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A survey of vitamin D status in a northern suburbs practice in Johannesburg, South Africa

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A SURVEY OF VITAMIN D STATUS IN A NORTHERN SUBURBS PRACTICE IN JOHANNESBURG, SOUTH AFRICA

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of

Master of Medicine in the branch of Internal Medicine

Johannesburg, 2014

Declaration

I, Kim Roberg declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.



.....

.....28.....day of.....April.....2014

To my mother, Christa Roberg and fiancé, Laurent Pieton

Presentations Arising From This Project

Oral Presentation:

Roberg, K., Govind, N., Bhana, S. A Survey of Vitamin D Status in a Northern Suburbs Pratice in Johannesburg, Gauteng. Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) Congress, Johannesburg, April 2013.

Poster Presentation:

Roberg, K., Govind, N., Bhana, S. A Survey of Vitamin D Status in a Northern Suburbs Pratice in Johannesburg, Gauteng. Federation of Infectious Diseases Society of South Africa (FIDSSA) Congress, Drakensberg, October 2013.

Abstract

Background: Hypovitaminosis D is endemic worldwide. With the discovery of extra skeletal receptors for 1,25-dihydroxyvitamin D, the influence of vitamin D ($25(OH)D_3$) deficiency has been investigated in metabolic diseases. In South Africa, little is known about $25(OH)D_3$ status.

Aim: To investigate the $25(OH)D_3$ status in patients in Johannesburg, and to assess for any correlation between $25(OH)D_3$, metabolic diseases and patient demographics and seasonal variation.

Methods: A retrospective study of 1000 patients attending a northern suburb practice in Johannesburg was performed. Serum $25(OH)D_3$ levels, demographics and metabolic data were collected.

Results: The mean $25(OH)D_3$ level was 24.45ng/ml and 74.3% were vitamin D deficient. There was no difference in mean $25(OH)D_3$ levels between age or gender groups. The lowest mean $25(OH)D_3$ was in the Indian race (p=0.001), HOMA-IR >2 (p=0.001), fasting glucose >7 (p=0.016) and highest was measured during the summer (p=0.001). There was a significant correlation between $25(OH)D_3$ level and cholesterol (p=0.001), however no correlation was found with hypertension or diabetes.

Conclusion: This study reports a high incidence of hypovitaminosis D especially among Indians. In this study there was no correlation between hypovitaminosis D and metabolic factors except for a negative correlation with the cholesterol level.

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Table of Contents

Declaration II

Presentations Arising From This Project IV

Abstract V

Acknowledgements VI

Table of Contents VII

List of Figures IX

List of Tables X

Nomenclature XI

1. Chapter 1. Introduction and Literature Review 1

1.1. Introduction 1

1.2. Metabolism of Vitamin D 1

1.3. Functions of Vitamin D 3

1.3.1. Vitamin D and Bone Health 3

1.3.2. Vitamin D and Extra-Skeletal Manifestations 5

1.3.2.1. Vitamin D and the Immune System 5

1.3.2.2. Vitamin D and Hypertension 7

1.3.2.3. Vitamin D and Diabetes Mellitus 8

1.3.2.4. Vitamin D and Dyslipidaemia 11

1.3.2.5. Vitamin D and Malignancy 12

1.4. Factors Determining Vitamin D Status 13

1.5. Global Estimates of Vitamin D Status 14

1.6. Optimal Vitamin D Status 15

1.7. Measurement of Vitamin D 17

1.8. Confounding Vitamin D Measurements: Underestimation of $25(OH)D_3$ 19

1.9. Recommended Vitamin D Dietary Intake 20
1.10. Supplementation of Vitamin D as Prophylaxis.....21
1.11. Aims of the Study 22
2. Chapter 2. Patients and Methods 23
2.1. Patients 23
2.2. Methods 23
2.3. Laboratory Analyses 24
2.4. Statistical Methods 25
3. Chapter 3. Results 26
3.1. Demographics of the Study Population 26
3.2. Vitamin D Status According to Sub-Group.....28
3.3. Vitamin D Mean.....29
3.4. Correlation and Regression Testing 31
4. Chapter 4. Discussion 35
4.1. Study Limitations 37
5. Chapter 5. Conclusion.....39
5.1. The Way Forward 40

List of Figures

Figure 1.1 Vitamin D synthesis and metabolism 2

Figure 1.2 Vitamin D and extra-skeletal functions 5

Figure 3.1 Vitamin D correlation with variables.....32

List of Tables

Table 1.1 Estimates of minimum optimal serum 25(OH)D_3 for fracture prevention 17

Table 3.1 Participant demographics by gender 26

Table 3.2 Three way table of 25(OH)D_3, gender and age.....27

Table 3.3 Three way table of 25(OH)D_3, age and race.....27

Table 3.4 Prevalence of vitamin D deficiency by gender, age, race, suburb, co-morbidities, chi-squared test. 29

Table 3.5 Mean vitamin D levels across sub-groups 31

Nomenclature

$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D
$25(OH)D_3$	25-hydroxyvitamin D
ALTM	All-laboratory Trimmed Mean
BMD	bone mineral density
DEQAS	International Vitamin D External Quality Assessment Scheme
EIA	enzyme immunoassay antibody
GIT	gastro-intestinal tract
HDL	high density lipoprotein
IFNγ	interferon γ
IL	Interleukin
MTB	Mycobacterium tuberculosis
NASH	non-alcoholic steatohepatitis
NO	nitric oxide
PTH	parathyroid hormone
RAAS	renin-angiotensin-aldosterone system
RANKL	receptor activated nuclear factor-κβ ligand
RDI	Recommended Daily Intake
RIA	radio-iodine assay
SD	standard deviation
T2DM	Type 2 diabetes mellitus
Th1/ CD_4	T-helper cell 1/ CD_4 cell
Th2/ CD_8	T-helper cell 2/ CD_8 cell
TLR1/2	toll-like receptor 1/2
VDR	vitamin D receptor

1. Chapter 1. Introduction and Literature Review

1.1. Introduction

Vitamin D, unlike the name suggests, is in fact not a vitamin, but rather a hormone. McCollum et al discovered Vitamin D in 1922, and termed it 'D' because it was the fourth known 'vitamin'(1).Vitamin D exists in two forms, vitamin D2 (25-hydroxylated ergocalciferol) and vitamin D3 (cholecalciferol). Cholecalciferol is a prohormone which is produced in the skin through ultraviolet radiation. It is a lipid soluble compound with a four ringed cholesterol backbone. This hormone form of vitamin D3 acts through a nuclear receptor to carry out its functions (2).

1.2. Metabolism of Vitamin D

Humans derive vitamin D through exposure to sunlight (vitamin D3), from their diet (vitamin D2), as well as from dietary supplements (vitamin D2 and/or D3). Since very few natural foods contain vitamin D some dietary sources are now fortified with vitamin D, such as fortified dairy products. One of the few foods containing natural vitamin D is oily fish. Ultraviolet B (solar) radiation penetrates the skin and converts 7-dehydrocholesterol (a derivative of cholesterol) to previtamin D3 (precholecalciferol). This then undergoes conversion to vitamin D3, which is the natural form of vitamin D in the skin.

Vitamin D2 is produced by irradiation of ergosterol. In the liver, vitamin D3 and D2 are metabolised to 25-hydroxyvitamin D ($25(OH)D_3$), which is the stored, metabolically inert form of vitamin D, and is used to determine a patient's vitamin D status. To activate $25(OH)D_3$, the kidneys metabolise this form to the active state, 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), which exerts it's effects by binding to the vitamin D receptor (VDR) in

target tissues (3).

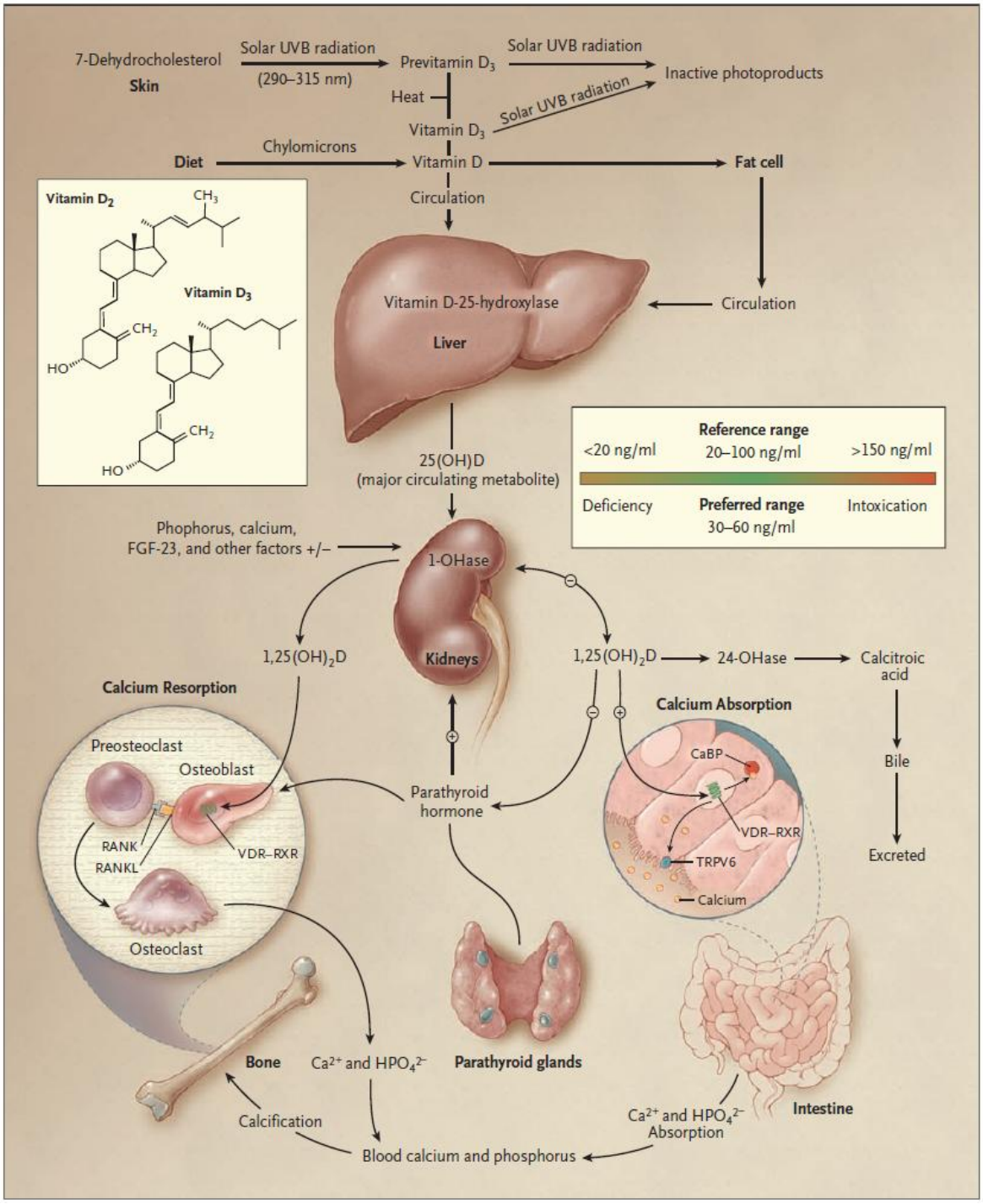


Figure 1.1 Vitamin D synthesis and metabolism (3).

1.3. Functions of Vitamin D

The main function of Vitamin D is to maintain calcium, phosphorous and bone homeostasis. Vitamin D functions to maintain adequate and steady calcium levels in the serum. Vitamin D deficiency therefore plays a major role in the pathogenesis of bone mineralisation diseases, such as rickets in children, and osteomalacia, osteopaenia, osteoporosis and fractures in adults.

A single vitamin D receptor is responsible for vitamin D hormone functions, and is present in the target cells of enterocytes, osteoblasts and renal tubular cells.

It has recently been recognised that tissues and cells not responsible for regulating calcium and phosphate metabolism have specific receptors for $1,25(OH)_2D_3$. This includes cells of the parathyroid gland, skin keratinocytes, colon, ovaries, pituitary gland, promyelocytes and lymphocytes. This suggests that the consequences of vitamin D deficiency are not limited to bone metabolism alone (4).

Vitamin D deficiency has been linked to the pathogenesis of many other diseases such as hypertension, diabetes mellitus, tumour growth (vis. colon, prostate, breast), autoimmune diseases and infections (5).

1.3.1. Vitamin D and Bone Health

The most well studied and understood function of vitamin D relates to bone health.
The most important function of vitamin D is to maintain a steady serum calcium concentration. Calcium is essential for many vital processes, some of these include myocardial and skeletal muscle contractility, action potential generation and conduction, and calcium dependant intracellular second messenger systems. Vitamin D maintains calcium levels through three main pathways. Firstly, vitamin D optimises calcium absorption from the intestine through active mechanisms. It also acts to absorb phosphate.

Without vitamin D, only 10-15% of dietary calcium is absorbed. Secondly, when dietary calcium is inadequate, vitamin D acts to maintain steady levels by mobilising calcium from bone. Vitamin D, together with parathyroid hormone (PTH), stimulates osteoblasts to produce receptor activated nuclear factor-κβ ligand (RANKL), which promotes bone resorption by stimulating the formation and activation of osteoclasts. Serum calcium is maintained at the expense of bone mineralisation. Lastly, vitamin D increases renal calcium absorption. Vitamin D, via stimulation of PTH, increases the absorption of calcium from the distal convoluted tubule in the kidney. This is approximately 7g/day, and is thus a significant contribution to the serum calcium pool (2).

If dietary calcium is not available, internal stores, from bone, are utilised. Prolonged vitamin D deficiency results in hypocalcaemia and hypophosphataemia. In children, this leads to rickets, a deforming bone disease secondary to impaired bone mineralisation. Adults develop osteomalacia and osteopaenia, and with time, osteoporosis. The majority of these patients are elderly females. The main concern in both adults and children with vitamin D deficiency is the risk of bone fractures. In both groups, fractures may cause considerable bone pain and often deformity. Fracture healing is frequently slow, with significant morbidity and mortality. Patients with fractures frequently have prolonged hospital admissions, with associated increased risk of hospital acquired infections such as pneumonia, or complications such as heart failure (6). Side effects from osteoporosis treatment are common and the economic burden is significant. Many elderly patients die prematurely as a result of fractures, particularly that of the hip (7).

The optimum vitamin D level to prevent fracture is not clear, but most experts agree that it should be 30ng/ml (8).

1.3.2. Vitamin D and Extra-Skeletal Manifestations

Biological mechanisms linking vitamin D deficiency with a multitude of non-bone related diseases have been proposed for many years. The identification of the $1,25(OH)_2D_3$ receptor in tissues other than bone (2, 3, 5) have prompted investigation into causal relationships between vitamin D deficiency and other disease pathogenesis. Some of the better recognised associations are discussed below (Figure 1.2).

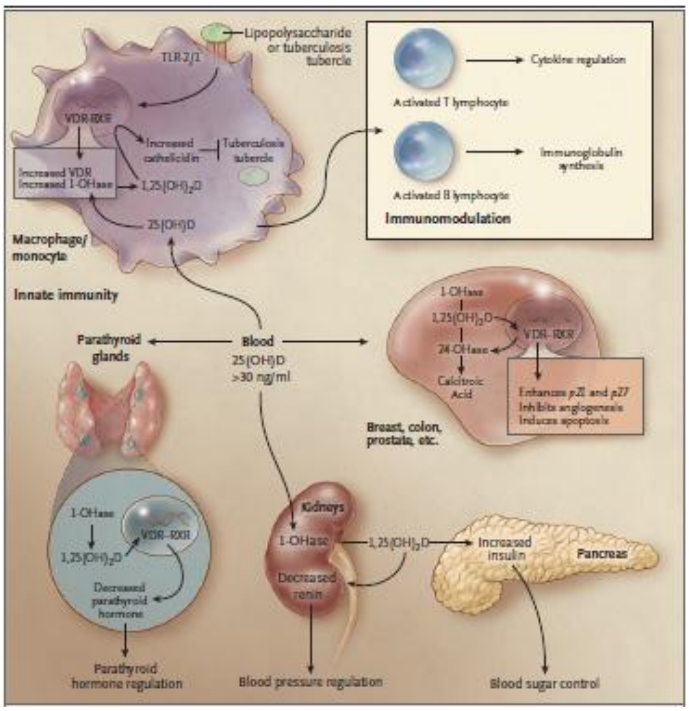


Figure 1.2 Vitamin D and extra-skeletal functions (3).

1.3.2.1. Vitamin D and the Immune System

One of the most well studied extra–skeletal effects of vitamin D is the interaction between vitamin D and the immune system, both the innate and adaptive systems.

a. The Innate Immune System

The macrophage expresses the VDR and produces $1,25(OH)_2D_3$ through the synthesis of 1α-hydroxylase, triggered by interferon γ (IFN γ). The VDR and 1α-hydroxylase gene are

specifically induced when toll-like receptor1/2 (TLR1/2) is activated. This receptor is the specific recognition receptor for Mycobacterium tuberculosis (MTB). The TLR1/2-$25(OH)D_3$ combination stimulates the expression of cathelicidin, an antibacterial protein, which promotes killing of MTB and other bacteria. Cathelicidin is also produced by other cells, and together with induction of TLR1/2 and high concentrations of $1,25(OH)_2D_3$, is able to prime the innate immune response to pathogens (9).

In addition to this, $1,25(OH)_2D_3$ also enhances the phagocytic action of all dendritic cells, promoting pathogen elimination (3).

This effect of vitamin D on the innate immune response has been shown to reduce bacterial, but not viral or fungal, infections. Vitamin D deficiency has been identified as a risk factor for tuberculosis in several case control studies (10, 11).

In southern Africa, tuberculosis is endemic. This is largely due to the Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS) epidemic. It has been noted that African and Indian population groups are at higher risk for developing tuberculosis (12). African people generally have lower vitamin D levels (and are often vitamin D deficient) due to high skin melanin levels. This may be one of the explanations why this race group is more prone to contracting tuberculosis, and often develop a more aggressive form of the disease (3).

b. The Adaptive Immune System

T helper 1 (Th1,CD_4) and T helper 2 (Th2,CD_8) cells are direct targets of $1,25(OH)_2D_3$. Once activated, the expression of the VDR on Th1 cells increases 5-fold. $1,25(OH)_2D_3$ acts to decrease the proliferation of CD_4 cells, therefore decreasing the production of interferon gamma (IFNγ), interleukin 2 (IL2) and interleukin 5 (IL5). In contrast, $1,25(OH)_2D_3$ increases the production of IL4 by CD_8 cells, ie. a shift from a Th1, to a Th2 response.

In vitamin D deficiency, reduced VDR activation leads to a dampening in the shift from Th1 to Th2, and a Th1 response dominates. This may promote the development of autoimmune diseases such as multiple sclerosis, Type 1 diabetes mellitus and inflammatory bowel diseases such as Crohn's disease (13).

1.3.2.2. Vitamin D and Hypertension

Hypovitaminosis D has been associated with hypertension through its lack of inhibitory effects on the renin-angiotensin-aldosterone system (RAAS) (14). A study showed that both $25(OH)D_3$ and $1,25(OH)_2D_3$ were inversely associated with plasma renin and angiotensin II concentrations, with $25(OH)D_3$ deficiency causing augmented renal vascular RAAS activity, as well as increased angiotensin II concentrations (15). Later it was shown that $1,25(OH)_2D_3$ inhibits renin expression in mice by inhibiting the renin gene expression (14).

It is further postulated that vitamin D has direct affects on the vascular endothelial tone by influencing the calcium concentration in the vascular smooth muscle cells.

Vitamin D may also have a vascular protective effect by reducing the harmful consequences of glycation end products on the endothelium, improving activity of the nitric oxide (NO) system, and by decreasing atherosclerotic and inflammatory mediators (16).

In addition to this, vitamin D deficiency leads to increases in PTH. High PTH levels may increase the blood pressure, as shown during trials of PTH infusion in healthy volunteers (1).

Lower plasma $25(OH)D_3$ levels in non-obese young women independently predicted the odds of subsequently developing hypertension. In another study, Forman et al. (17, 18)

reported an association between measured vitamin D deficiency, and increased risk for incident hypertension, which was independent of other risk factors.

Using data from the NHANES III survey (a national survey conducted between January 1998 and December 1994 from 89 survey locations in the USA), it was found that the prevalence of hypertension was significantly higher in those patients with low vitamin D levels, with up to 30% of these individuals having hypertension (19). One of few prospective studies demonstrated that patients with untreated, mild hypertension who were subjected to an artificial UVB source (not UVA) showed a significant reduction in both the systolic and diastolic blood pressure (-6/-6 mmHg), as well as a 162% rise in plasma $25(OH)D_3$ levels (20).

A meta-analysis of all data published before November 2013 reported an inverse relationship between $25(OH)D_3$ and hypertension (21).

In summary, optimal vitamin D levels have beneficial effects on the blood pressure by inhibiting the RAAS, modulating vascular tone and indirectly affecting vascular endothelium.

1.3.2.3. Vitamin D and Diabetes Mellitus

In global reports it has been noted that plasma $25(OH)D_3$ is a marker of insulin resistance in non-diabetic patients, with $25(OH)D_3$ being inversely associated with fasting glucose, fasting insulin, the HOMA-IR index, even when adjusted for other cardiovascular risk factors such as obesity and smoking (22). In the SENECA study, no association was found between fasting plasma insulin or the HOMA-IR index after adjusting for demographic, lifestyle factors and calcium intake. However there was an inverse correlation between fasting plasma glucose and the $25(OH)D_3$ levels (23). A meta-analysis of the effect of vitamin D supplementation on glycaemia, insulin resistance and progression to diabetes

found that although there was a small improvement on fasting glucose and insulin resistance, there was no effect on HBA1C and no improvement in outcome in patients with normal fasting glucose. This study concluded that there was no evidence of benefit to recommend vitamin D supplementation to improve glycaemic control (24).

a. Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is an autoimmune disease, where destruction of β cells by auto-antibodies in the pancreas leads to progressive insulin deficiency and ultimately β cell failure.

$1,25(OH)_2D_3$ has been recognised as an immune-modulator that targets T cells by decreasing their recruitment and activation. Furthermore, it has the ability to stimulate phagocytes to kill bacteria. Conversely, $25(OH)D_3$ suppresses the antigen-presenting capability of these cells, as well as suppressing important cytokines such as IL12 . Phagocytes also produce the enzyme 1 α-hydroxylase which converts vitamin D to its active form.

$1,25(OH)_2D_3$ administration has been shown to be protective against β cell destruction and reduces the incidence of both diabetes and insulitis in mice. This seems to be due to a predominantly Th2 immune response (shifted from a Th1 response in mice treated with $1,25(OH)_2D_3$), which reshapes the immune system to increase the elimination of autoimmune effector cells (25, 26).

The efficacy of supplementing $1,25(OH)_2D_3$ after the onset of β cell destruction, in order to arrest this progression, is uncertain (26).

b. Type 2 Diabetes Mellitus

It has been long known that the β cells in the pancreas express the receptor for $1,25(OH)_2D_3$. The effector protein for this pathway, calbindin–D_{28k}, is also present in the pancreas.

When comparing vitamin D levels between patients with type 2 diabetes mellitus (T2DM), and those without, those with T2DM have lower $25(OH)D_3$ levels than those without and have a higher incidence of vitamin D deficiency. Patients at risk for T2DM were also found to have lower $25(OH)D_3$ levels (27).

Studies on animal models have shown that pancreatic insulin secretion is impaired by vitamin D deficiency. This is due to loss of calbindin–D_{28k} β cell protection, which protects these cells from cytokine-mediated cell death. However, another factor which plays a role in glucose intolerance and insulin resistance in animals with abnormal VDRs is the genetic background, as not all animals with VDR knockout develop diabetes mellitus (26).

$1,25(OH)_2D_3$ appears to promote insulin release by causing a rise in intracellular calcium, via PTH. Low vitamin D increases PTH which may impair glucose tolerance and decrease insulin sensitivity. Secondly, vitamin D may enhance insulin responsiveness by stimulating the expression of the insulin receptor. Vitamin D's effect on the immune system, as a genetic modulator, is the final mechanism (22).

The correction of vitamin D deficiency may improve glucose tolerance. However, in the case of vitamin D sufficient patients, the supplementation of additional $1,25(OH)_2D_3$ has shown conflicting results. Some studies showed improvement in insulin and c-peptide secretion, whilst others demonstrated no change. The beneficial effect was, however, greatest in those patients who received combined vitamin D and calcium (28).

1.3.2.4. Vitamin D and Dyslipidaemia

In recent years, the metabolic syndrome (increased waist circumference, hypertriglyceridaemia, hypo-HDL-cholesterolaemia, hypertension and high fasting serum glucose) has become an increasing problem in many countries worldwide, with reports of up to 1 in 5 people having this syndrome (29). Many people have been treated with statins as part of the metabolic syndrome management, and it has been noted that those on statins have an associated reduced fracture risk (30). It has also been shown that statins may improve bone mineral density (31, 32). Another study shows that statins do not affect vitamin D status, but rather that low vitamin D levels are associated with dyslipidaemia (30). However, it has been shown that patients with replete $25(OH)D_3$ stores generally have better lipograms – lower fasting cholesterol levels, and higher HDL levels, and therefore improved cardiovascular risk (29).

Studies based on vitamin D effects on blood lipids are contradictory (33), and this needs to be further investigated.

In a systematic review by Zittermann et al.,(34) the influence of vitamin D on serum cholesterol levels was not demonstrated.

The reason for this seems to be due to the anti-atherogenic effects of vitamin D – via down-regulation of the renin-angiotensin system, and inhibition of the production of pro-inflammatory cytokines (29). Another mechanism is reduced expression of mRNA and protein levels of plasminogen activator inhibitor-1, and thrombospondin -1, both of which are implicated in the pathogenesis of atherosclerosis (30).

It is also thought that vitamin D increases the activity of lipoprotein lipase by regulating adipocytes, and therefore decreases triglyceride levels.

In general, there seems to be an inverse relationship between vitamin D levels, and components of the metabolic syndrome such as waist circumference, hypertriglyceridaemia and hyperglycaemia (35).

1.3.2.5. Vitamin D and Malignancy

It has been noted that people exposed to sunlight have lower malignancy rates and mortality. This seemingly protective effect was presumed to be as a result of $25(OH)D_3$ metabolism by UVB exposure. However, the presence of the VDR in breast, colon, prostate and ovarian tissue has prompted further research and an inverse relationship between these cancers and vitamin D status has been elicited (36).

In vitamin D's role as a genetic-modulator, $1,25(OH)_2D_3$ plays a part in regulating both normal and malignant cell growth, differentiation and proliferation. It also plays an important role in apoptosis and induction of terminal differentiation, so that hypovitaminosis D may lead to reduced apoptosis and cellular differentiation with resultant tumorigenesis (37).

In addition to these mechanisms, it has been proposed that Vitamin D controls multiple ion channels, including calcium and chloride channels, as well as modulating Protein Kinase C activity. Low vitamin D levels may lead to impaired control of the above mechanisms and lead to lack of inhibition of cell proliferation and differentiation and promote tumour growth (38). To illustrate this, the following has been noted:

1. Sunlight exposure and vitamin D intake were associated with a reduced risk of developing breast cancer (39, 40).
2. Both high and low levels of vitamin D have been implicated as a risk factor for the development of prostate cancer (41).

3. Many studies have shown a reduction in colorectal cancer risk in patients with increased vitamin D intake. However, there are conflicting reports with other studies showing no difference for colorectal cancer risk in patients on vitamin D supplementation (42, 43).

1.4. Factors Determining Vitamin D Status

The human $25(OH)D_3$ serum concentration is dependent on many factors, namely latitude, sun exposure and aging.

Latitude and serum $25(OH)D_3$ concentration has a significant positive correlation in Europe. This is because latitude determines the degree of sun exposure. The highest prevalence of vitamin D deficiency is in central and southern Europe. The lower levels of deficiency in Northern Europe and North American countries (high latitude, low sun exposure) has been attributed to the greater consumption of oily fish, as well as dairy product vitamin D fortification (44). However, vitamin D levels in countries with high degrees of sun exposure have reported higher than expected levels of vitamin D deficiency (45-48). At higher and lower latitudes, where sun exposure is far less, the prevalence of vitamin D deficiency is even greater (49-51). This is far less surprising than the low levels measured in countries closer to the equator.

Vitamin D levels decline in the elderly (52) and hypovitaminosis D is particularly common in post-menopausal women. These lower levels are because aging decreases the ability of the human skin to produce vitamin D. Also, the elderly are often confined indoors – whether due to illness, reduced mobility or institutionalization (49). Due to reduced earning capacity and low pensions, the elderly may also have a diet low in vitamin D and may not be able to afford supplementation.

With respect to race, definite differences in vitamin D status have been demonstrated. Light skinned people generally have higher vitamin D levels than their darker skinned counterparts.These differences are due to the fact that people with higher degrees of melanin, and therefore pigmentation, with comparable UV light exposure, produce less $25(OH)D_3$ than do people with light skins. It is necessary to have these greater levels of melanin as the darker skin provides protection from the intensity and amount of sunlight in their country of origin (50). It has also been reported that Black people in the USA use less dietary supplements than do Whites (53).

1.5. Global Estimates of Vitamin D Status

Globally, vitamin D deficiency is widespread, and is emerging as a major health problem. One of the main indicators of vitamin D status is sun exposure, which is predicted based on latitude (relation to the equator). In countries further north and south, such as Canada, Asia and Latin America, lack of sun exposure is considered the major risk factor for deficiency (49). Even in countries considered to have high UVB indices, such as parts of Australia and the USA, high sunlight exposed states in the USA such as Hawaii and Florida, the Middle East and Africa, the levels of hypovitaminosis D is far higher than would be expected (46, 47, 51).

The reasons for this are multi-factorial. In areas with high sun exposure, hypovitaminosis D may be due to protective clothing and sunscreen, as well as less time spent outdoors, as a result of greater awareness of the risk of skin malignancies. In Muslim countries, covering clothing for religious reasons is practiced, resulting in low sun exposure. In Africa, pigmentation, from high levels of skin melanin, results in decreased cutaneous synthesis of vitamin D_3. In fact, Black population groups usually have far lower vitamin D levels than their White contemporaries (53).

In addition to latitude, seasonal variation in sunlight and outdoor activity levels also plays a major role in vitamin D status. In the majority of studies, the highest $25(OH)D_3$ levels were recorded in the summer and autumn months, with the lowest recorded in the winter months (45, 54).

In developing countries, another factor leading to hypovitaminosis D is prolonged breastfeeding without vitamin D supplementation to the mother or child, compared to infants receiving bottle feeds (49).

The elderly, hospitalised and institutionalised also commonly have low vitamin D levels – this is due to poor dietary levels (little or no fish) as well as little outdoor activity (55).

Surprisingly, countries with little sunlight exposure such as the Netherlands and Scandinavian countries such as Sweden have relatively good $25(OH)D_3$ levels. It is presumed that this is from a diet containing large amounts of oily fish (49).

1.6. Optimal Vitamin D Status

There is currently no definition of the optimal vitamin D level (3, 56, 57). The possible criteria for which optimal $25(OH)D_3$ may be defined include that level for which there is:

1) Maximal suppression of circulating PTH

2) Maximal absorption of calcium from the gastro-intestinal tract (GIT)

3) Highest bone mineral density (BMD)

4) Lowest rates of bone loss

5) Reduced rates of falling and reduced incidence of fractures (56)

These criteria may, however, be problematic as:

1) Serum PTH is only an indirect measure and is not optimally sensitive. Parathyroid

hormone is more directly affected by and related to calcium metabolism. Often, an individual with deficient vitamin D status may not exhibit a perceptible PTH response (58).

2) When one compares calcium absorption with vitamin D levels, the lower limits of optimal vitamin D sufficiency is difficult to define as calcium absorption initially rises as vitamin D rises, but then plateaus at levels >80nmol/l. This makes calcium absorption an inefficient marker of serum $25(OH)D_3$ (57). Also, at 'normal' plasma $25(OH)D_3$ levels, calcium absorption varies, so that even at so called current 'normal' vitamin D concentrations, the calcium absorption is not necessarily optimal.

3) Reports of higher $25(OH)D_3$ concentrations correlating with higher BMD have also been shown to have non-significant differences in other trials, or to have small positive relationships between BMD and $25(OH)D_3$ (56).

4) Studies in elderly women indicate that vitamin D supplementation decreases net bone loss. The optimum level at which bone loss is minimal remains under debate and has not yet been defined (44).

5) Studies done on the elderly showed a reduction in fall and fracture risk with patients on vitamin D supplementation, however again, there is debate as to what the optimal serum level to prevent this is (59).

Table 1.1 Estimates of minimum optimal serum $25(OH)D_3$ for fracture prevention by different authors, adapted from Dawson-Hughes et al. (56).

Author	Optimal $25(OH)D_3$ level, ng/ml	Oral vitamin D3 dose needed to reach average optimal 25(OH)D3 level	
		µg/day	IU/day
Lips	20	10-15	400-600
Holick	30	25	1000
Heaney	32	40	1600
Meunier	30	20	800
Vieth	28	25	1000
Dawson-Hughes	32	25	1000

The Endocrine Society of America and the International Osteoporosis Foundation suggest that a $25(OH)D_3$ level of 30ng/ml is the optimal lower limit for adequate bone health (60-61).

Another concern is whether a $25(OH)D_3$ level of 30ng/ml is also adequate for vitamin D to achieve its extra-skeletal effects, as higher levels than this may be required (4).

Other markers which have been suggested for estimation of adequate vitamin D status include muscle strength, serum calcium and phosphorus levels, bone turnover markers, cell proliferation and metabolic function (62). Many of these are impractical, time consuming and expensive to perform. In addition to this, optimal levels have only been studied with respect to bone health, with little consensus reached on optimal levels or markers with regard to the extra-skeletal manifestations of vitamin D deficiency.

1.7. Measurement of Vitamin D

Vitamin D status is generally assessed by measuring the $25(OH)D_3$ level (63).

Various assays to measure circulating $25(OH)D_3$ levels have been developed over the years. Currently, two of the most widely used assays are a radioiodine-based (RIA) kit,

such as the DiaSorin assay (which is the most widely used assay), and an enzyme immunoassay antibody (EIA) type test (64).

There is an urgent need for standardisation of $25(OH)D_3$ test assays as variation between different methods have made defining optimal vitamin D levels difficult (65). The total $25(OH)D_3$ level may be underestimated when vitamin D_2 constitutes a large part of the total vitamin D level (66). This is most apparent in the EIA method where the primary antibody reacts unequally with vitamin D_2 and vitamin D_3, compared to the DiaSorin assay, which does not detect vitamin D_2 entirely. Therefore, the RIA based tests have emerged as the test of choice for most laboratories (64, 67). This has clinical implications both for the detection of hypovitaminosis D, as well as for monitoring of replacement in patients on supplementation and vitamin D related disease.

Binkley et al., (65) sent multiple samples to 6 different laboratories, which all used different assays. Results for the same specimens differed by as much as 2-fold.

Because of this, the International Vitamin D External Quality Assessment Scheme (DEQAS) has been monitoring the performance of various assays, and attempts to provide information on the accuracy and specificity of these different assays, as well as providing an All-Laboratory Trimmed Mean (ALTM) and Standard Deviation (SD) for each method. The overall accuracy of the different methods can then be assessed from the percentage bias of the method mean from the ALTM (66).

In addition to the RIA and EIA tests, mass spectrometry is able to measure $25(OH)D_3$ accurately, and to distinguish between vitamin D2 and vitamin D3. Until recently, mass spectrometry has been used only in research laboratories due to the complexity of the analysis. Because mass spectrometry may be a more sensitive and specific test for vitamin D analysis, has less inter-laboratory variation than the RIA and EIA tests, as well as being

able to independently distinguish between the various forms of vitamin D, it is now starting to be routinely used in laboratories around the world (68).

1.8. Confounding Vitamin D Measurements: Underestimation of $25(OH)D_3$

The differences between different assay methods have been discussed in the section above, however another cause of underestimation of $25(OH)D_3$ needs to be highlighted.

Studies suggest that patients who are obese or who have non-alcoholic steatohepatitis (NASH) have significantly lower vitamin D concentrations than age-matched controls. This, however, is not as a result of decreased sun exposure, or enhanced production of the active $1,25(OH)_2D_3$ metabolite, but rather because vitamin D is a fat soluble pro-hormone. This means that it gets deposited in body fat compartments and stored in adipose tissue, such as subcutaneously, as well as in the liver in obese patients. This means that there is decreased bioavailability of vitamin D_3 and assays performed in obese patients, or those with NASH, may show erroneously low levels. Some studies have demonstrated a >50% decreased bioavailability of cutaneously synthesised vitamin D_3 (70).

$25(OH)D_3$ level may also be affected by an age-related decline in renal function (59).

Inducers of the cytochrome P450 system in the liver, such as anti-epileptic drugs (71), rifampicin (tuberculosis treatment) (72), as well as anti-retrovirals such as efavirenz (73) (the latter two are especially important in the South African setting) promote the metabolism of $25(OH)D_3$ to less biologically active analogs. Again, patients who are taking these, and other cytochrome P450 inducers, may have lower vitamin D levels and may require higher doses of supplementation (3).

In addition to this, the season that the test was performed may have important implications for $25(OH)D_3$ estimation. It has been suggested that a $25(OH)D_3$ level performed in the

spring or autumn months may be the best indication of average of $25(OH)D_3$ over a one year period (74).

More than 85-90% of vitamin D in serum is bound to vitamin D-binding protein, with a further 10-15% being bound to albumin. This renders <1% of free circulating vitamin D available to act on target cells, and measurements of $25(OH)D_3$ may therefore be erroneously low. Between race groups, genetic polymorphisms for vitamin D-binding protein change the affinity of the binding protein for vitamin D, and may partially explain the racial differences in levels of total $25(OH)D_3$ (75).

1.9. Recommended Vitamin D Dietary Intake

Vitamin D is uniquely synthesised in the skin and as such there is variation in synthesis due to the seasonality of the sun exposure, the degree of skin pigmentation, individual genetic factors, use of sunscreen, latitude of area of living and amount of outdoor activity. Because of this, recommended dietary intakes (RDIs) are advised based on an assumption of minimal or no sun exposure. The RDIs are based on the amount of vitamin D required for good bone health (76).

On average, for adequate bone health, it is advised that adult males and females should receive 600IU/d of vitamin D to correspond with a serum $25(OH)D_3$ level of 20ng/ml. Males and females over the age of 71yr should have 800IU/d of vitamin D, to correspond to the same serum level of $25(OH)D_3$. The upper limit for vitamin D supplementation is 4000IU/d (76).

The determinants of the upper limits of RDI for vitamin D include evidence of a U-shaped relationship for mortality from cardiovascular disease, certain cancers, falls and fractures, as well as hypercalcaemia, hypercalciuria, vascular and soft tissue calcifications and nephrolithiasis (76).

Therefore, the upper limit of RDI is not a target intake, but rather advises at what levels risk for harm begins to increase.

1.10. Supplementation of Vitamin D as Prophylaxis

Despite the association between hypovitaminosis D and multiple skeletal and extra-skeletal diseases, the therapeutic window for beneficial, positive effects from vitamin D supplementation is narrow. This is due to the potential for toxicity with supplementation. These include mainly calcaemic effects such as bone demineralisation and hypercalcaemia, which may result in hypercalciuria and renal failure from nephrocalcinosis or renal stones. These effects are usually only seen with massive doses of vitamin D >10 000IU/d, however caution should be taken not to exceed the recommended daily allowance (76).

Excess sunlight exposure does not result in vitamin D toxicity. This is because excess precholecalciferol will photoisomerise into the biologically inactive photoisomers lumisterol and tachysterol. Another efficient sunscreen is melanin, which reduces the efficiency of sun-mediated photosynthesis of precholecalciferol. This explains why Black people have lower circulating levels of $25(OH)D_3$ compared to Caucasians (5). In addition to this, the 24-hydroxylase pathway will breakdown excess vitamin D2 and D3, and hepatic production of $25(OH)D_3$ is decreased by negative feedback from $1,25(OH)_2D_3$ (4).

1.11. Aims of the Study

1. To investigate the overall status of vitamin D levels in a northern suburbs practice in Johannesburg, Gauteng.

2. To describe the vitamin D status in different age, gender and race groups.

3. To assess for any possible correlation between the vitamin D level and cholesterol level, glucose level, hypertension, diabetes mellitus and HOMA-IR index.

4. To describe seasonal variation of vitamin D levels.

2. Chapter 2. Patients and Methods

2.1. Patients

This study was a cross-sectional, retrospective, descriptive study involving review of patient records. Patient data was collected from files from a private practice in the northern suburbs of Johannesburg.

A random sample of 1000 files from patients attending this practice from December 2010 until December 2012 was collected. Only data from patients living in the greater Johannesburg area was captured, and no exclusions were made based on age, race, gender or reason for consultation.

Ethics approval was obtained for this study (M120957) (Appendix A).

2.2. Methods

Data collected included the patient's age, race, gender, basal pre-supplement 25(OH)D_3 level (ng/ml), as well as the date the test was performed, fasting glucose level (mmol/l), fasting cholesterol level (mmol/l), HOMA-IR score, and whether or not the patient had known, pre-existing history of diabetes mellitus (no distinction was made for Type 1 or Type 2) or hypertension. Also documented was the suburb of residence.

Data was further grouped into the following categories:

Age -

Youth : <17 years old

Young Adult : 18 – 25 years old

Adult : 26 – 65 years old

Elderly : >65 years old

Race:

Black

White

Indian

HOMA-IR:

Abnormal >2

Normal ≤2

Cholesterol:

Hypercholesterolaemia >5mmol/l

Normal ≤5mmol/l

Area of Residence:

North – Parktown, Randburg, Fourways, Sandton

South – Lenasia, Alberton, Vereeniging, Vaal Triangle

East – Boksburg, Benoni, Germiston, Bedfordview

West – Krugersdorp, Roodepoort

Pretoria – Midrand, Pretoria

Season (according to the World Meteorological Organisation, for South Africa):

Summer – 22 December – 19 March

Autumn – 20 March – 20 June

Winter – 21 June – 22 September

Spring – 23 September – 21 December

Data was captured onto an Excel spread sheet for further analysis.

2.3. Laboratory Analyses

All patient blood samples were drawn before 10h00 and were performed under fasting conditions, after a minimum 8 hour fast.

The 25(OH)D_3 testing was performed using a Diasorin assay and the laboratory specific levels were used as reference ranges (Lancet Laboratories).

The reference ranges for $25(OH)D_3$:

Normal >30ng/ml

Moderate Deficiency = 10-29.9ng/ml

Severe Deficiency <10ng/ml

Cholesterol and glucose levels were tested using an enzymatic method.

$$\text{HOMA-IR score} = \frac{\text{Fasting Insulin X Fasting Glucose}}{22.5}$$

This was used to indicate insulin resistance.

2.4. Statistical Methods

Descriptive statistics were used to describe the sample in terms of: $25(OH)D_3$ level, age, gender, race, fasting glucose level, fasting cholesterol level, HOMA-IR index, hypertension and diabetes mellitus. Frequencies for these variables were calculated, as well as mean $25(OH)D_3$ levels for each group. Differences in means between independent samples were tested by using Student's t test or oneway analysis of variance (ANOVA). The Chi- squared test was performed to determine if the difference in mean vitamin D levels between variables was statistically significant. Correlation and regression testing was performed using the Pearson Correlation test. The limit for statistical significance was set to be <0.05. STATA IC 11 was used for data analysis.

3. Chapter 3. Results

3.1. Demographics of the Study Population

Of the 1000 subjects, 71.4% were female. The majority of subjects were White in ethnicity (75.8%), and were between the ages of 26-65 years (83.2%). Forty percent of patients lived in the Northern suburbs, with the remainder residing in the rest of Johannesburg and Pretoria. Of all the subjects, 14.7% had hypertension and 6.8% had diabetes mellitus (Table 3.1).

Table 3.1 Participant Demographics by Gender

Variable	Men (n=286)	Women (n=714)
Race		
Black (n=24)	8 (0.8%)	16 (1.6%)
Indian (n=218)	66 (6.6%)	152 (15.2%)
White (n=758)	212 (21.2%)	546 (54.6%)
Age		
Youth (n=27)	12 (1.2%)	15 (1.5%)
Young Adult (n=97)	27 (2.7%)	70 (7%)
Adult (n=832)	230 (23%)	602 (60.2%)
Elderly (n=44)	17 (1.7%)	27 (2.7%)
Suburb		
North (n=400)	107 (10.7%)	293 (29.3%)
South (n=202)	57 (5.7%)	145 (14.5%)
East (n=138)	39 (3.9%)	99 (9.9%)
West (n=88)	20 (2%)	68 (6.8%)
Pretoria (n=172)	63 (6.3%)	109 (10.9%)
Hypertension (n=147)	55 (5.5%)	92 (9.2%)
Diabetes Mellitus (n=68)	35 (3.5%)	33 (3.3%)

Table 3.2 Three way table of $25(OH)D_3$, gender and age

$25(OH)D_3$	Gender and Age							
	Female				Male			
	Youth n=15	Young Adult n=70	Adult n=602	Elderly n=27	Youth n=12	Young Adult n=27	Adult n=230	Elderly n=17
Severe Deficiency	1 (7%)	11 (16%)	51 (8%)	6 (22%)	0	2 (7%)	11 (5%)	0
Moderate Deficiency	8 (53%)	40 (57%)	398 (66%)	12 (44%)	9 (75%)	18 (67%)	164 (71%)	12 (71%)
Normal	6 (40%)	19 (27%)	153 (26%)	9 (33%)	3 (25%)	7 (26%)	55 (24%)	5 (29%)

Table 3.3 Three way table of $25(OH)D_3$, age and race

$25(OH)D_3$	Age and Race											
	Youth			Young Adult			Adult			Elderly		
	Black n=2	Indian n=11	White n=14	Black n=1	Indian n=23	White n=73	Black n=21	Indian n=174	White n=637	Black n=0	Indian n=10	White n=34
Severe Deficiency	0	1(9%)	0	0	9(39%)	4(5%)	2(10%)	45(26%)	15(2%)	0	4(40%)	2(6%)
Moderate Deficiency	1(50%)	10(91%)	6(43%)	1(100%)	11(48%)	46(63%)	16(76%)	115(66%)	431(68%)	0	3(30%)	21(62%)
Normal	1(50%)	0	8(57%)	0	3(13%)	23(32%)	3(14%)	14(8%)	191(30%)	0	3(30%)	11(32%)

3.2. Vitamin D Status According to Sub-Group

The mean 25(OH)D_3 level for the study population was 24.45ng/ml (SD11.81). According to the laboratory specific reference ranges, this is classified as moderate deficiency.

Overall, 74.3% of subjects were 25(OH)D_3 deficient, with 8.2% being severely deficient (Table 3.4).

Of particular note, three quarters (75.5%) of male subjects were vitamin D deficient, of which 71% were moderately deficient. 9.7% of the females, compared to 4.5% of males, had severe vitamin D deficiency, although there was no statistically significant difference between mean vitamin D levels between the gender groups.

In the race category, 69.3% of the White subjects were 25(OH)D_3 deficient, of which 22 (3%) were over the age of 65years. This is compared to 83.4% of Black subjects, and a staggering 90.8% of Indian subjects who were 25(OH)D_3 deficient. Again, in both groups, the majority of these subjects were in the adult category. Interestingly, none of the Indian subjects in the youth category had a normal 25(OH)D_3 level (n = 11) (Table 3.3).

Although there was no significant difference between 25(OH)D_3 means and deficiency categories between different age groups ($p = 0.745$ and $p = 0.149$), it is interesting to note that 75% of adults compared to 68.2% of the elderly were classified as 25(OH)D_3 deficient.

Table 3.4 Prevalence of Vitamin D Deficiency by Gender, Age, Race, Suburb, Co-morbidities, Chi-Squared test.

Variable	Normal (>30ng/ml)	Moderate Deficiency (10-29.9ng/ml)	Severe Deficiency (<10ng/ml)	P value
***Total* (n=1000)**	257 (25.7%)	661 (66.1%)	82 (8.2%)	
Gender				
Male (n=286)	70 (24.5%)	203 (71%)	13 (4.5%)	0.017
Female (n=714)	187 (26.2%)	458 (64.1%)	69 (9.7%)	
Race				
Black (n=24)	4 (16.6%)	18 (75%)	2 (8.4%)	
Indian (n=218)	20 (9.2%)	139 (63.8%)	59 (27%)	0.001
White (n=758)	233 (30.7%)	504 (66.5%)	21 (2.8%)	
Age				
Youth (n=27)	9 (33.3%)	17 (63%)	1 (3.7%)	
Young Adult (n=97)	26 (26.8%)	58 (60%)	13 (13.2%)	0.149
Adult (n=832)	208 (25%)	562 (67.5%)	62 (7.5%)	
Elderly (n=44)	14 (31.8%)	24 (54.6%)	6 (13.6%)	
Suburb				
North (n=400)	112 (28%)	265 (66.3%)	23 (5.7%)	
South (n=202)	45 (22.2%)	128 (63.4%)	29 (14.4%)	
East (n=138)	36 (26.1%)	93 (67.4%)	9 (6.5%)	0.034
West (n=88)	25 (28.3%)	58 (66%)	5 (5.7%)	
Pretoria (n=172)	39 (22.7%)	117 (68%)	16 (9.3%)	
Hypertension				
(n=147)	31 (21.1%)	102 (69.4%)	14 (9.5%)	0.354
Diabetes Mellitus				
(n=68)	14 (20.6%)	45 (66.2%)	9 (13.2%)	0.219
HOMA				
>2 (n=193)	32 (16.6%)	139 (72%)	22 (11.4%)	0.003
<2 (n=341)	98 (28.7%)	220 (64.5%)	23 (6.8%)	
CHOL				
>5 (n=434)	88 (20.3%)	306 (70.5%)	40 (9.2%)	0.001
<5 (n=384)	123 (32%)	231 (60.2%)	30 (7.8%)	
GLUCOSE				
>7 (n=26)	3 (11.5%)	20 (77%)	3 (11.5%)	0.265
<7 (n=832)	211 (25.4%)	551 (66.2%)	70 (8.4%)	
SEASON				
Summer (n=227)	82 (36%)	136 (60%)	9 (4%)	
Autumn (n=256)	56 (21.8%)	184 (71.8%)	16 (6.4%)	0.005
Winter (n=373)	68 (18.2%)	258 (69.2%)	47 (12.6%)	
Spring (n=144)	51 (35.4%)	83 (57.6%)	10 (7%)	

There was no statistically significant difference in mean $25(OH)D_3$ levels between gender and age groups ($p = 0.597$ and $p = 0.745$ respectively). Similarly, there was no significant difference between mean $25(OH)D_3$ levels between patients who have diabetes mellitus ($p = 0.087$) and between different suburbs of residence ($p = 0.069$). There was, however, statistically significant differences between the mean $25(OH)D_3$ levels between different race groups ($p = 0.001$), those who had hypercholesterolaemia ($p = 0.001$), and those who had a HOMA-IR index >2 ($p = 0.001$). As BMI may be strongly associated with T2DM, insulin resistance (HOMA-IR index) and hypercholesterolaemia, and BMI was not one of the variables measured, the negative correlation between vitamin D status and hypercholesterolaemia may become insignificant should BMI be corrected for. It is worth noting that although there was a significant difference between mean $25(OH)D_3$ between those patients who had hypertension and those who did not ($p = 0.007$), the absolute mean $25(OH)D_3$ level differed only marginally: 22.03 ng/ml in those with hypertension, and 24.87ng/ml in those without.

Although mean serum $25(OH)D_3$ levels in diabetic and non-diabetic patients was different, this was insignificant ($p = 0.087$) in this study. However, there was a significant difference between fasting glucose levels and mean $25(OH)D_3$ levels, with those patients with fasting glucose >7mmol/l having lower $25(OH)D_3$ means ($p = 0.016$). There was no significant correlation between fasting glucose and $25(OH)D_3$ level (correlation co-efficient -0.467, $p = 0.432$). Moreover, with insulin resistance, there were similar findings, those patients with high HOMA-IR indices (>2) had lower serum $25(OH)D_3$ levels ($p = 0.001$).

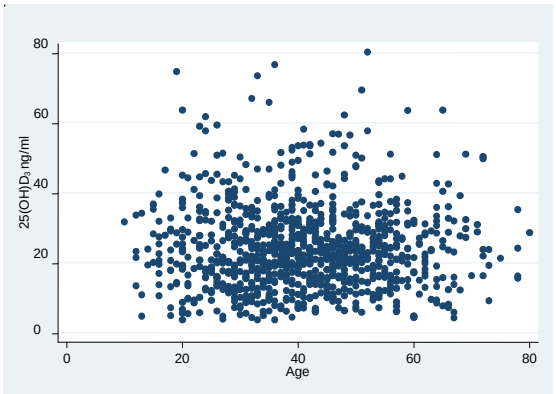
Mean $25(OH)D_3$ levels taken during different seasons was also statistically significant ($p = 0.001$), with the highest mean $25(OH)D_3$ being measured during the summer months, and the lowest during the winter months (Table 3.5).

Table 3.5 Mean Vitamin D levels across sub-groups

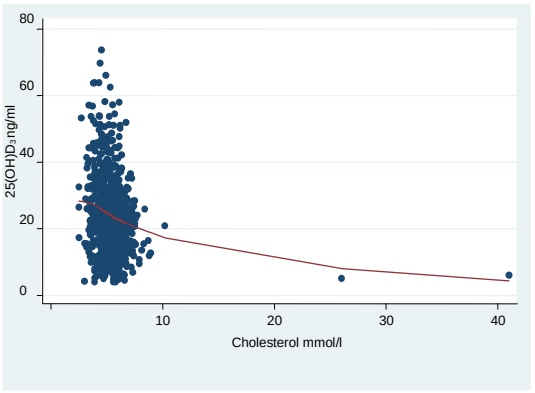
Variable	$25(OH)D_3$ Level (SD) ng/ml	P value
MEAN 25(OH)D	24.45 (11.81)	
GENDER		
Male (n=286)	24.76 (11.83)	0.597
Female (n=714)	24.32 (11.80)	
RACE		
Black (n=24)	20.78 (8.45)	
Indian (n=218)	16.40 (11.42)	0.001– Indian vs. White
White (n=758)	26.88 (10.94)	
AGE		
Youth (n=27)	25.30 (10.00)	
Young Adult (n=97)	25.29 (13.89)	
Adult (n=832)	24.27 (11.52)	0.745
Elderly (n=44)	25.58 (13.3)	
SUBURB		
East (n=400)	24.50 (11.21)	
North (n=202)	25.25 (11.97)	
Pretoria (n=138)	24.39 (12.39)	0.069
South (n=88)	22.39 (12.16)	
West (n=172)	25.54 (9.37)	
HYPERTENSION		
Yes (n=147)	22.03(10.4)	
No (n=853)	24.87 (11.90)	0.007
DIABETES MELLITUS		
Yes (n=68)	22.08 (13.89)	
No (n=932)	24.62 (11.63)	0.087
HOMA		
<2 (n=193)	25.89 (11.88)	
>2 (n=341)	20.89 (10.42)	0.001
CHOLESTEROL		
<5mmol/l (n=384)	26.08 (12.14)	
>5mmol/l (n=434)	22.49 (10.34)	0.001
GLUCOSE		
<7mmol/l (n=832)	24.34 (11.90)	
>7mmol/l (n=26)	18.67 (7.90)	0.016
SEASON		
Summer (n=189)	27.23 (11.87)	
Autumn (n=155)	24.24(10.84)	0.001- Winter vs.
Winter (n=476)	21.98 (11.18)	Summer
Spring (n=150)	26.81 (13.53)	

The possibility of a correlation between 25(OH)D_3 and variables such as age, cholesterol level, glucose level and HOMA level was investigated.

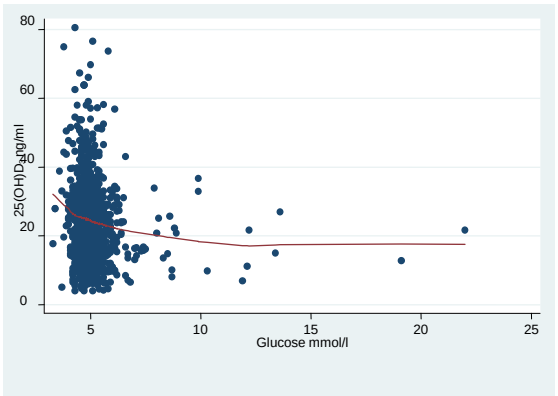
With increasing age, there was no significant change in 25(OH)D_3, no correlation was found (r = 0.0017, p = 0.958). There was a negative correlation between 25(OH)D_3 and cholesterol levels (r = -0.187, p = 0.001). Similarly, there was a negative correlation between 25(OH)D_3 levels and the glucose level (r = -0.118, p = 0.001). There was also a slight negative correlation between 25(OH)D_3 levels and the HOMA-IR level (r = -0.096, p = 0.026) (Figure 3.1).



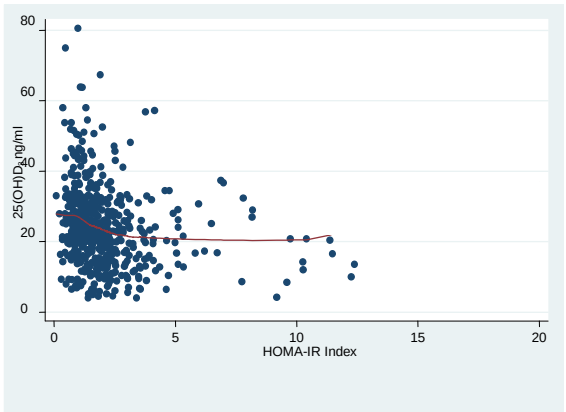
Vitamin D Correlation with Age



Vitamin D Correlation with Cholesterol



Vitamin D Correlation with Glucose



Vitamin D Correlation with HOMA

Figure 3.1 Vitamin D Correlation with Variables

Using regression testing, the estimated change in variable levels was investigated (Tables 3.6 and 3.7). When measured using univariate regression testing, there were statistically significant changes in cholesterol levels, glucose levels and the HOMA-IR indices. In all cases, the higher the $25(OH)D_3$ level was, the more optimal these levels were.

However, when investigated using multivariate regression testing, these differences were, in most cases, statistically insignificant.

The effect of changes in $25(OH)D_3$ levels on the subjects' clinical characteristics revealed that only cholesterol showed a statistically significant inverse relationship (coefficient -0.028, $p = 0.001$). As the $25(OH)D_3$ level increases, so the cholesterol levels decrease, and become more optimal. In addition to this, backward stepwise regression models were used. This did not demonstrate any other significant relationships.

The effects of changes in the subjects' clinical characteristics on the $25(OH)D_3$ level was also examined. Again, there was a statistically significant negative correlation between the cholesterol level and the $25(OH)D_3$ level (coefficient -1.022, $p = 0.001$). An inverse relationship was also noted between different race groups and the $25(OH)D_3$ (coefficient -8.357, $p = 0.001$). Again, although backward stepwise regression models were performed, no further relationships were evident.

Table 3.6 Regression analysis of the effect of the subjects' clinical characteristics on the $25(OH)D_3$ level

	Univariate		Multivariate	
	Coefficient	**P value**	**Coefficient**	**P value**
Cholesterol	-0.052	0.001	-1.022	0.001
Glucose	-0.041	0.001	-0.467	0.432
HOMA-IR	-0.025	0.035	-0.309	0.282
Age	-0.009	0.791	+0.029	0.492
Race	-0.335	0.001	-8.357	0.001
Gender	-0.034	0.382	-1.302	0.234
Hypertension	-0.070	0.161	-0.838	0.588
Diabetes	-0.109	0.119	+3.329	0.211

Table 3.7 Regression analysis of the effect of the $25(OH)D_3$ level on the subjects' clinical characteristics

	Univariate		Multivariate	
	Coefficient	**P value**	**Coefficient**	**P value**
Cholesterol	-0.522	0.001	-0.028	0.001
Glucose	-0.192	0.001	-0.004	0.256
HOMA-IR	-0.338	0.035	-0.011	0.121
Age	-0.007	0.791	+0.086	0.077
Hypertension	-0.028	0.161	-0.001	0.584
Diabetes	-0.022	0.119	+0.001	0.615

4. Chapter 4. Discussion

In this study, a strikingly high incidence of vitamin D deficiency among patients attending a practice in the northern suburbs of Johannesburg was observed. This is consistent with worldwide reports of high levels of hypovitaminosis D. To my knowledge, this is the first study to assess vitamin D status in a random cohort in South Africa (not related to specific or bone related diseases), as well as with respect to related health correlates and patient characteristics.

In this study, there was no overall difference between mean vitamin D levels between males and females or different age groups. In addition, there was also no correlation between gender or age and vitamin D levels. This differs from other studies where females had lower levels, and higher incidence of vitamin D deficiency, compared to males (44, 49, 54, 78). This has been reported as being particularly common in postmenopausal women, as many studies indicate a higher level of hypovitaminosis D amongst the elderly (44, 49, 54). The difference in vitamin D status between age groups was not demonstrated in this study. Possibilities for this may be: firstly, as the practice from which this study was performed is in an affluent region of the city, the elderly subjects were more likely to be retired, and probably able to spend more time in outdoor pursuits such as gardening, golf, walking etc. Secondly, a less likely possibility is that some of these patients may have already been on vitamin D supplementation, in the form of over the counter vitamins, or prescriptions from previous doctors, which they did not disclose to the physician at the time of consultation. However, when considering these results one should also take into consideration the fact that there were very small numbers in especially the youth and elderly age group. Greater numbers in these age groups may have demonstrated a difference.

There was a significant difference between vitamin D means measured between different race groups. The lowest levels were recorded amongst those population groups with darker skins, ie. Black and Indian. Again, this is consistent with other studies (49, 53, 54). Cultural practices with respect to clothing are another factor related to the low vitamin D levels, demonstrated particularly in the Indian group. Many Muslims wear covering clothing such as a veil (hijab or niqab), and long sleeves and dresses, covering almost the entire body. This practice greatly diminishes the skin's synthesizing ability (77). This study shows similar differences between vitamin D levels between race groups as do other worldwide reports.

A significant difference between mean $25(OH)D_3$ levels due to seasonal variance was observed. The highest means were recorded during the summer months, and the lowest during the winter months. This is expected as the highest sun exposure (UVB index) and outdoor activity rates are during the longer daylight hours experienced in the summer months. This correlation is noted in most vitamin D status studies (47, 49, 51, 78)(47, 49, 51, 78).

This study demonstrated a significant difference between vitamin D levels between specifically the northern and southern suburbs of Johannesburg. The lowest levels were recorded in the south, and the highest in the north. As the municipality of Lenasia was included in the southern suburbs, and given that the majority of the subjects' data collected from this area were Indian, this difference in vitamin D levels was presumed to be on the basis of the cultural and ethnic differences – greater skin melanin and pigmentation, protective and covering clothing for religious and cultural reasons, rather than on an area/ regional basis. Johannesburg covers an area of $1645km^2$ (Wikipedia), and as such the degree of UVB exposure difference is considered negligible.

There was a significant difference between mean vitamin D levels between subjects who had underlying hypertension, and those who did not. Patients with hypertension had lower mean $25(OH)D_3$ levels.

Although this study showed that the mean $25(OH)D_3$ level was significantly lower in those subjects with hypertension compared to those without, it did not demonstrate the negative correlation suggested by the previously mentioned studies and meta-analysis.

No significant correlation was found in this study between vitamin D levels between subjects who had diabetes mellitus and subjects who did not. In addition, no significant difference was found between mean vitamin D status between subjects with well controlled glucose levels and those with high glucose levels. Since the numbers of subjects in these groups were small, perhaps a significant correlation may have been found if the numbers were larger.

Of note in this study, a significant correlation was found between the $25(OH)D_3$ level and the cholesterol level, as well as a significant difference in mean $25(OH)D_3$ between subjects with hypercholesterolaemia and those without. The reason for this is unclear and should be further investigated.

4.1. Study Limitations

As this was a retrospective study, some variables which may have had an influence on the bioavailability of vitamin D were not available to be captured. These include factors such as:

1. The body mass index (BMI) of each individual.

2. Presence of NASH.

3. The renal function – specifically a decline in renal function.

3. The presence of a cytochrome P450 inducing drug, such as an anti-epileptic, anti-retroviral, or rifampicin. However, the contribution from these drugs was presumed to be small as less than 5 patients had epilepsy, only 2 were HIV positive, and none were known to have active tuberculosis, or to be on rifampicin.

A further limitation to this study was that the numbers of young (n = 27) and elderly (n = 47) patients was relatively small. The demographic profile of South Africa was not representative in this study, as Black patients made up only 2.4% (n = 24) of the cohort.

In terms of co-morbid diseases, there was a small number of patients with hypertension (n = 147) and diabetes mellitus (n = 68). In addition, no distinction between T1DM and T2DM was made, as the number of patients with this disease was very small. Although no correlation was found in this study, a correlation may exist should the numbers have been bigger.

In addition, only patients with a pre-existing history of diabetes mellitus or hypertension were captured. Because of this, there may have been a number of patients with undiagnosed disease in this cohort. Furthermore, the vitamin D status was not correlated to the absolute blood pressure levels.

5. Chapter 5. Conclusion

This study demonstrates that a significant majority of subjects were vitamin D deficient. The demographics of this vitamin D deficient population are in keeping with similar studies around the world. The major variable which was out of keeping with worldwide reports was that in this study, the elderly did not have significantly lower serum $25(OH)D_3$ compared to other age groups. Furthermore, there was no difference noted between serum vitamin D levels between males and females.

Risk factors for vitamin D deficiency, as from the results of this study in the Johannesburg region were:

Indian ethnicity

Residence in the southern suburbs (Lenasia)

A fasting cholesterol >5mmol/l

A fasting glucose >7mmol/l

A HOMA-IR index >2

$25(OH)D_3$ level taken in the winter months.

This group is a representative sample of the White and Indian populations living in Johannesburg and gives a reasonable indication of the state of vitamin D levels in these groups in Johannesburg as a whole, and may be stratified to predict the status of vitamin D in the rest of the people living in Johannesburg. One may expect similar levels of deficiency despite this city's high UVB index.

Given the high incidence of hypovitaminosis D in this population, it would be interesting to follow these subjects up over their lifetimes to document the $25(OH)D_3$ trend over many

years as well as the incidence of especially bone disease, but also possible extra-skeletal manifestations such as diabetes mellitus, hypertension and malignancy.

5.1. The Way Forward

Given that hypovitaminosis D is re-emerging as a global pandemic, and with the evidence that this is also a concern in South Africa, the future requires the focus to be placed on the following areas:

1. For the public:
    a. Awareness of the existence and extent of the problem
    b. Education on the complications and consequences of vitamin D deficiency
    c. Education on how to prevent deficiency
    d. South African guidelines as to who should be tested for deficiency
2. For the Practitioner:
    a. South African guidelines on who to screen for deficiency
    b. Training on interpretation of results
    c. South African guidelines as to vitamin D supplementation: dose and when to prescribe
3. For the laboratory / Endocrine advisory society:
    a. Emphasis on the importance of assay standardisation
    b. Emphasis on the need for standardised reference values

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Appendix A: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Kim Roberg

CLEARANCE CERTIFICATE	**M120957**
PROJECT	A Survey of Vitamin D Status in a Northern Suburbs Practice in Johannesburg, Gauteng (revised title)
INVESTIGATORS	Dr Kim Roberg.
DEPARTMENT	Department of Internal Medicine
DATE CONSIDERED	28/09/2012
DECISION OF THE COMMITTEE*	Approved uncondtionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07/06/2013 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Sindeep Bhana

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...