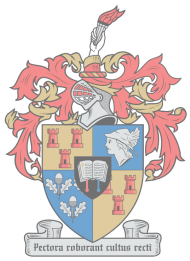
Determinants of serum 25-hydroxyvitamin D levels in healthy young adults living in the Western Cape

by
Kimberley Knight



Thesis presented in fulfilment of the requirements for the degree of Master of Nutrition in the Faculty of Medicine and Health Sciences at Stellenbosch University

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Declaration

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Date: 13/12/2017

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English Abstract

Background: The prevalence of vitamin D deficiency is fast emerging as a global pandemic. In South Africa, however, few studies have been conducted to determine the vitamin D status of the healthy population. While the effects of vitamin D on the body have been known for centuries, vitamin D has recently garnered great attention, with new evidence surrounding vitamin D and health emerging at a rapid rate. Previously thought to only be important for bone health, new research into the role of vitamin D in the body has revealed the importance of sufficient vitamin D levels for overall health.

Aim: The main aim of this study was to investigate the vitamin D status of adults in the Western Cape. Objectives sought to determine whether skin tone, gender, basic anthropometrical measurements, diet, and lifestyle factors had an effect on serum 25(OH)D levels.

Methods: This descriptive, cross-sectional study investigated the vitamin D status of healthy, undergraduate students (mean age: 20.41 ± 2.29 years) at Stellenbosch University. Serum 25(OH)D was collected and analysed, along with basic anthropometrical measurements (weight, height, BMI and waist circumference). A food frequency questionnaire was used to estimate dietary vitamin D intakes. Skin tone was measured using the Fitzpatrick Skin Type (FST) Classification, and a skin reflectometry device was used to measure dermal melanin content.

Results: A total of 242 undergraduate students (with equal gender representation) were included in this study during September 2016. The results showed a mean serum 25(OH)D of 63.80 ± 41.35 ng/ml and a high prevalence of vitamin D sufficiency (90% of participants). The relationship between gender and serum 25(OH)D was found to be significant ($p<0.01$), with more females experiencing suboptimal vitamin D levels than males (18% vs. 5%). Just over half of the participants identified themselves as skin type IV when using the FST classification, and participants with lighter skin tones had higher levels of 25(OH)D than those with darker skin tones ($p=0.02$). The majority of the participants (60.74%) had normal BMIs, although the relationship between BMI and serum 25(OH)D was not statistically significant ($p=0.09$). Total mean dietary vitamin D intake was 7.99 ± 13.81mcg, with 87.2% of participants consuming less than the recommended daily intake of vitamin D (15mcg). The relationship between total vitamin D intake and serum 25(OH)D was found to be weak, but statistically significant ($p=0.003$). Sun exposure and lifestyle factors were not found to have an effect on serum 25(OH)D levels in this study.

Conclusion: This study found a low prevalence of vitamin D deficiency amongst young adults, despite low dietary vitamin D intakes. Significant relationships were found between serum

25(OH)D and gender, skin tone, and vitamin D intake. While this study population was homogenous, it encompassed a very specific group of young, healthy undergraduate students and further studies need to be done before the results are applied to the greater public.

Afrikaans Opsomming

Agtergrond: Die prevalensie van vitamien D tekort is vinnig besig om te groei tot ‘n globale pandemie. Beperkte aantal studies is al uitgevoer in Suid-Afrika om die vitamien D status van die gesonde populasie te bepaal. Alhoewel die effek van vitamien D op die liggaam al bekend is vir eeue, het vitamien D onlangs nuwe belangstelling gelok met nuwe inligting rakende vitamien D en gesondheid wat na vore kom. Voorheen was vitamien D bekend vir die verband met been gesondheid, terwyl onlangse navorsing die belang van voldoende vitamien D vlakke vir algehele gesondheid beklemtoon.

Doel: Die hoofdoel van hierdie studie was om die vitamien D status van volwassenes in die Wes-Kaap te ondersoek. Addisionele doelwitte het die verband tussen velkleur, geslag, antropometriese metings, dieet en lewensstyl faktore op serum 25(OH)D vlakke ondersoek.

Metodes: Hierdie dwarssnit, beskrywende studie het die vitamien D status van gesonde, voorgraadse studente (gemiddelde ouderdom 20.41±2.29 jaar) by die Universiteit van Stellenbosch bepaal. Serum 25(OH)D is versamel en bepaal, tesame met basiese antropometriese metings (gewig, lengte, LMI en middel omtrek). ‘n Voedselfrekwensie vraelys is gebruik om dieet vitamien D vlakke te bepaal. Velkleur is gemeet deur die Fitzpatrick Skin Type (FST) klassifikasie en ‘n vel reflektometer is gebruik om dermale melanieninhoud te meet.

Resultate: 'n Totaal van 242 voorgraadse studente (gelyke geslagsverteenwoordiging) is ingesluit in hierdie studie gedurende September 2016. Die resultate het 'n gemiddelde serum 25(OH)D van 63.80 ± 41.35 ng/ml en 'n hoë voorkoms (90% van deelnemers) van voldoende vitamien D vlakke getoon. Die verwantskap tussen geslag en serum 25(OH)D was betekenisvol ($p<0.01$), met meer vrouens wat suboptimale vitamien D vlakke toon as mans (18% teenoor 5%). Net meer as die helfte van die deelnemers het hulself geïdentifiseer as veltipe IV deur die FST klassifikasie te gebruik. Deelnemers met ‘n ligter veltoon het hoër vlakke van 25(OH)D getoon as dié met ‘n donkerder veltoon ($p=0.02$). Die meerderheid van die deelnemers (60.74%) het normale LMI gehad, alhoewel die verhouding tussen LMI en serum 25(OH)D nie statisties betekenisvol was nie ($p=0.09$). Die totale gemiddelde dieet vitamien D inname was 7.99 ± 13.81mcg, met 87.2% van deelnemers wat minder as die aanbevole daaglikse inname van vitamien D (15mcg) inneem. Die verhouding tussen totale vitamien D inname en serum 25(OH)D was swak, maar statisties betekenisvol ($p=0.03$). Lewensstyl faktore en son blootstelling het nie ‘n effek op serum 25(OH)D gehad nie.

Gevolgtrekking: Hierdie studie het 'n lae voorkoms van vitamien D tekort in jong volwassenes getoon, ten spyte van lae dieet vitamien D inname. Beduidende verhoudings was gevind tussen 25(OH)D en geslag, velkleur en vitamien D inname. Alhoewel hierdie studiepopulasie homogeen was het dit 'n baie spesifieke groep van jong, gesonde voorgraadse studente ingesluit en verdere studies moet gedoen word voordat die resultate toegepas kan word op die breë publiek.

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Abbreviations

1,25 $(OH)_2$ D	1,25-dihydroxyvitamin D
25 (OH)D	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
AVOVA	Appropriate analysis of variance
BMI	Body mass index
CANSA	Cancer Association of South Africa
CRP	C-reactive protein
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
EI	Erythema Index
FFQ	Food frequency questionnaire
FMHS	Faculty of Medicine and Health Sciences (Tygerberg Campus)
HIV	Human immunodeficiency virus
HREC	Human Research Ethics Committee
IOM	Institute of Medicine
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MI	Melanin index
PTH	Parathyroid hormone
RCT	Randomised controlled trial
RSSME	Root mean square standardized effect
SOPs	Standard operating procedures
SPF	Sun protection factor
TB	Tuberculosis
TSS	Tygerberg Studente Sentrum (Tygerberg Student Centre)
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
VDBP	Vitamin D binding protein
WHO	World Health Organisation

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1. Introduction and Background

In the last few years, vitamin D has become a nutritional “buzz-word”, with new evidence surrounding vitamin D emerging at a rapid rate. However, its effects on the body have been known for centuries. Hippocrates was one of the first to recognise the importance of sunlight on health, noting that those who lived on mountainsides with plenty of sunlight were in better health than those living in poor sunlight.[1]Theodore Palm, during missionary trips to Africa in the late 1800s, noted that children who lived closer to the equator had a lower prevalence of rickets. He theorised that the variances in sunlight exposure in different countries affected the incidence of rickets in these regions.[1]In 1921, Alfred Hess studied the effect of sunlight on infants with rickets, and found a marked and rapid improvement in the condition after exposure to direct sunlight.[2] Hess also deduced that direct skin exposure to sunlight, without the confines of glass or clothes, was essential in the treatment of rickets.[2] Edward Mellanby altered the diets of puppies to determine the effect of the diet on the development of rickets. He concluded that rickets, similarly to scurvy and beriberi, is a direct cause of the dietary deficiency of an essential component, in this case vitamin D.[3] His results also included one of the first lists of foodstuffs which prevented rickets, including milk, butter, margarine, meat, and cod liver oil.[3] Thus, adequate exposure to sunlight and a diet rich in vitamin D have been proven throughout history to have a positive effect on health.

2. Literature Review

2.1. Physiology of Vitamin D

As described above, the human body can obtain vitamin D in two ways. Firstly, through the exposure of cutaneous tissue to Ultraviolet B (UVB) radiation, which is most commonly found in sunlight, and secondly, through the consumption of vitamin D-rich foods. Sun exposure is the main source of vitamin D, with UVB exposed skin contributing to almost 90% of serum 25-hydroxyvitamin D (25(OH)D) levels.[4] Studies have shown a correlation between cutaneous exposure to UVB radiation and an increase in circulating 25(OH)D levels.[4,5]

When skin is exposed to sunlight, it is also exposed to Ultraviolet A (UVA) radiation. UVA radiation is, however, responsible for only a miniscule amount of vitamin D formation.[4]

2.1.1. Cutaneous production of Vitamin D_3

When the skin is exposed to UVB radiation (either naturally or artificially), UVB photons react to convert 7-dehydrocholesterol (7-DHC), found in the epidermis and dermis, to pre-

vitamin D_3, which is then converted to vitamin D_3 via non-enzymatic isomerization.[5,6] This isomerization process is dependent on temperature and time, and happens rapidly – pre-vitamin D_3 can be cutaneously converted to vitamin D_3 in less than 3 hours. Because of this quick conversion, circulating vitamin D_3 levels are at their highest 12-24 hours after exposure to UVB radiation.[4] Vitamin D_3 then undergoes a further transformation to 25(OH)D in the liver. A final conversion takes place in the kidneys, where the hormone 1-alpha-hydroxylase converts 25(OH)D to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) - the form needed by the body to assist with intestinal calcium absorption and ensure bone health (Figure 2.1).[6,7]

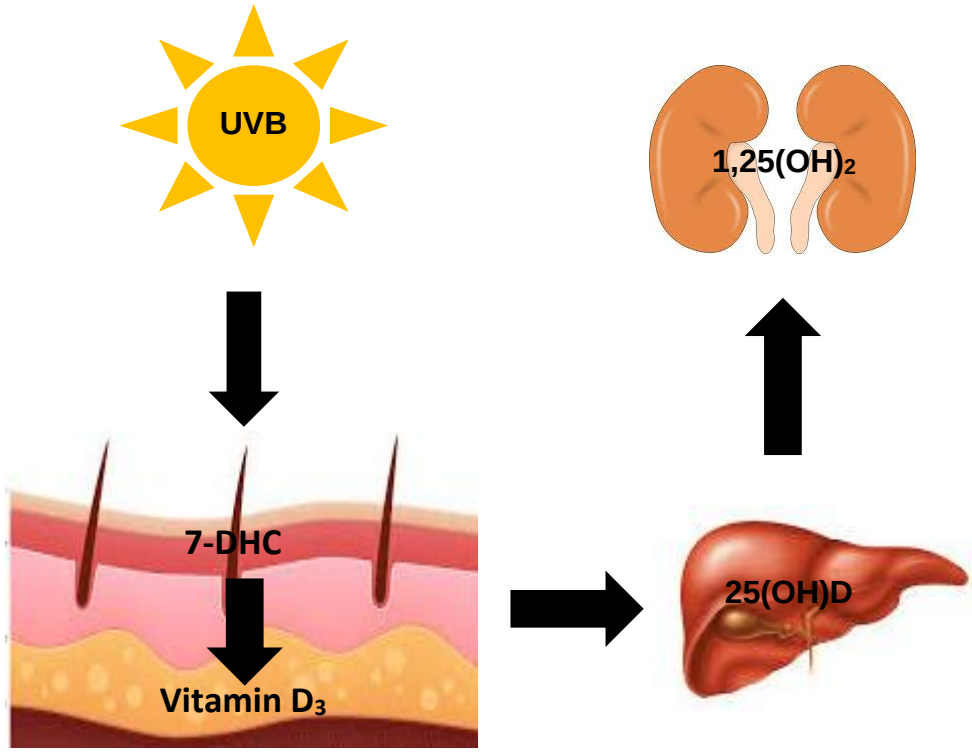

Fig 2.1. Cutaneous synthesis of vitamin D via exposure to ultraviolet-B radiation

Abbreviation: UVB: Ultraviolet B; 7-DHC: 7-dehydrocholesterol; 25(OH)D: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D

$1,25(OH)_2D$ supports bone health by improving the reabsorption of UVB-absorbing molecules, such as chromophores, which can hinder the success of cutaneous vitamin D_3 production. Melanin, found in the epidermis, is an example of a UVB absorbing chromophore.[6]

2.1.2. Prolonged cutaneous exposure to UVB radiation

While UVB radiation is essential for the synthesis of vitamin D_3, UVB exposure also results in undesirable effects, such as erythema and DNA damage. Unfortunately, the amount of UVB exposure needed to synthesise vitamin D_3 overlaps with the amount of UVB exposure responsible for skin damage. This means that in order to produce the correct amount of vitamin D_3 needed by the body, skin damage is inevitable.[7] A silver lining to this catch-22, however, is that prolonged exposure to UVB radiation does not increase vitamin D_3 synthesis once a maximum level has been reached. Maximum vitamin D_3 synthesis with minimal cutaneous damage is therefore possible. The success of vitamin D_3 production also hinges on many other factors including how much 7-DHC is available in the skin, the energy of the photons needed for the conversion, the angle of the sun at the time of exposure, the time of day, the use of sunscreens, and age.[4,7]

2.2. Factors influencing vitamin D levels

2.2.1 Age

Vitamin D affects all stages of the lifecycle, and as such, age plays a prominent role when looking at vitamin D status. Physiologically, the aging process affects many aspects of vitamin D, including the synthesis and metabolism of vitamin D.[8,9] The amount of 7-DHC in the skin decreases over time and this results in a decreased synthesis of vitamin D in the skin.[8] Added to this, there is less vitamin D absorption from the gut as the concentration of VDR found in the gut also decreases with age.[8] Physically, the elderly are less mobile than their younger counterparts, and many may be institutionalised, reducing their exposure to sunlight.

In young adults, the prevalence of vitamin D deficiency various widely from country to country.[10] Many studies have shown a high prevalence of vitamin D deficiency among this age group.[11-13] This prevalence is particularly high in the Middle East, especially in females.[14] Knowledge surrounding vitamin D also plays a role in the vitamin D status of young adults. In a Chinese study of medical students, it was found that the general knowledge surrounding the role of vitamin D in health was not widely known.[15] As the transition from adolescence to adulthood paves the way for future and lifelong habits, more education surrounding vitamin D aimed at young adults may be necessary.

2.2.2 Body Weight

Both obesity and inadequate vitamin D levels have been associated with poor health.[16] Low 25(OH)D serum levels have been observed in people with excess body weight in many studies.[16-18]

A systematic review and meta-analysis of 21 studies found obese participants were 35% more likely to be deficient in vitamin D when compared to their healthy weight counterparts.[16] Contrastingly, a South African study in Johannesburg found no association between body fat distribution and 25(OH)D levels.[19] This study did not consider vitamin D supplementation, however, which may account for these results.[19]

Obesity and vitamin D deficiency is associated with an increased risk in similar non-communicable diseases, such as cardiovascular disease (CVD), hypertension, diabetes, and certain cancers.[16] Pereira-Santos et al of the meta-analysis discussed above concluded that obesity's link to vitamin D deficiency is independent of other factors that influence vitamin D status.[16]

There are many theories for this. Higher body weight is usually linked to social stigma and obese individuals are more likely to stay indoors and cover up their bodies which can reduce their exposure to sunlight, which therefore lowers the cutaneous synthesis of vitamin D_3.[6] It has also been postulated that the excess fat under the skin affects the synthesis of vitamin D_3. Adipose tissue contains higher levels of 1-α-hydroxylase, the enzyme responsible for the activation of vitamin D, meaning excess adipose tissue uses more vitamin D.[16,17]

Vitamin D is a fat-soluble vitamin and as such, is stored in fat tissue. Due to the excess of fat tissue found in obesity, an explanation for lower circulating levels of vitamin D is that there is more fat tissue available to store vitamin D. 25(OH)D becomes trapped in the adipose tissue and cannot travel to the sites where it is needed.[17,18] However, the results of a meta-analysis by Vimaleswaran et al found that the bioavailability of vitamin D is not affected even when it is stored in adipose tissue.[17]

While obesity is often linked to lower serum 25(OH)D levels, there is little evidence showing that low vitamin D levels leads to obesity.[17]

Interestingly, it has been thought that the low vitamin D levels in obesity activates an increase in PTH levels, which in turn increases lipogenesis and thus, encourages weight loss.[20] Studies have also shown that low levels of $1,25(OH)_2D$ can lead to excessive

adipogenesis, as $1,25(OH)_2D$ plays a vital role in regulating the production of adipose tissue in the body.[16]

Vitamin D supplementation is thought by some to be a novel approach in preventing and treating the obesity pandemic.[21] A newer strategy for encouraging weight loss is the encouragement of adipose tissue death, as adipose tissue is comprised of both an excess in the number of adipocytes as well as adipocyte mass.[21] The effect that $1,25(OH)_2D$ has on adipocytes, through the activation of proteases, encourages apoptosis.[21] This is a novel approach, however, and more research is needed in this area.

2.2.1. Seasons

Exposure to sunlight is vital for the maintenance of serum 25(OH)D levels and this importance can be seen specifically in regions where seasonal fluctuations in serum 25(OH)D levels are present.[22] Serum 25(OH)D levels can lag up to 2 months behind exposure to high levels of solar UVB exposure.[22] The Physicians' Health Study found that those participants whose blood samples were provided during summer had higher serum 25(OH)D levels than those who provided samples in winter.[23]

A summary of investigations into the seasonal variations in 25(OH)D levels can be seen in Table 2.1.

Table 2.1. Seasonal variations in serum 25-hydroxyvitamin D levels

Country & reference	Study population	Outcome	Seasonal variations in serum 25(OH)D (results)
New Zealand, Rockell et al; 2008 [24]	n=342	Seasonal variations in vitamin D and PTH	25(OH)D levels higher in autumn than spring
Italy, Hanwell et al; 2010 [22]	Winter n=47 Summer n=23	Evaluate relationship between sun exposure and serum 25(OH)D during winter and summer	There was no difference between serum 25(OH)D in summer vs. winter
Australia, Daly et al; 2012 [25]	n=11 247	Evaluate the vitamin D status of Australian adults and risk factors associated with vitamin D deficiency	Vitamin D deficiency was more common during winter (42% of women and 27% of men were deficient at the end of summer compared to 58% and 35% at the end of winter)
Tanzania; Luxwolda, 2012 [26]	n=60 (35 Maasai + 25 Hadzabe)	Vitamin D status of traditionally living tribes in West Africa	Prevalence of vitamin D deficiency and insufficiency was found to increase in winter and decrease in summer.
South Africa, George; 2014 [19]	n=730	Vitamin D status of adults in Johannesburg and factors that influence vitamin D status	Seasonal variations in serum 25(OH)D were observed, with concentrations 40-60% higher in autumn than in spring

Abbreviation: PTH: Parathyroid Hormone; 25(OH)D: 25-hydroxyvitamin D

2.2.2. Gender

Many studies have shown evidence that female participants are more likely to experience low vitamin D levels than males, while others have found no correlation between gender and 25(OH)D levels. In a review of 194 studies to determine the vitamin D status in various countries, it was found that there were no statistically significant differences in 25(OH)D values between males and females.[27]

While the gender-related results were not statistically significant, the reviewers did observe that in certain regions, namely the Middle East/Africa and Asia/Pacific, lower serum 25(OH)D levels were found in women than in men. It has been suggested that this could be related to cultural clothing differences that could hinder cutaneous exposure to UVB radiation.[27,28]

The evaluation of the data collected during the NHANES III study showed that women in Northern America had lower serum 25(OH)D levels than men in the same region.[29] Daly et al found that in Australia, women were more likely to suffer from vitamin D deficiency.[25] In another Australia study, Gill et al's findings supported this.[18]

The lower levels of 25(OH)D experienced by females could be due to hormone-related differences in vitamin D binding protein (VDBP) levels, as well as body fat variations.[30] Body weight may play a larger role when it comes to supplementation of vitamin D, however. Waterhouse found that while women with normal body weights had a greater reaction to supplementation, supplemental dose-responses for all weights were the same.[31] Interestingly, South African studies have shown little evidence to support the variations in serum 25(OH)D between genders.[32,33]

2.2.3. Skin Tone

The Fitzpatrick skin type classification system was created in the 1970s to predict how the epidermis will react to photochemotherapy.[34] Today it has a plethora of uses, including a self-reported skin tone analysis.[34] It asks participants to classify their skin tone into six types, ranging from I (always burn, never tan) to VI (deepest brown skin), by asking 10 multiple choice questions based mostly on how the participant's skin reacts to UVB exposure.[34] A study by Cargill et al investigated the link between self-reported sun exposure questionnaires and melanin density in the skin and found that self-reported questionnaires are valid and accurate.[35]

Melanin in the skin acts as a natural sunscreen; the more you are exposed to sunlight, the more melanin is produced over time to protect your skin from the harmful UVB rays.[36] This phenomenon is explained in the climatic theory.

The climatic theory states that the skin tones of certain populations adapted to accommodate variations in their climate including exposure to the sun, and the harshness of the sun that they are exposed to. A difference in skin tone between population groups, therefore, has long been thought to be part of the adaption and evolution process.[26] People closer to the equator had an increased exposure to harsher UVB radiation and, as such, developed more melanin in their skin to protect them. Populations who migrated away from the equator and who were exposed to less sunlight and lower UVB indexes, developed less melanin to ensure that when they did were exposed to sunlight, they could make the most of it.[26]

A study by Luxwolda et al sought to investigate what the serum 25(OH)D levels were in populations who followed more traditional lifestyles (in this case, those who spent more time outside, and wore little to no sunscreen).[26] Two groups were studied, namely the Maasai and the Hadzabe of Tanzania, and it was found that none of the participants had a serum 25(OH)D lower than 50nmol/l.[26] Interestingly enough, there was very little difference in 25(OH)D levels between the groups, despite the Maasai group eating more traditionally vitamin D-rich foods.[26] Table 2.2 shows the results of other studies investigating skin tone and vitamin D deficiency.

Table 2.2. Effect of skin tone on serum 25-hydroxyvitamin D levels

Study	Study size	Outcome	Results
NHANES III, Zadshir; 2005 [29]	n=15390 (adults)	Prevalence of adequate vitamin D status in US adults	White men and women had higher serum 25(OH)D levels than black and Hispanic ($p<0.0001$)
Chen et al; 2007 [36]	Unknown	To determine 25(OH)D of different skin types after repeated exposure to UV radiation	Type II skin tones 5 -10x more likely than the type V skin tone to convert 7-DHC to pre-vitamin D_3.
Hannan et al; 2008 [37]	N=1114 (adult men)	Investigated link between serum 25(OH)D levels and bone mineral density in men	Men with darker skin tones had low serum 25(OH)D but these were not associated with lower bone mineral density
Au et al; 2013 [38]	n=307 (school children)	Determine whether differences in skin tone affect serum 25(OH)D levels	Overall, poor vitamin D status were worse in children with darker skin (prevalence of 25(OH)D inadequacy was 47% (white), 74% (black), 65% (Hispanic), 89% (Asian))
Powe; 2013 [39]	N=2085 (adults)	Determine whether VDBP and serum 25(OH)D differs between white and black Americans	Dark skin tones had lower 25(OH)D levels and lower VDBP levels than Caucasian counterparts, which might be an advantage of evolution to protect against low 25(OH)D levels.

Abbreviations: US: United States of America; UV: Ultraviolet; 7-DHC: 7-dehydrocholesterol; 25(OH)D: 25-hydroxyvitamin D; VDBP: vitamin D binding protein

The more sun exposure one has could be linked to a decrease in vitamin D production as the body's way of ensuring a failsafe method against vitamin D toxicity.[40] Those who spend lots of time in the sun may build up a tolerance to UVB exposure and have less vitamin D producing abilities than those who only spend time in the sun sporadically.[40,41] It may be inaccurate, therefore, to assume that those who spend lots of time in the sun are not at risk for vitamin D deficiency.[40] Powe et al also deduced that people with darker skin tones may

have developed protective bodily processes. They found that ethnically black people have lower levels of VDBP than their Caucasian counterparts, which might be an advantage of evolution to protect against low 25(OH)D levels.[39]

As described above, the bioavailability of serum 25(OH)D levels may be higher in people with darker skin tones. Less circulating 25(OH)D may be needed for bone health in darker skinned people than lighter skinned people.[39] The standard cut off values for vitamin D deficiency, therefore, might not apply to all races.[39] Vitamin D levels need to be looked at as part of a holistic picture, instead of an indicator of health in isolation.

While both darker and lighter skinned people experience seasonal variations in 25(OH)D levels, there is a greater variation in levels observed in lighter skinned people.[6] It has been suggested though, that latitude and climate may influence seasonal variations of 25(OH)D levels more than skin tone itself.[42] In areas where seasonal climate changes are temperate and the region is closer to the equator, skin tone does not have as big an effect on seasonal 25(OH)D variations as in lower latitude areas with more extreme weather variations.[42]

2.2.4. Diet

Vitamin D can enter the body via diet or oral supplementation, although even a diet highly rich in vitamin D does not allow the body to produce as much 25(OH)D as exposure to sunlight does. The diet is responsible for the provision of vitamin D_3, from animal sources, and vitamin D_2, a less bioavailable form of vitamin D, from plant sources.[43,44]
When either form of vitamin D is ingested, it is absorbed through the small intestine. As vitamin D is a fat-soluble vitamin, dietary fat facilitates this process.[44] The absorbed vitamin D is incorporated into chylomicrons, which are transported first by the lymphatic system and then in the blood to the liver for further conversion.

Despite the diet being a less effective source of vitamin D, sources of vitamin D-rich foods become vital when exposure to UVB radiation is scarce.[28] While foods naturally containing vitamin D are scarce, foods naturally rich in vitamin D_3 include fish liver, fish liver oils, oily fish, and egg yolks (Table 2.3).[45,46] The National Health Institute have also released the vitamin D content of food sources rich in vitamin D, which the Cancer Association of South Africa (CANSA) have used in a factsheet they released in 2017, to educate the South African public about vitamin D (Table 2.4).[47]

Table 2.3. International food sources rich in vitamin D, adapted from Endocrine Society Clinical Guidelines[46]

Food item	Vitamin D content (IU)
Cod liver oil	~400–1,000 IU/teaspoon vitamin D3
Salmon, fresh wild caught	~600–1,000 IU/3.5 oz. vitamin D3
Salmon, fresh farmed	~100–250 IU/3.5 oz. vitamin D3, vitamin D2
Salmon, canned	~300–600 IU/3.5 oz. vitamin D3
Sardines, canned	~300 IU/3.5 oz. vitamin D3
Mackerel, canned	~250 IU/3.5 oz. vitamin D3
Tuna, canned	236 IU/3.5 oz. vitamin D3
Shiitake mushrooms, fresh	~100 IU/3.5 oz. vitamin D2
Shiitake mushrooms, sun-dried	~1,600 IU/3.5 oz. vitamin D2
Egg yolk	~20 IU/yolk vitamin D3 or D2
Fortified milk	100 IU/8 oz. usually vitamin D3
Fortified orange juice	100 IU/8 oz. vitamin D3
Infant formulas	100 IU/8 oz. vitamin D3
Fortified yogurts	100 IU/8 oz. usually vitamin D3
Fortified butter	56 IU/3.5 oz. usually vitamin D3
Fortified margarine	429 IU/3.5 oz. usually vitamin D3
Fortified cheeses	100 IU/3 oz. usually vitamin D3
Fortified breakfast cereals	~100 IU/serving, usually vitamin D3

Abbreviation: IU: International Unit; oz.: ounce

Table 2.4. Food sources rich in vitamin D: National Health Institute[47]

Food item	Serving Size	Vitamin D content (IU)
Cod liver oil	1 Tablespoon	1360
Swordfish, cooked	90ml	566
Salmon, cooked	90ml	447
Tuna (canned in water, drained)	90ml	154
Orange juice (fortified with vitamin D)	125ml	137
Milk (fortified with vitamin D)	125ml	115 -124
Yoghurt (fortified with vitamin D)	180ml	80
Margarine (fortified with vitamin D)	15ml	60
Sardines (canned in oil)	Unspecified	46
Beef liver (cooked)	90ml	42
Egg	1 large	41
Cheese (Swiss)	30ml	6

Abbreviation: IU: International Unit

While mushrooms can be a good plant-based source of vitamin D, not all mushrooms contain a high level of vitamin D. Only mushrooms that have been exposed to UVB radiation during the growing process are a good plant-based source of vitamin D_2.[45] Similarly, the environment that fish has been reared in can have an effect not only on the amount of vitamin D found in the tissue, but also the type of vitamin D. Wild salmon contains more vitamin D_3 than farmed salmon, which may contain more vitamin D_2. Farmed oily fish also contains vitamin D_2 instead of the more bioavailable vitamin D_3.[36] Farmed salmon has been shown to contain only a quarter of vitamin D found in wild salmon, while other oily farmed fish have half the vitamin D when compared to wild salmon.[36] When it comes to cooking oily fish, the method employed can also affect the vitamin D content of the end product. Microwaving, baking and steaming all have little effect on the vitamin D content but when oily fish is fried in vegetable oil, half of the vitamin D can be lost.[36]

While oily fish is an excellent source of vitamin D, consumption is not always preferred due to high levels of mercury and carcinogenic compounds found in the muscle tissue of certain fish which can lead to neurotoxicity.[6] Fortification of staple foods is another way to increase

vitamin D in the diet.[45] Certain foods can be fortified with vitamin D, although often, as found by Chen et al, labels can inaccurately state (and usually overestimate) the amount of vitamin D in the product.[36]

While the preferred intake of any micronutrient is dietary sources, when these are scarce, or in the case of vitamin D, not sufficient, supplementation is an alternative way of meeting nutritional needs. Many studies have demonstrated a link between vitamin D supplementation and the increase in circulating 25(OH)D, as well as the decreased risk of bone fractures.[45]

Similar to calcium supplementation, the type of vitamin D supplemented is crucial for optimal absorption. Orally supplemented vitamin D_3 is more effective at increasing serum 25(OH)D levels than vitamin D_2.[48] Some studies have also shown that vitamin D_2 supplementation can suppress 25(OH)D_3 synthesis in the body, and as such, it is apparent that there is a clear distinction between supplementation efficacy in the two forms of Vitamin D, with vitamin D_3 supplementation being superior.[48]

2.2.4.1. Effect of vitamin D supplementation

A meta-analysis of 13 articles documenting the effects of vitamin D supplementation on muscle strength and balance in patients over 60 years old found that supplementing 800 to 1000IU of vitamin D daily improved both muscle strength and overall balance.[49] Many studies have shown that supplementing vitamin D to prevent bone fractures is dose dependent (Table 2.5).[45,50,51]

Table 2.5. Effect of oral vitamin D supplementation on health

Study and Reference	Study type and population	Outcomes	Amount supplemented	Effect of supplementation
Bischoff-Ferrari, 2009 [50]	Meta-analysis of RCTs (n=20) Patients ≥65 years	Efficacy of vitamin D supplementation to reduce fracture risk	Low dose <400IU and high dose >400IU	Fracture prevention was dose dependant and higher dosages of supplemental vitamin D should reduce risk of fractures (20% reduction)
Muir, 2011 [49]	Meta-analysis (n=13) Patients ≥ 60 years	Effects of supplementation on muscle strength and balance	800 -1000IU	Improved muscle strength and overall balance
Bischoff-Ferrari, 2012 [51]	Meta-analysis, double bind RCTs n=12 (≥ 65 years)	Effects of actual vitamin D supplementation on fracture risk	0-2000IU	High dose (>800IU/day) may reduce the risk of hip fractures
Woman's Health Initiative, Prentice, 2013 [52]	Double blind RCT Post-menopausal women	Effects of calcium and vitamin D supplementation on cancer risk and bone health	1000mg Calcium + 400IU Vitamin D_3	Increases bone density but does not reduce fracture risk
Bjelakovic, 2014 [53]	Meta-analysis n=159 RCTs (18-70years of age)	Beneficial and harmful effects of vitamin D supplementation on mortality	400-2000IU daily (D_3) 200-10000IU daily (D_2)	Vitamin D_3 supplementation reduces mortality in elderly

Abbreviation: RCT: randomised controlled trial; IU: International units

It is important to note that just as calcium requires vitamin D to work effectively, the same is true for vitamin D requiring adequate calcium levels to function optimally.[45] This phenomenon becomes contradictory when looking at micronutrient supplementation and its effect on parathyroid hormone (PTH) levels. While vitamin D lowers PTH levels, calcium also lowers PTH levels, independently of vitamin D, and is equally responsible for bone

health.[45] Perhaps a level of symbiosis is achieved between vitamin D and calcium in the case of PTH levels.

With regards to supplementation, individual reactions can differ vastly which means that one recommendation for the whole population might not be effective.[31] Waterhouse investigated the role of genetics in 25(OH)D levels.[31] The large study was conducted in Australia on elderly adults over summer, and considered diet, sun exposure, supplementation, anthropometry, lifestyle habits, and genetics. With regard to genes, they found that seven genes were associated with 25(OH)D levels, and not all people had the same gene expression.[31] Oral vitamin D supplementation may need a more comprehensive and inclusive holistic approach to be effective.

For every 100IU of vitamin D ingested, the serum 25(OH)D concentration increases by 1µg/l.[43,54] A study concluded that to raise serum 25(OH)D levels to sufficient ranges, external supplementation of 1500 – 2000 IU daily needs to take place – far more than is currently recommended.[43] In children with deficiencies, studies have shown that 2000IU vitamin D supplemented daily over a year has raised 25(OH)D levels into the sufficient range.[43] Waterhouse et al found, however, that as serum 25(OH)D levels increase, the body's response to supplementation decreases.[31]

With these higher recommended supplementation levels, toxicity becomes a concern. While the concern regarding vitamin D toxicity is valid, true vitamin D toxicity is rare and difficult to reach.[43] The effects of toxicity are often only observed when 25(OH)D serum levels exceed 200µg/l and this is often the result of vitamin D ingested in excess of 10 000IU per day over a period of months.[43]

With regard to the side effects of high dose vitamin D supplementation, a study conducted on many postmenopausal women found that when supplementing calcium and vitamin D together, there was an increase in renal calculi. The researchers were, however, unable to determine if the calcium or the vitamin D supplementation was the cause.[6]

2.2.5. Sunlight exposure

It has been widely acknowledged that in order for the skin to produce vitamin D_3, it needs to be exposed to UVB radiation. The best time for the skin to be exposed to the correct wave length is between 10am and 3pm.[43] Unfortunately, this is also the time that many cancer associations suggest skin remains the least exposed to sunlight, as 10am – 3pm is when the risk of skin damage that can lead to skin cancer is highest.[43] There is some data that suggests that exposure to morning or late afternoon sunlight, when the UVB exposure

index is less dangerous, is sufficient to produce adequate vitamin D_3 but more research is needed to confirm this.[6]

CANSA released a statement in 2016 regarding vitamin D and sun exposure, stating that most people can meet their daily vitamin D needs by following a balanced diet rich in vitamin D and getting some sunlight exposure. It does not, however, specify the amount of time the skin needs in the sun to produce vitamin D, nor does it differentiate between sun exposure times for different skin tones. It also fails to quantify whether exposure to sun earlier or later in the day can produce enough vitamin D_3 needed for good health.[55]

2.2.6. Sunscreen use

The harmful effects of both UVA and UVB radiation exposure on the skin have been well documented. Excessive exposure to these elements can lead to sunburn, DNA damage, aging, and skin cancers.[56,57] Sunscreen, among other things, is an effective way to counter these effects while still enjoying sunlight. A review by Burnett et al sought to determine the efficacy of sunscreens as well as their biological effects.[56] They reported that sunscreen inhibited vitamin D production in the skin and suppressed 25(OH)D levels.[56] The data from the US National Health and Nutrition Examination Survey (2003-2006), however, found no statistically significant difference in serum 25(OH)D levels in those who wore sunscreen as opposed to those who did not.[58]

To play a significant role in decreasing vitamin D_3 synthesis, sunscreen would have to block 100% of UVB rays. The highest SPF only absorbs 99% of UVB radiation and as such, vitamin D_3 synthesis should be possible even when sunscreen is applied accurately.[58]

However, people often apply much less than the recommended amount of sunscreen, thereby reducing the SPF power. People also do not often heed the application instructions, and when they do apply sunscreen, it may give them a false sense of security, encouraging them to spend more time in the sun.[6] This may give insight as to why the theoretical assumption that sunscreen decreases vitamin D_3 production has not yet been proven.

2.3. Vitamin D Assessment

2.3.1. Assessment techniques

The generally accepted indictor of vitamin D status is serum 25(OH)D.[28,45,59] $1,25(OH)_2D$ levels are preferred to diagnose renal disorders and disorders of vitamin D metabolism.[43] $1,25(OH)_2D$ is not an ideal indicator of overall vitamin D status, however, as it has a short

half-life, whereas 25(OH)D has a longer half-life of 2 weeks. $1,25(OH)_2D$ levels can also be normal or even raised when vitamin D deficiency is present.[45]

There is, however, some confusion regarding how best to analyse this 25(OH)D, as many assays are available, and each has their own advantages and disadvantages.[45] Because vitamin D is a fat-soluble nutrient, many compounds that can interfere with results are present. This makes it difficult to get an accurate, reliable method that will successfully report solely on 25(OH)D levels. One study found that results can also differ widely depending on many variable characteristics of the laboratory and their preferred method used.[60]

Common assessment methods include the Nichols automated assay, liquid chromatography-tandem mass spectrometry (LC-MS/MS), radioimmunoassay (RIA), and automated chemiluminescence-based immunoassay.[61,62]
The Vitamin D Quality Assessment Scheme (DEQAS) is an international, regulatory body that strives to ensure analytical reliability when it comes to testing serum 25(OH)D and $1,25(OH)_2D$. Carter et al looked at the data collected by DEQAS and reported that while the Nichols automated assay gave higher results than other assays; this method also gave the most consistent results.[61]
For the assessment of $25(OH)D_3$, Ouweland et al compared LC-MS/MS to RIA and ECLIA and found that the results from LC-MS/MS compared best to the results from RIA.[62] also found that the LC-MS/MS method to be the most efficient when it came to both reagent costs and labour.

According to the literature, the most widely accepted method for measuring serum 25(OH)D levels is LC-MS/MS.[63-65]

2.3.2. Normal serum values

There is much controversy surrounding the optimal serum values of 25(OH)D. Normal circulating 25(OH)D levels are varied, with anything between 25-200nmol/L being considered sufficient for health. Many studies have used the recommendation that in order to support bone and mineral health, a minimum of 50nmol/l must be maintained.[16,31,66,67] Other studies have argued that serum levels between 75-110nmol/l are needed for optimal bone health and cancer prevention.[26,28] Further studies have concluded that serum 25(OH)D levels should be maintained at a minimum of 60nmol/l to reduce fracture risk.[31] 25(OH)D serum levels of above 28ng/ml have also been associated with a decreased risk in cancer, mental disorders, and non-communicable diseases.[45]

Looking at reports from health committees, there is debate here as well. The Institute of Medicine released a report in 2011 with vitamin D guidelines for bone health in populations in the United States of America (USA) and Canada.[67] The report states that a serum 25(OH)D level of at least 20ng/ml will meet the daily needs of 97.5% of the population.[67] They suggest that this is achievable through the dietary intake of 600IU of vitamin D per day.

In contrast, the Endocrine Society also released a report in 2011 with guidelines to treat and prevent vitamin D deficiency in patients who are at risk of inadequacy. These guidelines state much higher serum 25(OH) levels for sufficiency as well as higher recommended daily requirements of up to 2000IU per day to meet the basic needs of those who have been found deficient.[46] Table 2.6 shows the 25(OH)D interpretation from each committee.

Table 2.6. Optimal serum 25-hydroxyvitamin D (25(OH)D) levels

Interpretation	Endocrine Society[46]	Institute of Medicine[67]
Deficiency	<20ng/ml (<50nmol/l)	<12ng/ml (<30nmol/l)
Insufficiency	20 – 29ng/ml (50-72.5nmol/l)	12-20ng/ml (30-50mnol/l)
Sufficiency	30-60ng/ml (75-150nmol/l)	>20ng/ml (>50nmol/l)
Toxicity	>150ng/ml (>374nmol/l)	>50ng/ml (>125nmol/l)

The IOM responded to the report released by the Endocrine Society, condemning them for being overcautious with screening groups and the level of 25(OH)D which they considered deficient when, in their opinion, the current evidence did not support this.[68] The IOM also took issue with the Endocrine Society for recommending that the same deficiency definition for at risk populations is being recommended for the general population.[68]

It is important to remember that these bodies were commissioned to provide guidelines for two different sets of populations, and the intent of the reports need to be kept in mind when using their guidelines.

2.4. Prevalence of Deficiency

2.4.1. Risk factors for deficiency and insufficiency

Traditionally, pregnant women, children, the elderly, and the institutionalised have formed part of the risk groups associated with low serum 25(OH)D levels.[26,45] As humanity has evolved technologically, we have moved away from the natural exposure of our skin to sunlight. Deskbound jobs, indoor activities, and prominent changes in clothing over the

centuries have resulted in our skin being less exposed to the essential UVB radiation needed to produce the all-important vitamin D_3.[26]
The environment can also play a vital role in cutaneous vitamin D production. Air pollution can block UVB rays and in regions with a thicker ozone layer, less UVB radiation will reach the skin.[45]

The elderly are more at risk for deficiency, not only because they spend less time exposed to natural UVB radiation but also because of the skin changes that occur over time. As the skin ages, there is less 7-DHC present in the skin, and as this is essential for the production of vitamin D_3, there is less produced in the elderly.[45]
Other individuals who are particularly at risk, are those with absorption disorders like bowel diseases, cystic fibrosis, diseases of the liver and kidneys, and those on certain medications, such as antiretrovirals.[69-71] Risk factors for the development of vitamin D deficiency and insufficiency include melanin levels, sunscreen use, cultural clothing norms, reduced UVB exposure, and a diet low in vitamin D.[28]
Smoking is also a risk factor for vitamin D deficiency as smoking can lower vitamin D levels, with one study finding that non-smokers and ex-smokers have higher levels of serum 25(OH)D than their smoking counterparts.[18]

2.4.2. Global prevalence

Vitamin D deficiency has been recently recognised as a global pandemic. In India, a study found that 70% of the population had vitamin D levels that were either insufficient or deficient.[72] A systematic review by van Schoor et al found that globally, the vitamin D status of healthy adults is low. The study observed that an insufficiency in serum vitamin D is especially prevalent in adults residing in the Middle East and Asia.[28]
In Europe, this study found serum 25(OH)D levels are influenced by latitude and skin tone, and 25(OH)D levels are higher in Northern and Western Europe than Southern and Eastern Europe.[28] While the high levels of 25(OH)D in Northern Europe might seem strange due to the higher latitude which is usually associated with lower UVB exposure, the Northern European countries usually have a higher dietary intake of vitamin D in the form of oily fish. The lower serum 25(OH)D levels in Southern Europe may be due to higher melanin levels in the skin.[28]

Table 2.7 shows the prevalence of vitamin D deficiency globally. Despite the sunny and warm climates of Australasia, suboptimal 25(OH)D levels are common.[18,25]

Table 2.7. Global prevalence of vitamin D deficiency

Region and year	Study size	Serum 25(OH)D classified as deficient	Prevalence
Hawaii, 2007 [40]	n=93 (Young adults; mean age 24.0)	Low vitamin D status <30ng/ml	51% low vitamin D status
Canada, 2011 [73]	n=1912 (middle aged adults)	Suboptimal <75nmol/l Deficiency <50nmol/l	20% insufficient and/or deficient 50% suboptimal levels
Australia, 2012 [25]	n=11 247 (healthy adults)	Deficiency <50nmol/l Suboptimal <75nmol/l	31% deficiency 73% suboptimal
South Australia, 2014 [18]	n=126 (healthy adults)	Suboptimal <75nmol/l Deficiency <50nmol/l	0.9% deficiency 38.5% insufficient

Abbreviation: 25(OH)D: 25-hydroxyvitamin D

Insufficiency is also high in Canada, and Hawaii. Those who live in Hawaii have abundant exposure to natural UVB radiation and the varying levels of vitamin D status found in the Binkley study are interesting.[40] This could mean that other factors play a more prominent role in vitamin D levels than just sunlight exposure. It might also mean that the widely accepted recommendation of 15 minutes of sun on hands and face to reduce skin cancer risk might not be sufficient to promote adequate vitamin D synthesis and good health.[40]

2.4.3. South African prevalence

South Africa has a diverse population when it comes to skin tone and ethnicities.[74] South Africa is also diverse when it comes to weather patterns and seasonal variations, which could potentially affect serum vitamin D levels. Johannesburg has a higher latitude and a sun-rich winter, as opposed to Cape Town, which has wet winters that scarcely sees sunlight.[42] This difference could result in Capetonians experiencing a greater seasonal change in their serum 25(OH)D levels.[42]

Table 2.8 summarises the studies that have been conducted to investigate the prevalence of vitamin D deficiency in South Africa. While many studies investigating vitamin D status

in South Africa have been done, few have been conducted on healthy adults – most studies investigate specific diseased states and/or children.[44]

Table 2.8. Prevalence of vitamin D deficiency in South Africa

Region and year	Study size	Serum 25(OH)D classified as deficient	Prevalence
Cape Town, 2011 [75]	n=370 (196 HIV-uninfected, 174 HIV-infected)	Deficiency <50nmol/l	Deficiency: 62.7%
Johannesburg, 2011 [32]	n=385 (Urban children)	Deficiency <50nmolL Insufficiency 50-74nmol/l	Deficiency: 7% Insufficiency: 19%
Soweto, 2013 [76]	n=247 (98 HIV neg, 74 HIV-positive non-ARV's, 75 HIV-positive, pre-ARV)	Deficiency <50nmol/l	Deficiency: 26.5% overall (26.5% HIV-negative; 29.7% non-ARV; 33.3% pre-ARV)
Johannesburg, 2014 [19]	730 (adults, African and Asian-Indian)	Deficiency <30nmol/l Insufficiency 30-49.9nmol/l	Deficiency: 28.6% Asian-Indian; 5% Africa
Cape Town, 2015 [74]	n=100 (young adults 18-24 years)	Deficiency <50nmol/l Severe deficiency >30nmol/l	Severe deficiency: 18% Xhosa; 12% Cape mixed race Deficiency: 33% (winter) 56% (summer) Xhosa; 70% (winter) 16% (summer) Cape mixed race

Abbreviation: 25(OH)D: 25-hydroxyvitamin D; HIV: human immunodeficiency virus; ARV: Antiretroviral treatment

The cohort study by Poopedi et al on urban children in Johannesburg, found that lighter skinned children experienced seasonal variations in their serum 25(OH)D levels whereas their darker skinned counterparts did not.[32] The researchers also found that the majority of their study population was found to have sufficient vitamin D levels.[32] This finding could be due to the fact that Johannesburg has abundant daily sunshine throughout the year. The study was also done on children who lived in urban homes with gardens, allowing them the opportunity to be exposed to the sun when they were at home. Vitamin D status results may be different in those living in flats with minimal access to sunlight exposure when at home.[32] This study also supported findings that females are more likely to experience a vitamin D deficiency than males.

As part of a Master of Medicine degree, Roberg sought to determine the vitamin D levels of the adult patients in a private practice in Johannesburg.[33] They found that almost three quarters of patients included in the study had a vitamin D deficiency.[33] Roberg used lower serum 25(OH)D cut off values (normal >30ng/ml; moderate deficiency 10-29.9ng/ml; severe deficiency <10ng/ml) to interpret the results, and if the higher standard cut offs described in other studies were used, the percentage of deficiency might even be higher. This is interesting as previous studies have not found as high a prevalence of vitamin D deficiencies in the Johannesburg area, particularly because of the climate which results in a high degree of sun exposure year-round.[32]

Roberg also found that darker skinned participants were more likely to be found deficient, although this study found people of Indian ethnicity to be the most likely of deficiency, even though their skin tone can be lighter than those of black ethnicity.[33] This could be due to the cultural and religious clothing choices in the Indian culture. The study sample was also not equally distributed amongst racial groups, with the black race underrepresented. Another study conducted in Johannesburg by George et al found that African participants had higher 25(OH)D levels when compared to Asian and Indian participants.[19] The participants of dark ethnic background were found to be the least deficient in this study.

2.5. Vitamin D in Health and Disease

2.5.1. Vitamin D and its effect on health

Once thought to be the only function of vitamin D, the regulation of calcium, phosphorous and PTH in the body is one of the vital roles vitamin D plays. The correct balance of calcium and phosphorous in the body is crucial for bone health, and vitamin D plays a huge role in skeletal health.[77] When $1,25(OH)_2D$ binds with its receptor in the small intestine, intestinal calcium absorption is increased by approximately 20-25% and intestinal phosphorous absorption is increased by 20%.[45] $1,25(OH)_2D$ also encourages the reabsorption of calcium from the glomerular filtrate in the kidneys.[77]

Vitamin D receptors (VDR) are needed to activate and regulate $1,25(OH)_2D$ and are found throughout the body. This substantiates evidence that vitamin D plays a much bigger role in the body than just skeletal health.[77] $1,25(OH)_2D$ regulates itself by stimulating the expression of 25(OH)D-24-hydroxylase in the kidneys, which metabolises $1,25(OH)_2D$ and 25(OH)D to inactive forms which are excreted in the bile.[77,78]

It is clear that the kidneys are the main regulators of $1,25(OH)_2D$ and 25(OH)D in the body. Extrarenal synthesis of $1,25(OH)_2D$ is also possible, however.[78] There is evidence that

nonrenal tissues, such as bone, placenta, and certain cancer cells, as well as brain, prostate, and breast tissue also contain VDR and can actively convert 25(OH)D to 1,25$(OH)_2$D, independently of renal involvement.[45,78] This success of this process is dependent on the availability of 25(OH)D circulating in the blood, however, and shows once again how valuable the correct serum levels of vitamin D are. [35,78]

Chronic insufficient levels of vitamin D in adults have been linked to diseases of the bone and bone weakening.[27] Proximal muscle weakness, and the resulting bone fractures that arise from this weakness, are common when vitamin D levels are low.[45]

2.5.2. Vitamin D and its role in disease

While there is a definite proven causal link between vitamin D and bone health, the strong causal links between vitamin D and other diseases, specifically extra skeletal diseases, is controversial.[21] An insufficiency of vitamin D in early life may increase the risk of mental health disorders and diabetes later in life.[45] Low serum 25(OH)D levels have been associated with an increased risk of cancer, infections, cardiovascular diseases, and autoimmune diseases as well as mental health and mood disorders, allergies, and complications during pregnancy.[27,54]

2.5.2.1. Immunity

Vitamin D has been linked to increased innate immunity, through the expression of VDR-containing cells in the innate and adaptive immune systems, as well as the prevention of certain autoimmune diseases.[21] Vitamin D regulates the innate immune system and balances the differentiation and activity of T-helper cells (Th1 and Th2) and through this is thought to supress autoimmune disease pathways. Specific innate immune modulatory cells, such as macrophages and dendritic cells, also contain VDR, and have the ability to convert circulating 25$(OH)D_3$ to 1,25$(OH)_2$D which plays a role in antigen binding. VDR are also present in those cells of the body involved in barrier protection (skin, lungs, GIT, etc.) and low circulating levels of 25(OH)D may play a role in the development of infections, such as tuberculosis.[21]

2.5.2.2. Cancer

As many as 2000 genes have been estimated to be influenced either directly or indirectly by vitamin D status.[79] An improvement in serum vitamin D levels will greatly affect the expression of genes that in turn have an effect on the development of certain diseases, such as cancers and cardiovascular disease (CVD). Recently, vitamin D deficiency has been linked to an increased risk for certain cancers. It has been suggested that the possible

mechanism of action for this revolves around the conversion of 25(OH)D to 1,25$(OH)_2$D in the tissues where cancers occur.[79] When this conversion happens in healthy colon, breast, and prostate cells, it can help prevent cancerous cell formation through the control of cellular proliferation. As mentioned above, the amount of 1,25$(OH)_2$D converted in extrarenal tissue depends on the circulating levels of 25(OH)D. If these are not high enough, the conversion cannot take place, and cancerous cell formation might not be controlled as effectively.

CYP3A4 is a vitamin D regulated gene that releases a protein which detoxifies lithocholic acid.[79] This bile acid is thought to play a role in colon cancer as it damages the DNA of intestinal cells. The stimulation of the gene that releases a detoxifying enzyme to counteract this DNA damage could explain the reasoning behind the protective effects that vitamin D has against colon cancer.

A meta-analysis by Chung et al found mixed results regarding the role vitamin D plays in cancer, however. The RCTs they analysed found that vitamin D supplementation reduced the risk of cancer but the observational studies they included stated that increased serum levels of 25(OH)D might also be linked to an increased risk in cancer.[80] The authors found that while the reduction in risk for colorectal cancer was inversely proportional to serum 25(OH)D levels, there was no significant dose response for breast and prostate cancer.[80] They concluded that there was not enough evidence regarding the positive and negative outcomes of vitamin D supplementation in the prevention of cancer.[80]

A meta-analysis that included the Physicians' Health Study found that there was a stronger inverse link between serum 25(OH)D levels and rectal cancer than with colon cancer.[23] An explanation for the weaker association might have been that the studies included only collected vitamin D blood samples in the summer months, and did not take into account the potential lower serum levels in the winter months.[23]

2.5.2.3. Mortality

A study by Durup et al found that higher levels of serum 25(OH)D were associated with a reduced risk in mortality up to a point.[81] They also found that if the serum levels of 25(OH)D were too high, the risk of mortality increased again. While some studies have found that supplementing vitamin D alone can reduce the risk of mortality, others found that mortality risk is only decreased when vitamin D is supplemented in conjunction with calcium.[81]

2.5.2.4. Cardiovascular Disease

Vitamin D, due to VDR and vitamin D activating enzyme found in the cardiac cells, can play a role in maintaining healthy cardiovascular function.[21] In rat trials, a deficiency of vitamin

D in the body has been linked to increased blood pressure, cardiac hypertrophy, congestive heart failure, and coronary artery disease.[21]

A study done on diabetic female outpatients found that low circulating vitamin D levels (<20ng/ml) were associated with higher levels of cardiovascular health indictors (A1C, triglycerides, CRP, fibrinogen) when compared normal circulating levels of 25(OH)D.[82] They also found that patients who had lower vitamin D levels were more likely to need lipid and insulin-lowering drugs.

It has been postulated that seasonal variations in serum 25(OH)D levels may be responsible for increased cardiovascular disease events observed during winter.[82,83] McCarty and Mark found that vitamin D lowers inflammatory markers and may explain why elderly patients have higher levels of CVD and inflammatory markers in the winter months.[83] In a study that investigated the effects of short term vitamin D supplementation on inflammatory cytokines after a myocardial infarction, they found that even in the short term, supplementation with vitamin D can lower levels of inflammatory cytokines.[84] Arnson concluded that although more study is needed, there is evidence to suggest that vitamin D has an anti-inflammatory effect and through this, contains protective cardiovascular properties.[84]

2.5.2.5. Diabetes Mellitus

In patients with both type 1 and type 2 diabetes, low serum 25(OH)D levels are common.[85] Low 25(OH)D levels are also associated with increased blood glucose levels in both diabetic and non-diabetic patients.[85] Vitamin D is thought to alter glucose metabolism in many ways. Firstly, the anti-inflammatory and immunomodulatory properties of vitamin D could protect the body from the low-grade inflammation that has been postulated as a possible cause of diabetes.[85] When serum levels of vitamin D are low, PTH is increased. These higher levels of PTH are thought to reduce the ability of the βcells in the pancreas from releasing insulin. A systematic review by George et al found that vitamin D supplementation had a small effect on fasting glucose and insulin resistance in patients with impaired glucose tolerance, but the same could not be said for those with normal glucose tolerance.[85] They also found that supplementation had no significant effect on HbA_{1C} levels.

Circulating 25(OH)D levels have been inversely associated with hyperglycaemia in type 2 diabetics.[21] This association gets stronger in the winter months, when vitamin D levels are lower and hyperglycaemic levels are higher. Sufficient vitamin D levels may also reduce the risk of developing type 2 diabetes. The results from Christakos are based on mostly

observational studies, however, and conclusions drawn from this need to be made with caution.[21]

2.5.2.6. Neurological Diseases

Many studies have indicated a link between depression and low serums values of 25(OH)D.[86]

Vitamin D has been linked to many neurological disorders, including depression and schizophrenia.[87] This may be due to the presence of vitamin D receptors in the brain and the ability of these receptors to produce $1,25(OH)_2D$ from circulating 25(OH)D, independently of renal processes.[86]

In a case control study by Umahu et al, the researchers investigated low serum 25(OH)D and suicide risks in US military. They found that the lower the level of serum 25(OH)D, the higher the risk of suicide.[87] Of the suicides committed, 33.5% had 25(OH)D below 20ng/ml.

The protective properties of vitamin D are also observed in neurological tissue. A diet rich in vitamin D_3 has been linked to a decrease in inflammation in the brain as well as an increased clearance of amyloid plaques. Because of this, it has been thought that an increase of vitamin D_3 in the diet can help reduce the risk of Alzheimer's disease as well as depression.[45]

2.5.2.7. Vitamin as an anti-inflammatory modulator

As seen above, vitamin D may play a role in inflammation in the body. In animal studies, it has been found that vitamin D supplementation lowers inflammatory cytokines and can improve cognitive function in fatty liver disease.[88] An experimental study by Adzemovic et al observed less severe inflammatory responses in rats with multiple sclerosis when supplementation took place before adulthood.[89]

In human studies, the relationship between 25(OH)D and inflammation is inconclusive. Whether vitamin D is an anti-inflammatory in its own right, or if inflammation reduces 25(OH)D levels, is unknown.[90] Amer et al examined the relationship between 25(OH)D levels and CRP levels in CVD patients and found that when 25(OH)D dropped below 53nmol/ml, CRP levels increased.[91] When 25(OH)D was above 53nml/ml, there was no statistically significant relationship between CPR and 25(OH)D, however, and the researchers concluded that vitamin D supplementation is only beneficial to inflammation when serum 25(OH)D levels are low.[91] Bellia et al found that in obese patients, CPR, IL-6 and TNF were all inversely related to 25(HO)D levels.[92]

2.5.2.8. Infectious diseases

Vitamin D deficiency has been linked to the development of communicable diseases, such as tuberculosis (TB) and HIV.[71,74] In a study conducted on healthy young adults in Cape Town, they investigated if vitamin D status and supplementation influenced the development of HIV. The researchers found that the darker skin tone participants had higher serum 25(OH)D levels in the summer months, while they found no significant difference between 25(OH)D levels in the different skin tones during the winter season.[74] This study also found that women were more likely to have lower levels of serum vitamin D than men. It was found that oral supplementation in winter decreased HIV infection progression. The authors suggested that vitamin D supplementation could be a cost-effective way to decrease HIV progression, especially in the early stages of infection.[74]
A study in Soweto done on HIV-negative and -positive women found that HIV infection and ARV use was not associated with lower 25(OH)D levels. Most participants had sufficient levels of 25(OH)D in their blood.[76]

In a study done on TB patients, the researchers reported that the prevalence of severe vitamin D deficiencies in patients with TB is much lower in tropical Africa than it is in Europe.[71] A study by Martineau sought to investigate the seasonal variations of vitamin D deficiency among TB sufferers in Cape Town. They found that regardless of whether the TB patient was infected with HIV, vitamin D deficiency was rife.[71] A stronger link was found between vitamin D deficiency and TB infection in those patients who had already been infected with HIV than those who were HIV-negative.[71] The study also observed seasonal variations in TB infection, along with variations in 25(OH)D levels, and found in seasons where 25(OH)D was lower, TB infection rates were higher. This suggests that vitamin D deficiency may leave the body more susceptible to TB infection.

2.5.3. Management of deficiency and insufficiency

As described above, vitamin D inadequacy could potentially cause numerous unfavourable conditions. Globally and locally, the literature has shown that the prevalence of insufficiency and deficiency is high. It is therefore imperative to have guidelines for how to restore vitamin D levels.

Table 2.9 shows the recommended daily intakes of vitamin D for healthy populations as recommended by various health committees.

Table 2.9. Vitamin D Recommended Daily Dietary Intakes

Deciding Board, Year	Infants	Children	Adults	Pregnancy and lactation	Elderly
Institute of Medicine, Food and Nutrition Board, 2010 [67]	400IU (10mcg)	**1-18 years:** 600IU (15mcg)	600IU (15mcg)	600IU (15mcg)	**>70 years**: 800IU (20mcg)
UK; Scientific Advisory Committee on Nutrition, 2016 [93]	**0-11months:** 340-400IU (8.5-10mcg)	**1-18 years**: 400IU (10mcg)	400IU (10mcg)	400IU (10mcg)	Unspecified
World Health Organization/Food and Food and Agriculture Organization of the UN [94]	200IU (5mcg)	200IU (5mcg)	200IU (5mcg)	200IU (5mcg)	**51-65 years:** 400IU (10mcg) **>65 years:** 600IU (15mcg)

Abbreviation: IU: International Units

The Endocrine Society suggestion for the management of vitamin D deficiency and insufficiency can be seen in Figure 2.2.[39]

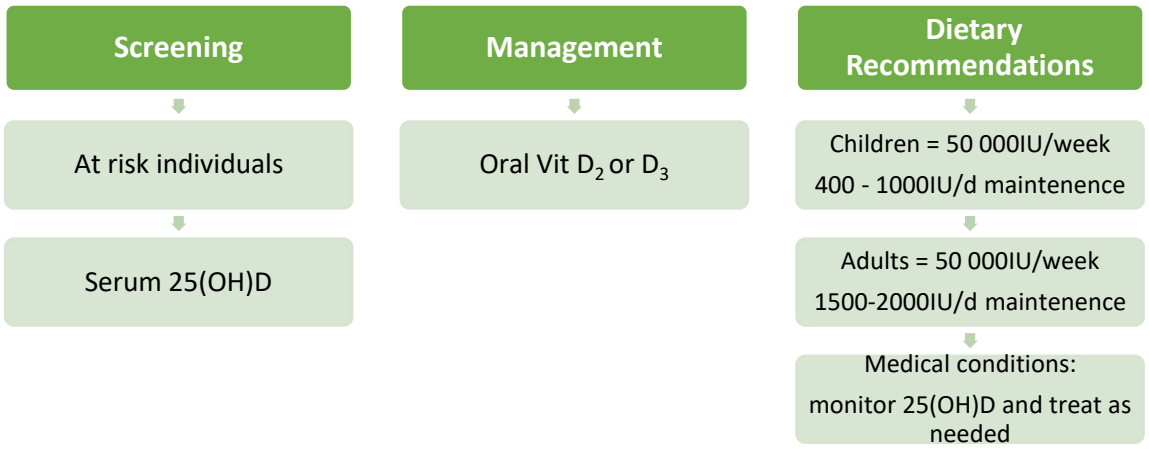

Fig 2.2. Management of vitamin D deficiency and insufficiency[46]

Abbreviation: 25(OH)D: 25-hydroxyvitamin D; Vit: vitamin

They suggest that screening complete populations is unnecessary and costly at this stage and that only those who are at risk should be screened for suboptimal 25(OH)D levels. Those who are at risk include those with bone health problems, absorptive problems, hyperparathyroidism, renal and hepatic failure, and the elderly. Recommendations for treatment of those with serum 25(OH)D levels <50nmol/l include the oral supplementation of either vitamin D_2 or D_3 in weekly high doses until serum levels improve, followed by a daily maintenance supplement.[46]

2.6. Motivation for study

Based on the literature, it can be expected that the prevalence of vitamin D insufficiency and deficiency will be increased in individuals with darker skin tones, and that individuals with lighter skin tones will have a greater seasonal variation in serum 25(OH)D levels. It is also expected that the prevalence of vitamin D deficiency after the winter season will be high.

Based on this, as well as South Africa's high level of darker skin tones, there is a good possibility that many participants in this study will be found to experience low serum 25(OH)D levels. Latitude and seasonal sun exposure also play a role in both the amount of sun one is exposed to and the effectiveness of this exposure. As Cape Town has a lower latitude, it is expected that the levels of 25(OH)D in the study group (based in Cape Town) will be lower than in other parts of the country. The data collection period was conducted post winter, and as the Cape Town climate results in intermittent sun exposure in the winter months, lower levels of 25(OH)D are also expected.

Vitamin D deficiency has been linked to many adverse health conditions (diabetes, CVD, cancer) and in a country where the healthcare system is already overburdened, investigating a way to potentially ease the burden could have positive effects on the country.

There is, however, always a possibility that the study will not yield the expected results. In the event that the study population are found to have an adequate vitamin D status, the results of this study will still be valuable and can have numerous applications. Researchers in areas where vitamin D deficiency is high may use these results to further investigate the reasons behind the good vitamin D status in these populations, and from this, develop their own prevention and treatment interventions. This study can be used as a baseline to many, more intensive studies surrounding the causes, relationships, and associations regarding vitamin D, sun exposure, and skin tones, as well as clinical trials using vitamin D supplementation to prevent, treat, and inhibit the progression of cancer. There is great emphasis placed on vitamin deficiencies in populations with chronic and debilitating diseases, with the healthy population falling through the cracks. This study aims to quantify whether the vitamin D status of South Africans is a cause for concern and which populations, based on demographic and dietary factors, are most at risk for poor vitamin D status.

3. Methodology

3.1. Research question

1. What is the vitamin D status of healthy young adults living in the Western Cape Province of South Africa?
2. Which factors influence serum 25-hydroxyvitmain D levels?

3.2. Aim

To determine the vitamin D status of healthy adults living in the Western Cape, South Africa

3.2.1. Objectives

1. To determine the relationship between gender and serum 25(OH)D levels

2. To determine the relationship between skin tone and serum 25(OH)D levels

3. To determine the relationship between basic anthropometrical variations and serum 25(OH)D levels

4. To determine dietary intake of Vitamin D and its relationship with serum 25(OH)D

5. To determine if lifestyle factors (smoking, alcohol use, exercise) have an effect on serum 25(OH)D levels

3.3. Hypotheses

Null hypothesis 1: Gender has no effect on serum 25(OH)D levels

Null hypothesis 2: Skin tone has no effect on serum 25(OH)D levels

Null hypothesis 3: Basic anthropometrical variations have no effect on serum 25(OH)D levels

Null hypothesis 4: Dietary intake of vitamin D has no effect on serum 25(OH)D levels

Null hypothesis 5: Lifestyle factors (smoking, alcohol, exercise) have no effect on serum 25(OH)D levels

3.4. Study Plan

3.4.1. Study outline

This Masters' study forms part of a bigger study to investigate, among other things, the differences in seasonal variations in serum 25(OH)D levels. The larger study was conducted in Cape Town, South Africa (latitude: 33°55'33", altitude: 42m) and had two phases: a baseline (6 weeks post winter solstice) and a follow-up phase (6 weeks post summer solstice) The data collected, analysed and reported on in this study was collected 6 weeks post winter solstice and, therefore, forms the baseline data for the larger study. For the purpose of this study, only the baseline data will be described.

3.4.2. Study type

Observational Descriptive Cross-Sectional Study with an Analytical Component

3.4.3. Study population

This was a single-centre study and undergraduate students from the Tygerberg Faculty of Medicine and Health Sciences (FMHS) campus of Stellenbosch University were included. The FMHS has an estimated 3000 students enrolled at the faculty and has the most diverse profile of all the faculties in the university, including students of both genders and various ethnicities and skin tones. The students on campus were assumed to have a more homogenous lifestyle, as they followed similar programmes and schedules and, therefore, this population was naturally able to minimise some confounding factors as far as possible. Postgraduate students were excluded from the study as they generally do not follow the same schedules as one another, and the profile of postgraduate students can differ significantly from undergraduate students.

3.4.3.1. Sample Size

Stratification for this study was done based on gender and skin tone. Because one of the main aims of the study was to determine the effects of skin tone, it was decided that the main strata would be skin tone, with secondary strata for gender. The size of the sample population was determined using a power calculation with 90% power for 1-Way analysis of variance (ANOVA) for continuous variables. Based on a RMSSE (root-mean-square standardised effect) of 0.35, the population size required for each stratum (male: light skin; male: dark skin; female: light skin; female: dark skin) was approximately 50 participants, bringing the total study size to 200 participants. As this study was part of a bigger study with a follow up phase, oversampling was completed during data collection for this study (the baseline stage of the bigger study) to compensate for loss to follow up ratio. The study population was oversampled by 20% bringing the final total study population size to 240 participants who were equally distributed amongst the strata.

3.4.3.2. Sample Selection

Sampling was multistage, and included a screening phase and a final selection phase. Participants were screened, based on inclusion and exclusion criteria, and those who met the inclusion criteria were stratified for skin tone and gender. Screening continued until all strata were filled and oversampled. In order to select the final study group, simple random sampling was completed within these strata to ensure equal representation of skin tone and gender in each stratum, as well as to prevent selection bias. (Figure 3.1)

3.4.3.3. Inclusion and exclusion criteria

Inclusion criteria

- Undergraduate students at the Faculty of Medicine and Health Sciences
- Participants 18 years of age or older
- Participants who were healthy (self-reported)
- English and/or Afrikaans speaking

Exclusion criteria

- Current final year students at the Faculty of Medicine and Health Sciences
- Postgraduate students
- Participants suffering from a chronic illness and/or disease that may have an impact on Vitamin D status, such as
    - Gastro-intestinal absorption disorders (Celiac disease, Crohn's disease, Cystic Fibrosis)
    - Renal disease or renal failure
    - Hepatic disease or failure
    - Osteopenia and/or osteoporosis
    - Diabetes Mellitus
- Participants taking medications that may interfere with Vitamin D mechanisms in the body such as
    - Antiepileptic drugs
    - Antineoplastic drugs
    - Antihypertensive drugs
    - Antiretroviral drugs
    - Bisphosphonates
    - Herbal therapies, such as Kava Kava and St John's wort
- Female participants that were pregnant and/or lactating at the time of the study
- Participants who did not give consent

3.5. Methods of data collection

Data collection consisted of a screening phase and a data collection phase. The screening phase was completed first and took place in August 2016 for period of 4 weeks. The data collection phase was done in September 2016 over a period of 4 weeks, and occurred directly after the period where the UVB exposure risk was the lowest in the year (6 weeks post winter solstice). Figure 3.1 shows a graphical representation of the data collection phase.

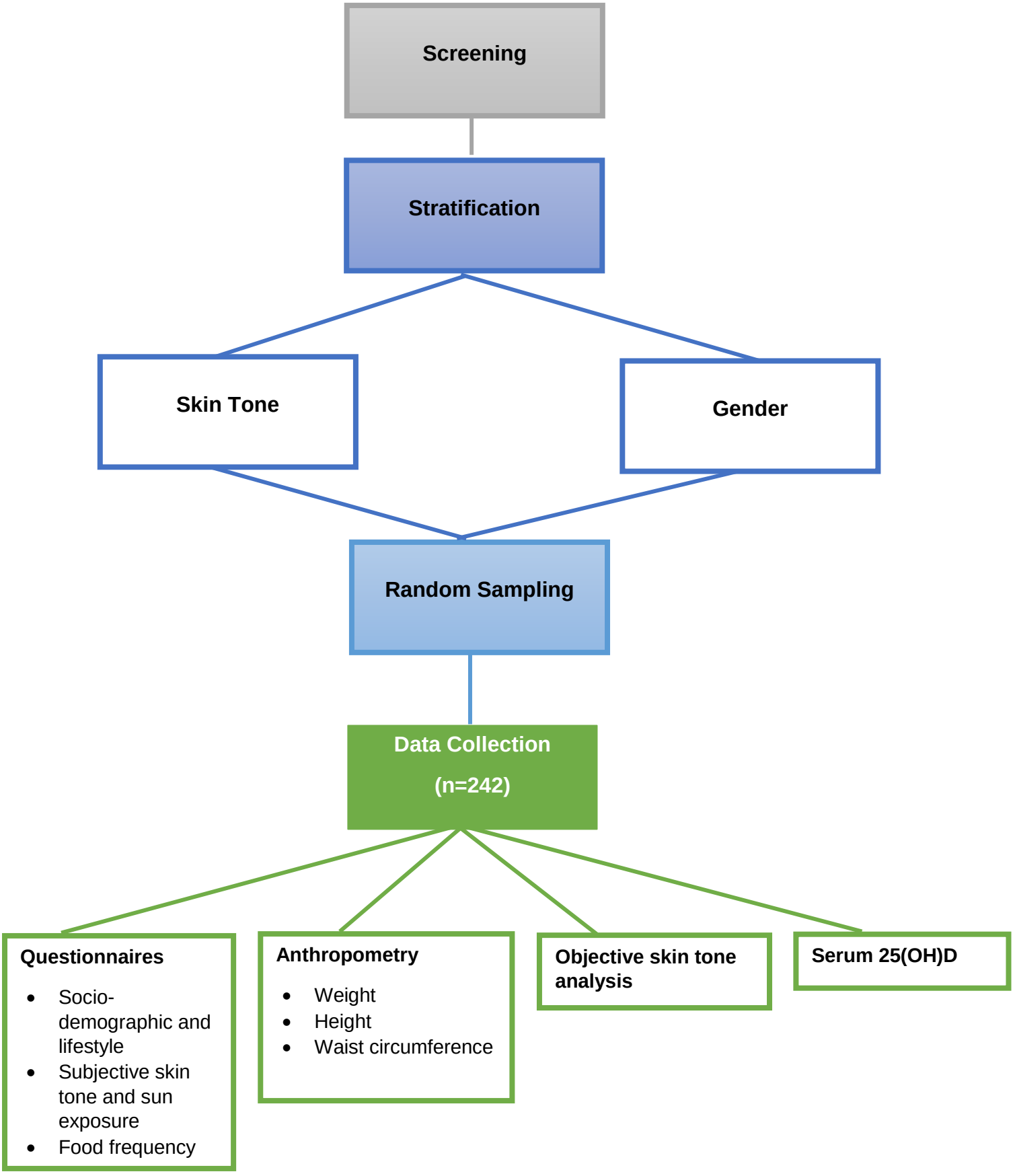

Fig 3.1. Data Collection

Abbreviation: 25(OH)D: 25-hydroxyvitamin D

3.5.1. Screening phase

Screening was done to increase the suitability of the sampling frame for the study, as well as to ensure a suitable sampling frame from which a random sample could be drawn. During this phase, participants were invited to one of two screening locations: in the cafeteria area of the Tygerberg Student Centre (TSS) between 10am – 3pm, and outside the coffee shop on the ground floor of the teaching block in the same hours. During screening, the purpose of the study and an overview of what it entails was explained to the participants. The concepts of informed consent were explained to them, and participants were given an opportunity to ask questions before they completed and signed a consent form if they were willing to participate (Addendum A). This form was completed in duplicate, with one form kept by the researchers and one given to the participant for their own records. Once consent was obtained, the participants were allocated a random three-digit number to ensure confidentiality, and a screening questionnaire was completed, along with a skin tone measurement using a skin reflectometer. The screening questionnaire (Addendum B) was used to determine participant eligibility to the study, based on inclusion and exclusion criteria. The questionnaire consisted of one section, with four questions. Participants were asked to provide their preferred language, so the correct number of questionnaires could be prepared for data collection and their preferred contact details on the screening questionnaire, so the participant could be contacted if they were selected to partake in the study, or if they had won a prize. The contact details were captured on a separate data sheet to ensure anonymity of the data collected. While the participant completed the screening questionnaire themselves, data collectors were available to help with the facilitation of the completion of the questionnaires. Data collectors also completed the skin tone analysis with each participant.

3.5.2. Data Collection for main study

Participants who were selected to take part in the main study phase were contacted for their approval, and given a suitable date and time to attend data collection. Date and time slots were given to ensure efficiency and to ease any potential congestion.

During the main data collection phase, the researchers and data collectors again explained the study and its aims to the participant, explained the concepts of voluntary participation and confidentiality, and answered any questions or concerns the participants had. A consent form was completed (addendum C) and a copy was given to the participant for their records.

A total of three questionnaires were completed by the participants during data collection: sociodemographic questionnaire, sun exposure questionnaire and skin tone questionnaire,

and food frequency questionnaire. In addition, anthropometry (weight, height, waist circumference) was done and a serum sample to determine 25(OH)D levels was collected for each participant.

Each participant spent between 25 and 50 minutes completing all phases of data collection.

3.5.2.1. Sociodemographic Questionnaire

The sociodemographic questionnaire (see Addendum D) consisted of 3 sections, each with 4 questions and took approximately 10-15 minutes to complete. The first section dealt with general sociodemographic information and helped the researchers to determine how accurately the study sample replicated the general population as well as to differentiate between subgroups (i.e. gender). The second section dealt with medical information and included questions on general health, mediation use, and oral contraceptive use. Section three gathered lifestyle information, such as exercise, smoking, and alcohol use. This section asked mainly yes/no questions and allowed participants to elaborate on frequency and amount. As these factors were not the main objectives of the study, detailed data regarding the specific type and description of the factors was not collected (e.g. type of tobacco used, type of exercise done, etc). Exercise was included as part of general lifestyle factors as it has been shown in the literature to have an impact on vitamin D levels.

3.5.2.2. Skin tone

The skin tone was determined using two ways: firstly, subjectively in the form of a self-administered skin tone questionnaire, and secondly, objectively using a handheld skin reflectometry device.

The skin tone questionnaire (Addendum E) followed the Fitzpatrick skin type classification and was self-administered. The Fitzpatrick skin typing system was developed in 1975 by Thomas Fitzpatrick and strives to determine a person's skin type based on how their skin tans and/or burns in response to sun exposure. It is commonly used in studies relating to skin cancer and UV radiation, as well as in cosmetic dermatology for estimating laser treatment dosages.[95] It can also be used to determine tolerance to chemical peels and topical bleaching agents, with darker skin tone (types IV-VI) most at risk of experiencing prolonged hyperpigmentation.[95] The self-reported skin tone questionnaire included 10 questions which asked participants mostly about their skin's reaction to the sun, as well as general genetic markers of skin tone, such as eye and hair colour.[96] Each question gave five possible answers, ranging from a score of 0 to 4. The minimum score possible was 0 and the maximum score was 40. These scores corresponded to one of six categories (Table 3.1):[95]

Table 3.1. Fitzpatrick skin type categories

Total Score	Category	Skin Colour	Description
0-6	Type I	Pale white	Always burns, never tans
7-13	Type II	White	Usually burns, minimally tans
14-20	Type III	Cream white	Sometimes mild burn, tans uniformly
21-27	Type IV	Moderate brown	Burns minimally, always tans well
28-34	Type V	Dark brown	Very rarely burns, tans very easily
35-40	Type VI	Dark brown to darkest brown	Never burns, never tans

The objective skin tone analysis was conducted using a portable handheld skin reflectometry device (DSM II ColorMeter, Cortex Technologies) and was expressed as EI (erythema index) and MI (melanin index). As no cut-offs exist for either EI or MI, the cut-offs were determined based on the screening group findings. The researchers determined the median MI value for males and females and used this to allocate participants to groups. Those participants whose MI readings measured less than the median were classified as “light” skinned and those whose MI readings measured more than the median formed part of the “dark” skinned group. Random sampling was then done to determine the final study group. The reflectometer was calibrated at the beginning and end of each day, as well as before each participant, using the supplied calibration technology. The reflectometer was placed on healthy, intact skin only. Measurements were taken by placing the probe section of the reflectometer on the skin site to be measured and pressing the measurement trigger button, recording the measurement on the device. Two skin sites were measured on each participant; one site where the skin is the darkest (forehead) and one site where the skin is the lightest (underside of the upper arm). Each measurement was completed three times, which amounted to a total of six skin tone readings taken for each participant. The average skin colour was then calculated for the MI value and this was used as the participant’s overall skin colour.

By using both the questionnaire and the reflectometer, this allowed the researchers to correlate the participants perceived subjective skin tone with the objective skin tone reading collected by the skin reflectometer device. While the reflectometer was used to stratify the

study population into groups during screening, the Fitzpatrick questionnaire was used to answer the objectives of the study.

3.5.2.3 Sun exposure

Participants were asked to complete a sun exposure questionnaire (Addendum E) which included a section on tanning bed usage, sunscreen usage, and religious and/or cultural clothing requirements that decreased cutaneous exposure to sunlight. The questionnaire also made provision for participants who had travelled to other countries in the 3 months prior to data collection. Daily sun exposure data was collected using a validated self-reported questionnaire that was adapted for the South African context. It investigated how long a participant was exposed to the sun, the amount of skin exposed, and at what time the exposure took place. The results from the sun exposure questionnaire were analysed using the rule of 9's for the amount of skin exposed to the sun, with the torso and each leg counting 18%, the arms and head as 9% and the face as 5%. This percentage was multiplied by the total amount of sun exposure per week to determine how much UVB radiation each participant is exposed to.

3.5.2.4 Anthropometry

Weight, height, and waist circumference were recorded for all participants (Addendum F). All anthropometrical measurements were taken in a private area where participant privacy and dignity was respected. A trained dietician performed all measurements. As data collectors rotated between the stations during the data collection period, all data collectors were trained in accordance with the methodology as set out in this protocol (Addendum G). All anthropometrical equipment was calibrated at the start of each day of data collection. Each measurement was taken three times for accuracy and acceptable discrepancies between the readings were 0.5kg for weight, 0.5cm for height and 0.5cm for waist circumference.[96]

Weight

A digital scale (Seca 874 flat scale) was used for weight and weight was measured in kilograms, with measurements rounded off to the nearest 0.1kg. As the room that was used for data collection was carpeted, a flat thick piece of plastic was placed under the scale to ensure a flat surface. The scale was calibrated at the start of each day using calibration weights of 1kg, 2kg, and 5kg. Participants were weighed without shoes and were asked to remove jackets, belts, scarves, and other extra, bulky pieces of clothing. The participant was asked to stand upright, facing the number display of the scale, with weight evenly distributed between both feet, and to refrain from leaning against anything. The measurement was only recorded once the digital numerical value had stabilised for at least

three seconds.[96] A total of three readings was taken for each participant and an average of the three was used as the final weight.

Height

Height was measured on a flat, stable surface, using a platform and stick stadiometer, and measured and recorded in centimetres, rounded off to the nearest 0.5cm. The participant was measured without shoes and with no head coverings; where head coverings were required due to cultural or religious beliefs, head coverings were minimal. The participant stood upright with their heels, buttocks, shoulders, and head against the stadiometer. Weight was evenly distributed between the feet to prevent leaning to one side. The participant's chin was parallel to the ground, in the Frankfurt plane. The headpiece was lowered until it touched the crown of the participant's head. In cases where head coverings or excess hair height was present, light pressure was applied to compress the hair or head covering. The participant was asked to inhale and at the point of maximum inhalation, the measurement was taken.[96] An average of three measurements were taken.

BMI

Weight and height measurements were used to calculate the BMI of each participant. This was calculated using the World Health Organisation (WHO) formula for BMI (weight (in kilograms) divided by height (in metres) squared) and was interpreted using the WHO reference values for BMI (see Table 3.2). [96]

Table 3.2. International classification of nutritional status according to body mass index (BMI)

BMI (kg/m^2)	Interpretation
<18.5	Underweight
18.5 – 24.9	Normal range
25.0 – 29.9	Overweight
30.0-34.9	Obese: Class I
35.0-39.9	Obese: Class II
≥40	Obese: Class III

Waist circumference

Waist circumference was taken for all participants. A non-stretching tape measure measuring 2 metres was used. Participants stood upright on a flat surface without shoes

on. The participant was asked to remove any bulky clothing and waist belts. The participant's shirt was raised to expose the abdomen up to the level of the diaphragm. The tape measure was placed horizontally at the midpoint between the last rib and the iliac crest. The tape measure was kept straight, parallel to the floor, lay flat against the skin, and did not dig into the skin at any time. The participant was asked to exhale and at the point of maximum exhalation, the measurement was read and recorded to the nearest 0.5cm.[95] (96)An average of three measurements was taken.

Waist circumference was interpreted as an individual measurement. Cut-off and interpretation values for this measurement can be found in Table 3.3.[96]

Table 3.3. Waist circumference interpretation

Gender	Waist circumference	Interpretation
Men	<94cm	Low risk of metabolic complications
Women	<80cm	
Men	94-102cm	Increased risk of metabolic complications
Women	80-88cm	
Men	>102cm	High risk of metabolic complications
Women	>88cm	

3.5.2.4 Food frequency questionnaire

A food frequency questionnaire (Addendum G) that focused on dietary sources of vitamin D was compiled and validated for content and face validity. Face validity was determined during the pilot study, where fourth year dietetics students completed the questionnaire and gave their feedback regarding it. Feedback included pointing out ambiguous questions, the time it took to complete, making questions more or less detailed, and pointing out where questions had either two answers, or no answers possible (e.g. the addition of an N/A option in the medical questionnaire). This input was valuable in ensuring the researchers collected the correct data to fulfil the study objectives. Two independent experts in the field of food and nutrition were approached and helped with content validity. The comments from both the face and content validity were reviewed and incorporated into the final questionnaire. The final food frequency included common vitamin D rich foods, especially those found in the South African environment.[97] The final food frequency questionnaire consisted of two sections, one for food items and a second for supplementation use. Section one consisted of seven sub-sections, a total of 52 food items, 4 frequency options for servings, and a comments section for each food item. A medium portion size was used as the average and the amount of the medium portion sizes was given for each food item.

Portion size options were small, medium, and large. Participants needed to determine if they ate a lot more, a lot less or about the same as the given portion size. If they ate less than half the medium portion size per serving, the researcher marked “small”. If they ate more than double the medium portion size per serving, the researcher marked “large”. If they ate about the same as the medium portion size, the researcher marked “medium”. Food models were used by the researchers to help the participants visualise a medium portion, and how much they ate in relation to this portion. This helped to standardise the responses from the participants.

Section two investigated the participant’s nutritional supplement use. Only micronutrient supplements and boosters containing vitamin D were included in the study – protein and meal replacement supplemental drinks and food products were excluded. Participants were asked to answer the food frequency based on what they usually eat in a typical month over winter. The questionnaire was completed in an interview format with a trained dietician asking the participant about each food item to ensure quality data was obtained. All data collectors involved in this section of data collection were trained on standardisation and how to accurately and consistently complete the food frequency questionnaire. The food frequency questionnaire took approximately 10 – 15 minutes to complete.

The data collected from this questionnaire was analysed using a validated software programme that details how much vitamin D each food item contains per serving (FoodFinder MRC III). This was used to estimate the average daily dietary intake of vitamin D per participant. As the software programme only provides nutritional reports per day and not per week or per month, the results from the questionnaire had to be calculated back to a per day serving. In the case of a participant taking a vitamin D containing supplement, the supplement was researched (either from the brand’s webpage or via an email sent to the company requesting the nutritional composition) and the amount of vitamin D provided by that supplement per day was added to the value provided from the FoodFinder report, to give a final average daily consumption of dietary vitamin D for each participant. The Dietary Reference Intake value of 15mcg (600IU) of vitamin D per day was used to determine if dietary sources of vitamin D were sufficient.[96]

3.5.2.5 Biochemistry

Phlebotomy for Vitamin D status was performed by a registered nursing sister, in a private area. Addendum H accompanied all blood work to the laboratory. 25(OH)D is universally accepted as the best indicator of vitamin D status and was used to determine vitamin D status in this study.[20,36] Blood samples (5ml whole blood) was collected from participants into plain tubes (red/yellow top vacutainer). The blood samples were clearly marked with

each participant's number and each sample was allowed to clot for a minimum of 20 minutes. The serum was then centrifuged and aliquoted daily, and stored in Eppendorf tubes at -20˚C until analysed. Once all the samples were collected, they were couriered together for batch analysis to the University of Witwatersrand's Developmental Pathways for Health Research Unit for analysis, who was awarded a certificate of efficiency by the International Vitamin D External Quality Assessment Scheme.

While liquid chromatography-tandem mass spectrometry (LC-MS/MS) tests are considered the gold standard for measuring serum 25(OH)D, these tests are costly, labour intensive and not widely available.[43,62] The LIASION automated assay has been validated and shown to have the most accurate results when compared to the LC-MS/MS methods and was used in this study.[43,62] 25(OH)D was measured using a chemiluminescent assay using DiaSorin Liasion kits (DiaSorin, Stillwater, MN, USA).

Table 3.4 shows how serum 25(OH)D levels were interpreted.[46]

Table 3.4. Interpretation of serum 25-hydroxyvitamin D (25(OH)D) levels

Interpretation	Endocrine Society[46]	Institute of Medicine[67]
Deficiency	<20ng/ml (<50nmol/l)	<12ng/ml (<30nmol/l)
Insufficiency	20 – 29ng/ml (50-72.5nmol/l)	12-20ng/ml (30-50mnol/l)
Sufficiency	30-60ng/ml (75-150nmol/l)	>20ng/ml (>50nmol/l)
Toxicity	>150ng/ml (>374nmol/l)	>50ng/ml (>125nmol/l)

While the Institute of Medicine's guidelines are more in line with those required by the healthy population (as opposed to the at-risk population that the guidelines proposed by the Endocrine Society suggests), the Institute of Medicine's proposed guidelines are specifically for bone health. [67] Many experts have argued that the serum 25(OH)D levels needed for good overall health are higher than those required for bone health.[46,98] Thus, both of these guidelines were used in the interpretation of overall vitamin D status for the total population, as well as in the interpretation of serum 25(OH)D in the main study objectives (i.e. gender and skin tone). Even though the Endocrine Society reference values are recommended for those at risk, and the current study population was not expected to be at risk, the levels used in these guidelines encompass more than just bone health. The Endocrine Society reference values were, therefore, used for the interpretation of serum 25(OH)D for the remaining objectives.

3.6 Quality Assurance

3.6.1 Training of data collectors

Data collectors were needed during all phases of screening and data collection. Three data collectors were recruited to join the student researcher during data collection plus a project manager. Data collectors were needed as follows: one for the informed consent discussion, signing of consent forms and random number allocation, one for anthropometry, and two for the completion of the food frequency questionnaires. A registered nursing sister was recruited for the phlebotomy aspect of the study. The appointed project manager was needed to oversee and supervise the collection of data, and to assist with data collection were needed. Training of data collectors was done to ensure accuracy during data collection and to ensure quality data was collected. Trained dieticians were approached to conduct the anthropometrical measurements, as well as the food frequency questionnaire, as they had been previously trained on the basic concepts of both of these. These techniques were revised according to the standard operating procedures (Addendum I).

Training of data collectors took place one week before the screening phase of the study, at the Division of Human Nutrition, 3rd Floor clinical building, Tygerberg Medical and Health Sciences campus of Stellenbosch University, for one day.

As dietitians have already been trained on how to take anthropometrical measurements, those involved underwent a session to revise the procedures on how to take weight, height, and waist circumference measurements, as set out in the methods section of this study.

Those involved in the skin tone aspect of data collection were trained, with the input from a dermatologist, on how to use the handheld skin colorimeter device, and where to measure the skin tone.

All data collectors were trained in all aspects of data collection and could be interchangeable if the needed arose to ensure efficiency of the data collection process.

3.6.2 Data storage

Signed consent forms and all study questionnaires were stored in the office of the principal investigator of the larger study. Personal information was kept confidential and separate from the data collected, and only used when blood abnormalities arose or when the participant asked to be informed of their results. The participant's personal and contact details were not found on any of the data collection tools, apart from the screening tool. Data collectors were trained on the importance of informed and voluntary consent, confidentiality, privacy and anonymity. All hard copies of raw data were treated with the

strictest confidentiality and privacy. All data from this study will be kept for 10 years before it is disposed of ethically and responsibly.

3.7 Pilot study

A pilot study was conducted to test the face and content validity of both the screening and the data collection questionnaires. This allowed the researchers to test if methods of data collection were inclusive and exhaustive and that the results obtained were those we wanted, as well as if data collection templates and data capturing procedures were efficient and practical. One of the researchers, a dermatologist, provided input in terms of data collection tools. The comments and concerns presented by these experts were reviewed and incorporated into the tools before face validity took place.

To test face validity, a pilot study was conducted in August 2016, after ethical approval had been obtained from the Health Research Ethics Committee of Stellenbosch University. The pilot study was conducted on the final year undergraduate students on the FMHS campus of Stellenbosch University, as this population was similar to the study population. As the tools were not changed substantially, a motivation sent to the Health Research Ethics Committee of Stellenbosch University for review was not required. To ensure that no participants who took part in the pilot study were included in the main study, the screening tool used during the screening phase of the main study included a question on whether or not the potential participant took part in the pilot study. If so, the participant was not included in the study. The data received from this pilot study, and its participants, was not included with the data collected and analysed for the main study.

3.8 Analysis of data

Statistical analysis was completed by the researchers with assistance and input from a statistician. MS Excel was used to capture the data collected and STATISTICA version 13 was used to analyse the data. Data was captured on pre-prepared Excel spreadsheets at the end of the data collection period. Both discrete and continuous quantitative methods were used to categorise data. Nominal scales were used for the categorisation of the strata. Summary statistics were used to describe the variables. Measures of central tendency were used to determine a middle point for the data, and where mean was calculated, standard deviation was also calculated and shown alongside the mean value. This gave an indication of extreme outliers which may have skewed the mean data value. Relationships between two continuous variables were analysed with regression techniques and where the variables were not normally distributed, Spearman's correlation was done. Kruskal-Wallis tests were performed to test relationships between non-normative data.

ANOVA tests were used to analyse the relationships between continuous and nominal variables. Mann-Whitney tests were performed to analyse the relationships between randomised designs.

Data was weighted where necessary to ensure appropriate conclusions could be drawn from the data collected.

Anthropometric measurements, subjective and objective skin tone measurements, FFQ data, and vitamin D status were interpreted according to the reference values and cut-offs provided under Methodology.

P-values of $p<0.05$ were calculated to represent statistical significance during hypothesis testing and a confidence interval of 95% was used to estimate unknown parameters.

3.9 Role of the student researcher

The data used in this study forms part of a Master of Nutrition project, and the same data was used as the baseline data for part of a larger study. Table 3.5 shows the role of the student researcher in this study.

Table 3.5. Role of the student researcher

Role and responsibility	Explanation
Aims and objectives	The development of study aims and objectives for the current study
Recruitment and screening of potential participants	Screening of potential participants was done to increase the suitability of the sampling frame for the study, as well as to ensure a suitable sampling frame whereby a random sample could be drawn. Potential participants completed a screening questionnaire and a self-administered skin tone questionnaire. The student researcher completed screening consent forms, allocated random numbers to participants, and facilitated the completion of the questionnaires. Once screening had been completed, the participants were stratified for skin tone. Random selection took place within these strata and the student researcher helped to contact those who were included in the main study.
Training of data collectors	Data collectors were needed during screening and data collection to ensure efficient and effective use of resources. The student researcher developed the SOP's set out in the protocol to help with the training of the data collectors.

Role and responsibility	Explanation
Collection of data during data collection phases	The student researcher was involved in all aspects of data collection, including: Consent: explanation of the study to participants; completion of consent forms; allocation of random numbers to participants Anthropometry: weight, height, and waist circumference measurements Food frequency questionnaire: the completion of interview-style food frequency questionnaires with participants Skin tone analysis: the measurement of skin tone with a handheld skin reflectometer and the upload of this data to spreadsheets Sun exposure questionnaire: the facilitation of the self-administered sun exposure questionnaire
Capturing of data **Data cleaning**	The student researcher was involved in the capturing, management and cleaning of raw data collected during data collection.
Analysis and interpretation of data	The student researcher was involved in the analysis and interpretation of data collected during this study including the calculation of skin type classifications and allocation of strata in the screening phase, the calculation and interpretation of anthropometrical measurements (BMI, waist circumference and waist-hip ratio), the interpretation of serum 25(OH)D levels, the calculation and interpretation of participant sun exposure index, analysis and interpretation of the food frequency questionnaire data.
Study tools	The student researcher was involved in the identification and adaptation of tools and questionnaires for the study.
Ethical aspects	The student researcher was involved in upholding good ethical research principles based on the 3 pillars of ethics as set out in the Belmont Report, as well as ensuring the confidentiality and privacy of participants, and ensuring good quality data and the safe storage of study data.

Role and responsibility	Explanation
Write up of a peer reviewed article	A peer reviewed article will be completed by the student researcher with the intention of submission to be published in a peer reviewed journal.
Write up of a thesis	In partial fulfilment of the student researcher's Master of Nutrition degree, a thesis was completed and submitted.

3.10 Budget

Table 3.6 shows the budget used for the study. The majority of the budget was used for the testing of serum 25(OH)D, a purchase of a handheld skin colorimeter and the compensation of data collectors. Funding for this study was provided by CANSA.

Table 3.6. Budget

Item	Price per item (R)	Amount needed	Total Amount (R)
Phlebotomy			
LIAISON 25(OH)D assay	175	242	42350
Analysis of 25(OH)D samples	37.50	242	9075
Transport of blood samples to WITS for analysis	15	242	3630
Laboratory materials			5000
Phlebotomist	100/hour	6 hours/day (20 days)	12000
Printing Costs and Stationery			
Consent forms (screening and data collection)	1.00/page (5 pages)	1000 copies	5000
Pens	1.00	50	50
Other printing costs			2000
Equipment			
Portable Stadiometer	1500	1	1500
Measuring tape	50	1	50
Digital scale	1000	1	1000
Yearly calibration of instruments	1000	2	2000
Calculators for BMI calculation	100	6	600
Handheld colorimeter (DSM II Colormeter, Cortex Technologies)	55000	1	55000
Data collectors			
Data collector compensation (screening)	R150/hour (10 days)	3 (5 hours/day each)	22500
Data collector compensation (data collection)	R150/hour (20 days)	3 (6 hours/day each)	54000
Training costs			
Manuals	5.00 per manual	5	25
Pens	1.00	10	10
Training venue hire	1000 per day	1 days	1000
Refreshments	100 per day	1 days	100
Refreshments during data collection	100 per day	20 days	2000
Publication Costs			
Language Centre Analysis			8000
Translation costs			5000
Other			
Incentives for participation during study			2500
Participant compensation	50	242	12100
SMS reminders for participants	1.00/sms	242	242
Total			**246732**

Abbreviation: 25(OH)D: 25-hydroxyvitamin D; WITS: University of Witwatersrand; BMI: body mass index

3.11 Ethical and legal aspects

This study was conducted in full conformance with the ethical pillars set out in the Declaration of Helsinki and followed the laws and regulations of research in South Africa. Ethical approval was sought from the Health Research Ethics Committee of Stellenbosch University (Ethics reference number: N16/01/013) before the study commenced. Ethical renewal was applied and granted for during each year of the study.

3.11.1 Consent, anonymity and confidentiality

All willing participants were required to sign an information and consent form before participating in the study. The consent form was available in English and Afrikaans, and consent was done during both the screening phase and the main data collection phase. The consent forms were completed in duplicate, with one copy kept by the researchers and the other copy given to the participant for their own records. Participants were informed of what the study was about, in a language of their choice, and in a way that they understood, before consent was signed. After the study had been explained to them, participants were asked to paraphrase why they were asked to participate, so that the researchers and data collectors could gauge the level of understanding and ensure that the participants made an informed decision. All participation was voluntary, and this was communicated to participants at the beginning of screening and data collection, as well as at predetermined intervals throughout data collection. Voluntary participation was confirmed both verbally and in writing by completing and signing the information and consent forms provided. Participants could withdraw from the study at any time, without any repercussions. Confidentiality was maintained through the random allocation of numbers to each participant and random selection of participants from each stratum. After informed consent was obtained, the participant became an active part of the study and was given randomly generated three-digit number. This number was used to identify all the data collected for that participant. As the study had an aspect of phlebotomy involved, participants could have been found to have an abnormality in their serum 25(OH)D levels, which could be detrimental to their health if not addressed. Due to this, and fulfilling the ethical principle of beneficence, in the event that a participant's serum 25(OH)D test results showed an abnormality, it was the ethical responsibility of the researchers, as responsible health care professionals, to inform the participant of this and to offer assistance in the form of referral to a registered health care professional for further analysis. Because of this, participants were asked on the screening questionnaire if they would like the researchers to inform them of their vitamin D status at the completion of the study. There was also a clause attached to this question that stated if an abnormality was found, the participant would be informed. For this reason, participants were asked to disclose their contact details on the screening

questionnaire. A separate spreadsheet containing the participant contact details and randomly assigned numbers was kept by the researchers. This information was kept confidential and separate from the data collected, and only used when blood abnormalities arose or when the participant asked to be informed of their results. The participant's personal and contact details were not found on any of the data collection tools, apart from the screening tool. Data collectors were trained on the importance of informed and voluntary consent, confidentiality, privacy and anonymity. All hard copies of raw data were treated with the strictest confidentiality and privacy, and data was stored in a secure storage facility and accessed only by those directly involved in the study. All data from this study will be kept for 10 years before it is disposed of ethically and responsibly.

3.11.2 Selection of participants

Participant selection was random, with all potential participants that met the inclusion criteria given equal opportunity to be chosen and included in the study.

3.11.3 Risk-benefit ration

This study posed minimal risk to the participant. There was a risk of discomfort while bloods were drawn, as well as bruising, bleeding, and light-headedness, but as this was a routine procedure that was conducted by a qualified phlebotomist, the risks were small. The benefits of this study outweighed the risks. To the participant, the knowledge of their vitamin D status can help them pursue optimal health. The greater population of the South Africa benefits from this prevalence study as the results can determine whether interventions are necessary and can form part of the baseline for further studies to investigate the reasons and causes behind high or low vitamin D status in this particular population.

3.11.4 Privacy and dignity of participants

During all phases of screening and data collection, the privacy and dignity of the participants was respected. All stages of data collection, particularly anthropometrical measurements and phlebotomy, took place in separated, cordoned off areas to ensure privacy, with only the participant and data collector occupying the space at that time. Sensitive data was not communicated to other participants. Data collectors were trained on the importance of discretion and respect for participant's dignity.

3.11.5 Incentives

Participants were incentivised to attend both the screening and data collection sessions. Participants that participated during screening were entered into a lucky draw to win a gift voucher to the value of R1000 for a local department store. Incentives for data collection also consisted of entry into a lucky draw to win a voucher for a local department store, to the value of R1500. The lucky draw took place after the final data collection period. The

participants' study numbers were randomly drawn by a staff member from the Division of Human Nutrition at Stellenbosch University. The participant whose number was drawn was contacted using the contact details provided. Participants who were chosen to take part in in data collection were compensated for their time by means of a food voucher. As data collection took place over lunch periods, participants received a R50 food voucher for the coffee shop on campus.

4 Results

4.1 Demographics

A total of 242 participants took part in the study during the 2016 data collection period: 121 males and 121 females. The median age was 20.00 years (mean age: 20.41 ± 2.29 years), with ages ranging between 18 and 28 years. The majority of participants were between 19 (26%) and 20 (23%) years of age. One participant of 45 years was included in the study.

The demographics of the study population can be seen in Table 4.1.

Table 4.1. Participant demographics by gender

Variable	Total, % (n=242)	Male, % (n=121)	Female, % (n=121)
Race			
African	23.6 (n=57)	19.8 (n=24)	27.27 (n=33)
Asian	2.5 (n=6)	2.48 (n=3)	2.48 (n=3)
Coloured	29.8 (n=72)	29.75 (n=36)	29.75 (n=36)
Indian	5.4 (n=13)	6.61 (n=8)	4.13 (n=5)
White	38.4 (n=93)	41.32 (n=50)	35.54 (n=43)
Language			
Afrikaans	28.9 (n=70)	31.40 (n=38)	26.45 (n=32)
English	46.3 (n=112)	49.59 (n=60)	42.98 (n=52)
Xhosa	6.2 (n=15)	3.31 (n=4)	9.09 (n=11)
Other	18.6 (n=45)	15.70 (n=19)	21.49 (n=26)
Province			
Eastern Cape	9.5 (n=23)	9.92 (n=12)	9.09 (n=11)
Free State	2.1 (n=5)	1.65 (n=2)	2.48 (n=3)
Gauteng	9.1 (n=22)	6.61 (n=8)	11.57 (n=14)
Kwazulu Natal	11.9 (n=29)	11.57 (n=14)	12.40 (n=15)
Limpopo	3.3 (n=8)	2.48 (n=3)	4.13 (n=5)
Mpumalanga	1.7 (n=4)	0.83 (n=1)	2.48 (n=3)
Northern Cape	2.5 (n=6)	3.31 (n=4)	1.65 (n=2)
Northwest Province	0.8 (n=2)	1.65 (n=2)	0
Western Cape	59.1 (n=143)	61.98 (n=75)	56.20 (n=68)

The race distribution of the study population was predominantly White, Coloured, and African, with Asian and Indian races only accounting for 2.5% and 5.4% respectively.

The most commonly spoken home language of the participants was English (46.3%). Xhosa accounted for 6% of the total population, with other African languages being more predominant. More than half of the participants came from the Western Cape (59.1%), with Kwazulu Natal (KZN) (11.9%) and the Eastern Cape (9.5%) accounting for the second and third most common home province (Table 4.1). Only one participant was from a foreign country (Democratic Republic of Congo).

4.2 Vitamin D status

The mean serum 25(OH)D level for the study population was 63.80 ± 41.35 ng/ml. Using the Endocrine Society (ES) interpretation of serum 25(OH)D, almost three quarters of the participants had sufficient or above sufficient levels of 25(OH)D in their blood. When using the Institute of Medicine (IOM) interpretation, however, this level rose to 90% (n=214). Toxicity was experienced by 8% (n=19) of participants when the ES interpretation was used and increased to 54% (n=127) when using the IOM interpretation. Insufficiency and deficiency only accounted for 11% (n=28) of the total population when the IOM reference values were used (Figure 4.1).

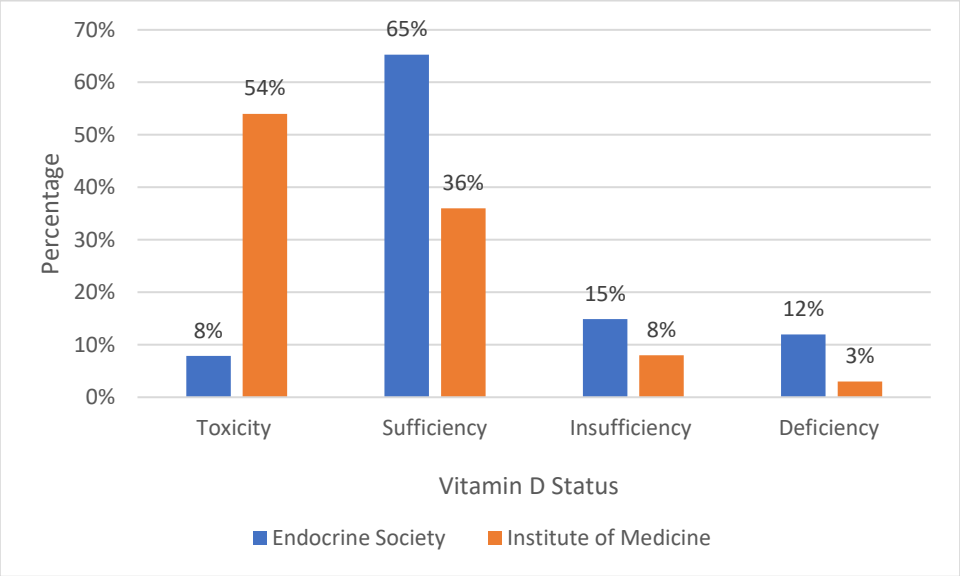

Fig. 4.1. Interpretation of serum 25-hydroxyvitamin D for total study population: Endocrine Society and Institute of Medicine

4.2.1 Vitamin D Status and Gender

Overall, males had a better vitamin D status than females. Figure 4.2 shows the vitamin D status interpretation using both the ES and IOM reference values for this subgroup.

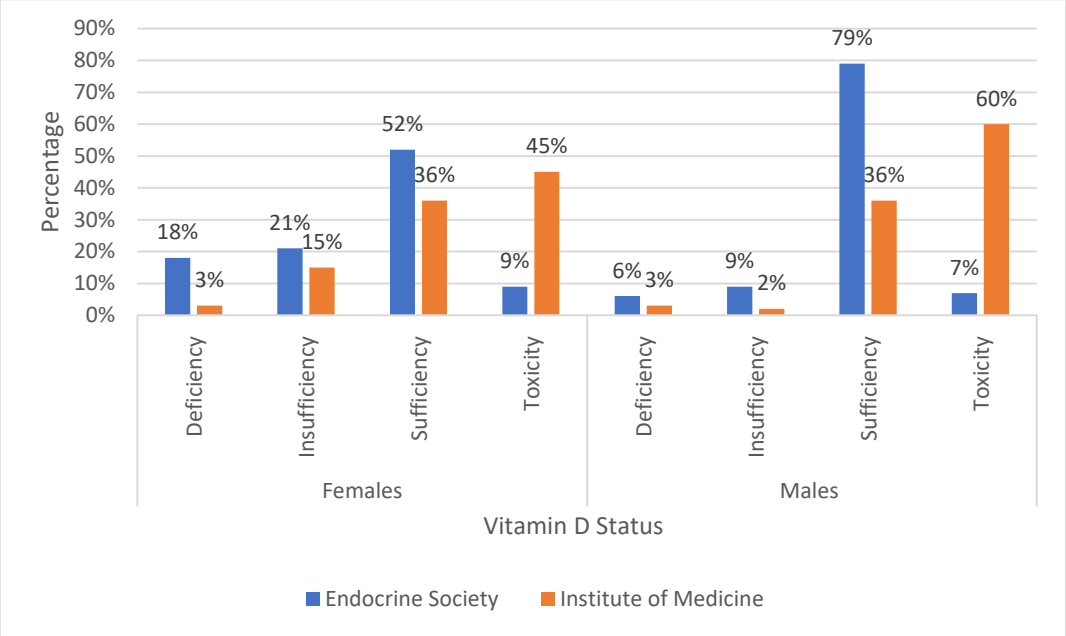

Fig 4.2. Vitamin D Status for gender: Endocrine Society and Institute of Medicine

Males had significantly higher mean serum 25(OH)D levels than females (68.99 ± 38.33 ng/ml and 58.60 ± 43.70 ng/ml respectively) ($p<0.01$) (Figure 4.3). The majority of males were found to have sufficient and above sufficient levels of vitamin D when using both ES and IOM reference values. When it came to vitamin D deficiency and insufficiency, more females had a suboptimal vitamin D status than males, with 39% (n=47) of females experiencing suboptimal 25(OH)D levels when using the ES reference values and 18% (n=22) when using IOM reference values (Figure 4.2).

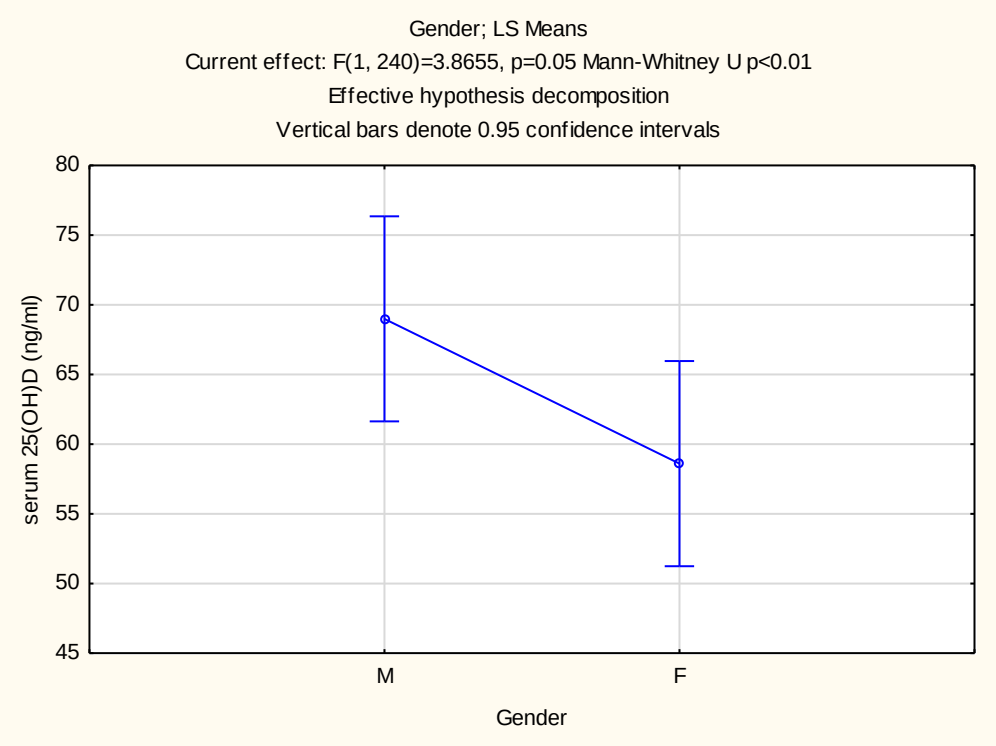

Fig. 4.3. Serum 25-hydroxyvitamin D and gender

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; M: Male; F: Female

4.2.2 Vitamin D status and Race

The mean serum 25(OH)D level was lowest for participants in the Indian population (38.42 ± 22.51 ng/ml) and highest for those in the White population (79.90 ± 42.47 ng/ml) (Table 4.2). The relationship between race and vitamin D status was found to be statistically significant (p=0.02; Chi^2=24.02) when using the ES reference values, with participants from races of darker skin tones more likely to experience low serum 25(OH)D levels. Participants in the White population were found to have the highest levels of toxicity (12.77%; n=12) while those in the Indian population had the highest levels of deficiency (23.08%, n=3).

Table 4.2 Mean serum 25-hydroxyvitamin D (25(OH)D) levels and interpretation of levels (Endocrine Society): Race

Variable	Mean serum 25(OH) in ng/ml (n=242)	25(OH)D interpretation, total population (%)				p-value
		Toxicity (>150ng/ml)	Sufficiency (>30ng/ml)	Insufficiency (20-30ng/ml)	Deficiency (<20ng/ml)	
Race						
African (n=57)	52.63 ± 38.18	5.23 (n=3)	57.89 (n=33)	17.54 (n=10)	19.3 (n=11)	**p*<0.01 (Kruskal -Wallis)**
Asian (n=6)	64.52 ± 40.58	0	66.67 (n=4)	16.67 (n=1)	16.67 (n=1)	
Coloured (n=72)	56.13 ± 38.32	5.56 (n=4)	63.89 (n=46)	13.89 (n=10)	16.67 (n=12)	
Indian (n=13)	38.42 ± 22.51	0	61.54 (n=8)	15.39 (n=2)	23.08 (n=3)	
White (n=94)	79.90 ± 42.47	12.77 (n=12)	71.28 (n=67)	13.83 (n=13)	2.13 (n=2)	

* p-value refers to mean serum 25(OH)D

Abbreviation: 25(OH)D: 25-hydroxyvitamin D

4.2.3 Vitamin D Status and Home Province

Participants from the Northwest Province had the highest levels of serum 25(OH)D (113.80 ± 51.19 ng/ml), followed by those from Mpumalanga (92.45 ± 43.42 ng/ml) and Free State (83.22 ± 40.90 ng/ml). Participants from the Northern Cape had the lowest mean 25(OH)D levels (55.12 ± 20.66 ng/ml). Mean serum 25(OH)D for participants from the Western Cape was 63.56 ± 41.03ng/ml (Table 4.3).

Table 4.3 Mean serum 25-hydroxyvitamin D (25(OH)D) levels and interpretation of levels (Endocrine Society): Home Province

Variable	Mean serum 25(OH) in ng/ml (n=242)	25(OH)D interpretation, total population (%)				p-value
		Toxicity (>150ng/ml)	Sufficiency (>30ng/ml)	Insufficiency (20-30ng/ml)	Deficiency (<20ng/ml)	
Province						
Eastern Cape (n=23)	64.41 ± 41.40	8.7 (n=2)	65.2 (n=15)	26.1 (n=6)	0	
Free State (n=5)	83.22 ± 40.90	20 (n=1)	80 (n=4)	0	0	
Gauteng (n=22)	57.29 ± 40.74	9.1 (n=2)	59.1 (n=13)	13.7 (n=3)	18.2 (n=4)	
Kwazulu Natal (n=29)	56.80 ± 42.82	10.3 (n=3)	58.6 (n=17)	10.3 (n=3)	20.7 (n=6)	
Limpopo (n=8)	76.64 ± 52.61	0	75 (n=6)	0	25 (n=2)	**p*=0.46 (Kruskal -Wallis)**
Mpumalanga (n=4)	92.45 ± 43.42	25 (n=1)	75 (n=3)	0	0	
Northern Cape (n=6)	55.12 ± 20.66	0	83.3 (n=5)	16.7 (n=1)	0	
Northwest Province (n=2)	113.80 ± 51.19	50 (n=1)	50 (n=1)	0	0	
Western Cape (n=142)	63.56 ± 41.03	6.3 (n=9)	65.5 (n=93)	16.2 (n=23)	12.0 (n=17)	

* p-value refers to mean serum 25(OH)D

Abbreviation: 25(OH)D: 25-hydroxyvitamin D

Although there was no statistically significant relationship found between vitamin D status and home province (p=0.39; Chi^2=28.83), participants from the Northern Cape and Free State had the highest percentage of vitamin D sufficiency (83.3%; n=5 and 80%; n=4

respectively), while those from KZN and Gauteng had the highest percentage of vitamin D deficiency (20.7%; n=6 and 18.2%, n=4, respectively).

4.3 Skin tone

Figure 4.4 shows the results from the self-administered Fitzpatrick skin tone questionnaire. These scores show that more than half of the participants placed themselves in the type IV skin tone group (light brown skin). Twenty-seven percent (n=67) identified themselves as type III (dark white skin), while 5% (n=11) identified themselves as type II (fair skin). No participants identified themselves as type I (pale white skin) and only one participant identified as type VI (dark brown skin). The Fitzpatrick scores were well distributed among the genders.

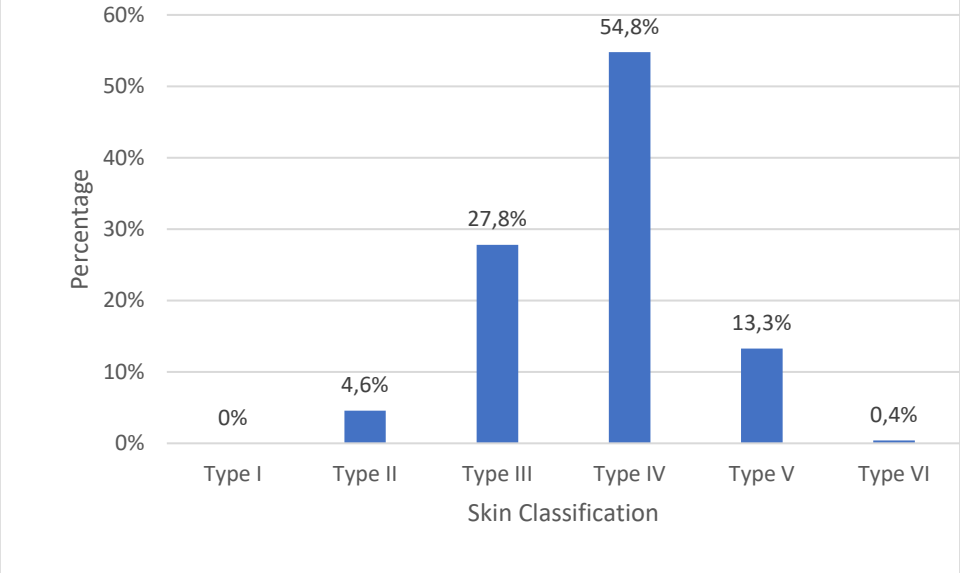

Fig. 4.4. Fitzpatrick skin type classification for total population

As expected, the average MI values (mean = 39.34 ± 13.37) (measured by the skin reflectometer) differed significantly between the subjective Fitzpatrick skin type groupings (Kruskal Wallis; p=<0.0001) (Figure 4.5). The relationship between MI and race was also significant, with the participants from races who traditionally have darker skin tones also having higher MI scores (P<0.01) (Figure 4.6).

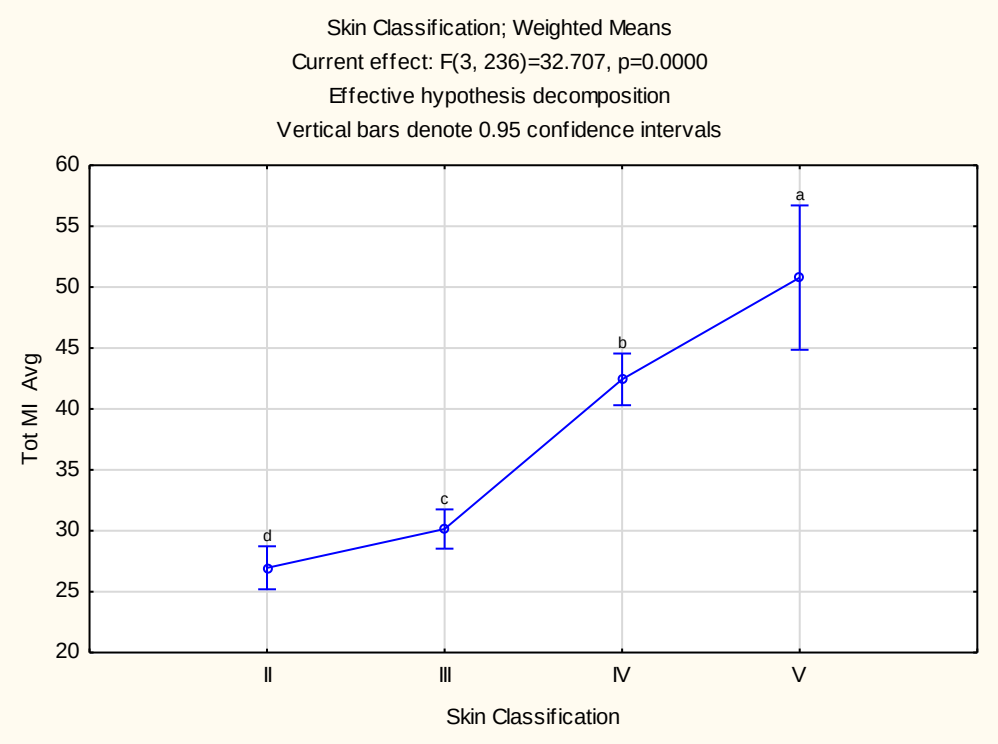

Fig 4.5. Relationship between Fitzpatrick skin type classification and average melanin index

Abbreviations: MI: Melanin Index; Avg: Average; II: Fitzpatrick skin type II; III: Fitzpatrick skin type III; IV: Fitzpatrick skin type IV; V: Fitzpatrick skin type V

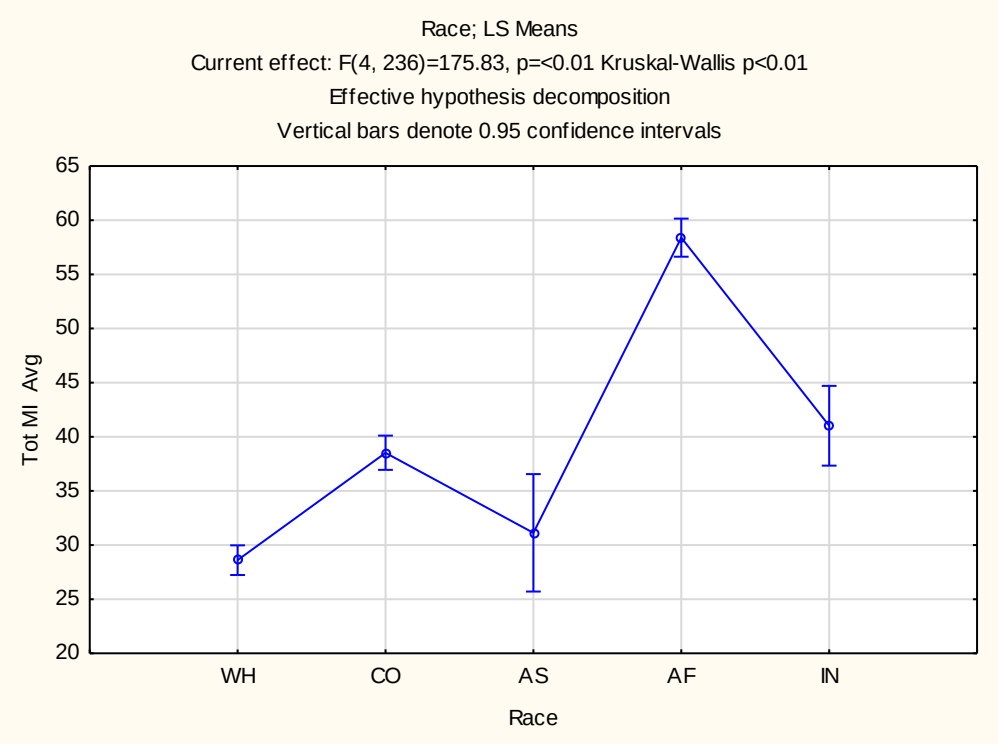

Fig 4.6. Relationship between race and average melanin index

Abbreviations: MI: Melanin Index; Avg: Average; WH: White; CO: Coloured; AS: Asian; AF: African; IN: Indian

A Chi-squared test between race and skin classification showed a significant relationship between the two ($p<0.05$; $Chi^2=91.12$), with the participants from races who have traditionally darker skin tones classifying themselves into darker skin types.

Participants from Fitzpatrick skin type II had the highest mean serum 25(OH)D (76.89 ± 46.35 ng/ml), while those from skin type V had the lowest mean serum 25(OH)D (48.60 ± 63.48 ng/ml) (Table 4.4). Interestingly, there was not much difference in serum 25(OH)D between the participants in types II and III, but a larger decrease in serum 25(OH)D was observed in participants who had darker skin types (Figure 4.7).

Table 4.4 Mean serum 25-hydroxyvitamin D (25(OH)D) levels and interpretation of levels (Endocrine Society and Institute of Medicine): Fitzpatrick skin type classification

Variable	Mean serum 25(OH) in ng/ml (n=242)	p-value	25(OH)D interpretation, total population (%): Endocrine Society				25(OH)D interpretation, total population (%): Institute of Medicine			
			Toxicity (>150ng/ml)	Sufficiency (>30ng/ml)	Insufficiency (20-30ng/ml)	Deficiency (<20ng/ml)	Toxicity (>50ng/ml)	Sufficiency (>20ng/ml)	Insufficiency (12-20ng/ml)	Deficiency (<12ng/ml)
Fitzpatrick skin type classification										
Type II (n=11)	76.89 ± 46.35	**p*=0.01 (Kruskal -Wallis)**	0	90.91 (n=10)	9.10 (n=1)	0	0	45.45 (n=5)	54.55 (n=6)	0
Type III (n=67)	75.42 ± 45.29		13.43 (n=9)	64.18 (n=43)	13.43 (n=9)	8.96 (n=6)	62.69 (n=42)	28.36 (n=19)	8.96 (n=6)	0
Type IV (n=133)	60.60 ± 38.49		6.02 (n=8)	66.92 (n=89)	14.29 (n=19)	12.78 (n=17)	52.63 (n=70)	35.34 (n=47)	7.52 (n=10)	4.51 (n=6)
Type V (n=30)	48.60 ± 63.48		6.67 (n=2)	50 (n=15)	23.33 (n=7)	20 (n=6)	30.00 (n=9)	50.00 (n=15)	13.33 (n=4)	6.67 (n=2)

* p-value refers to mean serum 25(OH)D

Abbreviation: 25(OH)D: 25-hydroxyvitamin D

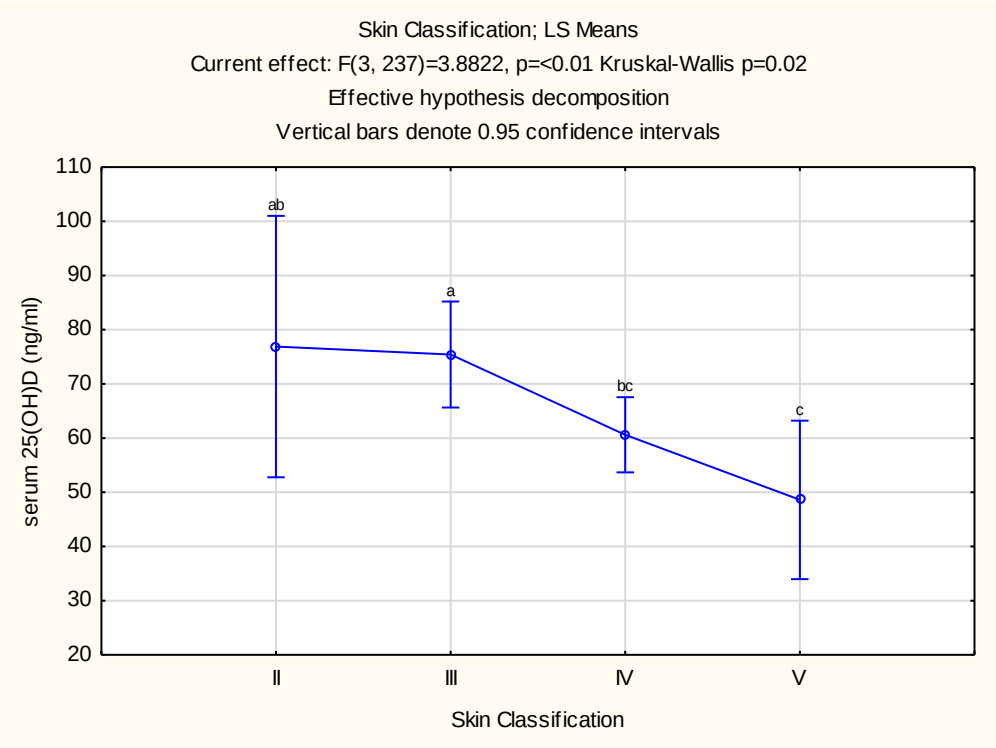

Fig 4.7. Serum 25-hydroxyvitmain D and Fitzpatrick skin type classification

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; II: Fitzpatrick skin type II; III: Fitzpatrick skin type III; IV: Fitzpatrick skin type IV; V: Fitzpatrick skin type V

Similarly, when it came to vitamin D status among skin tone, the majority of those who tested as deficient when using IOM reference values fell into the darker skin types (Figure 4.8). Interestingly, the relationship between vitamin D status and skin tone was not found to be statistically significant when using either of reference values (ES: $p>0.05$; $Chi^2=13.91$ and IOM: $p>0.05$; $Chi^2 = 18.83$).

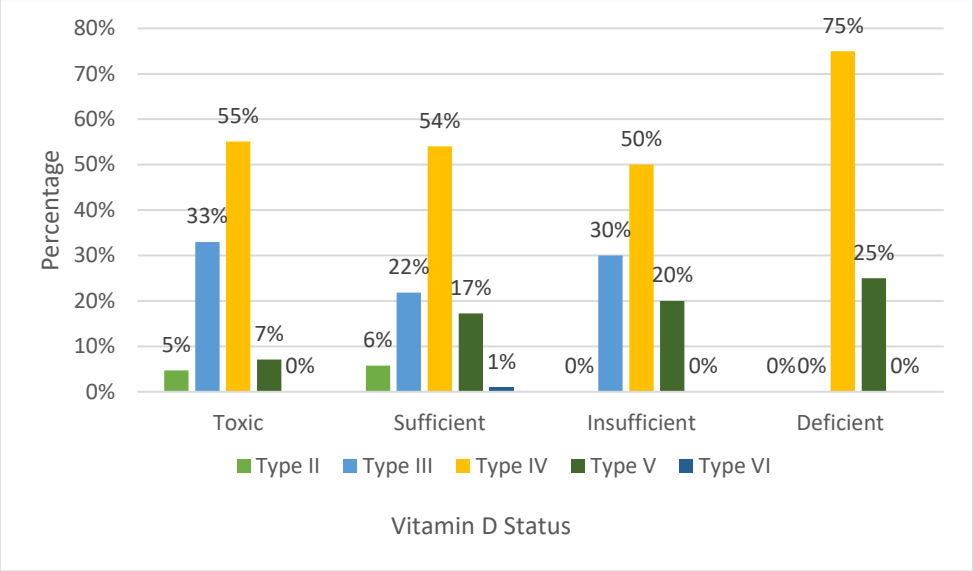

Fig 4.8. Fitzpatrick skin type classification vs. vitamin D status (Institute of Medicine)

4.4 Anthropometry

Table 4.5 shows the mean anthropometric measurements per demographic. The mean weight for male and female participants was 77.57 ± 14.04kg and 64.61 ± 11.84kg respectively. Mean BMI for the total population was 24.52 ± 4.01 kg/m^2, with very little difference in mean BMI between the genders. The participants from the Northwest Province had the highest mean BMI (25.17 ± 2.59 kg/m^2), while those from the Northern Cape had the lowest (22.21 ± 1.54 kg/m^2). The mean BMI for the participants from the Western Cape was 24.69 ± 4.02 kg/m^2.

Table 4.5. Mean anthropometrical measurements per demographic

Variable	Weight (kg) mean ± SD	Height (cm) mean ± SD	BMI (kg/m^2) mean ± SD	Test and p-value*	Waist circumference (cm) mean ± SD	Test and p-value**
Total (n=242)	**71.1 ± 14.03**	**170.0 ± 9.49**	**24.52 ± 4.01**		**81.54 ± 9.74**	
Gender						
Male (n=121)	77.57 ± 14.04	176.55 ± 6.84	24.85 ± 3.84	**Mann-Whitney U; p=0.07**	84.23 ± 9.57	**Mann-Whitney U; p<0.01**
Female (n=121)	64.61 ± 11.84	163.36 ± 6.80	24.19 ± 4.17		78.86 ± 9.19	
Race						
African (n=57)	70.36 ± 14.06	167.49 ± 9.99	25.08 ± 4.52	**Kruskal-Wallis; p=0.21 (p>0.05; weighted data)**	80.77 ± 9.74	**Kruskal-Wallis; p=0.15**
Asian (n=6)	63.24 ± 11.41	170.84 ± 7.88	21.51 ± 2.26		74.59 ± 6.52	
Coloured (n=72)	70.02 ± 16.47	166.27 ± 8.71	25.14 ± 4.77		82.19 ± 11.89	
Indian (n=13)	67.04 ± 12.12	169.96 ± 8.80	23.18 ± 3.85		80.41 ± 10.49	
White (n=94)	73.42 ± 12.08	174.22 ± 8.33	24.07 ± 2.89		82.12 ± 7.73	
Province						
Eastern Cape (n=23)	73.90 ± 11.25	170.32 ± 8.77	24.69 ± 4.02	**Kruskal-Wallis; p=0.40**	84.37 ± 10.48	**Kruskal-Wallis; p=0.33**
Free State (n=5)	66.70 ± 15.61	164.59 ± 11.37	24.73 ± 6.09		78.05 ± 11.09	
Gauteng (n=22)	68.26 ± 10.30	167.33 ± 8.58	24.40 ± 3.62		79.91 ± 7.66	
Kwazulu Natal (n=29)	68.67 ± 15.99	169.48 ± 9.35	23.89 ± 5.00		78.62 ± 10.47	
Limpopo (n=8)	69.68 ± 18.67	171.77 ± 15.92	23.14 ± 2.71		80.65 ± 8.21	
Mpumalanga (n=4)	67.35 ± 10.62	168.83 ± 8.94	23.62 ± 3.45		79.77 ± 7.99	
Northern Cape (n=6)	68.12 ± 9.45	174.74 ± 8.49	22.21 ± 1.54		80.41 ± 6.81	
Northwest Province (n=2)	81.16 ± 15.40	179.08 ± 7.85	25.17 ± 2.59		89.48 ± 13.74	
Western Cape (n=142)	71.85 ± 14.54	170.16 ± 9.38	24.69 ± 4.02		82.07 ± 9.89	

*p-value refers to mean BMI **p-value refers to mean waist circumference

Abbreviations: BMI: body mass index, SD: Standard deviation

Eighty percent of participants (n=194) had a normal waist circumference. Male participants were more likely to have a normal waist circumference (44%, n=106) when compared to female participants (36%, n=88).

The majority of participants had a normal BMI (60.74%, n=147) and just over a quarter were classified as overweight (Figure 4.9). The rest were classified as obese class I (8.26%, n=20) and obese class II (2.07%, n=5). Only one participant was classified as obese class III.

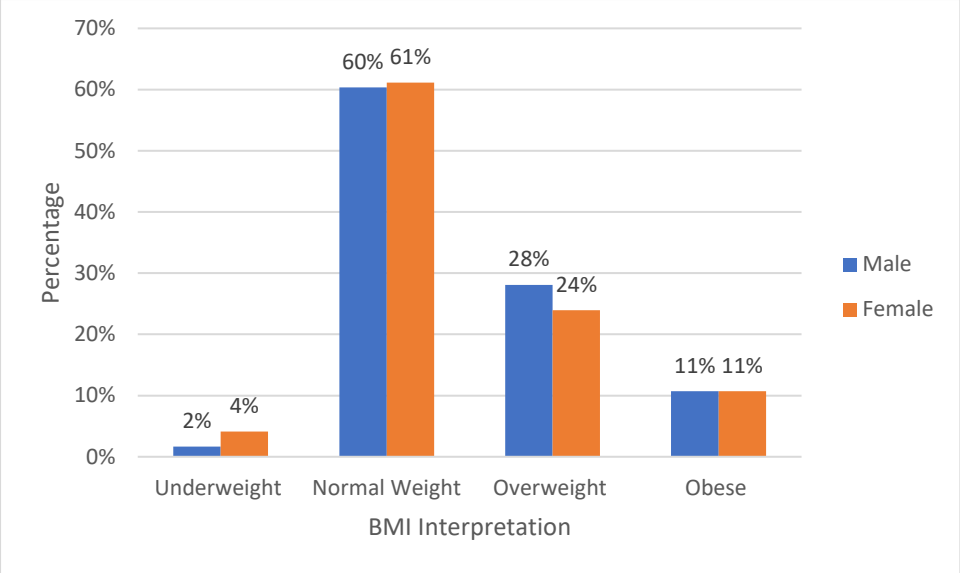

Figure 4.9. Body mass index (BMI) classification by gender

As mentioned above in Table 4.5, BMI scores differed between races. While the participants from Coloured and African races in this study had higher BMIs than their counterparts, this difference was not found to be statistically significant (Figure 4.10).

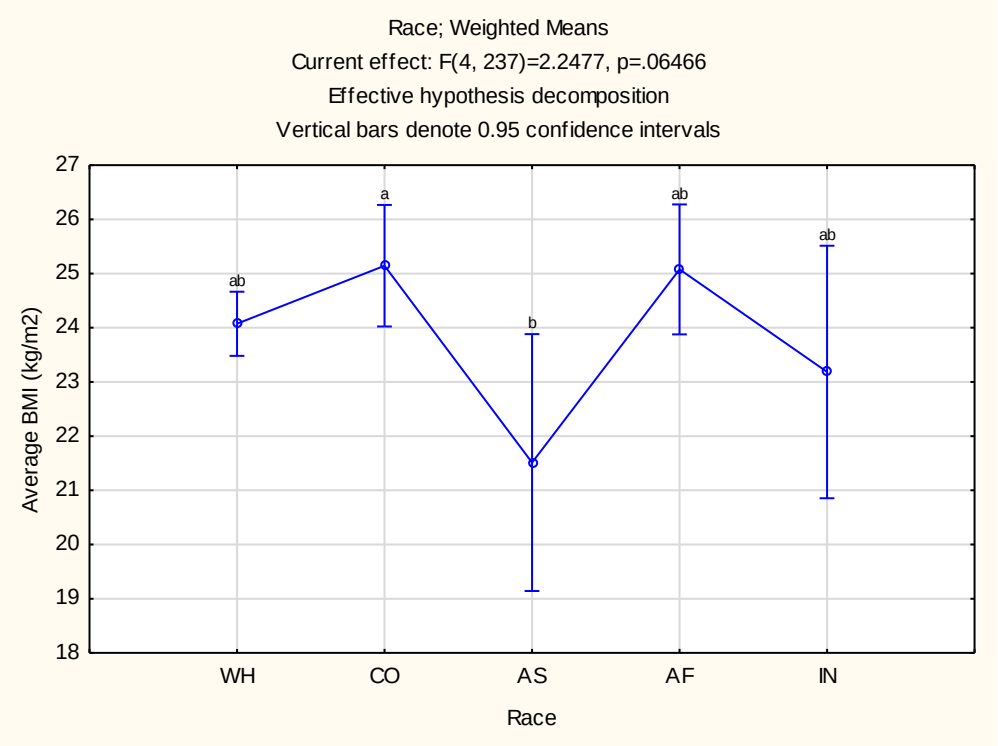

Fig 4.10. Body mass index and race

Abbreviations: BMI: Body mass index; WH: White; CO: Coloured; AS: Asian; AF: African; IN: Indian

There was no statistical significance found between the Endocrine Society's interpretation of vitamin D deficiency and BMI (Kruskal-Wallis; p=0.08), even when the data was weighted (figure 4.11). While the relationship was not significant, those with higher BMIs were more likely to have an insufficient vitamin D status. Similar results were found for waist circumference (Figure 4.12).

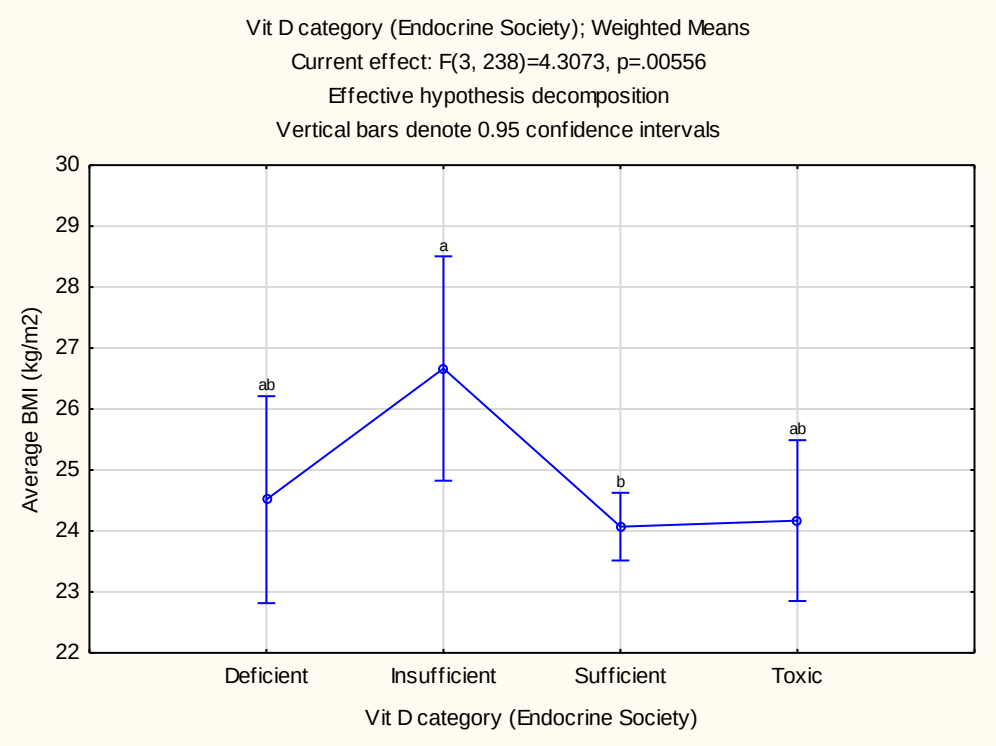

Fig 4.11. Body mass index and vitamin D status: Endocrine Society (weighted data)

Abbreviations: BMI: Body mass index, Vit D: Vitamin D

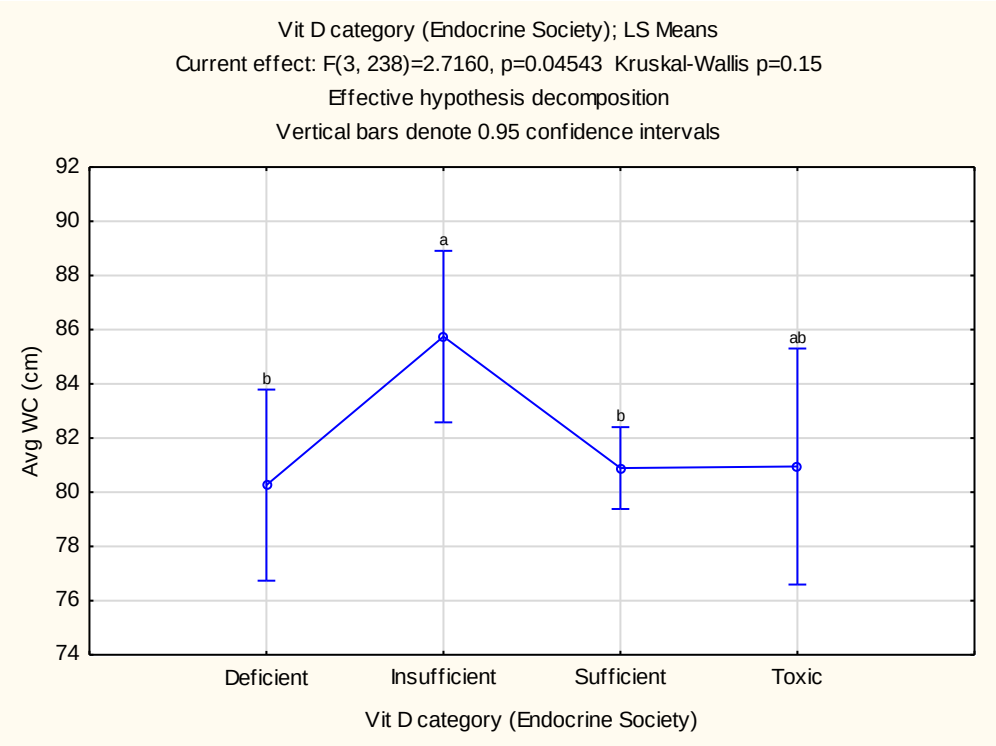

Fig 4.12. Waist circumference and vitamin D status: Endocrine Society

Abbreviations: Avg WC: Average waist circumference; Vit D: Vitamin D

There was a weak, negative relationship found between serum 25(OH)D levels and BMI but this was also not statistically significant (Spearman's r=-0.11; p=0.09) (Figure 4.13). Even though the relationship between BMI and serum 25(OH)D was not found to be significant, the relationship between height and serum 25(OH)D was (Figure 4.14), with taller participants having higher serum 25(OH)D levels.

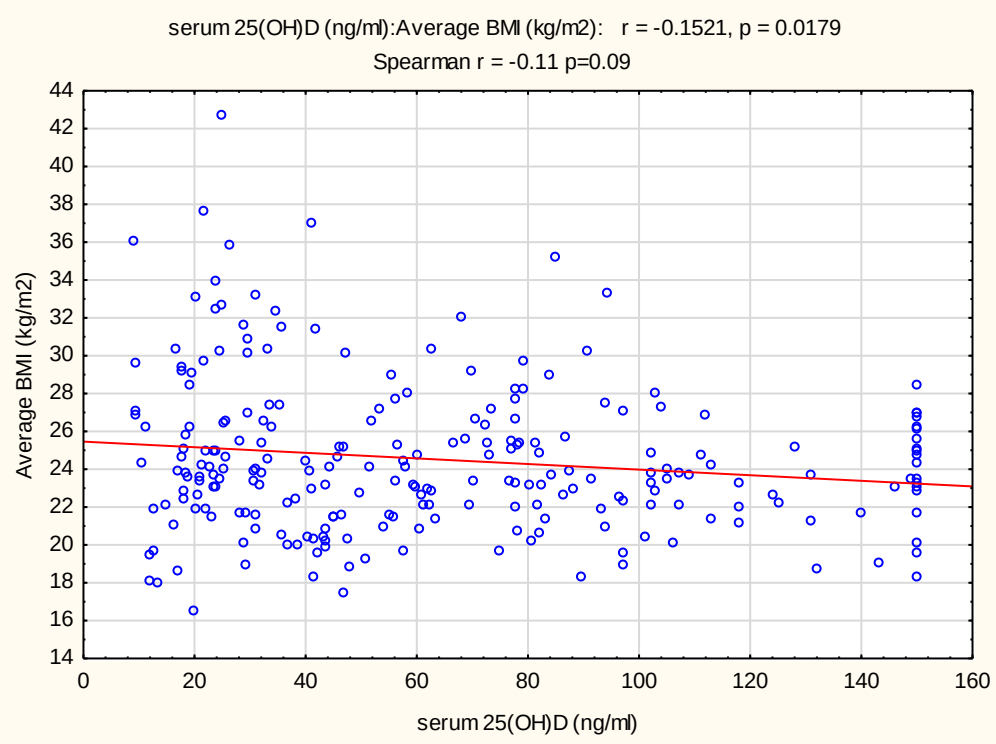

Fig 4.13. Relationship between body mass index and serum 25-hydroxyvitamin D

Abbreviations: BMI: Body mass index, 25(OH)D: 25-hydroxyvitamin D

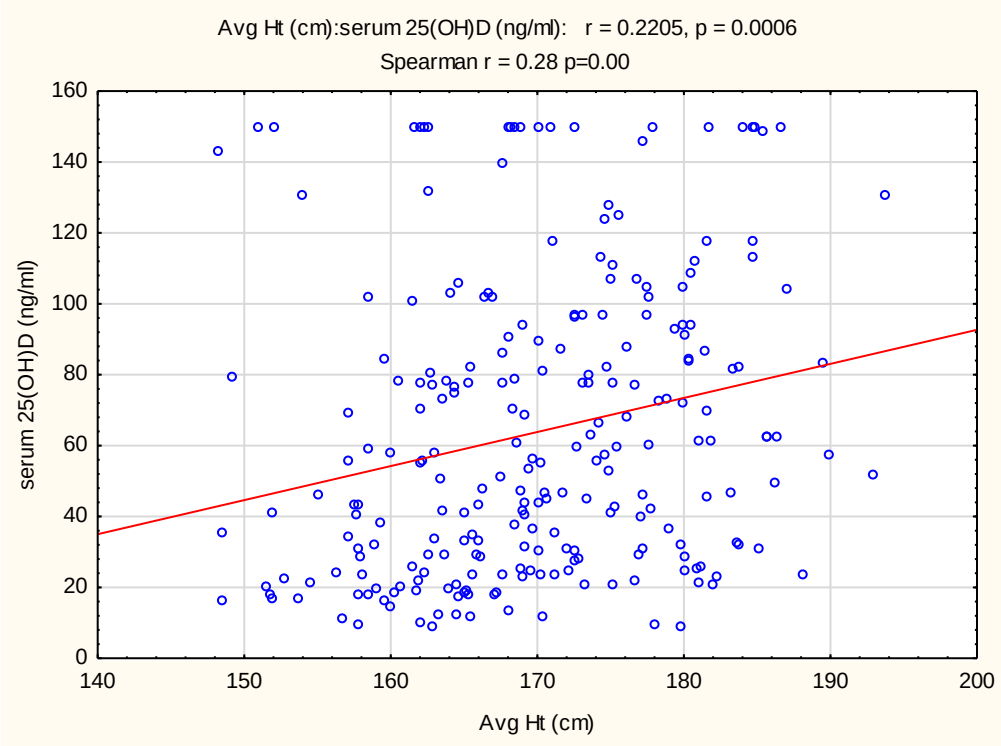

Fig 4.14. Height and serum 25-hydroxyvitamin D

Abbreviations: Avg Ht: Average height, 25(OH)D: 25-hydroxyvitamin D

4.5 Diet

The mean energy consumption from vitamin D rich food sources was 3604.97 ± 1732.73 kilojoules per day. The results of the food frequency questionnaire showed that the most commonly consumed vitamin D rich food groups were Milk and Milk Products, and Fish and Seafood (Figure 4.15).

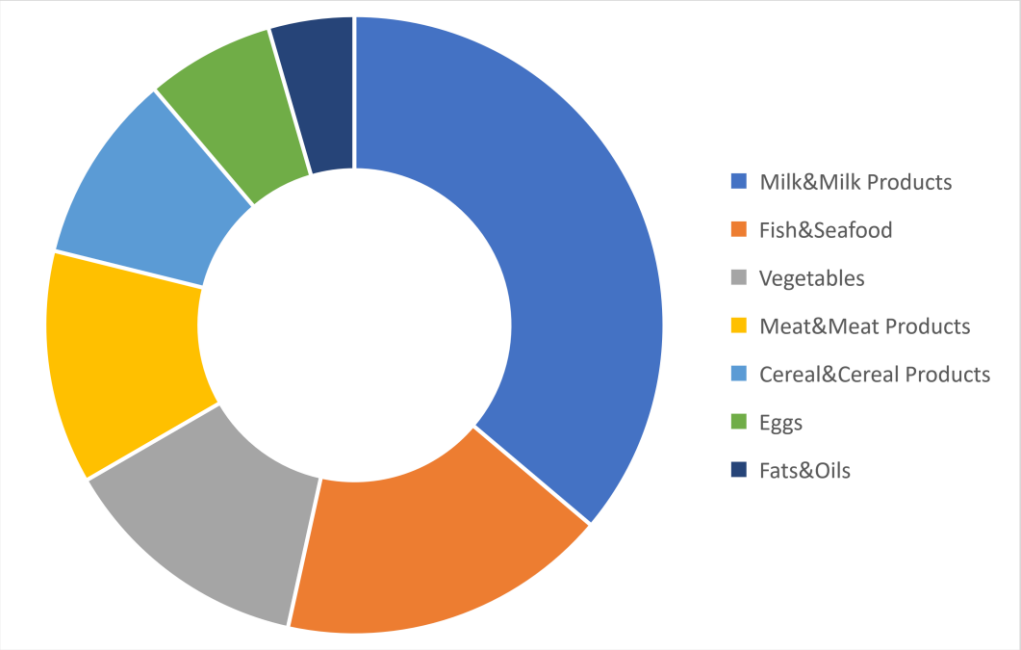

Fig 4.15. Food groups consumed by participants per day

Within the Milk and Milk Products food group, the most commonly eaten foods were plain milk, cheese, and yoghurt. The most commonly eaten Fish and Seafood products were canned tuna and low-fat fish. Broccoli, mushrooms and spinach made up the majority of the vitamin D rich vegetables consumed. More beef was consumed than pork.

Table 4.6 shows the total daily mean vitamin D intake, as well as the amount of vitamin D consumed daily from the diet and from supplements. The mean total vitamin D intake of the total population was 7.99 ± 13.81mcg. More vitamin D came from the diet than from supplemental sources. Males consumed more vitamin D than females (9.60 ± 9.46mcg and 6.33 ± 16.94mcg respectively). The participants from Mpumalanga consumed the least vitamin D and those from the Northwest province consumed the most.

Table 4.6. Mean vitamin D intake: Gender, Province, Fitzpatrick skin type classification

Variables	Total (mcg); mean ± SD (n=242)	Test and p-value	Dietary intake (mcg) mean ± SD	Test and p-value	Supplement intake (mcg) mean ± SD	Test and p-value
Total population	7.99 ± 13.81		5.04 ± 4.10		2.91 ± 12.95	
Gender						
Male (n=121)	9.60 ± 9.46	**Mann-Whitney U; p<0.01**	6.25 ± 4.74	**Mann-Whitney U; p<0.01**	3.27 ± 7.76	**Mann-Whitney U; p=0.22**
Female (n=121)	6.33 ± 16.94		3.81 ± 2.86		2.53 ± 16.59	
Province						
Eastern Cape (n=23)	6.45 ± 7.66	**Kruskal-Wallis; p=0.66**	4.94 ± 3.85	**Kruskal-Wallis; p=0.98**	1.52 ± 6.06	**Kruskal-Wallis; p=0.03**
Free State (n=5)	6.98 ± 4.78		5.18 ± 4.75		0 ± 0	
Gauteng (n=22)	6.32 ± 6.03		4.90 ± 3.56		1.42 ± 5.38	
Kwazulu Natal (n=29)	6.65 ± 7.30		5.54 ± 6.01		1.10 ± 2.87	
Limpopo (n=8)	10.08 ± 11.19		4.10 ± 4.38		5.09 ± 8.92	
Mpumalanga (n=4)	3.86 ± 2.41		3.86 ± 2.41		0 ± 0	
Northern Cape (n=6)	7.40 ± 10.12		2.81 ± 0.94		4.58 ± 10.05	
Northwest Province (n=2)	22.02 ± 12.70		4.17 ± 2.59		17.85 ± 10.11	
Western Cape (n=142)	8.63 ± 16.82		5.11 ± 3.89		2.28 ± 6.42	
Fitzpatrick skin type classification						
Type II (n=11)	7.21 ± 5.39	**Kruskal-Wallis; p=0.76**	5.85 ± 4.39	**Kruskal-Wallis; p=0.75**	1.35 ± 3.35	**Kruskal-Wallis; p=0.47**
Type III (n=67)	7.57 ± 8.81		4.61 ± 3.48		2.96 ± 7.32	
Type IV (n=133	8.58 ± 17.15		4.94 ± 3.81		3.57 ± 16.57	
Type V (n=30)	6.46 ± 7.07		6.02 ± 6.12		0.44 ± 1.96	

The total vitamin D intake of participants was mostly even amongst the skin type groups, with the participants in type IV consuming slightly more vitamin D and those in type V consuming slightly less (Table 4.6). The participants in type V also consumed the most vitamin D in the diet, but consumed the least vitamin D via supplementation of the groups.

Analysis of the food frequency questionnaires found that only 2.5% of participants consumed enough vitamin D in their diet to meet the recommended daily needs (15mcg). Of those who took vitamin D supplements, 16 participants (6.6%) took enough to exceed the recommended daily dosages. When it came to total vitamin D intake, only 31 participants (12.8%) consumed enough vitamin D to meet their recommended daily needs. Although one participant had a much higher daily intake of vitamin D (182.84mcg), no participants consumed toxic levels of vitamin D (250mcg – 1000mcg/day) either through the diet or through supplementation. Diet was the biggest contributing factor in those participants who consumed 1-14.99mcg of vitamin D per day (Figure 4.16).

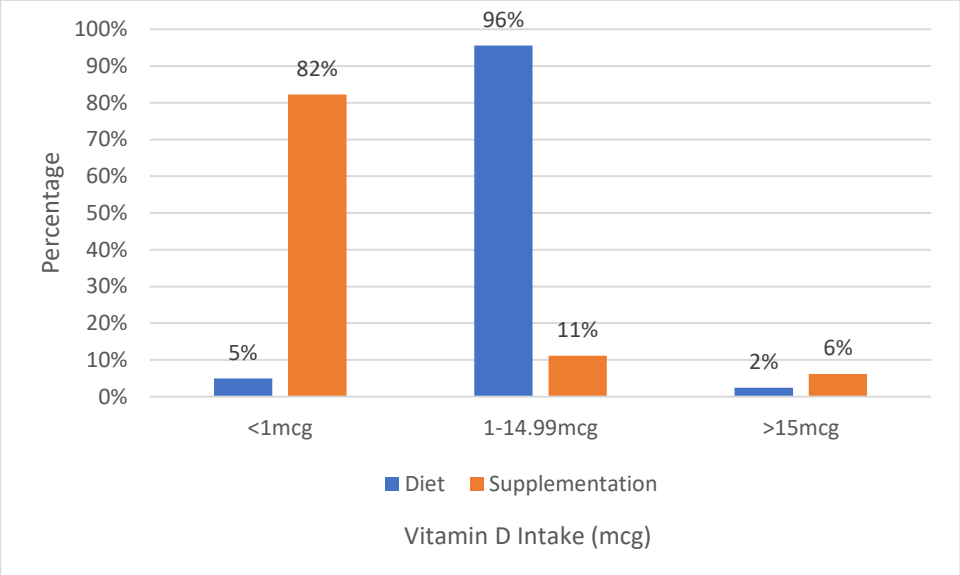

Fig 4.16. Vitamin D intake (in mcg) from diet and supplementation

The relationship between the vitamin D consumed in the diet and serum 25(OH)D levels was weak and not statistically significant (Spearman r=0.06; p=0.36), although a trend was observed that showed that serum 25(OH)D increased in relation to increased dietary vitamin D consumption. A weak, positive relationship between supplemented vitamin D and serum 25(OH)D levels was observed (Spearman r=0.19; p<0.01), although an extreme outlier was included in these results. Once the outlier was removed, the relationship was still weak but statistically significant (Spearman r=0.18; p=0.01) (Figure 4.17).

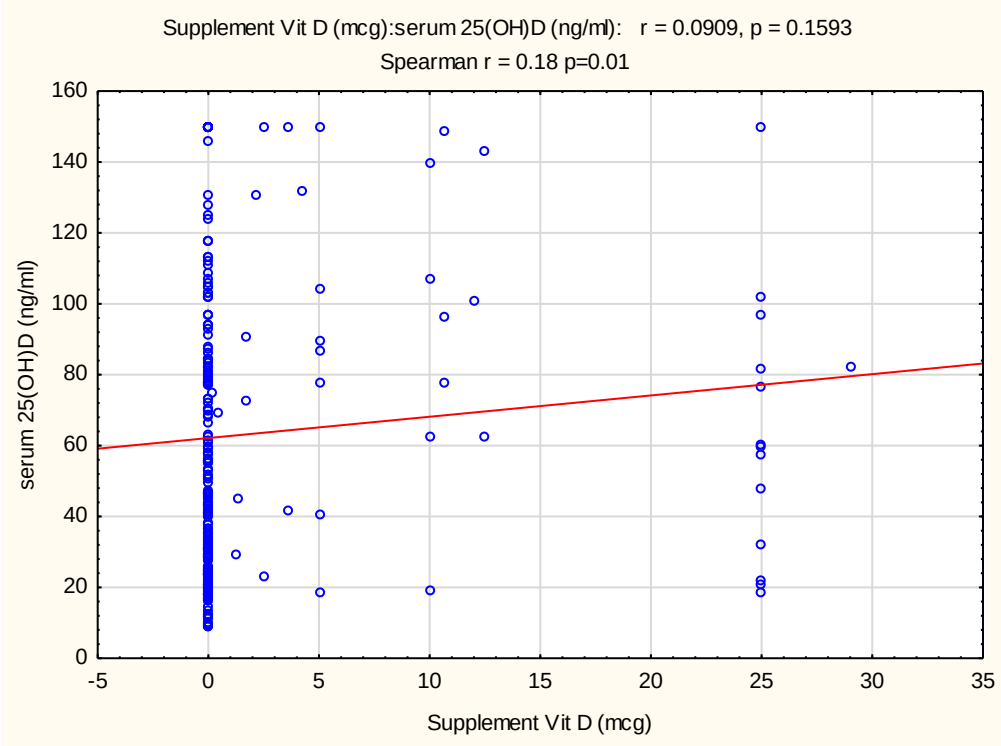

Fig 4.17. Serum 25-hydroxyvitmain D and supplemental vitamin D (outlier removed)

Abbreviations: 25(OH)D: 25-hydroxyvitamin D, Vit D: Vitamin D

The relationship between total vitamin D intake and serum 25(OH)D was positive and statistically significant, but weak. (Spearman r=0.14; p=0.03) (Figure 4.18). When a Kruskal-Wallis test was performed, the relationship between total vitamin D intake and vitamin D status was not found to be statistically significant (p=0.12), and the results showed very little difference between sufficient, insufficient, and deficient categories.

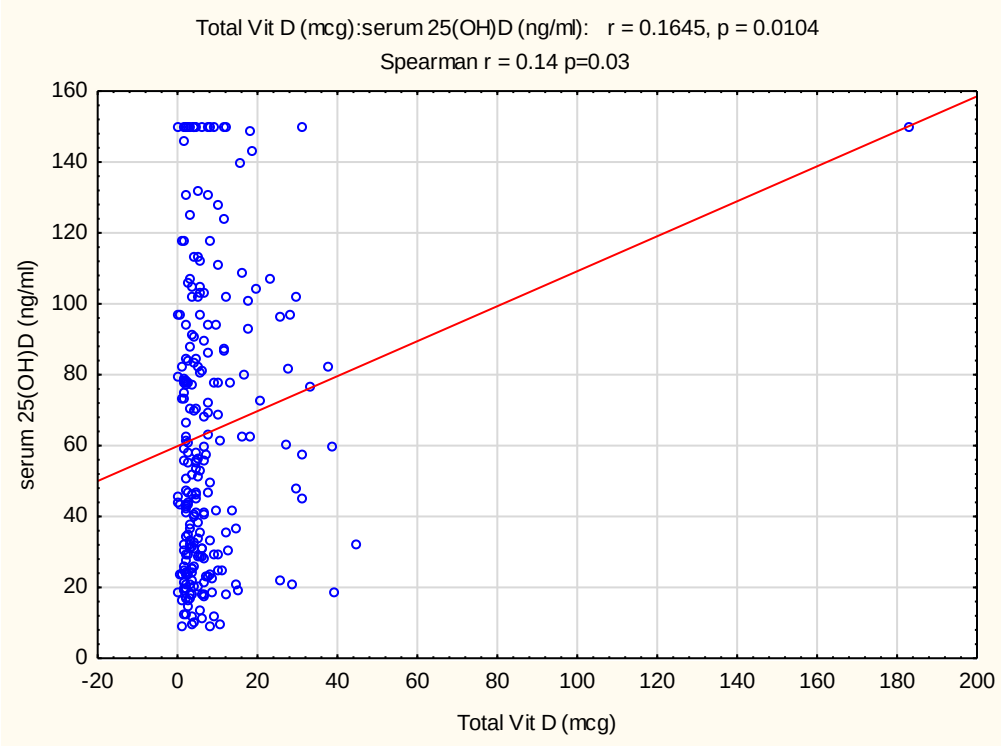

Fig 4.18. Total vitamin D intake vs. serum 25-hydroxyvitamin D

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; Vit D: Vitamin D

There was a significant relationship found between gender and dietary vitamin D (Figure 4.19), with males consuming more vitamin D from the diet than females. This was mirrored in total vitamin D intake when a Mann-Whitney U test was applied (Mann-Whitney U; $p<0.01$). While relationship between gender and supplementary vitamin D intake was not significant (Mann-Whitney U; $p=0.22$), the trend of males consuming more vitamin D than females was continued.

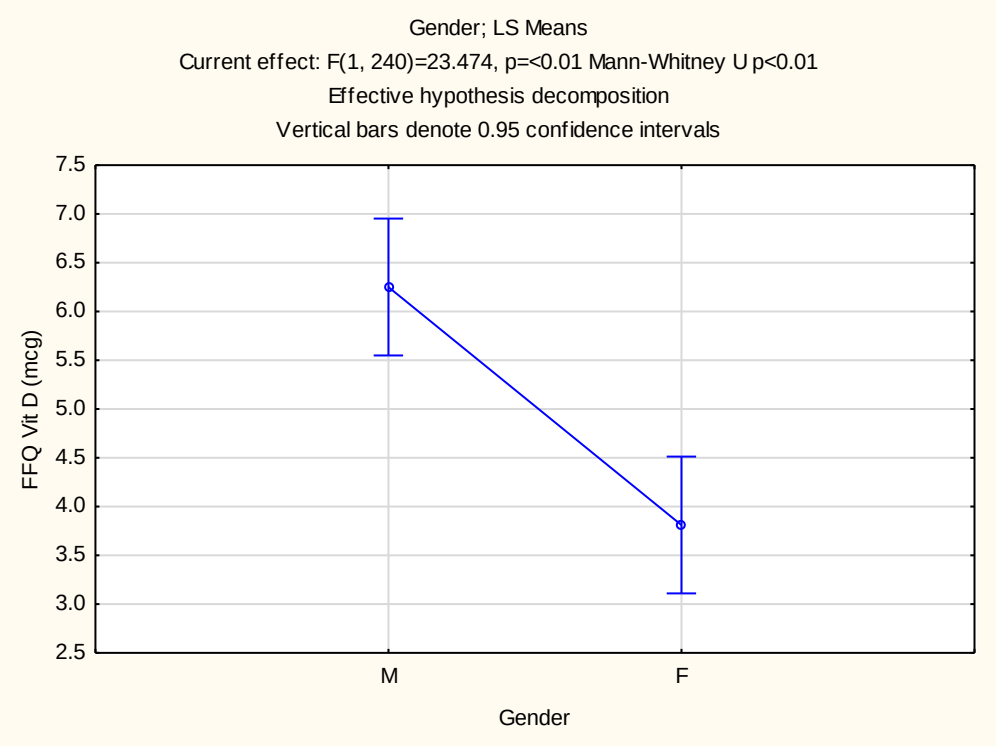

Figure 4.19. Dietary vitamin D intake and gender

Abbreviations: FFQ Vit D: Food frequency questionnaire vitamin D; M: Male; F: Female

While a Kruskal-Wallis test found no significant relationship between the variations of Vitamin D in the diet between participants from different home provinces (p=0.98), participants from KZN consumed slightly more vitamin D than those from the other provinces, and participants from the Northwest Province consumed slightly less. The total vitamin D intake of the participants did not differ significantly between provinces (Kruskal-Wallis; p=0.66), although those in the Northwest Province consumed slightly more total vitamin D than the other provinces.

The relationship between dietary vitamin D intake and skin classification was not found to be statistically significant (Kruskal-Wallis; p=0.75), nor was supplemental vitamin D (Kruskal-Wallis; p=0.47) or total vitamin D (Kruskal-Wallis; p=0.76). There was, however, a trend observed that showed a slightly higher intake of total vitamin D for those participants in the type IV skin type group.

4.6 Lifestyle factors

Twenty-seven participants (11.16%) were smokers, who smoked an average of 2.5 ± 3.46 cigarettes per day, and 147 participants (60.74%) consumed alcohol on a regular basis (2.88 ± 4.43 units per week).

Higher mean 25(OH)D levels were observed in those that used alcohol (Figure 4.20), although this relationship was not statistically significant.

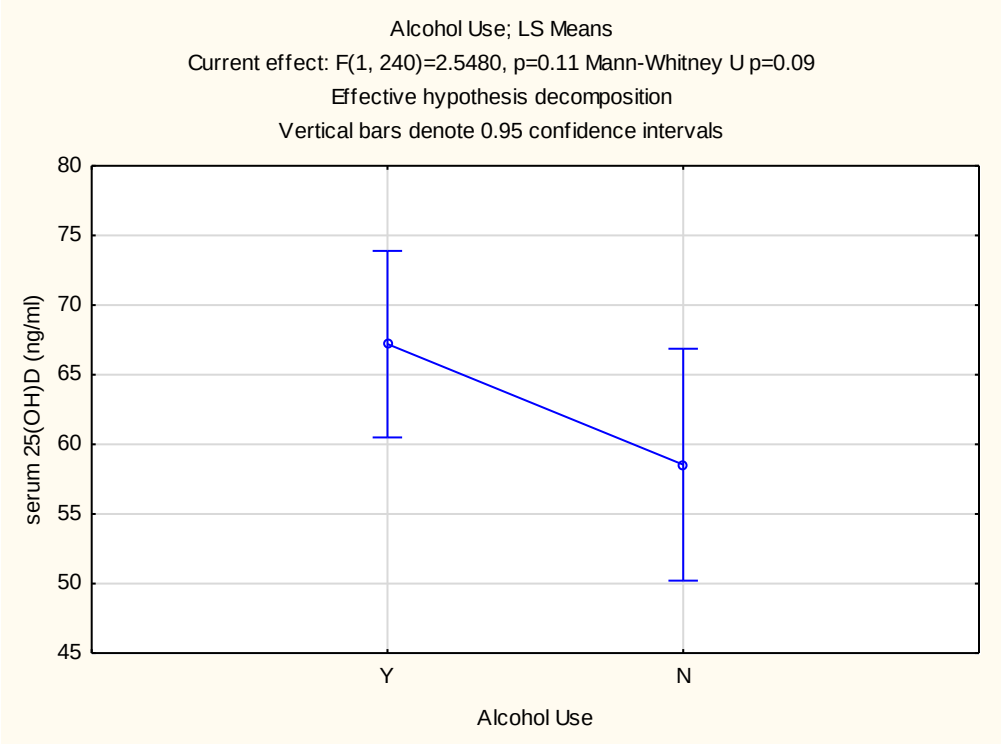

Fig 4.20. Alcohol use and serum 25-hydroxyvitamin D

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; Y: Yes; N: No

Tobacco use did not have a significant effect on serum 25(OH)D levels (Mann-Whitney U; p=0.48). It was observed, however, that the participants who used tobacco had lower serum 25(OH)D levels than those who did not.

The relationship between exercise and serum 25(OH)D was not significant (Mann-Whitney U; p=0.15) but those who partook in physical activity had higher 25(OH)D levels than those who did not. The relationship between total vitamin D intake and exercise was statistically significant, with those participants who exercised more likely to consume more vitamin D than those who did not (figure 4.21). The participants who exercised were also more likely to use supplements, but this relationship was not found to be statistically significant. (p>0.05).

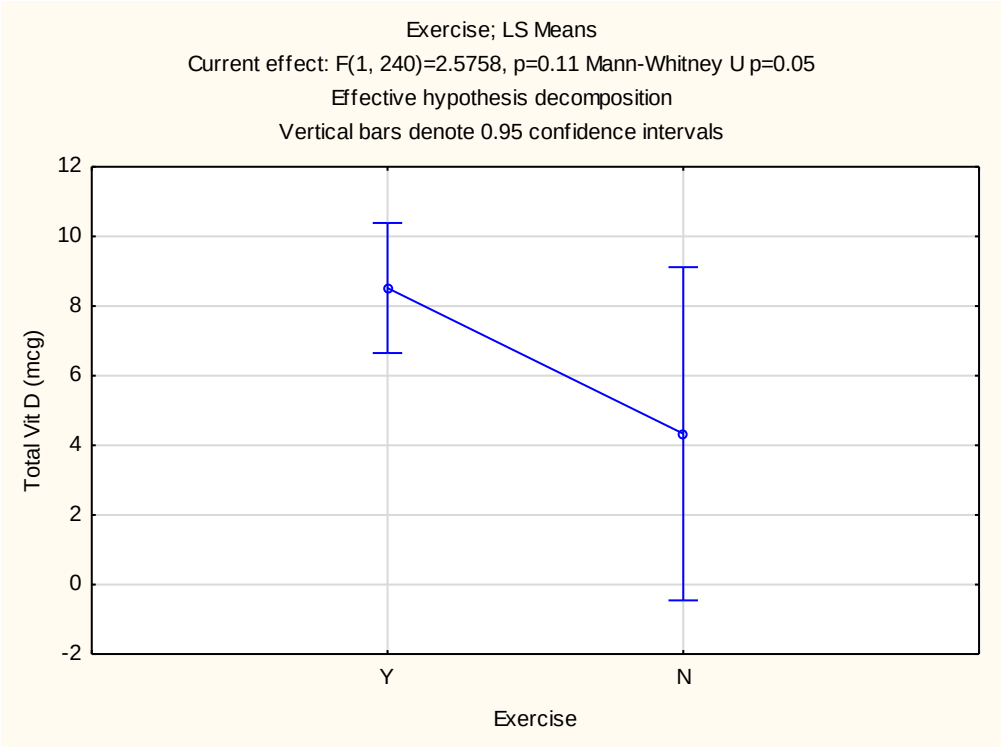

Fig 4.21. Total vitamin D intake vs. exercise

Abbreviations: Vit D: Vitamin D; Y: Yes; N: No

4.7 Sun exposure

Ninety-one percent of participants used sunscreen to some degree, with only 10% of participants applying sunscreen all the time (Figure 4.22).

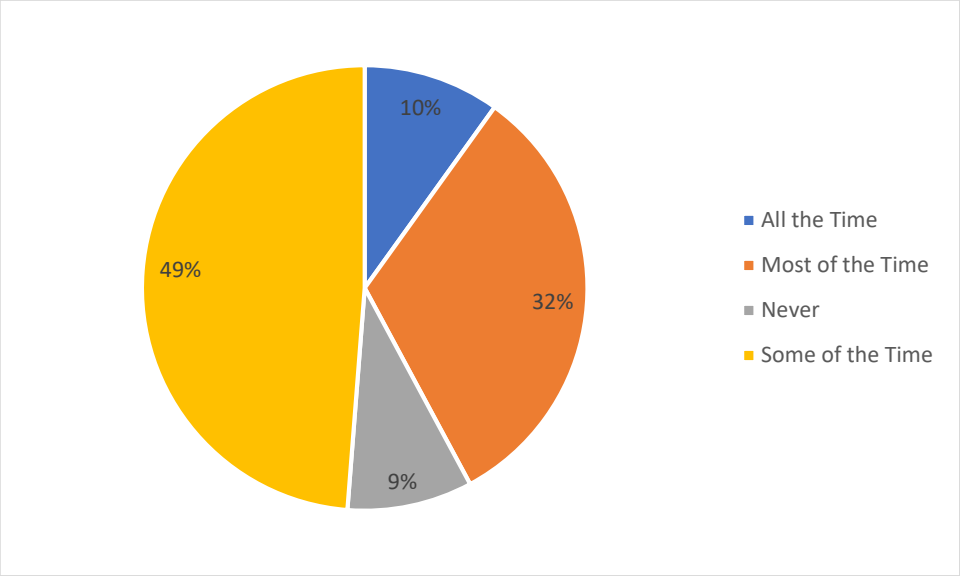

Fig. 4.22. Sunscreen usage of participants

Most participants spent an average of 5 – 30 minutes in the sun during the week, and were exposed to longer periods of sunlight (>30 minutes) on weekends (Figure 4.23). Most exposure took place in the afternoon, between 10h00 and 15h00, with few participants experiencing exposure before 10h00 (Figure 4.23). During the week, participants had more exposure to sunlight after 15h00 than on the weekends, when more sun exposure took place between 10h00 and 15h00.

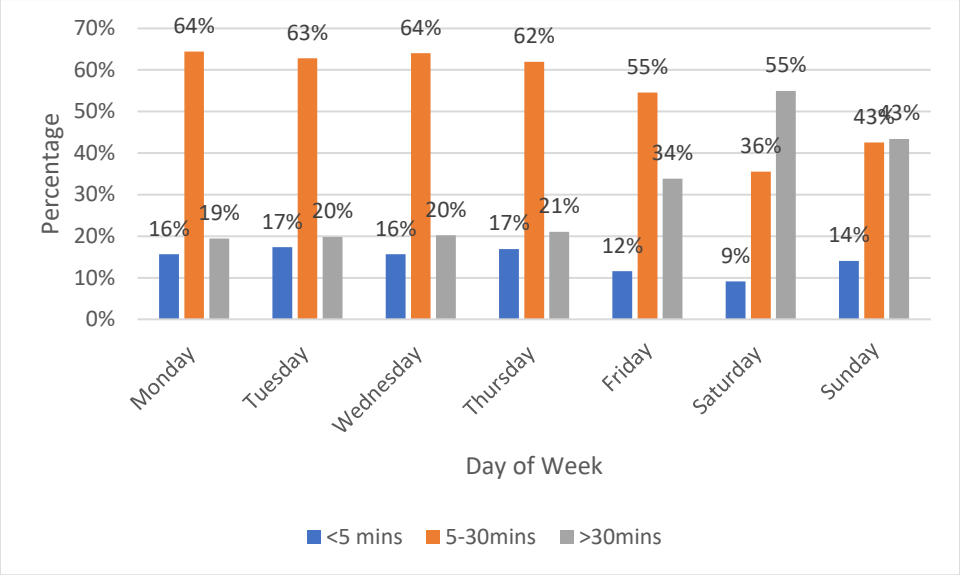

Figure 4.23. Time spent in the sun per day

Abbreviations: mins: minutes

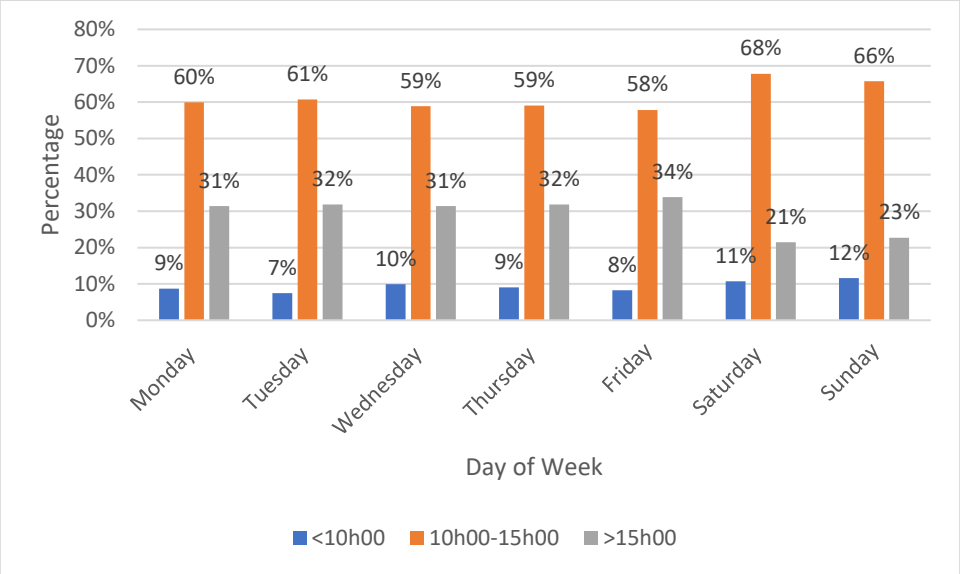

Figure 4.24. Time of day most sunlight exposure took place

The hands, face and arms were the areas of skin that participants exposed most during weekdays (Figure 4.25). Over the weekends, it was observed that participants had increased skin exposure, with more participants only covering their bathing suit area.

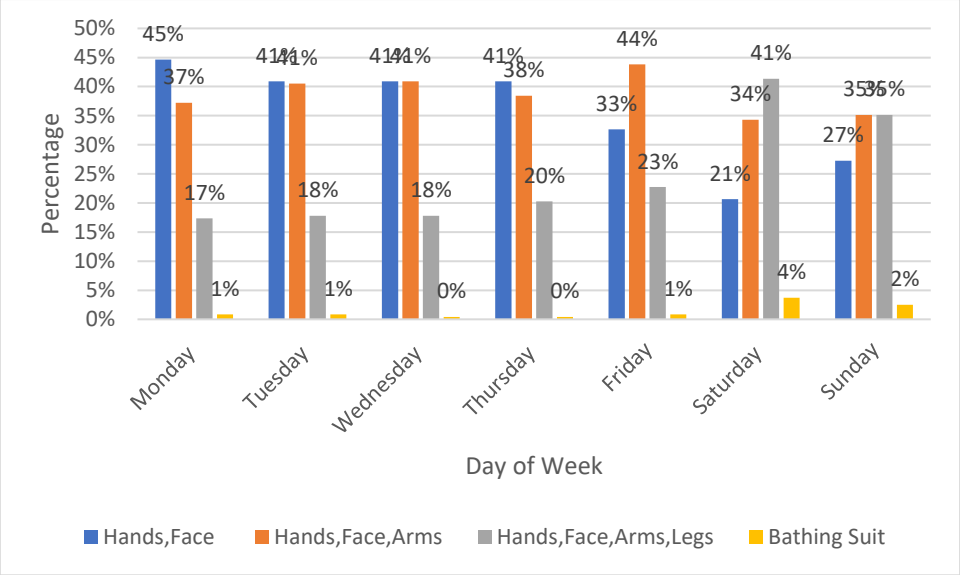

Figure 4.25. Amount of skin exposed to sunlight

The relationship between the duration of sun exposure and vitamin D status was not found to be significant (Chi^2=10.04; p>0.05) but most of the participants who were classified as having a sufficient vitamin D status were exposed to more than 5 minutes of sunlight per day (59.09%, n=143). The relationship between the amount of skin exposed and vitamin D status was also not statistically significant (Chi^2=11.51; p>0.05), although there was a trend of better vitamin D status when more skin was exposed.

5 Discussion

In this study, the researchers investigated the vitamin D status of young adults living in the Western Cape, as well as the potential factors that could influence vitamin D status. Factors investigated included gender differences, differences in skin tone, anthropometrical measurements, dietary intake of vitamin D, and lifestyle factors including smoking, alcohol use, and exercise. This study population, while homogenous, was specific in their profile (i.e. young, healthy, undergraduate students) and this needs to be taken into consideration when interpreting the results of this study.

5.1 Vitamin D status

The high prevalence of vitamin D deficiency worldwide is a global concern.[28,99] The results of this study, however, show a high prevalence of vitamin D sufficiency (73% using ES reference values and 90% using IOM reference values) in young adults living in Western Cape. Other South African studies vary when it comes to investigating and reporting vitamin D status. Some studies have found a high prevalence of vitamin D deficiency, while others support the results of this study of adequate vitamin D status.[32,33,75,100] The results of this study were in some cases expected, while in other cases not.

Firstly, the study was conducted at the end of winter (6 weeks post winter solstice), a season where many global studies, using <50nmol/L (20ng/ml) to define insufficiency and <25nmol/L (10ng/ml) to define deficiency, have reported a strong tendency towards vitamin D deficiency.[101-103] Karaguzel et al studied schoolchildren and found that, when using a cut off value of <50nmol/L (20ng/ml) for deficiency, serum 25(OH)D levels were lower in autumn, particularly in girls when compared to boys.[104] Alvares et al conducted a study in Sao Paulo to investigate serum 25(OH)D levels in healthy adults at the end of winter and found that hypovitaminosis D was more prevalent at the end of winter.[105] Based This result is particularly interesting as Brazil has a high level of sunlight throughout the year. Djennane et al also studied 25(OH)D levels (using cut off values of <50nmol/L (20ng/ml) for insufficiency and <30nmol/L (12ng/ml) for deficiency) in children and adolescents in Algeria and found similar results to Alvares – even though Algeria's climate is sunny year-round, there was still a higher prevalence of vitamin D deficiency at the end of winter.[106] As these studies, as well as the time of data collection, it was expected that a higher prevalence of hypovitaminosis D would be found. In the winter of 2016, however, Cape Town experienced low levels of rain and unseasonably high temperatures and UVB indexes as a result of El Niño that affected the country's natural rainfall patterns.[107]

As the majority of vitamin D is synthesised from natural exposure to UVB radiation, climate change may play a role in vitamin D status both at present and in the future. Increases in

surface sea temperature in the oceans surrounding South Africa has led to changes in the wind patterns of some coastal towns and cities.[108] These changes have been exacerbated by El Niño events and have led changes in the Cape Town weather pattern. Previously experiencing extreme rains during the winter season, Cape Town has in the last few years experienced much dryer winters. Studies have shown that these less extreme precipitation events will continue in the Cape Town region.[109] These changes in the South African climate have not only altered the seasonal rainfall patterns, but have also affected seasonal temperatures, making the weather warmer and more mild in seasons where it has been traditionally colder. Meteorological predictions for South African have shown temperatures increases of up to 4°C before the dawning of the next century.[110] These changes in seasonal weather patterns could perhaps have encouraged participants to venture outside instead of staying indoors, increasing their exposure to natural UVB radiation.

The participants of this study were young adults, and it was expected that they would have lower serum 25(OH)D levels. Tangpricha et al studied the vitamin D status of young adults and found that the 18 – 29 year age group was more likely to experience low levels of serum 25(OH)D than older groups, as well as more likely to experience greater seasonal variations in vitamin D status.[13] A study on Lebanese university students with a similar age range to current study found that while serum 25(OH)D levels were low, they were better than those groups who were not as educated.[111] In Iran, a study on university students found that almost half the population had vitamin D deficiency.[112] A Korean study found that the lowest serum 25(OH)D levels (and highest prevalence of vitamin D deficiency risk) are experienced between the ages of 20-29 years, compared to the peak 25(OH)D levels between 60-69 years.[113] From the above studies, we can see the prevalence of vitamin D deficiency is becoming increasingly common among the younger generation. Society is moving towards a more indoors lifestyle, and as such, may not spend as much time in the sun as previous generations.

The results of this study were also unexpected as Cape Town has a low latitude. Latitude has been found to have a significant effect on vitamin D_3 synthesis.[114] UVB intensity is linked to latitude, with those people living closer to the equator often experiencing sufficient levels of 25(OH)D.[115,116] Many studies have found that those living at low latitudes have lower levels of 25(OH)D when compared to those living at higher latitudes.[117] Hamilton et al studied the vitamin D status in pregnant women at a latitude of 32N and found higher levels of deficiency at lower latitudes when compared to studies done at higher latitudes, despite the study population being exposed to sunlight for most of the year.[118] South African provinces experience different levels of both altitude and latitude. While the data collection period for this study took place almost directly after the university's midyear break, allowing

those in residence to return to their home provinces with higher latitudes than Cape Town, it is unlikely to have had an effect on serum 25(OH)D levels. Datta et al investigated the half-life of serum 25(OH)D and found 25(OH)D to have a mean half-life of 89 days.[119] Interestingly, Datta et al also reported that the half-life of 25(OH)D increased as serum 25(OH)D levels decreased. The midyear break did not allow for long periods of time in other areas and, as such, is unlikely to have affected the results of this study.

This study also found no relationship between vitamin D deficiency and home province, which is in contrast to the results from other studies. Studies have found that there are less seasonal variations in serum 25(OH)D levels in Johannesburg, South Africa, than in Cape Town. The northern provinces of South Africa experience more hours of annual sunshine than the more southern provinces. This affects the UVB exposure index in that area, as well as the amount of time that people are exposed to sunlight each day.[44] Thus, those residing in Cape Town should be more at risk of low 25(OH)D levels in winter than those residing in Johannesburg.[42,114,120]

While there was only one participant who was born in a foreign country included in this study, intercountry differences in serum 25(OH)D can clearly be seen in the literature when people immigrate. Campagna et al studied the vitamin D status of immigrants and refugees compared to US born citizens in Minnesota and found those who were not born in the US were more likely to be vitamin D deficient.[121] A study conducted in Italy on native and immigrant mothers showed similar results, with immigrants more likely to experience low levels of 25(OH)D.[122] This shows that both genetics and country of origin might have long reaching health effects despite geographical relocation.

There was a high level of vitamin D toxicity found in this study, with ES reference values for toxicity (>150ng/ml) accounting for 8% of the population. When the IOM reference values were used (>50ng/ml), this percentage increased exponentially to 54%. This drastic increase was expected as the IOM reference values are much lower than those suggested by the ES, and what the IOM classifies as toxic 25(OH)D levels, the ES classifies as safe.

The DiaSorin LIAISON assay was used to analysed serum 25(OH)D levels. Research has shown that this method is the most efficient with regard to expense, labour, and accuracy when compared to LC-MS/MS.[123] Chemiluminescent immunoassays use a specific antibody to separate 25(OH)D from its binding protein.[124] While an accepted method of measuring 25(OH)D levels, these assays tend to overestimate serum 25(OH)D, with functional sensitivity for the LIAISON shown to be as much as 17.5nmol/l in one study.[124-126] It has also been found by some to be more sensitive to $25(OH)D_2$ than $25(OH)_3$.[127]

While rare, vitamin D toxicity is associated with life-threatening complications with symptoms of overdose including gastrointestinal complaints, weakness, and renal dysfunction.[187] Due to ethical considerations, vitamin D toxicity has not been extensively studied in the literature. Serum $1,25(OH)_2D$ toxicity is more common and, as such, more well documented in the literature than 25(OH)D toxicity, although vitamin D intake rarely results in increased $1,25(OH)_2D$ levels.[128] Because of this, the specific level of serum 25(OH)D required for symptoms of toxicity as reported in the literature is unknown. Some researchers have suggested that symptoms of toxicity only start showing at serum 25(OH)D levels of around 750nmol/L.[128] As the laboratory reports for serum 25(OH)D in the current study did not report specific 25(OH)D levels above 150ng/ml (merely stating >150ng/ml), the researchers were unable to determine if those participants who were classified as having toxic vitamin D levels experienced high enough serum 25(OH)D levels for the adverse health effects experienced with “true” toxicity.

As the main cause of hypervitaminosis D is dietary based, this relationship will be discussed further under “5.5. Diet”.

5.2 Gender and 25(OH)D

Males in this study had significantly higher levels of 25(OH)D than females, which rejects the null hypothesis of gender having no effect on serum 25(OH)D as put forward in the objectives. These results are in agreement with some South African and global studies.[25,100,129] Touvier et al found that in Caucasian adults in France, 25(OH)D levels were lower in women than in men.[130] Verdoia et al also found that females were more at risk of deficiency and severe deficiency than males when they studied the effect of gender on vitamin D status in relation to coronary artery disease.[131]

Not all studies support lower serum 25(OH)D in females, however. Forrest et al found that men were more likely to be deficient than women.[132] Hovsepian et al studied vitamin D deficiency in Iran and concluded that women in that region only experience more severe vitamin D deficiency than men because of the clothing restrictions imposed upon them.[129] However, this explanation loses strength when populations who have no gender related clothing restrictions are studied. Al-Horani et al studied young males and females (18-30 years of age) in Iraq and Jordan to determine the relationship between, among other things, gender and vitamin D status. They found that males had higher levels of 25(OH)D, even when compared to females who did not wear religious body coverings.[133] Iraqi females had higher 25(OH)D levels than Jordanian females, whether they were covered due to religious reasons or not.[133] They also found no difference in 25(OH)D between Iraqi and Jordanian males. Cultural and religious clothing restrictions that require one gender to cover more parts of their body than the other have long been thought as the reason why females in certain areas have low levels of 25(OH)D. Often, the amount of outdoor exercise and outdoor activity is also restricted in certain genders, allowing one gender to have more exposure to UVB radiation than the other. It is also interesting that in areas where cultural clothing restrictions are studied, traditionally darker skin tones are also studied. More clothing coverage prevents sun exposure but also limits melanin production. Based on this, it would be expected that when those who have lower melanin concentrations are exposed to sunlight, they would have increased 25(OH)D levels. This relationship between skin tone (melanin content) and 25(OH)D is further explored below (“5.3 Skin Tone”).

As there were few female participants in this study who reported that they were required to cover all parts of their body, cultural and religious clothing restrictions were unlikely to have influenced the serum 25(OH)D results of this study. Participants in the Indian population, however, were found to have the lowest 25(OH)D levels in this study. This population has been found to have lower levels of 25(OH)D when compared to other groups in the literature, and clothing choices have long been hypothesised to be the reason for this.[19,134] George et al found that in the Asian-Indian population, males were more likely to have

higher 25(OH)D levels than females, and though they could not determine concrete reasons for this, it was hypothesised that gender clothing choices played a role in this.[19] The low 25(OH)D level found in the Indian population will be further explored under "5.3 Skin Tone".

The relationship between the severity of vitamin D deficiency and gender in the literature is also inconclusive. The DRAGON study in the Netherlands studied the vitamin D status of Chinese participants living in the Netherlands.[135] They found that men were more likely to have serum 25(OH)D levels below 50nmol/l (20ng/ml) than women but that women were more likely to have 25(OH)D levels below 25nmol/l (10ng/ml) than men.[135] Conversely, a retrospective study investigating 528 African immigrants in New York and the risk factors associated with vitamin D deficiency that they experienced, found that females were more likely to have normal 25(OH)D levels, while males were more likely to experience severe vitamin D deficiency (25(OH)D <25nmol/l (10ng/ml)).[136] Sanghera et al measured the 25(OH)D levels of 3879 participants of Asian-Indian descent and found that men had consistently lower 25(OH)D levels than woman.[137] In a study that included 222 elderly adults (mean age: 72.0 ± 9.2 years), males had lower mean 25(OH)D levels than females.[138]

As mentioned above, males in this study had higher vitamin D levels than females. There is little conclusive evidence in the literature as to why one gender may have higher 25(OH)D levels than the other. As discussed above, clothing restriction is one reason for this. Another possible reason for males having increased 25(OH)D levels in the literature could be their higher testosterone levels.[139] Males also have higher nutritional needs than females and, as such, are more likely to consume more vitamin D than females (further discussed under "5.5 Diet").[140] While the relationship between gender and serum 25(OH)D was found to be significant in this study, it is clear that the relationship between these two entities is still up for debate globally. It is important to note, however, that the effect of gender on vitamin D status is rarely studied in isolation. Often, gender forms a secondary objective and studies investigating the effect of health conditions on vitamin D status usually include diseases and medications that are gender specific, and can impact findings. Conclusions that are drawn regarding the effect of gender on vitamin D status should be done so with care.

5.3 Skin tone

The relationship between skin tone and serum 25(OH)D has been the topic of many studies. The tone and colour of the skin is dependent on the amount of melanin present in the epidermis, and there are many tests available to assess skin tone and colour. In this study, both objective and subjective tests were used as an internal control measure, and the relationship between the subjective Fitzpatrick skin type classification and the objective MI results from the skin reflectometer was found to be statistically significant. While it was expected that these results would be similar, some studies have found a weak correlation between the results of the Fitzpatrick skin type and objective reflectometery devices.[141] The positive relationship found here is encouraging as skin reflectometers can be expensive to purchase and their results can be erratic (as discussed further under "5.8 Strengths and Limitations"), and if the Fitzpatrick skin type classification yields similar results, it could be a good alternative method for measuring skin tone. Holick et al found similar results between the Fitzpatrick skin type classification and MI results when studying vitamin D deficiency in children and concluded that the Fitzpatrick skin type classification could be used independently in studies where skin colour is studied in relation to vitamin D.[142]

As the results of this study show a negative, statistically significant relationship between skin tone (melanin content) and serum 25(OH)D, the null hypothesis of skin tone having no effect on serum 25(OH)D as stated in the objectives of this study is thus rejected. Interestingly, the relationship between vitamin D status and skin tone was not found to be statistically significant when using either the ES or IOM reference values. This result is contradictory, and not at all expected, as serum 25(OH)D is the gold standard for testing vitamin D status. Thus, the results from both tests should have been similar. Despite not being significant, the relationship between vitamin D status and skin tone showed the same as the results as skin tone and 25(OH)D levels – darker skin tones are more likely to have a suboptimal vitamin D status. This supports the results from other studies who have also found that those with a higher dermal melanin content experience decreased epidermal synthesis of vitamin D and have lower 25(OH)D levels.[36,143] Libon et al studied a similar age group (mean age = 23 years) when looking at the effect of skin tone on serum 25(OH)D and found similar results to this study.[143] Many studies have investigated the effect of artificial UVB radiation on different skin tones and found that lighter skin tones need less time to convert 7-DHC to previtamin D_3 than darker skin tones.[36,141] Chen et al found that the synthesis of previtamin D_3 in light skinned participants steadily increased with an increased duration of UVB exposure but in those with darker skin tones, the synthesis of previtamin D_3 decreased considerably after 10 minutes of UVB exposure.[36] The Chen et al study also concluded that Fitzpatrick skin type II skin tones were 5-10 times more likely

than the skin type V skin tones to convert 7-DHC to previtamin D_3.[36] Lighter skinned people may therefore require less UVB exposure to synthesise vitamin D_3 than their darker skinned counterparts.

In the current study, a significant relationship was also found between race and serum 25(OH)D, with participants from races of darker skin tones more likely to experience lower levels of 25(OH)D. George et al, however, found that Black participants had higher levels of 25(OH)D than their Asian-Indian counterparts, even though the Black ethnicity traditionally has a higher dermal melanin content than those of Asian and Indian descent.[19] While people with darker skin tones have been shown to have lower levels of total serum 25(OH)D, Aloia et al found that despite this, Black and White women both had the same amount of free serum 25(OH)D, and Black women had a higher bone density than White women.[144] This indicates that the bioavailability of 25(OH)D might be different for different ethnic groups. The relationship between circulating 25(OH)D versus the bioavailability of 25(OH)D and its effects on health needs to be further investigated.

Despite the significant relationship between race and serum 25(OH)D, participants from the Indian population had the lowest 25(OH)D levels. This finding was similar to that of Roberg, who also found that in Johannesburg, the Indian population were the most at risk of vitamin D deficiency.[33] Similar findings were found in two other South African studies.[19,145] A study conducted on healthy adults in the south of India also showed a high prevalence of vitamin D deficiency among this group.[146] Other studies comparing races of traditionally lighter skin tones have shown that the Asian-Indian population is more at risk for vitamin D deficiency than the Caucasian population.[147] Many reasons for this finding have been found in the literature. Low dietary calcium intakes along with diets rich in phytates and limited exposure to sunlight are all thought to be contributing factors to the high prevalence of vitamin D deficiency in this group.[19,146]

Based on the above, it may be prudent to have different recommendations for different skin types when it comes to sun exposure. Exposure to UVB radiation, however, is controversial, with many cancer organisations encouraging people to avoid sunlight at certain times of the day to decrease their risk of developing skin cancers. Sun exposure guidelines are often vague when it comes exactly how much time should be spent in the sun, and they often do not consider the melanin content of the skin.

While reducing the risk of skin cancer is imperative to good health, recent studies have shown that vitamin D deficiency has also been linked to the development of other cancers.[23,79,80] There is thus a delicate balance between being exposed to enough sunlight to produce enough vitamin D for good health while avoiding the level of sun exposure that

would increase the risk of skin cancer. Research into discovering and quantifying this balance in different skin types is vital for ensuring good health.

5.4 Anthropometry

Variations in anthropometrical measurements have many health-related effects and consequences on the body, and similar to skin tone variations, anthropometrical variations also have an effect on vitamin D status. The majority of this study population was classified as having a healthy body weight, with very few participants classified as obese. The relationship between anthropometrical measurements and serum 25(OH)D was not found to be statistically significant; therefore, the researchers of this study failed to reject the null hypothesis stated in the objectives. While no statistical significance in the relationship between the two was found, there was a trend observed showing that those participants with higher BMIs and waist circumferences were more likely to be classified as vitamin D insufficient. As mentioned above, the current study population had very few participants who were classified as obese (n=26), and while this was weighted during statistical analysis, this small number of participants could have played a role in the results.

These trends are supported by a study done by Forrest et al, who found that patients experiencing obesity were twice as likely to experience vitamin D deficiency.[132] Other studies also support Forest et al's findings linking weight to vitamin D status.[16.25.148] Participants from the Asian Indian Diabetic Heart Study were selected to have their 25(OH)D levels measured in order to determine if obesity had an effect on vitamin D status. The results showed that in those who were classified as obese, circulating 25(OH)D levels were significantly less than those with a normal body weight.[137] The Asian Indian Diabetic Heart Study also found that the relationship between vitamin D and BMI resulted in increased fasting glucose levels.[137] Snijder looked at the relationship between anthropometric markers and serum 25(OH)D in 250 obese men and women in New Zealand. [149] When it came to the relationship between waist circumference and 25(OH)D, they found an inverse but statistically significant relationship.[149] The mean waist circumference in Snijder et al's study, however, was 100.4 ± 12.8cm compared to a much lower mean waist circumference in this study (81.54 ± 9.74cm). The number of participants who had high waist circumferences in the current study was also low, and perhaps this could account for the lack of significance found in the current study.

Not all studies support the results found in this study, however. As vitamin D is a fat-soluble vitamin, it not only needs fat to be absorbed, but also to be stored. Datta et al found that higher BMIs were associated with higher 25(OH)D levels, as increased fat mass resulted in more storage space for vitamin D.[119] This increased "storage" has been the centre of studies hypothesising that people with higher BMIs might have the same amount, or even more, 25(OH)D than people with normal BMIs, but as most of their 25(OH)D is stored, low circulating levels of 25(OH)D are measured in the blood.[148]

If the above assumptions set forward by Datta are to be accepted, and body fat mass has a greater influence on serum 25(OH)D than body weight, BMI might not be the best indicator to use when determining body fat mass. In a review of BMI accuracy in children, Javed et al found that BMI did not accurately identify excess body fat in over 25% of the population.[150] Similarly, Romero-Corral et al found that BMI was not an accurate predictor of excess body fat, particularly in males and in those who are classified as overweight.[151] When comparing the relationship between 25(OH)D and fat mass, it might be more prudent to use a different means of quantifying total body fat percentage. Skin fold measurements and bioelectrical impedance analysis, although time consuming and costly, can help to accurately determine adipose tissue mass and can be used to further investigate the interesting relationship and interaction between vitamin D and body fat mass.

Recently, studies have shown gender differences when it comes to BMI scores, with women more likely to have more fat mass then men. [152,153] As vitamin D is stored in fat mass, women with higher BMI might have less circulating 25(OH)D than men with the same BMI, falsely classifying them as vitamin D deficient and their male counterparts as not. [153] While the males in this study did have higher BMI in the overweight and obese category compared to females, the study did not account for the differentiation of fat mass between the genders, and this topic might be valuable to study further.

BMI was also originally developed for the Caucasian population and new studies have found that BMI measurements can differ between ethnicities.[154,155] Carroll et al studied 185 men and women from Caucasian, Hispanic, and African American ethnic backgrounds to see if BMI was equally related to adipose tissue in all races.[156] They found that the African American demographic had less adipose tissue despite having similar BMIs to other ethnicities.[156] Therefore, different BMI reference values might be needed when working with participants from different ethnic backgrounds, and conclusions based on the relationships between serum 25(OH)D and traditional BMI reference values should be made with caution.

5.5 Diet

Oral intake of vitamin D, via food sources or supplementation, is one of the main ways, other than epidermal UVB exposure, that vitamin D enters the body. A food frequency questionnaire was used to evaluate the amount of vitamin D each participant consumed in this study, and it was found that 87.2% of the participants did not consume enough vitamin D daily to meet their recommended needs of 15mcg per day. These findings were expected as firstly, South Africa does not currently have any formal vitamin D food fortification programmes, nor do they prioritise vitamin D supplementation at a provincial or national level and, secondly, many of the participants in this study did not prepare their own food.

The FMHS has a residence option and, as such, some participants regularly ate most of their meals in the cafeteria. Other participants that stayed in residence also reported that they ate prepared, ready-meals, and of those participants who did not stay in residence, some reported that their meals were made for them, usually by parents. This not only meant that they had little control over their food choices, but also that participants did not know all the details of the food preparation methods. Therefore, the amount of food sources rich in vitamin D reported in the food frequency questionnaire could have been over or underestimated in some cases.

Food frequency questionnaires have been shown in some studies to underestimate intake, especially energy and protein consumption.[157] When it comes to nutrient specific FFQs, studies have shown a slight overestimation compared to real consumption. A Swedish study that looked at the validity of a vitamin D FFQ for children, found that while there was slight overestimation (0.6mcg) when compared to a three-day food record, FFQs were valid and reliable tools.[158] In Ireland, an interview-administered vitamin D specific FFQ was compared to a 14-day dietary recall and was found to overestimate vitamin D intake (0.5mcg).[159] Weir et al also reported overestimation in vitamin D when using a FFQ when cared to a 4-day weighted food record.[160] Overestimation may have been present in this study, especially as some of the food choices were not eaten regularly.

As mentioned above, South Africa does not have formal, government-regulated vitamin D fortification programmes. The efficacy of fortification programmes, however, is inconclusive. Some global vitamin D studies have found that in countries that have national vitamin D fortification programmes, the prevalence of vitamin D deficiency is low.[161,162] Not all studies support food fortification as a solution to vitamin D deficiency, however. Studies in the US and Canada have found that fortification does not increase the prevalence of vitamin D sufficiency. It is important to note that these countries only offer voluntary

fortification and formally fortify only a few staple foods, with Canada only allowing the national fortification of two staple food products (milk and margarine).[163,164]

In lieu of fortification, supplementation is another avenue used to increase serum 25(OH)D levels. In a study by Jolliffe et al conducted on elderly adults (mean age = 72.0 ± 9.2 years) that investigated the environmental factors that could impact serum 25(OH)D levels, those who took supplemental vitamin D had higher levels of 25(OH)D than those who did not (25(OH)D levels were found to be 17.9nmol/l higher in the supplemented participants).[138] Moore et al investigated how vitamin D intakes differ between demographics in 9719 participants and reported that while males had a higher dietary intake of vitamin D, females had a significantly higher supplemental intake, with White females consuming more than double the vitamin D from supplemental sources than Black and Hispanic females.[165] This was mirrored in the male population group. They also reported that higher income was associated with higher intakes of vitamin D in the White population, but not the Hispanic population.[165] In the current study, males also consumed more vitamin D than females. This was to be expected as males generally have higher dietary needs than females, and therefore consume more total food than females, making it more likely for them to consume higher levels of vitamin D.

As mentioned above, Datta et al studied the half-life of 25(OH)D in healthy adults in Denmark and found that when 25(OH)D was low, its half-life decreased faster in males than in females, but when 25(OH)D was at a medium-level, the relationship was insignificant.[119] Rapid and consistent supplementation in the face of vitamin D deficiency is, therefore, vital to ensure that the external sources of vitamin D provided is sufficient.

There were no statistically significant relationships found between dietary vitamin D intake and 25(OH)D, but as total vitamin D intake and 25(OH)D was found to be statistically significant, the null hypothesis of diet having no effect on 25(OH)D levels is rejected. This study also found a significant relationship between supplemental vitamin D intake and serum 25(OH)D levels, which was expected as most vitamin D deficiency intervention strategies focus their efforts on high dose oral vitamin D supplementation in an effort to increase serum 25(OH)D levels.[46] Despite this finding, of the participants studied here, few took supplements daily and most of the supplements taken were general multivitamins containing low levels of vitamin D. Only one participant took a micronutrient supplement specifically designed to increase vitamin D levels. More studies that investigate the use of consistent and adequate vitamin D supplementation and its effect on serum 25(OH)D levels in South Africa are needed.

As discussed above, vitamin D toxicity is rare. As toxicity from excess sunlight exposure is physiologically implausible, excessive vitamin D supplementation is the main cause of vitamin D toxicity.[46] A case series report of 16 patients with vitamin D toxicity (median 25(OH)D = 371 (175–1161) ng/dl) found that vitamin D overdose (either via the enteral or parenteral route) was the main cause of toxicity.[166] The IOM committee found that dietary intakes less than 10 000IU/day were unlikely to cause vitamin D toxicity (a finding supported by the Endocrine Society), while frequent dosages of 50 000IU/day over a period of months were associated with toxicity.[46] Other reports have stated that daily vitamin D intakes of above 40 000IU are linked to toxicity. [98,166] The reasons for excessive intakes of vitamin D are usually attributed to a lack of education of consumers which can lead an incorrect understanding of the measurement unit of vitamin D. Vitamin D has many measurement units (mg, mcg, IU) and, as such, it can become confusing for the consumer.[98] A dosage of 1000IU can be misinterpreted as 1000mcg, which can quickly lead to dangerously high 25(OH)D levels. Overdosing can also accidentally occur during the manufacture of foods that are fortified with vitamin D.[144]

The dietary reference intakes (DRI's) used to interpret the daily vitamin D intakes in this study were standard DRI's for South Africans. Hollis et al challenged the normal reference intakes of vitamin D in a review paper and proposed that much higher daily targets might need to be set for dietary vitamin D intake in order for people to consume the amount vitamin D associated with good health.[167] While the overall intake of dietary vitamin D in this study was low, most participants were found to have sufficient and above sufficient serum 25(OH)D levels. Recommendations involving the increase or decrease of the recommended DRI for this specific population group (i.e. healthy, young adults with some daily sun exposure) might not be prudent at this time.

5.6 Life style factors

5.6.1 Smoking

The results of this study showed no significant relationship between smoking and serum 25(OH)D. This contrasted with many other studies conducted on this topic. Participants from the HUNT study in Norway were recruited to determine the factors that had an effect on vitamin D status, and the researchers found that smoking increased the risk for vitamin D deficiency.[168] This result was echoed in a study by Cutillas-Marco et al, who studied the effect of smoking on serum 25(OH)D in 177 participants from eastern Spain.[169] Kassi et al found that in middle aged males, smoking lowered serum 25(OH)D by as much as 4.2ng/dl.[170] A study conducted on 194 acute ischaemic stroke patients found that those who were smokers had lower 25(OH)D levels and higher levels of depression.[171] In China, a cross-sectional study of 612 elderly males reported that smokers not only had lower levels of 25(OH)D, but serum 25(OH)D was dose-dependent on both the number of cigarettes smoked on a daily basis and the duration of smoking.[172]

The mechanics behind the relationship between smoking and serum 25(OH)D is unclear but it is thought that smoking is usually part of a less healthy lifestyle, which can lead to less vitamin D synthesis through reduced exposure to UVB radiation.[170,171] Thus, the low levels of 25(OH)D found in smokers may be attributed to factors other than the cigarette itself. The average number of cigarettes consumed daily by the participants in this study was low (mean=2.5 ± 3.46), as was the number of smokers in the study and the number of participants who smoked daily. This relatively low number of heavy smokers may account for this result.

5.6.2 Alcohol

The relationship between alcohol use and serum 25(OH)D in this study was not significant. Other studies have found the effect of alcohol on serum 25(OH)D to be inconclusive and this relationship is thought to be controversial. Touvier et al found that while excessive alcohol consumption was associated with low 25(OH)D levels, moderate alcohol consumption was associated with increased 25(OH)D levels.[130] A systematic review of 49 articles studying the effect of alcohol on vitamin D status reported that this relationship was inconclusive. The studies in the review were almost equally split between positive associations between alcohol and vitamin D, negative associations between alcohol and vitamin D, and no associations between the two.[173] Alcohol, when used in moderation, can have positive effects on the body, especially when it comes to cardiovascular health, and it will be interesting to see what more research into the relationship between vitamin D and alcohol use reveals.

5.6.3. Exercise

When it came to effect of exercise on serum 25(OH)D, this study found the relationship between exercise and 25(OH)D to be insignificant. There was a trend observed, however, that showed those who exercised more had both higher serum 25(OH)D levels and consumed more vitamin D. This result supports many studies investigating the relationship between physical activity and vitamin D status. A study by Koundourakis, who investigated the effect of serum 25(OH)D on the exercise performance of 67 Greek soccer players, found that good serum 25(OH)D was essential to good athletic performance.[174] Florez et al studied 291 elderly adults (mean age: 62 ± 13.48 years) to investigate the effect of outdoor physical activity on serum 25(OH)D levels.[175] They found a higher prevalence of hypovitaminosis D in those who did not take part in outdoor exercise.

A study conducted in Australia on elderly Vietnamese immigrants found that in men particularly, those who participated in physical activity had higher levels of serum 25(OH)D.[176] The amount and type of exercise was self-reported, however, and it was not clear whether the activity was done indoors or outdoors. A Korean study of 5847 adults that investigated the determinants of vitamin D status also found higher 25(OH)D levels in those who participated in physical activity versus those who did not.[177] Grimaldi et al looked at the effect of vitamin D on muscle strength in healthy adults, and found that higher 25(OH)D levels were associated with increased strength in the muscles of the arms and legs in all age groups and genders.[178]

The type of exercise that participants took part in the current study was not specified. Other studies have looked at the effect of specific types of exercise on serum 25(OH)D levels. One such study was conducted by Xiaomin et al on 20 young adults and investigated serum 25(OH)D levels in the time after endurance exercise (30 minutes cycling at 70% VO_{2max}).[179] They found that serum 25(OH)D levels were increased up to 24 hours post exercise, with men exhibiting greater levels than women. The relationships between the type of exercise and its effects on vitamin D status needs to be studied further so specific recommendations regarding this can be made.

As none of the relationships between lifestyle factors and vitamin D status were found to be statistically significant, the null hypothesis of lifestyle factors having no effect on serum 25(OH)D levels is thus accepted.

5.7 Sun Exposure

Cutaneous exposure to UVB radiation accounts for the majority of vitamin D found in the body. The most common way the majority of people experience UVB radiation, is through natural exposure to sunlight. Most participants in this study were exposed to sunlight during weekday afternoons, with moderate exposure (5-30 minutes) taking place on weekdays. This was expected as the study population attends classes indoors during the day and are given a daily one-hour lunch break. On weekends, the participants had more freedom when it came to sunlight exposure during the day, which was also expected. Studies have shown that lengthy exposure to sunlight may be ineffective at increasing serum 25(OH)D levels. In a study conducted by Webb et al, they found that when the skin is exposed to direct sunlight, shorter regular sun exposure intervals were more effective at increasing serum 25(OH)D than longer, intermittent exposure.[180] Edvardsen et al reported that 30 minutes of daily, direct sun exposure should be enough for most healthy adults to produce sufficient levels of 25(OH)D.[181] The participants of this study spent shorter, more regular intervals exposure to sunlight (most weekday afternoons) and as such, were expected to have increased vitamin D levels.

In the current study, the hands and face were the areas of the body that had the most daily exposure to sunlight, which was expected as the data collection period took place in winter. More participants wore clothes that covered more skin and left only the face and hands exposed to sunlight. As discussed above, the winter of 2016 was unseasonably mild, and certain summerlike winter days allowed a few participants to expose more of their skin to sunlight on the weekends. The limited amount of skin exposed (i.e. hands and face) experienced by most of the participants might not be sufficient to produce enough vitamin D for good health, however. A study in South Asia studied 15 participants of mixed ethnicity to determine the effect of UVB exposure and skin tone on vitamin D status.[182] They found that short simulations of UVB exposure, that mimicked frequent midday sun exposure, did not synthesise enough 25(OH)D to result in adequate vitamin D status. These findings were similar to Osmancevic et al who found that in light skinned people in particular (Fitzpatrick skin type II and III), the more skin exposed, the higher the 25(OH)D levels.[183] Contrastingly, Holick et al have reported on numerous occasions that one full body exposure to UVB radiation that results in the pinkening of the skin is equal to 250-625mcg of oral vitamin D, enough to meet the needs of a healthy adult. [78,186] This conclusion, of course, excludes many skin types which do not turn pink when exposed to sunlight.

Many factors influence the efficacy of dermal sunlight exposure, including the solar zenith angle of the sun at the time of exposure, cloud cover, surrounding surface reflection (snow, water, glass, etc), clothing worn during exposure, and outdoor behaviour patterns.[184] Bogh

et al investigated whether cholesterol or skin tone had a greater impact on serum 25(OH)D when the skin is exposed to UV radiation and found that total circulating cholesterol levels had a greater impact on serum 25(OH)D than skin tone during exposure to sunlight in winter.[185]

Many health boards claim that a few minutes of sun exposure on limited amounts of exposed skin (normally the hands and face) is enough to produce sufficient vitamin D needed for good health. There is, however, a contradiction between what health boards recommend regarding sun exposure and what research scientists advocate regarding adequate serum 25(OH)D. Many studies have shown that higher intakes of vitamin D are necessary for health than previously prescribed, and a few minutes in the sun is unlikely to achieve those levels.[186] Understandably though, health board recommendations err on the side of caution as longer durations of sun exposure can have their own detrimental side effects (namely sunburn and the corresponding increased risk of skin cancer that accompanies this).

As mentioned above, there is a great need to quantify and diversify sun exposure recommendations for the general population. The delicate balance between the sun exposure required for good health and the exposure that results in undesirable side effects is still unclear in the literature. The vague, one-size-fits all recommendations that are often disseminated to the public might not be sufficient to ensure that people of all skin tones experience enough exposure to natural UVB radiation to produce the levels of vitamin D required for good health. It is important to remember that this study group was specific and the results reported here might not be representative of the general public. Before conclusions regarding sun exposure and vitamin D status can be made for the greater population, more studies are needed.

5.8 Strengths and limitations

As with all research studies, there were both strengths and limitations to this study.

5.8.1 Strengths

1. The tools used in this study were validated for content and face validity (as seen under Methodology), allowing for less content bias in the results. The FFQ in particular was adapted to include commonly eaten South African foods, making it more relevant to the population group and allowing for greater accuracy when completing the FFQ. It also contained a large number of vitamin D rich food products and had enough frequency options to aid participants during the recall of information.
2. This study formed part of a bigger pilot study. While the sample size was small, it carried statistical power (determined using a power calculation with 90% power (ANOVA) and RMSSE of 0.35) and the strata (skin tone and gender) were equally weighted. When smaller subgroups were encountered, the data was weighted to give a more accurate representation of the results.
3. Screening for inclusion and exclusion criteria before data collection took place allowed for a large pool of potential participants from which a random sample could be selected. This ensured that the final sample selected was free from selection bias and ensured the researchers selected a representative sample to fulfil the aims and objectives.
4. Blood samples for serum 25(OH)D testing were taken by a qualified phlebotomist and blood samples were handled using accepted methods. The samples were sent to the University of Witwatersrand's Developmental Pathways for Health Research Unit for analysis, who was awarded a certificate of efficiency by the International Vitamin D External Quality Assessment Scheme. This certificate ensured that the tests were accurate and as free as possible from laboratory errors. The serum 25(OH)D samples were measured using a chemiluminescent assay using DiaDorin Liasion kits, and while liquid chromatography-tandem mass spectrometry (LC-MS/MS) tests are considered the gold standard for measuring serum 25(OH)D, LIASION automated assays have been validated and shown to have the most accurate results when compared to the LC-MS/MS methods.
5. The methods used during the data collection of this study were standardised, which allows the study to be replicated on different population groups and in different areas, and still produce comparable findings. Participants also completed data collection in person and the researchers checked all data collection tools before the participant left the data collection room. This ensured that the questionnaires used were completed fully and allowed for minimal gaps in the data collected.

5.8.2 Limitations

1. The skin reflectometry device was erratic in its results. Upon consultation with a dermatologist, it was explained that skin is not one-dimensional in colour and at any given time, depending on the tissues and blood beneath the measurement site, the colour of the skin is unique. It was, therefore, challenging to determine the exact amount of melanin in the skin. To overcome this, the researchers took the average of three measurements at each site, and when one result was clearly not in line with the rest, another measurement was taken. There were also no recognised reference values that could be used to classify the MI results received from the reflectometry device, so the researchers determined their own cut-off values from the data received during screening.
2. The above limitations of the skin reflectometry device also resulted in less diversity between racial groups in the final study population, and this led to some groups (namely Asian and Indian) being underrepresented. As mentioned above, statistical weighting was performed to ensure that these results were comparable.
3. As with all recall methods used to estimate dietary intake of nutrients, recall bias was present in the FFQ. Over or under reporting of specific foods is common when executing a FFQ, especially when it comes to those foods that are rarely eaten. Often foods that are used daily are also misreported due to the exact portion sizes being unknown. With this in mind, every effort was made by the researchers to minimise this recall bias by using food models to help participants visualise sizes, as well as ensuring the data collectors who performed the FFQ were trained and followed standardised procedures.
4. The amount of sunlight exposure participants were exposed to was also self-reported, and as with the FFQ, prone to recall bias. The tool used to determine sun exposure also only took into account the time of day when the most sun exposure took place (either <10h00, or 10h00-15h00, or >15h00). This proved difficult for the participants to answer as most participants had some exposure to sunlight in all three categories provided. Therefore, the overall duration of sun exposure, as well as the time that exposure took place, might not have been inaccurate.

5.9 Failure to reject or rejection of null hypotheses

Based on the above results, the following null hypotheses have been rejected by the researchers:

1. Gender has no effect on serum 25(OH)D levels
2. Dietary intake of vitamin D has no effect on serum 25(OH)D levels
3. Skin tone has no effect on serum 25(OH)D levels

The researchers accepted remaining two null hypotheses:

4. Anthropometrical variations have no effect on serum 25(OH)D levels
5. Lifestyle factors (smoking, alcohol, exercise) have no effect on serum 25(OH)D levels

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6 Conclusion

The main aim of this study was to investigate the vitamin D status of healthy young adults in the Western Cape, while the study objectives focused on investigating the effects of gender, skin tone, anthropometrical variations, dietary vitamin D intake, and lifestyle factors on serum 25(OH)D levels.

A high prevalence of vitamin D sufficiency (73%) was observed in the results of this study when using the Endocrine Society reference values, and the prevalence of sufficiency further increased (90%) when using the Institute of Medicine reference values. These results were not expected as the data was collected in winter, a season where most studies have shown a high prevalence of vitamin D deficiency, particularly in people with lighter skin tones. Males experienced higher mean serum 25(OH)D levels than females (68.99 ± 38.33 ng/ml and 58.60 ± 43.70 ng/ml respectively), and the relationship between serum 25(OH)D and gender was found to be statistically significant ($p<0.01$). The null hypothesis for the effect of gender differences on serum 25(OH)D is therefore rejected. The relationship between gender and serum 25(OH)D is widely debated in the literature, with some studies supporting the results found here, and other studies reporting that males are more at risk of poor vitamin D status than females.

Participants in the white population were found to have the highest mean serum 25(OH)D levels of the races (79.90 ± 42.47 ng/ml), and serum 25(OH)D and race was found to be statistically significant ($p<0.01$). The relationship between the Fitzpatrick skin type classification and serum 25(OH)D was significant ($p=0.01$) with those participants in the skin type II category (fair skin) having the highest mean serum 25(OH)D (76.89 ± 46.35 ng/ml), and those in the skin type V category (brown skin) having the lowest mean serum 25(OH)D (48.60 ± 63.48 ng/ml). The researchers, therefore, rejected the null hypothesis stating skin tone variations have no effect on serum 25(OH)D. As expected, there was a higher prevalence of vitamin D deficiency in the participants in the darker skin types (skin type IV=58.62% and skin type V=20.69%), although this relationship was not significant ($p>0.05$; $Chi^2 = 13.91$). While not significant, these results are in line with many studies that have shown that those with darker skin tones are more at risk of poor vitamin D status.

Anthropometrical results showed a mean BMI of 24.52 ± 4.01kg/m^2, with very little variation in BMI between the genders and races. Most participants had a normal BMI (60.74%, n=147), while more than a quarter of the participants were classified as overweight or obese. The relationship between BMI and vitamin D status was not significant (Kruskal-Wallis; $p=0.08$), although it was observed that those participants who had higher BMIs were more likely to be classified as having insufficient vitamin D levels. Similar tendencies were

observed between waist circumference and vitamin D status, although this was also not statistically significant (Kruskal-Wallis; p=0.15). Based on the above, the null hypothesis stated in the objectives is accepted.

Milk and Milk Products were the most commonly eaten sources of vitamin D (with plain milk, cheese, and yoghurt accounting for the most eaten foods within this food group), followed by Fish and Seafood. Mean total vitamin D intake for the total population was 7.99 ± 13.81mcg, with more vitamin D consumed from the diet than from supplements. Males consumed more vitamin D than females. The majority of participants (87.2%) did not consume enough vitamin D to meet their recommended daily need of 15mcg. As the relationship between total vitamin D intake and serum 25(OH)D was statistically significant (with those consuming more vitamin D having higher 25(OH)D levels), the null hypothesis set out in the objectives is rejected.

Few participants were smokers (11.16%) who smoked an average of 2.5 ± 3.46 cigarettes per day. Over half the participants moderately consumed alcohol on a regular basis (2.88 ± 4.43 units per week). While the relationships between smoking and alcohol and 25(OH)D were not significant, there was a trend observed that those participants who consumed alcohol were more likely to have increased 25(OH)D levels. The opposite was true for smoking and 25(OH)D levels. When it came to exercise, those participants who did physical activity were more likely to have higher 25(OH)D levels (p=0.15). Those who exercised were also more likely to consume more vitamin D than those who did not exercise (p=0.37). Based on the above, the null hypothesis set out in the objectives stating lifestyle factors have no effect on serum 25(OH)D is accepted. Other studies have shown mixed results when investigating the effect of lifestyle factors on serum 25(OH)D. This debate is seen the most when discussing the effect of alcohol on 25(OH)D levels, with little consensus observed in the literature.

Poor vitamin D status is a fast-growing pandemic, but few studies investigating the vitamin D status of healthy adults in South Africa have been conducted. Studies focusing on the role of vitamin D in disease, or the effect of diseased states on vitamin D status, are more common in the South African setting.

Conclusions based on the gender results of this study, as well as other studies, need to be made with caution, as this relationship between gender and serum 25(OH)D is rarely studied in isolation. Inherent gender characteristics could have an impact on results particularly when the effect of gender on serum 25(OH)D is studied in conjunction with co-morbidities and medications.

The effect of skin tone on 25(OH)D levels also requires careful analysis and explanation. While those who have darker skin tones often have lower 25(OH)D levels, some researchers have argued that serum 25(OH)D may be more bioavailable in people with darker skin tones, and studies have shown that darker skin toned people are not necessarily more at risk for poor bone health. The lower levels of 25(OH)D found in those with darker skin tones is thought to be a protective, evolutionary development to the climate one is exposed to, preventing those who are exposed to extended natural UVB radiation from developing undesirable side-effects.

There is a great need to quantify and diversify sun exposure recommendations for the greater public. The effect of melanin on cutaneous 25(OH)D synthesis is varied and those with more melanin in their skin often need longer exposures to synthesise the level of 25(OH)D associated with good health. In contrast, those with lighter skin tones need less time to synthesise 25(OH)D but are also more at risk of the undesirable side-effects of prolonged UVB exposure. More research is needed into the intricacies of this delicate balance so that specific recommendations can be made to ensure good health for all. The results of this study show a high prevalence of vitamin D sufficiency, despite low dietary vitamin D intakes. Based on this, it would not be prudent to make recommendations about formal vitamin D food fortification and supplementation programmes regarding this study population at this time.

The strengths of this study included the use of validated tools, a sample size that carried statistical power, sampling techniques that reduced selection bias, and the use of an accredited laboratory which used accepted methods for testing serum 25(OH)D. The limitations of this study included erratic results from the skin reflectometry device used and no accepted reference values for the melanin index readings, as well as recall bias during the completion of the food frequency and sun exposure questionnaires.

It is important to note that the current study population, while homogenous, was very specific. The study population included young, healthy, undergraduate students who usually follow different daily patterns to the working population. Further studies are needed to investigate different regions of South Africa, as well as a wider variety of population groups that may be more at risk for vitamin D deficiency than the current study population, before conclusions can be drawn. Studies investigating seasonal differences in 25(OH)D levels in this group or a similar group of participants would also be interesting as the results here show high levels of serum 25(OH)D in winter, a time where serum 25(OH)D levels are usually low. It would be interesting to see firstly, if different 25(OH)D levels are observed, and secondly, by how much these levels increase after the summer months.

7. Recommendations

1) Further studies need to be conducted. The results of this study are interesting, but only encompass a small region of South Africa, with a specific young, healthy, undergraduate student population group. Undergraduate students usually follow different daily patterns to the wider South African population, especially when it comes to sun exposure. Before wider conclusions can be drawn, similar studies need to be conducted in other areas of South Africa, where latitude and environmental factors vary, as well as on different population groups. Factors that need to be explored in greater detail in other areas include:
    a. Diet. The provincial differences in dietary patterns and commonly eaten foods are vast in South Africa. Other provinces may consume more vitamin D rich foods (e.g. morogo and spinach are more popular in other provinces than in the Western Cape) and this could influence daily dietary intakes of vitamin D.
    b. Age. While the current population group was young, serum 25(OH)D levels have been shown in the literature to decrease with age. Studies that look into the effects of aging on serum 25(OH)D levels in South Africa can help tailor specific recommendations for specific age groups during all stages of the lifecycle.
    c. Latitude. Latitude has been shown in the literature to have a large effect on 25(OH)D levels. South African studies have also shown that serum 25(OH)D levels in Johannesburg are higher than those in Cape Town. It would be interesting to perform the same study on a similar population group in another province to determine if the same environmental factors influence serum 25(OH)D levels.
2) Anthropometry and 25(OH)D. With the prevalence of obesity overtaking undernutrition globally, the effect of excess body weight on health is an important topic. Vitamin D deficiency has also been linked to many of the same diseases that overnutrition has been linked to, and this relationship and interaction should be further investigated. As mentioned in the Discussion, BMI is not always an accurate indicator of body fat. The relationship between adiposity and serum 25(OH)D should be studied further, with more accurate body fat measurement techniques (such as skin folds or bioelectrical impedance analysis) used.
3) Exercise and 25(OH)D. The effect of exercise on 25(OH)D levels was briefly discussed in this study, but the effect of specific exercise types was not elaborated on. The literature has shown little into the effect of specific types of exercise on 25(OH)D levels, and this needs to be investigated in more detail so more specific recommendations regarding exercise can be developed.

4) Seasons and 25(OH)D. Seasonal variations in serum 25(OH)D should be determined in similar populations. The prevalence of vitamin D sufficiency in this study was found to be high at the end of winter, a time where most studies have found an increased prevalence of vitamin D deficiency. If the summer season increases 25(OH)D levels even more, some participants may be at risk of toxicity, and this needs to be further investigated to ensure good health.
5) Sensitivity of tests to determine toxicity. In this study, the laboratory results did not specify serum 25(OH)D levels over 150ng/ml, merely stating >150ng/ml. The Endocrine Society suggests that 25(OH)D levels above 150ng/ml should be classified as toxic, but as seen above in the Discussion, the exact level of serum 25(OH)D where symptoms of toxicity are present is unknown. Therefore, this population group might not have experienced “true” toxicity. More specific, sensitive tests need to be done on similar population groups when determining vitamin D status to ensure participants are not nearing true toxic levels.
6) More research needs to be done into investigating official reference values for the MI values measured with skin reflectometry devices, so future results can be more accurate and standardised.
7) The participants in the Indian population were found to have the highest levels of vitamin D deficiency, which is similar to the findings in other South African studies. As few Indian participants were included in this study, further research investigating this finding as well as the causal relationships could be of benefit.
8) Sun exposure. This study briefly touched on sun exposure, but more research is needed in a similar setting regarding the effect of sun exposure on different skin tones and races. It would be interesting to see if people from different races and who have different skin tones have different sun exposure patterns and beliefs.
9) This study did not differentiate between the dietary intake of those who prepared their own meal versus those who ate at the cafeteria as part of residence. Future studies that investigate the vitamin D content of the meals prepared by food service units on campuses could be valuable in determining the vitamin D status of university attending young adults.

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9. Addenda

Addendum A

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Screening Phase

TITLE OF THE RESEARCH PROJECT:

Determinants of serum 25-hydroxyvitamin D levels in adults in the Western Cape Province of South Africa

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR:

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You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research project all about?

This research project will look into how skin tone effects vitamin D status in adults in South Africa. This is a pilot study and the only data collection site will be at Tygerberg campus. All participants for this project will be recruited from the Faculty of Medicine and Health Sciences, Stellenbosch University.

In conducting this study, the research team hopes to gain insight into the effect that skin tone has on the vitamin D status of healthy adults. The research team also wants to see whether factors such as gender, weight, height, waist circumference, and/or diet have an influence on vitamin D status. The results from this pilot project could potentially help motivate for larger studies, as well as to contribute to the development of interventions to potentially improve the health of all South Africans.

This project has a screening phase and two data collection phases. By completing this form, you are giving your consent to take part in the screening phase of the project. Please note that the final study selection is random and you may not be selected to take part the final study. The initial sample in the first data collection phase (baseline) will include 240 participants in total. This total includes over-sampling as to account for possible loss to follow-up in the second phase.

In this screening phase of the project, we will collect information from you to determine if you are suitable for the main phase of the study. This screening process should not take more than 10 minutes of your time. You will be asked to complete a questionnaire based on the inclusion and exclusion criteria of the study. You will also be asked to complete a short skin tone assessment (skin reflectometry) to determine your skin tone classification is. You will be asked to complete socio-demographic and contact details to enables the researchers to contact you should you be randomly selected to participate in the main phase of the study. If you meet the project criteria and are selected for the main study, you will be contacted with the details of the data collection. Please note that even if you are selected, participation is voluntary and you can decline to participate at any time.

All information will be kept confidential. If you choose to take part in this research project, you will be allocated a randomly generated number and this number will be used throughout the study to protect your identity.

Why have you been invited to participate?

A need has been identified for such a study to be performed based on the lack of current data on vitamin D status in health adults in South Africa. You have been invited to participate in the screening phase of this research project to determine if you will be suitable for the project.

What will your responsibilities be?

During this screening phase, a checklist will be completed by a member of the research team to assess your suitability for the study. If eligible, you will also be required to participate in a skin reflectometry screening assessment If you are selected for the main study, you will need to attend two data collection phases, one in August 2016 and one in February 2017. These phases will be approximately two – four weeks long and you will only have to attend one session, which should take between 1 -2 hours to complete.

Will you benefit from taking part in this research?

If you are selected for the main study, you will indirectly benefit from this research project by becoming aware of your vitamin D status via a free vitamin D biochemical analysis. Other people in South Africa can benefit from this pilot project as results of the main study will allow us some insight into the health status of adult South Africans regarding vitamin D and whether there is a need to develop interventions to improve the health of the general population.

Are there in risks involved in your taking part in this research?

This study has minimal risks to you as the participant during the screening phase. Your privacy and confidentiality will also be strictly protected at all times.

If you do not agree to take part, what alternatives do you have?

If you decide not to take part in this study, it will not negatively affect you in any way and it will not be held against you.

Who will have access to your medical records?

All information you provide and all information that is collected during this screening phase of the project will be treated as confidential and be protected at all times. Only those directly

involved in research project, i.e the researchers and the statistician, will have access to the information you provide. All records will be kept in a secure storage area. When the results of this research project are presented and/or published in a health sciences journal, the identity of all the participants will remain anonymous.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the screening phase of the study but you will be entered into a lucky draw to possibly win a retail voucher (to the value of R1000).

Is there any thing else that you should know or do?

- You can contact Janicke Visser on 021 9389473/0828298529 if you have any further queries or encounter any problems.
- Study sponsors, study monitors, auditors, or HREC members may need to inspect the research records.
- Participants will be informed of their rights to have any new information arising during the course of the study communicated to them. If needed, the informed consent form will be revised to incorporate this information.
- You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.
- This research project is funded by the Cancer Association of South Africa (CANSA).

Declaration by participant

By signing below, I …….…….. agree to take part in a research study entitled, *Determinants of serum 25-hydroxyvitamin D levels in adults in the Western Cape Province of South Africa.*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....…….. on (*date*) ……...…….. 2016.

……. .....

Signature of participant Signature of witness

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.

- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…….. on (*date*) ……...…….. 2016.

……. …….

Signature of investigator Signature of witness

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) …… to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…….. on (*date*) ……...……..

.....	.....
Signature of interpreter	Signature of witness

Addendum B

Screening form

Participant number				Date								
				Day			Month			Year		

SCREENING CHECKLIST

Vitamin D study

This questionnaire is used as a screening tool/checklist to determine your eligibility for the study. Please note that as the selection of the final study group is random, even if you are found to be eligible for the study, you may not be selected to take part.

Please indicate your choices by marking the answer of your choice with an 'X' (where relevant).

Please note:
The completion of this screening checklist is voluntary.
All information in this questionnaire is, and will be kept, confidential.

Did you take part in the pilot study of this project?

Yes	No

If yes, you need not progress with the remainder of this questionnaire.
If no, please continue.

Initial & Surname _____

Undergraduate course

- ☐ BSc Physiotherapy
- ☐ BSc Dietetics
- ☐ B Speech, Language and Hearing therapy
- ☐ B Occupational Therapy

☐ MB ChB

Gender	Male	☐	Female	☐
Contact details *	Telephone/cell number	_____		
	Email address	_____		
Informed consent given	Yes	☐	No	☐

** To allow prospective participants to be contacted if randomly selected to be part of the main study and to be entered into the lucky draw.*

Participant number | | | |

Please indicate your choices by marking the answer of your choice with an 'X' (where relevant).

Questions	Yes	No
Are you an undergraduate student at the Faculty of Medicine and Health Sciences, Stellenbosch University?		
Are you 18 years and older?		
Do you consider yourself to be healthy?		
Are you able to speak either English or Afrikaans?		

Are you a final year student in 2016?		
Are you a postgraduate student at the Faculty of Medicine and Health Sciences, Stellenbosch University?		
Do you suffer from ANY of the following chronic illnesses and/or diseases: • *Gastro-intestinal absorption disorders (Coeliac's disease, Crohn's disease, Cystic Fibrosis)* • *Renal disease or renal failure* • *Hepatic disease or hepatic failure* • *Osteopenia and/or osteoporosis*		
Do you take any of the following medications: • *Antiepileptic drugs* • *Antineoplastic drugs* • *Antihypertensive drugs* • *Antiretroviral drugs* • *Bisphosphonates* • *Herbal therapies (such as Kava, St John's wort)*		
Are you pregnant or breastfeeding?		

Completed by:

_____

Participant number

TO BE COMPLETED BY THE RESEARCHERS

If any of the shaded blocks are selected, the individual is not eligible to participate in the study.

Was informed consent obtained?	Yes	No

Eligible for participation	NOT eligible for participation

Skin Reflectometry readings (Dorsum):

EI	***MI***	**Average EI**	**Average MI**

Completed by:

_____

Addendum C

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Main study phase of project

TITLE OF THE RESEARCH PROJECT:

Determinants of serum 25-hydroxyvitamin D levels in adults in the Western Cape Province of South Africa

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR:

Janicke Visser

ADDRESS:

Division of Human Nutrition

Faculty of Medicine and Health Sciences

Stellenbosch University

CONTACT NUMBER:

021 9389473

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

This research project will investigate the effect of skin tone and other factors on vitamin D status in adults in South Africa. This is a pilot study and the only data collection site will be at Tygerberg campus. All participants for this project (undergraduate students) will be recruited from the Faculty of Medicine and Health Sciences (FMHS), Stellenbosch University.

In conducting this study, the research team hopes to gain insight into the effect that skin tone has on the vitamin D status of healthy adults as this plays a role in the availability and synthesis of vitamin D. The research team also wishes to investigate whether factors such as gender, weight, height, waist circumference, and/or diet have an influence on vitamin D status. The results from this pilot project could potentially help motivate for larger studies, as well as to contribute to the development of interventions to potentially improve the health of all South Africans.

This study has a screening phase and two data collection phases. By this time, you would have already completed the screening process and subsequently been randomly selected for participation in the main study. By completing this consent form, you are giving your consent to take part in the first and second phases (winter and summer respectively) of data collection. The initial sample in the first data collection phase (baseline) will include 240 participants in total. This total includes over-sampling as to account for possible loss to follow-up in the second phase.

In this phase of the study, we will collect the first round of information from you. This phase will consist of six stations. Each station will be separate and private from the other participants. At the first station, you will be asked to complete a socio-demographic questionnaire. At the second station, a member of the research team will weigh you, measure your height and waist circumference. At the third station, a member of the research team will ask you questions about what kinds of foods you eat, how much of each item you eat, and how often you eat the food item. At the fourth station, you will be asked to complete a short questionnaire telling us how much time you spend in the sun on a daily basis. The fifth station will determine your skin tone by means of a light reflecting machine (skin reflectometer) that painlessly scans your skin. At the final station, a nursing sister will take a blood sample from you to see how much vitamin D you have in your blood. The procedures conducted in the first phase of the study (winter) will be the same in the second phase of the study (summer). All information will be kept confidential. If you choose to take part in this study, you will be allocated a 3-digit number and this number will be used throughout the study to protect your identity.

Why have you been invited to participate?

A need has been identified for such a study to be performed based on the lack of current data on vitamin D status in health adults in South Africa. You have been randomly selected to participate in the main phase of this research project as you fulfil the inclusion criteria of the study.

What will your responsibilities be?

You will need to attend two data collection sessions – this data collection period (winter) and one in February 2017 (summer). These data collection periods will be between two to four weeks long and you only have to attend one session per phase. Each session should take between 1-2hours. At each of these sessions, you will be required to provide a small amount of blood (approximately 1 teaspoon – 5ml).

Will you benefit from taking part in this research?

If you are selected for the main study, you will indirectly benefit from this research project by becoming aware of your vitamin D status via a free vitamin D biochemical analysis. Other people in South Africa can benefit from this pilot project as results of the main study will allow us some insight into the health status of adult South Africans regarding vitamin D and whether there is a need to develop interventions to improve the health of the general population.

Are there in risks involved in your taking part in this research?

This study has minimal risks to you as the participant. There is slight risk of discomfort while bloods are drawn, as well as bruising, bleeding, lightheadedness, but as this is a routine procedure that will be conducted by a qualified phlebotomist, these risks are small. Weight, height and waist circumference measurements will be performed by trained professionals and therefore there is minimal risk of discomfort with these procedures. The skin reflectometer will be used to painlessly scan your skin to determine skin tone, and there are no risks involved with the use of the device. Your privacy and confidentiality will also be strictly protected at all times.

If you do not agree to take part, what alternatives do you have?

If you decide not to take part in this study, it will not negatively affect you in any way and it will not be held against you.

Who will have access to your medical records?

All information you provide and all information that is collected during this screening phase of the project will be treated as confidential and be protected at all times. Only those directly involved in research project, i.e the researchers and the statistician, will have access to the information you provide. All records will be kept in a secure storage area. When the results of this research project are presented and/or published in a health sciences journal, the identity of all the participants will remain anonymous.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the screening phase of the study but you will be compensated for your time through the provision of a lunch voucher to the value of R50 (at each phase) that can be redeemed on campus. Furthermore, you will be entered into a lucky draw to win 1 of 2 gift vouchers (to the value of R1500). There will be no costs involved to you, if you do take part.

Is there any thing else that you should know or do?

- You can contact Janicke Visser on 021 9389473/0828298529 if you have any further queries or encounter any problems.
- Study sponsors, study monitors, auditors, or HREC members may need to inspect the research records.
- Participants will be informed of their rights to have any new information arising during the course of the study communicated to them. If needed, the informed consent form will be revised to incorporate this information.
- You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.
- Please note: you will be notified of your vitamin D status after the samples have been analysed at the end of the study. It is the responsibility of the research ream to inform you of any abnormalities in your vitamin D blood results. If any abnormalities are found to be present, a member of the research team will offer assistance in the form of referral to a registered health care professional for further assessment and management as needed.

➢ This research project is funded by the Cancer Association of South Africa (CANSA).

Declaration by participant

By signing below, I ……..…….. agree to take part in a research study entitled, *Determinants of serum 25-hydroxyvitamin D levels in adults in the Western Cape Province of South Africa.*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2016.

……. .....

Signature of participant Signature of witness

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…….. on (*date*) ……...…….. 2016.

……. …….

Signature of investigator Signature of witness

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*)

…….…….. using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…..……. on (*date*) ……...……..

……. …….

Signature of interpreter Signature of witness

Addendum D

Questionnaire 1: Socio-demographic, medical and lifestyle questionnaire

Participant number | | | |

Date | | | | | | | | | Day Month Year

SOCIO DEMOGRAPHIC QUESTIONNAIRE

Vitamin D study

This questionnaire is to find out more about you and your current lifestyle. There are no correct or incorrect answers and all information disclosed here will be kept confidential.

Please indicate your choices by marking the answer of your **choice with an 'X'** (where relevant).

Please note:
The completion of this questionnaire is voluntary. All information in this questionnaire is, and will be kept, confidential.

SECTION ONE – BASIC SOCIO-DEMOGRAPHIC INFORMATION

Gender	Male			Female	
Age	years _____				
Race	African	Asian	Coloured	Indian	White
Home Language	Afrikaans	English	Xhosa	Other, please specify:	
Home province/country					

SECTION TWO – MEDICAL INFORMATION

1. Please indicate any medical condition/s you have been diagnosed with/or currently receiving treatment for:

- ☐ Cancer
- ☐ Diabetes
- ☐ Heart disease
- ☐ High cholesterol
- ☐ Hypertension
- ☐ Osteoporosis or osteopenia
- ☐ Gastro-intestinal absorption disorders (Coeliac's disease, Crohn's disease, Cystic Fibrosis)
- ☐ Renal disease or renal failure
- ☐ Hepatic disease or hepatic failure
- ☐ Other

If other, please specify:

2. Are you currently using any oral contraceptives?

Yes	No	Not applicable

3. Are you currently using any medication?

Yes	No

If yes, please indicate whether any of the medication/s are classified under the following:

- ☐ Antiepileptic drugs
- ☐ Antineoplastic drugs
- ☐ Antihypertensive drugs
- ☐ Antiretroviral drugs
- ☐ Bisphosphonates

- ☐ Herbal therapies (e.g. Kava Kava and St. John's Wort)
- ☐ Other

If other, please specify:

SECTION THREE – LIFESTYLE INFORMATION

1. Do you consume any alcohol?

Yes	No

If yes, how many units* of alcohol do you consume per day: _____ per week: _____

*One unit of alcohol =

- 355 ml beer
- 148 ml dry or semi-sweet wine
- 60 ml fortified wine
- 25 ml brandy, whiskey, liquor

2. Please specify when the majority of alcohol consumption takes place:

Week	Weekend

3. Do you smoke any tobacco products?

Yes	No

If yes, please indicate how many cigarettes per day?

4. Do you currently partake in any physical activity/exercise?

Yes	No

5. If yes, how much exercise do you do per week?

Less than 90min	90 – 150 min	More than 150min	I don't know

Addendum E

Skin type and sun exposure questionnaire

Participant nr | | | |

Date | | | | | | | | | | |
Day Month Year

SKIN TONE - FITZPATRICK SKIN TYPE CLASSIFICATION

Please circle the statement that applies the most to you in each block.

1. Eye colour 0. Light colours 1. Blue, grey, or green 2. Dark 3. Brown 4. Black	**6. Do you turn brown/darker?** 0. Never 1. Seldom 2. Sometimes 3. Often 4. Always
2. Natural hair colour 0. Sandy red 1. Blond 2. Chestnut or dark blonde 3. Brown 4. Black	**7. How brown/dark do you get?** 0. Never 1. Light tan 2. Medium tan 3. Dark tan 4. Deep dark
3. Your skin colour (unexposed areas) 0. Reddish 1. Pale 2. Beige or olive 3. Brown 4. Dark brown	**8. Is your face sensitive to the sun?** 0. Very sensitive 1. Sensitive 2. Sometimes 3. Resistant 4. Never have a problem
4. Freckles 0. Many 1. Several 2. Few 3. Rare 4. None	**9. How often do you actively tan?** 0. Never 1. Seldom 2. Sometimes 3. Often 4. Always
5. If you stay in the sun too long?	**10. When was your last tan?**

0. Painful blisters, peeling 1. Mild blisters, peeling 2. Burn, no/mild peeling 3. Rare 4. No burning	0. +3 months ago 1. 2-3 months ago 2. 1-2 months ago 3. Weeks ago 4. Days

SCORING TO BE COMPLETED BY THE RESEARCHERS	
Total Score	
Skin Type Classification	

Skin Reflectometry readings:

Forehead		***Upper Inner Arm***	
EI	***MI***	***EI***	***MI***

SUN EXPOSURE QUESTIONNAIRE

The questionnaire will be used to determine how much sunlight you are exposed to on a daily, and weekly, basis. It will also provide information on your general sun exposure behaviors. There are no correct or incorrect answers to this questionnaire. All information will be kept confidential.

Please mark your answer clearly with an "X"

GENERAL QUESTIONS

1. Have you used a tanning bed/visited a tanning salon in the last 3 months?

Yes	No

2. Have you visited another country in the last 3 months?

Yes	No

If yes, which country?

3. Do you use sunscreen when you are exposed to the sun?

Never	Sometimes	Most of the Time	All of the time

4. How often do you reapply sunscreen?

5. If you use sunscreen, which sun protection factor (SPF) do you most often use?

SPF20	SPF30	SPF40	SPF50	Unsure
Other, please specify: _____				

6. Does your make-up or moisturizer contain a SPF?

Yes	No	Not applicable

If yes, please specify the SPF:

7. Does your religion/culture require you to cover parts of your body?

Yes	No

If yes, which parts?

SUN EXPOSURE

Please identify which best suits your lifestyle for each day of the week by means of an “X”.
Please note that once you have completed each row, there should be three “X”’s in that row.

These questions should be answered with regard to the current season we are in.

Day	Amount of time in sun			Amount of skin exposed				Time of day		
	<5 mins	5-30 mins	>30 mins	Hands and face	Hands, face, arms	Hands, face, arms, legs	Bathing suit	<10h00	10h00–15h00	>15h00
Monday										
Tuesday										
Wednesday										
Thursday										
Friday										
Saturday										
Sunday										

Addendum F

DATA COLLECTION

ANTHROPOMETRY

Participant nr | | | |

Date | | | | | | | | | |
Day Month Year

MEASUREMENTS				
	Value 1	**Value 2**	**Value 3**	**Average**
Weight (kg)				
Height (cm)				
Waist circumference (cm)				

Addendum G

Food Frequency Questionnaire

Participant nr | | | |

Date | | | | | | | | | |
Day Month Year

FOOD FREQUENCY QUESTIONNAIRE

Vitamin D study

Thank you for your participation in the study to collect information on vitamin D intake. The aim with the questionnaire we are going to complete, is to estimate how often you eat and drink certain foods and how much. We require this information to estimate how much vitamin D you consume through your diet. You will not be judged or criticized on what you eat and drink. There are no right or wrong answers. All the information you provide will be kept confidential.

Should you have any questions or if something is unclear do not hesitate to ask me.

Section One – Food Frequency

- Please think about how often you usually eat and drink the foods, listed in the questionnaire, in a typical day/week/month. Do not just think about what you have eaten during the past week, as this may differ from what you usually eat or drink.
- Please inform me should you *never* eat a food item or if you eat it *less than once per month*.
- Please also indicate how much you usually eat per day/week/month. In order to determine how much you usually eat or drink the size of a medium portion is provided for each food item as a guideline. Please indicate whether you usually eat/drink:
    - the same amount as the medium portion;
    - less than half of the medium portion size;
    - more than twice the medium portion size.
- Please provide any additional information that you might regard as important, about the food you eat/drink.

Guidelines for interviewers

- When a food item is not consumed, make an “X” in the “never or less than once per month” column.
- When a food item is consumed record the number of times per day AND either the number of times per week OR per month. You therefore have to mark <u>two columns</u> (per day AND either per week or per month).
- Record a NUMBER in these columns (day/week/month).
- Once you have recorded this you record the amount consumed (serving size columns).
- Use the food models/cards as relevant.
- Make an “X” in the relevant serving size column.
- In order to determine how much was eaten or drunk, the size of a medium portion is provided for each food item as a guideline. Portion sizes are given to help you determine the usual size of portions. These are known as medium sized portions.
    - If the amount of food usually consumed is the same as the medium portion size make an “X” in the column marked M for the serving size.
    - If less than the medium portion size of a particular food item (less than half) is usually eaten, choose the small option (make an “X” in column S)
    - If much more than the medium portion of a particular food (more than double) is usually eaten, choose the large option (make an “X” in column L).
    - Please do not forget to indicate the portion size.
- Provide brand names, additional information regarding portion sizes and other details in the “comments” column.
- Rather provide too many comments where relevant, than too little!
- An example of how to complete the questionnaire can be found below.

If you drink a carton of chocolate milk (500ml) Monday through Friday, then choose L (large) because it is 2 times the size of the medium portion.

Types of food or drinks	**Complete IF applicable with an “x”**	**Complete (number of times)**	**Complete week OR month (number of times)**		**Medium serving size**	**Your serving size**		
	Never or less than	**Per day**	**Per week**	**Per month**		**S**	**M**	**L**

	once per month							
Milk: full cream, 2% (low fat), 1% or fat free					1 cup (250ml)			
Chocolate milk: full cream, 2% (low fat), 1% or fat free		1	5		1 cup (250ml)			X

Types of food or drinks	Complete IF applicable with an "x"	Complete (number of times)	Complete week OR month (number of times)		Medium serving size	Your serving size			Comments
	Never or less than once per month	Per day	Per week	Per month		S	M	L	
DAIRY									
Milk: full cream, 2% (low fat), 1% or fat free – ***Plain***					1 cup (250ml)				
Milk: full cream, 2% (low fat), 1% or fat free – ***with cereal***					½ cup				
Milk: full cream, 2% (low fat), 1% or fat free – ***in tea/coffee***					1 tablespoon				
Milk: full cream, 2% (low fat), 1% or fat free – ***Milo, Horlicks, hot chocolate***					½ cup				
Smoothies (with milk)					½ cup				
Chocolate milk: full cream, 2% (low fat), 1% or fat free					1 cup (250ml)				
Soy milk: Plain or flavoured					1 cup (250ml)				
Other plant milks (specify in "comments")					1 cup (250ml)				

Types of food or drinks	Complete IF applicable with an "x"	Complete (number of times)	Complete week OR month (number of times)		Medium serving size	Your serving size			Comments
	Never or less than once per month	Per day	Per week	Per month		S	M	L	
Milk shake					1 cup (250ml)				
Milk-based dessert **(ice cream)**					1 scoop ice cream				
Milk-based dessert **(baked custard)**					½ cup				
Milk-based dessert **(pudding)** i.e. instant pudding					½ cup				
Milk-based dessert sauce **(custard)**					½ cup				
Milk-based cheese sauce					½ cup				
Cream soups, made with milk					1 cup (250ml)				
Yoghurt (Plain)					½ cup (125g container)				
Yoghurt (Sweetened)					½ cup (125g container)				
Yoghurt (Frozen)					½ cup (125g container)				

Types of food or drinks	Complete IF applicable with an "x"	Complete (number of times)	Complete week OR month (number of times)		Medium serving size	Your serving size			Comments
	Never or less than once per month	Per day	Per week	Per month		S	M	L	
Smoothies (with yoghurt)					½ cup (125g container)				
Cheese: soft or spread (e.g. cottage cheese, cream cheese)					1 tablespoon				
Cheese: hard (e.g. cheddar, gouda)					Matchbox size (2 slices)				
Cheese: other (Brie, camembert, edam, mozzarella)					Matchbox size (2 slices)				
FATS AND OILS									
Cod liver oil					5ml				
Butter (specify brand in "comments")					1 teaspoon				
Margarine (specify brand in "comments" – e.g. Flora regular)					1 teaspoon				
FISH AND OTHER SEAFOOD									

Types of food or drinks	Complete IF applicable with an "x"	Complete (number of times)	Complete week OR month (number of times)		Medium serving size	Your serving size			Comments
	Never or less than once per month	Per day	Per week	Per month		S	M	L	
Canned salmon					2 tablespoons (e.g. on bread)				
Canned salmon					1 cup salmon casserole				
Canned tuna					2 tablespoons (e.g. on bread)				
Canned tuna					1 cup tuna casserole				
Canned sardines					2 sardines (1/2 can)				
Canned pilchards (Specify small or large can under "comments")					2 pilchards (1/2 can)				
Salmon steak					90g				
Other fish: white (e.g. hake)					90g				
Other fish: oily (e.g. mackerel, anchovies)					90g				

Types of food or drinks	Complete IF applicable with an "x"	Complete (number of times)	Complete week OR month (number of times)		Medium serving size	Your serving size			Comments
	Never or less than once per month	Per day	Per week	Per month		S	M	L	
Seafood: shrimp, prawn, lobster, crab					1 cup				
Shellfish: mussels, oyster					½ cup				
Sushi (salmon, tuna, crab, prawn)					6 pieces				
MEAT AND CHICKEN									
Liver, chicken					30g				
Liver, beef					30g				
Liver, lamb					30g				
Pork					120g				
Beef					120g				
Veal					120g				
EGGS									
Eggs, eaten alone					1 large egg				
Eggs, eaten in other foods					1 large egg				
VEGETABLES									

Types of food or drinks	Complete IF applicable with an "x"	Complete (number of times)	Complete week OR month (number of times)		Medium serving size	Your serving size			Comments
	Never or less than once per month	Per day	Per week	Per month		S	M	L	
Broccoli, kale, spinach, other greens (specify type in "comments)					1 cup raw or 1/3 cup cooked				
Mushrooms (specify type in "comments")					1 cup raw or 1/3 cup cooked				
OTHER									
Pizza					4 slices				
Tofu					1 cube				
Pasta with cheese (e.g. macaroni cheese, lasagne)					1 cup				
Fortified cereals (specify in "comments")					1 cup				
Any other known foods fortified with Vitamin D (describe in detail under "comments")									

Completed by: _____

Section Two - Nutritional supplements

Please list the nutritional supplements used in the past month:

Name of Supplement	Does the Supplement Contain Vitamin D?	Amount Taken	Frequency of Supplement Use

Completed by: _____

Addendum H

Study Code:		*For laboratory use*

Vitamin D Study

Division of Human **Nut**

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University of Stellenbosch

NHLS Tygerberg Laboratory Request Form

NHLS Account:		NHLS Location:	TYGERBERG

Patient Information

Participant number:		Age:	years
Name:	N/A	Gender:	M or F
Surname	N/A		

*PLEASE MARK THE ACCOUNT FROM NHLS FOR THE ATTENTION OF "**Mr Franklin Van Wyk**" WHEN SENDING VIA DIVISION OF HUMAN NUTRITION; FACULTY OF MEDICINE AND HEALTH SCIENCES; UNIVERSITY OF STELLENBOSCH! THIS WILL RESULT IN TIMEOUS PAYMENT.*

Clinical Information/Sample Information

Clinical notes:	**Markers to be taken**	Sample Taken	/ / 20
Centrifuging of Vitamin D analysis and storage at -20°C		Time:	
Red vacutainers with blood samples and pipettes provided. Need to be transferred to Cryovial tubes and stored		Specimen Type:	**Red:** Vitamin D sample (5ml)

Phlebotomist:		Signature:	

Instructions

Any queries, please contact:

***Janicke Visser** (Principal Investigator): 021 938 9473*

***Lauren Philips** (Project Manager) 021 938 9193*

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Addendum I

Standard Operating Procedures

General conduct

1. Respect for persons is an essential ethical principle. Each member of the research team should treat all interaction will study participants with respect. The participant does not have to take part in this study and is doing us a favour. If it wasn't for the participants, the study would not be possible. If the research team is not respectful of their right to autonomy, they will be less inclined to participant. Each person who is part of the research team should show respect for
    - The aims and objectives of this study
    - All members of the team
    - The individual study participants
    - The data collected in this study

The data collector should

- Always be polite and professional towards the participants, whether or not they have agreed to take part in the study
- Answer all questions the participant may have honestly and with the correct information. If you do not know they answer, please inform the participant to this and let them know that you are going to ask the researchers. Always come back to them with what you have found out.

2. All participation in this study is voluntary. Every participant has the right to refuse to join the study at any time in the study. If they are at the last data collection station, and they refuse to continue, that is there right and it must be respected. Please do not, in anyway, make the participant feel like that have to take part in this study. Respect the participant's decision, whether to participate or not, at all times, and do not treat those who have chosen to participate differently to those who have chosen not to.
3. All information is confidential. Participants will be given randomly generated numbers to protect their anonymity and these must be used on all data collection sheets. A participant's name, contact details, or any other identifying characteristics should not appear on any of the data collection sheets. Please inform all participants of privacy and confidentiality during the informed consent briefing.
4. All participants must sign a consent form before taking part in the study. It is every patient's right to informed consent. Please thoroughly go through the consent form with each participant before they sign, and provide them with correct, factual information,

in their language of choice. Answer any and all questions they may have regarding the study. If you do not know the answer to a question they have asked, please ask one of the researchers and they will either explain it to you, or come and explain it to the participant. Please do not let the participant sign the consent form until all their questions and concerns have been addressed. Please note that informed consent does not end once the participant has signed the consent form; it is an ongoing process that continues through all phases of data collection. Please check at each station if the participant wishes to continue.

5. These procedures are in place to help this study run as smoothly as possible, while ensuring good quality data. Unforeseen circumstances can occur, however. In the event of
6. As much as we take care to prevent mistakes, they do happen. If you find that you have entered a value incorrectly or made some other error, please inform the researchers as soon as possible so we can take steps to rectify this.
7. All participants have the right to privacy, regardless of age, gender, culture, etc. All members of the research team must respect each participant's right to privacy. All data collection stations must be conducted in separate, private areas. Please do not cause the participant any unnecessary embarrassment, uncomfortableness, or discomfort.

Data integrity

1. The data we collect during our data collection phases is the culmination of months of preparation. This data is crucial for our final product and it is vital that the information that is collected, recorded and stored is correct and accurate. If the data is inaccurate or incorrect, the results of this study will also be and all our efforts will have been wasted. People that could have benefited from this study could possibly be put at risk if we draw inaccurate conclusions from incorrect data. It is vitally important that the information is collected properly, recorded accurately and stored safely. If you make a mistake, please tell the researchers as soon as possible. We may be able to fix the problem, and if we can't, at least we know what data is usable and what is not.
2. The protocol and these procedures detail how each stage of the study must be completed. Please ask if you are unsure of or uncomfortable with any of the techniques or data recording.
3. As much as we try and plan for all eventualities, sometimes things happen and we have to deviate from the plan. As a data collector, sometimes you are not able to follow study procedures, through no fault of your own, and sometimes mistakes happen. It is very important to let the researchers know if you need to deviate off book or if you make a mistake because it is the researchers' responsibility to report these problems.

Problems are part of the learning curve; please don't feel bad or embarrassed if something goes wrong. These things happen. It is not good if you don't inform the researcher of these problems, however.

Anthropometry

There are many variations in how to perform anthropometrical measures, so it is important that we all follow the same methods. In order for good quality measurements to be taken and for standardisation amongst fieldworkers to occur, it is necessary to adhere to the following guidelines.

1. Please only use equipment that has been approved and calibrated for this study.
2. Please check your equipment for calibration at the beginning of each day.
3. Measurements will be randomly spot checked throughout data collection. When a participant's measurements are spot checked, the following will happen:
    a. Two data collectors will independently measure and record all the anthropometrical measurements on the data collection sheet
    b. Each data collector will perform the measurements twice
    c. The second measurements will be compared against the data collector's own first measurements
    d. Both measurements will be compared between the two independent data collectors.
    e. The following discrepancies are acceptable:
        - 0.5kg for weight
        - 0.5cm for height
        - 0.5cm for waist circumference
    f. If a greater discrepancy occurs, please tell the researchers immediately.
4. Please remember to respect the participant's right to privacy and voluntary participation at all times.
5. Please inform the participant of what you will be doing at all stages of anthropometrical data collection.
6. Always ask permission before you conduct a measurement or touch a participant.

Weight

1. Participants should be weighed wearing minimal clothing, but are allowed to remain dressed. Outer clothing, such as jackets, coats and jerseys should be removed, as should scarves and waistcoats.

2. Participants should be weighed without shoes. Socks may remain on.
3. Scales must be placed on a flat, stable floor surface.
4. Scale must be sprayed with alcoholic spray and wiped between participants.
5. Participants must stand facing the numerical display.
6. Participants must have both feet on the scale, with weight evenly distributed between the feet, and not lean against anything.
7. Participants should not move around once they have settled on the scale
8. Participants should stand upright, and look straight ahead.
9. The numerical display must stabilise for at least three seconds before the measurement is read.
10. The participant should step off the scale after the first measurement is recorded.
11. Record the weight in kilograms, rounded off to the nearest 0.5kg, in the correct place on the data collection sheet.
12. Please do not comment or make judgements on the participant's weight.

Height

1. Stadiometers must be placed on a flat, stable floor surface.
2. Ask the participant to remove their shoes and any head coverings.
3. Ask the participant to step onto the platform, facing away from the measuring stick.
4. Weight should be evenly distributed between the feet.
5. The participant should stand independently without leaning on anything
6. The participant's chin should be parallel to the floor.
7. Lower the head piece to it touches the crown of the participant's head
8. In the case of excess hair, compress lightly, to flatten the hair.
9. Ask the participant to breathe in, at the point of maximum inhale, read the measurement.
10. Raise the headpiece and ask the participant to step off the platform.
11. Record the measurement on the data sheet in centimetres, rounded off to the nearest centimetre.

BMI

1. Please use the following the formula to calculate BMI

$$\frac{\text{Weight (in kilograms)}}{\text{Height (in metres)}^2}$$

2. Record the BMI on the data sheet rounded off the nearest tenth

Waist circumference

1. Ask the participant to remove any shoes with heels.
2. Ask the participant to remove any bulky jerseys, jackets, waist coats, etc.
3. Ask the participant to remove any waist belts.
4. Ask the participant to stand up straight with weight evenly distributed between both feet.
5. Lift the participant's shirt to expose the abdomen.
6. Using your fingers, find the last rib and the top of the ileal crest. Mark each place with a pen.
7. Do the same of the other side.
8. Using the measuring tape, find the half way point between the two points on each side.
9. Place the measuring tape horizontally on the midway points.
10. Make sure the measuring tape is straight and flat against the skin.
11. Pull the measuring tape firmly but do not let it dig into the participant's skin. It should be snug against the skin.
12. Holding the measuring tape loosely, ask the participant to inhale and then exhale
13. On the exhale, pull the measuring tape firmly (but not so tight that it cuts into the skin) and read off the measurement.
14. Record the measurement in centimetres, rounded off to the nearest 0.1 centimetre.
15. Provide the participant an alcoholic swab to remove the ink marks.

Hip circumference

1. Ask the participant to remove any shoes with heels.
2. Ask the participant to remove any bulky jerseys, jackets, etc.
3. Ask the participant to remove any belts.
4. Ask the participant to stand up straight with weight evenly distributed between both feet.
5. Using your fingers, find largest part of the greater trochanter on the right side of the participant. Mark place with a pen.
6. Do the same of the other side.
7. Place the measuring tape horizontally on each of these points.
8. Make sure the measuring tape is straight and flat against the skin.
9. Pull the measuring tape firmly but do not let it dig into the participant's skin. It should be snug against the skin.
10. Read off the measurement.
11. Record the measurement in centimetres, rounded off to the nearest 0.1 centimetre.

12. Provide the participant an alcoholic swab to remove the ink marks.

Food Frequency Questionnaire

An important part of this study is the completion of the food frequency questionnaire to determine how much vitamin D participants are consuming from their everyday diets. The information and the results of the study is only as good as the information we collect, so accuracy is vital to the success of this project. When completing the questionnaire with each participant, please adhere to the following:

1. Please remember to fill in the participant number at the top of the questionnaire
2. Please do not record any of the participant's personal details on the questionnaire
3. Please not separate the pages and/or remove any of the pages from this questionnaire
4. Please mark each section with an 'X'
5. Please do not leave a question blank. If it does not apply to the participant, mark the "never or less than 1 time per month" option.
6. Should a mistake be made, please draw a horizontal line through the incorrect entry, sign next to the error and enter the correct information.
7. Before beginning, please explain to the participant what you will be doing with them i.e. asking them which foods they eat, how many times a month they eat these foods, and how big their average portion size is. Please inform the patient that there are no right or wrong answers and that we are not judging them on what they eat.
8. For each row, please follow the following steps:
    a. Ask the participant if they eat the provided food item. Please read out everything relating to that food item (i.e. Milk: full cream, 2%, 1% or fat free). If no, mark "never or less than 1 time per month". If yes:
        i. Ask the participant how many times they consume the item each month, by reading out the frequency options. Place an 'X' in the appropriate column.
        ii. Read out the medium portion size. Ask the participant if they eat more, less or the same as that portion at each sitting.
            1. If they eat the same, place an 'X' in the "medium" column
            2. If they eat less than the medium portion, ask them how much less. If they eat half or less of the medium portion, place an 'X' in the "small" column.
            3. If they eat more than the medium portion, ask them how much more. If they eat double or more of the medium portion, place an 'X' in the "large" column.
    b. Once each row has been completed, please check that each row has two 'X's.

i. If a participant does not eat a food item regularly, that row will only have one ‘X’. Every row must have at least one ‘X’.

9. For the nutritional supplementation section, please ask the participant which supplements, if any, they use.
    a. Name of the supplement
    b. How much is consumed each time
    c. How often the supplement is consumed
    d. The main nutrients in the supplement, if they participant knows, or if the supplement contains Vitamin D.
10. When the questionnaire has been completed, please check that the participant number has been filled in at the top of the questionnaire and thank the participant for their time.
11. Please ensure all pages are stapled and place completed questionnaires in the boxes provided.
12. The information we get here is vital to the study and it is imperative that participant’s feel comfortable and safe enough to be honest with us. Everyone is different and everyone eats and enjoys different foods. Please do not make any judgemental comments or make the participant’s feel like they cannot be honest with you.