

Vitamin D levels of anaesthetists in the Department of Anaesthesiology at the University of the Witwatersrand

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Declaration

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

..... (Signature)

..... day of, 20.....

Abstract

Background and Objective

There has been a recent resurgence of interest in vitamin D and its far-reaching effects in physiology and pathophysiology. Theatre personnel, and all indoor workers, should be cognisant of vitamin D deficiency as a real occupational hazard. Vitamin D deficiency is a global problem that has been studied extensively in colder climates and even been found in warmer climates. No research was identified among medical personnel in South Africa.

The primary objective of this study was to describe serum 25-hydroxyvitamin D (25(OH)D) levels of anaesthetists.

The secondary objective was to describe and compare factors influencing vitamin D levels in anaesthetists who are vitamin D insufficient to those who are not. These factors included: ethnicity, gender, body mass index (BMI), multivitamin use, calcium or vitamin D supplementation, sun exposure, vitamin D intake from diet alone, vitamin D intake from diet and supplementation and calcium intake (dairy).

Methods

Data was collected over a period of one month, in winter (mid-July to mid-August 2013).

On the morning of sample collection anaesthetists agreeing to participate signed the informed consent (Appendix 2), prior to enrolment in the study. The anaesthetists then completed the questionnaire (Appendix 5). The following data was obtained from the questionnaire: age, gender, ethnic group, dietary supplementation, sun exposure, sunscreen use, BMI and diet.

Each participant had 5 ml of blood collected in a standardised manner into a purple top ethylenediaminetetraacetic acid blood specimen tube. The processing of samples was done by qualified laboratory personnel using standard chemical pathology equipment and procedures. High Performance Liquid Chromatography

was performed to determine 25(OH)D levels using a Shimadzu® Nexera X2 Ultra performance liquid chromatography system with a photodiode array detector (Shimadzu®, Japan).

Results

The median 25(OH)D was 43.8 nmol/l (IQR 26-76), with 51 of 89 (57.30 %) anaesthetists being vitamin D insufficient.

There was a statistically significant association between ethnicity and vitamin D status ($p < 0.001$). Twenty-one (80.77 %) Indian anaesthetists and 14 (70.00 %) black anaesthetists were vitamin D insufficient, as compared to only 10 (28.57%) white anaesthetists.

There was no significant association between the other secondary objectives-gender ($p = 0.60$), sun exposure ($p = 0.93$), vitamin D intake from diet alone ($p = 0.07$), vitamin D intake from diet and supplementation ($p = 0.05$) and calcium intake ($p = 0.55$) and vitamin D status.

There was no significant difference between BMI and vitamin D status. When a comparison was made between the two groups of BMI < 25 and BMI ≥ 25 , using a Mann-Whitney test the two-tailed P value was 0.6791. There was a significant association between multivitamin use ($p = 0.01$) and vitamin D status.

Conclusion

Vitamin D should no longer be a forgotten vitamin. The insufficient vitamin D levels of anaesthetist in this study, puts them at risk for pathology far beyond bone health. Adequate vitamin D levels should be seen as essential, rather than optional, even in “sunny” climates.

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List of abbreviations

CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
HJH	Helen Joseph Hospital
HPLC	High Performance Liquid Chromatography
IOM	Institute of Medicine
NHANES	National Health And Nutrition Examination Survey
NHLS	National Health Laboratory Service
MED	Minimal erythematol dose
PTH	Parathyroid Hormone
RDA	Recommended Daily Allowance
RMMCH	Rahima Moosa Mother and Child Hospital
USA	United States of America
UVB	Ultraviolet B
UV	Ultraviolet
VDR	Vitamin D Receptor
Wits	University of the Witwatersrand
25(OH)D	Serum 25-hydroxyvitamin D

Chapter 1 Overview

1.1 Introduction

This chapter presents the background to the problem, problem statement, aim and objectives and research assumptions. Demarcation of the study field, ethical considerations, research methodology, significance of the study and validity and reliability are summarised. The study outline is also presented.

1.2 Background

There has been a recent resurgence of interest in vitamin D and its far-reaching effects in physiology and pathophysiology (1, 2). Theatre personnel, and all indoor workers, should be cognisant of vitamin D deficiency as a real occupational hazard.

Sun exposure is a major source of vitamin D in most people. The ultraviolet B (UVB) rays cause the conversion of 7-dehydrocholesterol in the skin to previtamin D₃, which eventually isomerizes to vitamin D₃. Sunscreens absorb UVB photons, reducing vitamin D synthesis by as much as 92-95% in the case of Factor 8 sunscreen (3). Melanin does the same, hence it significantly affects vitamin D levels (4).

Vitamin D produced in the skin has a half-life of at least twice that of orally ingested vitamin D (5). According to the Institute of Medicine (IOM) Guidelines the recommended daily allowance (RDA) of vitamin D for a 19 to 50 year old male or female is 600 IU. Sitting in the sun is a more convenient alternative to attain this RDA, as 5 to 10 minutes of sun exposure to the arms and legs produces about 3000 IU vitamin D, versus the average multivitamin tablet that contains only 400 IU. (1, 6) Dietary sources of vitamin D are limited, unless foods are specifically fortified (1).

Vitamin D deficiency may not only interfere with one's attainment of peak bone mass, but has numerous previously unrecognised effects which include immune modulation (7, 8), increased risk of certain cancers, autoimmune conditions, cardiovascular disease and mental illness (1, 9).

It is estimated that worldwide, there are one billion people who are vitamin D deficient (1, 10). In colder climates, people have been more aware of insufficient sun exposure leading to vitamin D deficiency. This is especially true amongst “at risk” populations, like the elderly in Europe, where mean vitamin D levels have been found to be in the deficient range (11). Doctors have only more recently been studied, and were found to have a high prevalence of deficiency. Amongst Boston, Massachusetts trainee doctors, 25% were deficient (12). In Portland, Oregon, the prevalence of deficiency was as high as 47% among resident doctors (13).

Studies in warmer climates found vitamin D deficiency to be even more common at times. Amongst a cohort of healthy Saudi Arabian men, 87% were found to be deficient of vitamin D (14). A study in Mexico, among resident doctors found a prevalence of deficiency of 75% (15).

This brings to light the multiple factors influencing vitamin D levels. These range from cultural norms to dietary supplementation and milk fortification. (16) Review of current literature revealed a paucity of data on vitamin D levels in adult South Africans. In addition, no research could be identified among medical personnel in South Africa.

1.3 Problem statement

Anaesthetists are indoors for most of their work hours and thus are at risk for vitamin D deficiency. Vitamin D is critical for calcium homeostasis and deficiency will hinder one’s attainment of peak bone mass (1). The other effects of vitamin D deficiency are less obvious, but still widespread in physiology, affecting immunity (7, 8), increasing risk of certain cancers, autoimmune conditions, cardiovascular disease and mental illness (1, 9).

Vitamin D deficiency is a global problem (1, 10, 17) that has been studied extensively in colder climates (9, 12, 13) and even been found in warmer climates (14, 15, 18). No research was identified among medical personnel in South Africa. In the Department of Anaesthesiology, University of the Witwatersrand (Wits), anaesthetists work on average 48 hours a week. All of these hours are spent indoors

and even though some are evening shifts, many daytime hours are spent doing preoperative visits, attending tutorials or studying. For these reasons the study population is at risk for vitamin D deficiency, despite South Africa commonly being thought of as a land of sunshine (19).

1.4 Aim and objectives

1.4.1 Aim

The aim of this study was to describe serum 25-hydroxyvitamin D [25(OH)D] levels during winter (mid-July to mid-August 2013), in anaesthetists working in the Department of Anaesthesiology, Wits.

1.4.2 Objectives

The primary objective of this study was to describe serum 25(OH)D of anaesthetists.

The secondary objective was to describe and compare factors influencing vitamin D levels in anaesthetists who are vitamin D insufficient to those who are not. These factors included:

- ethnicity
- gender
- body mass index (BMI)
- multivitamin use
- calcium or vitamin D supplementation
- sun exposure
- vitamin D intake from diet alone
- vitamin D intake from diet and supplementation
- calcium intake (dairy).

1.5 Research assumptions

The following definitions were used in this study.

Anaesthetist: in this study referred to a registrar or medical officer working in the Department of Anaesthesiology.

Medical Officer: a doctor employed by the provincial government in a medical officer position in the Department of Anaesthesiology.

Registrar: a doctor that is in the process of obtaining a specialist anaesthesiology qualification, endorsed by the Health Professions Council of South Africa.

Ethnic groups are defined below.

- **Arab-** person of Arabic descent.
- **Asian-** a non-Indian person of Asian descent.
- **Black-** black people of African descent.
- **White-** Caucasian or of European descent.
- **Indian-** Asian-Indians with ancestry from India.
- **Coloured-** a person of mixed origin who possesses ancestry from Europe, Asia and Black ethnic groups.

25(OH)D: serum 25-hydroxyvitamin D. This is the major circulating form of vitamin D and the form which is measured in the laboratory (6). In this study serum 25-hydroxyvitamin D will be referred to as 25(OH)D.

Calcitriol: physiologically active hormone- 1,25-dihydroxyvitamin D.

According to the IOM, vitamin D status in free living healthy adults, with regard to bone health is defined as:

- **vitamin D deficiency:** 25(OH)D < 30 nmol/l (<12 ng/ml) (6)
- **vitamin D inadequacy:** 25(OH)D of 30 to 50 nmol/l (12 to 20 ng/ml will be considered insufficient (6)
- **vitamin D sufficiency:** 25(OH)D > 50 nmol/l (>20 ng/ml) will be considered sufficient (6).

Most of the studies in doctors that were reviewed, combine the vitamin D deficient and inadequate categories into one (12, 13, 15, 20). For this study this group will be called "vitamin D insufficient".

The unit of measurement used by the IOM is ng/ml. The National Health Laboratory Service that was used for this study uses nmol/l as the unit of measurement. The conversion factor for ng/ml to nmol/l is 2.5 (2.496).

1.6 Demarcation of study field

This study was conducted in the Department of Anaesthesiology, Wits. The Wits Medical School is associated with: Chris Hani Baragwanath Academic Hospital (CHBAH), Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Helen Joseph Hospital (HJH) and Rahima Moosa Mother and Child Hospital (RMMCH).

1.7 Ethical considerations

Appropriate approvals from the relevant authorities were obtained.

The study was conducted adhering to strict ethical considerations.

The study was conducted according to the principles of the Declaration of Helsinki (21) and the South African Good Clinical Practice Guidelines and Good Laboratory Practice Guideline (22).

1.8 Research methodology

1.8.1 Research design

This study used a prospective, contextual, descriptive research design.

1.8.2 Study population

The study population was anaesthetists working in the Department of Anaesthesiology.

1.8.3 Study sample

In consultation with a biostatistician a sample size of 81 anaesthetists was calculated with nQuery Advisor® version 7. A convenience sampling method was used.

1.8.4 Inclusion and exclusion criteria

Specific inclusion and exclusion criteria for the study were defined.

Inclusion criteria for this study are listed below.

- Anaesthetists in the Department of Anaesthesiology at Wits.
- Consenting to take part in the study.

1.8.5 Data collection

Data was collected over a period of one month, in winter (mid-July to mid-August 2013).

The specimen taking, labelling, storage and transport and processing were done in a standardised manner.

1.8.6 Data analysis

Data was captured on Microsoft Office Excel 2010® spread sheets and analysed with the assistance of a biostatistician using Statistica® Version 12.5.

1.9 Significance of the study

Vitamin D deficiency is a global health problem, with an estimated 1 billion people deficient worldwide (1, 10). Deficiency has far-reaching effects in normal physiology and pathophysiology (1, 2) such as interference with one's attainment of peak bone mass, immune modulation (7), increased risk of certain cancers, autoimmune conditions, cardiovascular disease and mental illness (1).

If vitamin D deficiency and inadequacy were found to be prevalent amongst anaesthetists in the Department of Anaesthesiology, this may further highlight the magnitude of vitamin D insufficiency in sunny climates. Furthermore, actions could be taken to rectify the insufficiency, thereby contributing to the anaesthetists' well-being.

1.10 Validity and reliability of the study

Measures were taken to ensure the validity and reliability of the study.

1.11 Study outline

This study will be presented as follows:

- Chapter 1 – Overview of study
- Chapter 2 – Literature review
- Chapter 3 – Research methodology
- Chapter 4 – Results and discussion
- Chapter 5 – Limitations, recommendations and conclusion.

Summary

In this chapter the background to the problem, problem statement, aim and objectives and research assumptions as well as the demarcation of the study field, ethical considerations, research methodology, significance of the study and validity and reliability were summarised. The study outline was also presented.

The literature review is presented in the following chapter.

Chapter 2 Literature Review

2.1 Introduction

This literature review will address the physiology of vitamin D. It will then explore the epidemiology of vitamin D deficiency, the factors influencing vitamin D levels, the laboratory testing of vitamin D and then discuss the health consequences of vitamin D deficiency.

2.2 Vitamin D physiology

Vitamin D, also known as calciferol, is a fat-soluble vitamin that occurs in two major forms: vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). Vitamin D₂ is the main form manufactured for supplementation or to fortify food. Sun exposure supplies 50 to 90% of vitamin D in the body, through synthesis of vitamin D₃ in the skin (17). Vitamin D₃ can also be made commercially, although less commonly than vitamin D₂ (6).

The only difference between the two molecules is the structure of their side chains. This does not interfere with their effectiveness, as evidenced by the ability of both to treat vitamin D-deficient rickets. (23)

The following aspects of vitamin D will be briefly discussed: cutaneous vitamin D₃ synthesis, vitamin D metabolism and vitamin D's role in homeostasis.

2.2.1 Cutaneous vitamin D₃ synthesis

Through a process known as thermal isomerization, UVB rays from the sun result in conversion of 7-dehydrocholesterol in the skin to previtamin D₃. This molecule then isomerizes to vitamin D₃. Vitamin D production in the skin is dependent on the amount of UVB photons reaching the dermis, as well as the amount of 7-dehydrocholesterol in the skin. The absorption of UVB radiation is affected by season, latitude, clothing, sunscreen and skin melanin content. Age affects 7-dehydrocholesterol levels negatively. (1)

Ultraviolet (UV) radiation is quantified using the minimal erythema dose (MED) as a unit of comparison. One MED is defined as an adult wearing a bathing suit exposed to enough UV radiation to cause a slight pinkness of the skin 24 hours after exposure. This results in a vitamin D production equivalent to ingesting between 10,000 and 25,000 IU of vitamin D. (16) Exposure of arms and legs to sunlight between 10:00 and 15:00 for an average of 5 to 10 minutes, produces about 3000 IU vitamin D and is equivalent to 0.5 MED (1).

2.2.2 Vitamin D absorption

Vitamin D, being a fat-soluble vitamin, is absorbed with other fats from the diet in the small intestine (5). The absorption depends on the fat concentration in the intestinal lumen, which triggers bile acid and pancreatic lipase release. The bile acids emulsify lipids, while the pancreatic lipase hydrolyses triglycerides into monoglycerides and free fatty acids. The importance of bile acid and pancreatic lipase has been revealed by the significant reduction in vitamin D absorption in people with compromised bile release or pancreatic insufficiency. (6)

Vitamin D is bundled in the intestinal wall with other lipids, cholesterol, triglycerides and lipoproteins into chylomicrons. These chylomicrons reach the systemic circulation via the lymphatic circulation. (6)

2.2.3 Vitamin D activation

Dietary vitamin D₂ and D₃, as well as vitamin D₃ synthesized in the skin, all require activation via two enzymatic hydroxylation reactions. The first of these is catalysed by 25-hydroxylase in the liver to form 25-hydroxyvitamin D. (24) The second reaction takes place in the kidney, whereby 1 α -hydroxylase converts 25-hydroxyvitamin D to the physiologically active hormone 1,25-dihydroxyvitamin D (calcitriol). This reaction is accelerated by parathyroid hormone (PTH) and retarded by fibroblast-like growth factor-23. (6) The significance of the fact that this last reaction is able to take place in organs other than the kidney, which also contain the 1 α -hydroxylase enzyme, may lie in vitamin D's antiproliferative role in some cancers (1).

The major circulating form of vitamin D is 25(OH)D. In the blood it is bound to a specific carrier protein, vitamin D binding protein. (6, 25)

2.2.4 Vitamin D excretion

Metabolic destruction of vitamin D is catalysed by 24-hydroxylase. The by-products are excreted through bile into the faeces. Minimal amounts are excreted in the urine. (26)

The average lifetime of vitamin D in the body is about two months. 25(OH)D, remains in the body for about 15 days, whereas the active form, calcitriol has a lifetime of a few hours only (26). For this reason, laboratory measurement is usually of 25(OH)D.

2.2.5 Actions of vitamin D: role in calcium homeostasis

Calcium homeostasis is orchestrated predominantly by the vitamin D, PTH endocrine system. The main objective of this negative feedback system is to achieve optimal levels of ionized calcium in the blood. (24)

Calcium is the main electrolyte in bone, which contains more than 99% of total body calcium as calcium hydroxyapatite. Its effects are ubiquitous in human physiology as a second messenger. It also plays an integral role in extracellular fluid, muscle function, vasodilation and vasoconstriction, coagulation, as well as hormonal secretion. (24)

During periods of hypocalcaemia, calcium can rapidly be mobilised from bone. This begins with the calcium sensing receptor in the parathyroid gland detecting insufficient ionized calcium levels in the serum and triggering PTH release. The PTH then induces 1α -hydroxylase activity in the kidney to convert 25(OH)D to 1,25(OH)D. Calcitriol, in turn, provides negative feedback, decreasing further PTH release. (25)

Calcitriol stimulates intestinal calcium absorption, bone resorption and also stimulates the kidneys to reabsorb more calcium and phosphorous. (25)

There are two mechanisms of calcium absorption: active (transcellular) transport and passive (paracellular) diffusion across intestinal mucosa. (6) The former mechanism

is dependent on the interaction between calcitriol and the intestinal vitamin D receptor (VDR). During low and moderate calcium intake, absorption takes place via this mechanism. The VDR can be upregulated via genetic mechanisms during periods of deficiency. This receptor has the highest concentration in the duodenum. (27)

Passive diffusion is paracellular and as in other types of diffusion is dependent on a concentration gradient. As a result of this, diffusion takes place more commonly during periods of high calcium intake, which results in high intestinal calcium concentrations. Diffusion is also less selective than active transport and occurs throughout the small intestine. (28)

2.2.6 Non calcium effects of vitamin D

Vitamin D-responsive elements are an integral component of vitamin D's non-calcium effects. They are present in many human genes with effects ranging from regulating cell proliferation, differentiation and apoptosis to immunomodulatory effects (8). It is these effects that are thought possibly to play a role in cancer (29) and chronic conditions of infective, auto-immune and cardiovascular origin (1).

2.3 Vitamin D deficiency

2.3.1 Definition

There is controversy in the literature with regard to the level of 25(OH)D that is deemed deficient, inadequate and sufficient. The IOM definitions are for free living healthy adults. It is incorrect to use these definitions for populations that are at risk for vitamin D deficiency. Thus the definitions of the Endocrine Society would be more appropriate for these populations specifically.(16)

The definitions according to the IOM will be used for this study and were defined in Chapter 1 in the research assumptions.

2.3.2 Epidemiology

It is estimated that, according to the above definitions, 1 billion people worldwide have vitamin D deficiency or insufficiency, with very few regions excluded (1, 10).

When looking at the global prevalence of vitamin D deficiency or insufficiency, the numerous factors influencing its complexity come to light. The epidemiology and most important factors influencing vitamin D levels will be briefly discussed.

North America

Some of the most extensive epidemiological data regarding vitamin D status in the United States of America (USA) may be obtained from the National Health and Nutrition Examination Survey (NHANES) in the USA. This survey is conducted by the National Center for Health Statistics and the Centers for Disease Control and Prevention. Its aim is to assess the health and nutritional status, utilizing a representative sample of 20 289 non-institutionalised, civilian people. This survey takes place every four to six years and although it only pertains to the USA, it is very extensive, with sampling accounting for differences in latitude, seasonal variation and race. (30)

The NHANES 2000-2004 revealed the following: age standardised mean serum 25(OH)D was 65 nmol/l in non-hispanic whites (vitamin D sufficient) and 40 nmol/l (vitamin D insufficient) in non-hispanic blacks. Values were slightly lower in women than men. (30)

Looker et al. (30) identified three factors that negatively affected serum 25(OH)D. These were low milk intake, use of sun protection and BMI. The reason for this relationship is likely to be the fact that numerous foods in the USA are fortified with vitamin D. These include margarine, cereals, orange juice and milk. (9, 17)

The most recent analysis of the NHANES 2005-2006 data identified was by Forrest et al. (31). It showed an overall prevalence of vitamin D insufficiency of 41.6%, with the highest rate in black individuals (82.1%) and hispanics (69.2%).

Latin America

In the review by Lips et al. (17) on worldwide vitamin D nutrition, the following conclusion is drawn: "data on vitamin D status in Latin America are scarce and population studies are not available." A prior study by Lips et al. (32) amongst

osteoporotic postmenopausal women showed a mean serum 25(OH)D of 65 nmol/l in Mexico, 81 nmol/l in Brazil and 75 nmol/l in Chile. In comparison to similar populations in North America the vitamin D status is better. Using IOM definitions none of these is considered insufficient, however this is in a population at risk for vitamin D deficiency and thus the definitions of deficiency of the Endocrine Society would be more appropriate.(16)

Maeda et al. (33) studied vitamin D levels in healthy hospital workers in Brazil and also found reasonable vitamin D status. Resident doctors had mean 25(OH)D levels of 67 nmol/l (sufficient) and hospital workers and medical students had even higher levels (33).

Europe

Europe shows great diversity with regard to vitamin D levels. Some studies found severe deficiency (25(OH)D below 25 nmol/l) in 2 to 30% of adults. The prevalence increased to 75% or more in elderly, institutionalised people. (10) The European Action on Nutrition and Health-Survey was a multicentre study in 12 European countries. Overall, it showed that 36% of men and 47% of women had serum 25(OH)D levels of less than 30 nmol/l, with mean serum 25(OH)D levels of 20 to 30 nmol/l in Southern European centres and 40 to 50 nmol/l in Northern European centres. The reason for the difference between north and south was the high intake of fatty fish and cod liver oil in Northern Europe, despite lower UVB radiation at the higher latitude. (10, 11)

By IOM definitions the above European figures all fall into the vitamin D insufficient category. A compounding factor could be the advanced age of most of the study participants, with the average age in the European Action on Nutrition and Health-Survey trial being older than 70 years.

Asia

Mithal et al. (10) in their review, concluded that in South Asia there was widespread vitamin D deficiency, in both sexes and across all age groups. The factors influencing levels in Asia were hypothesised to be darker skin pigmentation, traditional clothing

and minimal outdoor activity. Air pollution as an additional factor amongst urban people was also elucidated (34).

Numerous studies across India have indicated a high prevalence of deficiency. A study based in Delhi, found the prevalence of vitamin D deficiency to be 90% in otherwise healthy school girls, across socioeconomic groups (35). Arya et al. (36) performed a study amongst healthy hospital staff members in Lucknow, India, and the prevalence of deficiency was 78%. Darker skin pigmentation and decreased sun exposure due to cultural attire were likely factors influencing the levels (36).

Middle East

The Middle East has been found to have rates of vitamin D deficiency as high as 87%, despite the sunny climate (14). In the study by Ardawi et al. (14), healthy Saudi Arabian men across all age groups were found to be deficient. Severe vitamin D deficiency (< 24.96 nmol/l) was most commonly seen in “at risk” populations like the elderly (10).

Contributing factors are decreased sun exposure due to cultural clothing, prolonged periods of breastfeeding, darker skin pigmentation and calcium deficiency (37). Amongst Turkish women, cultural clothing’s role was clearly delineated. Turkish women who wore a veil had a mean serum 25(OH)D of 32 nmol/l, while those who were completely covered had a mean of only 9 nmol/l. Turkish women who wore western clothing had a mean serum 25(OH)D of 56 nmol/l. (17)

Oceania

Literature from Australia and New Zealand has recently earmarked vitamin D deficiency as a significant healthcare problem. Seasonal variation as a compounding factor is an underlying theme, as well as geographical latitude, advanced age and genetic influence. (38, 39) Genetic influence has been noticed in many immigrant and indigenous people. This may be as a result of increased skin pigmentation or cultural avoidance of sun exposure, as opposed to actual genetic influence on vitamin D levels. (10) Sunscreen use would be expected to decrease vitamin D levels, however, when this was studied in Australia, no effect was seen across all age groups (40).

Van der Mei et al. (39) compared data from cross-sectional studies of three regions across Australia: southeast Queensland (27°S), Geelong region (38°S), and Tasmania (43°S). They found that the prevalence of vitamin D deficiency was 40.5% in southeast Queensland, 37.4% in the Geelong region, and 67.3% in Tasmania. They concluded that vitamin D insufficiency is prevalent over a wide geographical latitude range. They also found season to play more of a role than latitude. They emphasised the importance of behavioural factors, as season and latitude did not sufficiently account for the variation in serum 25(OH)D levels. (39)

Africa

In the review by Prentice et al. (41) it was concluded that studies in Africa have an important geographical gradient with regard to 25(OH)D levels. These range from deficiency in North African countries to high values in equatorial countries. Seasonal variation in sun exposure is only of significance outside the tropics (30°N and 30°S). Thus in countries like Morocco, Tunisia, Algeria, Libya, Egypt and South Africa, dietary sources of vitamin D are essential to prevent deficiency during winter. (41)

The central themes with regard to the factors influencing vitamin D levels in Africa are the following.

- 40% of Africa follow the Islamic faith, thus religious attire influences sun exposure (41).
- Low calcium intake (42) and the burden of infectious disease increase vitamin D utilization and turnover (41).
- Rates of malnutrition in Africa are the highest in the world (43) and this is commonly associated with vitamin D and calcium deficiency .
- In Africa dietary vitamin D intake is low and absorption is further hindered by the high prevalence of tropical enteropathy (41, 44).

South Africa

Most of the studies in South Africa have been done in children and women. Serum 25(OH)D levels ranged from deficient, in a few children studied with active rickets, to sufficient levels in the rest. Possible factors contributing to deficiency include skin pigmentation and low dietary calcium. (45-47)

In the study by Daniels et al. (48) in South African women, premenopausal black women had a mean serum 25(OH)D of 48.3 nmol/l; premenopausal white women 65.8 nmol/l; postmenopausal black women 47.5 nmol/l and postmenopausal white 64.5 nmol/l. The black women in this study were vitamin D insufficient and the white women sufficient. There was no difference in levels between pre and postmenopausal women. The reasons were not elucidated (48)

In a study by Pettifor et al. (49), among 232 elderly black women with femur neck fractures, mean serum 25(OH)D was found to be 44.3 nmol/l, with a significant seasonal variation. Van Papendorp (50) also showed that elderly nursing home residents in South Africa had a mean serum 25(OH)D of 32 nmol/l, thus being insufficient.

Using IOM definitions here would also lead to inaccuracies as these are for free living healthy adults. Most of these populations studied are at risk for vitamin D deficiency and thus the definitions of deficiency of the Endocrine Society would also be more appropriate.(16)

A recent study by George et al. (51) in healthy urban African and Asian-Indian adults revealed a prevalence of vitamin D deficiency of 28.6% in Asian-Indian subjects as opposed to just over 5% in African individuals.

Doctors

Studies among doctors are summarised in Table 2.1. The study sample and mean 25(OH)D in nmol/l and ng/ml are given. The percentage of the population that was insufficient, geographical location, season and study population are illustrated. The U.V index during the period in which the studies were conducted is also included. The U.V index will be discussed as a factor influencing sun exposure in the following section.

Study	Sample size	Mean 25(OH)D nmol/l	Mean 25(OH)D ng/ml	Vitamin D Insufficient (%)	Location	Season

Growdon (12)	102	67±26	26.84±10.41	25	Boston, USA	Winter
Tangpricha (20)	165	74.88±24.96	30±10	30	Boston, USA	End winter
Tangpricha (20)	142	87.36±24.96	35±10	11	Boston, USA	End summer
Haney (13)	35	50.92±18.97	20.4±7.6	47	Portland Oregon, USA	Spring (End winter)
Haney (13)	35	61.65±19.97	24.7±8.0	26	Portland Oregon, USA	Fall (End summer)
Mendoza (15)	20	42.18±12.73	16.9±5.1	75	Mexico City, Mexico	Summer

Table 2.1 Summary of studies amongst doctors

2.4 Factors affecting 25(OH)D levels

Studying the factors responsible for fluctuating vitamin D levels is essential to understand and combat the problem. Many of the factors have been named in the section on epidemiology, but further discussion will follow below.

The factors influencing vitamin D supply can be separated into three broad categories:

- dietary intake: diet, food fortification, supplementation
- sun exposure: geographical location, time of exposure, season, clothing, skin pigmentation and sunscreen use
- inadequate cutaneous synthesis, absorption and metabolism: age, BMI, fat malabsorption syndromes and medication use.

Finally, increased vitamin D turnover and utilization may also decrease vitamin D levels. This may result from inadequate calcium intake (42) or happen as a result of chronic disease (41).

2.4.1 Dietary intake

The two main sources of vitamin D are food and dietary supplements. Very few foods are naturally high in vitamin D. Foods that contain ample vitamin D are egg yolk, fish liver oil and oily fish (eg: sardines, herring, anchovies, salmon, trout and mackerel). Vitamin D fortification of certain foods also takes place, for example in the USA milk is fortified with 385 IU per litre. (9) Some cereals, orange juice, yoghurts and margarine are also fortified in the USA. In most European countries milk is not fortified, however, margarine and some cereals are. (9)

In South Africa very few foods are fortified with vitamin D, limited almost only to certain margarines. These are fortified with between 22.5 and 50 IU of vitamin D per 5 g serving. The reason for this limited fortification is most probably due to the fact that 25(OH)D levels of most South Africans are assumed to be adequate. An average South African diet, much like the rest of Africa, has minimal vitamin D but due to ample sunshine vitamin D deficiency is relatively uncommon. (41, 54)

The IOM (6) and The Endocrine Society (16) conclude that where sun exposure is inadequate, dietary supplements are the only way to ensure adequate vitamin D levels. Quantities needed for repletion of stores are higher than the usual RDA (6). Most people however, have unpredictable dietary intake of vitamin D rich foods (39). Inadequate calcium intake has also been found to decrease serum 25(OH)D levels, hence calcium supplementation may alleviate the problem even further. (37, 47)

As with all medication, supplementation is limited by the compliance of the patient. Elderly patients, who are most at risk for deficiency, are often the least compliant, likely due to the number of medications already being taken (55).

According to The Endocrine Society Clinical Guideline and the IOM, the RDA of vitamin D for a 19 to 50 year old male or female is 600 IU (15 µg). However, for patients at risk for vitamin D deficiency 1500-2000 IU is recommended as a daily requirement. (6, 16) If an average multivitamin contains 400-1000 IU and 100 g of salmon contains 100 -250 IU (1), it is clear that anyone at risk for deficiency needs to specifically quantify their vitamin D intake and ensure that it is adequate. It is also clear why sun exposure is such an attractive alternative, as 5 to 10 minutes of sun exposure to the arms and legs produces about 3000IU vitamin D with no risk of overdose (1, 6).

Overdosing vitamin D can result in toxicity (hypercalcaemia and hyperphosphataemia), however it is very rare as extremely high doses need to be ingested (50 000 IU per day). There is also a large margin of safety as 10 000 IU of vitamin D daily for five months was studied and found not to cause toxicity. (1) Excessive sun exposure cannot result in toxicity as a plateau in vitamin D levels is reached after about 15 minutes of exposure. Thereafter there is photo-degradation of vitamin D into inactive components, but not degradation of already synthesized vitamin D. (56)

A review by Cranney et al. (57) and a study by Aloia et al. (58) support the presumption that 25(OH) D levels increase with increased vitamin D intake. The relationship is however non-linear, possibly due to the number of factors that affect serum levels.

Calcium intake below the RDA for 19 to 50 year olds (1000 mg/day) (6) will result in increased 25(OH)D conversion to 1,25-hydroxyvitamin D, thus resulting in lower 25(OH)D levels. Insufficient calcium intake is also a known cause of rickets. (42)

2.4.2 Sun exposure

Factors affecting serum 25(OH)D levels include geographical location (latitude and altitude), season, clothing, time of exposure, sunscreen use and skin pigmentation.

Holick et al. (9), reported that at latitudes above 37°N and below 37°S, the UVB rays are insufficient to induce adequate vitamin D₃ production during winter months. The reason for this is the increased distance that UVB rays must travel through the ozone layer due to the oblique angle of the rays at high or low latitudes. This is known as the Zenith angle. (3) Inadequate vitamin D levels have been found to be present even when sun exposure is plentiful, as in Honolulu, Hawaii (latitude 21°N). Mean 25(OH)D levels in this study were not in the deficient range by current definitions, however they did border on insufficient (18).

An in vitro vitamin D formation study in Johannesburg (26°S) and Cape Town (35°S), revealed that in Cape Town very little vitamin D₃ production took place during winter. In contrast the vitamin D₃ formation in Johannesburg showed very little seasonal variation. These differences were presumed to be latitude related. (19) Other geographical factors increasing UVB radiation penetration are high altitude, minimal ozone or cloud cover and low pollution levels (3).

Seasonal variation in Australia's sun exposure has been reported to result in a 63% increase in vitamin D levels from winter to summer (39). The reasons for these variations extend beyond the amount of daylight hours. Season affects climate and this in turn influences behaviour, such as the amount of clothing, sunscreen use or time spent in the sun.

Cultural clothing practices range from religious attire leaving minimal skin exposed, to cultural practices whereby actual "sun-bathing" takes place (41). The time of day

also influences UVB radiation exposure, with maximal exposure between 10:00 and 15:00, but this too has considerable seasonal variation (3).

Sunscreens absorb UVB photons, reducing vitamin D synthesis by as much as 92 to 95% in the case of Factor eight sunscreen (3). Melanin does the same, hence increased skin pigmentation significantly decreases vitamin D levels (4).

The UV “index” was devised by the World Health Organization and World Meteorological Organization in 1994 as a standard indicator of UV levels. There are numerous factors influencing the UV Index. These are ozone, sun elevation, cloud cover, reflection by snow and pollution. (59) The UV Index is a linear scale. The higher the number, the greater the risk of radiation exposure due to UV rays. A UV index of zero corresponds to no UV radiation, for example, at night. A UV index above 10 is common in the tropics, mountainous altitudes, and regions with significant ozone layer depletion (60)

Skin cancers as a result of sun exposure

In a review by MacKie (61) it is stated that there is clear, strong evidence from epidemiological and animal studies that excessive exposure to UV radiation is a major cause of squamous cell carcinoma. Epidemiological studies also confirm that UV radiation is a major cause of basal cell carcinoma and three varieties of malignant melanoma. (61)

Thus, excessive sun exposure, particularly to the extent of burning in childhood, must be avoided. Sunscreens and protective clothing are essential during midday hours at most latitudes during summer. Mild sun exposure of 0.5 to 1 MED, three times a week, however, is still regarded as essential. (62)

2.4.3 Inadequate cutaneous synthesis, absorption and metabolism

As discussed under physiology, cutaneous vitamin D synthesis is dependent on 7-dehydrocholesterol in the skin. With increasing age there is a decrease in 7-dehydrocholesterol content in the skin, thus decreasing cutaneous vitamin D synthesis. (1, 3)

In a study of obese individuals it was found that increased BMI resulted in a decreased bioavailability of vitamin D from cutaneous synthesis. There was no difference in skin levels of 7-dehydrocholesterol, but instead inadequate release of vitamin D₃ from the skin into circulation. This resulted in a 57% decrease in serum 25(OH)D levels compared to non-obese individuals. Orally supplemented vitamin D did not show differences in bioavailability. (63)

As a result of vitamin D's fat solubility, fat malabsorptive syndromes, bile acid or pancreatic lipase deficiencies can all negatively influence its absorption and hence 25(OH)D levels. (9) There are also numerous chronic diseases known to interfere with vitamin D absorption (e.g. coeliac disease, irritable bowel syndrome and inflammatory bowel diseases).

With regard to medications, bile acid sequestrants (eg: cholestyramine), or enzyme inducers (anticonvulsants, rifampicin, protease inhibitors) may influence 25(OH)D levels, by retarding absorption or increasing metabolism. (9)

2.5 Laboratory testing of 25(OH)D

Laboratory measures of vitamin D consist of vitamin D₂ [25(OH)D₂] and vitamin D₃ [25(OH)D₃] Active vitamin D, calcitriol, can also be measured. In the USA, vitamin D assays measure the combination of 25(OH)D₂ and 25(OH)D₃ to obtain a total 25(OH)D. Traditionally, in Europe, only vitamin D₃ has been measured. (6)

Internationally several assay types are currently in use. In their review of vitamin D status in Africa, Prentice et al. (41) reported that 15 different assays were used across studies.

The two most common types of assays are:

- antibody-based methods
- liquid chromatography-based methods, which use automated equipment featuring either UV or mass spectrometric detection.

These methods are considered equivalent in terms of measuring a total 25(OH)D level in serum, but there is some controversy with regard to the performance of these

assays in clinical and research laboratories. When interpreting the literature, it is important to be aware of the numerous assays available, and that serum 25(OH)D levels should be interpreted with care. (6)

In this study High Performance Liquid Chromatography (HPLC), using UV-detection was used by the NHLS. Lensmeyer et al. (64) compared HPLC to the liquid chromatography–tandem mass spectrometry method, a competitive protein-binding assay and a radioimmune assay. They found the HPLC method to be reliable and robust which has an advantage over antibody based assays, since it is able to distinguish 25(OH)D₂ and 25(OH)D₃. (64)

2.6 Health consequences of vitamin D deficiency

2.6.1 Musculo-skeletal effects

Severe, chronic 25(OH)D deficiency in infants and children results in rickets. Rickets causes bone deformation. Interference with chondrocyte maturation results in inadequate mineralization of the epiphyses (due to inadequate calcium-phosphorous product). This is as a result of the secondary hyperparathyroidism which results from vitamin D deficiency. The hypocalcaemia also causes further renal loss of phosphate and hypophosphataemia. This results in widening of the epiphyses of long bones, resulting in classical bowing. It also causes the rachitic rosary of the ribs, because of the costochondral junction bulging. Milder deficiencies may not result in rickets, but may hinder adolescents from attaining their peak bone mass. (25, 65)

Adults who are vitamin D deficient, already have fusion of their growth plates. The secondary hyperparathyroidism that results from this deficiency increases osteoclastic activity in bones to mobilise calcium and phosphorous to the extracellular space. This results in porous bones with insufficient mineralisation, resulting in decreased bone mineral density and an increase in fragility-fracture risk. (9, 24) Osteomalacia and osteoporosis are both metabolic bone diseases in adults. The differentiation is normally a histological one, however, the presence of bone pain in osteomalacia has been found to be a common distinguishing feature (66). It has been suggested that when 25(OH)D levels are less than 10 ng/ml (25 nmol/l), osteomalacia is usually present (9).

Vitamin D's role in skeletal muscle function is explained by the presence of numerous calcitriol receptors within the muscle (65). Vitamin D deficiency is commonly associated with muscle weakness amongst children with rickets and adults with osteomalacia (67). A randomised control trial by Bischoff et al. (68) on treatment of the elderly with vitamin D and calcium versus calcium alone resulted in a 49% reduction in falls.

2.6.2 Non calcium-related health consequences

Breast, brain, colon, lung, skin, prostate and a multitude of other organs and tissues have been shown to express VDRs (69). In addition T and B lymphocytes and activated macrophages have also been shown to express VDR, revealing its role in immunity (70). It is difficult however, to supplement vitamin D for any condition other than osteoporosis, rickets or osteomalacia, as hypercalcaemia can result.

Cancer

Vitamin D's role in cancer was initially observed in people living at higher latitudes in the USA. (71) They were found to have increased risk for Hodgkin's lymphoma, in addition to colon, pancreas, ovarian and breast cancers in comparison to people living at lower latitudes (1, 9, 70). Latitude, however, is not always linearly-related to vitamin D levels (18). This is likely due to the great heterogeneity of factors influencing vitamin D levels.

Vitamin D has been found to exert a potent hormonal influence to regulate cell growth. Calcitriol induces differentiation into normally functioning cells and inhibits proliferation.(71) Much of the evidence for this, however, was obtained from in vitro studies. Despite this, evidence does exist to support the association between increased sun exposure and a lower incidence of many cancers (9). Holick (9), concedes that the level of evidence for this, is at times weak and is based on ecological cancer incidence studies. The IOM (6) corroborates this sentiment in their concluding statement on the association of vitamin D deficiency and cancers, where they state that "available evidence from randomised control trials and observational association studies for a relationship between vitamin D and/or calcium and the risk

for either incidence of or mortality from all cancers does not support the use of cancer mortality as an indicator for Dietary Reference Intake development.” (6)

Autoimmune diseases, osteoarthritis and diabetes

Vitamin D deficiency has been found to be associated with numerous autoimmune diseases (72). Amongst these are multiple sclerosis and inflammatory bowel disease. They occur because of an inappropriate immune-mediated attack against native tissue. Similar associations have been found with other autoimmune conditions like rheumatoid arthritis. Although non-immune related, osteoarthritis also has a similar association. As in the case of vitamin D's relation to increased cancer incidence, a higher incidence of type 1-diabetes, multiple sclerosis, and inflammatory bowel disease was initially discovered because of its association with people living at higher latitudes. (72, 73)

Pancreatic β cells possess VDRs and in response to calcitriol stimulation, increase insulin production (74). As discussed, vitamin D plays a role as an immune modulator and through this mechanism can possibly prevent autoimmune pancreatic β cell destruction and thus the development of type I diabetes (9). This was illustrated by a birth-cohort study by Hyppönen et al. (75), which included 10,366 Finnish children. Higher vitamin D supplementation (2000 IU daily) during the first year of life had a rate ratio of 0.22 (range, 0.05-0.89) of risk for type I diabetes compared to those who supplemented less vitamin D.

Cardiovascular diseases

The evidence for the association of cardiovascular diseases with vitamin D stems from data in patients with end-stage renal disease. Early in chronic kidney disease, secondary hyperparathyroidism ensues as a result of the decreased activation of 25(OH)D by the kidney. It is the elevated PTH that is the risk factor in the development of cardiovascular disease (76). Excess PTH increases blood pressure, cardiac contractility and also leads to cardiomyocyte hypertrophy and interstitial fibrosis of the heart (77). There are two hypotheses regarding the pathophysiology of vitamin D deficiency leading to hypertension. The first is that hypocalcaemia and elevated PTH could be contributing factors to the hypertension. The second is that

the vitamin D deficiency could result in an activation of the rennin-angiotensin system (76).

In addition to reduced insulin secretion, vitamin D deficiency can also result in insulin resistance and contribute to “Syndrome X”. (78). Krause et al. (79) exposed hypertensive patients to UVB radiation three times a week and showed that the increase in vitamin D levels to above 100 nmol/l was associated with a significant decrease in their blood pressures (both systolic and diastolic pressure were reduced by 6 mmHg). Outdoor exercise may very well be the panacea to “Syndrome X”, targeting numerous aspects of the pathology: BMI, vitamin D, blood pressure and insulin resistance. (79)

Mental illness

Vitamin D's association with mental illness goes beyond the logical link between sunshine and seasonal affective disorder (80). McGrath et al. (81) reported that there is robust epidemiological data describing the relative excess of schizophrenia births in winter and spring. This is thought to be an indicator of sun exposure during the ante/ perinatal period. (81) In the study by McGrath et al. (81), an association between long term trends in sunshine duration around the time of birth and schizophrenia birth rates was found. This was, however, only statistically significant for males, a fact that could be due to the male preponderance in schizophrenia (82). It also brings into question whether ecological studies of this nature, truly reflect an association.

Summary

This chapter addressed the physiology of vitamin D. It then explored the epidemiology of vitamin D deficiency, the factors influencing vitamin D levels, the laboratory testing of vitamin D and then discussed the health consequences of vitamin D deficiency.

In Chapter 3 methodology will be discussed.

Chapter 3 Research Methodology

3.1 Introduction

In this chapter the problem statement, aims and objectives are presented. Furthermore, the ethical considerations, detailed research methodology, validity and reliability are explained.

3.2 Problem statement

Anaesthetists are indoors for most of their working hours and thus are at risk for vitamin D deficiency. Vitamin D is critical for calcium homeostasis and deficiency will hinder one's attainment of peak bone mass (1). The other effects of vitamin D deficiency are less obvious, but still widespread in physiology, affecting immunity (7, 8), increasing risk of certain cancers, autoimmune conditions, cardiovascular disease and mental illness (1, 9).

Vitamin D deficiency is a global problem (1, 10, 17) that has been studied extensively in colder climates (9, 12, 13) and even been found in warmer climates (14, 15, 18). No research was identified among medical personnel in South Africa. In the Department of Anaesthesiology, University of the Witwatersrand (Wits), anaesthetists work on average 48 hours a week. All of these hours are spent indoors and even though some are evening shifts, many daytime hours are spent doing preoperative visits, attending tutorials or studying. For these reasons the study population is at risk for vitamin D deficiency, despite South Africa commonly being thought of as a land of sunshine (19).

3.3 Aim and objectives

Aim

The aim of this was study to describe serum 25-hydroxyvitamin D [25(OH)D] levels during winter (mid-July to mid- August 2013), in anaesthetists working in the Department of Anaesthesiology, Wits.

Objectives

The primary objective of this study was to describe serum 25(OH)D levels of anaesthetists.

The secondary objective was to describe and compare factors influencing vitamin D levels in anaesthetists who are vitamin D insufficient to those who are not. These factors included:

- ethnicity
- gender
- BMI
- multivitamin use
- calcium or vitamin D supplementation
- sun exposure
- vitamin D intake from diet alone
- vitamin D intake from diet and supplementation
- calcium intake (dairy).

Using the questionnaire in Appendix 4, sun exposure was quantified into adequate or inadequate for cutaneous vitamin D production. Anaesthetists were required to recall average sun exposure over the past month. This was then quantified in comparison to an MED into an adequate (more than 30 minutes a week) or inadequate (less than 30 minutes a week) category.

3.4 Ethical considerations

This study was approved by the Human Research Ethics Committee (Medical) (Appendix 9) and the Postgraduate Committee, Health Sciences of Wits (Appendix 10).

Approval for the study was obtained from the Chief Executive Officers of the following hospitals: CMJAH (Appendix 5), HJH (Appendix 7), RMMCH (Appendix 8) and the Medical Advisory Committee of CHBAH (Appendix 6).

Invited participants were given an information sheet (Appendix 1) and if they agreed to participate, written informed consent was obtained (Appendix 2). All the information in the survey was kept confidential. A separate 'Study number allocation sheet' (Appendix 3) contained study number, participants' names and contact details. This sheet was kept confidential and was only accessed by the researcher and his supervisors. After completion of the study, the researcher contacted participants with their 25(OH)D results, if they requested them on their questionnaire (Appendix 4). Provision was made for a dietician to be consulted by anaesthetists, if they so wished.

Data would be stored securely for six years following completion of the study.

The study was conducted in adherence with the principles of the Declaration of Helsinki (21) and the South African Good Clinical Practice Guidelines and Good Laboratory Practice Guideline (22).

3.5 Research methodology

3.5.1 Research design

Research design may be described as the blueprint for a study (83). The methods which the researcher uses to obtain subjects, interpret results, collect and analyse data are all determined by the research design (84).

This study used a prospective, contextual, descriptive research design.

Brink (84) defines a prospective study as one in which the researcher follows a selected population over time to determine outcomes (84). This study was prospective because the variables were measured at the time the study took place.

De Vos (85) describes context as a "small-scale world" which, amongst other things, can include clinics, wards or critical care units. This study was contextual because it was conducted in the Departments of Anaesthesiology at the following hospitals: CMJAH, CHBAH and HJH and RMMCH.

A descriptive study is one which is used when more information is required in a particular field, and involves identifying the characteristics of the observed phenomenon (86). This study was descriptive because it described anaesthetists' vitamin D levels.

3.5.2 Study population

The study population was anaesthetists working in the Department of Anaesthesiology at Wits.

3.5.3 Study sample

Sample size

In studies amongst medical personnel, the prevalence of vitamin D insufficiency is between 25 and 47%, with sample sizes ranging from 35 to 142 participants (12, 13, 20).

In consultation with a biostatistician it was calculated with nQuery Advisor[®] version 7 that a sample of at least 81 anaesthetists would estimate the expected proportion of insufficiency of 30% to an accuracy of within 10%. A slightly larger sample size to provide for laboratory error will be acceptable.

Sampling method

The sampling method is the process followed by the researcher to select a subset from the population to participate in a study (83, 84). Generally the population is too large to study in its entirety, thus the researcher works with samples (84).

Convenience sampling is referred to by Brink (84) as “accidental” or “availability sampling”. It entails the choosing of subjects who are readily available, as the objects for the study. This continues until the desired sample size is reached. (83, 84) The disadvantage of this sampling method is that data might not truly represent the population.

A convenience sampling method was used for this study as available anaesthetists were enrolled until the sample size was realised.

Inclusion and exclusion criteria

Inclusion criteria for this study were:

- anaesthetists working in the Department of Anaesthesiology.
- consenting to take part in the study.

Exclusion criteria for this study included:

- pregnancy
- known disorders of PTH
- renal disorders
- medication that interferes with vitamin D absorption or metabolism
(anticonvulsants, glucocorticoids, ketoconazole, certain anti-retrovirals)
- diseases known to interfere with vitamin D absorption (eg: coeliac disease, irritable bowel syndrome and inflammatory bowel diseases)
- travel to a sun-rich destination within the month prior to the study
- anaesthetists who joined the department in the month prior to specimen collection.

3.5.4 Data collection

Data was collected over a period of one month, in winter (mid-July to mid-August 2013).

The researcher e-mailed anaesthetists and gave a brief overview of the study and invited them to participate. The e-mail also included the information letter (Appendix 1), and dates that sampling would take place at the various hospitals. Posters were displayed in the tearooms of the departments, reminding the anaesthetists about the study. A reminder e-mail was also sent the night before the sampling day.

On the morning of sample collection, anaesthetists agreeing to participate signed the informed consent (Appendix 2), prior to enrolment in the study. The anaesthetists then completed the questionnaire (Appendix 4). The following data was obtained from the questionnaire:

- age
- gender
- ethnic group
- dietary supplementation
- sun exposure
- sunscreen use

- BMI
- diet

Furthermore, the anaesthetists were asked to indicate whether they would like to be informed of their results.

Specimen taking

Each participant had 5 ml of blood collected in a standardised manner into a purple top ethylenediaminetetraacetic acid blood specimen tube.

The standardised manner was as follows.

- The researcher washed hands and donned gloves.
- A tourniquet was applied to the upper arm.
- The blood drawing site was cleaned with a 70% isopropyl alcohol swab and allowed to dry.
- A 20 or 22 gauge Vacutainer® needle and Vacutainer® needle holder was used to withdraw 5 ml of blood into a purple top blood specimen tube.
- The blood specimen tube was then gently rotated for 30 seconds to ensure mixing of the blood with the ethylenediaminetetraacetic acid.

Specimen labelling

After filling the blood specimen tube with blood, a standard National Health Laboratory Service investigation request form was completed for every sample. Special attention was given not to use any participant information.

The information was recorded as follows.

- **Patient hospital number:** Study number was written as “VITD-01”. Where “VITD” was a designation for this study in particular. “01” referred to a unique number that was assigned to every study participant, running consecutively from “01”.
- **Surname:** Research
- **First name:** Dr. E. Kelly
- **Hospital:** CMJAH, CHBAH, HJH, RMMCH
- **Ward:** Theatre
- **Diagnosis/ Reason for request:** Research
- **Type of specimen:** Blood
- **Date taken:** dd-mm-yyyy

- **Time:** hh:mm
- **Taken by:** Dr. E. Kelly
- **Requesting health care worker:** Dr. E. Kelly
- **HPCSA/ SANC number:** MP0658847
- **Contact number:** 074 116 5133
- **E-mail address:** eugene.h.kelly@gmail.com
- **Investigation required:** 25-hydroxyvitamin D

The unique study number was written on the blood specimen tube as well as on the questionnaire form.

A separate 'Study number allocation sheet' (Appendix 3) contained study number allocation, participants' names and contact details. This sheet was kept separate from the raw data and would only be accessed by the researcher when the participants requested their results.

Specimen storage and transport

Specimens were delivered to the Chemical Pathology Laboratory, Department of Chemical Pathology at Wits as soon as it was possible, by the researcher.

If there was a delay in transporting the specimens to the laboratory and testing was not done within eight hours, the specimens were refrigerated in a portable cooler at 2 to 8 °C. Samples were centrifuged and separated immediately by laboratory personnel. Samples are stable for four days at 4 °C. If processing could not take place within this time, samples were centrifuged and frozen. A frozen aliquot of serum remains stable at -20 °C for up to four weeks.

Specimen processing

The processing of samples was done by qualified laboratory personnel, using standard chemical pathology equipment and procedures. HPLC was performed to determine 25(OH)D levels using a Shimadzu® Nexera X2 Ultra performance liquid chromatography system with a photodiode array detector (Shimadzu®, Japan). The samples were extracted and analysed as per the “25 OH Vitamin D Recipe® kit” (Recipe® Munich, Germany) method where the vitamin D₂ and D₃ fractions of 25(OH)D are separated and then combined to give total 25(OH)D. 50 ul of the extracted sample was injected onto the chromatographic system where the vitamin D fractions were detected at 264nm. A three point calibration curve was run with every sample and two controls (one low level and one high level) were run every ten samples.

The system was validated prior to running the sample with correlation coefficients $r^2=92\%$ for linearity. The limit of detection was 1.2 nmol/l for 25(OH)D₃ and 1.8 nmol/l for 25(OH)D₂. The limit of quantitation was 7 nmol/l for 25(OH)D₃ and 9 nmol/l for 25(OH)D₂. Intraday and interday precisions were reported as 7% and 9% for 25(OH)D₃ and 6.5% and 8% for 25(OH)D₂.

3.5.5 Data analysis

Data was captured on Microsoft Office Excel 2010® spread sheets. The data was analysed using Statistica® Version 12.5. Data was then analysed using descriptive and inferential statistics.

The proportion of vitamin D insufficient anaesthetists was determined. 25(OH)D levels were described, using frequency, median and interquartile range. To achieve secondary objectives, factors influencing vitamin D levels in anaesthetists were compared using Chi-squared tests.

3.6 Validity and reliability of the study

Validity is defined by Burns and Grove (83) as “a measure of the truth or accuracy of a claim.”

According to Bothma et al., reliability “represents the consistency of the measure achieved” (87).

Validity and reliability of this study were ensured by the following.

- A representative sample, as the study population and sample were close to the same size.
- Selection bias was minimized by approaching all anaesthetists in the department.
- The researcher collected, labelled, stored and transported all the samples in a standardised manner.
- The processing of samples was done by qualified laboratory personnel using standard chemical pathology equipment and procedures.

The Shimadzu® HPLC machine was calibrated according to manufacturer’s recommendations.

All data entry points in Microsoft Office Excel 2010® were checked for accuracy.

3.7 Summary

In this chapter the problem statement, aims and objectives were discussed. Furthermore, the ethical considerations, detailed research methodology, validity and reliability were explained. Chapter 4 will now discuss results.

Chapter 4 Results and discussion

4.1 Introduction

This chapter contains the results and discussion thereof. Results are presented as per the research objectives.

The primary objective of this study was to describe serum 25(OH)D of anaesthetists.

The secondary objective was to describe and compare factors influencing vitamin D levels in anaesthetists who are vitamin D insufficient to those who are not. These factors included:

- ethnicity
- gender
- BMI
- multivitamin use
- calcium or vitamin D supplementation
- sun exposure
- vitamin D intake from diet alone
- vitamin D intake from diet and supplementation
- calcium intake (dairy).

4.2 Results

During the one month data collection period (mid-July to mid-August 2013), 92 anaesthetists working in the Department of Anaesthesiology at Wits had blood samples taken and tested by HPLC to determine 25(OH)D levels.

Of the 92 anaesthetists that were tested, 3 were excluded owing to laboratory error resulting in a total sample of 89 (n=89).

In the descriptive analysis, all 89 anaesthetists were used. For the tests of comparison, on the advice of the statistician, the “others” ethnic group was excluded

from the statistical analysis. The “others” ethnic group consisted of coloured, Asian and Arabic anaesthetists and had only eight anaesthetists in it.

In consultation with a biostatistician, a sample of at least 81 anaesthetists would estimate the expected proportion of insufficiency of 30% to an accuracy of within 10%. A slightly larger sample size than was required was collected to provide for laboratory error. The sample size was not calculated to adequately power the secondary objectives and thus, these should be interpreted with caution.

Defining categories

These are repeated here for continuity and ease of interpreting results.

According to the IOM, vitamin D status in free living healthy adults, with regard to bone health is defined as:

- **vitamin D deficiency:** 25(OH)D <30 nmol/l (<12 ng/ml)(6)
- **vitamin D inadequacy:** 25(OH)D of 30 to 50 nmol/l (12 to 20 ng/ml)(6)
- **vitamin D sufficiency:** 25(OH)D of >50 nmol/l (>20 ng/ml)(6).

Most of the studies reviewed combine the vitamin D deficient and inadequate categories into one (12, 13, 15, 20). For this study this group will be called “vitamin D insufficient”.

4.2.1 Demographic characteristics of the study sample

The study sample had a mean age of 31.33 years (\pm 4.32). Gender distribution, ethnicity and BMI are shown in Table 4.1.

Table 4.1 Demographic characteristics

		Freque nc y	(%)
Gender	Female	45	(50.56)
	Male	44	(49.44)
Ethnic Group	Black	20	(22.47)
	Indian	26	(29.21)
	White	35	(39.33)
	Arabic	4	(4.49)
	Asian	3	(3.37)
	Coloure d	1	(1.12)
BMI	<25	55	(61.80)
	≥25	34	(38.20)

4.2.2 Primary objective: Describe 25(OH)D of anaesthetists and describe the proportion anaesthetists who are vitamin D insufficient.

25(OH)D levels of anaesthetists were not normally distributed and thus the median is reported. The median 25(OH)D is 43.8 nmol/l (IQR 26-76).

Vitamin D status is further categorised in Table 4.2

Table 4.2 Vitamin D status

Vitamin D status	Freque nc y	(%)	Frequency	(%)
Deficient	28	(31.46)	51	(57.30)
Inadequate	23	(25.84)		
Sufficient	38	(42.70)		

} **Insufficient**

Table 4.2 reveals that there were 28 (31.46 %) anaesthetists who fell into the deficient category. Twenty three (25.84 %) were in the inadequate category. The combination of the deficient and inadequate categories into an insufficient category, as is common in the literature, reveals that 51 (57.30 %) anaesthetists were vitamin D insufficient.

4.2.3 Secondary objectives

Describe and compare factors influencing vitamin D status in anaesthetists who are vitamin D insufficient to those who are sufficient.

The following factors influencing vitamin D status are described and compared where appropriate:

- ethnicity
- gender
- BMI
- multivitamin use
- calcium or vitamin D supplementation
- sun exposure
- vitamin D intake from diet alone
- vitamin D intake from diet and supplementation
- calcium intake (dairy)

4.2.3.1 Ethnicity

Description of vitamin D status

Vitamin D status according to the ethnicity of participants is shown in Table 4.3.

Table 4.3 Vitamin D status according to ethnicity

Vitamin D status	Black	Indian	White	Other	Total
Insufficient Frequency (%)	14 (70%)	21 (80.77%)	10 (28.57%)	3 (37.50%)	48
Sufficient Frequency (%)	6 (30%)	5 (19.23%)	25 (71.43%)	5 (62.50%)	41
Median (IQR) 25(OH)D	41.85 nmol/l (26.6-51.55)	23.05 nmol/l (18.53-44.45)	73.2 nmol/l (48.75-83.45)	37.00 nmol/l (23.83-47.13)	
Total	20	26	35	8	89

Comparison of Ethnicity and Vitamin D status

The “others” ethnic group was excluded from the statistical analysis it had only eight participants.

A comparison between ethnicity and vitamin D status is illustrated in Table 4.4.

Table 4.4 Comparison of ethnicity and vitamin D status

Vitamin D status	Black Frequency (%)	Indian Frequency (%)	White Frequency (%)	Total
Insufficient	14 (70%)	21 (80.77%)	10 (28.57%)	45
Sufficient	6 (30%)	5 (19.23%)	25 (71.43%)	36
Total	20	26	35	89

Chi² [2] = 18.706, p < 0.001

There is a statistically significant association between ethnicity and vitamin D status (p<0.001). Twenty one (80.77 %) Indian anaesthetists and 14 (70.00 %) black anaesthetists were insufficient, as compared to only 10 (28.57%) white anaesthetists.

4.2.3.2 Gender

The vitamin D status as per gender and a comparison are illustrated in Table 4.5.

Table 4.5 Comparison of gender and vitamin D status

Vitamin D status	Male Frequency (%)	Female Frequency (%)	Total
Insufficient	27 (33.3%)	24 (29.55%)	51
Sufficient	18 (40%)	20 (42.7%)	38
Total	45	44	89

Chi²[1]= 0.2705, p=0.60.

When gender and Vitamin D status were compared, there was no statistically significant association (p=0.60).

4.2.3.3 BMI

The description of BMI amongst the participants is illustrated in Table 4.6

Table 4.6 BMI of study participants

BMI	Frequency	(%)
<25	55	61.8
≥25	34	38.2
Total	89	100

Comparison of BMI vitamin D status

When a comparison was made between the two groups of BMI <25 and BMI ≥25, using a Mann-Whitney test, the two-tailed P value was 0.6791. This is not considered significant. Mann-Whitney U-statistic = 885.50 U' = 984.50.

Thus no significant difference between BMI with vitamin D status was found.

4.2.3.4 Multivitamin use

Multivitamin use is illustrated in Table 4.7

Table 4.7 Multivitamin use

Multivitamin use	Frequency	(%)
No	64	71.91
Yes	25	28.09
Total	89	100

Comparison between multivitamin use and vitamin D status

A comparison between multivitamin use and vitamin D status is illustrated in table 4.8.

Table 4.8 Comparison between multivitamin use and vitamin D status

Vitamin D status	No Multivitamin use	Multivitamin use	Total
	Frequency (%)	Frequency (%)	
Insufficient	42 (65.62%)	9 (36%)	51
Sufficient	22 (34.38%)	16 (64%)	38
Total	64	25	89

Chi²[1]=6.449, p=0.01

When a comparison was made between multivitamin use and vitamin D status, there was found to be a statistically significant association (p=0.01).

Of the 25 anaesthetists using multivitamins, 16 (64%) were vitamin D sufficient, whereas only 9 (34%) were insufficient. This is in comparison to the anaesthetists not using multivitamins, where 42 (65.62%) were insufficient and 22 (34.38%) were sufficient.

4.2.3.5 Calcium or vitamin D supplementation

Calcium or vitamin D supplementation is illustrated in Table 4.9.

Table 4.9 Calcium or vitamin D supplementation

Calcium or vitamin D supplementation	Frequenc y	(%)
No	85	95.51
Yes	4	4.49
Total	89	100

There were only four anaesthetists who took vitamin D or calcium supplementation. Three of them had sufficient vitamin D levels and one insufficient. Owing to the small numbers, no further analysis was done.

4.2.3.6 Sun exposure

Sun exposure amongst anaesthetists is illustrated in Table 4.10.

Table 4.10 Sun exposure amongst anaesthetists

Sun exposure	Frequency	(%)
Inadequate	66	74.16
Adequate	23	25.84
Total	89	100

Comparison between sun exposure and vitamin D status

A comparison between sun exposure and vitamin D status is illustrated in Table 4.11.

Table 4.11 Comparison between sun exposure and vitamin D status

Vitamin D status	Inadequate sun exposure	Adequate sun exposure	Total
	Frequency (%)	Frequency (%)	
Insufficient	38 (57.58 %)	13 (56.52 %)	51
Sufficient	28 (42.42 %)	10 (43.48 %)	38
Total	66	23	89

Chi²[1]=0.007745, p=0.93

No statistically significant association between sun exposure and vitamin D status could be found (p = 0.93).

4.2.3.7 Vitamin D intake from diet alone

Vitamin D intake was quantified into adequate or inadequate and illustrated in Table 4.12.

Table 4.12 Vitamin D intake from diet alone

Vitamin D intake	Frequency	(%)
Inadequate	77	86.52
Adequate	12	13.48
Total	89	100

Comparison between vitamin D intake from diet alone and vitamin D status

A comparison between vitamin D intake from diet and vitamin D status is illustrated in Table 4.13.

Table 4.13 Comparison between vitamin D intake from diet and vitamin D status

Status	Inadequate intake Frequency (%)	Adequate intake Frequency (%)	Total
Not sufficient	47 (61.04%)	4 (33.33%)	51
Sufficient	30 (38.96%)	8 (66.67%)	38
Total	77	12	89

Chi² [1]=3.257, p=0.07

No statistically significant association between dietary vitamin D intake and vitamin D status was found (p= 0.07).

4.2.3.8 Vitamin D intake from diet and supplementation

Vitamin D intake from diet and supplementation was quantified into adequate or inadequate and illustrated in Table 4.14.

Table 4.14 Vitamin D intake from diet and supplementation

Vitamin D intake	Frequency	Percent
Inadequate	57	64.04
Adequate	32	35.96
Total	89	100

Comparison between adequacy of vitamin D intake from diet and supplementation and vitamin D status

A comparison between vitamin D intake from diet and supplementation and vitamin D status is illustrated in Table 4.15.

Table 4.15 Comparison between vitamin D intake from diet and supplementation and vitamin D status

Vitamin D status	Inadequate intake	Adequate intake	Total
	Frequency (%)	Frequency (%)	
Insufficient	37 (64.91%)	14 (43.75%)	51
Sufficient	20 (35.09%)	18 (56.25%)	38
Total	57	32	89

Chi²[1]=3.751, p=0.05

When a comparison was made between vitamin D intake from diet and supplementation and vitamin D status, the association was not found to be statistically significant (p=0.05).

4.2.3.9 Calcium intake from dairy products

Calcium intake predominantly from dairy was quantified into adequate or inadequate and illustrated in Table 4.16.

Table 4.16 Calcium intake from dairy products

Intake	Frequency	(%)
Inadequate	75	84.27
Adequate	14	15.73
Total	89	100

Comparison between vitamin D status of anaesthetists with regard to calcium intake

A comparison between vitamin D status and calcium intake from dairy products is illustrated in Table 4.17.

Table 4.17 Comparison between calcium intake and vitamin D status

Vitamin D status	Inadequate dairy intake	Adequate dairy intake	Total
	Frequency (%)	Frequency (%)	
Insufficient	44 (58.67 %)	7 (50 %)	51
Sufficient	31 (41.33 %)	7 (50 %)	38
Total	75	14	

Chi² [1]=0.3622, p=0.55

When compared, no statistically significant association between calcium intake and vitamin D status was found (p=0.55).

4.3 Discussion

The integral role Vitamin D plays in homeostasis spreads far beyond calcium and bone health (1, 2). In apparently “sunny” climates, the impact of inadequate sun exposure is often overlooked or under-investigated. The aim of this study was to describe the Vitamin D levels of anaesthetists working in the Department of Anaesthesiology.

The vitamin D levels of anaesthetists in our study were not normally distributed with a median 25(OH)D of 43.8 nmol/l (IQR 26-76). Most of the studies reviewed had normally distributed 25(OH)D levels and thus reported mean 25(OH)D. In a normally distributed sample, the median is very close to the mean, so although a direct comparison between mean and median cannot be made, we are able to presume that our median 25(OH)D was generally lower than the median levels of 25(OH)D found in the majority of studies among doctors. The prevalence of vitamin D insufficiency in our study was found to be 57.3%.

Working indoors is known to be a risk factor for vitamin D insufficiency (10). Doctors also have increased risk due to long working hours. Studies among doctors like that of Growdon et al. (12) found 25% of trainee doctors to be insufficient. Haney et al. (13) found 47%, and Tangpricha et al. (20) found 30% to be insufficient. All three of these studies were performed at northern latitudes during winter.

Mendoza et al. (15) found 75% of the doctors in their study, during the Mexican summer, to be insufficient. This is despite the fact that Mexico City had the highest

UV index figures of studies identified among doctors. Season and latitude alone cannot explain these high rates of insufficiency. Other factors influencing vitamin D levels need to be addressed. These include sun exposure, ethnicity, age, vitamin supplementation, diet, and BMI.

Growdon et al. (12) concluded from their study during Boston winter that despite their cohort not displaying traditional demographic characteristics associated with vitamin D deficiency, their residents had a very high prevalence of vitamin D insufficiency. Long working hours may have played a role here, as well as the fact that many residents and registrars spend much of their free time studying. Our study and that of Mendoza et al. (15) in Mexico expand on this. They reveal that not only indoor workers residing at higher altitudes are at risk, but also those from “sunny” climates.

Our secondary objectives will now be discussed. This data should be interpreted with caution as the sample size was not calculated to adequately power these objectives.

Haney et al. (13) from their study in Portland Oregon found that sun exposure and multivitamin use were predictive of the 25(OH)D concentration. Sun exposure and multivitamin use were also considered as secondary objectives in our study. The former did not achieve statistical association with vitamin D status, but the latter did. The questionnaire that was used to determine this and other secondary objectives was based on literature review and discussion with a dietician and a vitamin D expert (88). Quantification of sun exposure was based on the participants answering of the questionnaire (Appendix 4) which collated area of skin exposure and duration. This was then compared to the unit of comparison of UV radiation- an MED. Cultural attire like “veiling”, was not specifically recorded in the questionnaire, but was incorporated into the aforementioned quantification of sun exposure.

Haney et al. (13) mentioned as a limitation to their study that they had a study sample that was predominantly Caucasian. In our study ethnicity was shown to be significantly associated with vitamin D status. The prevalence of insufficiency amongst Indian anaesthetists was 80.77%, as opposed to 70% of black anaesthetists and only 28.57% of white anaesthetists. This concurs with other studies that have shown darker skin pigmentation to be associated with lower 25(OH)D levels (10, 89, 90). Additional reasons for varying levels of sun exposure include avoidance of the sun and cultural attire. A recent South African study by George et al. (51) mentioned diet, clothing and sun exposure all as compounding factors with regard to vitamin D levels.

Our study population was relatively homogenous with regard to age. The mean age was 31.33 (\pm 4.32) years. Advancing age's effect on cutaneous vitamin D synthesis (1, 89) could not be shown. This makes the high prevalence of vitamin D insufficiency in our study striking, as advanced age has long been a recognised risk factor for poor vitamin D status (6).

Multivitamin use determined by the questionnaire (Appendix 4) revealed a significant association with vitamin D levels. The questionnaire also determined dietary intake of vitamin D, aside from supplementation, to be quite poor. The reason for this could be that vitamin D rich foods are not commonly consumed by South Africans. (91) Additionally, staple food fortification is not practiced in South Africa, aggravating the poor dietary intake of vitamin D.

Other physiological factors that are supposed to influence vitamin D levels negatively like BMI (63) were not revealed by our study. The reason for this could be that with regard to BMI our study population was not normally distributed.

As an essential part of human physiology, vitamin D has long been overlooked. Northern latitude countries have begun to realise its importance, but owing to conventional risk stratification and a paradigm that "sunny" climates are exempt from the problem, many individuals remain vitamin D insufficient. As discussed extensively in the literature review, many individuals may be incorrectly classified as sufficient, because of the reference values being used by their medical advisors. The

Endocrine Society defines sufficiency for individuals at risk for deficiency as >72.5 nmol/l. The IOM defines sufficiency for free living healthy adults as >50 nmol/l. From this it is clear that, although a prevalence of insufficiency of 57.3% amongst anaesthetists is quite high, this could be an underestimate, if anaesthetists were reclassified as a population at risk.

Chapter 5 Summary, limitations, recommendations and conclusion

5.1 Introduction

This chapter will summarise the aim, objectives, research methodology and results of this study. The limitations will be considered, recommendations made and a conclusion presented.

5.2 Study summary

5.2.1 Aim

The aim of this study was to determine 25(OH)D levels during winter (mid-July to mid- August 2013), , in anaesthetists working in the Department of Anaesthesiology.

5.2.2 Objectives

The primary objective of this study was to describe serum 25(OH)D of anaesthetists.

The secondary objective was to describe and compare factors influencing vitamin D levels in anaesthetists whoare vitamin D insufficient to those who are not. These factors included:

- ethnicity
- gender
- BMI
- multivitamin use
- calcium or vitamin D supplementation
- sun exposure
- vitamin D intake from diet alone
- vitamin D intake from diet and supplementation
- calcium intake (dairy).

5.2.3 Methodology

This study used a prospective, contextual, descriptive research design.

Data was collected over a period of one month, in winter (mid-July to mid-August 2013).

Anaesthetists agreeing to participate signed the informed consent (Appendix 2) prior to enrolment in the study. The anaesthetist then completed the questionnaire assessing factors influencing vitamin D levels (Appendix 4).

Furthermore, the anaesthetists were asked to indicate whether they would like to be informed of their results. Each participant had 5 ml of blood collected in a standardised manner into a purple top ethylenediaminetetraacetic acid blood specimen tube. Specimens were delivered to the Chemical Pathology Laboratory, Wits Medical school campus, as soon as possible by the researcher. The processing of samples was done by qualified laboratory personnel using standard chemical pathology equipment and procedures. HPLC was performed to determine 25(OH)D levels.

Data was analysed using descriptive and inferential statistics.

5.2.4 Main findings

The median 25(OH)D was 43.8 nmol/l (IQR 26-76), with 51 of 89 (57.30 %) anaesthetists being vitamin D insufficient.

There was a statistically significant association between ethnicity and vitamin D status ($p < 0.001$). Twenty one (80.77 %) Indian anaesthetists and 14 (70.00 %) black anaesthetists were insufficient, as compared to only 10 (28.57%) white anaesthetists.

There was no significant association between the other secondary objectives- Gender ($p = 0.60$), sun exposure ($p = 0.93$), Vitamin D intake from diet alone ($p = 0.07$), vitamin D intake from diet and supplementation ($p = 0.05$) and calcium intake ($p = 0.55$) and vitamin D status.

There was no significant difference between BMI and vitamin D status. When a comparison was made between the two groups of BMI <25 and BMI ≥25, using a Mann-Whitney test, the two-tailed P value was 0.6791. There was a significant association between multivitamin use (p=0.01) and vitamin D status.

5.3 Limitations

This study was performed contextually within the Department of Anaesthesiology at Wits, therefore, the results may not be generalisable to any other anaesthesiology community.

The questionnaire that was used to determine demographic variables and other secondary objectives was based on literature review and discussion with a dietician and a vitamin D expert. (88)

The secondary objectives should be interpreted with caution as the sample size was not calculated to adequately power these.

Owing to financial restraints PTH and calcium of subjects were not tested. Disorders of PTH, or hypo and hypercalcaemia may negatively or positively influence vitamin D levels.

5.4 Recommendations

5.4.1 Clinical practice

Anaesthetists spend most of their day indoors. This is compounded by long working hours as well as the fact that many of them spend much of their free time studying. It is thus recommended that anaesthetists spend more of their free time outdoors and that vitamin D supplementation should be taken, should levels be insufficient.

Although sun exposure did not influence vitamin D levels in my study (sun exposure was a secondary objective and thus possibly underpowered) it is known that exposure to sun increases vitamin D levels. A lecture to the Anaesthesiology Department will be held to highlight these concerns.

5.4.2 Further research

Further studies that are adequately powered for the variables included as secondary objectives in our study. These are ethnicity, gender, BMI, multivitamin use, calcium or vitamin D supplementation, sun exposure, vitamin D intake from diet alone, vitamin D intake from diet and supplementation and calcium intake from dairy products. PTH and calcium levels should also be tested when these studies are conducted.

5.5 Conclusion

Vitamin D should no longer be a forgotten vitamin. The insufficient vitamin D levels of anaesthetist in this study, puts them at risk for pathology far beyond bone health. Adequate vitamin D levels should be seen as essential, rather than optional, even in “sunny” climates.

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Appendices

Appendix 1: Participants' information letter

Dear colleague,

Hello, my name is Eugene Kelly and I am a registrar in the University of the Witwatersrand (Wits) Department of Anaesthesiology.

I would like you to invite you to participate in a research study: Vitamin D levels of anaesthetists in the Department of Anaesthesiology at the University of the Witwatersrand. This will be submitted to the Wits Faculty of Health Sciences towards my MMed degree.

The primary objective of the study is to determine the proportion of anaesthetists that have a vitamin D deficiency. A secondary objective is to describe and compare the factors influencing vitamin D levels amongst those that are deficient to those who are not.

Vitamin D deficiency is a global health problem, with an estimated one billion people deficient worldwide. Deficiency has far reaching effects in normal physiology and pathophysiology. To name a few: interference with one's attainment of peak bone mass, immune modulation, increased risk of certain cancers, autoimmune conditions, cardiovascular disease and mental illness.

I will also ask you to fill in a short questionnaire that will take less than 5 minutes to complete. The content of the questionnaires will only be viewed by me and my research supervisors. Your name will be kept on a separate data collection sheet, together with your phone number, so that you can be contacted with your results, should you request this.

After this 5 ml of blood will be collected and sent for serum 25-hydroxyvitamin D levels.

Participation in this study is entirely voluntary. I will ask you to sign a consent form. You are free to withdraw from the study at any time, without having to provide a reason and there will be no repercussions. Published results will have no identifying data and will be made available to participants.

The study offers the following benefit to participants. Knowledge of ones vitamin D status allows supplementation or modification of factors influencing vitamin D levels. Should you wish to see a dietician, Lizl Veldsman (079 520 2568) will be available for consultation.

The study has been approved by the Human Research Ethics Committee (HREC) (Medical) No.M130305 and the Postgraduate Committee of the University of the Witwatersrand.

Please ensure that you have read and understood all of the above information before giving written informed consent.

Thank you for taking time to read this letter. If you have any questions or concerns with regard to the study, you may contact the following people with your queries:

- Professor Cleaton-Jones (chairperson of the HREC): (011)717-1234
- Eugene Kelly (researcher): 074 116 5133

Yours sincerely

Eugene Kelly **Appendix 2: Informed consent**

Research Title: Vitamin D levels of anaesthetists in the Department of Anaesthesiology at the University of Witwatersrand

I, _____ understand what this study is about and consent to participate in the study. I have read and understand the participants' information letter and my questions have been answered. I acknowledge the fact that 5 mls of blood will be drawn from me. I am aware that I may withdraw from the study at any time without any prejudice toward me. I understand that my name will not appear in any of the results of the research.

Signature of participant

Date

Appendix 3: Study Number Allocation Sheet

Study Number	Name and telephone number	Want results
VTD-01		
VTD-02		
VTD-03		
VTD-04		
VTD-05		
VTD-06		
VTD-07		
VTD-08		
VTD-09		
VTD-10		
VTD-11		

Appendix 4: Questionnaire- Factors influencing vitamin D levels.

VTD-

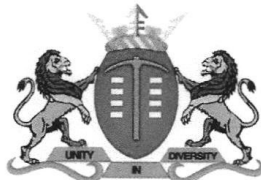
Study Number

PLACE x OVER OPTION OR FILL IN IF APPLICABLE

1. Age (in years)			
2. Gender	Male	Female	
3. Ethnic group (white, black, indian, coloured, Asian).			
4. Do you take a multivitamin? If yes state which	Yes _____	No	
5. Do you take calcium and vitamin D supplements? If yes state which	Yes _____	No	
6. Sun exposure: minutes of direct sun exposure to arms and legs between 10:00 and 15:00.	> 30 min cumulatively / week	< 30 min cumulatively / week	
7. Sunscreen use	Often	Rarely	
8. BMI	Height (metres)	Weight (Kilograms)	
9. Dairy product consumption (total cumulative)- [milk in coffee = 50 ml; 1 slice of cheese = 30mls]	> 750 ml daily	< 750 ml daily	
10. Oily fish consumption (salmon, sardines, mackerel, tuna, cod liver oil) , shitake mushrooms	Daily (>4/5x a week)	Weekly (<2x a week)	Monthly/ rarely
11. Margarine consumption (Flora, Sunshine D- serving of one slice bread = 5g)	_____g daily		
12. Egg yolks (number eaten weekly)			

13. Would you like your vitamin D results?	Yes	No
---	-----	----

Appendix 5: CEO permission-CMJAH



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:

Ms. L. Mngomezulu

Tell: (011): 488-3792

Fax: (011): 488-3753

11th July 2013

Dr. Eugene Harmerton Kelly
Anaesthetic Registrar
CMJAH

Dear Dr. Kelly

RE: "Vitamin D levels of Anaesthetists in the Department of anaesthesiology at the University of Witwatersrand"

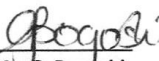
Permission is granted for you to conduct the above research as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic hospital will not in anyway incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the Head of Department and Unit Manager or Sister in Charge to agree on the dates and time that would suit all parties.

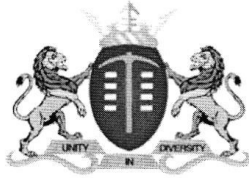
Kindly forward this office with the results of your study on completion of the research.

Approved not approved


Ms. G. Bogoshi
Chief Executive Officer

13/07/2013

Appendix 6: CHBAH permission from Medical Advisory Committee



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 16 July 2013

TITLE OF PROJECT: Vitamin D levels of anaesthetists in the Department of Anaesthesiology at the University of the Witwatersrand

UNIVERSITY: Witwatersrand

Principal Investigator: Dr EH Kelly

Department: Anaesthesiology

Supervisor (If relevant): Dr H Perrie

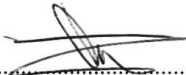
Permission Head Department (where research conducted): Yes


Date of start of proposed study: July 2013

Date of completion of data collection: January 2014

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.


.....
Recommended
(On behalf of the MAC)
Date: 16 July 2013


.....
~~Approved~~ / Not Approved
Hospital Management
Date: 2013/07/18

Appendix 7: CEO permission-HJH

Dr EH Kelly
Department of Anaesthesiology
University of Witwatersrand
eugene.h.kelly@gmail.com
July 2013

Re: Vitamin D levels of anaesthetists in the Department of Anaesthesiology at the University of the Witwatersrand

[Supervisor: H. Perrie (MSc) Co-supervisor: Dr S. Chetty MBChB (Natal) DCH (SA) DA (SA) FCA (SA) Cert. Crit. Care (SA)]

Abstract

My study is for the Master of Medicine in anaesthesiology and it entails describing and comparing vitamin D levels of anaesthetists in the Wits University training hospitals.

There has been a recent resurgence of interest in vitamin D and its' far reaching effects in physiology and pathophysiology. Theatre personnel, and all indoor workers, should be cognisant of vitamin D deficiency as a real occupational hazard.

Sun exposure results in a major source of vitamin D in most people.

In the Department of Anaesthesiology, University of the Witwatersrand anaesthetists work on average 48 hours a week. All of these hours are spent indoors and even though some are evening shifts, many daytime hours are spent doing preoperative visits, attending tutorials or studying. For these reasons the study population is at risk for vitamin D deficiency, despite South Africa commonly being thought of as a land of sunshine.

Vitamin D is critical for calcium homeostasis and deficiency will hinder one's attainment of peak bone mass. The other effects of vitamin D deficiency are less obvious but still widespread in physiology, affecting immunity; increasing risk of certain cancers, autoimmune conditions, cardiovascular disease and mental illness.

No research could be found amongst medical personnel in South Africa and the vitamin D levels of anaesthetists in the Department of Anaesthesiology, Wits University, are not known.

Ethics and Postgraduate approval.

The study has been approved by the Human Research Ethics Committee (HREC) (Medical) Clearance certificate no. M130305 and the Postgraduate Committee of the University of the Witwatersrand.



Dr N.L. Hlongwane
Senior Superintendent
Helen Joseph Hospital
11/7/2013

11/7/13

Accept (Date)

Decline (Date)

Appendix 8: CEO permission-RMMCH



health and
social development
Department: Health and Social Development
GAUTENG PROVINCE



RAHIMA MOOSA MOTHER AND CHILD HOSPITAL

ENQUIRIES: MRS. S. JORDAAN

TEL: (011) 470 – 9030/4

FAX: (011) 477- 4117

School of Human and Community Development
Private Bag 3
WITS 2050
JOHANNESBURG
2001

Re: "Vitamin D levels of Anaesthetists in the Department of Anaesthesiology at the University of Witwatersrand.

Dear Dr. E.H. Kelly

Permission is granted for you to conduct the above survey as indicated in your request:

1. The Rahima Moosa hospital will not in anyway incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study site.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.
5. NO file should leave the records department and/or the hospital premises.

Arrangement will be made with recordkeeping clerks so that you could occupy space in their department.

Kindly forward this office with the results of your research on completion of it.

I, Eugene Kelly accept the terms and conditions set-in this document

sign [Signature] date 10/7/13

Yours sincerely,

CHIEF EXECUTIVE OFFICER
SJ/mdp. 2013-07.10

Appendix 9: Ethics clearance certificate

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R14/49 Dr E.H. Kelly et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130305

NAME: Dr E.H. Kelly et al
(Principal Investigator)

DEPARTMENT: Department of Anaesthesiology
Chris Hani Baragwanath Academic Hospital


PROJECT TITLE: Vitamin D levels of Anaesthetists in the
Department of Anaesthesiology at the
University of Witwatersrand

DATE CONSIDERED: 05/04/2013

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr Helen Perrie

APPROVED BY: 

Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 03/05/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 10: Postgraduate Committee approval

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Faculty of Health Sciences
Private Bag 3 Wits, 2050
Fax:
Tel: 027117172040

Reference: Ms Mpumi Mngapu
E-mail: mpumi.mngapu@wits.ac.za

08 May 2013
Person No: 573335
PAG

Dr EH Kelly
P O Box 48
Florida Hills
1716
South Africa

Dear Dr Kelly

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Vitamin D levels of anaesthetists in the Department of Anaesthesiology at the University of the Witwatersrand* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

A handwritten signature in cursive script, appearing to read "Sandra Benn".

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences