OH)\textsubscript{2}D, is an inhibitor of EAE. Treatment with 1,25(OH)\textsubscript{2}D shortly before EAE induction results in no or milder disease activity and prolonged survival, and there is correlation between pathology and clinical signs of disease (137, 138). Further, onset of EAE is accelerated in vitamin D-deficient mice but pre-treatment with 1,25(OH)\textsubscript{2}D completely blocks EAE induction (139). Vitamin D modulates acute severe EAE by halting progression and stabilizing disease severity or reversing paralytic symptoms (139, 140).

The mechanism by which vitamin D inhibits EAE induction is not known. However, the immune effects are likely mediated via the VDR as 1,25(OH)\textsubscript{2}D does not prevent EAE in VDR null mice (141). CD8\textsuperscript{+} T-cells are likely not necessary for disease induction as 1,25(OH)\textsubscript{2}D is able to prevent EAE in CD8\textsuperscript{+}-null mice (142). Further, a role for calcium in disease inhibition is suggested by evidence that when there is lack of dietary calcium, increasing dose of 1,25(OH)\textsubscript{2}D fails to suppress EAE (143).

It is known that CD4\textsuperscript{+} T cells are key players in the pathogenesis in both EAE and MS, at least initially (112). In vitro data suggests that 1,25(OH)\textsubscript{2}D affects T-cells by variably inhibiting CD4\textsuperscript{+} Th1 development or enhancing Th2 development depending on the EAE model (144). In the classic MBP induction model 1,25(OH)\textsubscript{2}D--treatment does not affect IFN-\gamma or TNF-\alpha transcripts, but increases IL-4 transcripts (a key cytokine in the anti-inflammatory Th2 response) (145). In contrast, in a MOG induced mouse model, 1,25(OH)\textsubscript{2}D is a potent inhibitor of Th1 development without deviating to a Th2 response (101). In an adoptive transfer model, 1,25(OH)\textsubscript{2}D treated mice do not develop EAE, but lymph nodes have a high number of CD4\textsuperscript{+} Th1 cells that proliferate and produce IFN-\gamma (140). There is no increase in Th2 IL-4 transcripts either in the lymph nodes or in the
CNS. The variation in the mechanism of the effect of vitamin D in different EAE models suggests that a simple Th1 to Th2 pathway shift does not explain the modulating effects of vitamin D.

TGF-β1 and IL-10 are recognized as anti-inflammatory cytokines and are thought to play an important role in autoimmune disease; they may have a role in modulating EAE (144, 146). 1,25(OH)₂D is unable to prevent EAE in IL-10 null mice suggesting that IL-10 is necessary in vitamin D-mediated inhibition. (146). 1,25(OH)₂D treatment prior to EAE induction enhances TGF-β1 transcripts in lymph nodes in mice. Treatment after EAE induction increases transcripts in the CNS (145). In contrast, in a rat model there is no effect of short-term 1,25(OH)₂D treatment on TGF-β1 transcripts in the CNS after EAE induction (36).

Evidence that 1,25(OH)₂D inhibits DC maturation, activation and APC function, all components of T-cell priming, led investigators to explore the role of APCs in EAE. The Rag-1 gene is required for the development of T and B-cells and knocking out this gene results in impaired T and B-cells. 1,25(OH)₂D treatment before EAE induction does not inhibit EAE in transgenic mice that have a non-functional Rag-1 gene, indicating that Rag-1-dependent T or B cells are necessary for 1,25(OH)₂D mediated inhibition of EAE (102). This finding rules out a direct effect of 1,25(OH)₂D on APCs in EAE induction but does not disprove an indirect effect by 1,25(OH)₂D through APCs or a role at a later stage of the disease.

The conflicting evidence of the impact of 1,25(OH)₂D on the Th1/Th2 response has suggested that 1,25(OH)₂D might strengthen the function of suppressor lymphocytes, such as Tregs, that regulate autoreactive T cell responses. Currently there is limited data
on the effect of 1,25(OH)_2D on Treg involvement in EAE but in one study IL-10–producing CD4^+ Treg cells were induced by treatment with vitamin D_3 and the immunosuppressive steroid dexamethasone (147). This population of Tregs was only induced when the two agents were used in combination, while using the drugs individually did not lead to the generation of such Tregs. These Tregs generated by the combination treatment completely prevented EAE when they were adoptively transferred prior to disease induction.

1,25(OH)_2D treatment has a profound effect on clinical disease in EAE even once it is established. However, the mechanism by which it reverses EAE is unknown. Evidence supports that 1,25(OH)_2D may reverse EAE by inducing changes with respect to inflammatory cells within the CNS. 1,25(OH)_2D treated mice have 50% less white matter and meningeal inflammation and a 70% reduction in inflammatory cells (macrophages) in the spinal cord compared to placebo treated mice (140). 1,25(OH)_2D treatment after EAE induction increases proapoptotic genes and increases the proportion of apoptotic cells in inflammatory lesions (148). Further, 1,25(OH)_2D treatment after EAE induction reduces chemokines that recruit the inflammatory cells into the CNS along with actually reducing cell recruitment (149). Thus, these observations may account for the decline in macrophages in the CNS.

Ultimately, it is the inflammatory cells that secrete cytokines and other agents that result in the destructive damage to myelin, oligodendrocytes and even neurons. Nitric oxide (NO) is one such agent and its increased production is thought to be responsible for some of the CNS tissue destruction in MS and EAE (150). It is a free gaseous radical produced by inducible nitric oxide synthase (iNOS). 1,25(OH)_2D treatment after the
appearance of clinical signs of EAE inhibits the expression of iNOS by macrophages, microglia and astrocytes within the CNS (39). The inhibition of iNOS expression coincides with clinical improvement. Additionally, NO production facilitates inflammatory cell recruitment in MS and EAE (150). 1,25(OH)\textsubscript{2}D within the CNS could therefore facilitate reduction in the production of NO and could reduce the infiltration of inflammatory immune cells that contribute to demyelination.

Overall, the mechanism of 1,25(OH)\textsubscript{2}D in preventing EAE induction and suppression of disease progression is a complex interplay between many immune cells in different pathways at various stages of the disease. Collectively, these studies support a role for vitamin D both in the prevention and the ongoing treatment of MS.

2.5.3 Vitamin D in Multiple Sclerosis Patients

Currently, there is limited understanding of the impact of vitamin D on the course of MS once it has begun, although, preliminary studies are ongoing. Vitamin D status, however, is a concern even once a diagnosis of MS has been established because hypovitaminosis D is commonly seen in MS patients and is not desired for optimal health. A Finnish group of MS patients have significantly lower 25(OH)D serum levels than controls during the summer (58 nmol/L versus 85 nmol/L) although there is no difference in winter levels (41± 5 nmol/L versus 44 nmol/L) when both groups have significant insufficiency (151). MS patients in Tasmania, Australia are more likely to have vitamin D levels less than 40 nmol/L (40.5% vs. 31.4%) compared with age and sex matched community controls (132). Long-term vitamin D malnutrition is characterized by low bone mass and high fracture rates; 25(OH)D levels less than 50 nmol/l are seen in
69% of MS patients in a New York hospital with significantly reduced bone mass and this level is associated with a 10-fold higher fracture rate than controls (152, 153).

Current data on the correlations between 25(OH)D levels and disease activity in MS suggest a potential association. In a Finnish study, relapsing MS patients have lower mean 25(OH)D level than patients in remission, (44±4 nmol/L vs. 59 ±5 nmol/L), although there was no correlation with level of disability, various CSF parameters, or enhancing lesions on CNS MRI (151). Another study finds that during the months of the year when vitamin D levels are typically lower there is a corresponding increase in gadolinium enhancing lesions on MRI, further supporting an association between the immunomodulatory effects of vitamin D and MS (154, 155).

2.5.4 Vitamin D Supplementation in Multiple Sclerosis

The efficacy of vitamin D supplementation in MS has not yet been examined, but small, non-controlled trials with arbitrary doses have been undertaken. In one study 16 patients receive 5000 IU of vitamin D per day as cod liver oil along with magnesium and calcium over one to two years. In this open-label uncontrolled study there are fewer exacerbations than expected (156). The inability to actually estimate the number of expected exacerbations however means this outcome is of limited validity. In another study, short-term treatment with vitamin D₃ (1000 IU daily) and calcium over 6-months raises the levels of TGF-β1 which is an important anti-inflammatory cytokine in EAE (157). Other small studies using 1,25(OH)₂D₃ or related analogues for the most part reveal no significant changes in disease activity (clinical or MRI) (158-160). The studies primarily evaluated the safety and tolerability of using such compounds in MS. The occurrence of hypercalcemia is noted in the study using 1,25(OH)₂D. Unlike
1,25(OH)₂D, parent vitamin D₃ and 25(OH)D on their own have no reported direct calcemic effects and emphasize that using 1,25(OH)₂D may not be as safe as using vitamin D₃ itself because of the high risk of hypercalcemia (161). Vitamin D₃ is safer and during clinical trials with intake of 10,000 IU/day for up to 20 weeks and intake of 50,000 IU/day for eight weeks, there is no hypercalcemia (162). One pilot-trial currently underway is a one year matched-pair controlled design study looking at escalation of vitamin D₃ up to 40,000 IU/day for 6-months, followed by a downward titration over the second 6-month period. Preliminary safety results report no instance of hypercalcemia or other adverse effects. Follow-up data will explore immunological effects and the effect on several clinical outcome measures including EDSS, ambulation index and relapse rate (163).

Mounting evidence therefore converges to implicate lack of sunlight exposure and the resulting deficiency of vitamin D, as an important modifier of MS risk and pathogenesis. There is no single alternative interpretation proposed to explain the results of all of these studies supporting the role of vitamin D in MS.
2.6 Defining Optimal Vitamin D Status

Currently, there is no standard definition for optimal vitamin D status even for skeletal health. The notion that optimal status can simply be defined as the absence of osteomalacia has been largely rejected even though chronic vitamin D deficiency is the cause of rickets and osteomalacia. The substrate dependent synthesis of $1,25(OH)_2D$ starts to become affected when serum $25(OH)D$ levels drop below 30-40 nmol/L (164). As such, in adults, most experts have historically defined “vitamin D deficiency” as serum $25(OH)D$ levels less than 40-50 nmol/L (23). There is now also appreciation for a state of mild to moderate deficiency of vitamin D, termed “insufficiency”. Insufficiency is defined as a pre-clinical phase of deficiency that impacts skeletal health to a lesser degree than rickets and osteomalacia. There is considerable overlap in the biochemical and bone histologic changes found in vitamin D deficiency and insufficiency—which together have been referred to as hypovitaminosis D. Several physiological and clinical outcomes have been assessed in relation to serum $25(OH)D$ levels to better define a threshold for insufficiency related to skeletal function.

A common biomarker that has been used to define insufficiency is the serum $25(OH)D$ level that maximally suppresses serum parathyroid hormone (PTH). An inverse relationship is described between PTH concentrations and serum $25(OH)D$. PTH starts to rise when serum $25(OH)D$ falls below a certain threshold (165). Secondary hyperparathyroidism leads to increased bone resorption by decreasing the mineral and matrix content of the skeleton. This increases the risk and severity of osteoporosis (27). Secondary hyperparathyroidism also causes hypophosphatemia, which lowers the calcium-phosphorus product below normal, causing a mineralization defect leading to
osteomalacia. The serum 25(OH)D level at which PTH production begins to plateau varies, but it is usually at 25(OH)D levels of 75-80 nmol/L. This level has therefore been proposed as the minimum level for bone health (166).

Serum 25(OH)D levels also relate to calcium absorption. Increasing serum 25(OH)D levels from 50 to 80 nmol/L increase intestinal calcium absorptive efficiency by 45% to 65% (167). Studies of bone mineral density (BMD) and fracture prevention also contribute to estimating the lower limit of vitamin D insufficiency. In 13,432 men and women of different ethnic-racial backgrounds aged 20 and older who participated in the National Health and Nutrition Examination Survey (NHANES), a positive correlation is observed between serum 25(OH)D levels and total hip BMD (168). Being at the upper end of the range of serum 25(OH)D level, 90 to 100nmol/L, is advantageous for BMD.

Additionally, several randomized controlled trials show a positive effect of vitamin D supplementation on BMD. Prevention of hip (26%) and any nonvertebral fracture (23%) in older adults is observed in a meta-analysis of randomized controlled trials with vitamin D supplementation compared with calcium or placebo (169). No significant benefit is observed for trials evaluating doses of 400 IU vitamin D per day; only those evaluating a dose of 700-800 IU per day are associated with this benefit. Fractures are also associated with serum 25(OH)D levels; serum concentrations have to exceed 75 nmol/L to minimize fracture risk (170).

While accumulating evidence suggests a function for vitamin D beyond the scope of bone health. The level of vitamin D necessary to maintain such health outcomes is currently unknown. However, it seems best to assume that this recommended level must be at least as high as that first required to satisfy the skeletal needs of the body
(>80nmol/L). A recent review that evaluated outcomes including lower-extremity function, periodontal disease and colorectal cancer prevention, proposes a level of at least 100 nmol/L (171).

Much of the world’s population receives inadequate vitamin D; it is estimated that at least one billion people worldwide have vitamin D insufficiency (23). Vitamin D can be obtained from three sources: sunlight, artificial UVB light, or dietary supplements. Because the skin has a very large capacity to produce vitamin D, sunlight is the most efficient and cost-effective approach for its acquisition. For light-skinned individuals, exposing a considerable part of their bodies (e.g. swimsuit) to the point of minimum redness of the skin (1 minimal erythemal dose) is equivalent to getting between 10,000 and 25,000 IU vitamin D from an oral supplement (27). The individual serum 25(OH)D concentrations obtained from people living or working in sun-rich environments have been reported to be as high as 225 nmol/L (172). Studies also document increases of serum 25(OH)D levels by the use of artificial tanning (173-177). However, tanning beds and lamps are not consistent in the amount of UVB light they emit, making it difficult to quantify a dose-response relationship.

There are a number of factors that affect the cutaneous production of vitamin D. Sunscreens effectively absorb UVB rays and markedly diminish the total amount of UVB rays that can reach 7-dehydrocholesterol in the skin’s cells. A sunscreen with a sun protection factor (SPF) rating equal to or greater than 8 can reduce the amount of previtamin D produced in the skin by >95% (178, 179). Further, melanin is a skin pigment that acts as a natural sunscreen. Melanin competes with 7-dehydrocholesterol for UVB rays in the skin and increases the time of UV radiation exposure that is required to
maximize previtamin D formation (2). Dark-skinned individuals require more exposure
time to sunlight to produce the same amount of vitamin D in their skin as would a light-
skinned person (180). Also, the skin content of 7-dehydrocholesterol decreases by 50% 
from age 20 to age 80, indicating how age limits the ability of the skin to generate 
vitamin D (181). Clothing and remaining indoors also reduce access to vitamin D; these 
are also affected by climate, culture, age, gender and disability.

Latitude also influences vitamin D generation in the skin. Below 35° latitude, the 
angle of the sun (zenith angle) is more direct allowing previtamin D₃ synthesis in the skin 
to occur year-round. When the angle is very oblique (early morning, late afternoon and 
winter), sunlight must pass through more ozone, which absorbs UVB rays and very little, 
if any, reach the earth’s surface. Above 35° latitude, the angle of the sun is so oblique 
during the winter months that most, if not all of the UVB light below 315 nm is absorbed 
by the ozone layer, thereby either reducing or completely preventing the production of 
previtamin D in the skin. (182). This has resulted in the concept of a ‘vitamin D winter’ 
which is apparent in northern latitudes; in Edmonton, Canada (53° N) this ‘vitamin D 
winter’ extends from October to March (183).

These factors (skin color, sunlight exposure, sunscreen use, age, clothing, climate, 
culture, gender and latitude) combined with concerns about the increased risks of skin 
cancer and premature aging that are associated with sun exposure, means most people 
cannot rely on sun exposure for their vitamin D source. This supports an endemic of 
vitamin D insufficiency in northern latitudes such as Canada; indeed current prevailing 
data suggests that up to 97% of Canadians have insufficient vitamin D levels at some 
time during the year (184).
With the exception of the flesh of oily fish, very few foods that humans normally eat naturally contain vitamin D. Apart from milk, some cereals and bread, very few foods are fortified with vitamin D in North America (31). The high incidence of lactose intolerance in blacks reduces milk consumption and may decrease the likelihood of these people getting adequate vitamin D from their diet. Supplementation is a convenient and effective source of vitamin D in the absence of sun exposure.

The current recommendations for vitamin D intake from official food and nutrition agencies in the USA and Canada are as follows, a daily intake of: 200 IU for children and adults up to 50 years of age; 400 IU for adults 50-70 years of age; and 600 IU for adults older than 70 years of age (185). These recommendations are based on an assumption that the skin is also being exposed to at least some sunlight to produce vitamin D. However, these dose recommendations have been shown to have little effect on serum 25(OH)D concentrations in adults and using the current definitions of insufficiency (75-80 nmol/L) they are definitely outdated (172, 186, 187). Further, the present safe tolerable upper intake level (UL) is set at 2000 IU/day. This is essentially meant to be the highest chronic daily oral intake of vitamin D that will pose no risk of adverse effects for most healthy adults. The current UL for vitamin D is believed by many experts to be based on faulty evidence and requires revision based on current scientific evidence (162). A recent review presents a risk assessment of vitamin D based on relevant human clinic trials of vitamin D. It should be noted that hypercalcemia is the main endpoint as an adverse effect occurring at the lowest dosage in evaluating the safety of vitamin D. A no-observed-adverse-effect level (NOAEL) for vitamin D for the general healthy population was recommended as 10,000 IU/day and 220 nmol/L was selected as
the serum 25(OH)D concentration that occurs at this selected intake (162). Among individuals with low sun exposure, supplements providing 1000 IU/day often increase circulating levels to 80-100 nmol/L (166, 188) and supplements of 4000 IU/day maintain levels greater than 100 nmol/L (172, 189). There are no reports of hypercalcemia or other known adverse side effects in healthy individuals taking these doses. Also, 4000 IU/day results in circulating levels of 25(OH)D which are lower than the average levels among individuals living in sun rich environments (190).

Recommendations of high dose oral supplements come with the caution of toxicity, which although rare, is possible. Vitamin D toxicity (hypervitaminosis D) results in abnormally high serum calcium levels (hypercalcemia), which can lead to bone loss, kidney stones and calcification of organs like heart and kidney (191). The first sign of toxicity is the presence of excessive calcium in the urine (hypercalciuria) and then in the serum. There are no reported cases of vitamin D intoxication from sun exposure as the skin’s own intrinsic regulation prevents overproduction. All of the reported cases of hypercalcemia have been due to faulty industrial production, labeling errors, dosing errors or have occurred in patients with compromised health or other confounding factors treated medically with high doses of vitamin D$_2$ (ergocalciferol) (192). There are no reported cases of toxicity from vitamin D$_3$ (cholecalciferol) in humans other than from pharmaceutical manufacturing errors. Based on reports of patients intoxicated with vitamin D$_3$, 25(OH)D levels greater than 700 nmol/L may be needed to evoke hypercalcemia in normal adults (172, 193-195). There is no credible evidence in the literature of vitamin D toxicity in adults taking $\leq$ 10,000 IU daily of supplemental vitamin D (192). With chronic supplementation at doses exceeding the current upper
limit (2000IU/day), a reasonable strategy to avoid the possibility of toxicity would be to monitor serum 25(OH)D and calcium levels.
2.7 Measuring Serum Vitamin D Levels

Serum 25(OH)D concentration is the best clinical indicator of vitamin D status because it represents the combined contributions from cutaneous synthesis and dietary intake of vitamin D (10, 11). A number of different techniques have been developed over the years for the measurement of vitamin D levels including radioimmunoassay (RIA), high-pressure liquid chromatography (HPLC), mass spectrometry and competitive binding protein. Most of the assays are available as commercial kits for large-scale clinical laboratory testing. A major issue related to measuring vitamin D levels has been the variability of the assays (196). This variability had added to the challenge of establishing a single threshold value to define optimal vitamin D status and to the challenge of optimizing levels in individual patients. Nevertheless, efforts are being made to strive for an international standardization of 25(OH)D measurement (197). Most experts agree that external as well as internal quality assessment could maintain and increase the reliability of vitamin D testing (198).
2.8 Self-Management

Recent recognition of the widespread importance of vitamin D in maintaining health, recognition that higher serum levels than previously understood are required and growing appreciation of widespread hypovitaminosis D has led to a need to learn how to optimize vitamin D in individuals. This has resulted in an increasing number of vitamin D levels being ordered, challenging the already heavy service burden for healthcare personnel and adding to the cost of testing. Perhaps involving patients in the assessment and management of their own vitamin D needs may be effective, acceptable to patients, and may be a more efficient model of care.

Self-management of dosing according to laboratory values has been studied in the care of people on oral anticoagulation therapy since the early 1990’s (199). The need to monitor therapeutic control of anticoagulation therapy is important because of the risk of thromboembolic events and bleeding complications. The international normalized ratio (INR) is a measure of an individual’s anticoagulation induced clotting defect. INR target ranges have been developed for a variety of thrombotic conditions that best balance the risk of haemorrhage and thrombosis. The number of INR values within the target range is used as an indirect parameter of the risk of thromboembolic and bleeding complications (199). Self-management involves instructions on how to use a portable coagulometer for measurement of the INR and how to self-adjust treatment dose. Alternatively, conventional management entails advising patients of recommended dose adjustments after review of lab results by health practitioners, sometimes in a specialty anticoagulation clinic.
A systematic review of studies comparing randomized self-management vs. conventional management shows that self-management is at least equivalent to management by a specialized anticoagulation clinic (199). Moreover, a recent large (n=737) randomized controlled trial provides additional evidence for the benefit of patient self-management. Patients assigned to self-management of anticoagulation achieve a similar quality of INR control but have superior results in terms of reduction of total major complications compared to those assigned to conventional management (200).

Educating patients is an integral part of the self-management model. Most of the anticoagulation studies included structured training courses for patients (200, 201). Furthermore, studies in diabetes management have identified that patient education is necessary for long-term success of self-management and can play a crucial role both in control of the disease and in the quality of life of patients (202).

Self-management also influences healthcare from the patient perspective because patients are responsible for making clinical decisions themselves. Improved self-awareness of health status is believed to be the reason self-management strategies improve safety and adherence to therapy (203-205). In this way, patient empowerment relates to outcome. Self-management programs emphasize the central role of the patient in managing his or her own illness and offer patients the opportunity to collaborate with healthcare providers in optimizing their care (206).

One of the main concerns related to self-management is adherence. Menéndez-Jándula and colleagues attribute simple education programs as being useful in achieving good results (200). Even with an intervention group that includes many patients who were elderly or had lower levels of education (only a primary-school education or were
illiterate), at least 50% of patients are able to safely use the self-management strategy. The dropout rate of 21% in the self-management group is not different than seen in other anticoagulation studies, where drop out rates range from 17% to 33% (200). These findings suggest that self-management may not suitable for all patients. However, with evidence suggesting improved patient outcomes, and the drive to reduce health care costs, application and evaluation of the self-management model of care in other conditions should be explored.
2.9 Health Informatics

Health informatics is a growing field that is changing health care delivery and improving access to care globally. "Health informatics" refers to the use of information and communication technology in combination with information management concepts and methods to support the delivery of health care (207). The introduction of the Internet to clinical practice as an information-sharing medium has given rise to many opportunities for innovative interventions to manage chronic illnesses (208). The appeal of web-based therapies is that the format and structure of the interventions can be quite diverse, ranging from something simple like accessing health-related educational information, to something more interactive like on-line support (either individualized or to groups) (209).

Interestingly, combining web-based interventions with self-management has revealed promising outcomes. A recent web-based application for diabetes, MyCareTeam, demonstrates a significantly better treatment effect in terms of achieving glycemic goals in patients with poorly controlled, complicated diabetes (210). The patients entered their blood glucose and exercise data, and communicated with a clinical care provider via the web site on a weekly basis. The care provider recommended subsequent clinical interventions to optimize blood glucose control, and gave advice via MyCareTeam, including suggestions regarding diet, exercise, and medication changes. MyCareTeam also provided general diabetes, nutrition, and exercise information. The study shows significant trends in the reductions of hemoglobin A1C levels which corresponded to frequency of use of the web intervention. MyCareTeam is an example of
a more elaborate and interactive web-based application. It highlights the use of a web-based intervention to support healthcare.
Chapter Three: Methods

3.1 Study Design

This was a pilot study to examine the prevalence of vitamin D insufficiency in a sample of MS patients. It was also a prospective exploration into the process of optimizing serum 25(OH)D levels longitudinally in those patients who were insufficient by incorporating four different methods of optimizing serum vitamin D levels.
3.2 Subjects and Recruitments

3.2.1 Inclusion/Exclusion Criteria

Eligible participants included all MS patients, at least 18 years of age, who were participating in a longitudinal outcomes study (CIMS) and who had previously indicated their willingness to consider participation in additional research studies. This longitudinal study was established in 2002 and was designed to determine the clinical, biological, and demographic characteristics associated with MS outcomes. Only patients with a diagnosis of MS or a diagnosis of “clinically isolated syndrome” (CIS)-a first neurologic episode suggestive of MS are enrolled in this outcome study. Of this pool of patients, only those who had not previously had their serum 25(OH)D levels measured and optimized were included.

Patients with cognitive deficits that would preclude their ability to provide informed consent and/or complete the study questionnaire were excluded from the study. Also, patients with any of the following known conditions were excluded from the project as vitamin D supplementation may have been unsafe for them: primary hyperparathyroidism, sarcoidosis, tuberculosis, and lymphoma. Patients who were on the following specific medications: Digoxin, Verapamil, Diltiazem or thiazide diuretics were also excluded due to the possible risk of hypercalcemia with vitamin D supplementation.

While it is common to exclude pregnant and breastfeeding women from clinical studies, these women are also at risk of vitamin D insufficiency which may have implications on the health of their child including decreased birth weight and childhood bone-mineral accrual (211-213). Vitamin D supplementation studies in breastfeeding women, and a current clinical trial of pregnant women, indicate there is no adverse event
related to physiologic levels of supplementation (211, 214). As it is therefore important to optimize the vitamin D levels of these women as well, there was no reason to exclude them from this study which was just evaluating the process of optimization.

3.2.2 Recruitment

Patients who were scheduled to see an MS clinic physician during the recruitment period (September 2006 and January 2007) were informed of this study prior to their clinic visit by telephone to allow them time to consider participation. If the patient was interested in participating in the study, he or she was mailed or e-mailed an informed consent form. At the time of their clinic appointment, the patients’ MS clinic physician was advised of the patient’s interest in participating in the study. If the physician agreed that the patient should have their vitamin D level optimized, informed consent was obtained.

The Calgary MS clinic is the only source in Southern Alberta of multidisciplinary care for over 4000 registered patients. Over 1700 patients are currently followed in the CIMS project where active participants complete annual questionnaires. Approximately 60% of the CIMS population have provided e-mail addresses with their contact information so we expected a similar proportion of those recruited to this study to have Internet access. A sample size similar to that of a previous study of vitamin D levels in Calgarians (n=188) was thought to be adequate because that study included enough subjects to describe vitamin D levels throughout the year.

In addition, a sample size of 200 would have sufficient power to estimate the prevalence of vitamin D insufficiency within 3-8% (determined by a consultant from the Centre for Advancement of Health, Calgary) (Appendix A). This degree of precision was
considered adequate for the purposes of this study. Because of an anticipated loss of 20% of the total sample to follow-up of vitamin D levels at 6-months, 240 patients were recruited altogether. The 240 patients were equally divided for the four management groups, 60 participants were recruited for each group.
3.3 Procedures

Participants were asked to have their blood tested for 25(OH)D level, complete questionnaires and take oral vitamin D supplements and adjust their vitamin D dose according to the study protocol at baseline, 3-months and 6-months.

3.3.1 Measurement of Serum 25-Hydroxyvitamin D Levels

Lab requisitions were provided to participants at the baseline recruitment visit. As these were clinically indicated tests, the patient had their blood drawn at Calgary Laboratory Services (CLS) clinics. It was the responsibility of each participant to remember the dates for follow up blood tests as this was part of measuring adherence to the study protocol.

CLS used a commercially available radioimmunoassay (RIA) kit to measure serum 25(OH)D$_3$ (DiaSorin Inc., Stillwater, Minn). In this assay 25(OH)D$_3$ is extracted from each sample using acetonitrile, and an iodine I$^{125}$-labeled tracer is used to measure the amount of 25(OH)D$_3$ (215). The intra- and inter-assay coefficients of variation are approximately 15% and 10%, respectively (R. Krause, Section Head Scientific Operations, CLS, personal communication). All vitamin D metabolite tests for patients within the Calgary health region (CHR) are completed by CLS. Blood from all participants who lived outside of the CHR, was tested at the University of Alberta where an in-house assay using liquid chromatography and tandem mass spectrometry (LC/MS/MS) is used. To standardize the lab results of the study to the dominant method of RIA, the lab results generated by this method were converted into RIA values using a linear regression equation derived from the U of A lab’s own quality control data:

$$[25(OH)D]_{\text{RIA}} = 0.87 [25(OH)D]_{\text{LC/MS/MS}} + 2.96$$ (216).
Additionally, toward the end of this study, CLS changed its assay technique from the RIA to a non-RIA chemiluminescence immunoassay, Liaison platform (DiaSorin Inc., Stillwater, Minn) (217). It uses the same antibody detection method but there is a slight shift in results related to the absence of a using a radioactive tracer (13). To standardize the results, a linear regression equation from CLS lab’s own quality control data was applied to convert the data into RIA values: 

\[ [25(OH)D]_{\text{LIAISON}} = 0.625 [25(OH)D]_{\text{RIA}} + 7.9 \] 

(R. Krause, Section Head Scientific Operations, personal communication).

### 3.3.2 Questionnaires and Feasibility of Optimization Protocols

To assess factors that can influence 25(OH)D levels, participants completed self-administered questionnaires at baseline and at each of two follow up periods after a blood test (Appendix B and C). The following factors have been previously described to affect serum vitamin D levels and questions pertaining to each were asked of each participant: age, melanin pigmentation in the skin (race), body mass index (BMI), sun exposure, dietary vitamin D intake, travel, use of sunscreen, current prescription medications and artificial tanning (2, 179, 184, 218-223).

MS specific characteristics of the study sample were compiled from existing data collected from patient chart reviews with participation in the CIMS study. Age at MS onset is defined as the age that MS symptoms first appear and may be several years before a diagnosis of MS is made. Disease duration is defined as time from onset of first symptoms to specified date which for this study was the date of recruitment. Disease course is defined as either relapsing remitting, primary progressive, secondary progressive or uncertain. Expanded disability status scale (EDSS) is an estimate of the
severity of MS disability. Scores range from 0 to 10 with higher scores indicating greater disability. EDSS was assessed at the time of patient recruitment in the clinic by the patient’s MS physician. Disease modifying therapy status is defined as taking any of the following four approved MS disease modifying therapies: any three of the Interferon-beta formulations Rebif, Betaseron, Avonex or Glatiramer Acetate (Copaxone). Disease modifying therapy status was obtained in the questionnaire patients completed at their recruitment visit in the clinic.

In developing the questionnaires, questions were obtained, with permission, from several different sources. In the baseline questionnaire, questions on sex, date of birth, race, weight, height and prescribed drugs were taken from a previous CIMS study questionnaire developed at the University of Calgary MS Clinic by Dr. L Metz and her colleagues. Some questions on sun exposure were taken from the Canadian Metacenter Osteoporosis Study questionnaire (224), and other questions on sun exposure and vitamin intake were taken from a previous vitamin D study (184). The question on diet was developed by the graduate student. The questions on artificial tanning were taken from the Rapid Risk Factor Surveillance System (225). The question on medication adherence was from a senior care practice survey and modified for use in this study (226). The questions on the follow-up questionnaire were similar to the baseline questionnaire except the time-periods were modified to reflect the previous 3 months rather than the previous year.

At the end of the study at 6-months, all participants completed a questionnaire assessing satisfaction and acceptance of the study management method they were assigned (Appendix D). Also, an exploratory aim was to assess changes in participant
behaviours that may increase vitamin D levels as a result of participating in the overall optimization process. Behaviours included were: reducing the number of missed doses of vitamin D, increasing the sun exposure, including more vitamin D rich foods in the diet, reducing the amount of sunscreen worn and wearing less sun protective clothing when outdoors in the sun. This questionnaire was developed by the graduate student as existing satisfaction surveys were not applicable for this study given the exploratory nature of this outcome. An online survey tool, SurveyMonkey was used to administer questionnaires to those participants in the Internet access groups. An expanded description of the SurveyMonkey tool has been included as Appendix E.

The other purpose of this study was to explore the feasibility of the optimization protocols. Measures of feasibility were: adherence to the protocol, namely proportion of participants that complete all required blood tests and follow the optimization procedures (based on their report in the questionnaires), and an estimate of the time required for the graduate student to manage the interventions. A record was kept of participants that did not adhere to the protocol and the time that was required to inform each participant of their serum 25(OH)D level and dose adjustment, if applicable to their assigned management method was recorded. The graduate student used a stop watch to record the time for each participant as lab results were received over the duration of the study. The record to track time was started after baseline, to give patients the opportunity to request clarifications about their management method. Thus, from the 3-month time-point onward, it was expected all participants were familiar with the process for their assigned management method.
3.3.3 Optimizing Vitamin D Dose with Algorithm

As with all clinical samples, lab results are sent to the MS clinic to be first reviewed by MS clinic nurses. All 25(OH)D results were first reviewed by the nurses and then passed on to the graduate student for management. All of the lab results were reviewed and then entered into an electronic database for each participant. The patient’s MS clinic physician was advised of all results less than 40 nmol/L. Also, when a 25(OH)D level remained unchanged in a participant who reported taking his or her supplements, the principal investigator Dr. Luanne Metz was informed as well as the patient’s clinic physician so that appropriate follow up could be arranged if necessary. Otherwise all the lab results including the examples from situations described above that did not require physician follow up were used to optimize patient vitamin D level as per study protocol.

After commencing the study, two procedural amendments were introduced. In the circumstance that a patient increased their dose and the 25(OH)D level decreased (without any suitable explanation), the patient was asked to have his or her blood retested. In addition, patients who remained on the same dose but showed extreme increases in 25(OH)D level were also asked to have their blood retested. Only the results from the first lab result were used for data analysis.

Clinical experience in 2005 suggested that 1000 IU of vitamin D₃ increased the 25(OH)D level by approximately 20 nmol/L (L. Metz, unpublished observations). This was supported by data from studies of people living at various latitudes, suggesting that for every 40 IU of vitamin D intake, circulating 25(OH)D increased by approximately 0.70 nmol/L which is a 17.5 nmol/L level increase per 1000 IU dose increase (189).
Therefore, the following algorithm was developed for dose-adjustment of oral vitamin D supplements: For approximately every 20 nmol/L increment (or portion thereof) the 25(OH)D level was below the minimum target (80 nmol/L), the vitamin D dose was increased by 1000 IU. A simplified version of this algorithm given to patients and used by the graduate student is outlined in Table 3.1 (and as Appendix F).

Table 3.1: Dose-adjustment algorithm given to participants and used by the graduate student

<table>
<thead>
<tr>
<th>Lab Result 25-Hydroxy Vitamin D Level</th>
<th>Add the following amount to your Daily Dose (IU=International Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-30 nmol/l</td>
<td>3000 IU</td>
</tr>
<tr>
<td>30-50 nmol/l</td>
<td>2000 IU</td>
</tr>
<tr>
<td>50-80 nmol/l</td>
<td>1000 IU</td>
</tr>
<tr>
<td>&gt;80 nmol/L</td>
<td>Optimal Range-No Change in Dose</td>
</tr>
<tr>
<td>&gt;130 nmol/L</td>
<td>If you have 2 lab results 3 months apart like this, reduce daily dose by 1000 IU</td>
</tr>
</tbody>
</table>

3.3.4 Method of Assignment to Study Group

To manage their 25(OH)D levels, participants were stratified by having either Internet access or no Internet access, and then randomized into “self-management” or “directed management” groups. As patients consented to participate, they were asked whether or not they had Internet access and whether or not they were willing to participate using this method. Then the appropriate enrolment package—either “Internet”
or “Postal” was given to the participant. The participant and the administrators of the study did not know if the participant received the self-management or directed management method until the package was opened by the participant since the enrolment packages had been sealed and shuffled.

All participants received enrolment packages prepared by the graduate student; all of the packages included the following components:

1) Instructions based on the assigned management method (Appendix G);
2) Baseline questionnaire to be completed in the clinic (Appendix B);
3) Lab requisition;
4) MS clinic vitamin D brochure (Appendix H); and
5) Investigator contact information (Appendix G)

The four groups for vitamin D management were:

**Self-management with Internet Access:**

- This group was provided with the dose-adjustment algorithm and instructions describing its use to manage their vitamin D levels.
- Participants were provided with a username and password and were assigned a unique study ID to access a website restricted only to participants of this project.
- The website listed their lab results by study ID. It also included electronic versions of the contents of the enrolment package (except lab requisitions) (Website pages included as Appendix I).
• Participants were asked to check the website for their results about 2 weeks after lab tests had been completed. They were then supposed to adjust their dose as necessary according to the algorithm.

**Self-Management without Internet Access:**

• This group was provided with the algorithm and instructions describing its use to manage their vitamin D levels.

• The graduate student would receive the participants’ lab results after each blood test and mail this information to the participant.

• Participants in this group were supposed to adjust their vitamin D dosage as necessary according to the algorithm.

**Directed Management with Internet Access:**

• The graduate student would receive the participants’ lab result and interpret them based on the algorithm. The graduate student would advise the participants of their individual levels and recommended dose adjustments (if any) by e-mail.

**Directed Management without Internet Access:**

• The graduate student would receive the participants’ lab results and interpret them based on the algorithm. The graduate student would advise the participants of their individual levels and the recommended dose adjustments (if any) by mail.

At the end of the study, Dr. Metz advised each participant on the continued dose recommended and whether or not follow up monitoring of serum 25(OH)D levels was required.
3.4 Defining Insufficiency and Data Management

Current expert opinion suggests that the lower limit of the serum 25(OH)D level that maintains adequate bone health is 75-80 nmol/L (166). In accordance with these data, insufficiency for this study was defined as a serum 25(OH)D level less than 80nmol/L.

Participants were not contacted about unreturned 3-month questionnaires as this could have influenced participant adherence to the study protocol. However, participants were reminded about unreturned 6-month questionnaires within 2 weeks of being sent. The 6-month questionnaires were sent out after the patients lab result had been received by the graduate student, therefore contacting the participant would not have affected adherence to the study. Missing responses on questionnaires were treated as unknown values.

3.5 Statistical Considerations

Data from the baseline and follow-up questionnaires were initially analyzed using univariate analyses. This analysis was completed for the entire study sample at baseline and follow up periods. Mean 25(OH)D levels and total daily vitamin D dose were calculated for the entire group at each time interval and changes between time intervals were assessed with paired t-tests.

3.5.1 Objective 1

To test the primary study hypothesis (Objective one) the prevalence of vitamin D insufficiency among MS patients at baseline was determined with the associated 95% confidence interval. To assess if the prevalence of vitamin D insufficiency was \( \geq 50\% \) as hypothesized, a one-sided, one sample test of a proportion was conducted. The null
hypothesis was that the prevalence of vitamin D insufficiency would be <50% and the alternate hypothesis was that the prevalence would be ≥50%. These data were presented for the entire sample that had a baseline blood test.

Also part of objective one was to determine the selected correlates of vitamin D insufficiency. Associations between serum 25(OH)D level and demographic and questionnaire specific variables were analyzed using unpaired t-tests and ANOVA (Scheffé’s test for subsequent between-group comparisons). A general question about travel was asked of patients in the questionnaires but was analyzed specifically for travel to destinations below 42° N latitude.

3.5.2 Objective 2

To evaluate adherence to the study protocol, the proportion of participants that completed the required blood tests and the proportion of participants that adhered the study protocol was determined. This was done for the total participating group and also analysed by subgroup (study management method). Chi Square tests were used to assess if there were differences in the proportions of those that adhered to the study protocol.

3.5.3 Objective 3

The next step in the analysis was objective three; to determine if the proportion of participants who optimized their vitamin D levels differed across the management groups at the 6-month follow up. A Chi Square test was used to test the null hypothesis that the optimization of vitamin D levels was the same across the four groups. The alternate hypothesis was that optimization of vitamin D levels in at least one management group will differ from the others at 6-months.
3.5.4 Objective 4

Objective four was to determine the proportion of vitamin D insufficient patients who had their insufficiency corrected at 6-months. This analysis was descriptive. These data were presented for the entire sample that completed the study. One goal of the study was to show that 75% of the insufficient subjects would improve their serum 25(OH)D levels through the study, so the proportion who were optimized was simply contrasted with this projected proportion.

3.5.5 Objective 5

To evaluate the feasibility of the optimization protocols, the mean time required to manage each participant according to the different management methods by the graduate student was calculated. ANOVA was used to test if there was a difference in mean time to manage across the four groups and Scheffé’s test was used to evaluate between-group comparisons. Unpaired t-tests were used to test if there was a difference based on self or directed management and based on study communication mode (Internet and postal).

3.5.6 Objectives 6 and 7

Participants’ responses from the end of study questionnaire provided descriptive data and were presented using frequency distributions and proportions. This questionnaire included questions on patient satisfaction related to the study management group they were assigned to and also changes in participant behaviour that may increase vitamin D levels as a result of participating in the overall optimization process.

3.5.7 Objective 8

To assess the vitamin D dose-response relationship a simple calculation was carried out to estimate the change in serum 25(OH)D for every 1000 IU/day dose
increase. Two specific time periods were analyzed: baseline to 3-months and 3-months to 6-months for participants that completed the study. The dose-response was calculated for these two separate 3-month time periods and not the overall 6-months because participants would be taking a consistent single dose during these three month intervals.

Statistical significance was set at a p-value of 0.05. Statistical analyses were conducted using Intercooled Stata version 8.0 for Windows (Stata Corp, TX, USA).

3.6 Ethical Considerations

This study received approval by the conjoint health research ethics board (CHREB) of the University of Calgary and the Calgary Health Region (CHR) (Appendix J).

A participants' treating MS clinic physician had to approve the participants' involvement in the study. This way only those participants for whom optimization was indicated for clinical care, and who did not have an exclusionary condition, were included in this project. No lab tests were done that were not clinically indicated. All lab results were carefully monitored, as such, toxicity of vitamin D supplements at the recommended dosages was not of concern.

Since this study measured the process of optimization and corrected a deficiency in participants, pregnant and breastfeeding MS patients were included in this project as these women are also at risk of vitamin D insufficiency. Current evidence does not indicate any adverse events related to physiologic levels of supplementation in this group of participants.

Confidentiality was protected by assigning each patient with a unique study identification number with no personal information linked to it. All questionnaires were identified with the study ID and paper based versions were stored in a locked cabinet in a
locked office before and after the data were entered into a computer database, and only handled by the graduate student. The computer database was developed in Microsoft Access and data were stored on the CHR server. The project website was password protected with only participants in the project having access to the password. No personal information was stored on the website, as lab results for participants were listed by participant study ID. The website was created under the graduate student’s account which is stored on the University of Calgary web-server. No one other than the graduate student and his supervisor, Dr. L. Metz, had access to this account. The graduate student used his CHR issued e-mail account to correspond with participants as approved by the ethics board. The resulting e-mails were removed from the e-mail account and downloaded into a designated folder for the duration of the study under the graduate student’s CHR account.

With regard to the SurveyMonkey program, only participant e-mail addresses and study ID numbers were stored on the SurveyMonkey account and linked to the questionnaires that were completed. The results of the electronic version of the questionnaires were downloaded into the computer database. The SurveyMonkey privacy policy states that program operators will not use the data for their own purposes, and the collected data will be kept private and confidential. Only the principal investigator, Dr. L. Metz and graduate student had access to the SurveyMonkey account.
Chapter Four: Results

4.1 Recruitment and Study Completion

Recruitment began in September 2006 and was completed in January 2007. A total of 240 patients consented to participate in this study; all except one patient completed a baseline questionnaire at their clinic visit. There were 213 consented patients that went on to complete a baseline blood test (hereinafter any reference to “participants” indicates the 213 consenting patients that completed the baseline questionnaire and blood test). Of these 213 participants, 160 (75.1%) had a 3-month blood test and 151 (70.9%) returned a completed questionnaire. Lastly, 134 (62.9%) of the participants had a 6-month blood test and 130 (61.0%) returned a completed questionnaire. 6-month serum 25(OH)D levels and questionnaires were complete by August 31, 2008 in 91.8% (123/134) of completers. There were nine participants that completed baseline and 6-month blood tests but did not have a 3-month blood test.

Three out of the 27 consenting patients that did not participate provided notice of their withdrawal from the study. Four participants who withdrew at later times during the study provided a reason: one attributed withdrawal to active MS that they attributed to use of vitamin D, another reported that they were not able to consistently take their vitamin D, and two reported other health issues. The available data from these four participants were included in the analysis. The reasons why other patients discontinued participation are unknown. An overview of study completion is provided in Figure 4.1.
Figure 4.1: Overview of study recruitment and completion (% represents the proportion out of 213 participants)
4.2 Baseline Characteristics

Demographic and MS disease characteristics of the 213 participants that completed the baseline assessments, and the 134 participants that completed 6-months of study, did not differ (Table 4.1). Participants varied widely in age (21-72 years) and were mostly female (~80%). The majority had a relapsing remitting course (77.0%), most (64.8%) were taking MS disease modifying therapy, and most had mild to moderate disability although the sample included participants with severe disability (mean 3.2; range 0-8.5). The consenting patients that did not even have their baseline blood test however were younger at both the time of consent (mean 41.7 years vs. 45.6 years, p=0.05) and at MS onset (mean 28.5 years vs. 33.3 years, p=0.01), had slightly longer disease duration (mean 13.2 years vs. 12.3 years, p=0.03), were more likely to have secondary progressive course (30.8% vs. 15.0%, p=0.04), and were less likely to be taking MS disease modifying therapy (42.1% vs. 64.8%, p=0.02). Only two out of 213 participants identified themselves as being other than Caucasian: one was Asian and one was Aboriginal. One individual that was consented but did not have a baseline blood test was Hispanic.
Table 4.1: Baseline demographic and disease characteristics

<table>
<thead>
<tr>
<th></th>
<th>n=213 Completed Baseline</th>
<th>n=26 Non-Participating</th>
<th>Comparison of baseline completers and non-participants p-value</th>
<th>n=134 Completed 6-Months</th>
<th>Comparison of baseline completers and 6-month completers p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>45.6 (9.5)</td>
<td>41.7 (8.7)</td>
<td>0.05</td>
<td>45.9 (10.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Range</td>
<td>21–72</td>
<td>26-59</td>
<td></td>
<td>22-72</td>
<td></td>
</tr>
<tr>
<td>Gender, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>78.9 (168)</td>
<td>84.6 (22)</td>
<td>NS</td>
<td>79.9 (107)</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>21.1 (45)</td>
<td>15.4 (4)</td>
<td></td>
<td>20.1 (27)</td>
<td></td>
</tr>
<tr>
<td>Caucasian Race, %</td>
<td>99</td>
<td>96</td>
<td>NS</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SD)</td>
<td>26.1 (5.4)</td>
<td>26.4 (5.0)</td>
<td>NS</td>
<td>25.6 (5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Proportion taking at least 1000 IU vitamin D3 daily, %</td>
<td>60.6</td>
<td>42.3</td>
<td>NS</td>
<td>63.4</td>
<td>NS</td>
</tr>
<tr>
<td>Age at MS onset, years (SD) Range</td>
<td>33.3 (9.1)</td>
<td>28.5 (7.9)</td>
<td>0.01</td>
<td>33.4 (9.1)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12.0-55.2</td>
<td>13.0-43.9</td>
<td></td>
<td>15.5-55.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.3 (8.6)</td>
<td>13.2 (8.3)</td>
<td>0.03</td>
<td>12.6 (9.5)</td>
<td>NS</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------</td>
<td>------------</td>
<td>------</td>
<td>------------</td>
<td>----</td>
</tr>
<tr>
<td>Duration (SD) Range</td>
<td>0.74-50.7</td>
<td>1.1-32.9</td>
<td></td>
<td>(0.74-50.7)</td>
<td></td>
</tr>
<tr>
<td>EDSS (SD) Range</td>
<td>3.2 (2.0)</td>
<td>4.0 (2.3)</td>
<td>NS</td>
<td>3.2 (2.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Relapsing Remitting Course, %</td>
<td>77.0</td>
<td>69.2</td>
<td>NS</td>
<td>76.1</td>
<td>NS</td>
</tr>
<tr>
<td>Secondary Progressive, %</td>
<td>15.0</td>
<td>30.8</td>
<td>0.04</td>
<td>15.7</td>
<td>NS</td>
</tr>
<tr>
<td>Primary Progressive,%</td>
<td>4.2</td>
<td>NA</td>
<td>NA</td>
<td>3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Uncertain course, %</td>
<td>3.8</td>
<td>NA</td>
<td>NA</td>
<td>4.5</td>
<td>NS</td>
</tr>
<tr>
<td>On Disease Modifying Therapy, %</td>
<td>64.8</td>
<td>42.1</td>
<td>0.02</td>
<td>65.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

EDSS=Expanded Disability Status Scale (assessed in 89% of the baseline participants at their recruitment visit)
4.3 Baseline Vitamin D levels

The first objective was to determine the prevalence of vitamin D insufficiency and to test the hypothesis that at least 50% of the study participants would have insufficient (<80 nmol/L) serum 25(OH)D levels at baseline. The prevalence of vitamin D insufficiency was 62.4% [95% CI: 55.9 to 68.9]. A one-sided test of a proportion was used to test the null hypothesis that the prevalence of vitamin D insufficiency is <50% and the alternate hypothesis was that the prevalence is ≥50%. The proportion of 62.4% was significantly greater than 50%, p<0.01 (Figure 4.2). Nine participants had serum 25(OH)D levels measured with a different assay (LC/MS/MS). These levels were converted into approximate RIA values.

The mean serum 25(OH)D was 72.6 ± 26.7 nmol/L and values had a wide range from 17.9-160 nmol/L (Table 4.2). The mean total daily vitamin D dose was 964.0 ± 820.8 IU and the dose had a wide range from 0-4400 IU (Table 4.2). Approximately 60.6% of the baseline participants were taking at least 1000 IU/day vitamin D3. There were 23.0% (49/213) of participants not taking any vitamin D3 supplementation. The mean serum 25(OH)D level for this group was 53.5 ± 21.6 nmol/L and values had a range from 17.9-127.7 nmol/L. The prevalence of vitamin D insufficiency was 87.8% [95% CI: 75.2 to 95.4].

Bivariate analysis was conducted to assess variables that were associated with serum 25(OH)D levels at baseline which was part of the objective one. The following variables were assessed: age, body mass index (BMI), month of blood collection (from September-January), sun exposure, travel below latitude 42° N within the prior three months, any artificial tanning within the prior year, vitamin D rich foods, total daily
vitamin D dose and frequency of missed vitamin D doses (questionnaire included as Appendix B). Higher 25(OH)D levels were associated with earlier month of blood collection, any use of artificial tanning within the prior 12 months, higher vitamin D dose, fewer missed doses of vitamin D, and 'normal' BMI. There was no association between age, natural sun exposure, travel below 42° N latitude, or dietary ingestion of vitamin D rich foods and serum 25(OH)D levels.

There was a decreasing trend in serum levels from September to January with an overall global difference between the months (p=0.04) (Figure 4.3). There was no significant difference in serum 25(OH)D level with between month comparisons. However, mean serum level in September was 83.7±23.4 nmol/L and by January decreased to 65.8±27.4 nmol/L. BMI in the 'obese' category was associated with decreased serum 25(OH)D levels (p<0.01) (Figure 4.4). Artificial tanning was defined as use anytime in the 12 months prior to the blood test and average total time for those engaging in artificial tanning over the year was 184.7±181.1 minutes with a range of 7-624 minutes. Tanners had a significantly higher serum level than non-tanners (Table 4.3). As total daily vitamin D dose increased, mean serum 25(OH)D levels also increased (Figure 4.5). There was a significant difference in not taking any vitamin D and taking any amount greater than 1000 IU per day. Greater number of missed vitamin D doses corresponded with lower serum 25(OH)D levels (Figure 4.6). There was a difference between never missing a dose and missing more than 2 doses per week (p=0.05).
Figure 4.2: Vitamin D insufficiency in the 213 participants that completed the baseline blood assessment
Table 4.2: Baseline serum 25(OH)D level and total daily vitamin D₃ dose in the 213 participants

<table>
<thead>
<tr>
<th></th>
<th>Baseline participants (n=213)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>25(OH)D Level (nmol/L)</td>
<td>72.6 ± 26.7</td>
</tr>
<tr>
<td>Total Vitamin D₃ Dose (IU)</td>
<td>964.0 ± 820.8</td>
</tr>
</tbody>
</table>

IU=International units
Figure 4.3: Mean 25(OH)D level at baseline reported by month of collection

The vertical lines represent standard deviation of the means

ANOVA $p = 0.039$
Figure 4.4: Mean 25(OH)D level at baseline reported by body mass index
The vertical lines represent standard deviation of the means

ANOVA p<0.01 (The ‘underweight’ category was excluded in the analysis because of the small sample size). There was a difference between the ‘Normal’ and ‘Obese’ groups (p<0.01) but there was no difference between the ‘Normal’ and ‘Overweight’ groups (p=0.16).
Table 4.3: Mean serum 25(OH)D levels at baseline reported by artificial tanning use within the previous 12 months

<table>
<thead>
<tr>
<th>Artificial Tanning Use</th>
<th>25(OH)D level (SD) (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (n=39)</td>
<td>82.1±27.9</td>
</tr>
<tr>
<td>No (n=174)</td>
<td>70.4±26.0</td>
</tr>
<tr>
<td>t-test p-value</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Figure 4.5: Mean 25(OH)D level at baseline reported by total daily vitamin D intake. The vertical lines represent standard deviation of the means (IU=International units).

ANOVA p<0.01. There was a difference between not taking any vitamin D compared to taking any amount greater than 1000 IU/d (all p<0.01). And a difference in taking either ‘<1000’ or ‘1000-<2000’ compared to ‘>=3000’ (p<0.01, p=0.04).
Figure 4.6: Mean 25(OH)D level reported at baseline reported by missed total daily vitamin D dose category

The vertical lines represent standard deviation of the means

"Infreq"= 1-2 times per month, "Somewhat freq"=1 time per week, "Freq"= 2 or more times per week.

ANOVA p-value=0.04. There was a difference between ‘Never’ and ‘Freq’ groups (p=0.05).
4.4 Longitudinal Serum 25(OH)D Levels and Vitamin D dose

Serum 25(OH)D levels and daily vitamin D dose are presented in Table 4.4 and in Figures 4.7 and 4.8. There was no difference in the mean baseline serum 25(OH)D level or the total daily vitamin D₃ dose between the total participating group and the completers (p=0.28). There was an increase of both the mean serum 25(OH)D level and the mean vitamin D dose over the 6-month period (p<0.01). There was also a difference in the mean serum 25(OH)D level between baseline and 3-months (p<0.01) and in the total daily dose between baseline and 3-months (p<0.01). There was no difference in the mean serum level from 3-months to 6-months (p=0.54), although the mean daily dose did significantly increase during this period (p<0.01). Five completers had their serum 25(OH)D levels measured using the LC/MS/MS and two had their levels measured using the chemiluminescence assay; these were all converted into approximate RIA values.

Bivariate analysis was conducted to assess variables that were associated with serum vitamin D levels at 3- and 6-months. The following variables were assessed: baseline BMI, month of blood collection, travel below latitude 42°N and artificial tanning use within the prior three months of blood collection, vitamin D rich foods, total daily vitamin D dose and frequency of missed vitamin D doses (questionnaire Appendix C). Higher 25(OH)D levels at 3-months were only associated with use of artificial tanning and travel below 42° N latitude within the prior three months (Table 4.5 and Table 4.6). Most (21/23) traveling participants traveled to tropical destinations including Mexico, Hawaii, Florida, Cuba or Costa Rica. The reported travel duration ranged from six to 45 consecutive days with daily sun exposure. The mean total time for the participants who engaged in artificial tanning over the three months was 115.6±129.3 minutes with a range
of 7-540 minutes. Tanners had significantly higher serum 25(OH)D levels compared with non-tanners.

At 6-months, higher 25(OH)D levels was again associated with use of artificial tanning in the prior three months (Table 4.7). The mean total time for the participants who engaged in artificial tanning over the three months was 87.9±74.5 minutes with a range of 10-240 minutes. Tanners had significantly higher serum 25(OH)D levels compared with non-tanners. Twelve of the 14 participants who reported tanning use at 6-months had also reported it at 3-months. Obesity (BMI>30kg/m²) was again associated with decreased serum 25(OH)D levels (Figure 4.9).
Table 4.4: Mean serum 25(OH)D levels and total daily vitamin D₃ dose

<table>
<thead>
<tr>
<th></th>
<th>Baseline Participants (n=213)</th>
<th>Completers (n=134)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Mean 25(OH)D level (SD) (nmol/L)</td>
<td>72.6 ± 26.7</td>
<td>75.8 ± 27.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean total vitamin D dose (SD) (IU)</td>
<td>964.0 ± 820.8</td>
<td>986.6 ± 783.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-values correspond to comparisons between baseline and 3-months, and 3-months and 6-months.
Figure 4.7: Serum 25(OH)D levels by time-period in 6-month completers
Figure 4.8: Total daily vitamin D₃ dose by time-period in 6-month completers (IU=International units)
Table 4.5: Mean serum 25(OH)D levels at 3-months according to travel to below 42° N
latitude within the prior three months

<table>
<thead>
<tr>
<th>Travel below 42° N latitude</th>
<th>Mean 25(OH)D level (SD) (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (n=24)</td>
<td>99.5±7.1</td>
</tr>
<tr>
<td>No (n=127)</td>
<td>80.9±1.9</td>
</tr>
<tr>
<td>t-test p-value</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table 4.6: Mean serum 25(OH)D levels at 3-months according to artificial tanning use within the prior three months

<table>
<thead>
<tr>
<th>Artificial Tanning Use</th>
<th>Mean 25(OH)D level (SD) (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (n=19)</td>
<td>98.5±26.3</td>
</tr>
<tr>
<td>No (n=132)</td>
<td>81.7±23.9</td>
</tr>
<tr>
<td>t-test p-value</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Table 4.7: Mean serum 25(OH)D levels at 6-months according to artificial tanning use within the prior three months

<table>
<thead>
<tr>
<th>Artificial Tanning Use</th>
<th>25(OH)D level (SD) (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (n=14)</td>
<td>98.6±31.2</td>
</tr>
<tr>
<td>No (n=116)</td>
<td>83.2±22.1</td>
</tr>
<tr>
<td>t-test p-value</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Figure 4.9: Mean 25(OH)D level at 6-months reported by body mass index
The vertical lines represent standard deviation of the means

ANOVA p-value=0.01 (The ‘underweight’ category was excluded in the analysis because of the small sample size). There was a difference between the ‘normal’ and ‘obese’ groups (p=0.01) but there was no difference between the ‘normal’ and ‘overweight’ groups (p=0.98).
4.5 Adherence to the Study Protocol

The second objective of the study was adherence to the protocol. It was defined as participants correctly self-adjusting the vitamin D dose (self-management group) or correctly following the instructions they were given (directed-management group). Correct adjustment was inferred from the dose that the participant reported they had been taking during the previous 3 months. Out of the 6-month completers, 79.9% (107/134) were adherent. Out of those only completing 3-months, 82.9% (29/35) were adherent (Figure 4.10).

Adherence was associated with method of study management across the 4 groups at 6-months, $\chi^2=7.69$, $p=0.05$ (Table 4.8). There was a significantly greater proportion of adherence in the directed management group as opposed to the self management group, 88.4% vs. 70.8% ($\chi^2=6.47$, $p=0.01$) (Table 4.9). There was no difference between the Internet and postal groups ($\chi^2=0.98$, $p=0.32$) (Table 4.10). Adherence was associated with vitamin D status at 6-months; there was a much greater proportion of sufficiency in those that adhered to the protocol (60.8%) than those that did not adhere (29.6%), $\chi^2=8.41$, $p<0.01$. Vitamin D sufficiency at 6-months based on adherence is presented in Figure 4.11.
Figure 4.10: Overview of adherence to the study protocol
Table 4.8: Adherence to the study protocol based on the four study management groups

<table>
<thead>
<tr>
<th></th>
<th>Internet</th>
<th>Postal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Self-Management (n=30)</td>
<td>Directed-Management (n=36)</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>76.7</td>
<td>88.9</td>
</tr>
<tr>
<td>No (%)</td>
<td>23.3</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Self-Management (n=35)</td>
<td>Directed-Management (n=33)</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>65.7</td>
<td>87.9</td>
</tr>
<tr>
<td>No (%)</td>
<td>34.3</td>
<td>12.1</td>
</tr>
</tbody>
</table>


Table 4.9: Adherence to the study protocol according to self or directed study management group

<table>
<thead>
<tr>
<th></th>
<th>Self-Management (n=65)</th>
<th>Directed-Management (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (%)</td>
<td>70.8</td>
<td>88.4</td>
</tr>
<tr>
<td>No (%)</td>
<td>29.2</td>
<td>11.6</td>
</tr>
</tbody>
</table>
Table 4.10: Adherence to the study protocol according to mode of communication

<table>
<thead>
<tr>
<th></th>
<th>Internet (n=66)</th>
<th>Postal (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (%)</td>
<td>83.3</td>
<td>76.5</td>
</tr>
<tr>
<td>No (%)</td>
<td>16.7</td>
<td>23.5</td>
</tr>
</tbody>
</table>
Figure 4.11: Vitamin D sufficiency in the 134 participants that completed 6-months of study based on adherence to the study protocol.
4.6 Optimization of Vitamin D Level

The third objective was to test the hypothesis that optimization of 25(OH)D levels in at least one management group will differ from the others at 6-months. Vitamin D optimization at 6-months was not associated with management group across the four groups, $\chi^2=2.66$, $p=0.45$ (Table 4.11) or with either self or directed management, $\chi^2=2.34$, $p=0.13$. While there was a greater proportion of vitamin D sufficient participants at 6-months in the directed group (60.9%) than in the self-management group (47.7%), this was not statistically significant ($p=0.13$) (Table 4.12). As noted above however, management group did impact adherence which was related to successful optimization of vitamin D levels.

The fourth objective was to explore if 75% of all insufficient participants could have their 25(OH)D levels optimized by 6-months. Out of the 134 completers, 61.2% (n=82) had an insufficient baseline serum 25(OH)D level. At 6-months, 47.6% (39/82) of this sample became vitamin D sufficient. However, 34.6% (18/52) with adequate 25(OH)D levels at baseline became insufficient at 6-months. Out of the 18 participants, 10 participants had serum levels below 80 nmol/L at 3-months. Eight of these 10 participants correctly increased vitamin D dose from 3- to 6-months but were still insufficient at 6-months.
Table 4.11: Vitamin D status at 6-months in completers based on the four study management groups

<table>
<thead>
<tr>
<th>Optimized 25(OH)D level</th>
<th>Internet</th>
<th>Postal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Self- Management (n=30)</td>
<td>Directed- Management (n=36)</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>50.0</td>
<td>58.3</td>
</tr>
<tr>
<td>No (%)</td>
<td>50.0</td>
<td>41.7</td>
</tr>
</tbody>
</table>
Table 4.12: Vitamin D status at 6-months in completers according to self or directed study management group

<table>
<thead>
<tr>
<th>Optimized 25(OH)D level</th>
<th>Self-Management (n=65)</th>
<th>Directed-Management (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (%)</td>
<td>47.7</td>
<td>60.9</td>
</tr>
<tr>
<td>No (%)</td>
<td>52.3</td>
<td>39.1</td>
</tr>
</tbody>
</table>
4.7 Feasibility of Optimization Process

Objective five was the average time to manage participants within each study group by the grad student to evaluate the feasibility of each method of management. The process to get the lab result was the same for all participants. Normally, all MS clinic patient lab reports come to the MS clinic where a clerk confirms that they are a clinic patient by searching an electronic clinic database, then writes the patients identification number on the lab report, and gives the report to a clinic nurse. The clinic nurse identifies seriously abnormal results that require physician attention and places them in physician ‘work’ boxes for review over the next 1-2 weeks. A lab report demonstrating an abnormal level of 25(OH)D generates a phone call to a patient to determine their current vitamin D dose and to advise them of a dose adjustment and need for repeat testing according to the physician recommendation. During this study, the lab reports were instead passed on to the graduate student by the clinic nurse for review and management according to the study protocol.

The average work time for the initial clinic process is about five to ten minutes per report. The time for the graduate student to manage each participants result was defined as the time to generate postal information or post internet results and in some cases to provide dose-adjustment instructions. Only the graduate student time was included in the analysis. There was a difference in the mean time required to manage each participant according to the four management groups at both 3- and 6-months (p<0.01) (Table 4.13). The shortest times were seen for the Internet based groups; there was no difference within the Internet mode between the directed and self-management at either 3- or 6-months (p=0.88, 0.93). When the directed and self groups were combined,
there was no difference in time to manage per participant between the two groups, at either 3- or 6-months, (p=0.24, 0.27) (Table 4.14). However, there was a difference in time to manage per participant when analyzed by mode of communication. The postal mode took almost double the time to manage per participant than the Internet mode (Table 4.15).
Table 4.13: Mean time to manage each participant according to the four study management groups

<table>
<thead>
<tr>
<th></th>
<th>Internet</th>
<th>Postal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Self-Management</td>
<td>Directed-Management</td>
</tr>
<tr>
<td></td>
<td>(n=38)</td>
<td>(n=45)</td>
</tr>
<tr>
<td>Mean time per</td>
<td>1.37 ± 0.55</td>
<td>1.25 ± 0.56</td>
</tr>
<tr>
<td>participant (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(minutes)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td>(n=30)</td>
<td>(n=36)</td>
</tr>
<tr>
<td>Mean time per</td>
<td>0.95 ± 0.29</td>
<td>0.91 ± 0.28</td>
</tr>
<tr>
<td>participant (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(minutes)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td>(n=41)</td>
<td>(n=36)</td>
</tr>
<tr>
<td>Mean time per</td>
<td>2.21 ± 0.61</td>
<td>2.31 ± 0.73</td>
</tr>
<tr>
<td>participant (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(minutes)*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA (p-value<0.01)
Table 4.14: Mean time to manage each participant according to self or directed study management group

<table>
<thead>
<tr>
<th></th>
<th>Self-Management</th>
<th>Directed-Management</th>
<th>Comparison between groups p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=79)</td>
<td>(n=81)</td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean time per participant (SD) (minutes)</td>
<td>1.87 ± 0.71</td>
<td>1.71 ± 0.83</td>
<td>0.24</td>
</tr>
<tr>
<td>6-months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean time per participant (SD) (minutes)</td>
<td>1.62 ± 0.65</td>
<td>1.49 ± 0.68</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Table 4.15: Mean time to manage each participant according to mode of communication

<table>
<thead>
<tr>
<th></th>
<th>Internet (n=83)</th>
<th>Postal (n=77)</th>
<th>Comparison between groups p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-months</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean time per</td>
<td>1.30±0.55</td>
<td>2.26±0.66</td>
<td></td>
</tr>
<tr>
<td>participant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SD) (minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td>(n=66)</td>
<td>(n=66)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean time per</td>
<td>0.92±0.28</td>
<td>2.16±0.19</td>
<td></td>
</tr>
<tr>
<td>participant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SD) (minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.8 Patient Satisfaction

All participants that completed 6-months of study were asked to complete a questionnaire to assess overall satisfaction with their participation in the study (included as Appendix D). This analysis was the sixth objective of the study. Out of the 134 6-month completers, 130 questionnaires were returned. Greater than 95% of the completers at 6 months reported being comfortable with the management method to which they were randomized and that adequate instructions were provided to receive lab results and adjust dose. Of those subjects self-managing, 83% reported that they were comfortable adjusting their own vitamin D dose. Positive feedback was received in regard to use of the website:

- most felt the use of the website to communicate lab results worked well for them in the optimization process
- the website was easy to navigate and it could be used in other ways to support patient care in the MS clinic
- most participants in this group would consider participating in more web-based programs at the MS clinic.

Of those assigned to a directed-management group, 63.6% reported they would not have been comfortable adjusting their own vitamin D dose. The e-mail group was comfortable receiving the vitamin D lab results and dosage information by this mode. The participants reported high level of satisfaction; 92% rated their overall experience in the study as either positive or very positive.
4.9 Did Participation Affect Behaviour?

An exploratory aim of this study outlined as objective seven was to assess changes in participant behaviours that may increase vitamin D levels as a result of participating in the overall optimization process (questionnaire included as Appendix D). The main finding from this analysis was that 25.4% of the 6-months completers reported that they reduced the number of missed doses of vitamin D supplements. The following behaviours were also reported but on a less frequent basis:

- 14.6% increased the amount of time they spent outdoors in the sun
- 11.5% reported consciously including foods rich in vitamin D in their diet
- 7.7% reduced the amount of sunscreen worn when outdoors in the sun
- 5.4% reported wearing less sun protective clothing when outdoors in the sun
4.10 Estimated Dose-Response Relationship

That last objective of the study was to assess the dose-response relationship of vitamin D supplementation. Clinical observations and support from the literature suggested that 1000 IU of vitamin D$_3$ daily would result in an approximate increase of 20 nmol/L in serum 25(OH)D levels. A very simple approximate dose-response for each time period was calculated as follows:

Example baseline to 3-months

$$\frac{\text{Mean serum 25(OH)D change}}{\text{mean vitamin D dose change}} = \frac{5.7 \text{nmol/L}}{748.2 \text{ IU}} = 0.0076 \text{ nmol/L per 1 IU of vitamin D}$$

$$0.0076 \times 1000 = 7.6 \text{ nmol/L serum increase per 1000 IU of vitamin D}$$

The estimated dose-response calculated from baseline to 3-months was 7.6 nmol/L per 1000 IU/day vitamin D increase and from 3- to 6-months it was 5.8 nmol/L per 1000 IU/day vitamin D increase. The dose-response was calculated for these two separate 3-month time periods and not the overall 6-months because participants would have been taking a consistent single dose during these three month intervals. Over the 6-month period, with a mean increase in vitamin D dose of 1219.2 IU/day, the average serum level increase was 7.3 nmol/L. Mean changes in the total daily vitamin D dose, in the serum 25(OH)D levels, and in the dose-response for the participants who adhered to study protocol is shown in Table 4.16.
Table 4.16: Changes in serum 25(OH)D level and total daily vitamin D dose and calculated dose-response between study time points in 6-month completers that adhered to the study protocol

<table>
<thead>
<tr>
<th></th>
<th>Mean change in total vitamin D3 dose (SD) (IU)</th>
<th>Mean change in serum 25(OH)D level (SD) (nmol/L)</th>
<th>Estimated dose-response per 1000 IU/d (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline to 3-months</td>
<td>748.2 ± 703.2</td>
<td>5.7 ± 31.4</td>
<td>7.6</td>
</tr>
<tr>
<td>3-months to 6-months</td>
<td>498.2 ± 590.8</td>
<td>2.9 ± 27.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Baseline to 6-months</td>
<td>1219.2 ± 966.3</td>
<td>7.3 ± 30.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

 IU=International units
4.11 Summary of Main Results

- There was a 62.4% prevalence of vitamin D insufficiency in the 213 participants that had baseline 25(OH)D level tested.
- Higher baseline 25(OH)D levels were associated with earlier month of blood collection, any use of artificial tanning within the prior 12 months, higher vitamin D dose, fewer missed doses of vitamin D, and ‘normal’ BMI.
- There was no association between age, natural sun exposure, travel below latitude 42°N, or dietary ingestion of vitamin D rich foods and baseline serum 25(OH)D levels.
- Adherence was associated with method of study management across the four groups at 6-months. There was a significantly greater proportion of adherence in the directed-management group as opposed to the self-management group, 88.4% vs. 70.8%.
- There was a much greater proportion of sufficiency in those that adhered to the protocol (60.8%) than those that did not adhere (29.6%).
- Vitamin D optimization at 6-months was not associated with management group across the four groups, or with either self or directed management.
- At 6-months, 47.6% of participants that had insufficient 25(OH)D levels at baseline had their levels optimized.
- The shortest times for the graduate student to manage each participant's result were in the Internet based groups regardless of self or directed management.
• Reduced number of missed doses of vitamin D supplements was one of the main reported changes in participant behaviour that may increase vitamin D levels as a result of participating in the overall optimization process.

• The estimated dose-response calculated from baseline to 3-months was 7.6 nmol/L per 1000 IU/day vitamin D increase and from 3- to 6-months it was 5.8 nmol/L per 1000 IU/day vitamin D increase.
Chapter Five: Discussion

5.1 Vitamin D Insufficiency, Optimization Methods and the Effectiveness of the Optimization Algorithm

5.1.1 Vitamin D Insufficiency

A high prevalence of vitamin D insufficiency is present in Southern Alberta MS patients over the fall and early winter months (latitude 51° N, altitude 1077 m). When an 80 nmol/L minimum for serum 25(OH)D levels is used as the cutoff for insufficiency, 62.4% of the sample is insufficient. With 60.6% of the sample taking at least 1000 IU of vitamin D₃ daily, the mean serum 25(OH)D level is still only 72.6±26.7 nmol/L. The results confirm that most MS patients (≥50% was hypothesized) in this region require additional vitamin D supplementation simply to achieve the current minimum serum 25(OH)D levels recommended for the general population.

These findings are comparable with those of a study that assessed serum 25(OH)D levels in healthy subjects living in the same geographic location. That study excluded anyone consuming more than 200 IU of supplemental vitamin D per day and found that the mean serum level in the Fall (November or December) was 52.9±17.2 nmol/L and in the winter (February or March) it was 57.3±21.3 nmol/L (184). When a threshold of 80 nmol/L was applied, 94% (Fall) and 86% (Winter) were insufficient. In our study the mean serum level at baseline was substantially higher at 72.6±26.7 nmol/L, although 60.6% of subjects were taking at least 1000 IU vitamin D₃ daily. The mean serum 25(OH)D level in the unsupplemented subgroup of MS patients however was 53.5±21.6 nmol/L and 87.8% were insufficient. This is almost identical to that within the healthy population. This suggests that MS patients, at least those with mild
to moderate disability as was typical in this sample, are as likely as healthy people from the same region to have vitamin D insufficiency and are expected to have similar serum levels.

Prior reports of vitamin D levels in MS patients have been exclusively in non-supplemented patients. Low levels of vitamin D are commonly reported in MS patients. The mean serum 25(OH)D level in 52 patients in a New York hospital (latitude 40° N) was 42.9 nmol/L (153). In a group of 29 patients living in Northern Ireland (latitude 54° N) the mean level was 69.1±40.0 nmol/L in the wintertime (227). Further, in a cross sectional group of 40 Finnish patients (latitude 60-70° N), the mean wintertime level was 41±5 nmol/L (151). When the most conservative cut-off of 40-50 nmol/L was applied, all the studies reported a high prevalence of insufficiency and even reports of severe deficiency (<20-25 nmol/L) in both MS patients and healthy matched controls (153, 228). None of the studies used the current recommended (higher) cut-off of 80 nmol/L for defining insufficiency, which would have likely revealed even higher proportions of insufficiency. The vitamin D levels reported in these studies vary and the level found in our MS sample was within the range of these other reports. This suggests that the results of this study can be generalized to other populations of similar latitude (above latitude 40° N).

In a study of vitamin D supplementation in people with MS, the mean serum level in 17 patients increased from 42.5 ± 15 nmol/L to 70 ± 20 nmol/L (New York region, latitude 40° N) after 6-months of taking 1000 IU of vitamin D daily, (157). No other details were available on this sample of MS patients however, the mean serum 25(OH)D level is similar to what we report in our baseline data where 60.6% of the sample is
supplemented with at least 1000 IU per day. Both this study and ours suggest that 1000 IU/day of vitamin D is not adequate to ensure optimal serum levels for our MS patients living in northern latitudes over the winter months.

A number of variables were analyzed to assess their influence on serum 25(OH)D levels at each time point. Month of testing, obesity, and overall vitamin D acquisition based on the dose of supplementation, adherence to supplementation, artificial tanning, or sun vacations all impact levels. Factors which did not impact levels included: natural sun exposure, age, and dietary ingestion of vitamin D rich foods.

There was a decreasing trend in baseline serum 25(OH)D levels from September to January (p=0.04). The mean serum levels decrease from 83.7±23.4 nmol/L in September to 65.8±27.4 nmol/L in January. This difference of nearly 20 nmol/L reflects a clinically relevant difference. The decrease can be attributed to a recognized seasonal affect; sun irradiation is inadequate to generate vitamin D from October to March in northern latitudes (183, 229). Thus our data are congruent with the expected effect of season and support the greater need for supplementation over the winter.

We did find that the use of artificial tanning is associated with increased serum levels at all three time periods. This is consistent with reports in the literature that artificial tanning use increases serum 25(OH)D levels, despite tanning equipment having variable amounts of vitamin D producing UVB radiation (173-175, 177, 230). Also, at the 3-month follow up period participants that traveled to destinations below 42°N latitude had higher serum vitamin D levels than those that did not. Our findings are consistent with another study that reported travel below 42°N latitude was associated with significant increases in serum 25(OH)D levels (184). The significance of travel in
our study likely reflected a ‘sun vacation’, as a majority of the participants reported traveling to tropical destinations including Mexico, Hawaii or Florida with daily sun exposure. As such vacations are not common until December this factor would not likely have been identified at baseline because of the timing of enrolment which was largely before such common vacations occur. These findings confirm that exposure to UV radiation either natural or artificial raises the vitamin D levels of people with MS as expected.

Supplement dose and frequency of missed vitamin D doses were also associated with serum 25(OH)D at baseline. Missing more than two doses per week was associated with lower 25(OH)D levels. This supports the effectiveness of vitamin D supplementation in increasing 25(OH)D serum levels and the importance of adherence.

BMI is an indirect measure of body fat percentage which can be used in population studies although it is not currently recommended to diagnose obesity in individuals in clinical practice. We found that obesity, defined as BMI \(>30\text{kg/m}^2\) was associated with lower serum 25(OH)D\(_3\) levels. This is consistent with other reports in the literature (222, 231, 232). This relationship between obesity and lower serum vitamin D levels is hypothesized to be due to the increased sequestration of vitamin D in fat deposits as adipose tissue is the major storage site for vitamin D\(_3\) (232, 233). Obesity is therefore a factor that contributes to the risk of vitamin D insufficiency and should be considered when deciding to evaluate serum 25(OH)D levels and may influence supplement dose requirements.

We did not find that natural sun exposure during day to day life had a significant influence on serum vitamin D levels however, most patients (65%) reported seldom (less
than 3 months) or never exposing a considerable part of their body to sun exposure within the 12 months preceding their baseline blood test. This is probably a behavioural characteristic of Southern Albertans but the frequent inability of people with MS to tolerate heat may contribute to the limited sun exposure in our group of patients (234). This finding confirms that in daily life most people did not get much sun exposure.

Increasing age is associated with decreased serum levels; we did not find evidence of this in our study. This relationship is most pronounced when the source of vitamin D is from the sun, as there is a decrease in cutaneous vitamin D synthesis due to a decrease in 7-dehydrocholesterol in the skin with aging (181, 235). The reductions in vitamin D synthesis in the skin are most pronounced after the age of 60-70 years. Although, our participant age ranged from 21-72 years, 75% of participants were less than the age of 50. Therefore, limited sun exposure reported by our participants and a large proportion of the sample below 50 years of age likely explains the lack of an association between age and vitamin D levels.

Only two participants identified themselves as other than Caucasian, therefore, influence of skin pigmentation on serum levels was not possible. Lastly, ingestion of vitamin D rich foods was not associated with serum 25(OH)D levels. Weekly consumption of fish such as salmon, tuna, and herring was infrequently reported and therefore not analyzed. Milk in Canada is supplemented with about 100IU of vitamin D₃ per 250ml serving. An average of six milk servings per week was reported per participant at all time points. A total vitamin D contribution of approximately 600 IU/per week or 86 IU per day from such milk consumption would have contributed little to serum 25(OH)D levels, hence an association was not observed.
Overall, study completion was poor as only 62.9% of the total participants completed the study. The completers were however representative of the baseline sample of 213 participants with respect to demographic and MS characteristics. Previous studies have shown poor adherence to interferon safety lab testing in MS populations suggesting that the need for repeat lab testing to adjust vitamin D dose may have limited adherence to completing the study. Also, several participants spontaneously report to a clinic physician during an unrelated clinic visit that frequently missed vitamin D doses was a reason for not following through with the study (Metz, L, personal communication). The power calculation that estimated the need for a sample size of 200 patients only applied to the determination of the prevalence of vitamin D insufficiency at baseline; it was not applicable to the follow-up analyses.

Educating patients about MS is an important focus of the Calgary MS clinic. Patients are provided with written information about the disease and its symptoms, in addition to education by the clinic nurses. One brochure distributed to all clinic patients in a mailed newsletter in 2005, and provided to new patients since that time, includes information about vitamin D (Appendix H). It recommends supplementation with at least 1000 IU of vitamin D₃ daily and facilitates the educational objective. It may have contributed to the motivation of participants that completed the study but additional education may be required for some patients.

Therefore, future longitudinal studies need to improve adherence, possibly through enhanced education, or be designed to account for a high rate of drop-outs.
5.1.2 Methods of optimization

Another objective of this study was to assess if it was feasible to include patients in the assessment and management of their own vitamin D levels. Studies of self-management in anticoagulation therapy report drop out being a concern (200). We did not observe a significant difference in drop out rate at 6-months between the self- and the directed-management groups; rates were 38.1% vs. 36.1%, respectively. There was also no significant difference in the drop out rate between the Internet and postal groups; rates were, 40.0% vs. 34.0%, respectively.

Adherence to the study protocol at 6-months however, was associated with study management group. There was greater adherence with the directed-management groups (combined) compared to the self-managed groups (combined). Adherence also improved vitamin D status at 6-months. There were up to 15% more vitamin D sufficient participants in the directed-management groups (combined) than in the self-management groups. There was no difference in adherence between the postal and Internet groups. This study suggests that MS patients are more likely to adhere to a vitamin D dose that is recommended to them than they are to follow an algorithm and make their own dose adjustment. Furthermore, in this study Internet and postal methods of dose adjustment were equally adhered to.

We also determined if patients were comfortable with each of four methods of vitamin D optimization. At least 80% of the self-management group reported they were comfortable adjusting their own vitamin D dose. It was interesting that 63.6% of the directed group combined reported they would not have been comfortable adjusting their own vitamin D dose even though assignment of management method was randomized.
Participants became accustomed to the management method they were assigned. Regardless, the directed-management groups had a better outcome and those randomized to this management would not have been comfortable with the self-management method. Therefore, the self-management model is not suitable for all MS patients.

An initial motivating factor to explore self-management of vitamin D levels was to reduce management time. The process to get the lab result and be reviewed by the clinic nurses is one aspect but informing patients of their serum level and dose-adjustment is the other component. A lab report demonstrating an abnormal level of 25(OH)D generates a phone call to a patient to determine their current vitamin D dose and to advise them of a dose adjustment and need for repeat testing. If the patients could have direct access to their lab result this could reduce the initial clinic time for nurses and a more efficient alternative to phone calling would also reduce work time. There was no significant difference in the time to manage participants serum 25(OH)D levels based on self- or directed-management. However, the time required to inform participants in the postal groups was almost twice as much compared to the Internet groups (about two minutes versus one minute per patient). Postal contact was used as a mode of communication instead of phoning to keep the process unbiased by human contact in one group only. Additionally, the use of e-mail and the website was very well accepted by patients. Thus, using an Internet-based method is the most time-efficient way to optimize vitamin D levels and it is accepted by MS patients who already use the Internet.

Our findings suggest that we were realistic in our expectation that MS patients will be able to participate in self-management with the use of Internet-based strategies similar to those employed with diabetes and anticoagulation patient groups. Overall, directed
management is more effective in optimizing dose but direction regarding dose adjustment may be provided by mail or Internet. While Internet communication appears slightly more efficient, it is not an option for patients without Internet access and the most time consuming aspect of management is receiving and reviewing paper copies of lab results. In addition, e-mail is not secure enough to protect patient privacy so a password protected website would need to be maintained. This would increase complexity and reduce efficiency. At this time, postal communication is preferable. If the initial steps of reviewing paper copies of lab reports is automated, and secure Internet messaging becomes available, this would likely be much more efficient and is likely to be accepted by many MS patients. This method of dose adjustment would extend to management of other medication. Finally, it is unclear how widespread Internet availability is, and how its effectiveness and efficiency compare with telephone management.

5.1.3 The Effectiveness of the Optimization Algorithm.

Another objective of the study was to optimize the serum 25(OH)D levels above 80 nmol/L with oral supplement dose recommendations using a dose adjustment algorithm. Our objective was to optimize serum levels in 75% of the insufficient subjects by 6-months; only 47.6% had their insufficiency corrected. Adhering to the study protocol was an influencing factor. Adhering to the protocol was associated with a larger proportion of sufficient subjects at 6-months (60.8%), compared with not adhering to the protocol (29.6%). Participating in the optimization process also influenced behaviour. The most notable finding was that 25.4% (33/130) of the 6-month completers reported that they reduced the number of missed doses of vitamin D supplements. This was reflected in the findings at 3- and 6-months that very few participants reported missing
vitamin D doses even once per week (3-months 13.6% (20/147), 6-months 11.5% (15/130).

Another likely factor for the lack of optimization was that our dose-adjustment algorithm was too conservative. Our initial dose-response relationship data suggested that serum 25(OH)D rises on oral dosing with vitamin D₃ by 0.7 nmol/L for every microgram (=40 IU) of vitamin D per day or ~20nmol/L for every 1000 IU/day dose increment (189). However, reports in the literature support that the kinetics of the dose-response relationship are not linear; individuals with high starting serum 25(OH)D values will get less of an increase from the same steady-state oral dose than those with lower starting values (192). Our study data support this finding; with a mean serum level 72.6 ± 26.7 nmol/L at baseline we saw small changes in serum level at both follow up periods. Our estimated dose-response calculated for baseline to 3-months and 3-months to 6-months when participants would have been taking a consistent single dose was 7.6 and 5.8 nmol/L per 1000IU/day vitamin D increase, respectively.

Intrinsic factors of the vitamin D endocrine system may account for the non-linear dose-response curve. The serum kinetics of administered vitamin D, and the appearance of 25(OH)D in serum, are affected by the mode of transport of vitamin D in the circulation (236). A more efficient and sustained supply of vitamin D is associated with UV induced production in the skin. In contrast, oral vitamin D consumption leads to a rapid but less sustained availability of the metabolite (236). Vitamin D synthesized by exposure to the sun is largely bound to the vitamin D binding protein (237, 238). Diet derived vitamin D is absorbed from the intestine in association with dietary fat and then transported in chylomicron carriers in chyle (239). Some of the absorbed vitamin D is
presented to the liver on the chylomicron particles via specific receptor mediated uptake and some redistributes to carriers such as the vitamin D binding protein (236). Non-vitamin D binding protein transport results in a more rapid hepatic uptake of vitamin D which is then subjected to inactivating metabolism (236).

The variation in serum kinetics may also result from variable activity of the hepatic metabolic pathway. The amount of 25(OH)D produced by the liver is not directly proportional to the input of vitamin D. When the reserves are depleted, 25(OH)D is made with greater efficiency than when there is an abundance of vitamin D (6). The vitamin D endocrine system has evolved in humans with sun exposure induced production as the natural way to obtain vitamin D. This is supported by the finding that there are no reports of vitamin D toxicity from sun exposure likely because the human body has developed an intrinsic system of regulation preventing overproduction of previtamin D (2). The hepatic inactivation of vitamin D may serve as a protective mechanism, preventing the accumulation of a potentially ‘toxic’ molecule because as far as the body knows, this is an ‘unnatural’ way to obtain vitamin D (219, 237). Despite these issues related to oral supplementation, currently it is a safe and convenient way to achieve and maintain optimal serum levels of vitamin D as fears of the risk of skin cancer have caused a general reluctance to increase unprotected skin exposure to sunlight.

The dose-response relationship was also difficult to assess due to poor precision of the assay. We often observed serum 25(OH)D level changes without reason (DiaSorin RIA approximate intra-assay coefficient of variation ±15%). Finally, different assays for measurement result in a different normal range and variability. Therefore, health care practitioners should be aware that interpretation of 25(OH)D₃ levels is challenged by
differing methods that are not equivalent and by imprecision of the individual assays. This makes routine 25(OH)D level testing to adjust vitamin D dosing a challenge.

Finally, 34.6% of patients who had adequate levels at baseline became insufficient. This can likely be explained by poor assay precision and the seasonal impact that was not integrated into the algorithm. Also, the dose increase in some patients from 3- to 6-months was likely not enough to raise serum levels over this time period.

**5.1.4 Reflections**

Preventing vitamin D insufficiency among people with MS across Canada is warranted even though the possible effects of vitamin D on MS disease activity are not known. It is apparent that serum levels to maintain adequate bone health at a minimum should be above 80 nmol/L and higher levels (>100 nmol/L) may be required for other health outcomes (166, 170, 240). Our patients spend most of their daylight hours indoors and cannot generate adequate vitamin D stores with occasional sun exposure. Our data, and that of others, suggests that 1000 IU/day of vitamin D is not adequate to achieve optimal levels in many patients. Also, this data supports a fairly flat dose response curve above this dose (190). There is substantial evidence to support there is absolutely no change in calcium levels, let alone cases of hypercalcemia (the most basic sign of vitamin D toxicity) with an intake of 10,000 IU/day up to 20 weeks and an intake of 50,000 IU/day for eight weeks (162, 190). The risk of severe hypercalcemia only arises when high amounts of vitamin D (>40,000 IU/day) are consumed with 25(OH)D levels of greater than 700 nmol/L (194). Therefore, the recommended daily dose of vitamin D is likely to be well above 1000 IU daily.
The imprecision of the vitamin D assay may not support frequent testing and patients are not likely to adhere to frequent testing of serum 25(OH)D levels. Thus, a single oral vitamin D supplement dose sufficient to ensure that the total needs of most our patients are met without the burden of frequent monitoring is the best solution. It has been suggested that about 3000 IU/day of vitamin D is likely required to assure that 97% of Americans obtain levels >87.5 nmol/L (165). A Canadian expert suggests intakes ≥4000 IU per day to ensure desirable 25(OH)D concentrations >80 nmol/L (190). Further studies are needed to confirm that this dose is adequate, safe, and effective over the long term.

5.2 Important Aspect about the Experimental Studies Relating to Vitamin D

Accumulating research supports a potential role of vitamin D beyond bone metabolism in areas such as the nervous and immune system. The expression of the VDR and the activating enzyme, 1α-hydroxylase in many different organs and tissues supports that 1,25(OH)₂D has some undetermined function within these systems. A main criticism of the many cell culture systems or animal models in which vitamin D has been studied is that the concentration of 1,25(OH)₂D needed to produce a particular effect has been much higher than can be achieved at physiological serum concentrations of 1,25(OH)₂D. For example, the effects in most of the in vitro immune studies discussed in this thesis were at concentrations in the range of 5x10⁻¹¹ to 10⁻⁷ mmol/L (0.05 to 500 nmol/L). These levels are extremely high. In vivo circulating concentrations of 1,25(OH)₂D are in the picomolar/litre range (1000-fold less). Achieving such extreme circulating concentrations would result in hypercalcemia. This has been a major limitation to 1,25(OH)₂D therapy in vivo.
The natural phenomenon of extrarenal 1,25(OH)$_2$D synthesis may potentially explain and hold the key to the generation of high local concentrations of 1,25(OH)$_2$D. There is consensus that serum 1,25(OH)$_2$D is only a measure of the endocrine function of vitamin D and not an indicator of body stores or the ability of vitamin D to perform its many autocrine functions (192). Researchers were able to produce the first direct evidence for synthesis of 1,25(OH)$_2$D in the CNS, when vitamin D was used in the EAE model (241). Using circulating 25(OH)D as the substrate, the required higher local concentrations of 1,25(OH)$_2$D may be produced intracellularly, in an autocrine manner for example by cells in the CNS such as macrophages which hold this capacity (192). This potentially eliminates the need for exogenous 1,25(OH)$_2$D. It also stresses the importance of achieving and maintaining serum 25(OH)D levels that correspond to satisfying the skeletal needs of the body. This would then ensure that 25(OH)D as a substrate is available for the synthesis of 1,25(OH)$_2$D for the non-bone functions. Further study of the extrarenal synthesis of 1,25(OH)$_2$D will contribute to understanding the full scope of vitamin D action in non-skeletal areas like the CNS and the immune system.

5.3 Limitations

An oversight was made at the beginning of the study that patients living outside of the CHR have their blood tested at a facility that employs an in-house LC/MS/MS assay instead of the DiaSorin RIA. Additionally, toward the end of the study, CLS changed its assay technique from the RIA to a non-RIA chemiluminescence immunoassay, Liaison platform (DiaSorin Inc., Stillwater, Minn). In both situations, linear regression equations from the two labs' respective quality control data (LC/MS/MS vs. DiaSorin RIA, DiaSorin RIA vs. DiaSorin Liaison) were applied to convert the results from the non-RIA
methods into RIA values. It is unlikely that the conversion of data affected the overall conclusions of the study as the out of region oversight only applied to nine individuals out of the total 213 participating in the study and the CLS change only affected the 6-month lab results of two participants.

The duration of the study lasted during the fall-winter months, so we were unable to assess the seasonal influence of summer sun exposure on serum 25(OH)D levels. Our final dose recommendations in the study were based on serum levels which were during the winter. As heat intolerance is a well-recognized feature of MS it would likely lead to an avoidance of the sun and limit the vitamin D production from sun exposure for our MS patients. With the upper limit for optimal serum levels as 250 nmol/L, even if our patients do get the occasional summer sun exposure, their serum levels are unlikely to exceed this upper limit. Furthermore, serum 25(OH)D concentrations obtained from people living or working in sun-rich environments have ranged from 105-163 nmol/L with individual levels reported to be as high as 225 nmol/L (172). If patients do exceed this upper limit, it will only be for a short period of time and toxicity will unlikely be a concern.

The duration between the follow up time periods may not have been long enough to perhaps establish a true steady state concentration of 25(OH)D. Conventional pharmacology studies suggest that it should take 4 half-lives before a drug’s steady-state concentration is established. However, unlike a conventional drug, 25(OH)D is a metabolite whose concentration can be altered through balance between its production and clearance so that an equilibrium can be achieved earlier than would be expected from the half-life (172). The half-life of 25(OH)D in serum varies with estimates ranging from 10 to 30 days but is generally considered to be around 19 days (5, 6). Taking the half-life
data into consideration, at least three half-lives were likely covered by most of our patients when they had their serum levels measured at 3- and 6-months. It was necessary to compromise on classical pharmacology namely because of the length of the study. Having a longer study duration would have influenced patient willingness to participate and even complete the study.

The questionnaires were self-administered, so there is a reliability issue associated with recall for patients. However, our questionnaires only had two questions at baseline that required patients to recall information from one year prior. Otherwise, the questions asked patients to recall information at a maximum three months preceding the time of completing a questionnaire at 3- and 6-months.

5.4 Strengths

This was a study to assess vitamin D levels in a sample of MS patients in Canada. The sample of recruited patients was largely unselected in terms of patients visiting the Calgary MS clinic. The Calgary MS clinic is a population-based clinic. The participants in CIMS are representative of the MS population. The sample did include a high proportion of relapsing-remitting patients. These patients make up a majority of the visiting patients because they require to be seen annually if they are on disease modifying therapy. This study assessed vitamin D levels in a cohort advised to take a vitamin D supplement dose above the current Canadian nutrition board guidelines which some researchers have suggested are inadequate to achieve optimal levels while others believe this dose to be quite conservative (188, 242). Accordingly, this study adds novel data about vitamin D insufficiency in patients already taking an above average supplement dose and using the cut-off of 80nmol/L. Finally, we tested an innovative strategy
involving MS patients in the management of their own healthcare with the incorporation of Internet-based applications for the first time in our clinic.

5.5 Conclusions and Recommendations

- Vitamin D insufficiency is a global concern; individuals living in northern latitudes are especially vulnerable over the winter months.
- Active detection of vitamin D insufficiency among patients with MS and intervention to restore vitamin D status to adequate levels (>80 nmol/L) should be considered as part of the clinical management of MS.
- Engaging in a self-management model of care was acceptable for MS patients however, it may not be suitable for all patients and was less effective than a directed management approach.
- MS patients are comfortable using Internet-based strategies in the management of their vitamin D levels and incorporation of such strategies to enhance other aspects of patient care in the Calgary MS population may contribute to efficiency once secure messaging and electronic review of lab results is available.
- Variation in the different vitamin D assays may not be conducive to frequent monitoring of serum levels and practitioners should be aware of this.
- Adequate optimization of vitamin D levels will likely be best achieved by recommending a safe, relatively high oral dose at least 2000 IU per day, possibly as high as 4000 IU per day that all MS patients can take year round without the need for frequent monitoring of serum levels.
- Adherence to vitamin D therapy may be improved with less frequent dosing using higher dose formulations.
5.6 Unanswered Questions and Future Research

- Long-term supplementation of high doses requires further study to assess effectiveness and safety.

- Whether alternative routes of administration, such as parenteral, may be more efficient and overcome some of the issues associated with adherence to oral supplementation is unclear?

- The impact of vitamin D on MS progression and disease activity is unknown but laboratory studies support assessment of such benefits.

- A clinical trial or at least a longitudinal study evaluating the impact of vitamin D optimization on progression is needed but design will be challenged by lack of a control group as it will be unethical not to offer vitamin D optimization. Studies of pharmacologic doses of vitamin D are also indicated in MS.

- Population studies to evaluate the benefit of very early vitamin D optimization (from gestation through childhood and into adulthood) are needed to determine if MS and many other diseases are potentially related to vitamin D deficiency. These include autoimmune diseases such as MS, Type I diabetes, malignancy such as colon, breast and prostate cancer, and even some neurodevelopmental diseases such as autism and schizophrenia.

- Finally, the role of artificial tanning in optimizing vitamin D levels deserves further study.
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APPENDIX A: PRELIMINARY STATISTICAL ANALYSIS

Provided by the Centre for Advancement of Health, Calgary

Objective #1: To determine the prevalence of vitamin D insufficiency among MS patients.

The prevalence of vitamin D insufficiency (and 95% exact binomial confidence intervals) will be determined.

With a fixed sample size of 200:

<table>
<thead>
<tr>
<th>Number with vitamin D insufficiency</th>
<th>Estimated proportion</th>
<th>Exact 95% binomial confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>0.28</td>
<td>(0.21, 0.34)</td>
</tr>
<tr>
<td>70</td>
<td>0.35</td>
<td>(0.28, 0.42)</td>
</tr>
<tr>
<td>85</td>
<td>0.43</td>
<td>(0.36, 0.50)</td>
</tr>
<tr>
<td>100</td>
<td>0.50</td>
<td>(0.43, 0.57)</td>
</tr>
<tr>
<td>115</td>
<td>0.58</td>
<td>(0.50, 0.64)</td>
</tr>
<tr>
<td>130</td>
<td>0.65</td>
<td>(0.58, 0.72)</td>
</tr>
<tr>
<td>145</td>
<td>0.73</td>
<td>(0.66, 0.79)</td>
</tr>
<tr>
<td>160</td>
<td>0.80</td>
<td>(0.74, 0.85)</td>
</tr>
<tr>
<td>175</td>
<td>0.88</td>
<td>(0.82, 0.92)</td>
</tr>
<tr>
<td>190</td>
<td>0.95</td>
<td>(0.91; 0.98)</td>
</tr>
</tbody>
</table>

A sample size of 200 will allow you to estimate the prevalence of vitamin D insufficiency with precision of about +/- 3%-8%.

Use a one-sided, one-sample test of a proportion. The null hypothesis is that the prevalence of insufficiency is < 50% and the alternate hypothesis is that the prevalence is >= 50%.
APPENDIX B: BASELINE QUESTIONNAIRE

PILOT STUDY: Optimizing Vitamin D Levels in a Multiple Sclerosis Population

Study ID: ________________
Date: __________/________/________
       year   month   day

1. Sex:
   ☐ Female
   ☐ Male

2. Date of your birth:

   __________/________/________
   Year   Month   Day

3. What best describes your race? (Check all that apply)
   ☐ Caucasian
   ☐ Asian
   ☐ Native American
   ☐ African-American
   ☐ Middle-Eastern
   ☐ Other (Please specify) ________________

4. Weight: ________ kilograms

5. Height: ________ centimeters

The following questions ask about your exposure to sun

6. In the past 12 months, did you ever expose a considerable part of your body to direct sunlight?
   ***(Please refer to the box below for further clarification)

   ☐ Never
   ☐ Seldom
   ☐ Regularly
   ☐ Often
Considerable part of the body = part of the body exposed for 30 minutes or more in a swimsuit or equivalent (this includes a t-shirt/tank top and shorts).

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>Did not expose considerable part of my body to direct sunlight for at least 30 minutes any day</td>
</tr>
<tr>
<td>Seldom</td>
<td>Sometimes but less than 3 months of the year</td>
</tr>
<tr>
<td>Regularly</td>
<td>3 to 6 months of the year</td>
</tr>
<tr>
<td>Often</td>
<td>More than 6 months of the year</td>
</tr>
</tbody>
</table>

7. In the past 12 months, have you used sunscreen or face cream with any SPF (Sun Protection Factor) rating to protect your skin against sunlight?

- NO
- YES

If ‘YES’, was it

- Sometimes
- Usually
- Always

8. Have you spent any time outside of Canada in the last 3 months?

- NO (please go to question 11)
- YES

9. If ‘Yes’, please state where you went and estimate how much time you spent in the sun per day and the number of days.


10. While traveling did you wear sunscreen on a regular basis?

- NO
- YES

If YES, what SPF (Sun Protection Factor)?


The following questions are about use of artificial tanning equipment such as a tanning bed, sunlamp or tanning light. Tanning creams or lotions do NOT count as artificial tanning equipment.

11. Have you ever used artificial tanning equipment?
12. How long have you been using artificial tanning equipment?


13. In the past 12 months how many times have you used artificial tanning equipment?

  *One tanning session of any length of time would equal 1 time.

14. In the past MONTH how many times have you used artificial tanning equipment?

  *One tanning session of any length of time would equal 1 time.

15. What is the average number of minutes per tanning session?

16. In an average WEEK please indicate how many servings you would have of the following food items:

  *One serving equals the size of the palm of your hand.

  Herring

  Mackerel or Salmon

  Sardines or tuna

  Milk (250 mL=1 cup)

17. In the past 3 months, have you been taking any vitamin D or multivitamin supplements?

  NO

  YES

  If ‘YES’, what is the TOTAL DAILY amount of vitamin D in International units (IU) that you are currently taking?

18. It is common for people to forget to take vitamins occasionally. In the past 3 months, how often did you miss taking a dose of your vitamin D supplements?

  Never

  Infrequently, 1-2 times a month

  Somewhat frequently, 1 x week

  Frequently, 2 or more x week
19. Please list all of the prescribed drugs you are currently taking

You have reached the end of the questionnaire. Thank you for your participation.
APPENDIX C: FOLLOW-UP QUESTIONNAIRE

PILOT STUDY: Optimizing Vitamin D Levels in a Multiple Sclerosis Population

Study ID: ______________
Date: _______/______/______
year month day

Please answer the following questions based on the time period between your last blood test and the most recent one you just had.

The following questions ask about your exposure to sun

1. Have you spent any time outside of Canada in the last 3 months?

☐ NO (please go to question 4)
☐ YES

2. If ‘Yes’, please state where you went and estimate how much time you spent in the sun per day and the number of days.

3. While traveling did you wear sunscreen on a regular basis?

☐ NO
☐ YES
☐ If YES, what SPF (Sun Protection Factor)? ________

The following questions are about use of artificial tanning equipment such as a tanning bed, sunlamp or tanning light. Tanning creams or lotions do NOT count as artificial tanning equipment.

4. Have you ever used artificial tanning equipment?

☐ NO (please go to question 8)
☐ YES

5. In the past 3 MONTHS how many times have you used artificial tanning equipment? ________
*One tanning session of any length of time would equal 1 time.
6. In the past MONTH how many times have you used artificial tanning equipment?
   
   *One tanning session of any length of time would equal 1 time.

7. What is the average number of minutes per tanning session? ____________

8. In the last 3 months, in an average WEEK please indicate how many servings you would have of the following food items:
   *One serving equals the size of the palm of your hand.

   _____ Herring
   _____ Mackerel or Salmon
   _____ Sardines or tuna
   _____ Milk (250 mL = 1 cup)

9. In the past 3 months, have you been taking any vitamin D or multivitamin supplements?

   □ NO
   □ YES
   □ If 'YES', what is the TOTAL DAILY amount of vitamin D in International units (IU) that you are currently taking?
   **Please also include the amount of vitamin D that is in your multivitamin (usually 400 IU) and/or calcium supplement. __________

10. It is common for people to forget to take vitamins occasionally. In the past 3 months, how often did you miss taking a dose of your vitamin D supplements?

    □ Never
    □ Infrequently, 1-2 times a month
    □ Somewhat frequently, 1 x week
    □ Frequently, 2 or more x week

   **You have reached the end of the questionnaire. Thank you for your participation.**
APPENDIX D: END OF STUDY QUESTIONNAIRES

Study ID: 
Date: _____/_____/_____
year month day

Self-Management with Internet Access

Please check only one response to the following questions.

1. I was comfortable with the management method I was assigned.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

2. Adequate instructions were provided for the process of receiving lab results and adjusting vitamin D dosage.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

3. I was pleased to receive my vitamin D lab results rather than not receiving the results at all.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

4. I would prefer to always receive lab results for tests done in the MS clinic even if they are normal.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree
5. I was comfortable adjusting my own vitamin D dose rather than having someone from the MS clinic direct me.

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree

6. The use of a website to communicate lab results worked well for me in this process.

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree

7. I found the website easy to navigate.

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree

8. I feel the website can be used in other ways to support patient care in the MS clinic. (Please include any suggestions in question 12)

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree

9. I would consider participating in more web-based programs at the MS clinic.

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree
10. Over the duration of the project, did you consciously change your behavior in any of the following ways: (Please check all that apply)

- [ ] Reduced the number of missed doses of vitamin D supplements.
- [ ] Included foods rich in vitamin D in my diet.
- [ ] I increased the amount of time I would spend outdoors in the sun.
- [ ] I reduced the amount of sunscreen I wore when outdoors in the sun.
- [ ] I would wear less sun protective clothing when outdoors in the sun.

11. How would you rate your overall experience in this study? (Please check one)

- [ ] Very Positive
- [ ] Positive
- [ ] Neutral
- [ ] Negative
- [ ] Very Negative

12. Please provide any feedback you wish.

_____________________________________________________
_____________________________________________________
_____________________________________________________
_____________________________________________________

You have reached the end of the questionnaire. Thank you for your participation.
Self-Management without Internet Access

Please check only one response to the following questions.

1. I was comfortable with the management method I was assigned.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

2. Adequate instructions were provided for the process of receiving lab results and adjusting vitamin D dosage.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

3. I was pleased to receive my vitamin D lab results rather than not receiving the results at all.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

4. I would prefer to always receive lab results for tests done in the MS clinic even if they are normal.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree
5. I was comfortable adjusting my own vitamin D dose rather than having someone from the MS clinic direct me.

- Strongly agree
- Agree
- Neither agree nor disagree
- Disagree
- Strongly disagree

6. Over the duration of the project, did you consciously change your behavior in any of the following ways: (Please check all that apply)

- Reduced the number of missed doses of vitamin D supplements.
- Included foods rich in vitamin D in my diet.
- I increased the amount of time I would spend outdoors in the sun.
- I reduced the amount of sunscreen I wore when outdoors in the sun.
- I would wear less sun protective clothing when outdoors in the sun.

7. How would you rate your overall experience in this study? (Please check one)

- Very Positive
- Positive
- Neutral
- Negative
- Very Negative

8. Please provide any feedback you wish.

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

You have reached the end of the questionnaire. Thank you for your participation.
Directed Management without Internet Access

Please check only one response to the following questions.

1. I was comfortable with the management method I was assigned.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

2. Adequate instructions were provided for the process of receiving lab results and adjusting vitamin D dosage.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

3. I was pleased to receive my vitamin D lab results rather than not receiving the results at all.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

4. I would prefer to always receive lab results for tests done in the MS clinic even if they are normal.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree
5. I would have been comfortable adjusting my own vitamin D dose rather than having someone from the MS clinic direct me.

☐ Strongly agree
☐ Agree
☐ Neither agree nor disagree
☐ Disagree
☐ Strongly disagree

6. Over the duration of the project, did you consciously change your behavior in any of the following ways: (Please check all that apply)

☐ Reduced the number of missed doses of vitamin D supplements.
☐ Included foods rich in vitamin D in my diet.
☐ I increased the amount of time I would spend outdoors in the sun.
☐ I reduced the amount of sunscreen I wore when outdoors in the sun.
☐ I would wear less sun protective clothing when outdoors in the sun.

7. How would you rate your overall experience in this study? (Please check one)

☐ Very Positive
☐ Positive
☐ Neutral
☐ Negative
☐ Very Negative

8. Please provide any feedback you wish.

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________

You have reached the end of the questionnaire. Thank you for your participation.
Study ID: 
Date: ___/___/____ 
year month day 

Directed Management with Internet Access

Please check only one response to the following questions.

1. I was comfortable with the management method I was assigned.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

2. Adequate instructions were provided for the process of receiving lab results and adjusting vitamin D dosage.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

3. I was pleased to receive my vitamin D lab results rather than not receiving the results at all.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree

4. I would prefer to always receive lab results for tests done in the MS clinic even if they are normal.
   - [ ] Strongly agree
   - [ ] Agree
   - [ ] Neither agree nor disagree
   - [ ] Disagree
   - [ ] Strongly disagree
5. I would have been comfortable adjusting my own vitamin D dose rather than having someone from the MS clinic direct me.

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree

6. I was comfortable receiving the vitamin D lab results and dosage information by e-mail.

☐ Strongly agree  
☐ Agree  
☐ Neither agree nor disagree  
☐ Disagree  
☐ Strongly disagree

7. Over the duration of the project, did you consciously change your behavior in any of the following ways: (Please check all that apply)

☐ Reduced the number of missed doses of vitamin D supplements.  
☐ Included foods rich in vitamin D in my diet.  
☐ I increased the amount of time I would spend outdoors in the sun.  
☐ I reduced the amount of sunscreen I wore when outdoors in the sun.  
☐ I would wear less sun protective clothing when outdoors in the sun.

8. How would you rate your overall experience in this study? (Please check one)

☐ Very Positive  
☐ Positive  
☐ Neutral  
☐ Negative  
☐ Very Negative

9. Please provide any feedback you wish.
You have reached the end of the questionnaire. Thank you for your participation.
APPENDIX E: INFORMATION ON SURVEYMONKEY

- The 2 groups with Internet access will complete their follow-up and end of study questionnaires with this online survey tool.

*What is Surveymonkey?*
It is a software tool used to conduct online questionnaires.

*Why are we using an online survey tool in this project?*
Because you have Internet access and we thought this would be a great opportunity to test out this method of completing questionnaires in our patient population.

*What do you have to do?*
You will be e-mailed a link to complete the survey; this link will take you to the questionnaire, complete it and submit it.

*Is personal information linked to the questionnaires?*
Your e-mail address and unique study ID number will be the only information stored on the Surveymonkey account and linked to the questionnaires you complete.

*Does the questionnaire have to be completed in one sitting?*
You can leave the survey and then resume it later. The survey link will remember where you left off based on the last completed page. As you click on the “next” button in the survey, the survey page saves. You will need to access the survey link from the original computer in order for the survey link to remember the last completed page you left off from.

*How is the data in the questionnaires kept secure?*
The SurveyMonkey privacy policy states that program operators will not use your data for their own purposes. The data collected will be kept private and confidential. For more information on security and privacy you can visit the website at www.surveymonkey.com

*SurveyMonkey uses cookies*
"Cookies" are small text files a website can use to recognize repeat users. With cookies enabled, you will not need to fill in password or contact information. You can easily turn off cookies. Most browsers have a feature that allows the user to refuse cookies or issues a warning when cookies are being sent. However, the website will not function properly without cookies. Enabling cookies ensures a smooth, efficient visit to the website.

*If you have any other questions in regard to Surveymonkey please contact:*
  Pavan Ahluwalia (Graduate Student)
  *Telephone:* 944-2756   *E-mail Address:* pavan.ahluwalia@calgaryhealthregion.ca
APPENDIX F: ALGORITHM TO ADJUST VITAMIN D DOSE

Please use the following table to find the range your 25-Hydroxy vitamin D lab result falls in and look to the column on the right to get the amount to add or subtract from your current daily dose of vitamin D.

Do not increase the dose of other vitamins along with vitamin D.

<table>
<thead>
<tr>
<th>Lab Result 25-Hydroxy Vitamin D Level</th>
<th>Add the following amount to your Daily Dose (IU=International Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-30 nmol/l</td>
<td>3000 IU</td>
</tr>
<tr>
<td>30-50 nmol/l</td>
<td>2000 IU</td>
</tr>
<tr>
<td>50-80 nmol/l</td>
<td>1000 IU</td>
</tr>
<tr>
<td>&gt;80 nmol/L</td>
<td>Optimal Range-No Change in Dose</td>
</tr>
<tr>
<td>&gt;130 nmol/L</td>
<td>If you have 2 lab results 3 months apart like this, reduce daily dose by 1000 IU</td>
</tr>
</tbody>
</table>
APPENDIX G: INSTRUCTIONS FOR PARTICIPATION

Self-Management with Internet Access

What do you do now?

1. The lab requisition in your enrolment package has been filled out for you to include the 3 separate blood tests that you will have and details to where the results should be returned.
2. Take the lab requisition in your package to any Calgary Laboratory Services clinic and have your blood tested as soon as possible.
3. Within 2 weeks of having your blood test, your vitamin D lab result will be posted on the website.
4. You will then be able check your lab results on the following website:
   http://ucalgary.ca/~psahluwa/
5. When accessing the website, you will be asked for a username and password.
   USERNAME: vitamind   PASSWORD: sunlight06
6. Follow the instructions on the Vitamin D Project homepage to check your lab result and using the guidelines on the website to adjust your vitamin D dose as necessary.
7. Remember the aim of this process is to bring your 25(OH)D level above the optimal target of 80 nmol/L.

What happens next?

1. Write down the date of your first blood test.
2. With a calendar, go forward approximately 3 months and 6 months from this date, these are the dates of your next 2 blood tests.
3. You do not have to go exactly on these dates but anytime a week before or after that date will be fine.
4. Remember to write down the dates of the next 2 blood tests and put them in a place where you will not forget.
5. After each blood test within 2 weeks of the test your lab results should be posted on the website.
6. Go to the website and check your lab result and using the guidelines on the website you will adjust your vitamin D dose as necessary.
7. You will also be e-mailed a link to a short online questionnaire to complete.
8. This questionnaire is completed through the SurveyMonkey tool, for more information on this tool see the “SurveyMonkey Information” page that was included in your enrolment package.
9. If you need assistance or have any questions, contact the Graduate Student listed below.
10. Remember your vitamin D dose is adjusted only after a blood test, so you will take this dose everyday until your next blood test.
11. At the end of the project, after your last blood test, you will also be mailed a link to complete a short “End of Study” questionnaire.

12. When the project concludes, your MS clinic doctor along with Dr. Metz (Principal Investigator) will advise you on your vitamin D dose and if you require further blood tests.

If you have any questions or concerns during the course of the project, please feel free to contact:

Pavan Ahluwalia (Graduate Student)
Telephone: 944-2756   E-mail Address: pavan.ahluwalia@calgaryhealthregion.ca
Website Address: http://ucalgary.ca/~psahluwa/
Website Username: vitamind   Website Password: sunlight06
Self-Management without Internet Access

What do you do now?

1. The lab requisition in your enrolment package has been filled out for you to include the 3 separate blood tests that you will have and details to where the results should be returned.
2. Take the lab requisition in your package to any Calgary Laboratory Services clinic and have your blood tested as soon as possible.
3. Within 2 weeks of having your blood test, your vitamin D lab result will be mailed to you.
4. When you receive your lab result in the mail, refer to the guidelines “Adjusting Your Vitamin D dose” that were included in your enrolment package.
5. Adjust your dose according to these guidelines as necessary.
6. Remember the aim of this process is to bring your 25(OH)D level above the optimal target of 80 nmol/L.

What happens next?

1. Write down the date you had your first blood test.
2. With a calendar, go forward approximately 3 months and 6 months from this date, these are the dates of your next 2 blood tests.
3. You do not have to go exactly on these dates but anytime a week before or after that date will be fine.
4. Remember to write down the dates of the next 2 blood tests and put them in a place where you will not forget.
5. After each blood test within 2 weeks of the test your lab results will be mailed to you in a package.
6. Using the guidelines given to you, you will adjust your vitamin D dose as necessary.
7. In this package you will also get a short follow up questionnaire to complete.
8. Please return the completed questionnaire in the enclosed envelope. No postage is necessary.
9. **If you need assistance or have any questions, contact the Graduate Student listed below.**
10. Remember your vitamin D dose is adjusted only after a blood test, so you will take this dose everyday until your next blood test.
11. At the end of the project, after your last blood test, you will be mailed a follow up questionnaire and a second short “End of Study” questionnaire.
12. Please return the completed questionnaires in the enclosed envelope. No postage is necessary.
13. When the project concludes, your MS clinic doctor along with Dr. Metz (Principal Investigator) will advise you on your vitamin D dose and if you require further blood tests.

At any time during project if you have any questions or concerns please feel free to contact:

Pavan Ahluwalia (Graduate Student)

Telephone: 944-2756   E-mail Address: pavan.ahluwalia@calgaryhealthregion.ca
Directed Management without Internet Access

What do you do now?

1. The lab requisition in your enrolment package has been filled out for you to include the 3 separate blood tests that you will have and details to where the results should be returned.
2. Take the lab requisition in your package to any Calgary Laboratory Services clinic and have your blood tested as soon as possible.
3. Within 2 weeks of your blood test, your vitamin D lab result and new dosage of vitamin D will be mailed to you.
4. Remember the aim of this process is to bring your 25(OH)D level above the optimal target of 80 nmol/L.

What happens next?

1. Write down the date of your first blood test.
2. With a calendar, go forward approximately 3 months and 6 months from this date, these are the dates of your next 2 blood tests.
3. You do not have to go exactly on these dates but anytime a week before or after that date will be fine.
4. Remember to write these dates down and put them in a place where you will not forget.
5. After each blood test, within 2 weeks your lab result and new dose will be mailed to you in a package.
6. In this package you will also get a short follow up questionnaire to complete.
7. Please return the completed questionnaire in the enclosed envelope. No postage is necessary.
8. **If you need assistance or have any questions, contact the Graduate student listed below.**
9. Remember your vitamin D dose is adjusted only after a blood test, so you will take this dose everyday until you receive further information about adjusting your dosage.
10. At the end of the project, after your last blood test, you will be mailed a follow up questionnaire and a second short “End of Study” questionnaire.
11. Please return the completed questionnaires in the enclosed envelope. No postage is necessary.
12. When the project concludes, your MS clinic doctor along with Dr. Metz (Principal Investigator) will advise you on your vitamin D dose and if you require further blood tests.

If you have any questions or concerns during the course of the project, please feel free to contact:
Pavan Ahluwalia (Graduate Student)

Telephone: 944-2756    E-mail Address: pavan.ahluwalia@calgaryhealthregion.ca
Directed Management with Internet Access

What do you do now?

1. The lab requisition has been filled out for you to include the 3 separate blood tests to be taken and details to where the results should be returned.
2. Take the lab requisition in your package to any Calgary Laboratory Services clinic and have your blood tested as soon as possible.
3. Within 2 weeks of having your blood test, your lab result and your new dosage of vitamin D will be e-mailed to you.
4. Remember the aim of this process is to bring your 25(OH)D level above the optimal target of 80 nmol/L.

What happens next?

1. Write down the date you of your first blood test.
2. With a calendar, go forward approximately 3 months and 6 months from this date, these are the dates of your next 2 blood tests.
3. You do not have to go exactly on these dates but anytime a week before or after that date will be fine.
4. Remember to write these dates down and put them in a place where you will not forget.
5. After each blood test, within 2 weeks your lab result and new vitamin D dose will be e-mailed to you.
6. You will also be e-mailed a link to a short online questionnaire to complete.
7. This questionnaire is completed through the SurveyMonkey tool, for more information on this see the “SurveyMonkey Information” page that was included in your enrolment package.
8. If you need assistance or have any questions, contact the Graduate Student listed below.
9. Remember your vitamin D dose is adjusted only after a blood test, so you will take this dose everyday until you receive further information about adjusting your dosage.
10. At the end of the project, after your last blood test, you will also be mailed a link to complete a short “End of Study” questionnaire.
11. When the project concludes, your MS clinic doctor along with Dr. Metz (Principal Investigator) will advise you on your vitamin D dose and if you require further blood tests.

If you have any questions or concerns during the course of the project, please feel free to contact:

Pavan Ahluwalia (Graduate Student)
Telephone: 944-2756  E-mail Address: pavan.ahluwalia@calgaryhealthregion.ca
APPENDIX H: MULTIPLE SCLEROSIS AND VITAMIN D BROCHURE

Multiple Sclerosis and Vitamin D

What is Vitamin D?

Vitamin D is a fat-soluble vitamin that helps maintain normal blood levels of calcium and phosphorus in our body. It also plays a role in maintaining healthy immune function. Vitamin D is needed for normal calcium absorption to deposit of calcium in the bone. This leads to healthy strong bones. Vitamin D deficiency leads to rickets in children and osteomalacia (softening of the bones) in adults. There is now also evidence suggesting a link between low levels of vitamin D and an increased risk of tuberculosis, osteoarthritis, Type 1 diabetes (formerly called juvenile diabetes), some cancers (breast, ovarian, prostate and colorectal) and multiple sclerosis (MS).

Recently, there has been increased awareness of an early phase of vitamin D deficiency (low levels of vitamin D) with no obvious symptoms. This is called “vitamin D insufficiency”. Vitamin D insufficiency, when combined with not taking in enough calcium, may cause osteoporosis, a serious disease that can lead to disabling fractures (especially of the hip, spine and wrist). It is important to prevent, detect and treat osteoporosis as early as possible. Osteoporosis may be prevented by having enough calcium and vitamin D in our bodies.

Where Does Vitamin D Come From?

Sunlight is our best natural source of vitamin D. Ultraviolet (UV) rays from the sun stimulate our skin to make “pre-vitamin D” (vitamin D3), which is then converted to active vitamin D by our kidneys and liver. UV light from artificial sources (e.g. tanning beds) will also increase vitamin D levels, just as it increases our risk of skin cancer. Vitamin D is also found in some foods; however, sources from foods will not maintain necessary vitamin D levels. A vitamin D supplement is usually needed for people living in Canada, as our sun’s radiant energy is not strong enough most of the year.

Who is at Risk for Vitamin D Deficiency and Insufficiency?

Every Canadian is at risk for vitamin D deficiency. There is not enough radiant energy from the sun during the fall and winter to create enough vitamin D production in the skin, no matter how long the exposure. People living in the tropics often have vitamin D (25-hydroxyvitamin D) levels over 100 nmol/L. Even in sunny Alberta, we cannot reach that level without taking vitamin D supplements. A minimum level of 80 nmol/L is considered adequate. A recent study found that 97% of healthy Calgarians were deficient in vitamin D at some time during the year.

Calgary gets more hours of sunshine per year than any other Canadian city. This means that other Canadians may be at an even higher risk of insufficiency. Taking vitamin D supplements is therefore recommended for everyone, especially during the fall and winter months. Having darker skin, using sunscreen, staying indoors and increasing age further limit our ability to make vitamin D.
How Can Vitamin D Affect Multiple Sclerosis?

It is not clear if taking vitamin D will have an impact on MS or slow the disease progression. Most of the evidence linking low vitamin D levels with MS relates to a person's risk of getting MS, but even this evidence is uncertain:

- There appears to be a link between sunlight and MS. MS is more common at higher latitudes (the further you live away from the equator). For example, in Canada and Scandinavian countries, where high-energy sunlight is in short supply and MS is common.
- In Australia, people with the greatest amount of sun damage to their skin had the lowest rates of MS.
- In 2004, it was noted that Americans who had been taking vitamin D supplements had lower rates of MS; doses over 400 IU daily were better than lower doses.

This circumstantial evidence is supported by recent understanding that vitamin D plays an important role in immune function. Low levels of vitamin D may alter immune responses. Mice that had low levels of vitamin D developed a form of lab MS much earlier than those with enough vitamin D. These mice also showed more severe symptoms than those with a normal dietary intake of vitamin D.

There is very little evidence that vitamin D actually affects MS once you have it; however, one small study showed that there were more new MS lesions on MRI during the months that vitamin D levels are usually low.

Osteoporosis is very common in people with MS. This may be partly related to the use of steroids or a change of lifestyle that sometimes affects people with MS, such as becoming less active and spending more time indoors. When these situations happen, the vitamin D levels in our body may be lowered. Please see the pamphlet MS and Osteoporosis for more information.

Taking a vitamin D supplement is recommended. Although it is unclear if it will help your MS, the evidence exists that vitamin D has an important role in immune function, and in preventing osteoporosis. Further research is needed to understand fully the exact nature of the link between MS and vitamin D.

How Much Vitamin D and Calcium Should I Take?

<table>
<thead>
<tr>
<th>Amount Needed</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td></td>
</tr>
<tr>
<td>Adults - 1000 IU/day</td>
<td>• Helps body absorb and deposit calcium in the bones</td>
</tr>
<tr>
<td>Children (under 18 years) - 400 IU/day</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>Before age 50</td>
<td>supports normal bone growth 1,000 mg/day</td>
</tr>
<tr>
<td>After age 50 1,000-1,500 mg/day</td>
<td></td>
</tr>
<tr>
<td>(As suggested by the Osteoporosis Society of Canada)</td>
<td></td>
</tr>
</tbody>
</table>

Few Albertans will get enough calcium and vitamin D from their diet or through exposure to the sun. Most people will need to use a supplement. Vitamin D comes in two forms: vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). D₂ is commercially produced from ergosterol found in plants or yeast. The more abundant natural vitamin D₃ is much stronger with better absorption. Choose calcium sources from calcium citrate and calcium carbonate, as they may be more easily absorbed.

The recommended amount may need to be adjusted, and a vitamin D blood test can be checked to find out the best dose for you. Please discuss this with your doctor or nurse. In Alberta, a higher dose may be needed over the winter (from October to April).
What are the Best Sources of Vitamin D?

The chart below shows how you could get 1000 IU of vitamin D from your diet. It is unlikely you will get enough vitamin D from your diet alone.

<table>
<thead>
<tr>
<th>Source</th>
<th>Vitamin D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring (100 g)</td>
<td>900</td>
</tr>
<tr>
<td>Mackarel or salmon (100 g)</td>
<td>650</td>
</tr>
<tr>
<td>Sardines or tuna (100 g)</td>
<td>250</td>
</tr>
<tr>
<td>Milk, fortified (250 mL)</td>
<td>90</td>
</tr>
<tr>
<td>Soy beverage, fortified (250 mL)</td>
<td>88</td>
</tr>
<tr>
<td>Eggs (1 large)</td>
<td>25</td>
</tr>
<tr>
<td>Margarine (5 mL)</td>
<td>56</td>
</tr>
<tr>
<td>Liver, beef or pork (60 g)</td>
<td>32-45</td>
</tr>
</tbody>
</table>

Can Too Much Vitamin D be Harmful?

Yes, taking too much supplemental vitamin D can have serious side effects. It is a fat-soluble vitamin, so it can build up in your body. In people with primary hyperparathyroidism, sarcoidosis, tuberculosis or lymphoma, any amount of vitamin D can cause toxicity. Early signs and symptoms of toxic (extremely high) vitamin D levels include nausea, vomiting, poor appetite, constipation, weakness and weight loss. Too much vitamin D also raises blood calcium levels, leading to high blood pressure, abnormal deposits of calcium in bone and soft tissue, and abnormal kidney function.

What Should I Do Now?

1. Find out how much vitamin D you are already taking. Add up the amount in all your supplements. Write the total dose on your calendar or in a diary with today’s date.
2. Think about switching to vitamin D3 (cholecalciferol) if you are taking vitamin D2 (ergocalciferol).
3. Talk to your doctor about having your vitamin D (25-hydroxyvitamin D) level checked.
4. Adjust your dose as per your doctor’s recommendation. Levels may be lower during winter and spring, so you may be advised to take a higher dose during these months. Remember to take a daily calcium supplement. See the pamphlet MS and Osteoporosis.

For More Information Call:
Calgary MS Clinic (403) 944-4253

This material is designed for information purposes only. It should not be used in place of medical advice, instruction and/or treatment. If you have specific questions, please consult your doctor or appropriate health-care professional.
APPENDIX I: WEBSITE PAGES

Welcome Page

Vitamin D Project Website

December 12, 2006

Welcome

- Please remember this website is only for the participants in the Vitamin D project.

- Please use the links on the left side of the page to navigate through the website.

- Click on the "Lab Results" link to check your lab results.

- Then click on the "Adjusting your Vitamin D Dose" link to see how to adjust your dose after a blood test.

<table>
<thead>
<tr>
<th>Instructions for Participation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D Brochure</td>
</tr>
<tr>
<td>Lab Results</td>
</tr>
<tr>
<td>Adjusting your Vitamin D Dose</td>
</tr>
<tr>
<td>Copy of Project Consent</td>
</tr>
<tr>
<td>Contact Information</td>
</tr>
</tbody>
</table>

http://udacity.cs/p1o0062/20061212.6.78.22 PM
1. In the table below locate your study ID and look to the columns on the right to get the your 25(OH)D lab level.

2. Next click on the link on the left hand side of the page "Adjusting your Vitamin D Dose"

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Baseline</th>
<th>3 month follow-up</th>
<th>6 month follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>00100</td>
<td>73.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00102</td>
<td>127.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00113</td>
<td>76.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00114</td>
<td>97.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00115</td>
<td>91.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00120</td>
<td>47.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00123</td>
<td>64.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00124</td>
<td>65.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00126</td>
<td>68.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00128</td>
<td>74.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00129</td>
<td>91.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00134</td>
<td>70.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00136</td>
<td>75.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00139</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>00140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>00141</td>
<td>117.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00142</td>
<td>33.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00147</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Adjusting Your Vitamin D Dose

1. Please use the following table to find the range your 25-Hydroxy vitamin D lab result falls in and look to the column on the right to get the amount to add or subtract from your current daily dose of vitamin.

2. Do not increase the dose of other vitamins along with vitamin D.

<table>
<thead>
<tr>
<th>Lab Result 25-Hydroxy Vitamin D Level</th>
<th>Add the following amount to your Daily Dose (IU=International Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-30 nmol/l</td>
<td>3000 IU</td>
</tr>
<tr>
<td>30-50 nmol/l</td>
<td>2000 IU</td>
</tr>
<tr>
<td>50-80 nmol/l</td>
<td>1000 IU</td>
</tr>
<tr>
<td>&gt;80 nmol/L</td>
<td>Optimal Range-No Change in Dose</td>
</tr>
<tr>
<td>&gt;130 nmol/L</td>
<td>If you have 2 lab results 3 months apart like this, reduce your daily dose by 1000 IU</td>
</tr>
</tbody>
</table>
Contact Information

If you have any questions or concerns please feel free to contact Pavan Ahluwalia (Graduate Student)

Telephone: 403-944-2756

E-mail Address: pavan.ahluwalia@calgaryhealthregion.ca
APPENDIX J: ETHICS APPROVAL

2006-08-10

Dr. Lianne M. Metz
Department of Clinical Neurosciences
Foothills Hospital
Calgary, Alberta

Dear Dr. L. Metz:

RE: Pilot Study: Optimizing Vitamin D Levels in a Multiple Sclerosis Population

Ethics ID: E-20320

Student: Pawanjit S Ahluwalia

The above-noted proposal including the Research Proposal, Baseline Questionnaire, Follow up Questionnaire, End of study Questionnaire - Self Management with Internet Access, End of study Questionnaire - Self Management without Internet Access, End of study Questionnaire - Directed with Internet Access, End of study Questionnaire - Directed without Internet Access, Information of Survey Method, Consent Form (Version 1, dated: July 05, 2000). Instructions for Participants - Self Management with Internet Access, Instructions for Participants - Self Management without Internet Access, Instructions for Participants - Directed with Internet Access, Instructions for Participants - Directed without Internet Access, Mail Out Cover Letters - Self Management with Internet Access, Mail Out Cover Letters - Self Management without Internet Access, Mail Out Cover Letters - Directed with Internet Access, Mail Out Cover Letters - Directed without Internet Access, Internet Pages: Welcome page, Lab Results, and the Contact Information has been submitted for Board review and found to be ethically acceptable.

Please note that this approval is subject to the following conditions:
(1) appropriate procedures for consent for access to identified health information have been approved;
(2) a copy of the informed consent form must have been given to each research subject, if required for this study;
(3) a Progress Report must be submitted by August 10, 2007, containing the following information:
   i) the number of subjects recruited;
   ii) a description of any protocol modification;
   iii) any unusual and/or severe complications, adverse events or unanticipated problems involving risks to subjects or others, withdrawal of subjects from the research, or complaints about the research;
   iv) a summary of any recent literature, finding, or other relevant information, especially information about risks associated with the research;
   v) a copy of the current informed consent form;
   vi) the expected date of termination of this project.
4) a Final Report must be submitted at the termination of the project.

Please note that you have been named as the principal collaborator on this study because students are not permitted to serve as principal investigators. Please accept the Board's best wishes for success in your research.

Yours sincerely,

Michael King, PhD ABPP (C/CN)
Acting Chair, Conjoint Health Research Ethics Board

MICID
c.c. Adult Research Committee Dr.G. Colmanos (information) Research Services Pawanjit S Ahluwalia (Student)
Office of Information & Privacy Commissioner

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