Diet and lifestyle factors associated with vitamin D status in healthy women living in the United Kingdom and the Kingdom of Saudi Arabia.

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Declaration

I declare that this thesis is my own work, and no portion of the work has been submitted in support of an application for another degree or qualification of this university or any other learning institutions.

Finally, I have acknowledged all results and quotations from the published or unpublished work of other people.

Acknowledgements

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I give thanks for my amazing family, who provided support and encouragement during this journey, and my beloved daughters, Nadeer and Dhay, who have been there with me through thick and thin. Sometimes, all I needed was to think of you both to be motivated and continue. Thanks also to my mum and siblings, who have been supportive in many ways and inspired me along this journey by providing unending reassurance that I can achieve and complete this goal.

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Abstract

Background: Vitamin D is integral to bone health and skeletal growth. There is now growing evidence that vitamin D deficiency is one of the most common diseases worldwide, not only in countries with limited sunshine, such as the United Kingdom (UK) but also in countries with substantial sunshine, such as the Kingdom of Saudi Arabia (KSA).

Aims: The aim of the research was to study vitamin D intake and lifestyle factors that may affect vitamin D production in women adopting different clothing styles within two countries, Saudi women in the KSA, as well as the UK covered (UKc) and the UK uncovered (UKun) women.

Methods: The study methods were designed to collect participants' dietary vitamin D intake, sun exposure routine, other influential lifestyle factors, and vitamin D status. Participants were asked to complete, a vitamin D questionnaire (which included: demographic information, food frequency questionnaire (FFQ) and sun-exposure questionnaire), a 3-day food diary, and a blood sample was taken. A total of 192 participants were recruited and of these 145 completed the vitamin D questionnaire. The 3-day food diary were completed by 57 women, and 79 women gave a blood sample. Data were explained as mean±standard-deviations or percentage(frequency). Statistical test including ANOVA and Chi-square was used to determine differences between groups. Regression modelling was used to determine predictors of doctor-diagnosed vitamin D deficiency.

Results: The FFQ estimation of dietary vitamin D intake was 3.6±3µg/day, 9.2±11µg/day, and 8.6±6.5µg/day, for the KSA, the UKc and the UKun group respectively ($p<0.01$). The 3-day food diary estimation of dietary vitamin D intake was 1.4±1.3µg/day, 1.0±1.0µg/day, 3.3±3.2µg/day, for the KSA, the UKc and the UKun group respectively ($p=0.03$). Bland-Altman plot showed the two methods had low agreement, mean difference -3.93µg.

The sun-exposure assessment at peak-time was 2.3±2.8hour/day, 3.0±2.4hour/day, 1.2±1.4 hour/day for the KSA, the UKc and the UKun group respectively ($p<0.01$). Whereas, assessment of fractions of exposed body surface area (BSA) at peak-time was 0.11±0.04, 0.09±0.03, 0.14±0.09 for the KSA, the UKc and the UKun group respectively ($p<0.01$). The reported data of vitamin D status showed that previous diagnoses of vitamin D deficiency reported by 28.3%(n=41) of the participants, KSA 8.3%(n=12), UKc 17.9%(n=26) and UKun 2.1%(n=3). The collected blood samples showed that 79%(n=15) of the KSA group had vitamin D deficiency, with average level 7.53±6.91ng/ml. However, vitamin D level of the UK groups could not be obtained. Logistic regression modelling identified that supplements use and reasons for supplementation, log of average BSA exposed at peak hours and residency were predictors of being diagnosed with vitamin D deficiency of the total population (n=192).

Conclusion: Dietary vitamin D intake was very low for all the study groups regardless of residency. Sun-exposure habits varied between the group and this was most significant factor in previously diagnosed vitamin D deficiency. It may be difficult to change sun-exposure habits due to cultural or religious reasons and therefore dietary factors need to be studied to identify alternatives to sun-exposure in those who do not wish to expose their skin.

Table of Abbreviation

Abbreviation	The full name
BSA	Body Surface Area
BMD	Bone mineral density
BMI	Body Mass Index
CV	Coefficient of variation
DBP	Vitamin D-Binding Protein
HPLC	High Performance Liquid Chromatography
IU	International Units
KSA	Kingdom of Saudi Arabia
LC MS-MS	Liquid Chromatography Tandem Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantitation
MED	Minimal Erythema Dose
oz	Ounces
PTH	Parathyroid hormone
FFQ	Food Frequency questionnaire
SD	Standard deviation
SE	Standard error
SPF	Sun Protection Factor
UV	Ultraviolet
UK	United Kingdom
UAE	United Arab Emirates
UL	Upper Intake Level
1,25(OH)2D3	1,25-Dihydroxyvitamin D3
25(OH)D3	25 Hydroxyvitamin D3
7-DHC	7-dehydrocholesterol

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1 Introduction

1.1 Background

The recognition of the importance of vitamin D for bone health and skeleton growth began to increase many decades ago. There has been increasing evidence to support the relationship between general health benefits in the body and sufficient vitamin D levels (Holick 2007 ; Alshahrani et al., 2012). Vitamin D deficiency in childhood can cause rickets, skeletal deformity and retardation of growth, as well as increase the chance of hip fractures in the future, while in adults it can cause osteomalacia or osteoporosis, muscle weakness, and increase the possibility of fractures (Insel et al., 2003 ; Holick 2007). However, vitamin D deficiency has recently been linked to many other diseases, which include, but are not limited to, cancers, autoimmune deficiency, cardiovascular disease, and diabetes (Holick 2007).

An individual may suffer from severe vitamin D deficiency at times, but symptoms can be unclear and misleading. Warning signs of vitamin D deficiency can be very common, such as general fatigue and pains in muscles and joints, although an individual may not actually show any outward symptoms (Holick and Chen 2008 ; Pearce and Cheetham 2010 ; The Vitamin D Council 2015a). Hence, lack of outward symptoms may help to develop other health risks related to vitamin D deficiency.

The main source of vitamin D is adequate sun exposure, followed in second place by dietary intake. However, a multitude of factors which can interrupt sources of vitamin D in the body, and which could affect vitamin D levels in the body, can be divided into two main categories (Glerup et al., 2001 ; Holick 2007; Lee et al., 2008; Holick et al., 2011; Tsiaras and Weinstock 2011). Firstly, there are internal factors such as: aging, skin colour, chronic diseases, and obesity. Secondly, there are external factors such as: clothing, sun cream, indoor lifestyles, latitude, and the weather.

The second most important source of vitamin D is an individual's diet. In the case of low sun exposure, sufficient vitamin D intake may protect an individual from vitamin D deficiency (Ott et al., 2012). Yet, there are limited sources of food that are naturally rich in vitamin D, as it is generally restricted to oily fish, eggs, sun-dried mushrooms, and animal liver (Holick 2007). Therefore, most food products that are rich in vitamin D are actually fortified foods with vitamin D added to them such as milk and cereals.

Recommendations of vitamin D intakes vary from country to country, and these are not the only areas of disagreement concerning vitamin D. Indeed, definitions of vitamin D status in the body are still widely debated (The Vitamin D Council, 2015c). Moreover, methods of assessing vitamin D levels in the body are diverse, and the precision of the outcomes of methods varies too (Harrison et al., 2009 ; Sadat-Ali et al., 2014).

People with pigmented skin, particularly Asians, are found, in many countries, to have low vitamin D levels (Siddiqui and Kamfar 2007). In the UK, a high prevalence of vitamin D deficiency amongst the Asian and south Asian population, comparing them with Caucasian population, has been reported as an on-going problem since the early seventies (Ford et al., 1972 ; Ford et al., 1976 ; Kift et al., 2013). Nevertheless, vitamin D deficiency in Asian people has been acknowledged not only in the high latitude countries such as the United Kingdom (UK), but a high prevalence of vitamin D deficiency has been found even in countries with sunny climate throughout the year. For instance, the Kingdom of Saudi Arabia (KSA) is one of these countries with high vitamin D deficiency in despite sufficient sunlight (Sedrani et al., 1983). Recent studies have suggested that vitamin D deficiency in the Saudi population is still common (Bin-Abbas et al., 2011 ; Al-Othman et al., 2012 ; AlQuaiz et al., 2014). Meanwhile, despite attempts by both countries (the UK and the KSA) to increase awareness of the importance of vitamin D, figures still indicate a range of vitamin D deficiency problems.

1.2 Statement of the problem

Clothing is perceived as an obstacle to obtaining adequate vitamin D from exposure to the sun (Tsiaras and Weinstock 2011). Consequently, this study is concerned with vitamin D amongst women who adopt clothing styles that cover the whole body for reasons of cultural and religious choice. People who have adopted such a style were reported in the UK, by the Department of Health (2012), as one of the groups most at risk in the UK.

The dress that is commonly popular amongst Muslim women is the dress of Islamic law, namely a hijab, which, according to Islamic law, covers the whole body and sometimes includes a face veil, namely a niqab. In a country such as the KSA which follows Islamic law, such a form of dress is compulsory for women. It should be noted that females in the KSA appear to be affected by vitamin D deficiency at different stages of life as an adult and as a child (Siddiqui and Kamfar 2007 ; Siddiqui 2010). The KSA is a Muslim country, which imposes a dress code for women. “Abaya” must be worn in public, and it is enforced by the

religious police. A headscarf and face cover could be applied too but not compulsory for foreigners and non-Muslim women. Therefore, modest clothing for women in the KSA is considered a law, and in public places it is hard to find women with revealing clothing.

Clothing styles as a contributing factor have been excluded by Sedrani et al. (1983) as one of the main reasons for vitamin D deficiency in such countries. However, Mishal (2001) emphasized the role of clothing styles as an influential factor for vitamin D deficiency, and suggested more factors such as limited vitamin D intake through foods and supplements, indoor lifestyles and modern living conditions. In spite of the fact that the dominant results from all previous studies have demonstrated a clear prevalence of vitamin D deficiency among healthy women in the KSA, only a few studies have been published about healthy Saudi women's vitamin D status, and their vitamin intake between the periods of 1980 to 2015. In addition, there is insufficient data available to allow general comparisons of factors that influence vitamin D status between the KSA and the UK.

1.3 Hypothesis

The study aims to test the following hypothesis:

- Muslim women in the UK and the KSA, who adopt the dress of Islamic law, namely the Hijab (which covers the entire body and sometimes together with a face veil, known as a Niqab), are more likely to follow a certain lifestyle practices leading to vitamin D deficiency than those who do not adopt this form of dress.

The basis for this hypothesis stems from the fact that this form of dress completely covers the skin, which presents an obstacle to obtaining adequate vitamin D from exposure to the sun. Subsequently, the current hypothesis is formulated together with the secondary hypothesis, that

- Dietary intake is not sufficient to allow covered women to meet vitamin D requirement.

To investigate the "covered" theory's validity, the researcher has compared three groups of women: covered women in the UK, uncovered women in the UK, and covered women in KSA.

1.4 Aims

The aims of this research project with respect to the two hypotheses are to:

- Assess the dietary vitamin D intake of healthy Saudi women and healthy UK covered women, as well as healthy UK uncovered women.
- Compare data from FFQ and food diary to determine reliability and validity of the methods.
- Identify potential lifestyle factors that could affect the vitamin D status of healthy women in the UK and the KSA.
- Assess vitamin D status of healthy Saudi Arabian women, healthy UK covered women, and healthy UK uncovered women.

1.5 Research objectives

The objectives of this study are to

- Estimate and compare vitamin D intake from FFQ and diet diaries for covered and uncovered women in the UK and covered in the KSA.
- Use two dietary assessment methods to determine validity of the methods outcomes by conducting Bland-Altman plot.
- Estimate and compare sun exposure habits of the three study groups by using self-completed questionnaire.
- Test the vitamin D level in serum via literature review and blood sampling.
- Examine the lifestyle factors that could affect the vitamin D status in the two countries through the regression modelling.
- Highlight similarities and differences between the study groups' lifestyles that may influence the vitamin status by distinguishing the results of each group separately.

1.6 Research questions

The following questions were investigated in this study:

- Is there a difference in dietary intake measure by FFQ and food diary?
- What is the degree of agreement between the results of the two methods of dietary assessment (FFQ and food diary)?
- What is the association between study groups in terms of their usual sun exposure habit?

- Is there difference between study groups in terms of their holiday sun exposure habit?
- Is there a significant difference in vitamin D 25(OH)D levels when compared between covered women in the UK and covered women the KSA?
- Is there a significant difference between vitamin D 25(OH)D levels when compared in covered women in the UK and uncovered women in the UK?
- To what extent can sun exposure and dietary intake of vitamin D predict vitamin D 25(OH)D levels?
- What the other factors that can predict participants vitamin D level?

The expected outcomes of the research will go some way to answer the above questions after analysing the results.

1.7 Contribution to Knowledge

The current research project attempts to

- Provide a new knowledge in vitamin D research concerning healthy women in the KSA.
- Produce a clear comparison data between two covered groups, sharing similar values.
- Emphasise lifestyle factors that could improve the vitamin D status between covered women in both countries.

1.8 Outline of the Upcoming Chapters

The present thesis has been structured into a set of nine different chapters.

In chapter one, the background to the relevant literature is provided before defining the details of the research. The research aims, hypothesis, objectives and questions are presented and a summary of all the thesis chapters is outlined.

Chapter two details a range of literature comprising the most relevant works which currently exist on the topic at hand. The chapter illustrates three main themes. Firstly, a description of vitamin D compounds, production, metabolism, functions, resources, the global prevalence of vitamin D deficiency, as well as relevant methods of vitamin D measurement was presented. Secondly, the problem of vitamin D deficiency in the UK

is addressed in detail. Finally, another focus in chapter two is the prevalence of vitamin D deficiency in the KSA. The chapter concludes with a short summary.

The research methodology is the focus of chapter three. This part of the thesis explains, evaluates and justifies the methodology that was used within each stage of the research. Primary data investigated the participants' vitamin D statuses, vitamin D intakes, and lifestyles. Each method that was used in primary data collection is explained in clear detail, which includes a questionnaire, food diary, and an instrumental method of blood analysis. Furthermore, sampling, the choice of participants and the ethical considerations are all set out and justified within the chapter. Likewise, the data analysis plan and the methods of validation are clarified, followed by a short summary of the chapter.

Chapters four, five, six and seven describe the findings of the study and discuss the interpretations and implications of the findings. Overall, the chapters display the primary results of the current study. The analysis chapter is designed to answer the research aims. These four chapters were divided into three main sections: introduction, results and summary.

Chapter four presented descriptive results; and chapter five displayed the findings concerning vitamin D intake in healthy Saudi women and healthy UK covered women, as well as concerning healthy UK uncovered women by setting out the food diary outcomes, and FFQ findings. Chapter six showed an attempt to identify potential lifestyle factors that could affect the vitamin D status of healthy women in the UK and the KSA. Chapter seven showed the results for the vitamin D status study groups (healthy Saudi Arabian women, healthy UK covered women, and healthy UK uncovered women). Chapter eight demonstrated the compound data results, before presenting the concluding section of results in the last part of the chapter.

Chapter nine provides the discussion - the implications of the study's key findings are interpreted and explained. Chapter ten concludes the current research project. This chapter concluded the study's main findings, and outlines the research's contribution to knowledge. Moreover, and finally, it explains the study's limitations and makes suggestions for future research.

2 Literature review

The following chapter highlights and explains the variables in the study. It begins with an overview of vitamin D, and then moves on to the study of vitamin D deficiency factors. This is followed by a focus on the prevalence of vitamin D deficiency in the UK and the KSA.

2.1 Overview of vitamin D production and synthesis

There are two groups of vitamins in food: water-soluble and fat-soluble. Vitamin D is a fat-soluble vitamin, and is considered to be a unique nutrient because human beings are able to manufacture it. For this reason, it is not considered an essential dietary nutrient; however, insufficient exposure to sunlight combined with a shortage of the vitamin in the diet results in vitamin D deficiency (Insel et al., 2003).

There are various distinct forms of vitamin D, the focus of the present review is vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol), as these are the important nutritional forms for the human, and are converted to a bioactive form of the vitamin (see Figure 1) (Thacher and Clarke 2011). Vitamin D_2, or ergocalciferol, is formed naturally by activating ergosterol in plants through ultraviolet (UV) radiation, while vitamin D_3, or cholecalciferol, is created in the skin by UV absorption (Bender 2003 ; Gropper et al., 2005).

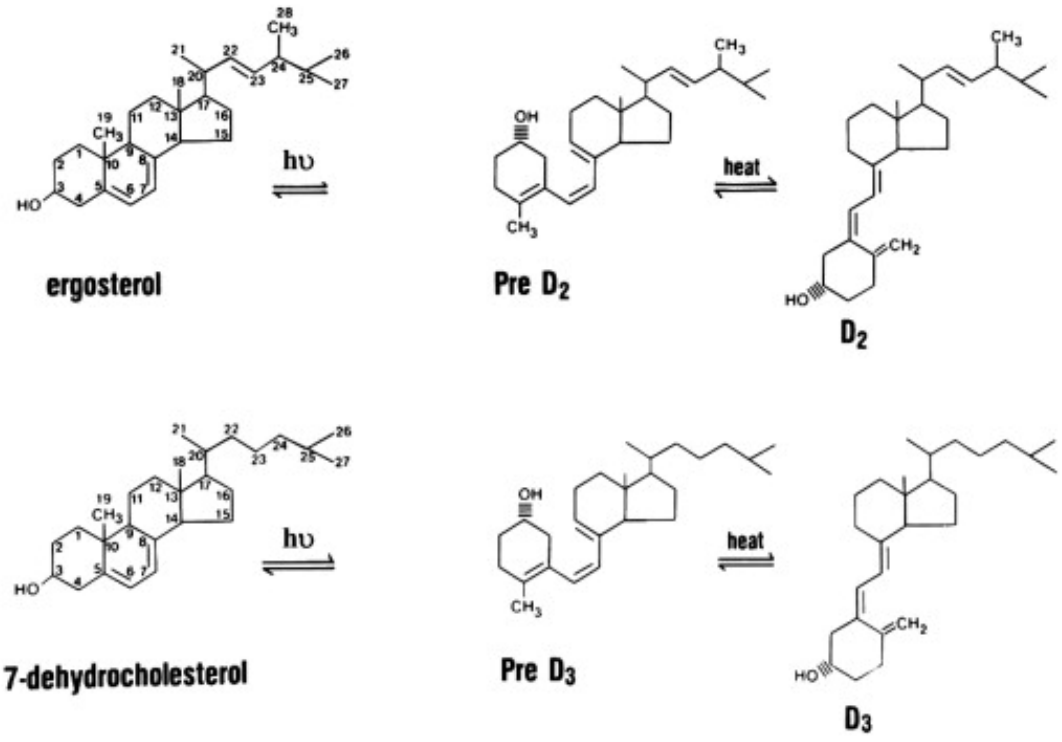

Figure 1 The Production and Metabolism of D_2 and D_3 (Bikle 2014).

2.2 Vitamin D metabolism and production

Vitamin D needs to go through a long process before it becomes fully bioactive. Moreover, the body obtains vitamin D from two separate sources: firstly, by the synthesis of the vitamin in the skin, and, secondly, from the diet (Gropper et al., 2005). An attempt to illustrate vitamin D sources and metabolism in the body is shown Figure 2.

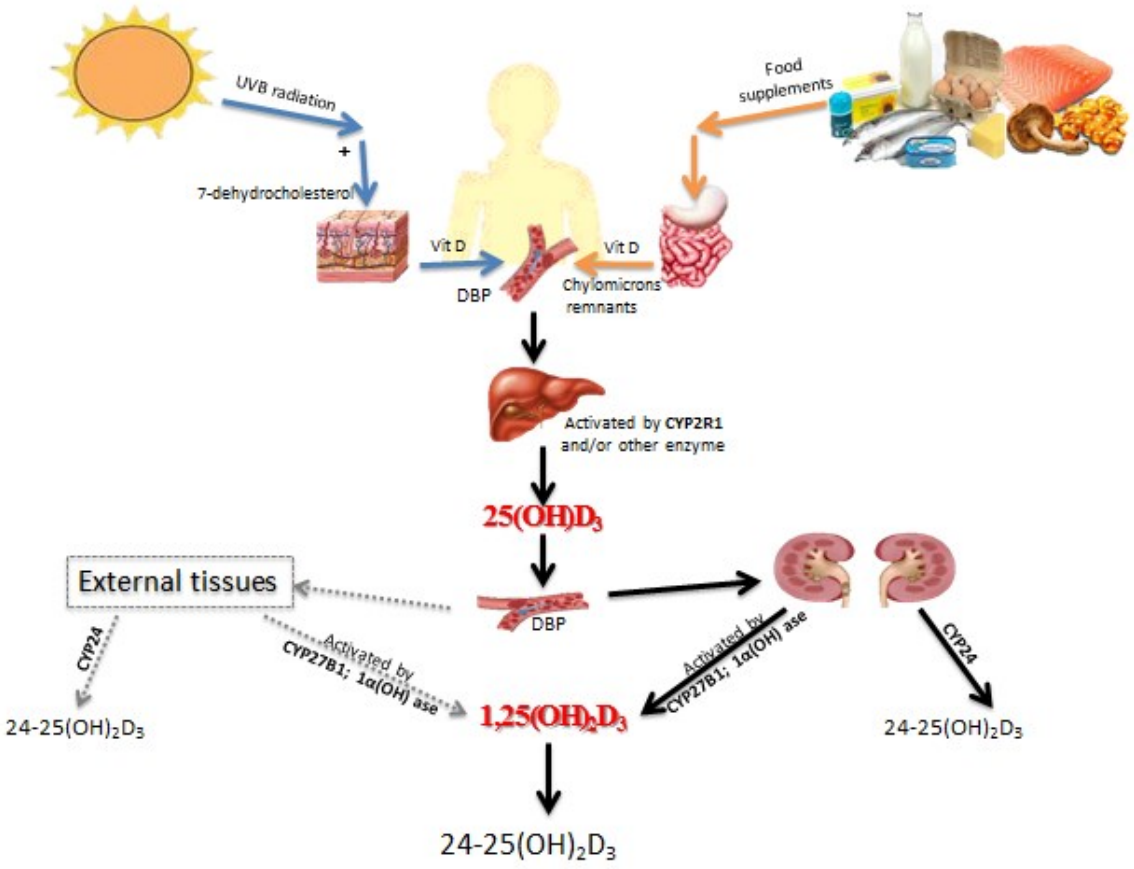

Figure 2 vitamin D metabolism in the body

2.2.1 Vitamin D production in the skin

In animals and in humans, 7-dehydrocholesterol (7-DHC) acts as a mediator. By exposing the skin to UV radiation, 7-DHC is synthesised in the sebaceous glands, and is then secreted onto the skin surface before it is absorbed into the epidermis. This step converts 7- DHC into pre-vitamin D_3 (precalciferol), and also produces some other components. Slowly, over 2-4 days, precalciferol will be isomerised thermally at 37°C to cholecalciferol (vitamin D_3) before it diffuses into the bloodstream. Vitamin D_3 in the blood will combine with DBP (vitamin D-binding protein) to be transported to the liver, where synthesis of 25 Hydroxyvitamin D (25(OH)D) occurs (Bender 2003 ; Gropper et al., 2005).

7-DHC is synthesised in the skin by exposure to UV radiation. However, production of the vitamin precursor is influenced by the level of UV radiation, with an optimal wavelength

range of 290–315 nm (Tsiaras and Weinstock 2011). The transformation that converts 7-DHC to vitamin D_3 occurs in two steps. Firstly, 7-DHC is photolysed by ultraviolet light in the B ring-opening electrocyclic reaction. The resulting product is pre-vitamin D_3. Secondly, pre-vitamin D_3 spontaneously isomerizes to vitamin D_3 (cholecalciferol) in an antarafacially sigmatropic hydride shift. At room temperature, the transformation of pre-vitamin D_3 to vitamin D_3 takes about 12 days to complete (Tsiaras and Weinstock 2011).

Following this, cholecalciferol diffuses slowly into blood circulation and bonds with DBP. A large percentage of the cholecalciferol is then transported to the liver, while the cells of other organs may also initially acquire a small amount, such as muscle tissue, before the majority of the vitamin D finally reaches the liver to become biologically active with enzyme intervention (Gropper et al., 2005).

2.2.2 Vitamin D ingested from the diet

Dietary vitamin D begins its journey in the intestine. Initially, the vitamin D intake is absorbed from the small intestine with help from lipid micelles and bile salts. Then, dietary vitamin D absorption in the small intestine will prepare the vitamin for joining the blood circulation, and a small percentage of vitamin D may bind with DBP, although most of the vitamin is transported to the liver by chylomicron remnants for bio-activation (Bender 2003 ; Gropper et al., 2005 ; Christakos et al., 2010). Moreover, the vitamin is fat-soluble and can, therefore be stored in adipose tissue following the blood circulation process. Storage in adipose tissue can be for up to two months, thus allowing the vitamin to be used when required, even if there is lower current production (Tsiaras and Weinstock 2011).

Subsequently, the resulting product of the vitamin from skin synthesis and from the ingested diet, pre-vitamin D, goes through a process in both the liver and, then, the kidney to allow vitamin D to be biologically activated. Initially, the liver converts the primary formula of vitamin D to $25(OH)D_3$, which illustrates the levels of stored amounts of vitamin D in the body, in comparison to the active form. The active form of the vitamin ,$1,25(OH)_2D_3$ (1,25-dihydroxyvitamin D_3), has an impact on the body's physiological functions, and is produced by converting 25(OH)D to $1,25(OH)2D_3$ in the kidney.

2.2.3 Vitamin D in the liver

Vitamin D_3 reaches the liver through the blood, carried by DBP. Then, vitamin D hydroxylated at carbon 25by two or more cytochrome P450 hydroxylases, which results in

the production of 25 hydroxyvitamin D_3 ($25(OH)D_3$) (Christakos et al., 2012). Examples of cytochrome P450 hydroxylates are CYP2R1, CYP2C11, CYP3A4, CYP2D25, and CYP2J3, which are recognised as generating $25(OH)D_3$ in the liver (Christakos et al., 2010 ; Lehmann and Meurer 2010). However, Christakos et al. (2010) mention that CYP2R1 is the key enzyme for hydroxylation of vitamin D. The formula of $25(OH)D_3$ in the blood is considered the definition of the level of storage of vitamin D in the body, and it has a half-life of about fifteen days (Lehmann and Meurer 2010).

2.2.4 Vitamin D in the kidney

Blood transports $25(OH)D_3$ to the kidney bound to DBP, where another hydroxylation cycle takes place to produce the bio-active form of the vitamin. Cytochrome P450 monooxygenase 25(OH)D 1α hydroxylase hydrolyses $25(OH)D_3$ in the A ring at carbon 1 to form calcitriol, $1,25(OH)_2D_3$, which is the vitamin form that is responsible for bio-functions in the body, (Christakos et al., 2010; Lehmann and Meurer 2010).

The enzyme 1α hydroxylase occurs mainly in the kidneys, but it also appears in other tissues such as the placenta (Bender 2003 ; Christakos et al., 2010). Consequently, other tissues are able to convert $25(OH)D_3$ to the active form $1,25(OH)_2D_3$, although only renal calcitriol, which is $25(OH)D_3$, makes a substantial contribution to the 1,25(OH)2D in circulation (Ott et al., 2012).

In addition, there is another form of vitamin D, $24\text{-}25(OH)_2D_3$, manufactured in the kidney. However, it is a comparatively ineffective metabolite in comparison to $1,25(OH)_2D_3$. Both $25(OH)D_3$ and $1,25(OH)_2D_3$ can interact with 24-hydroxylase (CYP24), which is also a mitochondrial P450 enzyme that is formulated in the kidney and other external organs to produce $24\text{-}25(OH)2D_3$.

2.2.5 Regulation

Vitamin D increases calcium absorption in the intestines and maintains sufficient serum calcium and phosphate concentrations to ensure normal mineralization of bone, and to prevent low serum calcium levels in the blood. $25(OH)D_2$ has an inverse relationship with $1,25(OH)_2D_3$, which means that the $25(OH)D_2$ level is low when $1,25(OH)_2D_3$ is sufficient. The renal $1,25(OH)_2D_3$, once activated, will produce a steroid hormone in the body as well as the vitamin. The major role of $1,25(OH)_2D_3$ is to sustain the calcium level in serum, which is consistent with certain other factors, such as phosphorus levels and parathyroid

hormones (Tsiaras and Weinstock 2011). However, $1,25(OH)_2D_3$ is an inducible hormone, which will become high when calcium levels are inadequate, but, conversely, will become low when calcium intake is high. Additionally, $1,25(OH)_2D_3$ has a short half-life of fifteen hours. $1,25(OH)_2D_3$ is the bio-form of vitamin D, although, it is not a good indicator of vitamin D status in the body, as it is an adaptive hormone, and normally does not reduce until vitamin D deficiency is serious (Holick 2007 ; Holick 2009).

2.3 Vitamin D functions

The classical function of this vitamin is to control the absorption and metabolism of calcium, which is closely associated with skeletal growth and bone health (Gropper et al., 2005 ; Alshahrani et al., 2012). A lack of the vitamin causes several damages, which are different depending upon the age of the person affected. For instance, deficiency in childhood can cause: rickets, skeletal deformity and retardation of growth, and increase the chance of hip fracture in later life, while in adulthood it can cause osteomalacia or osteoporosis, muscle weakness and an increase in the possibility of bone fractures (Insel et al., 2003 ; Turpeinen et al., 2003).

Additionally, there is growing evidence that an optimal level of vitamin D is needed to achieve general good health. Vitamin D has been shown to have a strong relationship to the prevention or reduction of the risk of many chronic diseases, including cancers, autoimmune deficiency, cardiovascular disease, and abnormal cell conditions such as psoriasis (Insel et al., 2003 ; Holick 2007 ; Alshahrani et al., 2012).

2.4 Vitamin D Sources

There are two natural sources of vitamin D: sunlight and diet. Supplementation and ultraviolet B (UVB) sunbeds can be artificial substitute sources of the vitamin. However, this study focused mainly on the natural sources.

2.4.1 Sunlight and vitamin D

Human requirements for vitamin D are largely met by sufficient exposure to sunlight. Holick (2007) cited the optimal wavelengths needed to synthesise the vitamin as being between 290 to 315 nm, while Tsiaras and Weinstock (2011) reported that the optimal range for vitamin D production is between 295 nm and 300 nm, with a peak at 297 nm. However, insufficient exposure can lead to vitamin D problems, and extensive exposure to UV can result in skin cancer, although it never leads to vitamin D toxicity (van der Mei et al., 2007).

It has been stated by Holick et al. (2011) and Nair and Maseeh (2012) that total exposure to sunlight of between 1000 hours and 1500 hours per season is sufficient to maintain adequate levels of the vitamin in all seasons except winter. According to Nair and Maseeh (2012), the human body, through sunbathing, when people wear a swim suit, synthesizes vitamin D in quantities equal to taking oral vitamin D at levels of between 10,000 and 25,000 international units (IU). IU is used to measure a biologic activity of a substance such as vitamins, which is agreed upon internationally for each substance (National Institutes of Health, 2015). Nair and Maseeh (2012) proposed that in order to be adequate, a dose of UVB radiation causes minor pinkness to the skin, which lasts for one day. Tsiaras and Weinstock (2011) explained that casual exposure of the body to 25% of the Minimal Erythema Dose (MED) of UVB radiation would be equivalent to an oral dose of vitamin D of 1000IU, although agreed that the MED differs from person to person and from minute to minute. Nevertheless, Pearce and Cheetham (2010) suggested that casual exposure of the body to the noontime sun for around half an hour would be sufficient to supply the body with the vitamin D equivalent to an oral dose of 2000IU. Moreover, Tsiaras and Weinstock (2011) defined MED as the lowest dose of radiation capable of producing a light pinkness of the skin within a period of 24h post-exposure. Consequently, the same review paper explains that by exposing 100% of the body to one full MED of sunlight, the amount of vitamin D produced will be equivalent to 16,000 IU given orally, and could even be more. However, vitamin D production and the ability for it to have an active effect in the body relies on many factors, including age and weight (Tsiaras and Weinstock 2011).

A Saudi study observed pre-vitamin D_3 production by placing borosilicate ampules that contained 7-dehydrocholesterol in ethanol in direct sunlight from sunrise to sunset, and analysed hour intervals using high performance liquid chromatography (HPLC) to observe the cutaneous production of pre-vitamin D3 (Alshahrani et al., 2012). Conclusively, the

study observed the peak production for pre-vitamin D3 is between sun peak hours 9:00 am to 3:00 pm (Alshahrani et al., 2012). However, Alshahrani et al., (2012) indicated that peak sun hours are different from country to country depending on latitude, seasons and the time of day. Additionally, they added that timing is vital to obtaining the vitamin, whilst avoiding sunburn and skin cancer is imperative. The Cancer Council Australia (2015) agreed, and reported that healthy people with fair skin are able to obtain the optimal amount of vitamin D, and avoid the dangers of excessive exposure by exposing 15% of the body to UVB all day for casual exposure that does not exceed a few minutes daily. However, dark-skinned people are more resistant to skin damage because of the levels of melanin in the skin, and, hence, need greater exposure to sunlight - between three to six times more than those with a light skin colour to generate adequate levels of vitamin D (Cancer Council Australia 2015). Moreover, a British study confirmed that casual exposure to the summer sun in the UK is insufficient for dark skin types to generate sufficient vitamin D (Farrar et al., 2011). Pearce and Cheetham (2010) also confirmed these results, stating that people with dark skin tones need up to ten times longer to produce an adequate amount of vitamin D.

2.4.2 Dietary vitamin D

Food is the second most important source of vitamin D, although, in terms of variety, food in general is a limited source of the vitamin. There are actually few foods which are naturally rich in vitamin D. These include oily fish (e.g., salmon, mackerel, and herring), egg yolks, mushrooms, and animal livers (e.g., those from cows, pigs and chickens) although there is as yet no proof to confirm that livers are a good source of the vitamin (Holick 2010 ; Merewood et al., 2010 ; Holick et al., 2011) . Foods that are high in vitamin D are presented in Table 1 and Table 2.

Table 1 Foods that are high in vitamin D.

Food	Serving	Vitamin D (IU)
Salmon Wild	85g	1000
Cod Liver Oil	5 ml (1 teaspoon)	450
Salmon Farmed	85g	275
Tuna Blue Fin	85g	170
Tuna Canned In Water	85g	135
Shrimp	85g	120
Milk	200 ml (1 cup)	100
Cod	85g	80
Mushrooms	57g	50
Eggs Whole	70 g (1 egg)	25
* The original table was from America and used oz as a unit. The researcher converted the oz to grams, the international unit that is commonly used in the UK (Chen et al., 2007).		

Otherwise, most dietary vitamin D comes from food that is fortified with vitamin D such as milk, margarine, soya, and cereals (Christakos et al., 2010 ; Larson-Meyer and Willis 2010). More importantly, the majority of common food product such as milk is not fortified with vitamin D, because it is not compulsory to fortify the food with vitamin D in most countries. Therefore, people may consume insufficient amounts of vitamin D. Studies have shown that people may not consume sufficient fortified food for more than one reason. This includes the dietary choices people make, and addition of insufficient vitamin D to fortified foods (Calvo et al., 2005 ; Holick 2006 ; Wagner et al., 2008 ; Kennel et al., 2010a). In addition, it has been shown that people's consumption of fortified products does not cover 100% of their daily requirement for vitamin D, because of the variability in the amount they consume per serving each day (National Osteoporosis Foundation 2014). For this reason, the National Osteoporosis Foundation (2014) stated that supplements must be sufficient to cover the daily requirements for the vitamin.

Kennel et al. (2010a) stated that the bioavailability of the vitamin from food sources, whether a food which is naturally rich in the vitamin or one which is fortified with vitamin D, does not alter, although the amount and type of fortification does change in level.

Table 1 and Table 2 demonstrate common vitamin D dietary sources (Chen et al., 2007 ; Holick 2007). The tables show differences in the vitamin content between wild salmon and farmed salmon. Wild salmon was found to be almost three times higher in vitamin D than farmed salmon. Interestingly, canned salmon was richer in vitamin D than farmed salmon. However, based on data found in tables 1 and 2, the vitamin content for the same product was different in the two tables. Table 1 shows 14% more fish (salmon) gives only the same

amount of vitamin D compared to table 2. Furthermore, the bioavailability of vitamin D needs to be tested in humans after using a dietary model which is rich in vitamin D to prove that the human body is able to overcome vitamin D deficiency through fortified food, not just by using supplements (Outila et al., 1999).

Table 2 Dietary, supplemental, and pharmaceutical sources of vitamins D_2 and D_3.

Source	Vitamin D Content
Natural Sources	
Salmon Fresh, wild (100 g)	About 600–1000 IU of vitamin D_3
Salmon Fresh, farmed (100 g)	About 100–250 IU of vitamin D_3 or D_2
Salmon, canned (100 g)	About 300–600 IU of vitamin D_3
Sardines, canned (100 g)	About 300 IU of vitamin D_3
Mackerel, canned (100 g)	About 250 IU of vitamin D_3
Tuna, canned (102.06g)	About 230 IU of vitamin D_3
Cod liver oil (1 tsp)	About 400–1000 IU of vitamin D_3
Shiitake mushrooms, fresh (100 g)	About 100 IU of vitamin D_2
Shiitake mushrooms, sun-dried (100 g)	About 1600 IU of vitamin D_2
Egg yolk	About 20 IU of vitamin D_3 or D_2
Exposure to sunlight, ultraviolet B radiation (0.5 Minimal Erythemal Dose)	About 3000 IU of vitamin D_3
Fortified Foods	
Fortified milk	About 100 IU/230ml, usually vitamin D_3
Fortified orange juice	About 100 IU/230ml, vitamin D_3
Infant formulas	About 100 IU/230ml, vitamin D_3
Fortified yogurts	About 100 IU/230ml, usually vitamin D_3
Fortified butter	About 50 IU/100 g, usually vitamin D_3
Fortified margarine	About 430 IU/100 g, usually vitamin D_3
Fortified cheeses	About 100 IU/100 g, usually vitamin D_3
Fortified breakfast cereals	About 100 IU/serving, usually vitamin D_3
Supplements Currently Available	
Prescription	
Vitamin D_2 (ergocalciferol)	50,000 IU/capsule
Drisdol (vitamin D_2) liquid supplements	8000 IU/ml
Over-the-counter	
Multivitamin	400 IU vitamin D, D_2, or D_3
Vitamin D_3	400, 800, 1000, and 2000 IU
*The original table used oz as a unit, the researcher converted these to g (Holick 2007).	

Gropper et al., (2005) mentioned that vitamin D_2 is used to fortify foods such as dairy products and supplements. Other scholars have reported that both forms of the vitamin (D_2, D_3) are used nowadays for food fortification and supplements, and deliver the same benefit as the actual vitamin to the body (Holick et al., 2011 ; National Osteoporosis Foundation 2014). Additionally, cooking methods should not affect vitamin content (Insel et al., 2003). Nevertheless, frying foods does decrease the vitamin D content of food (Chen et al., 2007; Ali 2010).

2.4.3 Vitamin D deficiency

There is still a debate around the definition of deficient, insufficient and sufficient levels of vitamin D in serum. Different organizations, reports and studies have suggested various ranges in vitamin D guidelines, see Table 3. Holick (2009) stated that deficiency is when the serum 25(OH)D level is less than 50 nmol/L; insufficiency is 52 to 72 nmol/L; the sufficiency level of 25(OH)D is at 73 nmol/L or over, which is the most desirable level in order to maintain the full health benefits conferred by the vitamin. On the contrary, other researchers have stated that serum 25(OH)D concentration is deficient at less than 20-25 nmol/L and insufficient between 25 and 75 nmol/L (Tsiaras and Weinstock 2011). Furthermore, another source defined the deficiency level as 20 nmol/L and less, and insufficiency as between 20 to 37 nmol/L, sufficiency as more than 37 nmol/L (Thomas et al., 1998).

Table 3 Vitamin D 25(OH)D range guidelines from various organizations (The Vitamin D Council 2015c).

	Vitamin D Council/USA ng/ml (nmol/L)	**Endocrine Society/USA** ng/ml(nmol/L)	**Food And Nutrition Board/USA** ng/ml(nmol/L)	**Testing Laboratories /USA** ng/ml(nmol/L)
Deficient	0-30 (0-75)	0-20 (0-50)	0-11 (0-28)	0-31 (0-77)
Insufficient	31-39 (77-97)	21-29 (52-72)	12-20 (30-50)	
Sufficient	40-80 (100-200)	30-100 (75-250)	>20 (>50)	32-100 (80-250)
Toxic	>150 (>374)			

Given the disagreement shown by these contradictory reports, it is not surprising that there is still no clear evidence as to what is the lowest sufficient level of vitamin D in serum required to maintain good health. A significant complication in establishing the level is the use of different units to report the levels. Some studies in the literature use ng/ml as a measurement unit for the vitamin, whilst others use nmol/L. This mixture of units can cause confusion, and, hence, it is important to use a single unit type and to show the conversion factors. For instance, 30 ng/ml is equivalent to the 75 nmol/L that could represent the initial acceptable level of the vitamin that allows the human body to maintain full health benefits (Lee et al., 2008 ; Holick 2010). Meanwhile, the Institute of Medicine in America and Canada reported that 40 nmol/L is sufficient to ensure that 50% of the study's population gain the full health benefits of the vitamin, while 95% of the candidates received the full health benefits of the vitamin at 50 nmol/L (Institute of Medicine 2011 ; Ross et al., 2011).

Conversely, it has been suggested that concentrations of 25 nmol/L or over are acceptable to avoid extreme hypovitaminosis D in adults (Hyppönen and Power 2007).

To conclude, the Vitamin D Council in US and US National Institutes of Health suggest that a level of 50 ng/ml is the ideal level to aim for to obtain the full health benefits of vitamin D (National Institutes of Health 2014 ; The Vitamin D Council 2015b). Indeed, less than 20 ng/ml (50 nmol/L) reflects insufficiency levels, and less than 12 ng/ml (30 nmol/L) presents a vitamin D deficiency level.

The measurement units that were used in the literature were usually one of either nmol/L or ng/ml. To convert the unit from nmol/L to ng/ml easily, it is necessary to divide by the number 2.496 (Thacher and Clarke 2011).

2.5 Issues influencing vitamin D deficiency

As foods are not the chief source of vitamin D (Ott et al., 2012), a shortage of the vitamin in food is not the main cause of any vitamin deficiency, although it can always exacerbate the problem. Different sources report that the main cause of vitamin deficiency is inadequate exposure to sunlight (Glerup et al., 2001 ; Holick 2007 ; Lee et al., 2008). However, there are some factors that contribute to reducing the ability of the skin to stimulate vitamin D production. Sunscreen with a sun protection factor (SPF) of 15 or more almost completely prevents synthesis of vitamin D (Lee et al., 2008), whilst Glerup et al. (2001) state that an SPF of 8 is sufficient to eliminate 95% of the skin's ability to synthesize the vitamin. Similarly, the time of the day, seasons and latitudes are important issues affecting the production of vitamin D (Glerup et al., 2001 ; Lee et al., 2008). The efficiency of UVB irradiation increases or decreases depending upon the sun's angle with respect to the earth's surface (Holick 2007). In addition, dark clothing, long-sleeved and covering clothing, fabric quality, glass and plastic covers, air pollution and cloud cover all prevent UVB radiation from reaching the skin (van der Mei et al., 2007 ; Tsiaras and Weinstock 2011 ; Alshahrani et al., 2012).

2.6 Groups at risk of vitamin D deficiency

In addition to the issues mentioned previously, there are some groups at greater risk of developing vitamin D problems than others. For instance, dark skin colour is a form of congenital sun protection and needs longer exposure to sunlight than light coloured skin does in order to synthesise an equivalent amount of vitamin D. Holick et al. (2011) reported

that any degree of darker skin needs 3-5 times longer than fair skin to achieve optimal vitamin levels. It has also been suggested that dark-skinned people could potentially need 6 times longer in the sun than fair skinned individuals, even though the degrees of skin tone have not been clarified (van der Mei et al., 2007 ; Kennel et al., 2010a).

In addition, various groups of individuals suffer from vitamin D deficiency due to the cause of different adverse health effects. There is an inverse relationship between an individual's body mass and vitamin D levels, as body fat becomes a significant factor in vitamin D deficiency when the BMI exceeds 30kg m^2 because the vitamin is fat soluble and hence high fat reduces the blood levels (Lee et al., 2008 ; Holick et al., 2011). Breast feeding infants who rely on their mothers that are deficient in vitamin D, can become vitamin D deficient too, as the milk lacks the vitamin (Holick 2007). Elderly people are normally at high risk of vitamin D deficiency, as the skin's ability to synthesize the vitamin decreases with age (Kennel et al., 2010a). Similarly, individuals who spend limited time out of doors, such as those who are housebound are at a high risk. Furthermore, individuals who suffer from chronic diseases, such as renal disease, hepatic failure, fat malabsorption syndromes, cancer; as well as individuals using certain medication, such as HIV-drugs, glucocorticoids, and those on multiple medication, all risk vitamin D problems, as they all can potentially have an inverse association with vitamin D level (Lee et al., 2008).

2.6.1 Vitamin D maintenance

Vitamin D deficiency treatment methods are various. Normally these are exposure to sunlight, the use of artificially produced UV radiation, consumption of vitamin D in dietary sources, using specific oral vitamin D supplements, or vitamin D injection (Lips 2001).

2.6.2 Recommended doses of vitamin D

Vitamin D intake doses can be divided into two types: the recommended daily intake, and the upper intake level (Hathcock et al., 2007). However, recommended doses of vitamin D vary according to age. A review by Thacher and Clarke (2011) said that to prevent, maintain, and protect skeletal health from disease and fractures, the daily vitamin intake should be: for children, especially those with pigmented skin, 400 IU, for adults: 600 IU, and for the elderly over 60 years: 800-2000 IU. However, Holick and Chen (2008) proposed that 800–1000 IU per day is the dose that may help to maintain full health benefits in both adulthood and childhood, and they include postmenopausal women in this group. The

National Osteoporosis Foundation (2014) stated that, in general, an adult's requirements for vitamin D varies, but 400-800 IU/day is an appropriate dose for most adults under 50 years old, and 800-1,000 IU/day is sufficient for those over 50 years old. The Institute of Medicine (2010) published that 600 IU/day is the recommended level for all age groups except for one-year-old infants and younger.

Similarly, upper-intake levels of vitamin D are still debatable because of reasons of caution and safety, as well as the risk of toxicity. The Institute of Medicine (2010), the Food and Nutrition Board (2011), and the US National Institutes of Health (2014) specified that 4000 IU/d is a safe and adequate upper-intake level (UL) for most adults. The European Commission Scientific Committee (2002) recommended 2000 IU/d, which is half of the vitamin D UL that was stated by the Food and Nutrition Board and US National Institutes of Health. The Expert Group on Vitamins and Minerals in the UK (2003) were even more cautious, and stated 1000 IU/d as the vitamin's UL.

Clinically, there are still no approved techniques that can be used to treat severe vitamin D deficiency (Kennel et al., 2010a). Nonetheless, Pepper (2009) detailed multi-strategies, which can be used to treat vitamin D deficiency. Patients suffering from a mild vitamin D deficiency can be treated with different methods and various doses. Despite the difference in the treatment approaches used clinically, patients who are diagnosed with hypovitaminosis D are most frequently treated with a high dose of vitamin D, which is identified as a "loading dose" for a period of time (Kennel et al., 2010a). One example of a "loading dose" could be 50,000 IU of the vitamin per week for 2-3 months, or oral vitamin D 50,000 IU 3 times a week for a month (Pepper 2009). However, some clinicians have used higher doses of vitamin D, such as 60,000, IU for severe deficiency (Kennel et al., 2010a).

2.6.3 The symptoms of vitamin D deficiency

Symptoms are normally used as indicators to locate the cause. However, in the case of vitamin D deficiency, the warning signs can remain unclear. The symptoms of vitamin D deficiency may be common to other conditions or non-specific, such as general fatigue and pains (The Vitamin D Council 2015a). Alternatively, patients may not show any symptoms at all (The Vitamin D Council 2015a). A lack of vitamin D in adults and children can present as muscle and joint pain, especially in the back, feet, thighs, ribs, hips, and pelvis (Holick and Chen 2008 ; Pearce and Cheetham 2010). Moreover, low bone density on x-rays indicates possible osteopenia, which normally arises as a result of severe vitamin D

deficiency (Pearce and Cheetham 2010). Parathyroid hormone production and calcium absorption are significantly affected by vitamin D deficiency (Holick and Chen 2008). It has been shown that people who suffer from pain in the muscles and bones should regularly examine their vitamin D levels because vitamin D deficiency is often misdiagnosed as illnesses with similar symptoms (Kennel et al., 2010a). Additionally, many studies have detected vitamin D deficiency in patients with aches, muscles weakness and atonality with ratios, which can reach as high as 90% (Kennel et al., 2010a).

2.7 Vitamin D measurement

In the case of vitamin D deficiency, calcium absorption in the intestine will decline once the calcium receptor in the parathyroid glands recognises that the secretion and production of the parathyroid hormone (PTH) has risen. An increase in PTH will help to regulate calcium metabolism (Holick 2009 ; Thacher and Clarke 2011) by:

- Increasing calcium reabsorption in the kidney
- Increasing calcium secretion from the skeleton
- Increasing production of the bio-active form of vitamin D (1,25(OH)2D) in the body.

As a consequence, due to the natural increase in the production of bio-active vitamin D, the 1,25(OH)2D measurement is not an efficient method for determining the actual vitamin D status within the body, as 1,25(OH)2 D levels can remain regular or high even in patients with vitamin D deficiency.

Nonetheless, there are several different alternative laboratory techniques that are used for assessing vitamin D levels; each of which could be a good indication of vitamin D status in the body (Clements et al., 1992 ; Lips 2001 ; Thacher and Clarke 2011). These include: 25(OH)D_3 in serum; low 24-hour urine calcium excretion; high parathyroid hormone levels; high total or bone alkaline phosphatase levels; low serum calcium; and the serum phosphorus level. To present a conclusive evaluation of the vitamin D storage level and confirm the individual's actual level or deficiency, all these methods need to be incorporated and connected to indicate the true vitamin D status (Kennel et al., 2010b)

However, the 25(OH)D_3 level represents both the vitamin D contribution synthesised in skin, and that obtained from dietary consumption. Thus, this study chose to measure 25(OH)D_3 in serum. A fasting serum 25(OH)D_3 measurement is more convenient and

common in comparison to other methods (Millen and Bodnar 2008 ; Holick 2009 ; Kennel et al., 2010a).

On the other hand, vitamin D measurement is rather challenging because of the nature of 25(OH) D as a fat-soluble vitamin and the vitamin's ability to attach strongly to vitamin D binding protein (DBP) (Roth et al., 2008). However, a number of well-recognised methods for measuring 25(OH)D levels were reported in the literature such as protein-binding assays, radioimmunoassays, high performance liquid chromatography (HPLC), and, more recently, Liquid Chromatography-tandem Mass Spectrometry (LC–MS/MS) (Harrison et al., 2009 ; Farrell et al., 2012 ; Sadat-Ali et al., 2014).

This research applies the methods of HPLC assay, and LC-MS/MS assay. LC-MS/MS was compared and tested in different studies, most agreeing it to be the definitive standard for measuring 25(OH)D levels (Farrell et al., 2012 ; Moon et al., 2012 ; Sadat-Ali et al., 2014). Roth et al. (2008) compared the six methods best known for vitamin D measurements. The study evaluated LC-MS/MS and HPLC methods as the best. Both methods were able to release the vitamin from DBP by extracting the vitamin chemically first before injecting the compound on to the chromatography system. Then, 25(OH)D is quantified by mass spectrometry in LC-MS/MS or UV detection in HPLC.

However, despite the accuracy of the assay, the LC-MS/MS assay is very expensive and the software requires highly trained operators (van den Ouweland et al., 2010 ; Farrell et al., 2012 ; Sadat-Ali et al., 2014).

2.8 Factors that influence the vitamin D level

2.8.1 General information

There are various factors that influence vitamin D levels in the human body such as age, dietary habits, weight, sun exposure and other factors, all of which have been stated in detail previously. Thus, it was necessary to comprehend the reasons, which may dominate, affect and alter the vitamin levels (both to increase or decrease) in the participant groups. Millen and Bodnar (2008) noted that the factors which can influence and control vitamin D levels were different from one study to another and could be predicted depending on the demographic of study groups, and certain contextual factors.

In the current study, an attempt was made to establish a number of facts about each participant, including age, BMI, skin colour, sun exposure, and vitamin D intake.

A questionnaire is the method most commonly implemented to collect this type of information (Guthrie 2010). A closed response questionnaire, which does not permit a participant to expand on their answer beyond the information required, was chosen to achieve the research goals because such questionnaires are considered to be good tools for gathering factual information regarding a large population within a limited time scale (Denscombe and Ebrary 2010).

2.8.2 Sun exposure

Vitamin D is limited in food sources, which means that the greatest natural source of vitamin D comes from sun exposure. Hence, in order to obtain accurate results as to the vitamin D status of individuals, sunlight exposure must be estimated alongside monitoring the intake of food. To assess sunlight exposure, a self-reporting sunlight exposure questionnaire is commonly used to estimate an individual's routine sun exposure (Barger-Lux and Heaney 2002 ; Macdonald et al., 2008 ; Gould et al., 2014). The general estimation of a country's latitude is not sufficient to determine the potential of the sunlight reaching the earth's surface for stimulating vitamin D production, nor is it an effective method of representing individual habits in relation to sun exposure (Millen and Bodnar 2008). Similarly, individual sun exposure self-assessment has been used for other forms of research such as skin cancer and eye research (Astner and Anderson 2004 ; McCarty 2008).

Even though there are no validated or standard questionnaires available for sun exposure self-assessment studies, researchers generally design and create newly adapted methods that specifically function for their research's aims (McCarty 2008). Previous published papers have indicated that survey questions need to include the following areas to help identify personal sun exposure: hours outdoors, clothing, sun screening, sunbathing, the weather and time on holiday (Macdonald et al., 2008 ; McCarty 2008 ; Gould et al., 2014).

The questionnaire method is low cost in comparison to serum 25(OH)D testing. However, it is not a very reliable method when utilised to test vitamin D levels without any confirmatory method. This has been shown in a previous study, where the sunlight questionnaire provided inaccurate estimations of ultraviolet radiation from sunlight, and unfortunately, thus provided an inadequate estimation of vitamin D levels (McCarty 2008).

Most studies report that skin reactions to sunburn are different depending upon skin pigmentation. There are various methods to identify types of skin colour such as verbal self-reporting, observation, ultraviolet-filtered photographs, and spectrophotometry readings (Daniel et al., 2009). Daniel et al. (2009) point out that self-reporting assessment is considered to be the most common method used to assess skin colour, damage, and correlated activities at cutaneous cancer research facilities. It has also been used in vitamin D studies to measure UVB damage (Armas et al., 2007). Moreover, this technique is commonly used to approximate UV levels, laser treatment, PUVA treatment, and some other cosmetic dermatology treatments (Astner and Anderson 2004 ; Sachdeva 2009). This common usage is related to the simplicity and cost-effectiveness of the technique. Fitzpatrick's classification of skin type is frequently used to assess skin sensitivity to sunlight (Pichon et al., 2010). Originally, the scale divided fair skin into four types (I, II, III, IV) depending on the degree of sunburn or suntan that the person defined themselves as having. Additionally, two types, V and VI, were added to Fitzpatrick's scale later to represent the darker skins. However, Pichon et al. (2010) argued that dark skinned people do not often use the terms sunburn or suntan; they often only define these conditions if their skin becomes significantly darker, itches, flakes, or becomes inflamed. Nevertheless, the Fitzpatrick Scale is considered to be the standard method for the self-reporting of skin colour (Sachdeva 2009). Its categories are as follows:

- Type I (Pale white): blonde or red hair, blue eyes, freckles — Always burns, never tans.
- Type II (White): fair, blonde or red hair, blue, green or hazel eyes — Usually burns, tans minimally.
- Type III (Cream white): fair with any hair or eye colour, quite common — Sometimes mild burn, tans uniformly.
- Type IV (Moderate brown): typical Mediterranean skin tone — Rarely burns, always tans well.
- Type V (Dark brown): Middle Eastern skin types — Very rarely burns, tans very easily.
- Type VI (Black) — Never burns, tans very easily.

Table 4 Determination of Fitzpatrick skin phototypes (He *et al.,* 2014)

Fitzpatrick Skin Phototype	Sunburn Tendency	Suntan Tendency

I	Always burns easily	Never tans
II	Always burns easily	Tans slightly
III	Burns moderately	Tans gradually
IV	Burns minimally	Tans moderately
V	Rarely burns	Tans profusely
VI	Never burns	Tans profusely

2.9 Measurement of dietary vitamin D intake

The most commonly used forms of dietary assessments are food Frequency Questionnaires (FFQ), food records, which are also known as a food diaries, and, finally, 24 hour food recall (Thompson et al., 2010). There is no gold standard method for assessing food intake; each method has its own advantages and disadvantages, and the capability of each dietary assessment method differs so as to cover a variety of research needs accurately (Thompson et al., 2010). Consequently, by understanding each method's strengths and weaknesses, and the research targets of the assessment, workers will be helped in choosing the "right/appropriate" technique for the specific study.

Furthermore, using and comparing more than one method is also considered to be a good way of validating this kind of assessment (Trabulsi and Schoeller 2001). One of the main objectives of this study has been to analyse the amount of vitamin D in the different foods people consume, to determine how much food of each type people eat, and to use this information to determine vitamin D intake from the diet. In order to do this properly, more than one dietary assessment had to be adopted. Hence, a combination of two methods for the dietary assessment, a three day estimated food diary and FFQ, were used to estimate vitamin D intake in the meals and the meal patterns.

The three day food diary, is an open ended form that allowed subjects to record food at the time they ate it - which should always reduce recording errors by avoiding reliance on the subject's memory (Thompson and Subar 2008). The basis of the technique is simple in that the subjects are able to weigh their food using scales, or assess the quantity using readily available household utensils. Additionally, this technique can be used with large populations, and does not restrict the participants' food choices. The interviewer's bias is not a concern in this type of method (Medical Research Council 2013). Moreover, this technique can generally help to gain detailed information on eating patterns, cooking methods, amounts consumed, brand names and portion sizes, provided it is previously stipulated that the participants should note this information down. On the other hand, the low response rate achieved using this technique is one of the method's biggest drawbacks.

Therefore, it is not advisable to use this method to measure consumption over long periods of time. Although, there was a risk that memory recall in the event of not making notes instantly might be used to record some consumption (Thompson and Subar 2008 ; Medical Research Council 2013).

It should be noted that, in most instances, a three day food record is not sufficient to measure the actual intake of vitamin D. Hence, in this study, an FFQ was used to provide additional data and create confidence in the diary results, even though the FFQ does not provide information on many aspects of dietary intake such as cooking methods and food mixtures in the meals being recalled. Nevertheless, the method is widely used to give an estimation of the typical intake of food and nutrients over long periods of time (Thompson and Byers 1994). This technique normally involves providing individuals with a list of food items from which they are able to identify what they have consumed over a specific period of time. Moreover, the FFQ can assist the researcher in assessing the amounts of general or exact nutrients consumed without monitoring actual eating day by day (Kristal et al. 1992). FFQ food lists can be adapted, modified or created depending on the proposed use (Thompson and Subar 2008).

2.10 Vitamin D status of populations from range of countries– evidence from published data

2.10.1 High latitude countries

Countries with a latitude higher than 40°N have insufficient or non-existent UVB for almost half the year, from mid-autumn to early spring, which would affect the production of vitamin D in the skin (Spiro and Buttriss, 2014). However, a low level of 25(OH)D was reported even in countries with ample sunshine (see Table 5). This table illustrates available published data about 25(OH)D levels from a range of countries. Notably, definitions of the cut-off value of the vitamin D level were different; therefore, the interpretation of vitamin D status may differ.

First, the table presents studies that assessed the vitamin D levels from countries at a high latitude. Webb et al (2010) measured the levels of 25(OH)D_3 of 125 healthy white adults in a study conducted in Manchester north west England. The study defined the level of vitamin D deficiency <12.5 nmol/L, insufficiency <50 nmol/L, sufficiency ≥ 50 and optimal ≥80. High serum levels of vitamin D were common in September 70.8±25.9 nmol/L (marked

as sufficient), whereas low serum levels of the vitamin was common in February 45.6±21.7 nmol/L (marked insufficient). The prevalence of vitamin D deficiency was 5% at the end of winter and 1% in the spring and summer. In Denmark, a study by Thuesen et al. (2012) evaluated annual vitamin D levels of 6146 healthy adults aged 30-60. Sixty-six percent (4056) had vitamin D deficiency (<25 nmol/L) or insufficiency (< 50 nmol/L). The study observed a seasonal effect on vitamin D serum level, which reported the highest levels of the vitamin in September, and the lowest levels of the vitamin in February.

A study in Sweden measured 25(OH)D levels among natives and immigrants (Andersson et al., 2013). The study definition of vitamin D status was as follows: deficiency <25 nmol/L (insufficiency 25–50 nmol/L and optimal levels >50 nmol/L. Of the total participants, 34% (n=21) were vitamin D deficient, 38%(n=23) were vitamin D insufficient and 28%(n=17) had an optimal level of vitamin D. However, vitamin D deficiency and insufficiency were statistically significant between the groups, p-value<0.001, which were more common in immigrant women than in native women. In Russia, a cross-sectional study was conducted in two latitudes (59–61°N) between 2009 and 2013 (Karonova et al., 2016). The study group ages ranged from 7-75 years. Adult residents of latitudes 61°N showed 25(OH)D levels lower (49.6 ± 1.6 nmol/L) than that of the residents of latitude 59°N (54.8 ± 0.7 nmol/L) , p-value < 0.05. Besides, female adults had a statistically significantly lower level (53.9 ± 0.8 nmol/L) than male adults (67.2 ± 2.2 nmol/L), p-value<0.01.

2.10.2 Low latitude countries

Table 5 shows that more studies were conducted in sun-rich countries with a latitude less than 30 °N.

A study conducted in Jeddah in the KSA compared the vitamin D level of pre- and post-menopausal women; 80% (n=934) of the study population had vitamin D levels of <50 nmol/L. The results showed a significant statistical difference among the study groups; the serum 25(OH)D level was 41.54±29.96 and 31.78±23.44 nmol/ for pre-menopausal women and for post-menopausal women respectively, p-value<0.001. The highest average levels of 25 (OH)D were detected after the winter season (April), which was 45.52±14.60 nmol/L, whereas the lowest average levels of 25(OH)D were detected at the end of the summer season (August), which was 25.46±7.68 nmol/L. The seasonal variation of the vitamin D level was statically significant, p-value=0.05.

An Emirati study assessed a 25(OH)D serum level among university students at Abu Dhabi, after the summer. Then a seasonal variation at the 25(OH)D level for females was tested after the winter (Al Anouti et al., 2011). The level of 25(OH)D in October was 27.3±15.7 nmol/L in the male group (n=70) and 20.9±14.9 nmol/L in the female group (n=70), p-value=0.02, whereas in April 25(OH)D the serum level for females (n=70) was 31.3±12.3 nmol/L, which showed an improvement, p-value=0.0005, but still below 50 nmol/L. In general, despite the insufficiency of the vitamin D level of the study group in all seasons, women had a lower vitamin D level than men.

A study from Kuwait measured a 25(OH)D serum level for 300 people between April to October, of which 150 used sunscreen and 150 did not use sunscreen (Al-Mutairi et al., 2012). The study considered a 25(OH)D level ≥75nmol/L as sufficient/optimal, and 25(OH)D level<75 nmol/L as deficient/insufficient. The result showed, 91% (n=136) of sunscreen users had a 25(OH)D level <75 nmol/L, while 76% (n=114) of non-sunscreen users had a 25(OH)D level <75 nmol/L, p-value<0.001. Of these who were vitamin D deficient in both groups, 173 (57.7%) had a vitamin D level ≤ 50 nmol/L. A sufficient level of 25(OH)D (≥75 nmol/L) was found in only 9% (n=14) of those who used sunscreen, and 24% (n=36) of non-sunscreen users, p-value<0.001. According to the study definition of vitamin D deficiency, the majority of the study population was either vitamin D deficient or insufficient, see Table 5.

In Oman a study assessed vitamin D serum level in 41 healthy female adults (Al-Kindi, 2011). The study result showed none of the study population had a sufficient level of vitamin D (≥50 nmol/L) and 49 % (n=20) were vitamin D deficient (< 25 nmol/L). The study had a small sample size, which may not represent the Omani population; however, the results on the vitamin D level were similar to those of other studies conducted on vitamin D levels at a similar latitude.

Table 5 Vitamin D status of populations from range of countries around the world (high latitude countries and low latitude countries) – evidence from published data.

Study	Population Number (age in years)	Country (latitude)	Season	Gender	25(OH)D3 level nmol/L	Study Definition of Vitamin D Status nmol/L
(Webb et al., 2010)	125 (20-60)*	England (52°N)	All seasons	M-F	End summer 70.8±25.9** End winter 45.6±21.7**	Deficiency <12.5 Insufficiency <50 Sufficiency≥ 50 Optimal ≥80
(Thuesen et al., 2012)	6146 (30-60)*	Denmark (56°N)	All seasons	M-F	48(16-100)***	Deficiency <25 Insufficiency < 50 Sufficiency ≥ 50
(Andersson et al., 2013)	61 (18-75)	Sweden (60°N)	Winter & early spring	F	Immigrant 22.2(13.7–28.6)*** Native 51.5 (39.6–66.5)***	Deficiency <25 Insufficiency 25–50 Optimal >50
(Karonova et al., 2016)	1226 (7-75)	Russia (59–61°N)	Not mentioned	M-F	Adults 54.8 ± 0.7 ** Children 46.8 ± 1.6**	Deficiency <50 Insufficiency 50–75 Sufficiency ≥75
(Ardawi et al., 2011)	1172 (20-79)*	KSA (21.2°N)	All seasons	F	35.84±26.86**	Severe deficiency <12.5 Moderate deficiency 12.5-25 Mild-deficiency ≥25 - <50 Insufficiency ≥50- ≤75 Sufficient >75 Optimal >100

Study	Population Number (age in years)	Country (latitude)	Season	Gender	25(OH)D3 level nmol/L	Study Definition of Vitamin D Status nmol/L
(Al Anouti et al., 2011)	278 (20.9±4.3)**	UAE (24°N)	Summer & winter	M-F	23.10±15.50**	Deficiency <25 Sufficiency 50–200 Toxicity >200
(Al-Mutairi et al., 2012)	300 (18-≥50)*	Kuwait (29.3°N)	Spring, summer& autumn	M-F	83.34(250) [+] < 75	Deficiency <50 Insufficiency 50–75 Sufficiency ≥75
(Al-Kindi, 2011)	41 (18-45)*	Oman (21.5°N)	Winter & autumn	F	27.61 ± 5.35 **	Deficiency<50

*(range)
**mean± standard deviation
*** median(range)
[+]% (n)

2.11 Vitamin D deficiency in the UK

Vitamin D deficiency has been proven by considerable research to be a common problem worldwide, and this despite the abundance of the sun in some of the countries where hypovitaminosis is found (Calvo et al., 2005 ; Hyppönen and Power 2007 ; Holick and Chen 2008). Hypovitaminosis is reported to be a common issue in all different age categories in America and Europe (Holick 2004). A study in North America confirmed vitamin D problems to be prevalent in the region in both white and black women (Hanley and Davison 2005). Other studies conducted in four European countries (Denmark, Ireland, Poland, and Finland), which are all positioned in northern Europe, stated that a lack of the vitamin causes general concern, especially during the winter season in adolescents and older women when sunlight is scarce (Burton and Dexter, 2005). In the UK, particularly, a nationwide study, which studied vitamin D status and the affected factors on a total of 7437 white middle-aged British respondents, reported that over half of the population presented vitamin D levels lower than the required level to maintain the full health benefits of the vitamin in all seasons (Hyppönen and Power 2007). The same study claimed that this percentage increased dramatically to reach 90% in the winter and spring seasons, while 16% of the population showed extreme deficiency (Hyppönen and Power 2007).

2.12 The causes of vitamin D deficiency in the UK

Some of the factors that have been reported to affect vitamin levels negatively in the UK are: low vitamin D intakes, indoor lifestyles, detrimental personal dietary habits, obesity and geographic location (Hyppönen and Power, 2007). Consequently, these factors have cost the UK billions of pounds in the past decade (Scarborough et al., 2011). In the UK, people tend to spend more than 8 hours per day seated, which constitutes more than a third of the day (Get Britain Standing 2015). Additionally, a review article attempted to evaluate lifestyles of UK South Asians, which presented the ethnicity and cultural beliefs of South Asian people as potentially promoting the acceptability of sedentary lifestyles in the UK more than other groups in the population (Lucas et al., 2013).

Many studies relating to vitamin D in the UK have shown that vitamin D intake was insufficient for most of the population with no significant difference between white people and people with darker skin colours (Webb et al., 2010 ; Farrar et al., 2011 ; Farrar et al., 2013 ; Kift et al., 2013). However, in general, vitamin D intake for Caucasians was slightly higher than for other ethnic groups such as Asians (Kift et al., 2013).

Obesity is widely reported as a risk factor for many human diseases (Allender and Rayner 2007). The Body Mass Index (BMI) is considered a good indication of body weight and obesity and studies have shown a negative relationship between high BMI and vitamin D sufficiency (Wortsman et al., 2000 ; Macdonald et al., 2008). A study by Wortsman et al. (2000) demonstrated an inverse relationship between obesity and vitamin D bioactivity in the body. In obese people, body fat may constrain vitamin D, which may reduce vitamin bioactivity and bioavailability in the body (Holick 2007). Obesity and obesity-related diseases cost the UK five billion pounds in 2006 and 2007 (Scarborough et al., 2011). Indeed, obesity in the UK in the general population is significant, with a higher ratio among women (Rennie and Jebb 2005). In England, the obesity ratio in women increased from 16% in 1993 to 24% in 2013 (Health and Social Care Information Centre 2013). Furthermore, Black and Asian women have been reported as one of the most affected groups (Rennie and Jebb 2005).

The geographic location of a country affects the amount of UVB photons arriving at the earth's surface (Holick 2007). The UK is located at above latitude 50–60° N, which reduces UVB solar in some seasons to very low or zero (Holick 2007 ; Pearce and Cheetham 2010). In the UK, it is difficult for the body to produce vitamin D for nearly 6 months every year from October to April, so people should normally rely on other sources of the vitamin to prevent vitamin D insufficiency (Pearce and Cheetham 2010). Indeed, a study by Macdonald et al. (2011) confirmed the impact of location on vitamin status, as it compared two groups of healthy Caucasian women living in the UK at two different latitudes: 57° N Scotland and 51° N, the South of England. The results revealed that women who resided at the higher latitude (57° N) had a lower vitamin D status than the other group.

2.12.1 Groups at risk in the UK

A global review highlighted the observation that in general older people in Europe, especially those who are housebound, and ethnic groups who come from non-European backgrounds such as the Middle East and North Africa, are the most common sufferers of vitamin D deficiency (Mithal et al., 2009). Separately, the UK Department of Health (2012), which coincided with the common findings from that previous global review, specified that the most prevalent groups to suffer from insufficient vitamin D levels are:

- Pregnant and breastfeeding women.
- Infants and pre-school children.

- The elderly (over 65 years old).
- People who cover their full body for cultural reasons.
- People with an indoor lifestyle.
- People with dark skin (South Asian, African, and African-Caribbean).

2.12.2 Vitamin D and ethnicity and culture in the UK

The UK is a country that has a very multicultural or diverse population, due to different waves of extended or permanent migration over the last 40 to 50 years (Home Office, 2014). For example, the nations of China, Eritrea, Syria, Iran, India and Somalia have all presented large groups of people who are reported to have acquired long term entry permits for a year or more (Home Office, 2014). Importantly, these specific nationalities practise different customs that result in avoiding the sun - for instance, dark skin tone, different clothing styles, conservative cultures, and simply coming from different places. Moreover, emigrating from sunny countries to a country of northern latitude, such the UK, without changing from a lifestyle, which involves avoiding direct sunlight exposure, could cause, or worsen, vitamin D deficiency (Farrar et al., 2011).

Dark-skinned people living in the UK, especially South Asians, have been documented as being at a greater risk of developing vitamin D deficiency (27%) than other migrant groups, compared to the white population (10%) (Farrar et al., 2011). Moreover, a study relating to non-European pregnant women who live in the UK showed 50% (n=80) presented serum vitamin D levels less than 8 ng/mL, while only 25% of native pregnant English women presented levels lower than 8 ng/mL (Datta et al., 2002). Dark skin needs more time than white skin to produce sufficient vitamin D to meet the body's requirements (Farrar et al., 2013). Additionally, genetic factors, dietary habits and lifestyle also negatively influence the vitamin D status of this group in the UK (Farrar et al., 2013).

Asian women and their infants were first recognised as one of the groups that are at risk of vitamin D deficiency in the UK in the 1970's (Ford et al., 1972 ; Ford et al., 1976). Since that initial recognition, the problem in this ethnic group has been reduced, as education has led to increased awareness of the risk and the availability of free vitamin D supplements for those at risk. However, in the mid-nineties, cases of rickets reappeared in a different minority group, black-African babies, and the re-emergence of cases of vitamin D deficiency in infants belonging to Asian mothers who were born in the UK (Shaw and Pal 2002). This may show ethnicity is still an influence on behaviour and diet, resulting in a lack of UVB

radiation exposure in higher latitude countries such as the UK even in second generation immigrants.

2.12.3 Vitamin D sources in the UK

2.12.3.1 Sunlight

The recommendation of the UK Department of Health for sufficient vitamin D levels throughout the year is casual exposure to summer sunlight. However, their statement was imprecise for the winter seasons. The Department of Health (2014b)stated that direct casual exposure to summer sun is adequate to produce sufficient levels of the vitamin and will not harm most people, and added that people living in the UK in the winter may not cover their need for the vitamin. People with a naturally dark skin colour can tolerate higher exposure to sunlight than those with fair skin. This leads to two results: pigmented skin is naturally more able to resist the negative effects of long exposure to the sun. However, this leads to the second result that this type of skin colour needs longer to produce vitamin D (Kift et al., 2013).

Two studies tested the recommendations of the UK Department of Health (Rhodes et al., 2010 ; Farrar et al., 2011). Participants had a UV treatment three times a week for 6 weeks. Each time, they received equivalent to 9-16 minutes of casual exposure to summer sunlight at the latitude of 53.5°N (see Table 6). Both studies resulted in substantial increases in the serum 25(OH)D level of the participants. Rhodes et al. (2010) claimed that 90% of the study's participants reached the "adequate" level of 20 ng ml of 25(OH)D, and nearly 30% reached the optimal level of 32 ng ml of 25(OH)D or higher, and there was no deficiency found among the participants. The study by Farrar et al. (2011) revealed that, regardless of a general increase in vitamin D levels in the South Asian volunteers, no one reached the "adequate" level of 20ng ml of 25(OH)D. More importantly, 25% still showed extreme deficiency levels of 25(OH)D of 5ng/ml. Consequently, even while following the UK Department of Health's recommendations, the South Asian population did not produce sufficient vitamin D levels, unlike the white population. This suggests that recommendations need to take skin colour into account in order to ensure that all ethnic groups achieve adequate levels.

Table 6 Comparing 25(OH)D level in the three study, between South Asian population and white population in the UK.

Study	Number	Participant Profile	Result
(Rhodes *et al.,* 2010)	109	• White Caucasian • 20–60 years old • Female/Male • Skin type I–IV	• The mean of 25(OH)D concentration value increased significantly. • Low vitamin D intake. • 90% of the population reached sufficient levels. • 26.2% reached optimal levels. • 0% of deficiency level of 5 ng ml.
(Farrar *et al.,* 2011)	15	• South Asian • 20–60 years old • Female/Male • Skin type V	• The mean of 25(OH)D concentration value increased significantly. • Law vitamin D intake. • 0% sufficient level of 20ng ml of 25(OH)D. • 25% had extreme deficiency levels of 25(OH)D of 5 ng ml.
(Farrar *et al.,* 2013)	51	• South Asian • 20–60 years old • Female/Male • Skin type V	• The mean of 25(OH)D concentration value increased significantly. • 25% of population reached sufficient levels of the vitamin at 20 ng ml. • All population members were above extreme deficiency levels of 25(OH)D of 5 ng ml.

Farrar et al. (2013) repeated previous experiments, and recruited only South Asian volunteers. However, the UV irradiation course was increased to three times more than in the two previous studies (see Table 6). UV imitation was equivalent to 15–90 minutes of clear sky, midday, summer sunlight, with 35% of the skin's surface exposed. The 25(OH)D concentration level showed a significant increase in all the participants. However, just 25% of the study population reached the "adequate" level of the vitamin at 20ng ml. In addition, a comparison of the starting baseline concentration of 25(OH)D in the three studies showed that the majority of the South Asian study population started with a concentration of 25(OH)D lower than that of the white study population.

2.12.3.2 Foods

Food can be rich in vitamin D in two ways: it can be natural or fortified. Natural foods that are rich in vitamin D are limited in variety – for example, oily fish such as salmon, animal livers, eggs, and mushrooms (Larson-Meyer and Willis 2010).

To enhance food products with nutrients, the food industry normally utilises food fortification. In the UK, a Department of Health leaflet stated that strengthening foods with vitamin D is optional for the food industry (Department of Health 2014a). When fortification does take place, according to the UK Department of Health, vitamins and minerals should be added to the food in small quantities, though the quantities have not been identified. In fact, choosing quantities for fortification was left to the judgment of each manufacturer.

In some countries, such as Canada and the USA, milk must be fortified with vitamin D, whereas, in the UK, vitamin D fortification is not compulsory for milk or any other food products, except margarine (Hyppönen and Power 2007). However, products that the law may allow to be fortified with vitamin D in the UK are common food products as mentioned by The Department of Health such as breakfast cereals, milk, butter and margarine, soya, and some dairy products. Of interest is that formula milk for babies is the only product that the UK Department of Health cited as needing to be fortified with vitamin D (Department of Health 2012 ; Department of Health 2014b).

The general vitamin D intake in the UK is half the general vitamin intake of the USA and Canada (Calvo et al., 2005 ; Hyppönen and Power 2007). This relates to the regulation differences in food fortification of vitamin D between these countries. Essentially, there is an obligation to fortify in the USA and Canada, but not in the UK. The USA's food has to be fortified and vitamin D therefore cannot be avoided, so the inevitable result is a higher intake of vitamin D (Calvo et al., 2005 ; Hyppönen and Power 2007).

The UK government's recommendation for daily vitamin D intake for healthy adults is not stated (Macdonald et al., 2011 ; Department of Health 2012 ; Department of Health 2014b). The only daily recommendation for vitamin D published by the government is intended to target people at risk of vitamin D deficiency. The recommended dose was 10 µg per day for all groups at risk of vitamin D deficiency, except for preschool children and babies, whose recommendation was set at 7-8.5 µg/day (see Table 7).

Table 7 People at risk of vitamin D deficiency and their daily recommended doses (Department of Health 2014b).

People at risk of vitamin D deficiency	Daily supplement
All pregnant and breastfeeding women	10 µg/day

• All babies and young children aged 6 months to 5 years. • Babies who are fed infant formula should not need a vitamin D supplement until they are having less than 500ml (about a pint) of infant formula a day. These products are fortified with vitamins and minerals and there is a risk of high intakes if they are consumed together. • Breastfed infants should be given a vitamin D supplement from one month of age if the mother did not take vitamin D supplements throughout her pregnancy.	7 to 8.5 µg/day
People aged 65 years and over (particularly those living in institutions or who are not regularly exposed to sunlight).	10 µg/day
People who are not exposed to much sun (e.g., housebound individuals and those who cover their skin for cultural reasons).	10 µg/day

2.13 Vitamin D deficiency in the KSA

Middle Eastern countries are positioned at a latitude of 15°-36°N, which means plentiful sunlight most of the year. Nevertheless, a high presence of diseases related to vitamin D deficiency has been reported in these areas (Mithal et al., 2009). In fact, Middle Eastern and African countries registered the highest number of cases of rickets worldwide (Mithal et al., 2009). Many studies showed that vitamin D deficiency prevalence in these areas was common in all age groups and in both genders (Dawodu et al., 1998 ; Al-Daghri et al., 2012 ; Elshafie et al., 2012). A study conducted in the UAE in the late nineties compared three groups of UAE residents: non-Gulf Arabs, Gulf Arabs, and, finally, Europeans (Dawodu et al., 1998). The study supported the finding that both Arab groups showed a mean of very low levels of the vitamin in serum, with a mean range 8.6 ng/ml – 12.6 ng/ml. By comparison, the European group had an adequate level of 64.3 ng/ml of vitamin D.

One of the early studies in the Gulf countries that studied vitamin D in healthy adults was conducted in the KSA (Sedrani et al., 1983 ; Fonseca et al., 1984). Both studies showed that there was a high prevalence of vitamin D deficiency among all groups of the population, particularly in women. A different study conducted in the KSA during the same period studied vitamin D levels in pregnant Saudi women and compared their findings with results of those researching pregnant Asian women who were living in the UK (Serenius et al., 1984). The research showed that all pregnant Saudi women were deficient in vitamin D, and more than 40% of them had undetectable or very low vitamin D. The results showed that the pregnant Saudi women had lower levels still than pregnant Asian women in the UK, regardless of the fact that both groups were using vitamin D supplements. Unfortunately, few studies have been published about healthy Saudi women's vitamin D status, and the intake of the vitamin since the problem began to be recognised and the first

studies were published in the 1980s. Nonetheless, the dominant results from all studies have demonstrated a clear prevalence of vitamin D deficiency among healthy women in the KSA.

2.13.1 The causes of vitamin D deficiency in the KSA

Some of the reported causes of vitamin D deficiency in the KSA are: indoor lifestyles and low physical activity, poor dietary habits, obesity, clothing style, and housing conditions (Al-Othman et al., 2012 ; Ardawi et al., 2012 ; Elshafie et al., 2012). Strong indicators from many studies pointed to low or limited activity and sun exposure in the lifestyles of the Saudi population (Al-Othman et al., 2012 ; Al-Daghri et al., 2012). Moreover, limited sun exposure and an indoor lifestyle were associated with the weather and culture in the KSA (Al-Othman et al., 2012). The weather in the KSA is sunny most of the year; however, the temperature can reach 50^{o}C in the summer seasons (Presidency of Meteorology and Environment 2015). Such high temperatures are best avoided if possible, and, thus, this constrains outdoor life and exposure to the sun amongst the Saudi population and it is this that leads to the associated deficiencies and lifestyle disorders, e.g., obesity as a result of a sedentary lifestyle (Ardawi et al., 2012). This finding is confirmed by a study conducted in Riyadh, the capital of the KSA, which compared vitamin D levels in cold and hot months and reported that, in the summer months, the baseline level of vitamin D level in participants was lower than in winter months, and participants' outdoor activity levels in hotter periods were kept to a minimum because of the high heat (Al-Daghri et al. 2012).

Dietary habits in the KSA have exhibited dramatic changes over recent decades (Hijazi et al., 2000). Daily consumption of whole grain products, fruit, vegetables, and dairy products such as milk and eggs, have gone down (Washi and Ageib 2010). Instead, consumption of high fat foods, such as fast food and western-style food, has gradually become more common. Eating patterns have changed too, as Saudi people tend to consume more take-out meals and eat in restaurants more often (Hijazi et al., 2000 ; Washi and Ageib 2010). In association with these dietary changes, it has been reported that there has been an increase in obesity rates among all population groups, a reduction in physical activity, and growth in the number of Saudis with chronic diseases (AlQuaiz et al., 2014).

Outdoor clothing styles in the KSA are mostly traditional and modest. Men commonly wear long white robes with long sleeves (Thobes) and have their head covered with a scarf or turban (Ghutra). Women usually wear long black dresses with long sleeves (Abayas) and

cover their heads with a scarf (Tarha). The face may be covered with a veil (Niqab). These clothing styles could be perceived as detrimental to health, as they present an obstacle to obtaining adequate vitamin D from exposure to the sun (Merewood et al. 2010). Interestingly, a study conducted in Jordan compared vitamin D levels in three groups of women (Mishal 2001). The first group was women who wore a hijab, which covers the entire body except the face and hands, the second group was women who wore a niqab, which covers the entire body including the face, and may also include the hands, and the third group was women who wore western-style clothing. The study reported that inadequate levels of vitamin D in all seasons reached over 50% in all groups. The most affected group were the women using the niqab, who had over 80% insufficiency in all seasons, followed by women wearing a hijab, who had over 50% insufficiency for vitamin D levels in all seasons. In addition, it was found that both covered groups had lower vitamin levels compared to the third group of women who adopted western-style clothing. However, there was no statistical difference between the three groups.

In addition, an early study of vitamin D deficiency in the Saudi population conducted in the capital of the KSA ruled out clothing style as a factor in vitamin D deficiency (Sedrani et al., 1983). The study explained this by emphasizing the results which demonstrated that young female adults, who were normally covered up and veiled had serum vitamin D which ranged between 4 to 11.5 ng/ml, whereas vitamin D levels in male subjects were between 3.1 to 8.4 ng/ml and levels for the elderly were between 1.3 to 3.6 ng/ml. Instead, the study put forward other causes for the vitamin deficiency such as limited exposure to the sun, low vitamin D intakes, and dusty weather. However, in contrast, a study conducted in 1984 reported that vitamin D insufficiency spread due to the Saudi clothing style, living space with no outdoor areas, and low vitamin intakes (Fonseca et al., 1984).

2.13.2 Groups at risk in the KSA

Recently, it has been reported that over 80% of the Saudi population suffers from vitamin D insufficiency, especially in the hot months (Al Faraj and Al Mutairi 2003; Siddiqui and Kamfar 2007; Ardawi et al., 2012). Previous research has shown the occurrence of vitamin D deficiency among large numbers of Saudi people. Some of the categories in the Saudi population that were earmarked as at risk of vitamin D deficiency were pregnant and breastfeeding women, children, infants, adolescents, obese people, people with chronic diseases, and elderly people, as well as people with low physical activity and indoor

lifestyles (Serenius et al., 1984 ; Al-Ali et al., 2009 ; Bin-Abbas et al., 2011 ; Al-Othman et al., 2012 ; Elshafie et al., 2012 ; AlQuaiz et al., 2014).

The Saudi Arabian Ministry of Health statements regarding vitamin D are fairly broad and unidentified. However, the Ministry of Health in the KSA has made multiple statements about osteoporosis and people who are at risk of osteoporosis (Kingdom of Saudi Arabia Ministry of Health Portal 2014b; Kingdom of Saudi Arabia Ministry of Health Portal 2012). Osteoporosis is one of the most common complications of severe vitamin D deficiency.

A statement in 2012 reported individuals at risk of osteoporosis as including those with a family history of the disease, people with low BMI, those with malnutrition, smokers, long-term users of steroids medication, those with limited sun exposure, and post-menopausal women (Kingdom of Saudi Arabia Ministry of Health Portal 2012). Moreover, the statement highlighted that sun exposure is important for avoiding osteoporosis, which will stimulate the production of vitamin D in the body to increase calcium absorption. The statement specified two groups who might face difficulties in producing vitamin D – the elderly, and people with limited sun exposure.

Another statement concerning osteoporosis from 2014 reported vitamin D as a controllable risk factor, and then briefly referred to the elderly with indoor lifestyles, and the winter season as issues that could limit vitamin D production (Kingdom of Saudi Arabia Ministry of Health Portal 2014b).

2.13.3 Vitamin D and women in the KSA

A review study highlighted a general increase in chronic diseases in women which was greater than that in men in the KSA (AlQuaiz et al., 2014). Moreover, many studies have reported that Saudi women at different stages of life were commonly suffering from vitamin D insufficiency problems, although, in many cases, this was not recognised due to an absence of symptoms (Serenius et al. 1984 ; Al Faraj and Al Mutairi 2003 ; Siddiqui and Kamfar 2007 ; Siddiqui 2010). Another study reported that 79% of Saudi women had a severe deficiency of vitamin D, while just over 20% had insufficiency or mild deficiency levels of the vitamin (Al-Mogbel 2012).

Saudi females who study or work would normally go out in the daytime, although, due to cultural and religious beliefs, they should always cover up (Al-Othman et al. 2012). However, a study of Saudi women's knowledge and practises in regards to vitamin D

deficiency showed that Saudi women choose to avoid the sun for numerous reasons such as cosmetic choice, tradition, and because of small living spaces (Christie and Mason 2011). In addition to these factors, there is also a prevalent lack of basic knowledge about the importance of sunlight exposure and vitamin D. For example, the research reported that large numbers of participants mistakenly believed that they did expose sufficient skin to the sun, or believed that exposing the skin to the sunlight in the presence of a barrier is sufficient to produce the vitamin (Christie and Mason 2011).

In addition, most of these studies showed that a low vitamin D intake was very common in the diet of the Saudi population all year round. Saudi women consumed products that were very low in vitamin D. In particular, milk was one of the least consumed products among women (Elshafie et al., 2012).

2.13.4 Sources of vitamin D in the KSA

Sunlight and diet are the main sources of vitamin D in the KSA, as for everywhere globally.

2.13.4.1 Sunlight

Noticeably, most Saudi studies would normally use available vitamin D recommendations for sun exposure and vitamin D supplements from previous literature, or USA guidelines (Hannan et al., 1984 ; Siddiqui and Kamfar 2007 ; Ali 2010 ; Al-Daghri et al., 2012). In fact, the commonly recommended dose of sunlight from most of the previous studies is not that different from the UK's general recommendation, which is casual exposure to direct sunlight twice a week for around 15 minutes. However, Mithal et al. (2009) argued for the inefficacy of current vitamin D current recommendations, and stated that this recommendation is failing to give desirable outcomes (Mithal et al., 2009).

The Ministry of Health in the KSA advised people to expose themselves to sunlight for fifteen minutes at peak times in order to obtain an adequate level of vitamin D (Kingdom of Saudi Arabia Ministry of Health Portal, 2014b). Nonetheless, the statement was too general and lacking in detail, as there was no reference to the defined peak time, recommended clothing, or the percentage of skin that should be exposed to sunlight. In addition, differentiations between the effects in individual skin tone were not addressed. However, the statement did mention that older people with perpetual indoor lifestyles, and people in the cold season need to rely on vitamin D supplements.

2.13.4.2 Foods

The website of the Saudi Arabian Health Ministry does not feature any specific statements regarding vitamin D. Meanwhile, however, the Saudi Arabia Health Ministry has twice released facts and instruction statements for osteoporosis in 2012 and 2014 (Kingdom of Saudi Arabia Ministry of Health Portal 20124b ; Kingdom of Saudi Arabia Ministry of Health Portal 2012). Within the statements, vitamin D was mentioned as an influential factor for osteoporosis, and vitamin D and sun exposure was explained very briefly within the statements. However, a daily dose of vitamin D intake was not mentioned at all. Indeed, the information stated that the use of vitamin D supplements should be for people with sedentary lifestyles, and elderly people and in the cold season. The Health Ministry in the KSA referred very briefly to milk as a good source of vitamin D in a separate statement called "Uses of Milk" (Kingdom of Saudi Arabia Ministry of Health Portal 2012b). In addition, another recent online statement from the Kingdom of Saudi Ministry of Health

Portal (2014a) called “Nutritional Value of Milk” directly referred to vitamin D daily intake. In the reference section, the amount of daily vitamin D intake by age groups, 15 µg/day, was the recommended amount for all ages except infants of 12 months and younger - their daily recommended amount of the vitamin was 10µg.

Conversely, the daily-recommended intake of vitamin D in several Saudi national studies seems to rely on the USA guidelines (Ali 2010 ; Al-Mogbel 2012 ; Al-Daghri et al., 2012). The commonly recommended daily dose of vitamin D was 5-15 µg (Hathcock et al., 2007). Indeed, the use of existing and common international guidelines such as those from the USA, the United Kingdom, and Canada was deemed acceptable by the Saudi Ministry of Health for Saudi dietary guidelines (Kingdom of Saudi Arabia Ministry of Health Portal 2012a).

2.14 Summary of the literature review

This chapter reviewed the literature surrounding the topic of vitamin D deficiency. The review began with an outline of significant types of vitamin D in the human body, vitamin D production, metabolism and regulation. A large proportion of this review explored natural sources of vitamin D, matters exacerbating the problems of vitamin D inadequacy, symptoms of vitamin D deficiency, and the common recommendations for vitamin D. The review concluded by focussing on vitamin D deficiency in two countries - the UK and the KSA. This section included a discussion of the causes of deficiency, people at risk of vitamin D deficiency, and, finally, vitamin D guidelines in both countries. However, the literature indicates that vitamin D status is poor worldwide. The KSA and the UK both have vitamin D deficiency as a common problem in the general population. Thus, the current study chooses to investigate vitamin D status, intakes, and lifestyles in women sharing similar ethics and religions, comparing them in two countries.

3 Methodology

This chapter outlines the methodological considerations applying to this study. It first considers the collection of secondary data, then the primary data, followed by the range of designs for the research methods. Finally, the ethics and research validation are discussed.

3.1 Research design

This study was based on new observational work, and its concept was to observe, measure and quantify individual factors which influence vitamin D, including age, sun exposure, and vitamin D intake. Two "covered" groups from different location were compared using individuals who held the same faith and followed the same rules of dress, and had similar cultural values. The second comparison were between two groups lived in the same location and have different clothing styles.

The investigative research was formulated and structured with the following variables in mind: serum vitamin D levels, and factors influencing vitamin levels in serum, including sunlight routine, exposure time to sunlight, geography, season, clothing, age, BMI, pigmentation, and vitamin D intake. Subsequently, in order to achieve all the aims of the research, various specific tools were implemented.

Sunlight exposure questionnaire and two food intake assessments were used, and were combined with the serum data to provide a full evaluation of vitamin D. Moreover, these questionnaires provided an insight into the participants' dietary habits and sunlight exposure routines.

3.2 Participants recruitment criteria

This study was designed to measure and compare the vitamin D levels in women who have adopted a covered way of dressing, as well as to analyse their vitamin D intake through food. It is specifically Muslim women who form the majority of those who follow this mode of dress. Healthy adult females were recruited on a numerically equal basis from both Manchester in the UK and Makkah, which is in the western region of the KSA. This set incorporated two groups of covered women in different location (Saudi covered women and British covered women) to be studied as part of this project. Additional comparison between two groups of women in the same location and latitude with different dressing style (UK covered women and UK uncovered women) was included.

The KSA participants have access to ample sunlight, especially UVB, whereas people in the UK are subject to extremely low level of UVB for approximately 6 months each year. Hence, expected vitamin D levels would become reduced based on this variable. Additionally, to confirm the theory that sunlight deficiency is the detrimental factor to levels of vitamin D, a third group of "uncovered" women in the UK were also tested. This 'uncovered' was believed to be a factor that may show that the UK women, who were not completely covered, would have higher levels of vitamin D than those who were. Thus, an analysis would be put forward to investigate to what extent clothing and exposure had an effect on vitamin D levels.

3.3 Sample size

There are various factors that determine a research sample size such as: the research purpose, the total population size, and methods used (Guthrie 2010). Guthrie (2010) has stated that it is not necessary to increase a sample size to more than 384 for survey-based research when a population is 1 million people or greater. Nevertheless, other scholars believe that an exploratory research sample size need not be as large as 384 participants, although many sources claim that the larger the sample population, the smaller the sampling error margin (Cottrell and McKenzie 2005 ; Guthrie 2010). At the time of this study, the general Muslim population in the UK was just over 2.7 million, which is about 5% of the entire population (Kern 2013; Office For National Statistics 2014), whereas, the population of the KSA is greater than 27 million.

Each group from a population size exceeds the 1 million figure, thus the expected sample size for each group should be 384 (which in total is 1152). However, because of the time limit and financial resources, the study had a total of 1152 participants, which is considered a very large sample size for this study. Therefore, the three groups were treated as one population and the targeted sample size was 384 in total for all three groups (128 for each group). Notably, a range of studies similar to this study have used a variety of sample sizes (see

Table 8).

The questionnaires were distributed to 384 persons in total. The food diary and blood samples could only be collected from a similar sample as they required an intensive collection and analysis process; thus, the three-day food diaries and blood sample collections were a voluntary option for all participants. The study aimed to collect as many blood samples and three-day food diaries as possible in order to ensure robustness of results

Table 8 Sample sizes and other factors in similar studies.

	Study	Elshafie *et al.* (2012)	Farrar *et al.* (2013)	Kift *et al.* (2013)	Ardawi *et al.* (2012)
Participants	**Number**	50	60	125	1722
	Dropout/exclusion number		9	38	888
	Age	16-60	20-60	20-60	20-74
	Sex	Female/male	Female/male	Female/male	Male
	Condition	Healthy married couple	Healthy South Asian adults	Healthy South Asian adults	Healthy
Profile Study Outline	**Design**	Questionnaire FFQ Serum 25(OH)D Circulating parathyroid hormone	6 weeks course of UV exposures (intervention) Minimal erythema dose and skin-color assessment 2 weeks diary of vitamin D–containing foods Serum 25(OH)D and circulating PTH were measured	7 day food diary Serum 25(OH)D and circulating PTH were measured in each season Measure personal UV exposure	FFQ 4 days dietary records Serum 25(OH)D test Urinary test PTH* measurement BMD* measurements
	Country	Riyadh/KSA	Manchester/UK	Manchester/UK	Jeddah/KSA
	Season	Winter	Winter/Spring	All seasons	All seasons
	Exclusion criteria	Any complaint related to the vitamin deficiency	Skin diseases Sunbed and sunbathing in last 3 months Vitamin D supplementation Pregnant/Breastfeeding	Skin diseases Sunbed and sunbathing in last 3 months Vitamin D supplementation Pregnant/Breastfeeding	Medication and disease affecting the vitamin Recent fracture Low bone mineral densitometry

*PTH: Parathyroid hormone
*BMD: Bone mineral density

3.4 Inclusion and exclusion criteria

The inclusion and exclusion criteria used in this study are similar to those used in the majority of research studies in this field (Armas *et al.,* 2007 ; Farrar *et al.,* 2011). All the participants in all three groups were healthy females between 18 and 60 years old.

Any volunteer with any of the following were excluded from blood sampling:

- A history of any chronic disease, such as: cancer, osteoporosis, intestinal disorder, liver disease, kidney disease, fat malabsorption, heart disease, high blood pressure, diabetes, milk allergy and lactose intolerance.
- Those who are taking vitamin D medication or prescribed supplements.
- Those who have had bone fractures in the last two years.
- Any volunteer who was aware of being more than 3 months pregnant.

3.5 Sample collection

First, all the participants were asked to fill out the questionnaire and three-day food diary, and to give a blood sample. However, fewer of the participants completed all three parts and the majority of them anticipated it to be daunting for them. Thus, the participants were asked to fill out the questionnaire, then asked if they were willing to go further with the food diary and blood sample. The majority filled out the questionnaire and some filled out the three-day food diary and/or the blood sample. Participant recruitment is illustrated in Figure 3.

Volunteers were recruited at different locations in Manchester in places where Muslim women were able to go. The most effective and straightforward locations at which participants could be recruited for this study were universities and mosques. In order to have a similar population profile, sampling in the KSA was also randomised and conducted amongst university students and staff, and at some types of social gatherings. However, recruiting covered women in the KSA was less challenging than in the UK, as the Hijab is mandatory for women in the KSA, which creates a greater percentage of potential volunteers for the study. The samples were collected in a season in which people, in each country, may tend to go outdoors less; the UK sample was collected in the winter season and the KSA sample was collected in the summer season.

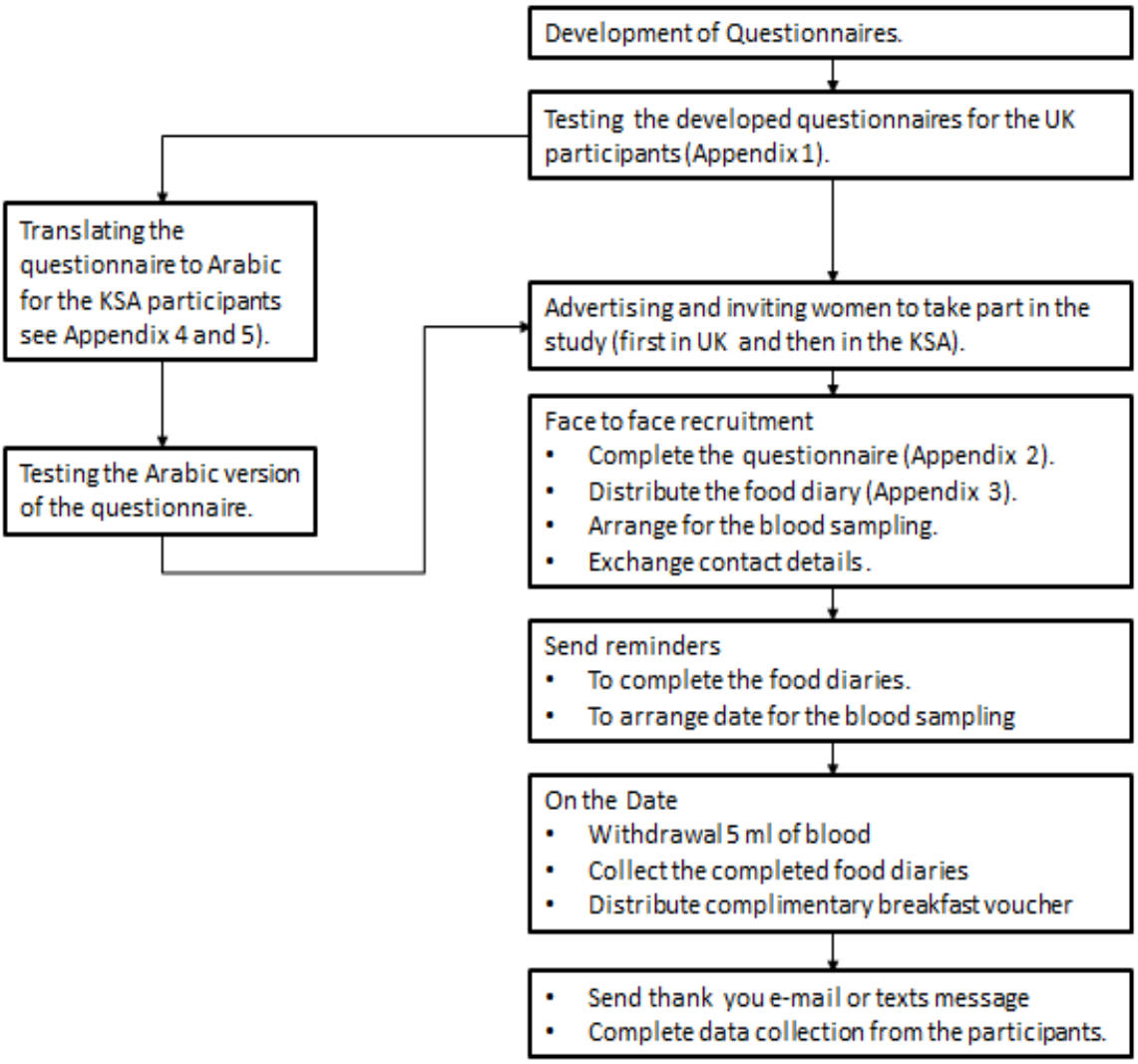

Figure 3 Recruitment Flow Chart.

Table 9 Summary of research methods - data collection and analysis techniques.

Method	Data Collected	Brief Details
Questionnaire	Demographic details	• This part was designed to gather general information relating to the participants. This includes age, weight, height, ethnicity, disease, number of children and skin colour (Fitzpatrick's skin colour scale).
Questionnaire	Sun exposure habits	• Questions were used to investigate sun exposure habits by estimating participants' previous exposure to the sunlight. • Previous week's exposure estimated by asking about time spent outdoors, sun protection methods, clothing and exposed areas of the body to sunlight between the usual daylight hours of 7 am and 7 pm.
Food Frequency Questionnaire(FFQ)	Vitamin D intake	• Questions in this section were intended to provide an estimation of vitamin D intake for the previous 6 months. • This section includes a table that lists 21 food items that are considered to be the richest sources of vitamin D, followed by second set of questions investigating participants' supplements intake, history of vitamin D deficiency, alcohol consumption, and smoking habits.
Food diary	Vitamin D intake	• The participants received a journal to fill in a three-day food diary.
Blood analysis by HPLC and LC MS-MS	Serum $25(OH)D_3$	• To measure vitamin D levels, a blood specimen was taken from willing participants. • The blood specimen was centrifuged to extract the serum. • A pre-treatment for the serum was applied to prepare the sample for being run in chromatography machines.

3.6 Data collection

The previous table presents a summary of the research methods and data collection. The analytical techniques and methods will be explained in more detail in the following sections.

3.6.1 Questionnaire

The questionnaire was divided into three sections: demographic information, the food frequency questionnaire, and the sun exposure questionnaire. Moreover, it contained closed questions, and, despite the constraints on the choice of answers, these were considered to be a highly reliable and repeatable method, which can be perpetually applied in order to measure the same thing (Guthrie 2010). Nevertheless, to overcome restrictions in the choice of answers, this study included the option "other" in some of the questions, which would accommodate elaboration. Additionally, in certain questions, adequate space was also added so the participants could add their own comments and thus provide an alternative method for respondents to avoid this limitation.

3.6.1.1 Demographic information

In the first section, the first fourteen items (see Appendix 2) were devised in order to gather general information relating to the participants. This included knowledge of the participants' backgrounds, as well as their previous and current health issues, including: age, weight, height, ethnicity, disease, number of children and skin colour. For the skin colour assessment, an established method known as Fitzpatrick's Skin Colour Classification was used (Astner and Anderson, 2004 ; Magin et al., 2012). This method is a self-assessment of sun sensitivity which has been widely used in the assessment of UV exposure, some cosmetic treatment doses, studies related to skin cancer, and protective behaviour (Sriprachya-anunt et al. 2002 ; Rokhsar and Fitzpatrick 2005 ; Sachdeva 2009).

3.6.1.2 Food Frequency Questionnaire

Questions 15 to 36 (See Appendix 2) were intended to provide an estimation of vitamin D intake for the previous 6 months.

Question 15, included a table that listed 21 food items that are considered to be the richest sources of vitamin D (Holick, 2007). Each item of food was labelled with a frequency-of-consumption ranking per month; this started from 1, which indicated never, to 7, indicating twice or more a day, to measure the frequency of vitamin D consumption.

This second set of questions (16-36) in the FFQ was adopted from the UK Women's Nutrition & Lifestyle Survey/University of Leeds and NHANES Food Questionnaire, and was moderated to measure vitamin D intake (Medical Research Council 2013). The questions asked about participants' consumption of meat products, milk, alcohol, and supplements intake.

3.6.1.3 Sun exposure questionnaire

In the third part of the questionnaire, questions 37 to 51 (see Appendix 2) were used to investigate sun exposure habits by estimating a participant's previous exposure to the sunlight (usual routines and holiday sun exposure).

The first set of questions in the third section of the questionnaire have been designed to estimate the usual sun exposure and was based on Gandini et al. (2005) ; Hanwell et al., (2010) ; Farrar et al., (2011) studies. The previous week's exposure was estimated by asking about time spent outdoors, whether sun protection was used, what clothing was worn and what areas of the body were exposed to sunlight between the usual daylight hours of 7 am and 7 pm (Gandini et al. 2005 ; Hanwell et al., 2010 ; Farrar et al., 2011). The next set of questions in the third section of the questionnaire inquired about holiday sun exposure. The questions asked about the destination, and time spent abroad, the purpose of the visit, season of the visit, and the weather within the last six months prior to the investigation. Additionally, the participant's sunbathing routine for the last six months was also questioned and documented (Whiteman et al., 2001 ; Gandini et al., 2005).

To estimate the exposed adult Body Surface Area (BSA) for participants, the researcher used an established method called the "rule of nines" (see Table 10) (Knaysi *et al.,* 1968 ; Barger-Lux and Heaney 2002). The original "rule of nines" is used to estimate the surface area of the body affected by burns by dividing the body to eleven areas plus the genital area. Each area presented nine percent of the body surface except the genital area which is one percent. Summing up the total of all areas adds up to 100% of the surface of the body (see Table 10). However, the methods were adapted to suit the study's nature by estimating usual skin exposure according to three main categories: arms, legs and head. From each category, one option was marked to be added up. Then, values were presented as an average for each participant for off-peak time and peak time (see Table 10).

Table 10 Original and adapted "Rule of Nines".

Rule of nines "%"						
Arms	**Legs**	**Anterior trunk**	**Posterior trunk**	**Head**	**Perineum**	**Total**
0.18	0.36	0.18	0.18	0.09	0.01	1
Adapted rule of nines "%"						
Arms			**Legs**		**Head**	
Hands	**Half arms**	**Full arms**	**Half legs**	**Full legs**	**Covered**	**Uncovered**
0.04	0.09	0.18	0.16	0.24	0.03	0.07
The study adapted the original "rule of nines" from similar studies. The categories were changed to match the study groups.						

A calculation was used to estimate hours per week of total skin exposure, this method is called "Total Sun Index" developed by Barger-Lux and Heaney (2002). The index is calculated by multiplying the reported average sun exposure per week by the exposed Body Surface Area (BSA) for each subject.

3.6.1.4 Pilot study (questionnaire)

In order to produce a well-structured and readable questionnaire which would be easy to complete but still retain significant validity and reliability, it is always vital to pre-test materials through the use of a pilot study. Hence, a draft questionnaire (see Appendix 1) was prepared and used to collect data. This trial provided results which were used to refine the questionnaire, and this improved the clarity of the layout and identified confusing and ambiguous questions. Saunders et al. (2009) suggested that ten is the minimum number of respondents needed in order to pilot a large questionnaire before implementing a full-scale test with a planned sample size of between 100 and 200. In fact, in this study, the pilot test was applied twice, in order to ascertain greater clarity of comprehension, and this was achieved by using fifteen volunteers on each occasion.

After the first 15 questionnaires were reviewed, the questionnaire was modified and retested (see Appendix 2). These changes involved modifications to parts of the questionnaire's structure and layout, as well as more sections being added. Furthermore, in the third part of the questionnaire, which consists of the sun exposure questions, some necessary modifications and explanations, which were identified through the pilot study, were included at the beginning of the section. The reason for these changes was that there was a low response rate in this section, even though this section was vital to the investigation, and also because it contained a significant percentage of the overall questions on the questionnaire. This part of the questionnaire contained a table with nine questions, and each question had six sections. Participants were required to fill in the

blanks with an approximate time (minutes and/or hours) indicating their levels of exposure to sunlight in the previous week. However, this was not clear to the participants, and, thus, in order to gain more clarity, an explanation and example was added to the second version of the questionnaire at the beginning of the table.

After revision, it was decided that, in contrast to the initial draft questionnaire, the revised questionnaire which was used in the second pilot test was readily accepted by the volunteers. Therefore, there was no need to implement any further alterations and modifications. Most of the respondents reported that the revised questions were clear and easy to follow. The final questionnaire, which was used in the main study, is shown in Appendix 2.

3.6.2 Food diary

All participants received a three day food diary to complete. The food diary was self-completed, and all the participants were educated on how to fill in the diary through examples and instructions in the first few pages (see Appendix 3). Following the instructions and the example sheets, the journal consisted of a slightly structured sheet of papers. Each diary included three blank tables, each table represent one main periods of the day (morning, afternoon and evening). The tables labelled with the day and date, then divided into four sections, time of the meal, food/drink description, brand name, portion size. At the end of the page there was a remainder to write only consumed foods/drinks. The participants were also requested to detail cooking methods to provide a more accurate estimation of nutrient intake.

Nutrient intakes were calculated using diet analysis software. Nutritics (2011, Ireland) is based on The UK Composition of Foods Integrated Dataset (COFIDS), The Irish Foods Composition Database from UCC, and the software allowed users to add foods and recipes to Nutritics account database for their own use.

3.6.3 Blood samples collection

In the UK, blood sampling took place in Manchester Metropolitan University, John Dalton laboratory, UK between October 2013 and February 2014 (latitude 53.46° N), whereas, in the KSA, blood sample took place in Makkah, in August 2014 (latitude 21.41° N), Umm Al-Qura University, in the university medical centre. The participants in both countries arrived at the laboratory after fasting over-night; they were allowed to have just water. 5ml of a venous blood sample was taken by a trained phlebotomist, and immediately placed into red-lidded tubes (which has no additive and normally uses for serum collection). Collected blood samples were left for around 30 minutes to an hour at room temperature to clot. The samples were centrifuged at 4000 rpm for 10 minutes to separate the serum (Alvi et al. 2012). Then, the serum was removed aseptically and transferred to plastic vials. The plastic vials were labelled with participants' first names, clothing styles, study codes, and date of collection and stored at -80 °C until they were required for the analysis. To keep confidentiality, each women was given a number as a code and the list of codes and women were kept safely and separately from other notes.

Printed adverts, emails, and face-to-face meetings were effective tools for attracting potential subjects. Still, many women declined to contribute mainly due to unwillingness to give blood samples and not wanting to fill out a food diary for three days.

3.7 Blood sample preparation and extraction

Of the 5ml blood sample collected, 0.5 ml of the serum was defrosted at room temperature for around 10 minutes. Then, the serum was vortexed with 350 µl of azeotrope mixture of methanol and 2-propanol (80:20) for half a minute. In order to extract 25(OH)D, 2 ml of hexane was added to the combined product of the previous step. The mixture was then centrifuged at 4000 rpm for ten minutes before transferring the upper phase to a 2.5ml microfuge. Extraction with hexane was repeated twice more (three time in total). The combined upper layers were then dried at 40° under gentle steam of nitrogen (Turpeinen *et al.,* 2003 ; Alvi *et al.,* 2012). The residue was dissolved in 1 ml of methanol, and filter before injecting samples to the HPLC system.

3.8 Solvents and regents

Chemicals and regents were obtained from Sigma-Aldrich, United Kingdom. All solvents and regents were HPLC grade. The 25(OH)D standards were obtained from Sigma-Aldrich, United Kingdom.

3.9 Chromatography

For analysing vitamin D in serum, two chromatography systems were implemented: High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-tandem Mass Spectrometry (LC MS-MS), with the HPLC system being utilised initially in the UK for the investigation.

HPLC methods have been used in many studies to analyse vitamin D levels in blood, and have been continuously developed in order to make the process easier and more straightforward. This method has often been reported as being capable of delivering reliable and acceptable results (Turpeinen et al., 2003 ; Carter et al., 2004 ; Lensmeyer et al., 2006).

3.9.1 HPLC methods in the UK

Initially, the HPLC method was carried out in the Hollings Faculty at Manchester Metropolitan University. Table 11 below presents the parameter of the equipment used, which followed by the methods details. Two HPLC methods were used. Firstly, the isocratic method of Turpeinen et al. (2003) was used, although results did not produce usable data following various attempts. Secondly, the gradient method by Alvi et al. (2012) was investigated as an alternative. However, this method did not provide reliable or reproducible results either.

Table 11 HPLC machine and methods used in the UK

System details	
Instrument	Shimadzu HPLC
Column	**Part Number:** 00E-3156-D0 (Phenomenex) **Description:** LiChrospher® 5 µm RP-select B 60 Å, LC Column 125 x 4 mm, Ea
Guard Column	**Part Number:** KJ0-4282 (Phenomenex) **Description:** SecurityGuard Guard Cartridge Kit, Ea
Detector	Ultraviolet Detection
The methods details	
First method	Turpeinen et al. (2003) Isocratic Method
Mobile Phase	76% Methanol
Flow Rate	1 ml/min
Temperature	40°C
Retention of times	**25 (OH)D_2:** 20.8–21.1 min **25 (OH)D_3:** 23.1 min
Injection Volume	50 µl
Concentrations	0-15-30-60-120 nmol/L
Detection wavelength	265 nm
Second method	Alvi et al. (2012) Gradient Method
Mobile phase	**A:** Acetonitrile, Methanol, Water (35:50:15) **B:** Acetonitrile, Methanol, Water (10:80:10) pH=3.0 phosphoric acid
Flow Rate	**A:** 1.2 ml/min for 19 min **B:** 1.5 ml/min for 25 min
Temperature	40°C
Retention times	**25 (OH)D_3:** 10.6 min **25(OH)D_2:** 12.0 min
Injection Volume	50 µl
Concentrations for both forms of 25(OH)D	0-15-30-60-120 nmol/L
Detection wavelength	265 nm

3.9.2 HPLC methods in the KSA

The serum was shipped to Manchester Metropolitan University, Manchester UK, for analysis, so that the analysis of blood serum could apply. The serum was frozen at -80°C and shipped to the UK in dry ice to prevent spoiling. However, as a precaution the vitamin D was analysed when analysing the samples for free from communicable disease certificate. The vitamin was measured in the laboratory of King Abdul-Aziz University Hospital, and the HPLC was used for analysis. An Eagle Biosciences Vitamin D HPLC kit was used for the analysis. Limited information was available, Table 12 below presents the obtained details of the HPLC, the detector, and the column. Then, details the available information of the methods.

Although the samples were dispatched by FedEx to the UK in dry ice to prevent spoiling, due to an error with the logistics company and confusion of receipt at MMU Hollings Faculty, which caused the samples to thaw and spoil before they were obtained. For this reason, the study was dependent on the vitamin results that were obtained in the laboratory of King Abdul-Aziz University Hospital.

The cut off level of 25(OH)D concentration that was used by the hospital in KSA to identify the participants vitamin D status is shown in Table 12.

Table 12 HPLC machine and method used in the KSA

System details	
Instrument	Agilent HPLC 1100
Column	Reversed Phase **Dimension:** 125 mm x 4 mm
Guard Column	Security Guard Cartridge Kit
Detector	Ultraviolet Detection
The methods details	
Mobile Phase	No information was given to the student from the hospital
Flow Rate	1 ml/min
Temperature	30 °C
Retention of times	15 min
Injection Volume	50 µl
Concentrations for both form of 25(OH)D	no information was given to the student from the hospital
Detection wavelength	264 nm

Table 13 Vitamin D levels that were used to identify participants status at the laboratory of King Abdul-Aziz University hospital

25(OH)D concentration, ng/mL	Vitamin D status
<10	Deficiency
10-30	Insufficiency
30-100	Sufficiency
>100	Toxicity

3.9.3 LC MS-MS methods

Subsequently, the LC MS-MS at John Dalton Chemistry Laboratory, Manchester Metropolitan University was obtained. Agilent Technology 25(OH)D standard methods were used (Agilent Technology, 2015). Then, the method was adjusted because the HPLC had technical problems, see Table 14. The table illustrates details of the instrument, the detector, and the column. The table then presents the method conditions of LC and MS parts. The original method is presented first then the adjusted method. In the adjusted methods, the iron source had to be changed.

Table 14 LC MS-MS details and methods used In the UK

The system details	
The instrument	HPLC Agilent 1260
Detector	Agilent 6540 UHD-QTOF-MS Ultra-high definition, quadrupole time of flight-mass spectrometer
Column	Extended C-18, 1.8 um, 2.1 x 50 mm, Batch: USHBDO3379; Product: 727700-902
Concentrations:	0 ,5 ,10 ,20,30,40,50 ng/ml
Methods details	
First method	Original method
LC Method	
Mobile phase	**A:** 0.1% Formic Acid in Water **B:** 0.1% Formic Acid in Methanol
Flow Rate	0.5 mL/min Flash 5 seconds 50% A- 50 % B 0 minute: 20% A - 80% B 2.3 minute 20% A - 80% B 3 minute 2% A - 98% B 3.9 minute 2 % A - 98% B Flash 5 seconds 50% A- 50 % B
Temperature	50^{o}C
Injection Volume	10µl
Running time	5 minutes

MS method	
25(OH)D3 Mass	401.341
Gas temperature	250^{o}C
Ion Source	APCI+
Dwell time	50 msec
Gas flow	5 L/min
Second method	Adjusted Method
LC Method	
Mobile phase	**A**: 0.1% Formic Acid in Water **B:** 0.1% Formic Acid in Methanol
Flow Rate	Flash 5 seconds 50% A- 50 % B 0 minute: 0.4 ml/min 10% A - 90% B 4.50 minute: 0.4 ml/min 10% A - 90% B 4.55 minute: 0.5 ml/min A 0 %- B 100% 5.53 minute: 0.5 ml/min A 0 %- B 100% 5.55 minute: 0.4 ml/min A 10% - B 90% 6.50 minute: 0.4 ml/min A 15 % - B 85 %
Temperature	20^{o}C
Injection Volume	40 µl
Running time	12
MS method	
25(OH)D_3 Mass	401.341
Gas temperature	320^{o}C
Ion Source	Dual AJS ESI
Gas flow	4 L/min

3.10 Data processing and analysis

The statistical analysis was conducted using Excel (2010) and IBM SPSS Statistics 21. For this study, three types of variables were considered: nominal, ordinal and continuous (numerical) variables. The outcome of interest which is vitamin D deficiency is presented as a nominal (binary) and a continuous variable. As a result, the binary outcome represents two groups of women previously diagnosed with vitamin D deficiency or not diagnosed with vitamin D deficiency. For the continuous outcome, vitamin D status was considered.

Exploratory result were demonstrated using mean and standard deviations for quantitative data, and percentages and frequencies were computed for nominal and ordinal data (Richard, et all, 2013).

3.10.1 Bland-Altman plot

A Bland-Altman plot was used to examine the agreement between the two measurements of dietary vitamin D intake methods, food diary and FFQ (Bland and Altman , 1986 ; Giavarina, 2015).

3.10.2 One way ANOVA and Chi-square test

Since the data was collected from three groups (UK un-covered, UK covered, KSA covered), there was an interest to examine statistical differences in the numerical variables (such as time of sun exposure) due to the three groups. Since the numerical variables needed comparing were found to normally distributed, one-way ANOVA was used to compare the means of variables due to the three groups. When the test showed significant differences, the aim was to examine which of the specific groups significantly differed, and therefore post hoc tests was conducted (Bland, 2000). For the nominal variables, proportion of nominal variables were compared due to the three groups using Chi-square test. P values of <0.05 were considered statistically significant.

3.10.3 Simple and Multiple Correlations

In order to measure the strength of the relationship between all variables of interest via correlation coefficients, simple and multiple correlations were used. Simple correlation measuring the strength of the association, which is ranging from -1 to +1. The relationship is said to be very weak as long as the correlation coefficient (r) is very close to zero. Multiple correlation coefficient was used to measure the effect of exploratory variables on the numerical dependent variable. The squared-multiple correlation determines the variation in outcome that explained by independent variables of model, (Draper & Smith, 1998). Notice that (r) is ranging from 0 to 1 whereas (R^2) is ranging from 0 to 100%.

3.10.4 Regression Model

Regression analysis was used to answer the key questions of the study concerned with the influence of a set of variables known as predictors on the outcome (vitamin D) (Draper & Smith, 1998). The researcher used two type of regression: linear and logistic regression. The relationship between level of vitamin D (dependent variable) and the independent variables, see Table 15, were modelled using multiple linear regression. It is important to check regression assumptions for fitted model, such the residual normality (Draper & Smith, 1998). For the binary outcome (vitamin D deficiency), logistic regression was

applied. Using logistic regression, the research aimed to estimate the odds ratio of vitamin D deficiency due to each category of nominal and ordinal variable (Hosmer et all, 2013).

Table 15 Variables of the prediction modelling

Variable	Defined as	Type
Vitamin D Level	Dependent	Numeric
Previous Vitamin D diagnosed	Dependent	Nominal (Binary)
Age	Independent	Numeric
BMI	Independent	Numeric
Has children	Independent	Nominal (Binary)
Use of fortified vitamin D products	Independent	Nominal
Use of sunscreen	Independent	Nominal
Travel abroad	Independent	Nominal (Binary)
Sun exposure during peak time hour/day	Independent	Numeric
Sun exposure during off-peak time hour/day	Independent	Numeric
Average of exposed BSA during peak time	Independent	Numeric
Average of exposed BSA in off-peak time	Independent	Numeric
Daily intake of vitamin D from supplements	Independent	Numeric
Daily vitamin D intake from common foods	Independent	Numeric
Daily vitamin D intake from uncommon foods	Independent	Numeric
Skin colour	Independent	Nominal (Binary)
Type of milk	Independent	Nominal
Use of food supplements	Independent	Nominal

3.11 Ethical considerations

Research ethics are about formulating and clarifying the morals and behaviour of the research topic, as well as about processing and ensuring that those individuals involved are not harmed (Saunders et al. 2009 ; Guthrie 2010). In fact, this is an important issue in any investigation because unethical practices will certainly adversely affect the research. The research was originally deemed ethical by the Research Committee of Manchester Metropolitan University / Hollings Department.

To avoid unethical behaviour and ensure that no person was actually or potentially harmed by this piece of work, the research was conducted through constraints on three particular issues: participant anonymity, the use of suitable language, and avoidance of any sensitive subjects (Guthrie 2010). Each participant signed a consent form, and for blood withdrawal, another consent form was provided to applicants to be signed (see Appendix 2 and Appendix 4).

Firstly, the privacy of all the individuals was maintained throughout this study, and this confidentiality was unequivocally respected. Hence, there are no questions in the

questionnaire that could reveal a participant's identity. Nevertheless, the participants were asked to sign consent forms, and in order to keep their identity confidential and anonymous, the researcher gave each participant a specific code that would connect the consent forms for each person with their blood sample, questionnaires, FFQ, and food diary, which were all marked with the same code.

Secondly, given the ethnic diversity among the Muslim population, and to allow all the respondents to understand the aims of the study, and to obtain fair and clear results, the questionnaire was produced in two languages. In the UK, the researcher conducted the survey in English, which is the common language of the participants (see Appendix 2 and Appendix 3). However, in the KSA, the questionnaire was translated into Arabic, which is the common language of the participants in that country (see Appendix 4 and Appendix 5).

A translated copy of the questionnaire was tested on 10 bilingual women to determine whether they were able to read and understand both the Arabic and English versions. This was done to ensure that the questionnaire was structured in such a way that when read in either language, there were no significant linguistic differences and that there were no differences in the questions in the different languages which would adversely change the meaning of the questions, and thereby confound the results obtained.

Thirdly, no sensitive subjects, such as financial matters and personal information that might make a person uncomfortable, were addressed. Specifically, no questions were directly asked in regards to any possible taboo issues such as the reasons for wearing the Hijab. According to Denscombe and Ebrary (2010) by avoiding taboo matters, it is possible to increase the response rate.

Thus, to increase willingness to partake in the research and ensure subjects felt comfortable, the questionnaire avoided direct questions relating to religion, ethnicity and identity. Therefore, the topic of the questions did not encroach on any religious issue that could offend any particular group of people, as the researcher ruled out any possible subject that would incline towards the essence of religion in any way. Instead, the questionnaire was strictly focused on the main aims, which were to estimate vitamin D levels, and identify factors that influence vitamin D levels in a group of people manifesting similar non-revealing clothing patterns. The fact of whether the participant wore a Hijab was clearly relevant to the overall study, but the reason for such a choice contributed

nothing to this form of research, as it was not required to distinguish cultural or religious practices.

Nevertheless, it must be noted that the researcher clearly explained in the consent forms (see Appendix 2) and the participants' information sheet that clothing is one of the factors affecting the vitamin level. The consent and information sheet explained the purpose of the research, gave the applicants an overview of the research subject, requested permission to use the information that they provided, and clarified their right to withdraw at any stage and simply to refuse to take part without any justification. In addition, for the blood sampling (see Appendix 2), the researcher gave a separate consent sheet with extra details to contributors which explained what they should expect for this step.

3.12 Summary of the methodology

This chapter has explained the philosophy of the study and the approach that was used for primary data collection to enable the achievement of research aims and answer study questions. The data collection technique was justified. Three main instruments were used. First, a questionnaire, which was designed in three sections: demographic information, a food frequency questionnaire, and a sun exposure questionnaire. Second, there was a food diary, which was intended to deliver detailed information about eating patterns and habits that may influence vitamin D consumption. Third and finally, blood collection and analysis was performed to measure vitamin D levels. The procedures for collection and analysis of the blood were outlined in detail above. Finally, attention was paid to validation issues and ethics in the research.

4 General characteristics of the study groups.

This chapter presents a general description of the study participants and general results.

4.1 Recruiting and participants

There were three groups of women in this study: UK covered, UK un-covered and KSA covered. To be included in the study, participants needed to have at least one of the following methods completed: a questionnaire, a food dairy and a blood sample. A total of 384 women were invited to participate in the baseline survey, of which 47 did not respond to the invitation, 74 withdrew and 56 did not complete the questionnaire. From the 207 who completed the baseline questionnaire, only 192 participants matched the research inclusion criteria. 145 completed questionnaires were returned. 57 participants submitted completed food diaries; and a total from both countries of 79 agreed to give blood samples (see Figure 4).

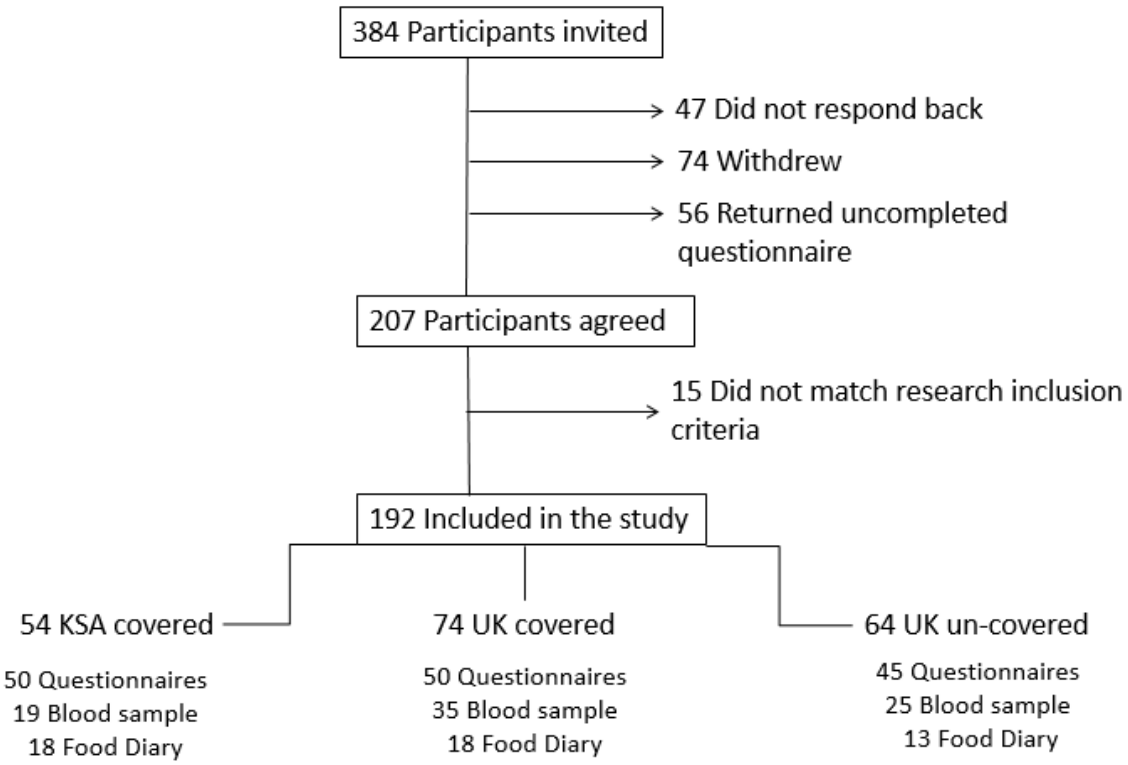

Figure 4 Recruiting and participant flow chart.

The current study used three main methods: questionnaire, food diary and blood sample. The study has 192 participants in total. Figure 5 shows the number of participants in each method, and how many of them participated in more than one method. Nineteen of the participants completed the three methods, 48 completed the food diary and the questionnaire, 20 completed the food diary and gave a blood sample, and 32 gave a blood sample and completed the questionnaire.

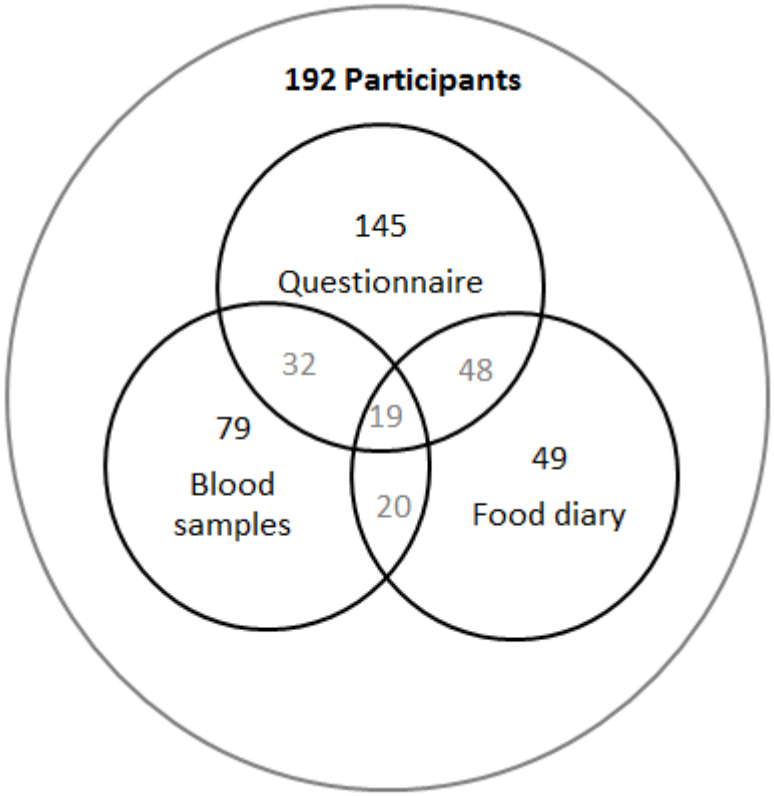

Figure 5 Distribution of participant number by participation methods, n=192.

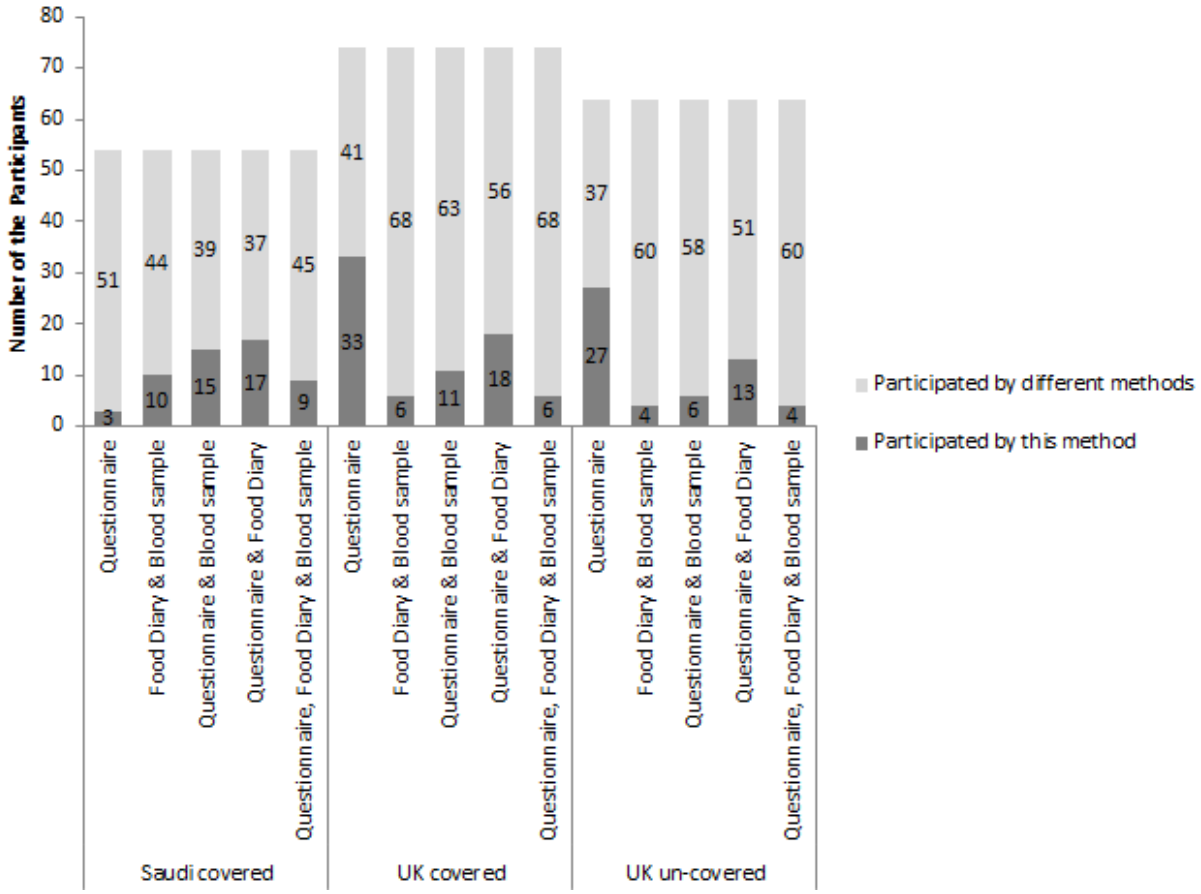

Figure 6 Distribution of the study groups by the participation methods, n=192.

Figure 6 shows the number of participants who completed each method in each group. The KSA group comprised 54 in total; only nine out of the 54 completed all three methods. The number in the UK covered group was 74 in total; six out of the 74 completed all three methods. Lastly, the UK uncovered group was 64 in total, four out of them completed all methods.

4.2 Distribution by all participants.

The following results highlight the main findings in the total population.

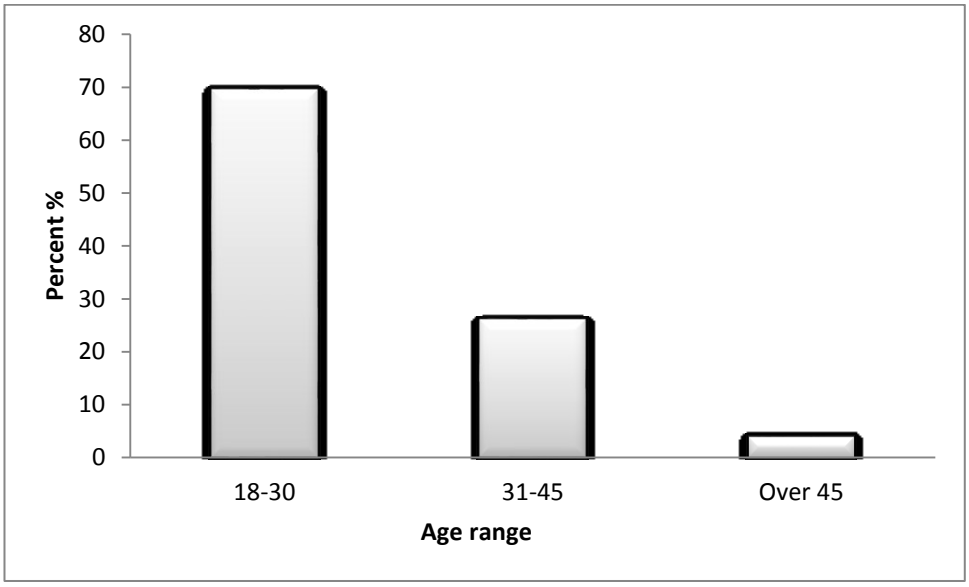

Figure 7 Distribution of participants by age, n=145.

The participants' ages varied. However, the largest group of participants in this study (70%) were young adults ages 18-30 years (Figure 7).

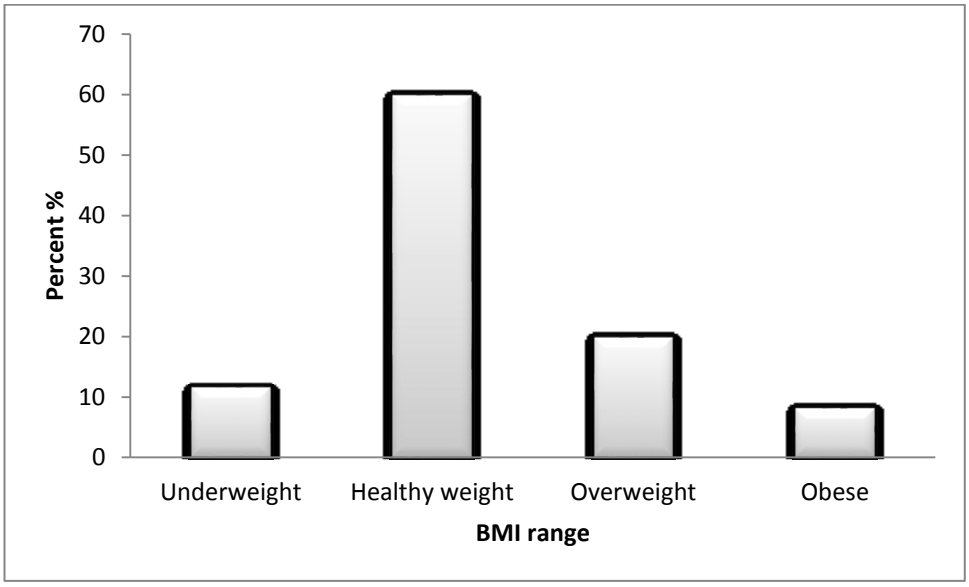

Figure 8 Distribution of participants by BMI, n=145.

Figure 8 shows the BMI distribution of the study participants. This analysis was based upon the self-reported height and weight data as part of the questionnaire. The data shows that 60% of the participants were in the healthy BMI range (18.5-24.9 kg/m^2). The 40% of the study group who were not in the healthy BMI range were divided into 3 categories, 11.72%

had a BMI which was below the healthy range (<18.5 kg/m^2), 20% were overweight (BMI >25 <30 kg/m^2), and 8.28% were classed as clinically obese (BMI > 30 kg/m^2)."

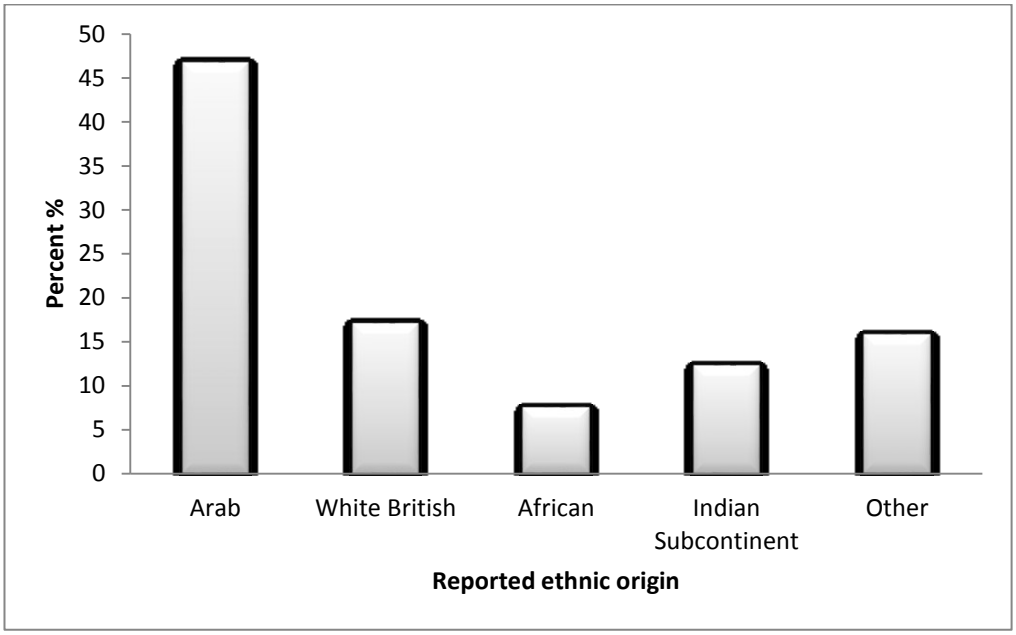

Figure 9 Distribution of participants by ethnic origin, n=145.

Figure 9 shows the ethnicity of the study participants as identified by the participants themselves in their questionnaire responses. Arab women represent the major group of "covered" women. Additionally, conducting the study partially in the KSA increased the Arab ethnic group. The other 53.10 % illustrate the multicultural nature of Manchester where the UK study was conducted - 17.24% of participants were White British, 12.41% from the Indian Subcontinent, 7.59% African, and 15.86% identified as other.

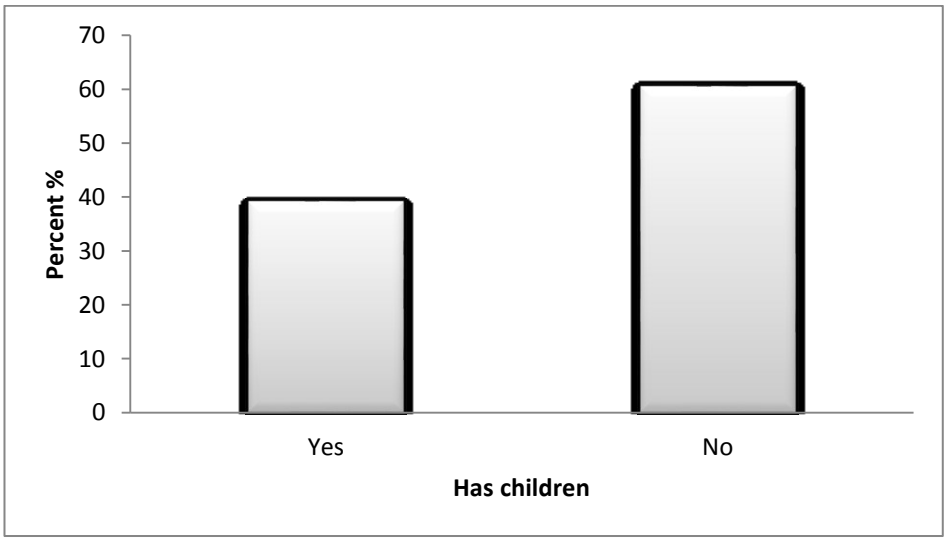

Figure 10 Distribution of participants who have children, n=145.

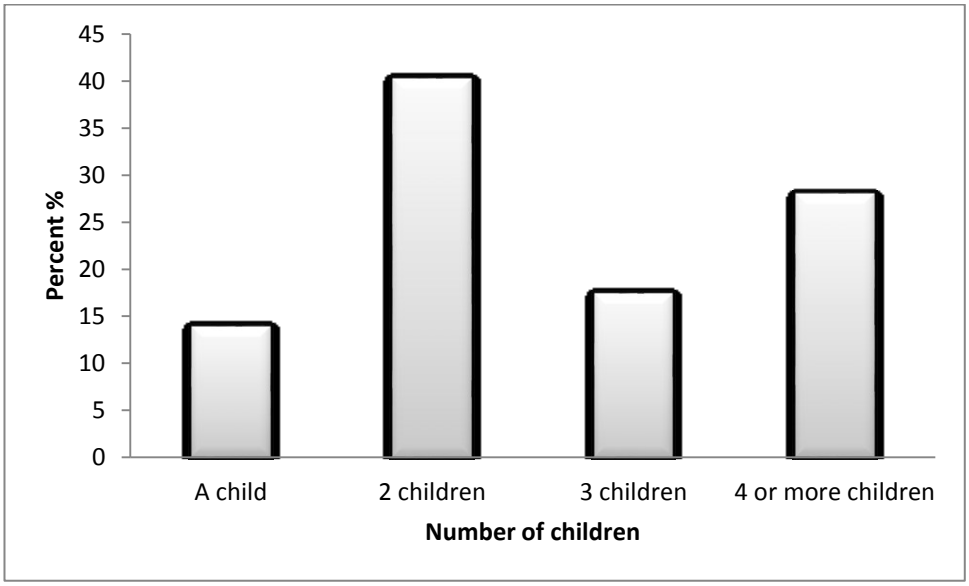

Figure 11 Distribution of participants who have children by the number of their children, n= 57.

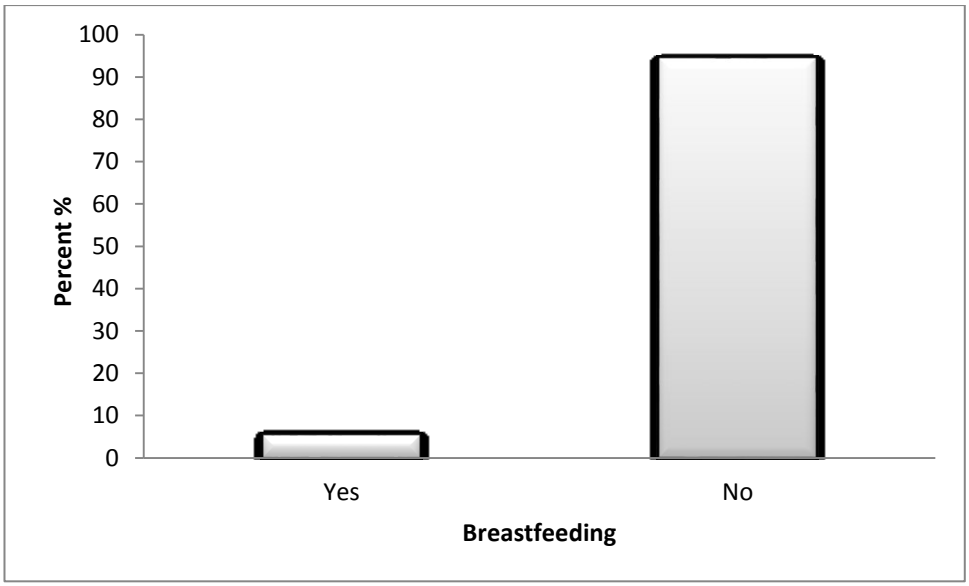

Figure 12 Distribution of participants who were breastfeeding, n=145.

In this study, nearly 61% of the participants did not have children, while nearly 39% had children (Figure 10). Of those who had children, 14.04% had 1 child, 40.35% had 2 children, 17.54% had 3 children, and 28.07% had 4 or more children (Figure 11). In addition, of those who had children, 5.52% were breastfeeding mothers (Figure 12).

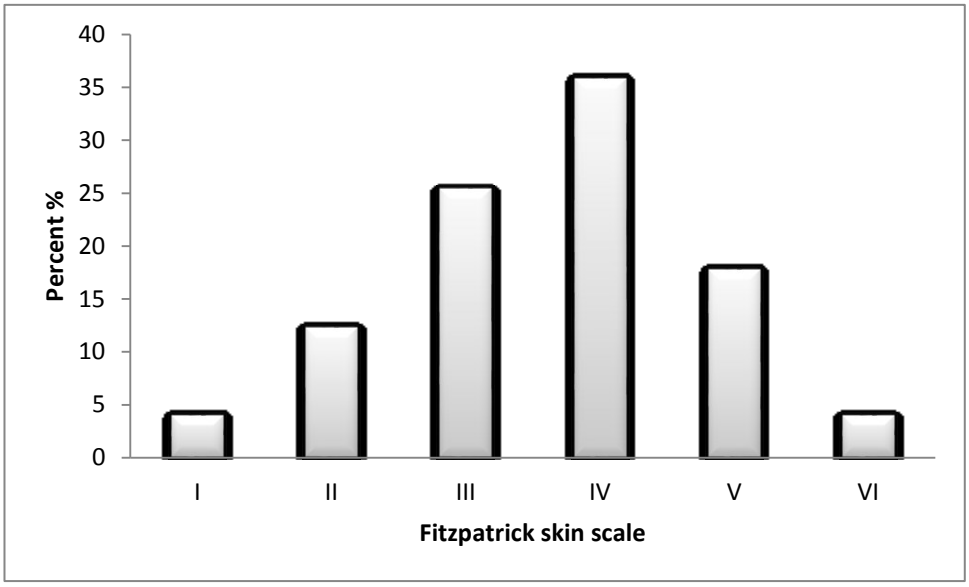

Figure 13 Distribution of participants by skin colour, n=145

Figure 9 illustrates the multi-ethnic nature of the participants; Figure 13 strongly evidences this diversity by presenting the reported skin colours. The study participants reported a variety of skin tones. The most common skin colours were “IV” (36%), followed by “III” at (25.5%). All other skin colours on the scale were reported by 38.5% of the participants in smaller frequencies.

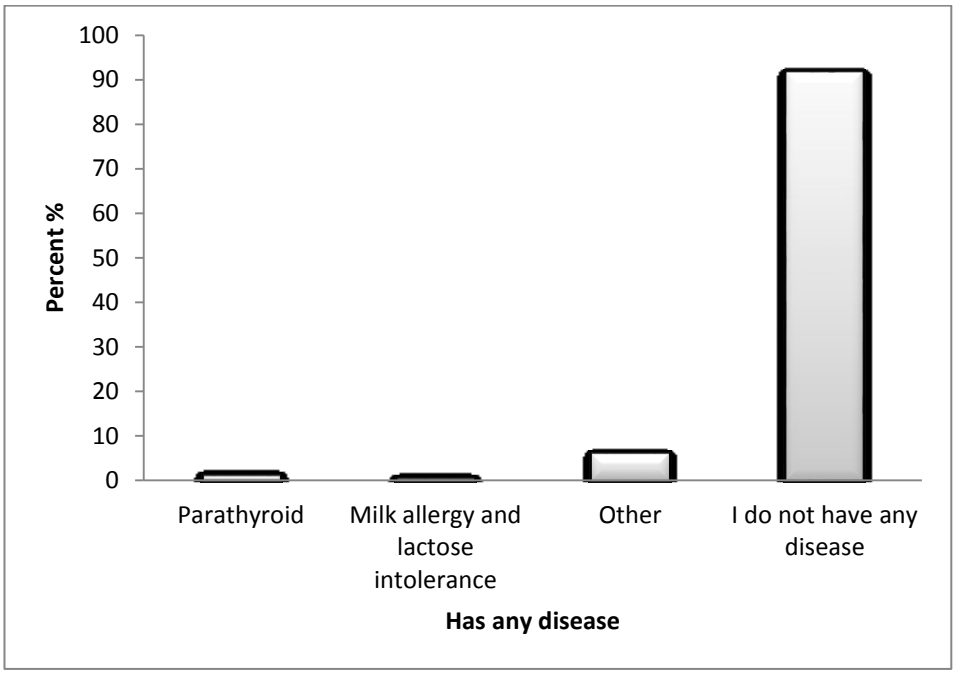

Figure 14 Percent of participants reported diseases related to vitamin d deficiency, n=145.

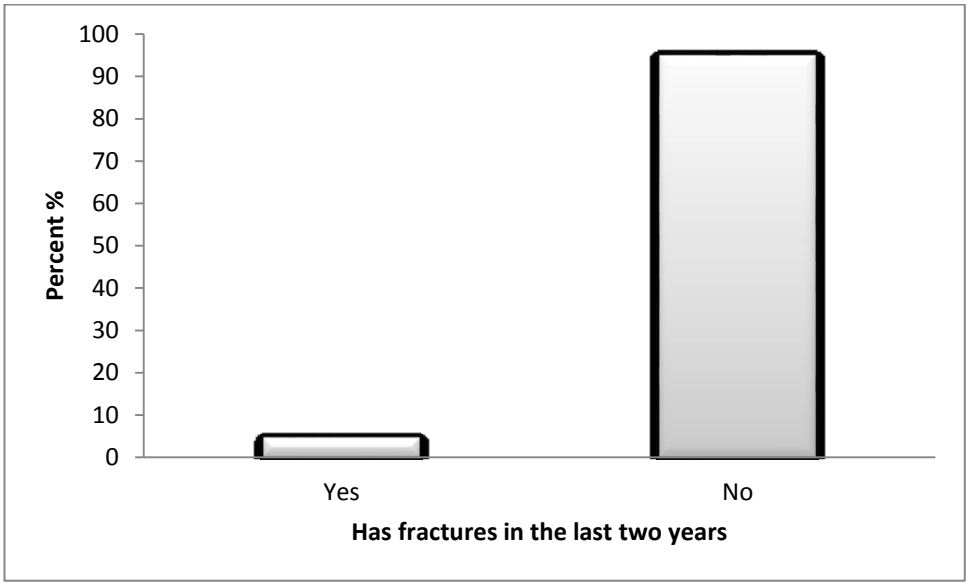

Figure 15 Distribution of participants who have had fractures in the last two years, n=145.

The study planned to measure vitamin D levels in healthy women. Therefore, some questions were asked directly about diseases that have a substantial relationship with vitamin D deficiency. These questions helped to eliminate affected participants from the blood test.

The results show nearly 92% of the study contributors are healthy and do not suffer from any diseases, and that 95.17% did not have any recent fractures in the last two years.

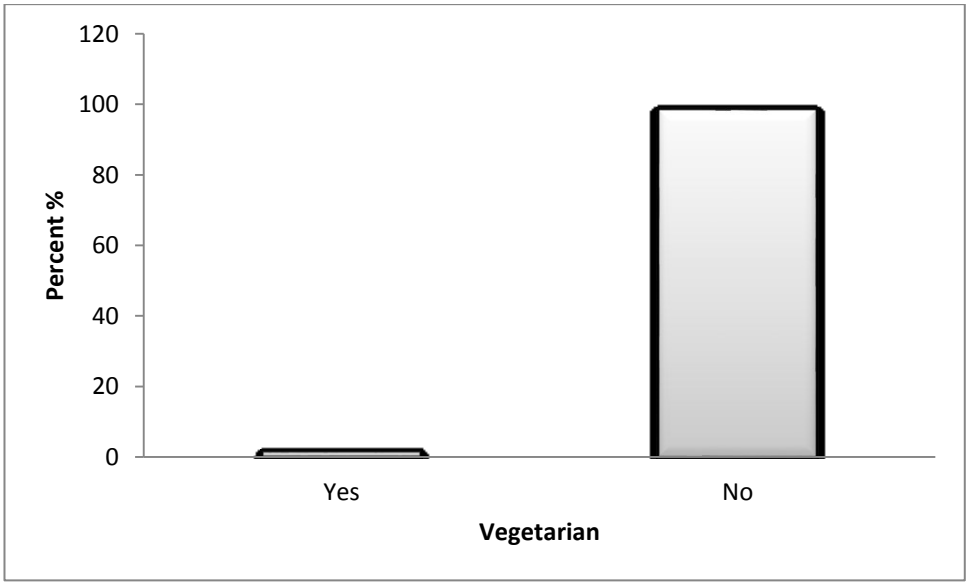

Figure 16 Distribution of vegetarians and vegans in the study, n=145.

Figure 16 shows that a minor proportion (1.38%) of the study's population described themselves as vegetarian or vegan.

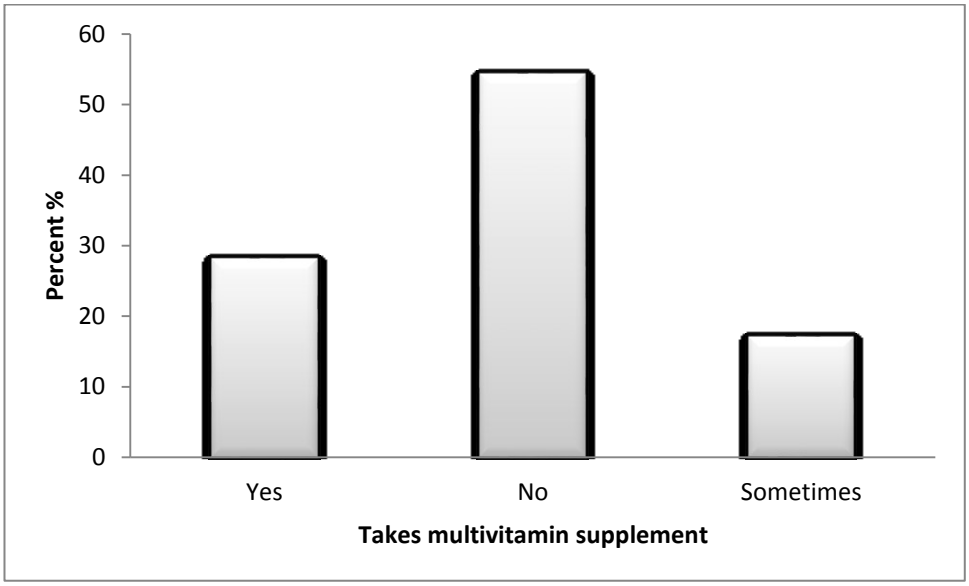

Figure 17 Distribution of participants who takes multivitamin supplements, n=145.

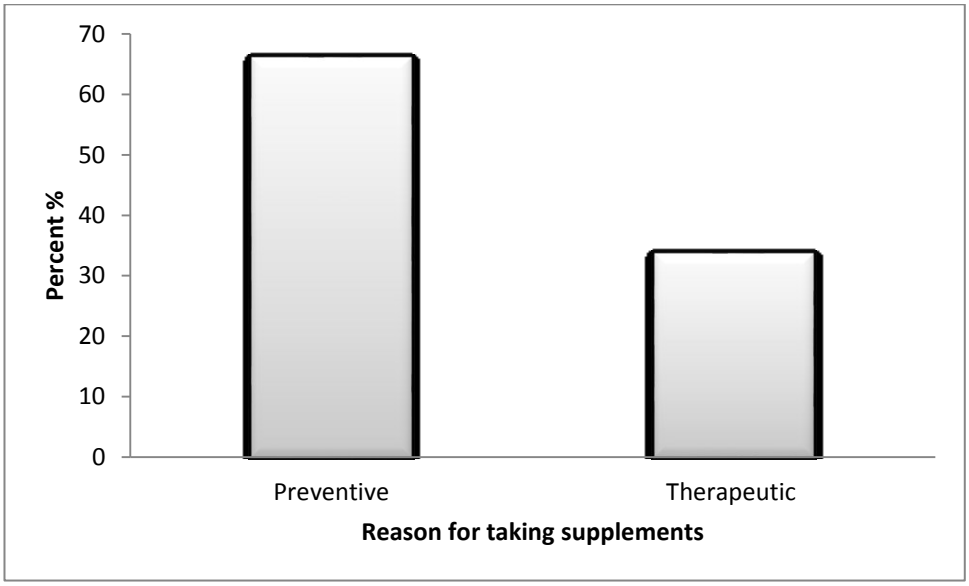

Figure 18 Distribution of participants by reasons for taking supplements, n=65.

Vitamin D supplements are considered an important compliment and substitute to reach good vitamin D level. However, the majority of the study's population denied taking food supplements, while nearly 46% stated that they take supplements always or sometimes (Figure 17). Of those who used food supplements 66% stated they take them for preventative reasons, whereas almost 34% stated the reason for taking supplements is therapeutic (Figure 18).

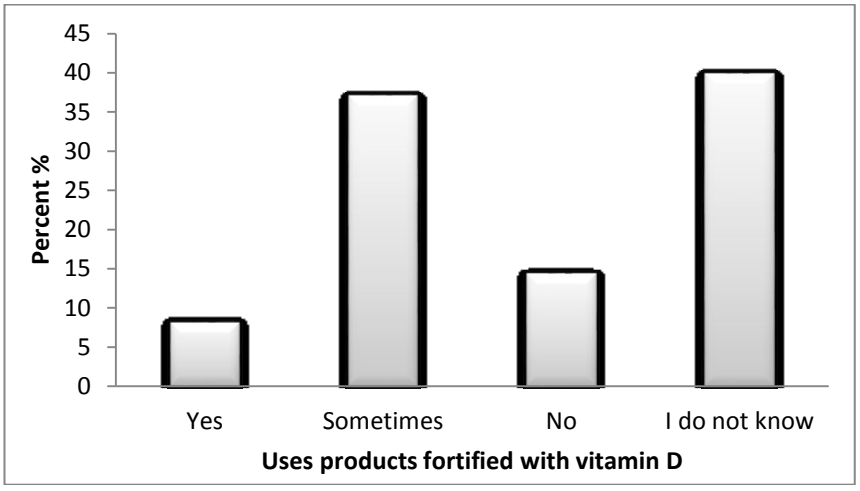

Figure 19 Percent of participants uses fortified products with Vitamin D, n=145.

The study asked participants about their knowledge of using food products fortified with vitamin D. Figure 19 shows that 40% of the study population were uncertain about using food products that are fortified with vitamin D, and 14.48% were certain of not using food products fortified with vitamin D. However, 45.52% stated that they always or sometimes use food products fortified with vitamin D. Of the population 58% believed their diet had not changed over the last year, whereas 42% stated that their diet had changed in the last year (Table 16).

Table 16 Changes in diet over the last 12 months and reasons for changing diet in the last 12 months.

Variables	Frequency	Percentage
Did not change their usual diet	83	57.2
Changed their usual diet	62	42.8
Reported reasons for changing diet		
Arthritis	1	0.7
Healthier lifestyle	1	0.7
I had a gastric bypass	1	0.7
I was breastfeeding	1	0.7
Improve food quality	10	6.9
Less money / student lifestyles	1	0.7
Moving country of residence	3	2.1
Other	4	2.8
Physical activity	2	1.3
Pregnancy - increased fruits, vegetable and milk	2	1.3
To lose weight	33	22.8
Travel	1	0.7
Wealthier lifestyle	1	0.7
Weight control	1	0.7

The reasons of those who changed their diet were various. Table 16 illustrates a list of reasons that were provided by the study contributors themselves. Most of the reported reasons revolved around weight and wellbeing. Table 16 shows that the most frequent reasons were “to lose weight” followed by “improve food quality”. However, less frequent reasons referred to diet changing due to financial reasons, health issues, and traveling etc.

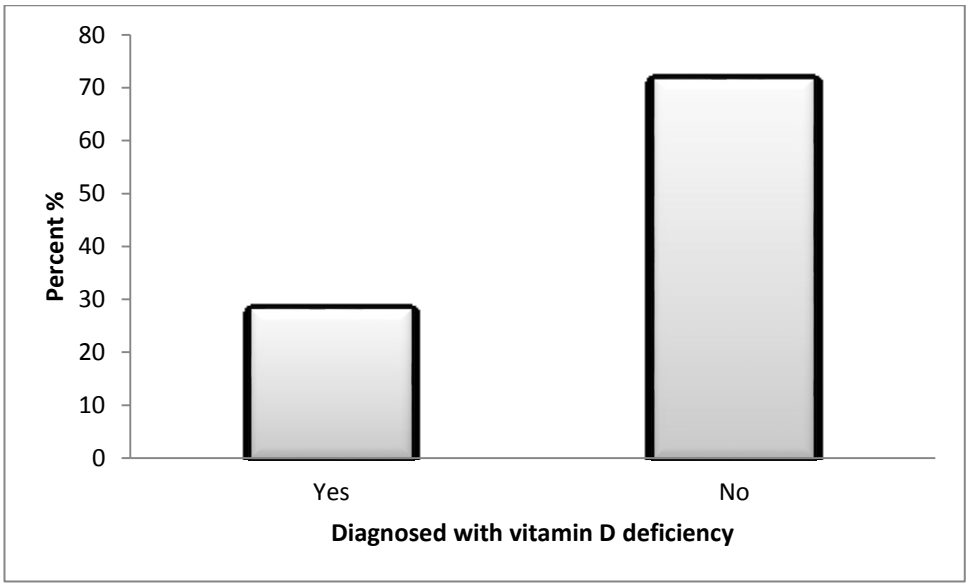

Figure 20 Percent of participants who had been diagnosed with vitamin d deficiency, n=145.

Participants were asked directly if they ever have been diagnosed with vitamin D deficiency. Of the study population 28% stated that they were clinically diagnosed with vitamin D deficiency at some time in their life (Figure 20).

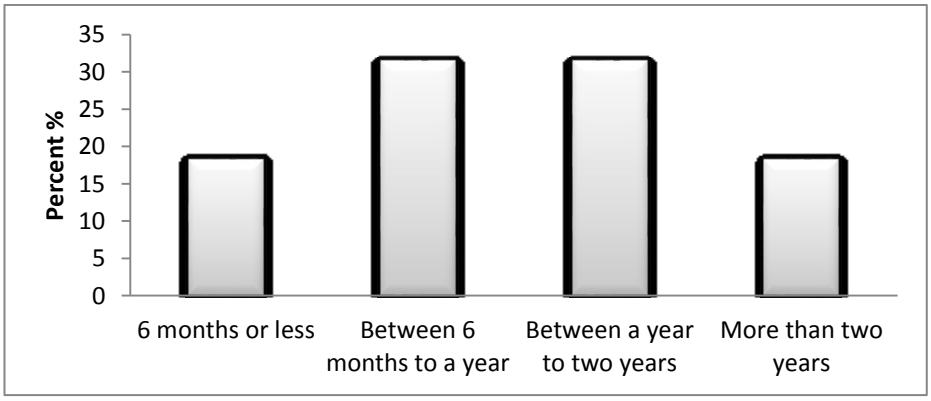

Figure 21 Distribution of participants by the time of being diagnosed with vitamin D deficiency, n=38.

Figure 21 illustrates those who had vitamin D deficiency according to their period of diagnosis. 31.6 % were first diagnosed with vitamin D deficiency two years ago, or more

than two years ago, and the same proportion found out they had vitamin D deficiency a year ago, or less than a year ago.

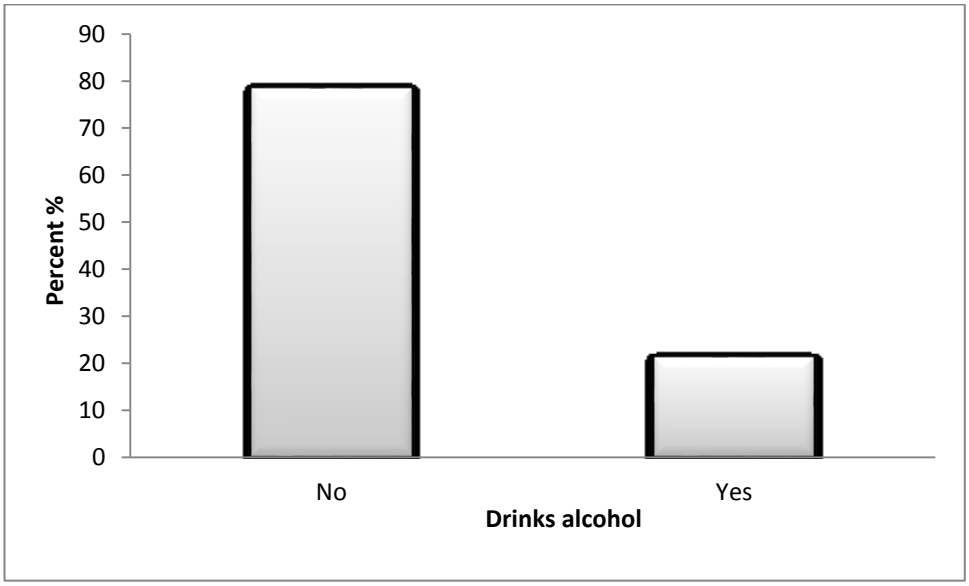

Figure 22 Percent of participants who consume alcoholic drinks, n=145.

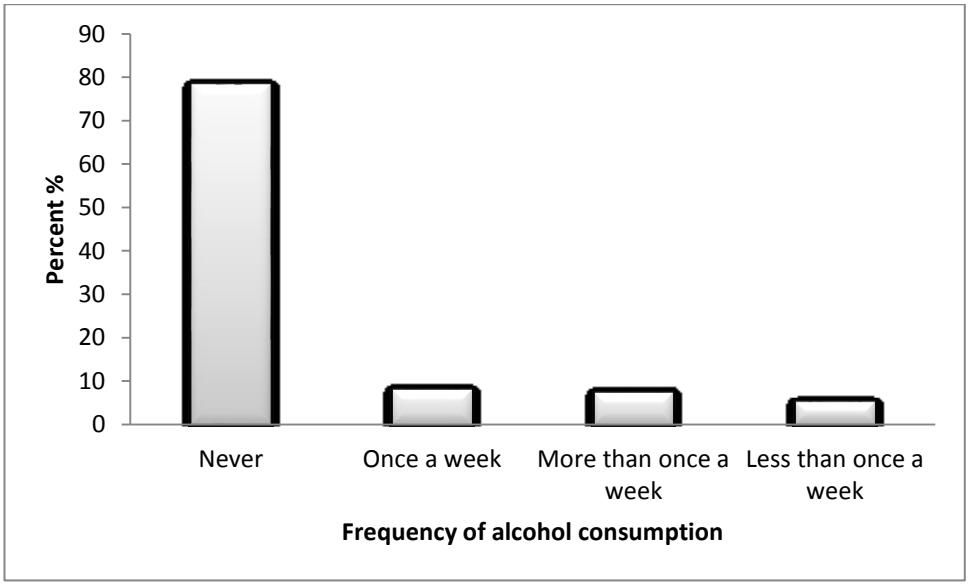

Figure 23 Frequency of alcohol consumption among the participants, n=145.

There is a low percentage of alcohol consumption among the applicants, and the majority of the study participants stated that they do not drink alcohol (Figure 22). Figure 23 shows how often participants consume alcohol.

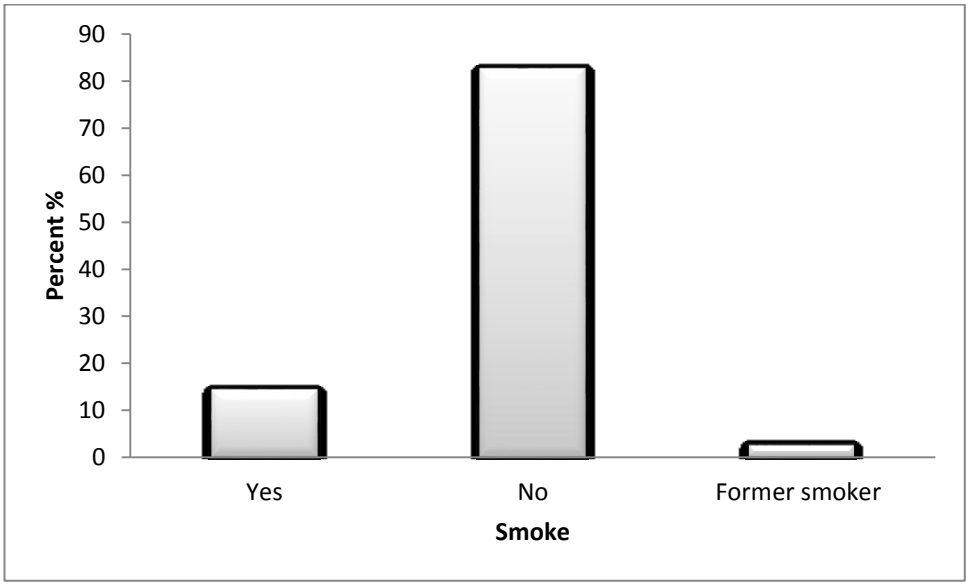

Figure 24 Distribution of participants smoking status, n=145.

The smoking habit of the study participants as identified by the participants themselves in their questionnaire responses shows that the majority of them were non-smoker (83%), whereas 17% stated that they smoke or used to smoke (Figure 24).

4.3 Distribution by study groups.

The above descriptions cover the entire participant population. However, this study aimed to compare three different populations and, hence, data was divided into these three categories and compared to determine whether the three groups were comparable (see Table 17). Details of the three study populations included in this research are given in Table 17. The values in the table are presented as mean ±SD or percentages (frequency).

Table 17 Data descriptions within the groups.

Variables	Groups			Total population
	KSA	UK Covered	UK Uncovered	
Study population, % (n)	34.5 (50)	34.5 (50)	31.0 (45)	100 (145)
Age range , % (n)				
18-30	21.4(31)	21.4 (31)	26.9 (39)	69.7(101)
31-45	11.7(17)	11.7 (17)	2.8 (4)	26.2(38)
> 45	1.4 (2)	1.4 (2)	1.4 (2)	4.1(6)
Weight kg, mean ±SD*	62.7±13.5	62.4±12.3	57.8±10.3	61.1±12.3
Height cm, mean ±SD*	159.6±6.1	161.6±6	164.3±7.1	161.8±6.6
BMI Range, % (n)				
<18.5	4.1 (6)	2.8(4)	4.8 (7)	11.7 (17)
18.5-24.9	17.2(25)	20.0(29)	22.8(33)	60 (87)
25 - >30	9.0(13)	8.3(12)	2.8 (4)	20.0(29)
≥30	4.1(6)	3.4(5)	0.7(1)	8.3 (12)
Skin colour, % (n)				
I	(0)	0.7(1)	3.4 (5)	4.1(6)
II	2.1(3)	2.1(3)	8.3(12)	12.4(18)
III	7.6(11)	9.0(13)	9.0 (13)	25.5(37)
IV	12.4(18)	15.2(22)	8.3(12)	35.9(52)
V	11.0(16)	5.5(8)	1.4(2)	17.9(26)
VI	1.4(2)	2.1(3)	0.7(1)	4.1 (6)
Has children, % (n)				
Yes	18.6(27)	15.9(23)	4.8(7)	39.3 (57)
No	15.9(23)	18.6(27)	26.2(38)	60.7 (88)
Number of children, % (n)				
1	8.8 (5)	1.8 (1)	3.5(2)	14(8)
2	12.3(7)	21.1(12)	7.0(4)	40.4(23)
3	5.3(3)	10.5(6)	1.8(1)	17.5(10)
≥4	21.1(12)	7.0(4)	(0)	28.1(16)
Alcohol consumption, % (n)				
Yes	(0)	(0)	21.4(31)	21.4(31)
No	34.5(50)	34.5(50)	9.7(14)	78.6(114)
Smoking, % (n)				
Yes	4.8(7)	4.8(7)	4.8(7)	14.5(21)
No	29.7(43)	29.7(43)	23.4(34)	82.8(120)
Former smoker	(0)	(0)	2.8(4)	2.8(4)

Variables	Groups			Total population
	KSA	UK Covered	UK Uncovered	
Taking multivitamin supplements, % (n)				
Yes	9.7(14)	9.7(14)	9.0(13)	28.3(41)
No	20.0(29)	16.6(24)	17.9(26)	54.5(79)
Sometimes	4.8(7)	8.3(12)	4.1(6)	17.2(25)
Reasons for taking supplements, % (n)				
Preventive	15.4(10)	26.2 (17)	24.6(16)	66.2(43)
Therapeutic	16.9(11)	12.3(8)	4.6(3)	33.8(22)
Diagnosed with vitamin D deficiency, % (n)				
Yes	8.3(12)	17.9(26)	2.1(3)	28.3 (41)
No	26.2(38)	16.6(24)	29.0(42)	71.7 (104)
Vegetarian, % (n)				
Yes	(0)	(0)	1.4 (2)	1.4 (2)
No	34.5(50)	34.5(50)	29.7(43)	98.6(143)
Using products fortified with vitamin D, % (n)				
Yes	2.1(3)	2.8(4)	3.4(5)	8.3(12)
No	8.3(12)	0.7(1)	5.5(8)	14.5(21)
Sometimes	4.8(7)	20(29)	12.4(18)	37.2(54)
Don't know	19.3(28)	11 (16)	9.7(14)	40.0(58)
Changed diet over the last 12 months, % (n)				
Yes	20.0(29)	13.1(19)	9.7(14)	42.8(62)
No	14.5(21)	21.4(31)	21.4(31)	57.2(83)
Weather condition, % (n)				
Cloudy	(0)	20.7(30)	12.4(18)	33.0(48)
Cloudy/Cold	(0)	2.1 (3)	1.4(2)	3.5(5)
Cloudy/Rainy	(0)	6.2(9)	4.8(7)	11.0(16)
Dark/Cold	(0)	(0)	3.5(5)	3.5(5)
Partly cloudy	(0)	4.8(7)	0.7 (1)	5.5 (8)
Rainy/Cold	(0)	0.7(1)	2.8(4)	3.5(5)
Sunny/Cold	(0)	0.7(1)	4.8(7)	5.5(8)
Sunny/Hot	34.5(50)	(0)	(0)	34.5(50)
Milk consumption habit				
Never	2.8(4)	2.1(3)	0.7(1)	5.5(8)
Monthly	9.0(13)	4.1(6)	2.1(3)	15.2(22)
Weekly	12.4(18)	14.5(21)	9.7(14)	36.6(53)
Daily	10.3(15)	13.8(20)	18.6(27)	42.8(62)

Variables	Groups			Total population
	KSA	UK Covered	UK Uncovered	
Average amount of consumed milk				
None	2.1 (3)	2.8 (4)	(0)	4.8 (7)
Less than 250 ml (1 large cup or less)	7.6 (11)	20.0 (29)	17.9(26)	45.5 (66)
Between 250 and 500 ml (1-2 cups)	23.4 (34)	9.0 (13)	8.3 (12)	40.7 (59)
Between 500 and 750 ml (2-3 cups)	0.7 (1)	1.4 (2)	4.8 (7)	6.9 (10)
750 ml (3 cups) or more	0.7 (1)	1.4 (2)	(0)	2.1 (3)
Type of milk				
Full cream	17.9(26)	9.0(13)	2.8(4)	29.7(43)
Skimmed	2.8(4)	8.3(12)	5.5(8)	16.6 (24)
Semi-skimmed	13.8 (20)	15.9 (23)	21.4 (31)	51.0 (74)
Soya	(0)	1.4 (2)	1.4 (2)	2.8 (4)
***SD:** standard deviation				

The table shows that the numbers in the three study populations were similar. The age range between the three groups was 18-30 years. However, the highest age mean went to the KSA group (29.32± 9.15 year olds), and the youngest age mean went to UK uncovered (24.29±8.39 year olds). The KSA group were the shorter (159.6±6.15 cm) among the study population. The study population had, in general, a healthy BMI. The UK uncovered participants had the highest percentage 22.8%(n=33) of healthy BMI range, followed by the UK covered 20%(n=29). The KSA participants had the lowest range of healthy BMI 17.2% (n=25), and the highest range of unhealthy BMI 17.2%(n=25).

The skin colours were varied between the three groups. However, III, IV and V were the commonest skin colours in the KSA group 31%(n=45) and the UK covered group 29.7%(n=43), while II, III and IV were the commonest skin colours in the UK uncovered group at 25.6% (n=37). The Saudi women stated the highest frequencies of having children 18.6% (n=27). Besides, they also yielded the highest frequencies in the range of number of children, whereby 21.1%(n=12) stated they have 4 or more children.

Alcohol consumption for the two covered groups was zero, and only 21.4%(n=31) of the UK uncovered group stated they drank alcohol. Smoking habits were similar within groups.

The three groups reported low use of food supplements: KSA covered 20.0% (n=29), UK covered 16.6% (n=24), and UK uncovered 17.9% (n=26). The most reported reason for using supplements was preventive: KSA covered 15.4%(n=10), UK covered 26.2% (n=17), and UK uncovered24.6%(n=16), which meant the use was without doctor's prescription. 33.8%

(n=22) reported supplement use as therapeutic and the highest frequencies of these were KSA Covered 16.9% (n=11).

Participants were asked if they have been diagnosed with vitamin D deficiency. This question was intended to exclude affected people from the blood analysis. Most respondents were not diagnosed with vitamin D deficiency before: 71.7%(n=104). However, from the 28.3%(n=41) that were aware of a deficiency, 17.9%(n=26) was from the UK covered group.

Of the population 1.4%(n=2) were vegetarian, which was reported within UK uncovered group, while none of the covered groups in either country reported themselves as vegetarian. 40.0% (n=58) of the participants were uncertain about whether they were using vitamin D fortified products, of which 19.3% (n=28) were KSA covered, 11.0% (n=16) UK covered, and 9.7% (n=14) UK uncovered. Most participants who have changed diet over the last 12 months were from the KSA covered group 20.0% (n=29). The majority of UK groups stated that the weather conditions, when the data was collected, were cloudy and cold, whereas, in the KSA group, all of the participants declared the weather was always sunny and hot.

Generally, milk consumption was common on a daily basis (42.8%, n=62), and weekly basis (36.6%, n=53). UK groups reported the highest habits of consumption. The milk consumption habits of the UK covered group were slightly equal on both a daily and weekly basis - 13.8% (n=20) and 14.5% (n=21) respectively. The UK uncovered group reported the highest of daily consumption of milk at 18.6% (n=27). Habits of milk consumption were less common among the Saudi group.

Concerning the average portion of consumed milk, UK groups reported that they consume an average portion equal to a cup or less of milk. The Saudi group reported one to two cups as the normal average portion of milk. The most common type of milk among UK groups was semi-skimmed milk - 15.9% (n=23) of the covered group and 21.4% (n=31) of the uncovered group. 17.9% (n=26) of the Saudi group reported full cream milk as the most common type of milk.

4.4 Summary of descriptive results

This chapter described the study's population from how the participants were recruited initially to the final characteristic differences between the three studied groups. The chapter began by providing general informative results to cover the entire participant population of the research, before a general description was provided to compare the three study groups.

The result of the current chapter showed that the KSA group presented the highest average age, and the lowest average of healthy BMI. In the UK, the uncovered group was the only group drank alcohol, although smoking habits was the same between the three groups. All the groups reported low use of multivitamin supplements. However, the covered group from the UK was shown to be the majority of those individuals who had been diagnosed previously with vitamin D deficiency. The KSA group had the highest percentage of individuals who were not sure whether they consumed vitamin D fortified products, and they were also the lowest in milk consumption. Therefore, all these descriptive characteristics help to produce a clearer understanding of the study groups.

5 Determination of vitamin D intake in healthy KSA women, UK covered women and UK uncovered women: comparison of methods.

5.1 Introduction

This chapter is intended to answer the following questions:

- Is there a difference in dietary intake measured by FFQ and food diary?
- What is the degree of agreement between the results of the two methods of dietary assessment (FFQ and food diary)?

5.1.1 Assessment of the FFQ

The FFQ contained a list of twenty-one foods items, which were identified by Holick (2007) as rich in vitamin D, and a selection of 7 categories ranging from "never" to "twice or more per day". FFQ results were reported as frequencies and percentages to identify commonly consumed food items on the list. By looking at the frequency and how frequencies of common and less common foods vary across the groups, to reduce error, due to quantification and error of composition.

These seven categories were condensed in the results into four, as this was considered to present a clearer and easier system for the reader to follow. The first category "never" remained the same, and then every two categories were condensed into one, as indicated below.

The original categories			**The new category**
"Never"			Never
"Less than once a month"	And	"1-3 times a month"	Monthly
"Once a week"	And	"2-4 times a week"	Weekly
"Once a day"	And	"Twice or more a day"	Daily

In addition, to estimate the amount of vitamin D intake, the reported frequency of consumption for each food item was multiplied by the vitamin D content in the food item per average portion, and then summing up the results. For example, the average portion of cooked egg has vitamin D content of 1.1 µg, and if a participant chose 2-4 times a week as frequencies of consumption, thus, 1.1 will multiply by the score of this category, which is 3. The same calculation was done for all twenty-one food, and subsequently the results were determined for each participant.

Nutrition analysis software "Nutritics" was used to evaluate the average portion and approximate content of vitamin D in certain servings (see Table 18). To obtain the weekly

average consumption of vitamin D, participants' frequencies of consumption were scored as follows:

Participants' frequencies of consumption	**Score**
Never	0.00
Less than once a month	0.25
1-3 times a month	0.50
Once a week	1.00
2-4 times a week	3.00
Once a day	7.00
Twice or more a day	14.00

Then, the results were divided by seven to yield the daily average of vitamin D.
Vitamin D intake from multivitamin supplements was calculated separately. The frequency of consumption of the supplements was recorded as the following: regular for participants who take supplements daily, and irregular for participants who take supplements in weekly and monthly basis, see appendix 2.

Table 18 Average portion for FFQ food items and vitamin D content based on Nutritics assumptions.

Food items	**Average portion** (g)	**Vitamin D content** (μg)	**Vitamin D content** (IU)
Cod liver oil	12	25.0	1000
Liver, cooked	85	0.8	32
Fresh salmon	122	11.2	448
Fresh sardines, cooked	85	10.5	420
Fresh mackerel, cooked	80	7.0	280
Fresh tuna, cooked	85	7.5	300
Herring, cooked	85	13.7	548
Canned salmon, drained	51	11.8	472
Canned sardines, drained	109	5.0	200
Canned mackerel, drained	109	6.1	244
Canned tuna, drained	45	1.6	64
Eggs, cooked	60	1.1	44
Fresh mushrooms	28	0.2	8
Sun-dried mushrooms	28	0.7	27
Milk	207	2.2	88
Processed orange juice	181	1.9	76
Yogurt, plain	125	0.1	4
Butter	12	0.1	4
Margarine	11	1.2	48
Cheese	45	0.1	4
Breakfast cereal	60	2.2	88
Multivitamin supplements	1	10.0	400

5.1.2 Assessment of the food diary

The response for the food diary was lower than the other methods as expected. Forty-nine full food diaries were returned for analysis. In the UK, thirty-one food diaries were returned, thirteen journals were from uncovered participants and eighteen journals came from covered participants. The KSA covered participants returned eighteen full food diaries for analysis. The Nutritic software was used to assess related intake of nutrients. Then, SPSS was used to test and compare the results statistically.

5.2 FFQ results

The list of vitamin D rich food contains 21 food items, and not all the items are equally common in everyday food. Therefore, the food list was divided into two categories: common food products, which are often consumed every day, and less common food products, which are consumed less often. The common food items included eggs, milk, processed orange juice, yogurt, butter, margarine, cheeses, and breakfast cereals, whereas less common food items included cod liver oil, liver, fresh salmon, fresh sardines, fresh mackerel, fresh tuna, herring, canned salmon, canned sardines, canned mackerel, canned tuna, fresh mushrooms, and sun-dried mushrooms. Thus, the frequency of consumption for vitamin D rich foods in daily, monthly and yearly frequencies can be reported, see Table 19.

Table 19 Frequency of vitamin D food items intake, n=145.

	Food items	**Never**%(n)	**Monthly**%(n)	**Weekly**%(n)	**Daily**%(n)
Less common foods	Cod liver oil	82.8(120)	10.3(15)	1.4(2)	5.5(8)
	Liver	46.9(68)	49.0(71)	3.4(5)	0.7(1)
	Fresh salmon	48.3(70)	39.3(57)	12.4(18)	0
	Fresh sardines	84.1(122)	13.1(19)	2.8(4)	0
	Fresh mackerel	76.6(111)	18.6(27)	4.8(7)	0
	Fresh tuna	63.4(92)	28.3(41)	6.9(10)	1.4(2)
	Herring	91.0(132)	8.3(12)	0.7(1)	0
	Canned salmon	83.4(121)	13.1(19)	3.4(5)	0
	Canned sardines	88.3(128)	11.0(16)	0.7(1)	0
	Canned mackerel	88.3(128)	9.0(13)	2.8(4)	0
	Canned tuna	16.6(24)	48.3(70)	35.2(51)	0
	Fresh mushrooms	39.3(57)	43.4(63)	15.9(23)	1.4(2)
	Sun-dried mushrooms	77.2(112)	16.6(24)	4.1(6)	2.1(3)
	Multi-vitamin supplements	55.2 (80)	8.3(12)	2.1(3)	34.5(50)
Common foods	Eggs	2.8(4)	32.4(47)	55.9(81)	9.0(13)
	Milk	5.5(8)	15.2(22)	36.6(53)	42.8(62)
	Processed orange juice	26.2(38)	31.0(45)	31.0(45)	11.7(17)
	Yogurt, plain	2.8(4)	30.3(44)	46.9(68)	20.0(29)
	Butter	13.1(19)	33.1(48)	40.0(58)	13.8(20)
	Margarine	46.9 (68)	22.1(32)	21.4(31)	9.7(14)
	Cheese	6.2 (9)	11.7 (17)	35.2 (51)	46.9 (68)
	Breakfast cereal	23.4 (34)	33.1 (48)	24.8 (36)	18.6(27)

Table 19 shows that consumption of common food products was frequently higher on a daily, weekly, and monthly basis, while consumption of less common food products was reported to be frequently higher on a monthly basis. For the common foods, cheese, milk and yogurt (46.9% 42.8% 20.0%, respectively) were reported as the foods most likely to be consumed daily. For the less common food products, the most frequently reported foods on a monthly basis were liver, canned tuna, fresh mushrooms, and fresh salmon (49.0%, 48.3%, 43.4%, and 39.3%, respectively). Although fish liver oil is one of the best sources of Vitamin D, the vast majority of women (82.8%) never take it. According to the results reported, in fact, it seems that fish is not commonly consumed.

Generally, the consumption of vitamin D-rich common foods was reported to be high on a weekly basis, where all common food items were reported with high frequency: eggs (55.9%), yogurt (46.9%), butter (40.0%), milk (36.6%), cheese (35.2%), processed orange juice (31.0%), breakfast cereal (24.8%), and margarine (21.4%). Canned tuna (35.2%) was

the most reported item on a weekly basis from the uncommon food products, followed by fresh mushrooms (15.9%) and fresh salmon (12.4%).

More than half the participants (55.2%) did not use supplements, whilst about one third of them did regularly (34.5%). The majority of those who take supplements used them regularly on a daily basis, 50 women.

5.2.1 Estimated daily vitamin D intake from the FFQ

The consumption frequency for foods rich in vitamin D was described above. Here, daily consumptions of vitamin D are investigated.

Table 20 shows the means for vitamin D intakes for three groups are compared using a one-way ANOVA. Since the initial result showed that the assumption of homogeneity (constant variance) is violated, Welch's F-statistics were used, and a Games-Howell test was adopted for multiple pairwise comparisons.

Table 20 Estimation of daily vitamin D intakes, n=145.

Variables	**KSA** mean±SD	**UK covered** mean±SD	**UK uncovered** mean±SD	**Total of population** mean±SD	**P-value**
Daily vitamin D intake for common food, µg/day	2.5±1.7	3.8±3.4	4.5±2.8	3.6±2.8	<0.01
Daily vitamin D intake for less common food, µg/day	1.2±2.1	5.3±9.4	4.1±5.7	3.5±6.6	<0.01
Daily intake of vitamin D from supplements, µg/day	3.5±4.7	3.9±4.9	3.2±4.6	3.5±4.7	0.24
Total Daily vitamin D intake (food), µg/day	3.6±3	9.2±11	8.6±6.5	7.1±7.8	<0.01
Total Daily vitamin D intake (food & supplements), µg/day	7.1±5.6	13.0±12.9	11.7±8.6	10.6±9.8	<0.01

Table 20 shows that the total vitamin D intake from food for the study groups was 7.1±7.8 µg/day. However, UK groups (covered 9.2±11 µg/day and uncovered 8.6±6.5 µg/day) had higher vitamin D intakes from food compared to the KSA group (3.6±3 µg/day). The KSA group consumed most of their daily vitamin D intake from common food products such as milk, yogurt, cheese and eggs. The UK covered group reported that most of their daily consumption of vitamin D came from less common food products such as fresh and canned oily fish, whereas the UK uncovered group reported that their daily vitamin D intake came equally from both food categories.

A one-way ANOVA was run to test total daily vitamin D intake from food in the study's population, Welch's $F(2, 75.5)= 15.2$, p-value<0.01 indicated that there was a statistically significant difference in intakes of vitamin D between the three groups. A post-hoc comparison using a Games-Howell test was conducted. The results show that KSA vitamin D intake has a significantly negative relationship with UK groups' intake at p-value <0.05.

A one-way ANOVA for daily vitamin D intake from common and uncommon food items indicated a statistically significant difference between the three groups, Welch's $F(2, 84.6)= 10.5$, p-value< 0.01 and Welch's $F(2,71.1)= 9.1$, p-value< .01 respectively. The Games-Howell post-hoc test revealed that KSA group had a significantly lower difference with both UK groups. However, both there was little difference between intakes for UK groups.

The three groups had similar daily intake of vitamin D from supplements with a total average of 3.5±4.7 µg/day. An ANOVA test shows there is no significant difference between the groups - p-value= 0.24.

Generally, it was noted that the KSA group consumed most of their daily vitamin D intake from common food products such as milk, yogurt, cheese and eggs, see Table 7. In contrast, the UK covered group reported that most of their daily consumption of vitamin D came from less common food products such as fresh and canned oily fish, whereas the UK uncovered group reported that their daily vitamin D intake came equally from both food categories.

Table 20 show that most of the vitamin D intake of the UK groups comes from foods more than from vitamin supplements, whereas the KSA group's vitamin D intake from supplements was equal to their vitamin D intake from food.

5.3 Vitamin D dietary intake of the population using a food diary

The food diary plays an important role in the evaluation and the validation of the FFQ results. In the following section, Kcal, carbohydrate, protein, fat, calcium and vitamin D intakes were investigated.

Table 21 Vitamin D and other daily nutrient intake of the population using a food diary, n= 49.

Variables Per day	**KSA** mean±SD	**UK covered** mean±SD	**UK uncovered** mean±SD	**Total of population** mean±SD	**P-value**
Energy, Kcal	1142±621	1175±296	1288±467	1193±475	0.7
Carbohydrate, g	128.4±61.1	138.1±30.6	160.7±64.1	140.5±53.4	0.4
Protein, g	53.2±27.9	48.4±15.2	59.4±20.9	53.1±22.1	0.4
Fat, g	49.4±34.1	51.9±19.6	48.6±18.4	50.2±25.2	0.9
Calcium, mg	489.7±294.4	533.9±173.8	558.1±296.8	524.1±253.4	0.8
Vitamin A, µg	624.4±1296.4	441.4±264.7	518.4±427.3	529.0±819.7	0.8
Vitamin D, µg	1.4±1.3	1.0±1.0	3.3±3.2	1.7±2.0	0.03

Table 21 illustrates the average daily intake of energy, carbohydrate, protein, fat, calcium, and vitamin D for the three groups in the study. The focus of the assessment was vitamin D intake, which was assessed to be very low in all three groups, with daily total averages of 1.7±2.0 µg. An ANOVA test indicated statistically significant differences between the three groups - Welch's $F(2, 21.850)=3.945$, p-value=0.03. A Games-Howell post-hoc test revealed that the difference was mainly between the UK uncovered group and both covered groups, with a p-value <0.05.

Table 21 clearly demonstrates that not only was vitamin D low, all recorded nutrients intakes were low and possibly underestimated. The total energy intake was 1193±475 Kcal, and the KSA reported lowest average energy intake of 1142±621 Kcal, followed by the UK covered group at 1175±296, and the UK uncovered group, which had the highest energy intake of 1288±467 Kcal. However, the difference was not great, and was insignificant between the groups, as supported by an ANOVA test: $F(2,46)=0.367$, p-value= 0.7 . Carbohydrate, protein, fat, vitamin A and calcium were low too compared with population recommendations. However, there were no statistical differences between the study groups for these nutrients – Carbohydrate: Welch's $F(2,24.239)=0.997$, p-value=0.4, Protein: $F(2,46)=0.946$, p-value= 0.4, Fat $F(2,46)=0.076$, p-value= 0.9, vitamin A: $F(2,46)=0.218$, p-value= 0.8 and Calcium $F(2,46)=0.288$, p-value= 0.8.

5.4 Testing the results of agreements for the FFQ and food diary using a Bland-Altman plot

A Bland-Altman plot was used to compare the agreement of two measurements of vitamin D intake (Food diary and FFQ) (Bland and Altman , 1986 ; Giavarina, 2015). However, the Bland-Altman plot only define the intervals of the agreement, the acceptable limits specify based on the study considerations(Bland and Altman , 1986 ; Giavarina, 2015).

The data from 48 participants who had completed in both the FFQ and food diary were used to visually evaluate the agreement between these two techniques. To produce a Bland-Altman plot, the difference of the two measurements used (FFQ and food diary) plot against the average of the two measurements. To find the limit of agreements, the mean difference and standard deviation (SD) of the differences between the two methods were calculated. Then, the upper limit of agreement was calculated, the mean+ (1.96*SD), and the lower limit of agreement was calculated, mean- (1.96*SD). IBM SPSS Statistic 21 was used to perform the Bland-Altman plot. Since the distributions of the two techniques were not normally distributed, log transformation was used to approximate normality.

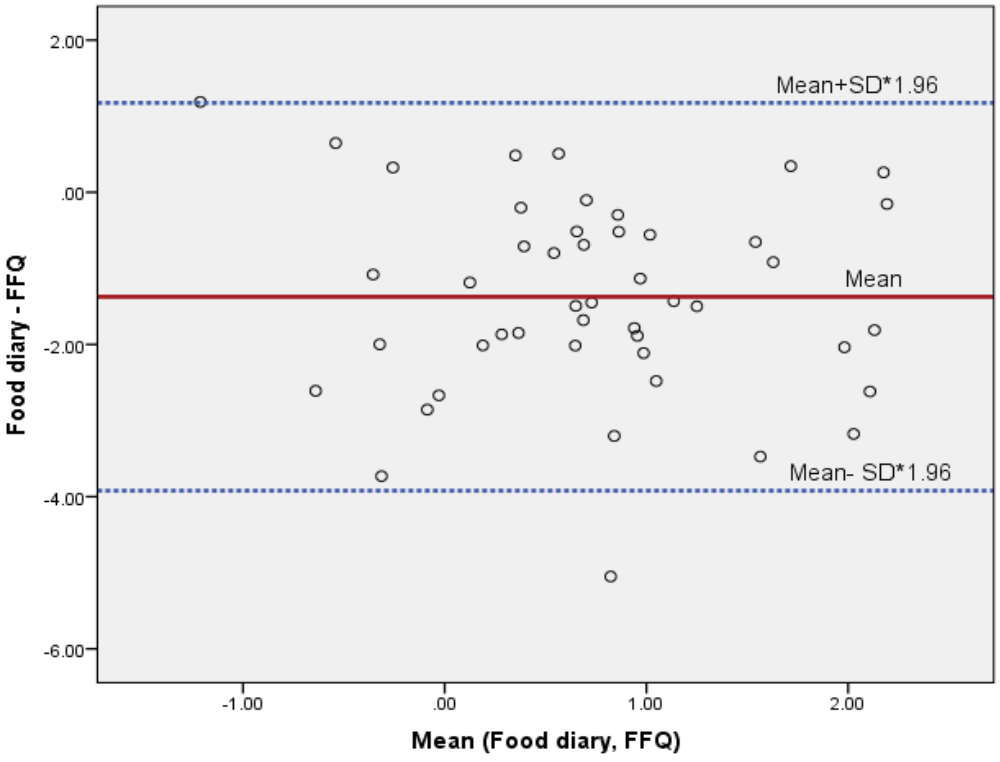

Figure 25 Bland-Altman plot for food diary and FFQ

Inspection of the Bland-Altman plot shows the following:

- The mean of the differences was -3.93 µg, which is not zero. This could mean, on average, the FFQ technique measures 3.93 µg more than the food diary technique.
- A negative mean indicates that the food diary underestimates vitamin D intake.
- There is no consistent variability (no clear trend) across the range of measurements.
- The 95% limit of agreement was defined by the study to be large as the current difference could change the clinical status of the patient. According to that the current study believes that the limit of agreement between the two methods seems to be weak, which indicates that the two methods are likely to be not equivalent.

5.5 Summary of vitamin D intake assessment

This chapter presented the participants' daily, weekly and monthly consumption of common vitamin D rich foods food. Overall, dairy products such as cheese, milk and yogurt were noted as the most likely to be consumed, whereas fish liver oil was reported as the least likely to be consumed by the participants.

The participants' vitamin D intake was estimated and compared through two methods, FFQ and food diary. Both methods reported low vitamin take for the three groups, although the food diary method reported lower vitamin D intake than the FFQ method. Finally, the Bland-Altman plot was used to compare the two methods agreement, and the key outcome was that the two measures of dietary vitamin D intake had low agreement.

6 Influence of lifestyle factors in vitamin D status in women.

6.1 Introduction

Factors that can reduce vitamin D synthesis in the body include: skin colour, clothing, suncream, indoor lifestyles, country location, weather, and vitamin D intake.

This chapter will examine participants' sun exposure habits and other related factors.

This chapter will answer the following questions:

- Is there an association between study groups in terms of their usual sun exposure habits?
- Is there an association between study groups in terms of their holiday sun exposure habits?

6.2 Results

The sun exposure questionnaire included two sets of questions (see appendix2). The first set was to identify usual habits of sun exposure, and the second set of questions concerned holiday sun exposure in the six months prior to the data collection.

Analysis of usual sun exposure habits was done by dividing the daytime periods that were recorded in the questionnaire into two groups: off-peak time and peak time. The off-peak times represented times of the day where UV radiation is normally low, and peak times represented the times of the day where UV radiation is normally high.

The length of sun exposure for each time group (peak time and off peak time) was calculated by adding up the reported times that were stated by the participants themselves. This was done as below:

Off-peak time = recorded times in the period (7am-9am) + recorded times in the period (3pm-5pm) + recorded times in the period (5pm-7pm).

Peak time = recorded times in the period (9am-11am) + recorded times in the period (11am-1pm) + recorded times in the period (1pm-3pm).

To estimate exposed adult body surface area (BSA), an established method called the "rule of nines" was used (see section 3.6.1.3)

The amount of sun needed to meet the recommended requirements for vitamin D varies significantly depending on location, weather conditions, the time of day (peak or off-peak),

and skin type. More cloud cover, as is the case in the UK, can result in less UV radiation. The optimal time to be in the sun for vitamin D production is approximately between 10:00 am and 2:00 pm. The amount of vitamin D from exposure to the sun can be affected by either use of clothing to cover exposed areas, or by sunscreen products, while activities such as sunbathing enhance the amount of vitamin D in the body.

The interaction between these factors of interest can result in different amounts of vitamin D between the study groups. A one-way ANOVA statistical test was employed to investigate any differences between the three groups (KSA covered, UK covered and UK uncovered). However, if the assumption of Levene's test of homogeneity of variances is violated, then Welch's adjusted F ratio was alternatively used.

For nominal variables, e.g., sunscreen use (yes/no), a Chi-square test was used to find the relationship between the three groups. If the assumption of the Chi-square test is violated, and the expected cell count was less than 5, Fisher's exact test was used as an alternative.

Table 22 Comparison of usual sun exposures across the three study groups using one-way ANOVA and Chi-square, n=145.

Usual sun exposure	**KSA covered**	**UK covered**	**UK uncovered**	**Total of population**	**P-value**
Sun exposure peak time hours per day, mean ±SD*	2.3 ±2.8	3.0 ±2.4	1.2 ±1.4	2.2 ±2.4	<0.05
Sun exposure off-peak time hours per day, mean ±SD*	1.3 ±1.3	1.6 ±1.5	0.9 ±0.7	1.3 ±1.3	<0.05
Fraction of average exposed BSA to sunlight in peak time, mean ±SD***	0.11 ±0.04	0.09 ±0.03	0.14 ±0.09	0.11 ±0.06	<0.05
Fraction of average exposed BSA to sunlight in off-peak time, mean ±SD*	0.12 ±0.04	0.1 ±0.06	0.15 ±0.09	0.12 ±0.07	0.001
Total sun index hours per week, mean ±SD*	4.3 ±4.2	5.9 ±4.6	6.0±12.9	5.4 ±8.0	0.5
Usual clothing, % (n)					
Uncovered	(0)	(0)	33.3(64)	33.3(64)	
Covered	9.9(19)	35.9(69)	(0)	45.8(88)	0.05
Veiled	18.2(35)	2.6(5)	(0)	20.8(40)	
Sun screen, % (n)					
Yes	9.7 (14)	3.4%(5)	4.8(7)	17.9(26)	
No	24.8 (36)	29.0(42)	26.2%(38)	80.0(116)	0.03
Sometimes	(0)	2.0(3)	(0)	2.0(3)	
Sunbathing, % (n)					
Yes	4.1(6)	0.7(1)	14.5(20)	19.3(27)	<0.01
No	30.4(44)	33.8(49)	16.6(24)	80.7(117)	

Usual sun exposure	KSA covered	UK covered	UK uncovered	Total	P-value
How often sunbathing, % (n)					
1-2 times a month	14.8(4)	3.7(1)	37.0(10)	55.5(15)	
More than twice a month	3.7(1)	(0)	25.9(7)	29.6(8)	0.9
Only on holidays	3.7(1)	(0)	11.1(3)	14.8(4)	
Usually clothed when sunbathing or using a sunbed, % (n)					
Nothing	(0)	(0)	11.1(3)	11.1(3)	
Bikini	(0)	3.7(1)	55.6(15)	59.3(16)	<0.01
Bra and shorts	11.1(3)	(0)	3.7(1)	14.8(4)	
T-shirt and shorts	3.7(1)	(0)	3.7(1)	7.4(2)	
T-shirt and long leggings	7.4(2)	(0)	(0)	7.4(2)	
Sunscreen use when sunbathing, % (n)					
Yes	10.7(3)	(0)	32.2(9)	42.9(12)	
No	10.7(3)	3.6(1)	25.0(7)	39.3(11)	0.6
Sometimes	(0)	(0)	17.9(5)	17.9(5)	

*__SD:__ standard deviation

** The BSA is a ratio

6.3 Usual sun exposure

6.3.1 Sun exposure at peak times

Table 22 shows the reported average sun exposure of the study population in peak time periods, which was between 9am to 3pm, and was 2.2±2.4 hour/day. However, UK covered women reported the highest average time of being out during the peak time periods with a mean of 3.0±2.4 hour/day. The reported time for the Saudi group put them second in terms of sun exposure during peak time, with a mean of 2.3±2.8 hours/day. The UK uncovered group reported the lowest exposure time during peak time periods, with a mean of 1.2±1.4 hours/day.

Using a one-way ANOVA, there was a significant difference in average hours of peak time exposure between the three groups, $F(2, 90.2)= 11.14$, p-value<.05. A post-hoc comparison using a Games-Howell test was conducted. The results show that the average hours of sun exposure during peak time for UK uncovered (1.2±1.4 hours/day) women exhibit a negative statistically significant difference from the two other groups - UK covered (3±2.4 hours/day) at p-value<0.05, and the Saudi group (2.3±2.8 hours/day) at p-value=0.05.

6.3.2 Sun exposure for off-peak time

The total reported average for sun exposure at off-peak time, which was considered the periods between 7am-9am and 3pm-7pm, was 1.3±1.3 hours/day and the total range was between 0-6 hours. Despite the general low sun exposure of all the study groups for these periods, UK covered women still reported the maximum amount of sun exposure with a mean 1.6±1.5 hour/day; the Saudi group came second with a mean of 1.3±1.3/day. Finally, the UK uncovered group reported the minimum amount of sun exposure time during off-peak periods (mean 0.9±0.7 hours).

A one-way ANOVA was also used to compare the study's three groups' average off-peak sun exposure time. However, the assumption of Levene's test of homogeneity of variances was violated, $F(2, 142)= 17.5$, p-value>0.05, therefore, Welch's adjusted F ratio was used instead.

The one-way ANOVA for the groups' average hours of off-peak time exposure yielded a statistically significant difference, Welch's $F(2, 86)= 5.3$, p-value<0.05, which indicated that the groups' average hours of off-peak time exposure was contradictory.

To identify which pairs of the study groups' means differed significantly, a Games-Howell post-hoc test, was used. The results revealed that the average hours of sun exposure during the off-peak time for the UK covered (1.6±1.5 hour/day) and UK uncovered (0.9±0.7 hour/day) groups differed significantly at p-value=0.008; the Saudi group's sun exposure during peak times (1.3 ±1.3 hour/day) was not significantly different from the other two groups - p-value=0.2 for both groups.

6.3.3 Fraction of exposed BSA to sunlight in peak time

Table **22** shows the calculated total average of BSA for all three groups, which was 0.11±0.06, and ranges between 0.04-0.63. The UK covered group exhibited the least exposed BSA during this period (mean 0.09±0.03 and a range from 0.07 to 0.24). The KSA group came next after the UK covered group with a mean of 0.11±0.04. However, the KSA group had a smaller range (0.04-0.16) than the UK covered women. Finally, the UK uncovered group featured the maximum average of exposed skin during peak time periods (mean 0.14±0.09 and range 0.11-0.63).

A one-way ANOVA was conducted between subjects to compare means for the three groups' exposed BSA at peak time. The results show statistically significant differences between average amounts of exposed BSA at peak time - $F(2,142)= 9.8$, p-value< 0.05.
Table **22** shows that the total mean of exposed BSA at peak time was 0.11±0.06 for the three study groups.
A Tukey HSD post-hoc test was used to find out where the statistical differences lay. The results yielded that the average exposed BSA for the UK uncovered group (0.14±0.09) and UK covered group (0.09±0.03) differed significantly at p-value< 0.05. Moreover, the UK uncovered group exhibited a higher average exposed BSA than the Saudi groups (0.11±0.04), at p-value<0.05. However, the UK covered group's average, and the KSA group's average exposed BSA were not statistically different.

6.3.4 Fraction of exposed BSA to sunlight during off-peak time

The total average exposed BSA during the off-peak periods was 0.12±0.07 and the range was 0.05-0.63, which is slightly higher than the total average of exposed BSA for peak time. This could be explained as the study population wearing fewer clothes or more revealing clothes during off-peak periods. All three groups reported that they exposed more skin

during off peak periods; the UK uncovered group revealed the most skin from the study's population with a mean of 0.15±0.09 and a range of 0.11-0.63. The UK covered group (0.1±0.06) and the KSA group (0.12±0.04) had similar averages. However, the UK uncovered group featured a greater range of exposed skin (0.07-0.32) than the Saudi group (0.05-0.27).

A one-way ANOVA was conducted between subjects to compare means for the three groups' exposed BSA at off-peak times. The results show a statistically significant difference between average amounts of exposed BSA at off-peak time, $F(2, 142)= 7.1$, p-value=0.001. Table **22** shows that the total mean of exposed BSA at off-peak times was 0.12±0.07 for the three study groups. A Tukey HSD post-hoc test suggested that the average exposed BSA for the UK uncovered group (0.15±0.09) and UK covered group (0.1±0.06) differed significantly at p-value<0.05. However, there were no statistically significant differences between the exposed BSA for the UK uncovered group and the Saudi group (p-value=0.07), and the exposed BSA for the UK covered group and the KSA group (p-value= 0.26).

6.3.5 Sun index

By multiplying the reported average of sun exposure per week by exposed BSA for each subject, was obtained an estimation of time that the skin is exposed to the sun each week. Table **22** shows that, in total, the women expose their skin to the sun, on average, 5.4±8 hours per week. The Saudi group and the UK covered group, on average, had the least time of skin exposure to the sun (mean 4.3±4.2 hour/week; mean 5.9±4.6, respectively). The UK uncovered group had the most time of skin exposure to the sun (mean 6.0±12.9 hour/week). A one-way ANOVA was used to contrast the three groups' sun indexes. However, this showed there are only statistically insignificant relationships, $F (2, 142) =0.696$, p-value=0.5.

6.3.6 Usual clothing

In this study, usual wear was divided into three options: veiled, covered and uncovered. The usual clothing style for the Saudi participants were vailed, 35 (out of 54), whereas, in the UK the majority were covered, 69 (35.9%) or uncovered, 64 (33.3%). A Chi-square test was used to investigate the association between usual wear and the study's three groups. There was a significant association between usual wear and the groups in the study $\chi^2 (4) = 265.5$, P-value<0.05.

6.3.7 Sunscreen

26 (18%) women out of the study groups indicated that they use sun protection, of which 14 (9.7%) women were from the KSA group and 7 (4.8%) were from the UK uncovered group. 8 (3.4%) women from the UK covered group, which represented around 5% of the group, stated that they use sun cream continuously or from-time-to-time. However, the majority of the research population (116 women, or 80% of the study population) reported that they do not use sunscreen (see
Table **22**). A Chi-square test was used to investigate the association between sunscreen use and the study's three groups. However, the assumption of the Chi-square test was violated, and the expected cell count was less than 5. Therefore, Fisher's exact test was used instead - Fisher's exact test p-value=0.03, which is <0.05. Thus, there is a statistically significant association between sunscreen use and the groups in the study.

6.3.8 Sunbathing

The same table shows that 81% of the study population, which was 117 women, do not sunbathe at all. 49 (34%) were from the UK covered group, 44 (30%) were from the KSA group, and 24 (17%) were from the UK uncovered group. On the other hand, 27 (19%) women from the total population reported that they work on a tan. The majority of them, which is 20 women (15%), were from the UK uncovered group, 6 (4%) were from the Saudi group, and only one woman (less than 1%) was from the UK covered group. A Chi-square test was used to investigate the association between sunbathing and the study's three groups. The result shows a significant association between sunbathing and the study groups χ^2 (2) = 32.9, p-value<0.05.

6.3.9 Frequency of sunbathing

Fifteen women out of those who sunbathe (n=27) reported that they sunbathe 1 to 2 times monthly. 10 of these (37.0%) were from the UK uncovered group, and 5 (18.5%) women were from the two covered groups. Additionally, 8 women indicated that they worked on their tan more than two times monthly - 7 (25.9%) were from the UK uncovered group, and 1 (13.7%) woman was from the KSA group. Finally, 4 (14.8%) of those who suntan stated that they do it occasionally. A Chi-square test was used to investigate the association between the frequency of sunbathing use and the study's three groups. However, the assumption of the chi-square test was violated because the expected cell count was less than 5, and, therefore, Fisher's exact test was used instead: p-value=0.9, which is >0.05;

accordingly, there is no statistically significant association between the frequency of sunbathing undertaken and the groups in the study.

6.3.10 Usual clothing when sunbathing or using a sunbed

Three of the participants (out of the 27) stated that they go nude while they sunbathe; all three were from the UK uncovered group. Sixteen women indicated that they wear a bikini – 15 (55.6%) were from the UK uncovered group and one was from the UK covered group. Four wear a bra and shorts, 3 (11.1%) from the Saudi group, and one (3.7%) from the UK uncovered group. Two women stated that they normally wear a t-shirt and shorts, one (3.7%) was from the UK uncovered group and the other (3.7%) was from the Saudi group. Finally, two (7.4%) of the Saudi group stated they wear a t-shirt and long leggings when they work on their tan. A Chi-square test was used to investigate the association between usual wear when sunbathing and the study's three groups. However, the assumption of the Chi-square test was violated, and the expected cell count was less than 5, so, therefore, Fisher's exact test was used instead: p-value=0.001, which is <0.05; the test shows a statistically significant association between clothing type when sunbathing, and the groups in the study.

6.3.11 Sunscreen use when sunbathing

17 women usually use sunscreen when they sunbathe; 14 (50.1%) were from the UK uncovered group, and 3 (10.7%) were from the Saudi group. 11 (39.3%) stated they do not use sun protection when they sunbathe. A Chi-square test was used to investigate the association between sunscreen use when sunbathing and the study's three groups. The assumption of the Chi-square test was violated, and the expected cell count was less than 5, and, therefore Fisher's exact test was used instead: p-value=0.63, which is >0.05; hence, there was no statistically significant association between sunscreen use when sunbathing and the groups in the study.

Table 23 A Comparison of holiday sun exposure across the three study groups using One-way ANOVA and Chi-square.

Holiday Sun Exposure	KSA covered	UK covered	UK uncovered	Total of population	P-value
Travel abroad in the last 6 months, % (n)					
Yes	11.7(17)	14.5(21)	17.9(26)	44.1(64)	0.06
No	22.8(33)	20.0(29)	13.1(19)	55.9(81)	
Purpose of the trip, % (n)					
Holiday	23.4(15)	25.0(16)	40.6(26)	89.0(57)	
Work	3.1(2)	7.8(5)	(0)	10.9(7)	0.02
Season, % (n)					
Spring	9.2(6)	(0)	(0)	9.2(6)	
Summer	12.3(8)	27.7(18)	36.9(24)	76.9(50)	<0.01
Autumn	(0)	(0)	1.5(1)	1.5(1)	
Winter	6.2(4)	4.6(3)	1.5(1)	12.3(8)	
Time of Going Out , % (n)					
Mostly daytime	9.4(6)	9.4(6)	25.0(16)	43.8(28)	
Mostly night-time	(0)	20.3(13)	1.6(1)	21.9(14)	<0.01
Day and night	18.8(12)	1.6 (1)	14.1(9)	34.4(22)	
Holiday clothing, % (n)					
Uncovered	1.5(1)	(0)	40.0(26)	41.5(27)	
Covered	24.6(16)	26.2(17)	(0)	50.8(33)	<0.01
Veiled	1.5(1)	6.2(4)	(0)	7.7(5)	

6.4 Holiday sun exposure

6.4.1 Travel abroad in the last 6 months

64 (44%) of the study population informed the researcher that they travelled out of their country of residency in the last six months, of which the majority were from the UK groups: 26 (17.9%) from the uncovered group and 21 (14%) from the covered group. However, just over 55% (81) of the population did not travel in the last six months; most of these were from the KSA group (33 (22.8%), see Table 23. A Chi-square test was used to investigate any association between traveling abroad and the study's three groups. The result shows an insignificant association between traveling abroad and the study groups: χ^2 (2) = 5.6, p-value=0.06.

6.4.2 Purpose of the trip

Holidaying was the most frequent reason for traveling among the three groups. Only 7 (10.9%) women from both covered groups stated that they travel for work reasons (see Table 23). A Chi-square test was used to investigate any association between the purpose of the trip and the groups in the study. However, the assumption of the Chi-square test was violated, and the expected cell count was less than 5, so, therefore, Fisher's exact test was used instead: p-value=0.02, which is <0.05; the test shows a statistically significant association between the purpose of the trip and the groups in the study.

6.4.3 Season during which trip was taken

Although all seasons were reported, summer was the most common season for traveling for all three groups (see Table 23). A Chi-square test was used to investigate any association between the season of the trip and the three groups. The assumption of the Chi-square test was violated, and the expected cell count was less than 5, and, therefore, Fisher's exact test was used as an alternative. Since p-value<0.05, the test shows a statistically significant association between the season of the trip and the groups in the study.

6.4.4 Time of going out

The most preferred time to go out when on holiday reported for the UK covered group is night-time. For the UK uncovered group, it is daytime, and for the Saudi group, it is both day and night-time. A Chi-square test was used to investigate any association between the time of going out and the study's three groups. However, the assumption of the chi-square test was violated, and the expected cell count was less than 5. For this reason, Fisher's exact test was used as an alternative. The test showed a statistically significant association between the time of going out and the study's groups, p-value <0.05.

6.4.5 Holiday clothing

Noticeably, the clothing styles on holiday were different from the usual wear for the Saudi group - they reveal more skin when they are out of their country of residency. Sixteen (24.6%) women from the KSA group stated that they are covered, and one (1.5%) stated that she was uncovered when they travelled (see Table 23) A Chi-square test was used to investigate any association between holiday attire and the groups investigated here. However, the assumption of the Chi-square test was violated, and the expected cell count was less than 5. As such, Fisher's exact test was used as an alternative. The test showed a statistically significant association between holiday clothes and the study groups, p-value <0.05.

6.5 Summary of potential lifestyle factors that could affect vitamin D status

This chapter set out the sun exposure behaviour of the study's population and highlight the factors that could affect vitamin D production in the body. The chapter presented an estimation of the total average of usual sun exposure for the three groups, exposed BSA, and the results of the sun index calculation. The average of sun exposure and exposed BSA was presented in peak times (times of the day where UV radiation is normally high) and off-peak times (times of the day where UV radiation is normally low). The UK covered women reported the highest average of exposure for both periods, while UK uncovered women reported the least. The UK covered group exhibited the least fraction of exposed BSA during both periods, while the UK uncovered group presented the highest fraction of exposed BSA. The sun index were statistically insignificant among the groups. Moreover, other factors reported in the chapter include sunscreen use and sunbathing habits. Additionally, holiday sun exposure habits were also described and tested statistically to present.

7 Vitamin D blood levels: data from a selection of healthy KSA and UK women.

7.1 Introduction

The purpose of this experiment was to determine the 25(OH)D levels in serum to assess vitamin D status for the study groups. Known concentrations of 25(OH)D and extracted samples were run through both the HPLC and LC-MS, and full details of the procedures were set out in section 3.7.

7.2 HPLC results (KSA)

The 25(OH)D_3 serum samples were analysed in the KSA at the hospital at the King Abdul-Aziz University. Then, the serum samples were shipped for comparison analysis to the UK, but, due to a logistical error, the samples were not delivered to the Manchester Metropolitan University laboratory on time, and, therefore, the sample was spoiled and had to be destroyed. As a result, the 25(OH)D_3 serum results for the KSA participants presented here are those that were obtained from the laboratory of the King Abdul-Aziz University Hospital. These results were used to demonstrate the vitamin levels for the KSA participants. Table 24 shows the number, the mean, and the standard deviation for the KSA participants, see Appendix 7 for the full results.

Table 24 Mean average vitamin D level for the KSA group.

Number of participants	Mean (ng/ml)	Standard deviation
19	7.53	6.91

Table 25 Provides the cut-off values of vitamin D levels that were used to identify participants' statuses by the laboratory of King Abdul-Aziz University Hospital, and the frequency of participants for each vitamin D status, n=19.

25(OH)D concentration, ng/mL	Vitamin D status	% (n)
<10	Deficiency	79(15)
10-30	Insufficiency	16(3)
30-100	Sufficiency	5(1)
>100	Toxicity	0(0)

According to the cut-off values for vitamin D levels that were identified by the hospital, the majority of the KSA participants exhibited insufficient or deficient vitamin D statuses (Table 25). The average 25(OH)D_3 status for this group was 7.53±6.91 ng/ml, which is considered a deficient level.

7.3 HPLC calculations and results (UK)

The UK groups samples (covered group n= 35, uncovered group n=25) went on to be analysed for vitamin D serum level using HPLC, section 3.9.1.

A series of five calibration solutions, (0, 15, 30, 60, 120 nmol/L), see section 3.9.1, were detected at a 265 nm wavelength using a UV detector. All calibration solutions were prepared in the mobile phase, they were prepared from Sigma-Aldrich 25(OH)D_3 standard and had them in 76% methanol. The first method indicated a retention time of 23 minutes for 25(OH)D_3, and the second method indicated a retention time of around 11 minutes for 25(OH)D_3, see section 3.9.1 for the methods details. Eluting peaks were not observed at these retention times for both methods. Multiple attempts were made to produce a calibration model, as this was repeated more than twelve times from November 2012 to March 2013. However, all attempts produced inconsistent results (see Appendix 6). The outputs of vitamin D calibrations from both methods showed a great deal of "noise", and, for the retention time, there was no peak or a very small peak compared to the statistical noise. This was just for the vitamin standard, and not for the serum samples. Therefore, the researcher could not produce the required calibration curve (see example of the attempts in Figure 26 and Figure 27). The standard solution was thought to be undetectable because the available UV director sensitivity was insufficient to recognise the required levels of the vitamin. Therefore, the LC MS-MS system was used to carry on with the analysis.

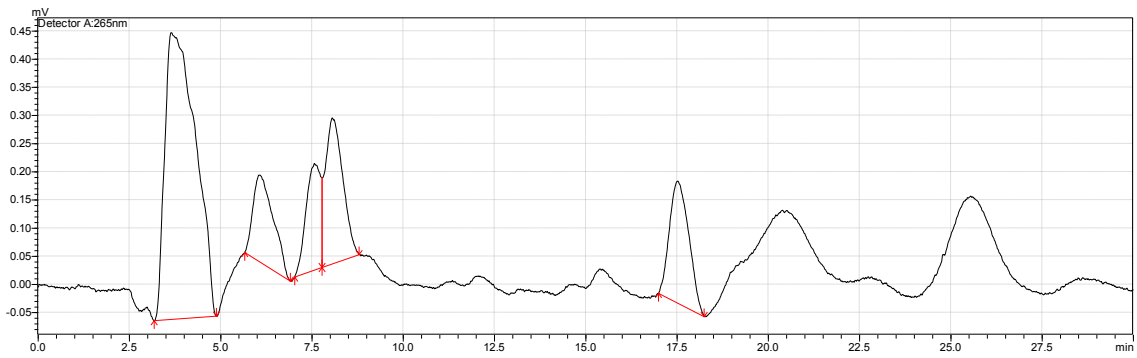

Figure 26 Example of the first method's outputs for HPLC. (This output was for vitamin D standard concentration 120 nmol/L).

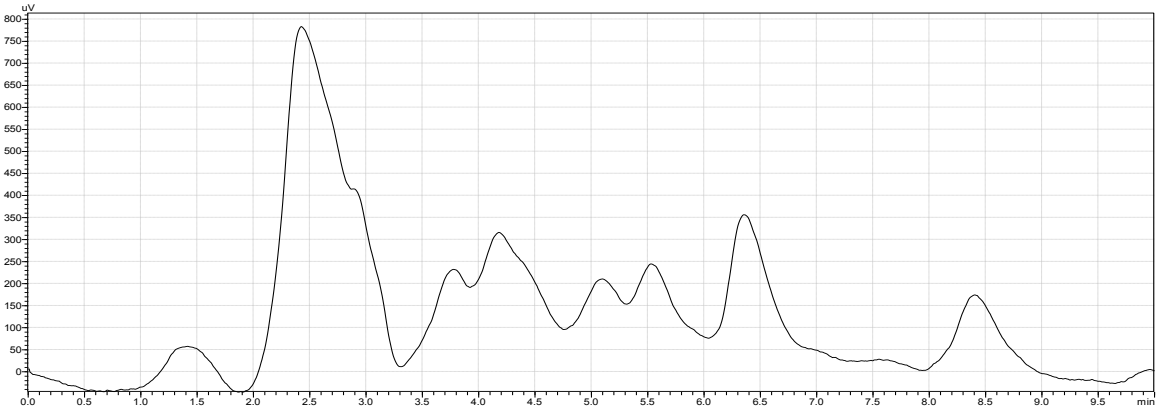

Figure 27 Example of the second method's outputs for HPLC. (This output was for vitamin D standard concentration 120 nmol/L).

7.4 LC MS-MS calculations and results

The UK blood sample were centrifuged then serum extracted. The serum was stored at -80^{o}C to keep it from degrading. The UK samples were collected in Autumn/Winter 2013-2014. The analysis was done in January 2015. Sample preparation and extraction were addressed in detail in the methods chapter in section 3.9.3. Subsequently the samples and the calibration standards were injected into the LC MS-MS system. A calibration curve was used for quantification.

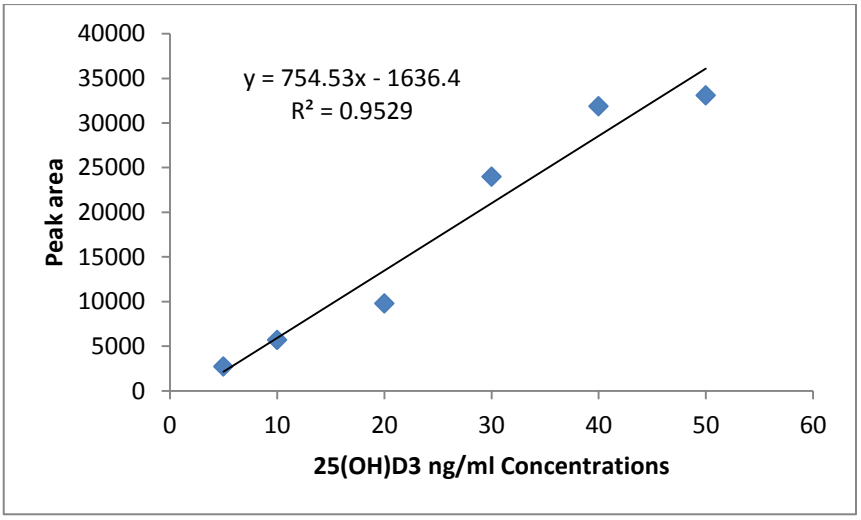

Figure 28 25(OH)D_3 calibration curve produced by LC MS-MS.

Concentrations of 25(OH)D_3 ranging from 5 to 100 ng/ml analysed by LC MS-MS to construct the calibration curve. The standard curve was linear between 5 and 50 ng/ml. To confirm repeatability, each concentration was repeated three times and the mean, and the standard deviation (SD) were calculated. Linearity of the calibration curve was determined

using Microsoft Excel 2010 (Figure 28). The calibration curve was produced by plotting the standard concentrations against the means of the areas.

Table 26 Method validation for 25(OH)D_3 standards.

Parameter	Value
Accuracy, ng/ml	100.71 ±15.41
Slope	754.53
Intercept	-1636.40
Linearity range, ng/ml	5 - 50
Correlation coefficient	0.95
Coefficient of variation, %	26.80, 3.75, 86.52, 12.21, 5.65, 12.66
SE of intercept	2543.80
SD of intercept	6229.77
LOD, ng/ml	27.24
LOQ, ng/ml	82.56

Table 26 shows the validation of the methods, (Cazes, 2004). The table contain the following values: accuracy, slope, intercept, linearity range, the correlation coefficient, the coefficients of variation (CV), the standard error (SE) of intercept, the standard deviation (SD) of intercept, limit of detection (LOD), and limit of quantitation (LOQ). The accuracy value is the mean of the recovery± the SD of the recovery; the recovery was calculated by dividing the found concentrations by the claimed concentrations and multiplying the answer by 100. From the calibration curve, the slope, the intercept and correlation coefficient (R^2) were reported. The coefficient of variation was calculated using the following equation (SD/mean)*100. LOD was calculated by dividing the SD of intercept by the slope, and multiplying the answer by 3.3. Finally, the LOQ was calculated by dividing the SD of intercept by the slope and multiplying the answer by 10.

The standard curve for 25(OH)D_3 was linear over the used range of concentrations, with R^2= 0.95. However, the results showed low accuracy, and the quantification of coefficients of variation for the standard exceeded 5%. The recovery value was higher than the recommended level of 100±2. The limit of detection (LOD), which is the minimum concentration that can be differentiated from the blank, was 27.24 ng/ml. Concentrations of 25(OH)D_3 levels in serum were expected to be much lower in the samples. Samples could not be processed because the calibration was invalid.

Subsequently, attempts were made to produce more consistent and accurate results. Calibration curves were constructed for 25(OH)D_3 using serials of concentrations from 1 to 100 ng/ml. A different linearity was obtained for 25(OH)D_3, with varied R^2. However, repeating the calibrations mostly produced poor linearity. This was thought to be caused by various technical problems with the available machine, and a final obstacle the researcher faced with the machine was the atmospheric pressure ionisation source, typically known as (ESI), which is the one that is normally used for low analyte levels. The instrument was used to analyse different compounds and mixtures; the vitamin is very sensitive to any traces of oil, lipids or any contaminant, and the cleaning procedures that had been used were thought to be inadequate. Moreover, on two occasions, the 25(OH)D_3 standard, from Sigma-Aldrich/ United Kingdom, turned out to be contaminated by the supplier. Finally, the LC MS-MS is a highly demanding system, which requires high level of technical competence and maintenance of the instrumentation. The researcher had to cut short the analysis due to temporal and financial constraints.

7.5 Summary of examining vitamin D status among the study groups

The current chapter presented the outcome of 25(OH)D_3 analysis that was done for the study groups. The result of the KSA group showed that the majority had low level of vitamin D. In fact, 95% of the Saudi group has either deficient or insufficient level of vitamin D

Then, the chapter outlined the different attempts that was made to analyse 25(OH)D_3 serum samples of the UK groups. First, the HPLC was used and two methods was applied. However, the results were not usable. Then, a different HPLC was used, LC MS-MS. The outcome of the LC MS-MS analysis was not satisfactory either. The time limitation for the study had to stop the experiment.

8 Prediction modelling of vitamin D status

8.1 Introduction

The current chapter will examine to what extent sun exposure habits and FFQ results can predict vitamin D status for the women in the study.

Regression modelling was used to fit the relationships between the variables of the study was used. The relationships was investigated by constructing a multiple linear regression, and multiple logistic regression models.

The multiple linear regression models, were used to examine whether there was an effect of predictors on the response variable. Correlation was used to measure the strength of relationship between two variables of interest. The aim of the first model (multiple linear regression models) was to determine a statistical model between a given numerical variable (dependent/response variable, see section 3.10.4, Table 15) and a set of predictors (independent variables). In terms of this study, sun exposure and FFQ results were predictors for vitamin D levels in the blood.

The second models included unadjusted and forward logistic regression, where the dependent variable was binary, taking two values only. This binary variable relates to the question of "Have you ever been told by a doctor that you have, or had, vitamin D deficiency?" (see appendix 2). Hence, the categories of being diagnosed with vitamin D is split into: yes (had been diagnosed with vitamin D deficiency) and "no". Sun exposure and FFQ results were used as predictors for vitamin D deficiency diagnosis. These approaches were applied to whole population and then applied to each population (UK and KSA).

8.2 Linear regression model

8.2.1 Sample and outliers

Of the 19 participants who provided blood samples for analysis, 15 had also completed the FFQ and sun exposure questionnaire. Extreme values were checked using boxplot, and hence seven participants were found to be extreme. After removing outliers, only 12 observations were retained for the analysis.

8.2.2 Variables selection

Pearson's correlational analysis was used to examine the direction and strength of relationship between the study variables (sun exposure, exposed BSA, and food and supplement intake) and vitamin D blood serum levels. Since the number of independent variables was considerably large compared with sample size, predictors showing significant correlations were retained for fitting the regression model.

To improve the value of the relationship, a transformation using a natural logarithm was performed on both dependent and numerical independent variables. The normality was examined for the regression model, and it met normality assumptions, see Table 27. The correlation (Pearson's r value) with log average exposed BSA to sunlight in peak time and log daily vitamin D intake for uncommon food were improved to be -0.70 and 0.54 rather than -0.65 and 0.48, respectively (Table 27 and Table 28). Hence, the multiple regression for vitamin D levels in blood was fitted based on using log average exposed BSA to sunlight in peak time and log daily vitamin D intake for uncommon food. Vitamin D level was insignificantly correlated with the remaining independent variables, therefore, it was not included in the regression analysis.

Table 27 Correlations (Pearson's r) between vitamin D levels and the independent variables (for KSA group), n= 12.

Independent variables	Pearson	p-value(2-
Age	0.35	0.25
BMI	0.12	0.70
Has children	-0.07	0.82
Had diagnosed vitamin D deficiency	-0.50	0.09
Use of fortified vitamin D products	-0.16	0.60
Use of sunscreen	-0.11	0.72
Travel abroad	0.06	0.83
Sun exposure during peak time hour/day	0.30	0.33
Sun exposure during off-peak time hour/day	0.06	0.83
Log average of exposed BSA during peak time	-0.65*	0.02
Log average of exposed BSA in off-peak time	-0.24	0.43
Daily intake of vitamin D from supplements	0.36	0.24
Log daily vitamin D intake from common foods	-0.01	0.97
Log daily vitamin D intake from uncommon	0.48	0.11
Skin colour, two groups	0.09	0.76
Type of milk ,two Groups	0.13	0.66
Use of food supplements, new	-0.07	0.82
*statistically significant p-value<0.05		

Table 28 Correlations (Pearson's r) between Log (vitamin D level) and the natural logarithm of the two independent variables (for KSA group), n= 12.

Independent Variables	Pearson Correlation	p-value(2-tailed)
Log of average exposed BSA in peak time	-0.70	0.01
Log of daily vitamin D intake for uncommon foods	0.54	0.06

The normality of the residual of the regression model was examined and found to be valid using the Shapiro test of normality (p-value=0.62), see Table 29. In addition, from Figure 28, the relationship between the predicted vitamin D levels and the residuals are random, indicating the residuals' homogeneity.

Table 29 Tests of normality for log vitamin D levels

	Shapiro-Wilk		
	Statistic	Df	p-value
ZRE Standardized	0	12	0.62
Residual	0.94		

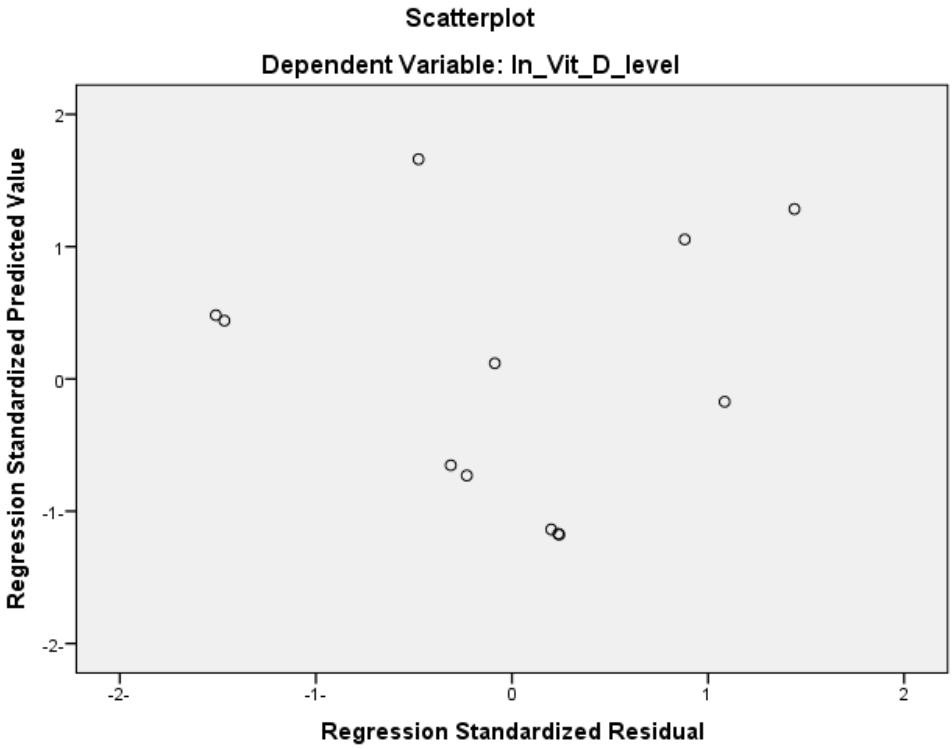

Figure 29 Scatter plot of predicted vitamin D levels and residuals

The fitted model is statistically significant (ANOVA: F=6.137, p-value=0.02), and explained 57.7% of variation in the vitamin D levels, see Table 30. Based on the estimated coefficients, only log of average exposed BSA to sunlight in peak time shows a significant effect on log the vitamin D level (β=-0.58, t=-2.42, p-value=0.03, 95% CI -1.1307-0.039).

Table 30 The fitted model of log vitamin D levels in blood

Model	95% CI			t-test	p-value
	β	Lower limit	Upper limit		
Log of average exposed BSA to sunlight in peak time	-0.58	-1.1307	-0.039	-2.42	0.03
Log of daily vitamin D intake for uncommon food	0.08	-0.103	0.268	1.007	0.34
R-squared=0.57, ANOVA for fitted model: F-statistic=6.13, F(p-value)=0.02					

8.3 Logistic regression

Initially, the logistic regression was fitted for each variable using the whole population. Table 31 demonstrates that the sample size for each country is small. Due to the small sample size, fitting a multiple logistic regression model using all the predictors will lead to redundant degrees of freedom. Consequently, the estimated regression coefficients for all the predictors cannot be estimated.

To overcome the issue of redundant degrees of freedom, only the most important predictors were retained for the vitamin D deficiency model using a forward selection procedure. Frequencies of categories for some nominal variables, such as skin colour, were very small, and, hence, those categories were combined into appropriate categories. In addition, the average BSA exposed at peak hours was extremely skewed, and, therefore, it was log transformed.

8.3.1 Unadjusted logistic regression model for all data

Table 31 shows the results for the predictors associated with medically diagnosed of vitamin D deficiency. Milk type had a significant effect on being diagnosed with vitamin D deficiency, where the women who drink skimmed/fat-free soya milk are more likely to be diagnosed with vitamin D deficiency compared with those who drink full cream milk (odd ratio=0.16, 95% CI: 0.04 0.55, p-value=0.004). Also, the risk of being diagnosed with vitamin D deficiency was likelier for the semi-skimmed group compared with the full cream group (odd ratio=0.32, 95% CI: 0.12 0.97, p-value=0.02). The likelihood of being diagnosed with vitamin D deficiency was significantly low when log average of exposed BSA at peak hours increased (odd ratio=2.96, 95% CI: 1.13 7.76, p-value=0.03). In addition, the women in the KSA were less likely to be diagnosed with vitamin D deficiency compared with women in the UK (odd ratio=0.29, 95% CI: 0.12 0.68, p-value=0.01). Other variables such as skin colour, using supplements and reasons for using the supplements, sunbathing, using fortified products, daily vitamin D intake, and sun exposure did not reach statistical significance.

Table 31 Results of an unadjusted regression model for vitamin D deficiency using whole data (KSA & UK)

Variable	%(n)	Odds Ratio	95% C.I Lower and upper limit		p-value
Age		0.96	0.92	1.10	0.15
BMI		1.01	0.91	1.10	0.75
Using supplements and reasons for use,					
Therapeutic (Ref)*	11.5 (22)				
No	66.1 (79)	2.75	0.94	8.03	0.06
Preventive	22.4 (43)	2.75	0.81	9.24	0.10
Has children,					
Yes (Ref)	39.3 (57)				
No	60.7 (88)	1.18	0.52	2.66	0.68
Skin colour,					
Pale white- white- cream white (Ref)	42.1 (61)				
Moderate brown- dark brown- black	57.9 (84)	1.04	0.43	2.49	0.99
Milk Type,					
Full cream (Ref)	24.5 (43)				
Skimmed/ fat free Soya	22.4(28)	0.16	0.05	0.55	0.004
Semi-skimmed	38.5(74)	0.32	0.12	.871	0.02
Sunscreen,					
Yes (Ref)	20 (29)				
No	80(116)	0.70	0.25	1.93	0.50
Travel abroad,		0.92	0.40	2.12	0.85
Yes (Ref)	44.1(64)				
No	55.9(81)				
Sunbathing,					
Yes / sometimes (Ref)	19.3 (28)	0.25	0.02	2.18	0.21
No	80.7(117)				
Using fortified products,					
Yes/sometimes (Ref)	45.5(66)				
(No, I don't know)	54.5(79)	1.58	0.70	3.58	0.27
Daily vitamin D intake from common food products		0.34	0.93	1.07	0.34
Daily vitamin D intake from less common food products		0.97	0.91	1.02	0.33
Sun exposure in peak hours		0.86	0.74	1.11	0.08
Sun exposure in off peak hours		0.94	0.71	0.12	0.94
Log (Average BSA exposed at peak hours)		2.96	1.13	7.76	0.03
Log (Average BSA exposed at off peak hours)		1.43	0.51	4.03	0.49
Residency,					
UK (Ref)	28.1(54)				
KSA	71.9(138)	0.29	0.12	0.68	0.01
(Ref): The reference/baseline category of the factor in the regression model.					

(C.I.): Confidence Intervals

8.3.2 Forward selection logistic regression for all data

Since the number of predictors was somewhat large compared with the sample size, which caused redundancy in the degrees of freedom, forward selection was used. Notice that including all predictors in the same model can result in controlling some predictors in the effect of other predictors. From Table 32, milk intake was not selected, whilst using supplements and reasons for use were retained in the final selection model. Average log BSA exposed at peak hours and residency variables were also retained in the model. From the table, women used supplements as preventive were six times less likely to diagnosed with vitamin D deficiency compared with those used supplements as therapeutic (odd ratio=6.04, 95% CI: 1.44 25.19, p-value=0.01). Also, using supplements for no reason were four times more likely to have lower risk of having vitamin D deficiency compared with using supplements as therapeutic (odd ratio=4.26, 95% CI: 1.22 14.79, p-value=0.02). The likelihood of being diagnosed with vitamin D deficiency was significantly low as long as the log average BSA exposed at peak hours increased in the groups (odd ratio=3.88, 95% CI: 1.13 11.27, p-value=0.02). Moreover, women in the UK were less likely to be diagnosed with vitamin D deficiency compared with women in the KSA (odd ratio=0.23, 95% CI: 0.09 0.61, p-value=0.003).

Table 32 Results of multiple regression selection model for vitamin D deficiency using all data (KSA & UK)

Variable	**%(n)**	**Odd Ratios**	**95% C.I.**		**p-value**
Using supplements and reasons for use,					
Therapeutic (Ref) *	11.5(22)				
No	66.1(79)	4.26	1.22	14.79	0.02
Preventive	22.4(43)	6.04	1.44	25.19	0.01
Log (Average BSA exposed at peak hours)		3.88	1.28	11.77	0.02
Residency,					
UK (Ref)	28.1(54)				
KSA	71.9(138)	0.23	0.09	0.61	0.003
(Ref): The reference/baseline category of the factor in the regression model. **(C.I.):** Confidence Intervals					

8.3.3 Unadjusted logistic regression model for covered groups in each country

Applying unadjusted logistic regression only for the covered groups in the two countries, Table 33 shows that sunscreen was found to be significant for only the UK; no sunscreen

was likely to decrease the risk of being diagnosed with vitamin D deficiency (odd ratio=0.09, 95% CI: 0.01 0.86, p-value=0.04). On the other hand, for the KSA, an increase in Log average BSA exposed at peak times was likely to reduce the risk of being diagnosed with vitamin D deficiency (odd ratio=5.30, 95% CI: 1.36 20.63, p-value=0.02).

Table 33 Results of a simple regression model for vitamin d deficiency for KSA & UK covered

Variable	*KSA(n=54)*				*Covered UK(n=74)*			
	Odd Ratio	**95% C.I.**		***P*-value**	**Odd Ratio**	**95% C.I.**		**p-value**
Age	1.06	0.99	1.14	0.08	1.02	0.96	1.09	0.44
BMI, kg/m^2	1.01	0.88	1.16	0.79	0.98	0.86	1.12	0.84
Using supplements and reasons, **Therapeutic (Ref)**								
Preventive	0.13	0.01	1.44	0.09	0.29	0.05	1.91	0.29
No	0.31	0.07	1.38	0.12	0.31	0.05	1.82	0.31
Has children, **Yes (Ref)**								
No	0.50	0.12	1.94	0.31	0.98	0.32	3.01	0.98
Skin colour, **pale white- white-cream white (Ref)**								
(moderate brown- dark brown- black)	0.81	0.18	3.60	0.79	-	-	-	-
Milk Type, **Full cream (Ref)**								
Skimmed/ Soya	7.66	0.76	76.45	0.08	2.88	0.60	13.74	0.18
Semi-skimmed	4.12	0.90	18.76	0.06	1.75	0.43	6.97	0.43
Sunscreen, **No (Ref)**								
Yes / Sometimes	3.75	0.94	14.82	0.06	0.09	0.01	0.86	0.04
Travel abroad, **Yes (Ref)**								
No	1.04	0.26	4.11	0.95	1.34	0.43	4.17	0.59
Sunbathing, **Yes / Sometimes (Ref)**								
No	1.66	0.17	15.85	0.65	-	-	-	-
Using fortified products, **Yes / Sometimes (Ref)**								
(No, I don't know)	0.94	0.29	3.04	0.08	2.00	0.16	23.77	0.58
Daily vitamin D intake from common food products	0.96	0.64	1.43	0.85	1.02	0.87	1.21	0.73

Variable	*KSA(n=54)*				*Covered UK(n=74)*			
	Odd Ratio	**95% C.I.**		***P*-value**	**Odd Ratio**	**95% C.I.**		**p-value**
Daily vitamin D intake from less common food products	0.88	0.55	1.39	0.59	1.01	0.94	0.11	0.81
Sun exposure in peak hours	1.23	0.96	1.56	0.08	1.03	0.81	1.31	0.79
Sun exposure in off-peak hours	0.91	0.55	1.51	0.73	1.05	0.73	1.51	0.79
Log (Average BSA exposed at peak hours)	5.30	1.36	20.63	0.02	1.10	0.22	5.48	0.91
Log (Average BSA exposed at off-peak hours)	2.81	0.38	20.72	0.31	0.51	0.12	2.07	0.34
(Ref): The reference/baseline category of the factor in the regression model. **(C.I.):** Confidence Intervals								

8.3.4 Forward selection logistic regression for each covered groups

Forward selection was applied to the multiple logistic regressions. From Table 34, for the KSA women, no supplement intake was 32 times more likely to yield a diagnosis of low vitamin D than for those women taking supplements therapeutically (odd ratio=32.05, 95% CI: 1.69 606.43, p-value=0.02). In addition, the increase in log average BSA exposed at peak was likely to reduce the risk of being diagnosed with vitamin D deficiency (odd ratio=12.43, 95% CI: 2.04 75.68, p-value=0.01). In terms of the UK covered women, sunscreen use was likely to decrease the risk of being diagnosed with vitamin D deficiency (odd ratio=0.09, 95% CI: .01 0.86, p-value=0.04).

Table 34 Results of multiple regression selection model for vitamin D deficiency for KSA & UK covered women

Variable	*KSA (n=54)*				*Covered UK (n=74)*			
	Odd Ratio	**95% C.I.**		***P*-value**	**Odd Ratio**	**95% C.I.**		**p-value**
Using supplements and reasons, **Therapeutic (Ref)** Preventive No	 6.314 32.059	 0.96 1.69	 41.24 606.43	 0.05 0.02	-	-	-	-
Sunscreen, **No (Ref)** Yes / sometimes	-	-	-	-	 0.09	 0.01	 0.86	 0.03
Log (Average exposed BSA at peak hours)	12.43	2.04	75.68	0.01	-	-	-	-
(Ref): The reference/baseline category of the factor in the regression model. **(C.I.):** Confidence Intervals								

9 Discussion

9.1 Introduction

The present chapter states and explains the implications of the study's findings, and how these findings fit in with existing knowledge of the topic.

Each results chapter represented one of the research aims; in this part of the thesis, the researcher will discuss to what extent to which these findings answer the study questions and meet the study aims and objectives. Therefore, the current chapter will follow same sequence of results chapters. Each section of the discussion will concentrate on one section of the results chapter and attempt to answer the research questions that related to the specific aim of that chapter.

9.2 Discussion of descriptive results

The descriptive chapter represented the baseline of the participant's nature and characteristics, and some general information about their diet. This study was designed to assess factors affecting vitamin D in young women that generally have good health, do not suffer from chronic diseases or obesity, but tend to follow a particular dress code, which may not allow their body to be exposed sufficiently to the sunlight most of the time.

The general findings demonstrated that most of the study's population were young adults. However, the KSA group and the UK covered group were slightly older than the UK uncovered group (29.3± 9.1 years, 27.8±8.7 years, and 24.2±8.3 years respectively). They have generally healthy BMIs, but both covered groups had a higher range of unhealthy BMIs than the uncovered group. Moreover, the number of women with children was reported as higher in both covered groups.

Clearly, the study includes a preponderance of ethnically Arab participants. This was anticipated because the study was conducted partially in the KSA, which increased the prevalence of the Arab ethnic group.

It is illustrated in the literature that the degree of melanisation of the skin has a significant influence on a person's ability to produce vitamin D (Cancer Council Australia 2015). Therefore, it was considered important for this study to identify the colour of the participants' skin. The results show that both covered groups had slightly darker skin than

the UK uncovered women, see Table 17 in chapter 4, which is entirely predictable given the ethnic background of participants in the study.

The literature showed that diet is the second most important source of vitamin D, and it is believed to have an impact on vitamin status (Holick 2010 ; Merewood *et al.,* 2010). Some of the dietary habits, which may affect vitamin D level were questioned. As most vitamin D rich foods come from animal products and sea foods, asking participants about their consumption of animal products was essential. The study illustrate none of the covered groups were vegetarian. Serenius *et al.* (1984), which is one of the earlier studies of vitamin D in women in the KSA and one of the few studies featuring a comparison between two covered groups, supported this finding in their paper.

Nutritional supplement intakes can maintain and boost levels of vitamin D, so the participants reported their intake of supplements and the reasons for using them. Supplement intakes were similar in all study groups. However, the difference was in the reasons for taking supplements. The KSA group mostly used supplements for therapeutic reasons - these kinds of supplements are normally prescribed by doctors because of some degree of deficiency for one or more nutrients, while both the UK groups used supplements for maintaining good health. On the other hand, the findings showed (see chapter 4, Table 17) the UK covered group reported themselves as being the most diagnosed with vitamin D deficiency. More than the half of UK covered groups stated that they had been diagnosed with vitamin D deficiency at some stage in their life.

Finally, over half of the Saudi group were not sure if they were using vitamin D fortified food products or not, and this could be explained by a lack of nutritional knowledge in this group.

None of the covered groups drink alcohol. This can be explained by Islamic law prohibiting intoxicant consumption. The KSA follows Islamic law, which considers alcohol as an illegal product. Additionally, this study includes a great number of conservative Muslim participants, and this was noticeable by their clothing styles. This was clear, as only a small proportion of the participants drink alcohol, and all of them were from the UK uncovered group (see chapter 4 Table 17).

9.3 Discussion of vitamin D intake

This section of the discussion attempts to evaluated participants' vitamin D intake and to consider to what extent an FFQ and dietary intake can predict vitamin D status.

9.3.1 Assessment methods for vitamin D intake

In this study, two methods were used to assess vitamin D intake, an FFQ and a food diary. Using two methods for assessment was intended to improve internal validity and minimize bias. Methods used for relative validation should have proportionality. In this case, each method has different causes and direction of error, as an FFQ is restricted by memory, the list of items, and by the participants' ability to estimate portion size, while measuring portion size and memory are not a concern for food diaries, which are not restricted by lists, as they are an open-ended method. Willett (2013) agreed that a food diary can be a good method for validating an FFQ, as both methods have low correlated errors.

An FFQ is the most commonly adopted method in similar studies, and has been reported to be an efficient tool in terms of response rates, and the cost of administration and processing (Taylor *et al.,* 2009 ; Pritchard *et al.,* 2010). Still, none of the methods are known to be a standard method, so the current study cannot guarantee that any of the used methods for tracking vitamin D intake is more accurate than any other, as the FFQ overestimated intake and the food diary underestimated the vitamin D intake of the participants. A Bland-Altman plot, see chapter 5, showed there was a low agreement between the outcomes of the two methods, and that there was large bias. The low agreement of the results of the methods attributed to:

- A three-day food diary may not be sufficient to assess vitamin D intake, so vitamin intake could be underestimated.
- Applying a three-day food diary once will not assess variation in vitamin D intake throughout the year.

A pilot validation study by Pritchard *et al.* (2010) using a five-day food diary to validate an FFQ to assess different nutrient intakes including vitamin D showed similar results. However, Pritchard *et al.*'s (2010) study attributed the variation in the results of the two methods to participants' awareness of the aims of the FFQ, which caused them to

overestimate vitamin intake; the food lists in an FFQ should be focused on the aims of the study with consideration of participants' culture, economic level, and the locality of the food items. Finally, collecting the five days of data at the same time will not reflect annual vitamin intake. In spite of the low agreements between the current study's vitamin D intake assessment methods, both methods agreed that vitamin D intake was less than the daily recommended amount in both countries.

9.3.2 Daily intake of vitamin D

The literature demonstrated that, until now, the UK Department of Health has not stated a reference amount for daily vitamin D intake for healthy adults (18-50 years) who do not fall into any of the vitamin D risk groups (Department of Health 2014b ; British Nutrition Foundation 2015). On the other hand, covered people in the UK were reported to be one of the groups at risk of vitamin D deficiency by the government, and the advisable daily vitamin D intake for this category is 10 µg, which is equal to 400 IU (Department of Health 2014b). The Saudi Ministry of Health declared that 15 µg, which is equal to 600 IU, is the daily reference amount of vitamin D intake for adults aged 18-50 (Kingdom of Saudi Arabia Ministry of Health Portal 2014a), whereas the National Osteoporosis Foundation (2014) recommended that healthy adults under the age of 50 should have 400-800 IU/day of vitamin D intake to maintain vitamin D levels, which is equal to 10-20 µg/day.

The results of this study showed that none of the study groups reached any of the recommended levels of vitamin D daily intake through foods (see chapter 5, Table 20 and Table 21). The Food Frequency Questionnaire showed the groups' average intake of vitamin D from food was 7.1±7.8 µg/day (see chapter 5, Table 20). However, the average amount of vitamin D intake slightly improved for those who take supplements, to 10.6±9.8 µg/day, particularly in the KSA. This is important because, as this study's results show Saudi women rely on supplements to satisfy their requirements for vitamin D. The Saudi Arabian's average daily intake of vitamin D that comes only from foods was 3.6±3 µg/day, which is very low compared to the UK groups, and to the recommended daily intake of vitamin D generally. For the Saudi group the amount doubled but was still low when supplement consumption was added - 7.1±5.6 µg/day. Indeed, the Saudi Health Ministry has noticeably encouraged the Saudi public to cover their needs of vitamin D, if sunlight is

not an option, through supplements and not food (Kingdom of Saudi Arabia Ministry of Health Portal 2014b ; Kingdom of Saudi Arabia Ministry of Health Portal 2012).

9.3.3 Common vitamin D food products and frequency of used

The results of the study showed that vitamin D-rich foods are divided into two groups according to reported frequency of use: commonly used and less commonly used (see chapter 5, Table 19). Common food groups comprise of breakfast cereals, processed orange juice and dairy products, which are used frequently on a daily basis. The other group contains mushrooms, fish and other animal products, and these foods were used on a weekly or monthly basis.

The Saudi Ministry of Health Portal (Kingdom of Saudi Arabia Ministry of Health Portal 2012a) reported that the consumption of meat and meat substitutes per day is two to three portions, and advised the public to eat different kinds of meat and meats substitutes with an emphasis on fish, legumes, and lean meat. However, they did not state a clear consumption amount for fish. Public Health England (2014) and the NHS (2013) recommendation for fish consumption is two portions per week, and for oily fish, it is one portion per week. The single portion size of fish reported by NHS (2013) and Public Health England (2014) was 140g. However, the current study shows that the general consumption of oily fish was common on a monthly basis, and low on a weekly basis for both canned and fresh oily fish, except for canned tuna. Public Health England (2014) supported this finding and reported that oily fish consumption was lower than the recommended amount for the UK public across all age groups. The NHS (2013) reported that fresh tuna, herring, mackerel, salmon and sardines are all good sources of vitamin D, and added more kinds, including sprats, trout, whitebait, anchovies, carp, jack and pilchards.

The British Nutrition Foundation (2014) reported a consumption guide for milk and dairy products of two to three times a day for an average portion; the average portion for milk was stated to be 200 ml, the average portion for yoghurt is 150g, and the average portion for cheese is 30g. The Kingdom of Saudi Arabia Ministry of Health Portal (2012a) reported a slightly higher guidance amount for milk and dairy products for people over 18 years old: two to four portions a day. The average portion of milk and yogurt is 240 ml, and cheeses is 30g. The results of the study show that dairy products were more highly consumed on a

weekly basis than on a daily basis, except for milk and cheeses, which were slightly more highly consumed on a daily basis. However, both UK groups reported greater habits of daily milk consumption than the Saudi group. Still, the average portion of milk consumed by the UK groups was reported to be cup or less, which is less than the recommended amount.

The NHS (2015a) and the British Nutrition Foundation reported (2014) that fat in milk is an important source for vitamins, particularly vitamin B. The same references add that full fat in milk and dairy foods can also increase energy intake. Thus, the NHS (2015a) recommended that semi-skimmed or skimmed milk can be a good substitute for full fat milk with the same nutritional benefits. However, the report did not mention milk as source of vitamin D, and the effect of reducing milk fat on fat-soluble vitamins such as vitamin D. The Saudi Health Ministry Portal (2014a) stated that not all dairy products have the same nutrient values and composition as milk, and reported the average nutrient values for skimmed, semi-skimmed, and full fat milk. However, the report did not mention which type of milk is better to use. The results of this study showed, Table 17, that low-fat milk is the most common type of milk consumed by the participants in the UK, and that full fat milk is the most common milk type consumed by the participants in the KSA.

Milk is not naturally rich in vitamin D. Therefore, many countries use vitamin D fortification for milk and other common food products. Vitamin D fortification for some food products is recommended in the UK, but is not compulsory (Department of Health 2014a). It is advised that small amounts of vitamin D be added to food products, but the fortification amount is left to manufacturers to choose (Department of Health 2014a). The NHS (2015b) cited that fortified fat spreads, fortified breakfast cereals, and some powdered milks are good vitamin D sources in the UK. However, the same source noted that cow's milk is not normally fortified in the UK, and, therefore, milk itself is not a good source of vitamin D in the UK. The current study looked at some milk brands in the UK and find out that only Marks & Spencer website report their milk as a good source of vitamin D. The Kingdom of Saudi Arabia Ministry of Health Portal (2012b) and The Kingdom of Saudi Arabia Ministry of Health Portal (2014a) describe twice that milk is a good source of vitamin D, which could be an indirect indication of milk fortification in the KSA. Two of the most common milk and dairy companies in the KSA, Almarai and Alsafi Danone, state on their official website that the amount of vitamin D in their milk and dairy products varies. For example, the nutritional

information of Almarai fresh milk showed the content of vitamin D is 400 IU/mL, while nutritional information of Alsafi Danone fresh milk did not reported vitamin D contents. On the other hands, Alsafi Danone nutritional information of other dairy products such as yogurt (Fresh Laban) report vitamin D contents, 40 IU/g.

9.4 Discussion of sun exposure

This section of the discussion evaluates the most important source of vitamin D. This study used developed methods to assess and estimate participants' sun exposure habits. This helped to study the similarities and differences between the study groups in terms of sun exposure habits, and to estimate their general sun exposure times.

Self-reported sun exposure habits for the three study groups (UK uncovered, UK covered, KSA covered) were explored to identify potential areas that may influence vitamin D levels negatively. McCarty (2008) mentions that there are no validated or standard questionnaires available for self-reported sun exposure assessment. However, Macdonald et al., 2008, McCarty 2008 and Gould et al., 2014 indicate that the questions in the self-assessment questionnaire should cover the following areas: time of outdoors sun exposure, clothing, sun screening, sunbathing habit, the weather and holiday sun exposure routine. This are the areas that the current study used to design and create the study questionnaire. The study questionnaire addressed outdoor exposure, holidays, clothing, sun protection use, and sunbathing habits. The time of day has an important effect on the production of vitamin D, and, because of that, sun exposure results were divided into two periods: off-peak time and peak time.

One of the study's key results (see chapter 6,

Table **22**) was that the UK covered group reported the maximum sun exposure time in both periods. However, skin exposed to sunlight was limited. The UK uncovered group reported limited exposure time but more exposed skin during the same periods. The KSA group had limited sun exposure time and limited exposed skin. This was clear when multiplying sun exposure time by the percentage of reported exposed skin. The results show that the KSA group had the lowest sun index of the groups, followed by the UK covered women. Generally, the average total of sun index for participants was 5.4±8 hours per week. Sun

index is a concept that was used initially by Barger-Lux and Heaney (2002) to estimate hours per week of total skin exposure, which comes from multiplying the reported average of sun exposure per week by exposed BSA. A Saudi study using the same calculation reported the sun exposure index for healthy Saudi women was 6.91±3.93 hour/week (Ardawi *et al.,* 2011), whereas this study reports 4.3±4.2 hours/week for the same group. However, the current study believes the difference in sun index values between this study and Ardawi et al. (2011) study comes from differences in the calculation. For example, the calculated values that was given for the common exposed part of BSA was different. The highest cut value for each category in Ardawi et al. (2011) study was arms (0.18) + legs (0.36) + head (0.09). Whereas, in the current study was arms (0.18) + legs (0.24) + head (0.07); as it believed this values will present better reflection of the study groups BSA.

However, the participants in this study may not fully benefit from their reported time of sun exposure. There are two possible reasons for this; the weather and the location of a country are two of the factors that can limit sun exposure and UVB radiation, which consequently reduce vitamin D synthesis. The UK data was gathered in the winter season, October to February. The weather was mostly reported by UK study groups to be cloudy, rainy and cold. Very importantly, the latitude of the UK, according to Pearce and Cheetham (2010) and Macdonald et al. (2011), decreases the UVB radiation every year from October to April to very low, or zero. As a result, the UK group may produce very low or zero vitamin D during these months from this source. Conversely, the Saudi part of the study was conducted in the summer, August, and the weather was reported as being sunny and hot by all KSA participants. However, temperatures in the KSA, especially in Makkah where the data was collected, can reach over 43^{o} C, and the UVB index can be over 11, which is very high, and is a dangerous level of UVB radiation (BBC 2015 ; General Presidency of Meteorology and Environment Protection 2015). Such excessive heat can reduce outdoor activity during the daytime, according to Ardawi *et al*., (2012) and Al-Daghri *et al*., (2012).

On the other hand, both covered groups had a higher sun exposure time in peak and of-peak periods than the uncovered group did. A probable explanation is that the influence of children upon adult/parental behaviour is significant. Women with children would do some activities that women without children would not do, such as school trips, which can lead to more sunlight exposure. 39.3% (n=57) of the study population had children, and most of

them were from the UK covered group and the KSA group. However, this time outside may not actually represent efficient exposure because of outdoor clothing styles, which may not expose more than the face and hands in many cases.

The Saudi group reported more exposed skin than the UK covered women during peak periods. In the KSA, schools, universities and many workplaces have segregated communities. This can give the women in the KSA more freedom in their attire than for the UK covered group. However, the KSA women showed lower exposed skin ranges than the UK covered women in both periods (see chapter 6,

Table **22**), which could be explained by the fact that KSA women often choose to wear conservative clothing styles most of the time. However, another likelihood is that this is attributable to the UK data being collected in the winter season, which makes people cover up more.

The use of sun protection was reported as generally low among all three groups (20%), although this was slightly higher among the Saudi population than for the UK groups, which is understandable because the KSA data was collected in the summer season.

Besides low reported amounts of sun exposure and limited skin contact with sunlight among the covered women in this study, skin colours for covered populations were observed to be slightly darker than for the UK uncovered group. Darker skin tones are able to tolerate longer sun exposures according to Pearce and Cheetham (2010), Farrar *et al*., (2011) and the Cancer Council Australia (2015). Farrar et al., (2011) tested the theory of casual exposure to midday summer sunlight. The study showed that the concentration of 25(OH)D before the UV radiation course was significantly higher in the white participants (with mean ±SD 17.6 ± 7.6 ng/mL) than the South Asian participants (with mean ±SD 6.4 ± 0.5 ng/mL). After the UV radiation course, 25(OH)D concentration of the white participants had increased to 28.0 ± 0.6 ng/mL, and the concentration of 25(OH)D of the South Asian participants had increased to 10.7 ± 0.7ng/mL, with $P < 0.001$. Farrar et al., (2011) study concluded that short casual exposure to midday summer sunlight did not cover the need of South Asian participants to reach the sufficient level of vitamin D. Thus, the current study believes the stated recommendation by Department of Health (2014b) in the UK of casual

sun exposure may fail to yield sufficient vitamin D for covered women with darker skin tones.

Sunbathing was not common in both covered groups, as only 4% reported getting a tan, and these were mostly from the KSA group. Moreover, the Saudi group revealed that they tended to wear conservative clothing even when they suntan.

To compare usual wear and holiday wear for all three groups, usual wear for outdoors and holidays was classified into three selections:

- Uncovered, women who wear a Western style of clothing.
- Covered, women who wear long, concealing clothing and head scarves, but expose the face and the hands.
- Veiled, people who wear long concealing clothing, a head scarf, and cover the face.

Both UK groups did not show any difference in their usual wear and holiday clothing styles. However, the holiday wear of the Saudi group was shown to be less strict than their usual wear. The veil was common among Saudi women for everyday wear; however, fewer women are veiled when they are on holiday abroad, and one woman reported being totally uncovered whenever she is out of the KSA. In any case, most KSA women cover their bodies and wear a hijab, but expose their face and hands.

Travelling in the summer and going out in the daytime would increase the chance of vitamin D production. The study groups reported summer as the most common season for traveling abroad, and other seasons were reported less frequently. The UK groups preferred to travel in the summer and less commonly in the cold season, whereas the KSA group reported the summer as the most common time to travel, reporting spring and winter less frequently. Going out times varied for the three groups. Daytime was mostly common for the UK uncovered group. Night-time was common in UK covered group, whereas the KSA group chose to go out during the day and at night.

However, previous assumptions did not guarantee the level of vitamin D for any of the three groups. Therefore, measuring vitamin D levels is essential to determine to what extent the reported factors may affect the vitamin levels in each group.

9.5 Assessment of vitamin D status

This step attempted to assess the participants' current levels of vitamin D. This would help to determine their general vitamin D status, and to compare the vitamin status of the study groups.

9.5.1 KSA results

The 25(OH)D_3 results that were analysed at the King Abdul-Aziz University Hospital showed that 15 (79%) out of the 19 participants suffered from vitamin D deficiency (see chapter 7) and that three (16%) had insufficient levels of the vitamin. Only one (5%) was reported with a sufficient level. This means that 95% of the Saudi participants in this study had vitamin D deficiency; however, the sample size of the Saudi participants applied in the current study was very small. Nevertheless, this is in line with other studies that have reported a prevalence of vitamin D deficiency among Saudi women. Ardawi *et al*. (2011) assessed 1172 healthy female adults in the KSA and reported that 90.2% of them had vitamin D levels less than 20 ng/ml. Siddiqui and Kamfar (2007) tested vitamin D levels of 433 healthy Saudi teenage girls and reported that 81% of them had vitamin D levels of 10 ng/ml or less. Despite this study's small sample size, the results of the study can be argued to be largely representative of healthy Saudi women in general and in agreement with other larger studies.

The storage form of vitamin D in the blood is 25(OH)D_3, and it has a half-life of about 15 days. Thus, this method does not provide an indication of fully comprehensive annual vitamin D storage in a participant, as it only shows the vitamin storage level at the time of blood collection (Millen and Bodnar 2008). Hence, to overcome and expand on this limitation, blood samples should ideally be analysed and compared during each season. However, more recently Kanan *et al*. (2013) tested 968 women of child-bearing age in the KSA and stated that 80% to 85% of them showed vitamin D levels of less than 20 ng/ml in both summer and winter seasons.

9.5.2 UK results

Vitamin D is not an easy compound to measure and this was reported in the literature (chapter 2, section 2.7). The vitamin has water-repelling characteristics, and strong

connections to vitamin D binding proteins (DBP). Thus, this bond needs to be broken so that 25(OH)D_3 can be extracted. The current study used the developed technique, see chapter 3, to prepare the serum and extract the compound. Lensmeyer *et al*. (2006) agreed that vitamin D analysis is a challenge and indicated some factors that would affect the compound negatively, which were light and temperature. These can gradually degrade 25(OH)D_3. Moreover, the vitamin could have a chemical reaction when the HPLC system and the column are not clean (Lensmeyer et al*.,* 2006).

This study tried to use the best available instrument for vitamin D analysis. The literature suggested that HPLC and LC MS-MS assays are the best available assays nowadays for vitamin D analysis (Farrell et al., 2012 ; Moon et al., 2012 ; Sadat-Ali et al., 2014). However, they are not the easiest assays to use. The LC MS-MS assay requires substantial technical skill, more than a HPLC essay (Lensmeyer *et al.,* 2006). Lensmeyer *et al*. (2006) and Turpeinen *et al*. (2003) reported that HPLC assay is less demanding and more straightforward, but that detector sensitivity is crucial for consistent and reliable results. The current study faced exactly this challenge with the available HPLC, as the sensitivity was believed to be inappropriate for the low concentrations that were used.

For the LC MS-MS, the researcher followed different methods, none of which produced good results. The method that did produce a good calibration line was reported in the methodology chapter. However, despite the good calibration line, R^2= 0.95, the method validation was poor. Different concentrations of the 25(OH)D_3 standard was used. However, high concentrations, above 50 ng/ml, normally did not present common vitamin D levels in the blood, so such concentrations was excluded.

The aim of this part of the study was to identify the vitamin levels of participants, not to develop or improve vitamin D methods of analysis. Despite all the efforts and attempts to produce good results, time restrictions forced the researcher to stop 25(OH)D_3 analysis.

9.6 Prediction modelling of vitamin D status

This part of the study tried to ascertain to what extent the study's assessed factors can predict vitamin D deficiency. Different regression models were applied to test this.

First, a simple linear regression model was planned to examine whether there was an effect of predictors on the response variable (level of $25(OH)D_3$). However, as the actual level of $25(OH)D_3$ was not identified for the majority of the study's population, the researcher used only the vitamin D level for the Saudi group to apply the linear regression model.

The next regression models were unadjusted logistic regressions, and the researcher substituted the response variable with a question that asked the participants directly if they had been diagnosed with vitamin D deficiency before. The question was dichotomous, yes was the reference. One model was applied to the whole population, and then the other model was applied only to the covered groups.

9.6.1 Linear regression model for the KSA group

The outcome from this model show that the average of exposed body surface area has a negative effect on vitamin D serum level. It would normally be expected that vitamin D would have a positive association with exposed body surface area. Al-Othman *et al*. (2012) reported a positive correlation between sun exposure and vitamin D serum levels. However, the study categorised sun exposure into none, daily and weekly according to time range, but did not report the actual amount of exposed skin. Besides the model shows that vitamin D levels were insignificantly associated with all other variables. However, the small sample size could reduce the statistical power of the model, which can produce unrepresentative data to support 17 predictors in the current model.

9.6.2 Unadjusted logistic regression model for all population

This model was devised to assess individual factors for the three study groups in order to increase the statistical power of the model. From the simple logistic regression model for all groups, there were three main predictors which appeared to contribute towards being diagnosed with vitamin D deficiency, and these were residency, type of milk used by participants and exposed body surface area at peak hours.

The current model showed that residency was one of the main contributors to vitamin D deficiency among women in the three groups. Thus, women who live in the UK are more likely to be deficient than those who live in the KSA. This is entirely expected because of the geographical location of the two countries, and the nature of the climate. This could

allow the KSA women to have ample sunlight almost all year. Whereas, the UK women have a lack of UVB, according to Pearce and Cheetham (2010), for almost half the year, and are presented with diverse weather conditions.

Second, the type of milk was a probable predictor for vitamin D levels, which could indicate that the chance of having vitamin D deficiency is high for women who tend to use semi-skimmed milk. This would raise questions about the bioactivity of nutrients, vitamin D in particular, and the use of low-fat and fat-free milk. Another possible interpretation is that the Saudi group preferred full-fat milk, whereas UK groups preferred semi-skimmed and other milk types to the full fat milk. Thus, this predictor the type of milk is explained by the residency and this was therefore just another marker of residency and not because this was a true association.

Log of exposed body surface areas at peak hours was one of the probable predictors in this model (see chapter 8). The logistic regression model for the whole population showed that the greater the exposed body surface area at peak hours, the lower the likelihood of diagnosis with vitamin D deficiency. This is a clear indicator of the effect of clothing on the body.

A study by Mishal (2001) showed the effect of clothing styles on women vitamin D level. The study was done in Jordan where three groups of women wearing three different clothing style were compared with men (western clothing, Hijab covered the whole body except the face and hand, and Niqab covered the whole body include the face and hand). The study showed that vitamin D levels for both covered groups were significantly lower than vitamin D level in the men (p-value <0.05).

9.6.3 Forward logistic regression model for the full population

Including all variables in the same model can help to make the choice of predictive variables automatically by the SPSS software package. Thus, this resulted in controlling some predictors in the effect of other predictors, and this was clear when the milk type was not selected (see chapter 8, table 2). This therefore suggest that this predictor could be affected by the residency factor.

Exposed BSA at peak hours and residency variables were retained in the model. Moreover, supplement use and reasons for this were factored into the final selection model.

The results showed (chapter 8, Table 32) that the chance of being diagnosed with vitamin D deficiency was less likely in women using preventive supplements, followed by women not using supplements as compared with those who used prescribed supplements.

9.6.4 Unadjusted logistic regression model for the covered groups

The study tried to find factors contributing towards vitamin D deficiency for each group. Building the regression model for the full data set increased the model's power and thus accuracy, especially with a small sample size, but will not allow for the comparison between the study groups to be made. Therefore, the current model was intended to expose the factors that predicted vitamin D deficiency the most for each group. However, the UK uncovered group was excluded here because their response levels to the independent question was very low - only three out of forty-five women reported that they were diagnosed previously with vitamin D deficiency. Nevertheless, excluding the UK uncovered group will not affect the purpose of the comparison, which was to compare the two-covered groups.

The outcomes of the current model showed that KSA women who had high exposure of body surface areas are less likely to be diagnosed with vitamin D deficiency. Still, the Saudi group had very low serum levels of vitamin D in any case.

UK covered women who reported no use of sunscreen were more likely to be diagnosed with vitamin D deficiency. This means, despite the lack of sunscreen use, covered UK women are still likely to be diagnosed with vitamin D deficiency.

9.6.5 Forward logistic regression model for covered groups

Forward selection for the logistic regression model showed the likelihood of being diagnosed with vitamin D deficiency is high for Saudi women who do not use supplements. This finding agreed with a Saudi study reported that 83% (n=299) of the of the study population (275 women and 25 men) had vitamin D level less than 7.5 ng/ml before taking vitamin D supplement (Al Faraj and Al Mutairi, 2003).

In addition, the model showed that the Saudi women who have a low average of exposed BSA have high risk of being diagnosed with vitamin D deficiency. The association between vitamin D level and clothing style has been discussed previously in section 9.6.2.

Applying forward selection for the UK covered women's data showed that an association between using sunscreen and being diagnosed with a vitamin D deficiency is existing. Sun protection was reported to reduce skin ability to synthesise vitamin D by more than 90% (Glerup et al., 2001 ; Lee et al., 2008). However, in this study the UK covered group may have other factors that influence the reported results.

9.7 Limitations, and suggestions for future research

The researcher has done her best to make sure that this study attained its aims. However, this study has a number of limitations, which need to be addressed. Further, the researcher has developed some ideas that could be considered in future research.

The difficulty in logistics of conducting data collection in two different countries by a single researcher restricted and slowed down the data collection process. One of the limitations of this study was the small sample, which was 192 in total. Besides, working in two countries reduced the study sample size because of the restricted timescale and budget that were planned for each country. A bigger sample size could increase the precision in such a study if repeated and refined in the future, by increasing the statistical power of the data to detect a sufficient difference between the groups. Furthermore, many participants avoided taking part and did not wish to contribute to the research because of the need for a blood sample, and the perceived inconvenience of keeping a food diary. To overcome this, the blood sample and food diary were made optional for the participants. However, this reduced the number of participants at each part, and not all completed all the study requirements. The food diary was done only for three days, which may not be sufficient for properly assessing vitamin D intake. This was done to increase participants' responses. However, future studies could apply a seven-day food diary which maybe more beneficial to estimate vitamin D intake. The researcher would then have to consider ways that participation could be better explained, consented to and, perhaps, incentivised – within the bounds of ethics constraints.

Data were collected once; the Saudi data collection phase was conducted in the summer season, while the British data collection phase was conducted in the winter season, which is a season in which people, in each country, tend to go outdoors less. However, repeating the sun exposure assessment and dietary assessment for each season will help to explore the effect of seasonal variation on dietary intake and sun exposure habits. This point could be considered in future studies.

This study attempted to compare the effect of diet and lifestyle factors on vitamin D status in certain types of women from two countries. However, one of the greatest limitations in this study was the logistical and technical challenges in relation to the blood analysis. Despite all attempts and efforts made to resolve this issue, time and finances constrained the researcher. Therefore, this study could be replicated, perhaps with more time and funding as a contingency, to compare other factors impacting on actual vitamin D levels.

Lastly, The Saudi women must wear abaya and respectable clothing, when they are in public. However, the current study showed that the Saudi women tend to maintain conservative appearance most times even when they are sunbathing. Therefore, investigating the level of knowledge and awareness towards vitamin D and sun protection in the Saudi population could be a fruitful idea for further study. Besides, investigate prevailing attitudes and behaviour toward sunlight will help to understand more factors that can affect vitamin D level among a population reported to be at high risk of low vitamin D level.

Finally, there are many studies on proposed or developed models for enhancing vitamin D through sunlight or supplements. However, the researcher did not come across any study building a model to study the effects of improving vitamin D status through diet and food only. This could be undertaken by a three-armed intervention study to test the effectiveness of a vitamin D-rich diet in one group, high sun exposure in another group, and a vitamin D-rich diet combined with high sun exposure and a control group. This could be a valuable area to investigate and may benefit the improvement of vitamin D fortification guidelines and laws, particularly in the KSA where there is a clear lack in this area.

10 Conclusion

10.1 Introduction

This thesis was carried out to study the effect of diet and lifestyle factors on vitamin D status in healthy female adults, who tended to follow certain dress codes, and came from two countries. Three aims were set to achieve this target, and these were attained by conducting secondary and primary research.

The literature review shaped the basis of the thesis and enabled the researcher to gain a deep understanding of the research background. The literature drew attention to a number of key issues. The most important conclusion that was derived from this section is that vitamin D deficiency is a continuing problem worldwide. Despite all the research that had been done on the topic of vitamin D, there is still disagreement on the level of the vitamin that will meet the body's needs and confer the vitamin's health benefits, the adequate amount of sun exposure that amounts to an essential minimum, and the appropriate intake of the vitamin through food and supplements without exposing individuals to harm. Food fortification with vitamin D is, as yet, optional in many countries, including in the UK and the KSA. The UK and the KSA governments have not stated a clear amount of vitamin D that manufacturers can use for food fortification.

In the UK, the government recommendation for sunlight as regards vitamin D did not address dark-skinned people's needs, and the general population's requirements for the winter season. While the recommendations for vitamin D daily intake are focused on people that are at risk of vitamin D deficiency, they ignore the general population that do not generally fall into these groups. People covered for cultural reasons are reported by the UK government to be at risk of vitamin D deficiency, and the UK government has stated that the daily vitamin intake for them should be 10 µg (Department of Health 2014b).

In the KSA, there was no public health statement dedicated particularly to vitamin D. The available recommendations from the government for sun exposure to obtain sufficient levels of vitamin D were very general and ambiguous, whereas the recommended daily intake is mentioned generally in different statements, but these are mostly not dedicated to vitamin D specifically. However, one of these stated directly that 15 µg/day is the advisable amount for all people from 1 year old to 70 years old (Kingdom of Saudi Ministry of Health Portal, 2014a).

Therefore, given the previous circumstances, the emphasis in the primary research was to assess women's sun exposure and the vitamin D intake of covered women living in the KSA and the UK to study similarities and differences that may cause vitamin D problems in these women.

10.1.1 Aim 1 and 2: Assess the dietary vitamin D intake of healthy Saudi women and healthy UK covered women, as well as healthy UK uncovered women; and compare data from FFQ and food diary to determine reliability and validity of the methods.

This aim was addressed by employing two dietary assessment methods to estimate and compare vitamin D intake for covered and uncovered women in the UK, and women in the KSA.

The primary research revealed and confirmed several key findings. The food frequency questionnaire showed the groups' average intake of vitamin D from food was 7.1±7.8 µg/day, and the food diary showed the groups' average intake of vitamin D was 1.7±2.0 µg/day. Both methods revealed that vitamin D intake is very low for all women studied who were living in the UK and the KSA compared to recommended levels for each country, which was 10 µg/day for UK covered women (Department of Health 2014b), and 15µg/ day for KSA women (Kingdom of Saudi Arabia Ministry of Health Portal 2014a). Therefore, governments in both countries should take steps to increase the daily intake, perhaps by encouraging food fortification in common food products.

10.1.2 Aim 2: Identify potential lifestyle factors that could affect vitamin D status in healthy women in the UK and the KSA.

To reach this goal, a questionnaire was used. The emphasis was on assessing sun exposure habits and other potential factors including age, BMI, skin colour, having children, smoking, and previous and current health issues. The main findings illustrated the following: The study groups showed different practices of sun exposure habits. In the UK, covered women reported high exposure time, but their skin exposure was limited. The UK uncovered women had high skin exposure, but low sun exposure time. In the KSA, women reported limited exposure time and exposed skin levels.

Therefore, the recommendations for each group should be based on the needs of each group, and any restrictions they are subject to. For example, covered women in the UK are restricted in terms of clothing, and advice must focus on increasing vitamin D through appropriate foods and supplements, while increasing exposure as much as possible during peak times. In the KSA, women, because of segregation in the community, may actually have more freedom in dressing, so advice may focus more on sun exposure. Additionally, the recommendations should consider women's skin colour, age, BMI, and previous and current health issues. The primary research showed the study groups generally had healthy BMIs and similar ages. However, the covered groups were slightly older and had slightly unhealthy BMIs. Skin colour for the covered groups were slightly darker than the uncovered group, and this was expected because the majority of covered groups were Asian.

10.1.3 Aim 3: Assess vitamin D status of healthy Saudi Arabian women, healthy UK covered women, and healthy UK uncovered women.

This goal was aimed at using chemical analysis to assess vitamin D levels in the blood. HPLC and LC MS-MS were the systems that were used. Clearly, the researcher faced challenges in this section of the study. The researcher did her best to produce sufficient data, and make sure that a lack of measurements of vitamin D levels in the UK would not affect the study's goals and outcomes.

The primary findings of the study showed that vitamin D deficiency was common among women in the KSA, as 7.53±6.91 μg/ml was the mean level of vitamin D. In the UK, more than half of the covered group (26 women) reported that they had been diagnosed with vitamin D deficiency before. This may indicate that vitamin D deficiency is widely spread among the covered groups in both countries.

Finally, the main outcomes of logistic regression models illustrated the factors that were found to be associated with vitamin D deficiency. The logistic regression of all groups associated residency, exposed BSA at peak hours, and supplement use and reasons for this with women who are diagnosed with vitamin D. A second model of logistic regression attempted to show the factors that were associated with vitamin D deficiency, but only for the covered groups. The Saudi group's chances of being diagnosed with vitamin D deficiency were associated with the reason for and use of supplements, and with exposed

BSA at peak time. The probability of the UK group for developing vitamin D deficiency was associated with not using sunscreen.

10.2 Research contribution and recommendation for public health.

This study is one of the few studies that has carried out a direct comparison between two countries, and compared two groups of healthy covered women, who are reported to be one of the at-risk groups in both the UK and KSA. This study evaluated differences and similarities between the groups to isolate the common causes of vitamin D deficiency for these women, and highlighted the recommendations and guidance that followed in each country via literature.

In the KSA to date, there has been limited research conducted on healthy women of child-bearing age in particular. In any case, most previous vitamin D studies existing in the KSA have tended to examine the effect of vitamin D on particular health issues, or the effect of a disease on vitamin D status. The current study contributes to the existing literature, and adds to a growing body of research on vitamin D, by purely focusing on vitamin D and vitamin D-influencing factors in healthy adults.

The proposed theoretical framework provided an insight into natural factors that influence vitamin D (diet and sun exposure were the focus in this thesis), which may help to improve the public health guidelines for vitamin D in general and in the KSA in particular.

Adequate vitamin D levels are best achieved through sufficient exposure to sunlight and could be complemented by a vitamin D-rich diet. Therefore, it is important to state clearly the sufficient time that the body needs to photosynthesise the vitamin without burning. Synthesising vitamin D in the body relies on many factors such as: latitude, season, time of the day, age, skin colour, BMI, clothing, physical activity, sunscreen and individual health status. All these factors must be taken into account when making public health recommendations and guidance. Additionally, the study groups, in the current study reported different habits despite having the same clothing style, religious values and living in the same country. The study shows, in the UK, healthy covered women have a higher exposure time than healthy uncovered women, but they may not benefit from this time because they are covered outdoors. While in the KSA, despite community segregation and

the greater freedom that covered women can have in some outdoor facilities, the study showed KSA women tend to cover up. Moreover, the study clearly shows vitamin D intake is very low in all groups.

Therefore, one broad statement on vitamin D sun exposure is inadequate; it may not suit individual requirements in all seasons, or encourage inhabitants to rely on vitamin D supplementation despite having a free natural source most of the year. This study recommends the following for future policy and guidance:

- Allow individuals to customize vitamin D recommendations according to their needs. For example, designing a points system can help individuals to calculate how much time they need to reach the required target of vitamin D. The calculation may include country of residency, season, the time of the day, the skin colour, exposed BSA (i.e. clothing style), BMI and any other factors that can influence the vitamin. Moreover, the system could consider that if calculated points are low and inadequate for the photosynthesis of vitamin D by sun exposure, then supplementation can be advised.
- Use of new technologies that are available to public education and engagement. Apps, for example, are easy to develop and design; and can reach large numbers of people, which makes the points system easy to be applied.
- Increase the knowledge and awareness of vitamin D, by using an appropriate channel to reach targeted groups. For example, social media would reach the younger generation faster than the older generation.
- Break the old habits of indoor lifestyle and low sun exposure by targeting the new generation, especially in countries with ample sunlight such as KSA. Schools are a good place to start; this could be accomplished by increasing fun outdoor activities for the students.
- Public Health recommendations in the KSA for fish and oily fish are not clear. Therefore, a clear statement on the required consumption amount for fish and oily fish must be provided. For example in the UK recommendation for fish consumption is stated precisely by Public Health England (2014) and the NHS (2013), which is two portions per week, and for oily fish, it is one portion per week.

- Policy for vitamin D food fortification, particularly in the KSA, needs to be specific and improved. For example, milk is not naturally rich in vitamin D, however, the Saudi Arabia Ministry of Health Portal (2014a) describes twice that milk is a good source of vitamin D. This is an indirect indication of milk fortification in the KSA; however, it is not specific or clear.
- The amount of vitamin D used for food fortification should be specified and standardised across manufacturers.
- Fortified products should be commonly consumed in the country. For example, breakfast cereal was consumed less frequently in the KSA, whereas cheese was commonly consumed.

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12 Appendices

12.1 Appendix 1: The first draft of the questionnaire

My name is Taqwa Bushnaq and I am a student at Manchester Metropolitan University, studying Human Nutrition at PhD level. This questionnaire forms part of my dissertation, which is entitled "Vitamin D status in women living in the KSA and the UK and dietary strategies to improve this (in the UK)" (Your views will be very useful on this subject matter, and will be treated confidentially).

Date: _____

1) **Where do you live?**
   a. The UK
   b. The KSA
2) **Your age in years _____**
3) **Your weight: _____ kg**
4) **Your height: _____ cm**
5) **What is your ethnic origin?**
   a. Arab
   b. White British
   c. African
   d. Indian subcontinent (Pakistan, Bangladesh and India)
   e. Other _____
6) **How would you describe your skin colour?**

I. White skin, blue or hazel eyes, and blond or red hair(always burn and never tan)
II. White skin, blue or hazel eyes, and blond or red hair(frequently sunburns but could show slight suntan)
III. White skin, dark hair or brown eyes (mildly suntan irregularly show slight sunburn).
IV. White skin, dark hair or brown eyes (regular suntan rarely sunburn)
V. Brown skin (often suntan rarely sunburn)
VI. Black skin(often suntan rarely sunburn)

7) **Are you pregnant?**
   a. Yes
   b. No
8) **Are you breast-feeding?**
   a. Yes
   b. No

9) **Do you have children?**

a. Yes

b. No (Escape the next question)

10) How many children do you have? _____

11) What level of education do you have?

a. High school

b. Undergraduate

c. Postgraduate

d. Basic level of reading and writing

e. Other _____

12) Do you smoke (e.g. cigarette, shisha)?

a. Yes

b. No (Escape the next question)

13) Are you normally:

a. Heavy smoker

b. Light smoker

14) Do you suffer from any of the following?

a. Osteoporosis

b. Parathyroid

c. Intestinal disorder

d. Liver disease

e. Kidney disease

f. Fat malabsorption

g. Heart disease

h. Cancer Hypertension

i. Type 1 diabetes

j. Milk allergy and lactose intolerance

k. Other, please list : _____

15) Do you use any pharmaceuticals drugs including supplements?

a. Yes "State please": _____

b. No

16) Do you drink alcohol?

a. Yes

b. No(Escape the next question)

17) How many units* of alcohol do you consume per week?

* refer to the alcohol unit's chart for reference _____

18) Do you use vitamin D supplements?

a. Yes
b. No (Escape the next question)

19) could you state please:

a. Which vitamin D supplements :_____
b. Name of supplier: _____
c. Proprietary name: _____
d. Quantity: _____

20) Do you have any recent fractures in the last 2 years?

a. Yes
b. No (Escape the next question)

21) When did you have fractures?

a. Less than 6 months
b. 6 months-a year
c. Less than 18 months
d. 18 months-24 months

22) Do you have a history of vitamin D deficiency?

a. Yes
b. No (Escape the next question)

23) When was that? _____

PART 2:	7 - 9am	9- 11am	11 am - 1pm	1 - 3pm	3- 5pm	5 - 7pm
How much time do you spend outdoors between the following period?						
Where would you be during this time period?						
What percent of this time did you spend under shade (e.g, Cloud, tree or beach shade)?						
How would you describe the weather conditions?						
What percent of time did you wear a hair cover (e.g, brimmed hat-hijab) ?						
What percent of time did you wear sunscreen?						
What is the sun protective factor (SPF)?						
Did it have UVA and UVB protection?						
What areas of your body are exposed to sunlight?	**[1]**Face. **[2]**Hands **[3]**Full arms. **[4]**Half arms. **[5]**Full legs. **[6]**Half legs. **[7]**Other "State please": ________	**[1]**Face. **[2]**Hands **[3]**Full arms. **[4]**Half arms. **[5]**Full legs. **[6]**Half legs. **[7]**Other "State please": ________	**[1]**Face. **[2]**Hands **[3]**Full arms. **[4]**Half arms. **[5]**Full legs. **[6]**Half legs. **[7]**Other "State please": ________	**[1]**Face. **[2]**Hands **[3]**Full arms. **[4]**Half arms. **[5]**Full legs. **[6]**Half legs. **[7]**Other "State please": ________	**[1]**Face. **[2]**Hands **[3]**Full arms. **[4]**Half arms. **[5]**Full legs. **[6]**Half legs. **[7]**Other "State please": ________	**[1]**Face. **[2]**Hands **[3]**Full arms. **[4]**Half arms. **[5]**Full legs. **[6]**Half legs. **[7]**Other "State please": ________

If you wish to collaborate in the next stage of the research please could you leave your information beneath.Your generosity is appreciated.

Contact Details (Email or mobile):

Name:

Thank you for your participation in this project ..

12.2 Appendix 2: The questionnaire, participants' information sheet and consent sheet to Participate.

Consent to Participate in Research Project

Researcher's name: Taqwa Bushnaq

Title of Research: Vitamin D status in women living in the KSA and the UK and dietary strategies to improve this (in the UK).

Brief statement of purpose
The aims of this study are to measure vitamin D deficiency among women and to identify the factors most strongly affecting their vitamin status.

In order to do this, the researcher will use a questionnaire to collect information on lifestyles, skin colour, sun exposure, medication and other factors normally affecting the body's ability to produce or store vitamin D. In addition, you will be asked to keep a food diary for three days and to complete a food frequency questionnaire (FFQ) to provide evidence of your vitamin intake and dietary habits.

If you are interested in participating further in the research, you will be asked to give a fasting blood sample to assess your vitamin D levels. The sample will be taken in privacy by a trained phlebotomist in a clinical room set aside for this purpose, at a date arranged with you later.

After approximately three months, you may be given a dietary intervention programme and you may be asked to give another blood sample to measure any change in your vitamin status.

Please read the following summary and if you agree to participate, sign the declaration below.

The objectives of this research have been explained to me:

- To estimate nutrient intakes by filling out the FFQ and food diary.
- To determine how different dietary and lifestyle factors may affect vitamin D status, a questionnaire needs to be filled in.
- To measure my vitamin D status by doing a blood test.
- To follow a dietary intervention programme (model) that could help to improve vitamin D intakes and level (for those interested in taking part in this stage of the research).

Under these circumstances, I agree to participate in the research

Name: …….. **Contact details (email or mobile):** ……..……..

Signature: …… **Date:** ……

Participant identification number: Uncovered Covered Veiled

Manchester Metropolitan University

Participants' information sheet

–

It is estimated that one billion people around the world experience vitamin D deficiency or insufficiency (Tsiaras and Weinstock, 2011). Many factors affect vitamin D levels, such as skin colour, age, chronic disease, clothing and exposure to sunlight. A deficiency of this vitamin can cause harm to the bones. Deficiency in adulthood can cause osteomalacia, osteoporosis or muscle weakness and increase the possibility of fractures (Holick, 2007; Insel, Turner & Ross, 2003). There is growing evidence of the role of vitamin D in preventing or reducing the risk of many chronic illnesses, including cancers, autoimmune deficiency and cardiovascular disease, and abnormal cell conditions such as psoriasis (Holick, 2007; Insel et al, 2003). According to previous research, the ideal measure of vitamin D status is made by measuring the primary form (25-hydroxyvitamin D) in blood.

Therefore, we would like to invite you to take part in a study to measure vitamin D levels to assess the factors affecting vitamin D status in healthy women.

- ✓ The Research Ethics Committee of Manchester Metropolitan University has reviewed the project and given its ethical approval.
- ✓ You will be asked to fill out a questionnaire to assess sun exposure, medication, skin colour and other factors affecting vitamin D level.
- ✓ You will be asked to give a fasting blood sample to measure vitamin D levels in your body at the beginning of the study and another at the end of the study to find out the effect of the dietary intervention model on your vitamin D status. These samples will be taken by a trained phlebotomist.
- ✓ You will be asked to keep a food diary for three days and to complete a questionnaire to measure vitamin D intake.
- ✓ By taking part in this study will be told about your vitamin D levels and whether you need to increase vitamin D by following an individualized rich foods dairy model.
- ✓ You have the right to change your mind and withdraw from the project at any time without explanation.
- ✓ All your information will be treated confidentially and used for research purposes only.
- ✓ We recognise that taking part will take up some of your time. We will do our best to minimise any inconvenience by ensuring that we meet at a time and place convenient for you. We do not expect anyone to suffer any harm or injury as a result of participating in this project. Blood collection may be uncomfortable or cause a small bruise.
- ✓ If you have any further questions please do not hesitate to contact the research student of the project.
- ✓ Thank you for reading this leaflet and for considering helping with this study.

Date:_____

Part 1: Questionnaire

1) **Where do you live?**
   a. The UK
   b. The KSA
2) **Your age in years_____**
3) **Your weight:** _____ kg
4) **Your height:** _____ cm
5) **What is your ethnic origin?**
   a. Arab
   b. White British
   c. African
   d. Indian subcontinent (Pakistan, Bangladesh and India)
   e. Other _____
6) **Are you pregnant?**
   a. Yes
   b. No
7) **Are you breast-feeding?**
   a. Yes
   b. No
8) **Do you have children?**
   c. Yes
   d. No (Skip the next question)
9) **How many children do you have?_____**
10) **How would you describe your skin colour?**
   I. Pale white skin (always burns, does not tan), blue/hazel eyes, blond/red hair
   II. Fair skin, blue eyes (Burns easily, tan)
   III. Darker white skin (Tans after initial burn)
   IV. Light brown skin (Burns minimally, tans easily)
   V. Brown skin (Rarely burns, tans darkly easily)
   VI. Dark brown or black skin (Never burns, always tans darkly)

11) Do you suffer from any of the following?

a. Osteoporosis
b. Parathyroid
c. Intestinal disorder
d. Liver disease
e. Kidney disease
f. Fat malabsorption
g. Heart disease
h. Cancer
i. High blood pressure
j. Diabetes
k. Milk allergy and lactose intolerance
l. Other (Please list)_____
m. I do not have any disease.

12) Do you use any pharmaceutical drugs, including supplements?

a. Yes (Please list)_____
b. No

13) Have you had any fractures in the last two years?

a. Yes
b. No (Skip the next question)

14) When did you last have a fracture?

a. Less than six months ago
b. Six months to a year ago
c. A year to 18 months ago
d. 18 months to 24 months ago.

Part 2: Food Frequency Questionnaire. **Please answer every question as best you can. If you are not certain, an estimate is better than no answer.**

15) Over the last 12 months, how often have you eaten the foods in the table below?

Food	How often have you eaten these foods in the last 12 months?						
	Never	Less than once a month	1-3 times a month	Once a week	2-4 times a week	Once a day	Twice or more a day
Cod liver oil							
Liver (such as cow's, pig's and chicken's)							
Fresh salmon							
Fresh sardines							
Fresh mackerel							
Fresh tuna							
Herring							
Canned salmon							
Canned sardines							
Canned mackerel							
Canned tuna							
Eggs							
Fresh mushrooms							
Sun dried mushroom							
Milk							
Processed orange juice							
Yogurt							
Butter							
Margarine							
Cheese							
Breakfast cereal							

16) Would you describe yourself as a vegetarian or vegan?

a) Yes Number of years _____
b) No

17) What type of milk do you use most often? Select one only

a) Full cream
b) Skimmed/ fat free
c) Semi-skimmed
d) Dried milk
e) Soya
f) None

18) How much milk do you drink each day, including with tea, coffee, milky drinks, cereals etc?

a) None
b) Less than 250 ml (1 large cup or less)
c) Between 250 and 500 ml (1-2 cups)
d) Between 500 and 750 ml (2-3 cups)
e) 750 ml (3 cups) or more

19) What breakfast cereals do you normally eat? Please list the types most often used.

Brand	Type

20) Do you usually spread butter/margarine on your bread / rolls / crackers?

a) Yes
b) No
c) Sometimes

21) How many slices of bread/rolls/crackers do you have with spread every day/ week?

_____slices per day / _____slices per week

22) How much spread do you use?

a) Just thinly spread
b) Medium

23) What kind of fat do you most often use for cooking (frying, roasting, grilling, baking)? Tick more than one if applicable

a) Butter
b) Margarine
c) Lard/dripping
d) Vegetable oil
e) Solid white vegetable fat
f) None
g) Other _____

24) Do you take any vitamins, minerals, fish oils or other food supplement?

a) Yes
b) No
c) Sometimes

25) If yes or sometimes, please fill in details below

Name and brand of supplement	**How much do you take at a time?**	**How often do you take these?**			
		Daily	*Weekly*	*Monthly*	*Less often*
		1	***2***	***3***	***4***
		1	***2***	***3***	***4***
		1	***2***	***3***	***4***

26) What are your reasons for taking supplements?

a) Preventive
b) Therapeutic (prescribed by your doctor)

27) Have you ever been told by a doctor that you have, or had, vitamin D deficiency?

a) Yes
b) No (Skip next question)

28) When was that?

Age_____ year_____

29) Do you use products fortified with vitamin D such as butter, orange juice, milk, breakfast cereals?

a) Yes, always
b) Sometimes
c) No
d) Don't know

30) Have you changed your diet over the last 12 months?

a) Yes
b) No

31) If yes, what were the reasons for the change?

a) Osteoporosis
b) Parathyroid
c) Intestinal disorder
d) Liver disease
e) Kidney disease
f) Fat malabsorption
g) Heart disease
h) Cancer
i) High blood pressure
j) Diabetes
k) Milk allergy and lactose intolerance
l) Other (please list)_____

32) How often do you drink alcohol?

a) Never
b) Once a week
c) More than once a week
d) Less than once a week

33) In a typical week, how many units do you drink? Please see the last page of the questionnaire to use the alcohol unit chart for reference

- Beer or cider _____ units
- Wine _____ units
- Spirits _____ units
- Other _____ units

34) Do you smoke?

a) Yes (go to question 36)
b) No (go to question 37)
c) I used to smoke, but not any more (go to question 35)

35) How long ago did you quit smoking?

_____ years
or _____ months

36) How would you describe yourself?

a) I smoke every day
b) I smoke occasionally, but not every day

Part 3: Sun exposure questionnaire

Please estimate your usual exposure to sunlight in the last week by completing the table on the next page. Please answer every question beginning "How much time…" by writing a number of minutes or hours in each space on that line. For example, in question 37, we want to know how much time you spent outdoors each day between the times shown at the top of each column. So if you spent about ten minutes outside between 7am and 9am each day, you should write "10 minutes" in the first box and so on. If you did not go out at this time you should write "0".

37) Sun exposure questionnaire	7-9 am	9-11 am	11am -1pm	1-3 pm	3-5 pm	5-7 pm
Write an answer for each time of day:						
1. How much time did you spend outdoors between these periods?						
2. Where would you be during this time?						
3. How much time did you spend in the shade (e.g. cloud, tree or sunshade)?						
4. How would you describe the weather conditions?						
5. How much time was your head covered (e.g. by a brimmed hat or hijab)?						
6. How much time did you wear sunscreen?						
7. Did the sunscreen you used have SPF and UVB protection?						
8. What was the sun protective factor (SPF)?						
9. What areas of your body were exposed to sunlight?	[1]Face. [2]Hands [3]Full arms. [4]Half arms. [5]Full legs. [6]Half legs. [7]Other (please specify) ________	[1]Face. [2]Hands [3]Full arms. [4]Half arms. [5]Full legs. [6]Half legs. [7]Other (please specify) ________	[1]Face. [2]Hands [3]Full arms. [4]Half arms. [5]Full legs. [6]Half legs. [7]Other (please specify) ________	[1]Face. [2]Hands [3]Full arms. [4]Half arms. [5]Full legs. [6]Half legs. [7]Other (please specify) ________	[1]Face. [2]Hands [3]Full arms. [4]Half arms. [5]Full legs. [6]Half legs. [7]Other (please specify) ________	[1]Face. [2]Hands [3]Full arms. [4]Half arms. [5]Full legs. [6]Half legs. [7]Other (please specify) ________

38) Where did you live during this time period? (Town or city and country, please)
_____

39) Have you been away in the last 6 months?
a) Yes (go to question 40)
b) No (go to question 48)

40) Where did you go?_____

41) How long did you stay there?_____

42) What was the purpose?_____

43) What was the season? _____

44) How would you describe the weather there? _____

45) What time of the day you would normally go out?_____

46) What would you usually wear?_____

47) How much time would you spend over water?_____

48) Have you been sunbathing or used a sunbed in the last 6 months?

a) Yes (go to question 49)
b) No

49) How many times did you do that per month? _____

50) What would you usually wear when sunbathing or using a sunbed?
_____

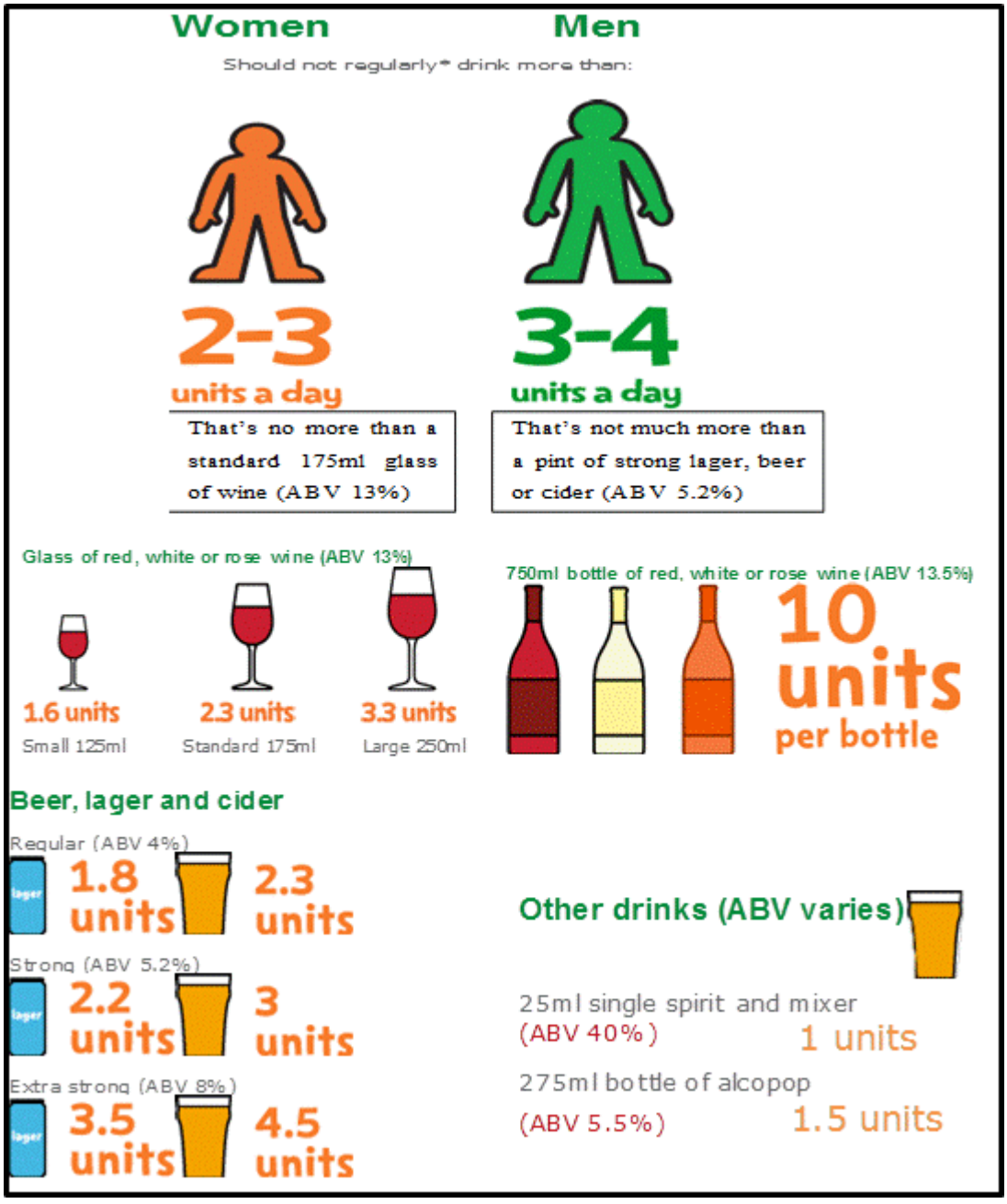
51) Do you use sun cream when sunbathing or using a sunbed? _____

Thank you very much for completing the questionnaire. Because we want to use the information you gave and not waste any of the effort and time you put into this, please take a moment to make sure you:

- have completed every space
- did not skip any questions, except when told to do so
- finish filling out everything (FFQ, Food Dairy, Questionnaire)

We are grateful to you for contributing to this project.

<u>If you wish to collaborate in the next stage of the research</u> please could you leave your information beneath, your generosity is appreciated.



If you do not know how to calculate alcohol consumption, you could also visit or download an app from the following website:

http://www.nhs.uk/change4life/Pages/understanding-alcohol.aspx

Blood withdrawal consent sheet:

The researcher name: TaqwaBushnaq

Title of Research: Vitamin D status in women living in the KSA and the UK and dietary strategies to improve this (in the UK).

Brief statement of purpose of work:

One of the research aims is to measure and acknowledge vitamin D deficiency among women. If you are interested in participating in the research, you will be asked to give fasting blood samples to assess vitamin D levels. 10-15 ml of blood will be withdrawal from you. Fully prepared room set for this purpose will be used to take the specimens by a trained phlebotomist.

We recognise taking part will take up some of your time. We will do our best to minimise any inconvenience by ensuring that we meet at a time convenient for you. We do not expect anyone to suffer any harm or injury as a result of participating in this project. However, blood collection may be uncomfortable or could cause a small bruise.

- The objectives of this research has been explained to me and I understand them.
- I agree to volunteer and give blood for this research to measure vitamin D level.
- Under these circumstances, I agree to participate in the research

Name: …….. **Contact Details (Email or mobile):** ……..……..

Signature: …… **Date:** ……

12.3 Appendix 3: The food diary Instructions and sheets.

Food and Drink Diary Instructions

Please read though these pages before starting your diary

We would like you to keep this diary of everything you eat and drink over three days. Please include all food consumed at home and outside the home, e.g. work, college or restaurants. It is very important that you do not change what you normally eat or drink just because you are keeping this record. Please keep to your usual food habits.

Day and date

Please write down the day and date at the top of the page each time you start a new day of recording.

Time slots

Please note the time of each eating occasion into the space provided. For easy use, each day is divided into sections, from the first thing in the morning to late evening and through the night.

What do you eat?

Please describe the food you eat in as much detail as possible.

Homemade dishes:

If you have eaten any homemade dishes, e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking method. Write this down in the recipe section at the end of the record day. Record how much of the whole recipe you have eaten in the portion size column.

Takeaway and eating out:

If you have eaten a takeaway or made-up dishes not prepared at home such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can, e.g. vegetable curry containing chickpeas, aubergine, onion and tomato.

Brand name

Please note the brand name (if known). Most packet foods will list a brand name, e.g. Birds Eye, Hovis, or supermarket own brands**.**

Labels/Wrappers:

Labels are an important source of information for us. It helps us a great deal if you enclose, in the plastic bag provided, labels from all ready meals and labels from foods of lesser known brands.

Portion sizes and foods quantity:

To describe the quantity or portion size you had you can use scales and household measures, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy. Be careful when describing amounts in spoons that you are referring to the correct spoon size.

Weights from labels is acceptable, e.g. 420g tin of baked beans, 125g pot of yoghurt, and number of items, e.g. 4 fish fingers, 2 chicken nuggets, 1 Rich Tea biscuit.

For drinks, quantity can be described using:

The size of glass, cup, etc. (e.g. large glass) or the volume (e.g. 300ml). Volumes from labels (e.g. 330ml can of fizzy drink).

We would like to know the amount that was actually eaten which means taking into account leftovers. You can do this in two ways:

1- Record what was served and note what was not eaten, e.g. 3 tbsp of peas, only 2 tbsp eaten; 1 Weetabix, ate ½.

2- Only record the amount actually eaten, i.e. 2 tbsp of peas; ½ Weetabix.

When to fill in the diary

- Please record the food you eat as you go, not from memory at the end of the day. Use written notes on a pad if you forget to take your diary with you. Each diary day covers 24 hours, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water.
- Please indicate if this is your normal eating pattern; if not please could you state the reasons, e.g. diet, special occasion.

On the first page you can see a 1-day example that has been completed. These examples show you how we would like you to record your food and drink, for example a ready meal and a homemade dish.

- If you have any queries about how to complete the diary please contact the researcher.
  Email: tooootei4@hotmail.com
- It only takes a few minutes for each eating occasion!

Food Diary **Example**

Day: Monday			**Date: 2/4/2013**
	Morning		
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**
7:30 am	Fresh milk semi-skimmed	Asda	½ cup
	Cornflakes	Kellogg's	20 g / ¼ cup
	White Sugar	Silverspoon	10 g / 1 tablespoon
9 am	Tea	Asda	1 cup
	with milk semi-skimmed	Asda	Splash
11 am	Biscuit water	 tap	2 2 cup

Afternoon			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size or quantity eaten**
12:30 pm	Tuna sandwich	Homemade	2 bowls
	Coffee	Nescafé Classic	1 cup
	Banana	Medium size	1
4 pm	Mixed nuts water	Sainsbury's Mixed Nuts tap	handful 2 cup

Evening			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**
7 pm	Seafood spaghetti	Homemade	1 bowl
	Orange Juice	Tesco Everyday Value Orange Juice	1 cup
8:30 pm	Chocolate	Kit Kat	4 fingers/ 250g

Did you finish all the food and drink that you recorded in the diary today?

◯ Yes

✓ No – If no, please go back to the diary and make a note of any leftover

---

Please record over the page details of any recipes or (if not already described) ingredients of made-up dishes or takeaway dishes.

Write in recipes or ingredients of made up dishes or takeaway dishes	
NAME OF DISH : Seafood spaghetti (2 bowls)	
Ingredients & quantity	**Brief description of cooking method**
200g dried spaghetti + pinch of salt in boiled water For the seafood sauce 2 tbsp olive oil/1 clove garlic, finely chopped/200g cherry tomatoes 300g ASDA Chosen by You Seafood Cocktail /3 tbsp Tesco crème fraîche small handful basil leaves, tarragon leaves – shredded, ½ lemon, juice/ pinch of sea salt, black pepper and dried chilli	Fry garlic in oil, add seafood and fry 2 mins Add pepper, tomatoes, crème fraîche simmer for 10 mins & add herbs before serving.

Name:

Date:	Day:	Morning	
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Afternoon			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Evening			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Did you finish all the food and drink that you recorded in the diary today?

Yes

No – If no, please go back to the diary and make a note of any leftover

---

Please record on next page details of any recipes or (if not already described) ingredients of made-up dishes or takeaway dishes.

Write in recipes or ingredients of made-up dishes or takeaway dishes	
NAME OF DISH :	
Ingredients & quantity	**Brief description of cooking method**
NAME OF DISH :	
Ingredients & quantity	**Brief description of cooking method**

Date:	Day:	Morning	
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Afternoon			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Evening			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Did you finish all the food and drink that you recorded in the diary today?

Yes

No – If no, please go back to the diary and make a note of any leftover

---

Please record on next page details of any recipes or (if not already described) ingredients of made up dishes or takeaway dishes.

Write in recipes or ingredients of made-up dishes or takeaway dishes	
NAME OF DISH :	
Ingredients & quantity	**Brief description of cooking method**
NAME OF DISH :	
Ingredients & quantity	**Brief description of cooking method**

Date:	Day:	Morning	
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Afternoon			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Evening			
Time	**Food/Drink description preparation**	**Brand name**	**Portion size (quantity eaten)**

Did you finish all the food and drink that you recorded in the diary today?

Yes

No – If no, please go back to the diary and make a note of any leftover

---

Please record on next page details of any recipes or (if not already described) ingredients of made-up dishes or takeaway dishes.

Write in recipes or ingredients of made-up dishes or takeaway dishes	
NAME OF DISH :	
Ingredients & quantity	**Brief description of cooking method**
NAME OF DISH :	
Ingredient & quantity	**Brief description of cooking method**

12.4 Appendix 4: The Arabic version of the questionnaire, participants' information sheet and consent sheet to Participate.

إقرار موافقة على المشاركة في مشروع البحث

اسم الباحثة: تقوى بشناق

عنوان البحث: حالة فيتامين (د) لدى النساء اللاتي يعشن في المملكة العربية السعودية والمملكة المتحدة , مع وضع الاستراتيجيات الغذائية لتحسين ذلك (في المملكة المتحدة)

وصف موجز للغرض من الدراسة:

ان الهدف من هذه الدراسة يتمثل في قياس نقص فيتامين (د) بين النساء بالإضافة الى تحديد العوامل الاساسية الاكثر تأثيرا على حالة الفيتامين لديهم.

لتحقيق هدف الدراسة سوف تستخدم الباحثة استبيان من أجل جمع المعلومات التي تتعلق بأنماط الحياة ولون البشرة والتعرض الى أشعة الشمس والادوية وعوامل اخرى التي قد تؤثر بصورة طبيعية على قدرة الجسد لإنتاج أو تخزين فيتامين (د). بالإضافة الى ذلك يتعين عليك الاحتفاظ بمذكرة غذائية لمدة ثلاثة أيام واكمال استبيان التقييم الغذائي للفيتامين والعادات الغذائية.

كخطوة اختيارية ، سوف يطلب منك إعطاء عينة دم أثناء الصيام لتقييم مستويات فيتامين (د) لديك. سوف تؤخذ العينة من قبل أخصائي في غرفة خصصت لهذا الغرض وبسرية تامة وفي موعد متفق عليه.

وبعد مايقارب ثلاثة أشهر تقريبا قد يتم اعطائك برنامج غذائي ويمكن أن يُطلب منك عينة دم مره أخرى وذلك بغرض قياس أي تغير يطرأ على حالة الفيتامين لديك.

يرجى قراءة الملخص التالي وفي حال موافقتك عليه نأمل منك التوقيع أدناه

أهداف هذا البحث شُرحت الي:

- تقدير المدخلات الغذائية وذلك عبر ملئ بيانات استبيان التقيم الغذائي ومذكرة الطعام.
- تحديد الى أي مدى يمكن أن تؤثر العوامل الغذائية وأساليب الحياة المختلفة على حالة فيتامين (د) ولهذا الغرض يتعين اكمال الاستبيانات.
- قياس مستوى فيتامين (د) عبر اجراء فحص دم.
- اتباع برنامج غذائي وذلك بغرض تحسين مستوى فيتامين (د).

وفي ظل هذه الظروف فإنني أوافق على المشاركة في هذا البحث

الاسم: ..... بيانات التواصل (ايميل أو جوال): .....

التوقيع: ..... التاريخ: .....

رقم هوية المشارك:
.....

Manchester Metropolitan University

ورقة معلومات للمشاركين

تشير التقديرات الى أن بليون شخص حول العالم يعانون من نقص أو عدم كفاية فيتامين (د) (Tsiaras and Weinstock, 2011). وحيث أن العديد من العوامل تؤثر على مستويات فيتامين (د) مثل لون البشرة والعمر والامراض المزمنة والملابس والتعرض الى أشعة الشمس. ونقص مستوى هذا الفيتامين يمكن أن يؤدي الى أذى العظام. كما أن نقص هذا الفيتامين في مرحلة البلوغ يؤدي الى لين العظام وهشاشتها أو ضعف العضلات وقد يزيد من احتمالية التعرض الى الكسور (Holick, 2007; Insel, Turner & Ross, 2003). وهناك أدلة متزايدة على دور فيتامين (د) في منع أو تقليص مستوى الاصابة بالعديد من الامراض المزمنة بما في ذلك الاصابة بمرض السرطان ونقص المناعة وأمراض القلب والاوعية الدموية بالإضافة الى بعض الامراض الجلدية مثل مرض الصدفية (Holick, 2007; Insel et al, 2003). ووفقا لبحوث السابقة فان الطريقة المثلى لقياس مستوى فيتامين (د) هي عبر قياس الصيغة الاولية (25 هيدروكسي فيتامين د) في الدم.

وبناء على ذلك نرغب في دعوتك للمشاركة في دراسة تعنى بقياس مستويات فيتامين (د) من أجل تقييم العوامل التي تؤثر على هذا الفيتامين في النساء الاتي يتمتعن بصحة جيدة.

- ✓ راجعت لجنة أخلاقيات البحوث في جامعة مانشستر متروبوليتان هذا المشروع البحثي واعتمدته.
- ✓ سيُطلب منك تعبئة استبيان لتقييم التعرض الى أشعة الشمس والادوية ولون البشرة والعوامل الاخرى التي تؤثر على مستوى هذا الفيتامين.
- ✓ سيُطلب منك اعطاء عينة دم أثناء الصيام من أجل قياس مستوى فيتامين (د) في الدم وذلك في بداية الدراسة, وعينة أخرى بنهاية الدراسة وذلك لتحديد تأثير برنامج التدخل الغذائي على مستوى فيتامين (د) لديك. وسيتم أخذ هذه العينات عبر أشخاص مدربين ومتخصصين.
- ✓ سيُطلب منك الاحتفاظ بمذكرة غذائية لمدة ثلاثة أيام بالإضافة الى اكمال استبيان لقياس مدخلات فيتامين (د).
- ✓ من خلال مشاركتك في هذه الدراسة سيتم اخبارك بمستوى فيتامين (د) لديك وهل يتعين عليك زيادة مستوى هذا الفيتامين لديك عبر تناول الاغذية الغنية به أو لا.
- ✓ لديك الحق في أن تغيري رأيك وأن تنسحبي من البحث في أي وقت وبدون أي سبب.
- ✓ سيتم التعامل مع جميع المعلومات التي تقدميها بسرية تامة وفي اغراض البحث فقط.
- ✓ نحن نعي بأن بمشاركتك في البحث ستطلب منك بعض الوقت للقيام بذلك, لذلك سنسعى لتقليص أي ازعاج عبر تحديد الموعد المناسب اليك. ونحن لا نتوقع اصابة اي شخص سيشارك في هذا البحث بأي ضرر من أي نوع, لكن خلال سحب العينة قد تتعرضي الى ألم بسيط او كدمة غير مؤذية بعد ذلك.
- ✓ اذا كان لديك أي استفسارات نرجوا عدم التردد في التواصل مع الباحث.
- ✓ نشكرك على قراءة هذا المستند ونتوقع منكم المساعدة في هذا البحث.

رقم هوية المشارك:

...

التاريخ:________________________

الجزء 1: الاستبيان

1- أين تعيشي؟

أ- في المملكة المتحدة

ب- في المملكة العربية السعودية

2- ما هو عمرك __________

3- ما هو وزنك __________ كجم

4- ما هو طولك __________ سم

5- ما هو أصلك العرقي؟

____

6- هل أنت حامل؟

أ- نعم

ب- لا

7- هل انت مرضعة؟

أ- نعم

ب- لا

8- هل لديك أطفال؟

أ- نعم

ب- لا

9- كم لديك من الاطفال؟ ____________________

10- كيف تصفين بشرتك؟

أ- بشرة شديدة البياض (دائما ما تتعرضي لحروق عند التشميس, لا تسمر ابدا), عينين زرقاء اوعسلي, شعر أحمراو أشقر

ب- بشرة بيضاء وعين زرقاء شعر بني (عند التشميس تتعرضي للحرق غالبا وبسهولة و نادرا تسمري)

ت- بشرة بيضاء (قد تتعرضي لحروق بصورة طفيفة ولكن تسمري).

ث- بشرة سمراء فاتحة (تسمري بعد التعرض الى أشعة الشمس و لا تتعرضي لحروق الشمس بكثرة)

ج- بشرة سمراء (نادر ما تحرق وتسمر بصورة غامقة بسهولة)

ح- بشرة بنية غامقة (لا تحرق وغالبا تسمر)

11- هل تعانين من أي من الامراض التالية؟

أ- هشاشة العظام
ب- الغدة الدرقية
ت- الاضطرابات المعوية
ث- مرض كبدي
ج- مرض كلوي
ح- سوء امتصاص الدهون
خ- مرض القلب
د- سرطان
ذ- ارتفاع في ضغط الدم
ر- مرض السكري
ز- حساسية الحليب و اللاكتوز
س- أخرى (حدد) _____
ش- لا أعاني من أي مرض

12- هل تتناولين أي أدوية طبية بما في ذلك المكملات الغذائية؟

أ- نعم (يرجى ذكرها) _____
ب- لا

13- هل تعرضت الى أي كسور على مر العامين الماضيين؟

أ- نعم
ب- لا (انتقلي الى السؤال التالي)

14- متى كان اخر كسر تعرضت له؟

أ- أقل من 6 أشهر ماضية
ب- من 6 أشهر الى عام
ت- من عام الى 18 شهر
ث- من 18 شهر الى عامين.

الجزء 2: استبيان التقيم الغذائي السنوي. يرجى الاجابة على جميع الاسئلة, واذا كنتي غير متأكدة فان الاجابة التقديرية أفضل من عدم الاجابة.

15- على مدى الاشهر 12 الماضية كم عدد المرات التي تناولت بها الاطعمة المذكورة أدناه؟

الطعام	على مدى الاشهر 12 الماضية كم عدد المرات التي تناولت بها الاطعمة المذكورة أدناه؟						
	ولا مرة	أقل من مره في الشهر	من مرة الى ثلاث مرات شهريا	مرة اسبوعيا	3-2 مرات أسبوعيا	مرة يوميا	مرتين أو أكثر أسبوعيا
زيت كبد الحوت	1	2	3	4	5	6	7
الكبدة (مثل كبد البقر والدجاج)	1	2	3	4	5	6	7
سمك السالمون الطازج	1	2	3	4	5	6	7
سمك السَّردين طازج	1	2	3	4	5	6	7
ماكريل طازج	1	2	3	4	5	6	7
سمك التونة الطازج	1	2	3	4	5	6	7
سمك الرنجة	1	2	3	4	5	6	7
سالمون معلب	1	2	3	4	5	6	7
سمك السَّردين معلب	1	2	3	4	5	6	7
ماكريل معلب	1	2	3	4	5	6	7
تونة معلبة	1	2	3	4	5	6	7
بيض	1	2	3	4	5	6	7
مشروم طازج	1	2	3	4	5	6	7
مشروم مجفف على اشعة الشمس	1	2	3	4	5	6	7
حليب	1	2	3	4	5	6	7
عصير برتقال المعالج	1	2	3	4	5	6	7
لبن رائب\زبادي	1	2	3	4	5	6	7
زبدة	1	2	3	4	5	6	7
المارجرين	1	2	3	4	5	6	7
جبنة	1	2	3	4	5	6	7
حبوب الافطار	1	2	3	4	5	6	7

16- هل أنت نباتية ؟

أ- نعم منذ _____ عام

ب- لا

17- ما هو نوع الحليب الذي تستخدمينه غاليا؟ نأمل اختيار واحد فقط

أ- كامل الدسم

ب- منزوع الدسم

ت- شبه منزوع الدسم

ث- مجفف

ج- الصويا

ح- لا أستخدم أي نوع

18- كم كمية الحليب التي تشربيها يوميا بما في ذلك المضافة للشاي والقهوة والمشروبات التي يدخل الحليب ضمن تركيبها؟

أ- لا شيئ

ب- أقل من 250 مل (1 كوب)

ت- ما بين 250 الى 500 مل (1-2 كوب)

ث- ما بين 500 الى 750 مل (2-3 كوب)

ج- 750 مل (3 أكواب) أو أكثر

19- ما هي حبوب الافطار التي تتناولينها في العادة؟

الماركة	النوع

20- هل تستخدمين المارجرين/ الزبدة على الخبز الذي تتناولينه؟

أ- نعم

ب- لا

ت- بعض الاحيان

21- كما عدد شرائح الخبز التي تتناولينها يوميا/ أسبوعيا مع االمارجرين/ الزبدة؟

أ- _____ شريحة يوميا

ب- _____ شريحة أسبوعيا

22- ما هي كمية المارجرين/ الزبدة التي تضعيها على الخبز؟

أ- كمية قليلة

ب- كمية متوسطة

23- ما نوع الدهون التي غالبا ما تستخدمينها في الطهي. يمكن اختيار اكثر من خيار

أ- الزبدة

ب- المارجرين

ت- السمن

ث- زيت نباتي

ج- سمن نباتي

ح- لا استخدم أي دهون

خ- أخرى _____

24- هل تتناولين أي فيتامينات أو معادن أو زيوت أسماك أو أي مكملات غذائية أخرى؟

أ- نعم

ب- لا

ت- في بعض الاحيان

25- في حالة الاجابة ب (نعم) أو (في بعض الاحيان) يرجى اكمال الجدول الموضح أدناه

اسم وماركة المكمل الغذائي	الجرعة التي تتناولينها	ما هي عدد المرات التي تتناولينها من هذه المكملات؟			
		يوميا	أسبوعيا	شهريا	أقل
		1	2	3	4
		1	2	3	4
		1	2	3	4

26- ما هي الاسباب وراء استخدامك للمكملات؟

أ- وقائية

ب- علاجية (تم وصفها من خلال الطبيب)

27- هل تم اخبارك من قبل طبيب أنك تعانين من نقص في فيتامين (د)؟

أ- نعم

ب- لا (انتقل الى السؤال التالي)

28- متى كان ذلك؟

أ- العمر _____ عام _____

29- هل تستخدمين منتجات مدعمة بفيتامين (د) مثل الزبدة وعصير البرتقال والحليب وحبوب الافطار؟

أ- نعم غالبا ما استخدمها

ب- في بعض الاحيان

ت- لا

ث- لا أعرف

30- هل قمت بتغيير نظامك الغذائي خلال الاثنا عشرة شهرا الماضية؟

أ- نعم

ب- لا

31- اذا كانت الاجابة بنعم فما هو السبب وراء هذا التغيير؟

أ- هشاشة العظام

ب- الغدة الدرقية

ت- الاضطرابات المعوية

ث- مرض كبدي

ج- مرض كلى

ح- سوء امتصاص الدهون

خ- مرض قلبي

د- سرطان

ذ- ارتفاع في ضغط الدم

ر- مرض السكر

ز- حساسية الحليب وعدم تحمل اللاكتوز

س- أخرى (حدد) _____

32- كم عدد المرات التي تتناولين بها المشروبات الكحولية؟

أ- لا أتناولها
ب- مرة في الاسبوع
ت- أكثر من مرة في الاسبوع
ث- أقل من مرة في الاسبوع

33- **كم عدد الوحدات التي تتناولينها؟** نأمل الاطلاع على أخر صفحة في الاستبيان وذلك لاستخدام جدول وحدات الكحول كمرجع

- ـــــــــ وحدة من النبيذ
- ـــــــــ وحدة من الجعة
- ـــــــــ وحدة من المشروبات الروحية
- أخرى (حدد) ـــــــــــــــــ

34- هل انت مدخنة (سجائر/ ارجيلة .....)؟

أ- نعم (اذهبي الى السؤال 36)
ب- لا (اذهبي الى السؤال 37)
ت- كنت مدخنة ولكن ليس الان (اذهبي الى السؤال35)

35- منذ متى أقلعت عن التدخين؟

أ- من ـــــــــ عام
ب- أو من ـــــــــ شهر

36- كيف تصفين نفسك كمدخنة؟

أ- أدخن كل يوم \كثيرا
ب- أدخن أحيانا ولكن ليس كل يوم \ في المناسبات

<u>الجزء 3: استبيان التعرض الى أشعة الشمس</u>

يرجى تقدير تعرضك الى أشعة الشمس خلال أخر أسبوع وذلك عبر اكمال الجدول في الصفحة التالية. ونأمل منك الاجابة على الاسئلة التي تبدأ بـ "كم المدة الزمنة.....؟" عبر كتابة عدد الدقائق أو الساعات في الفراغ المحدد. فعلى سبيل المثال في السؤال رقم 37, نود أن نعلم كم عدد الساعات التي تقضينها خارج المنزل يوميا خلال الفترات الموضحة في كل عمود. لذلك اذا كنت تقضي 10 دقائق خارج المنزل ما بين الساعة 07 ص. وحتى الساعة 9 ص. فانه يتعين عليك كتابة 10 دقائق في المربع المخصص. واذا كنت لا تذهبي الى اي مكان خلال تلك الفترة فتكتبي 0.

37- استبيان التعرض الى أشعة الشمس

	7-9 ص.	9-11 ص.	11 ص- 1 م	1-3 م	3-5 م	5-7 م
دوني اجابة لكل فترة خلال اليوم						
▪ كم المدة الزمنية التي تقضينها خارج المنزل خلال الفترات التالية؟						
▪ كم المدة الزمنية التي تقضينها في الظل؟						
▪ ما هو وصفك لحالة الطقس؟						
▪ كم المدة الزمنية التي يتم تغطية رأسك بها (على سبيل المثال عبر ارتداء قبعة أو الحجاب)						
▪ كم المدة الزمنية التي تستعملي كريم واقي من الشمس؟						
▪ هل الكريم الواقي من الشمس يوجد بها حماية من الاشعة فوق البنفسجة أو اشعة الشمس؟						
▪ ما هو عنصر الحماية الموجود في الكريم الواقي المستخدم (SPF)؟						
▪ ما هي مناطق الجسم المكشوفة عادة والتي تتعرض الى أشعة الشمس؟	1- الوجه 2- اليدين 3- الذراع بالكامل 4- نصف الذراع 5- كامل القدمين 6- نصف القدمين 7- أخرى حدد	1. الوجه 2. اليدين 3. الذراع بالكامل 4. نصف الذراع 5. كامل القدمين 6. نصف القدمين 7. أخرى حدد	1. الوجه 2. اليدين 3. الذراع بالكامل 4. نصف الذراع 5. كامل القدمين 6. نصف القدمين 7. أخرى حدد	1. الوجه 2. اليدين 3. الذراع بالكامل 4. نصف الذراع 5. كامل القدمين 6. نصف القدمين 7. أخرى حدد	1. الوجه 2. اليدين 3. الذراع بالكامل 4. نصف الذراع 5. كامل القدمين 6. نصف القدمين 7. أخرى حدد	1. الوجه 2. اليدين 3. الذراع بالكامل 4. نصف الذراع 5. كامل القدمين 6. نصف القدمين 7. أخرى حدد

38- أي تعيشين خلال هذه الفترة الزمنية التي تم تعبئة الاستبيان خلالها ؟ (المدينة\ البلد) ـــــــ

39- هل سافرتي الى اي مكان خلال 6 أشهر الماضية ؟

أ- نعم (يتعين الذهاب الى السؤال 40)

ب- لا (يتعين الذهاب الى السؤال 48)

40- أين ذهبتي؟ ـــــــــــ

41- كم المدة الزمنية التي قضيتيها هناك؟ ـــــــ

42- ما هو الغرض من السفر (اجازة\عمل..)؟ ـــــــ

43- في أي فصل من فصول العام كانت الرحلة؟ ـــــ

44- كيف تصفين الطقس هناك؟ ـــــــــ

45- ما هو الوقت الزمني الذي كنت تخرجين خلاله؟ ـــــ

46- ما هي الملابس التي كنت ترتدينها؟ ـــــــ

47- كم المدة الزمنية التي كنتي تقضينها بعيدا عن الماء(سباحة)؟ ـــــ

48- هل قمت بعمل حمام شمس او استخدمت السرير الضوئي (تشميس\ تان) خلال الستة أشهر الماضية؟

أ- نعم (اذهبي الى السؤال 49)

ب- لا

49- كم عدد المرات التي قمت بها بعمل حمام شمس خلال الشهر؟ ـــــ

50- ما هي الملابس التي غالبا ما كنت ترتدينها خلال حمام الشمس؟ ـــــ

51- هل تستخدمي كريم الحماية من أشعة الشمس؟ ـــــ

نشكرك لك اكمالك للاستبيان ، وحتى نتمكن من استخدام المعلومات التي قدمت الينا من قبلك دون ان اضاعة أيا من من الجهد والوقت الذي بذلتيه ، يرجى التوقف لحظة للتأكد من:-

- اكمال جميع الفراغات
- عدم تجاوز أي سؤال الا اذا كان ذلك من المتطلبات
- اكمال جميع الاستبيانات

كل التقدير والامتنان لمساهمتكم الفعالة في هذا المشروع البحثي

Manchester Metropolitan University

إقرار موافقة لسحب عينة الدم

اسم الباحثة: تقوى بشناق

عنوان البحث: *وضع فيتامين (د) لدى النساء التي تعيش في المملكة العربية السعودية والمملكة المتحدة , مع وضع الاستراتيجيات الغذائية لتحسين ذلك (في المملكة المتحدة)*

وصف موجز للغرض من الدراسة:

ان الهدف من هذه الدراسة يتمثل في قياس نقص فيتامين (د) بين النساء بالإضافة الى تحديد العوامل الاساسية التي تؤثر على حالة الفيتامين لديهم. إذا كنت ترغبي المشاركة في هذا البحث ، سوف يطلب منك إعطاء عينة دم أثناء الصيام لتقييم مستويات فيتامين (د) لديك. سوف يتم سحب من 10 الى 15 مل من الدم منك, و سوف تؤخذ العينة من قبل أخصائي في غرفة خصصت لهذا الغرض وبسرية تامة وفي الموعد المتفق معك عليه.

نحن نعي بأن بمشاركتك في البحث ستطلب منك بعض الوقت للقيام بذلك, لذلك سنسعى لتقليص أي ازعاج عبر تحديد الموعد المناسب اليك. ونحن لا نتوقع أن أي شخص سيشارك في هذا البحث سيتعرض الى أي ضرر من أي نوع. قد يسبب اجراء اخذ عينة الدم في شعورك ببعض الالم البسيط.

- أهداف البحث وضحت الي وقد فهمتها جيدا
- أوافق طواعية بأني أخضع الى فحص الدم وذلك بغرض قياس مستوى فيتامين (د).
- وفي اطار هذه الظروف فإنني أوافق على المشاركة في هذا البحث

الاسم: ..... بيانات التواصل (ايميل أو جوال): .....

التوقيع: ..... التاريخ: .....

12.5 Appendix 5: The Arabic version of : The food diary Instructions and sheets.

تعليمات اليوميات الغائية

نأمل قراءة التعليمات التالية قبل البدء في اليوميات

نود منك أن تذكر في هذه اليوميات جميع ما تأكله وتشربه لمدة ثلاثة أيام. مع ذكر جميع ما تم تناوله من طعام وشراب سواء كان داخل المنزل أو خارجه مثل الكلية أو المطاعم. فمن المهم أن لا تغير ما تأكله أو تشربه عادة فقط لغرض تعبئة هذا السجل. نأمل أن تحافظ على عادتك الغذائية التي تتبعها بصورة طبيعية.

اليوم والتاريخ

يرجى كتابة اليوم والتاريخ في أعلى الصفحة في كل مرة تبدأ يوم جديد من التسجيل.

الحيز الزمني

يرجى تدوين الوقت الزمني لكل وجبة تتناولها وذلك في الفراغ المخصص لذلك. ومن أجل سهولة الاستخدام تم تقسيم كل يوم الى عدة أقسام تبدأ منذ الصباح الى المساء وحتى أوقات الليل المتأخر.

ماذا تأكل؟

يرجى وصف الطعام الذي تتناوله بصورة تفصيلية كلما أمكن.

الوجبات الغذائية المعدة بالمنزل

اذا تناولت أي وجبات غذائية أُعدت في المنزل مثل وجبة الدجاج نأمل منك تسجيل طريقة الطهي والمكونات مع المقادير (بما في ذلك الماء أو السوائل الاخرى) لكامل الوصفة, بالإضافة الى عدد الاشخاص الذين تقدم اليهم الوجبة وطريقة الطهي. ويتعين تدوين ذلك في الجزء المخصص الى طريقة الطهي بنهاية السجل اليومي. كما نأمل منك تسجيل الكمية التي تناولتها من الوجبة وذلك في الجزء المخصص لحصة الفرد.

الوجبات الجاهزة (السريعة

إذا كنت قد تناولت الوجبات الجاهزة أو أطباق مختلقة ليست معدة في المنزل مثل تناول الطعام في مطعم أو منزل أحد الأصدقاء، يرجى تسجيل أكبر قدر من التفاصيل حول المكونات، على سبيل المثال الخصار بالكاري والتي تحتوي على الحمص والباذنجان والبصل والطماطم.

الاسم التجاري للوجبة

يرجى تدوين الاسم التجاري للوجبة حيث ان جميع الوجبات الجاهزة تكون مغلفة بطريقة ما ويكون على التغليف الاسم التجاري للوجبة مثل فطيرة لوزين بالجبن و الزعتر/ عصير المراعي 250 مل تفاح.

الغلاف

غالبا ما تكون اغلفة الاطعمة بمثابة مصدر هام للمعلومات . واذا استطعت ارفاق الغلاف الموجود في جميع الوجبات الجاهزة التي تتناولها فان هذا سيساعدنا كثيرا.

أحجام وكمية الطعام

لكي نتمكن من وصف كمية الطعام الذي تتناوله يمكن أن تستخدم الادوات والمعايير المنزلية, مثلا ملعقة صغير (ملعقة الشاي) من السكر, وشريحتين سميكتين من الخبز, وأربعة ملاعق كبيرة من البازلاء ونصف كوب من المرق. ويتعين أن تكون حريص عند وصف الكميات باستخدام الملاعق حيث انه يتعين عليك الاشارة الى الحجم الصحيح للملعقة(ملعقة شاي تعادل 5جم/ملعقة حلا جم10/ ملعقة طعام 15جم).

كما أنه من الممكن استخدام مسميات الاوزان, فعلى سبيل المثال يمكن أن تقول 420 جرام من الفاصوليا, و125 جرام من الزبادي بالإضافة الى عدد الاشياء مثل 4 قطع من فخذ دجاج و1 بسكويت شاي.

ومن اجل وصف المشروبات يمكن أن تستخدم:-

حجم الكأس الزجاجي (مثال كأس كبير) أو ذكر حجمه (مثال 300 مل), أو ذكر الاحجام المدونة على بطاقة المحتويات (330 مل من المشروبات الغازية)

نود أن نعرف مقدار الطعام الذي تناولته فعليا مع الأخذ بعين الاعتبار بقايا الطعام. ويمكنك القيام بذلك بطريقتين:

1- سجل ما قُدم اليك ودون ما لم تأكله, على سبيل المثال 3 ملاعق كبيرة من البازلاء، و, وتم تناول ملعقتين فقط.
2- فقط دون الكمية التي تناولتها بالفعل.

متى يتم تعبئة اليوميات:

- يرجى تسجيل الطعام الذي تتناوله أول بأول وليس عبر ذاكرتك في نهاية اليوم. كما تستطيع استخدام اي اوراق عينيوة او الكترونية لتدوين الملاحظات والكميات اذا نسيت أخذ دفتر اليوميات الخاص بالبحث. كل يومية تغطي اليوم على مدار 24 ساعة لذك يرجى منك تسجيل أي طعام أو شراب تتناوله خلال اوقات النهار و الليل. كما أنه يتعين عليك تسجيل الوجبات التي تتناولها ما بين الوجبات الرئيسية (التصبيرة) بما في ذلك الماء.
- ونأمل منك الاشارة عما اذا كانت هذه هي عاداتك الغذائية الطبيعية, وفي حال اذا لم تكن كذلك نرجوا ذكر ملاحظة بالاسباب (على سبيل المثال اتباع حمية غذائية).

في الصفحة الاولى ستجد مثال موضح على يوم قد تم اكماله وتعبئته. فهذا المثال يوضح اليك الطريقة التي نود أن تتبعها خلال تسجيلك للأطعمة والمشروبات التي تتناولها, على سبيل المثال الوجبات الجاهزة والوجبات التي يتم اعدادها في المنزل.

- في حال رغبت بالاستفسار عن أي شيء يتعلق بالمذكرات يرجى عدم التردد في الاتصال بالباحثة.

Email: tooootei4@hotmail.com

(مثال) مذكرة تدوين الطعام

اليوم: الاثنين	الصباح	التاريخ: 2013/04/02

الوقت	وصف الطعام / الشراب	اسم الماركة	الكمية التي تم تناولها
7:30 صباحا	حليب طازج شبه منزوع الدسم	المراعي	نصف كوب
	كورن فليكس	كيلوج	20 جران
	سكر ابيض	ملعقة فضة	10 جرام/ ملعقة كبيرة
9 ص	شاي	لبتون	كوب
	مع حليب شبه منزوع الدسم	المراعي	رشة
11 ص	بسكويت	دايجستيف	2
	ماء	صنبور	2 كوب

بعد الظهر			
الوقت	وصف الطعام / الشراب	اسم الماركة	الكمية التي تم تناولها
12:30 م	سندويتش تونا	أعد في المنزل	2
	قهوة	نسكافيه	كوب
	موزن	حجم متوسط	1
4 م	مكسرات مشكلة		ملئ اليد
	ماء	صنبور	2 كوب

المساء			
الوقت	وصف الطعام / الشراب	اسم الماركة	الكمية التي تم تناولها
7 م	معكرونة المأكولات البحرية	أعد في المنزل	1 طبق
	عصير برتقال	المراعي	كوب
8:30 م	شوكولاتة	كيت كات	4 أصابع/ 250 جرام

هل تناولت كمية الطعام التي دونها في هذه اليومية اليوم بصورة كاملة؟

☐ نعم

☐ لا

اذا كانت الاجابة بلا يرجى العودة الى اليومية وتدوين أي بقايا تركت.

يرجى تسجيل تفاصيل أي وصفات أو مكونات للأطعمة الجاهزة في الصفحة التالية

سجل أي وصفات أو مكونات الأطعمة الجاهزة التي تناولتها	
اسم الوجبة: معكرونة المأكولات البحرية (الكمية تكفي شخصين)	
المكونات	وصف مختصر لعملية الطهي
200 جرام معكرونة مجففة+ ذرة ملح في ماء مغلية ولعمل سوس المأكولات البحرية ملعقتين كبيريتين من الزيت, فص من الثوم و200 جرام طماطم , 300 جرام من المأكولات البحرية التي تختارها مع أوراق الريحان ونصف ليمونه وقليل من الملح.	يقلى الثوم في الزيت، وبعد ذلك يتم إضافة المأكولات البحرية وتقلى لمدة 2 دقيقة, ويتم بع ذلك إضافة الفلفل والطماطم ويترك على نار خفيفة لمدة 10 دقائق ومن ثم يتم إضافة الأعشاب قبل التقديم.

مذكرة تدوين الطعام

التاريخ: ــــ/ــــ/ــــــ

اليوم:	الصباح		**التاريخ:**
الوقت	وصف الطعام / الشراب	اسم الماركة	الكمية التي تم تناولها

	بعد الظهر		
الوقت	وصف الطعام / الشراب	اسم الماركة	الكمية التي تم تناولها

	المساء		
الوقت	وصف الطعام / الشراب	اسم الماركة	الكمية التي تم تناولها

هل تناولت كمية الطعام التي دونها في هذه اليومية اليوم بصورة كاملة؟

☐ نعم

☐ لا

اذا كانت الاجابة بلا يرجى العودة الى اليومية وتدوين أي بقايا تركت.

يرجى تسجيل تفاصيل أي وصفات أو مكونات للأطعمة الجاهزة في الصفحة التالية

مذكرة تدوين الطعام

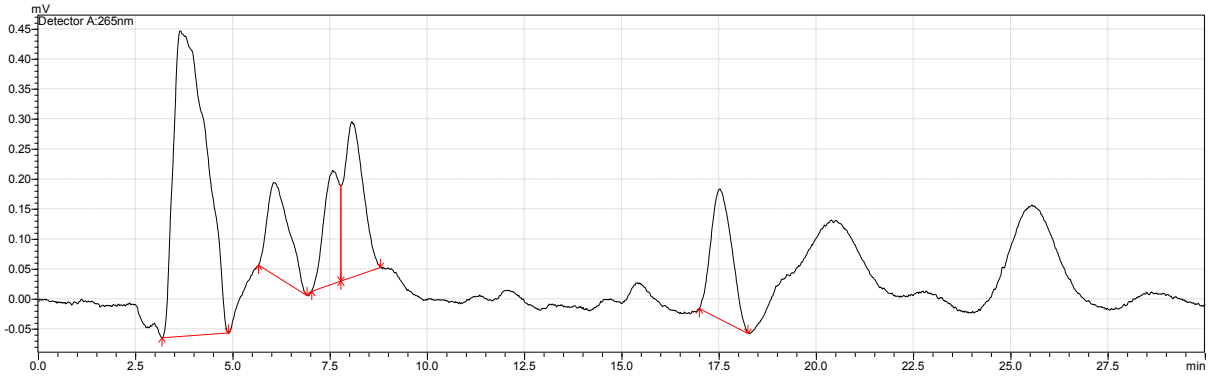
التاريخ: ــــ/ــــ/ــــــــ

سجل أي وصفات أو مكونات الأطعمة الجاهزة التي تناولتها	
اسم الوجبة:	
المكونات	وصف مختصر لعملية الطهي
سجل أي وصفات أو مكونات الأطعمة الجاهزة التي تناولتها	
اسم الوجبة:	
المكونات	وصف مختصر لعملية الطهي

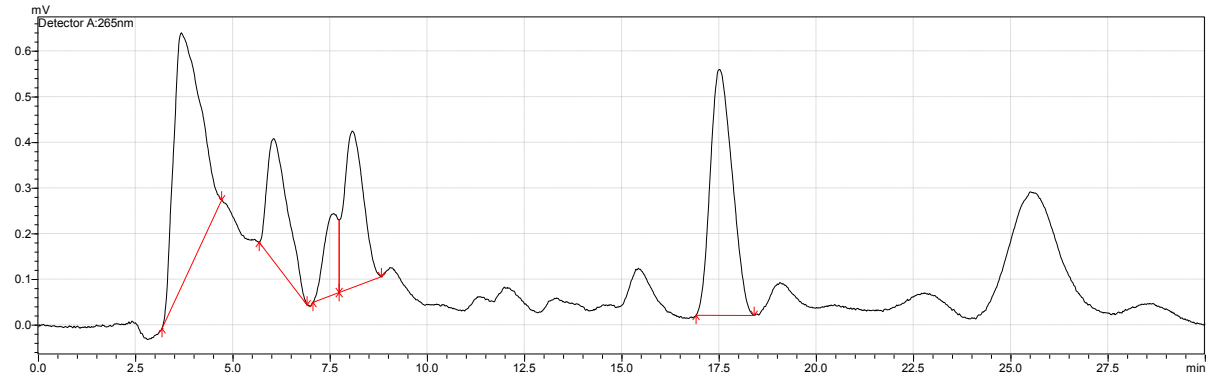
12.6 Appendix 6: The HPLC outputs of vitamin D standard.

HPLC Vitamin D Standard 21/1/2013 method 1

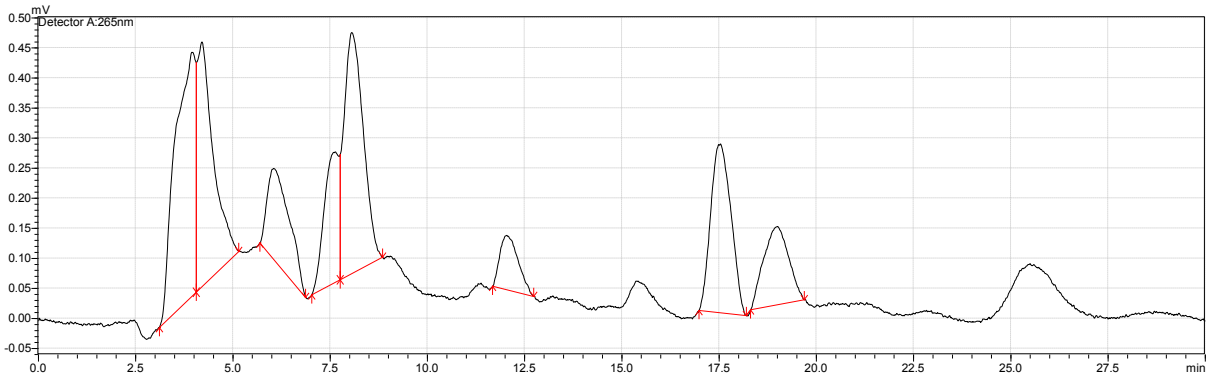
120 nml



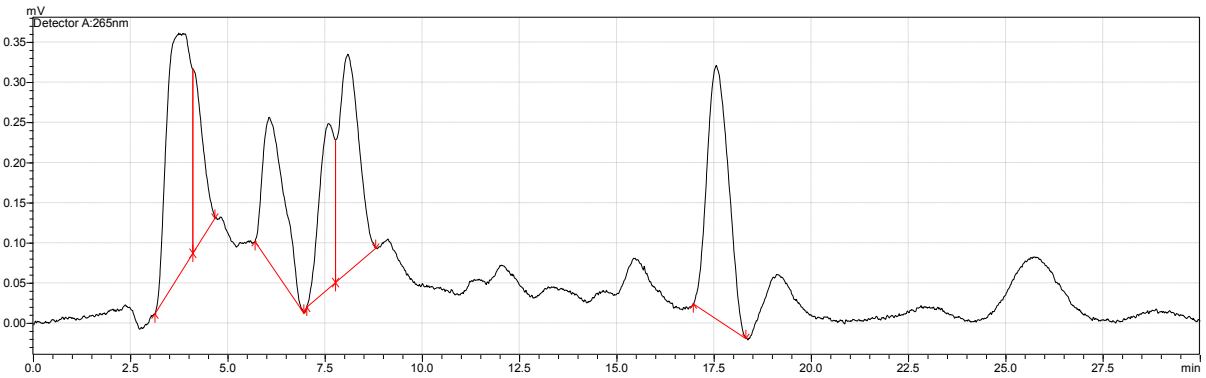
60 nml



30 nml

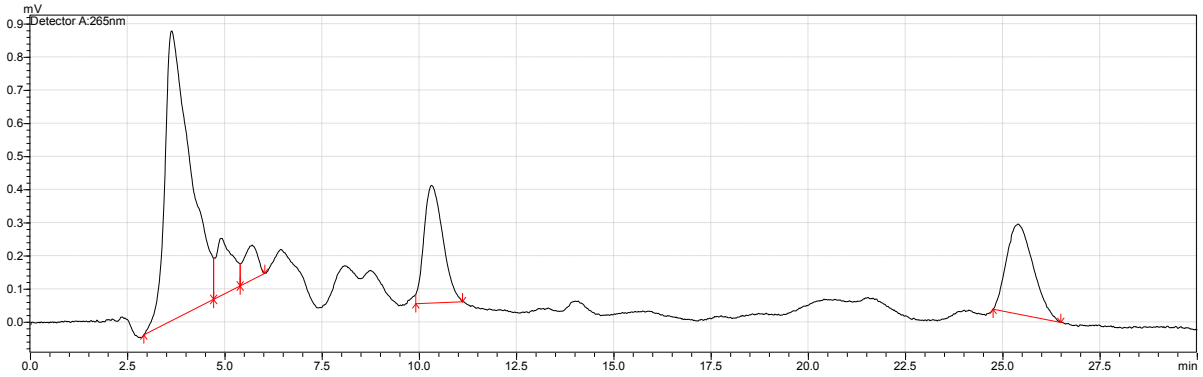


15 nml

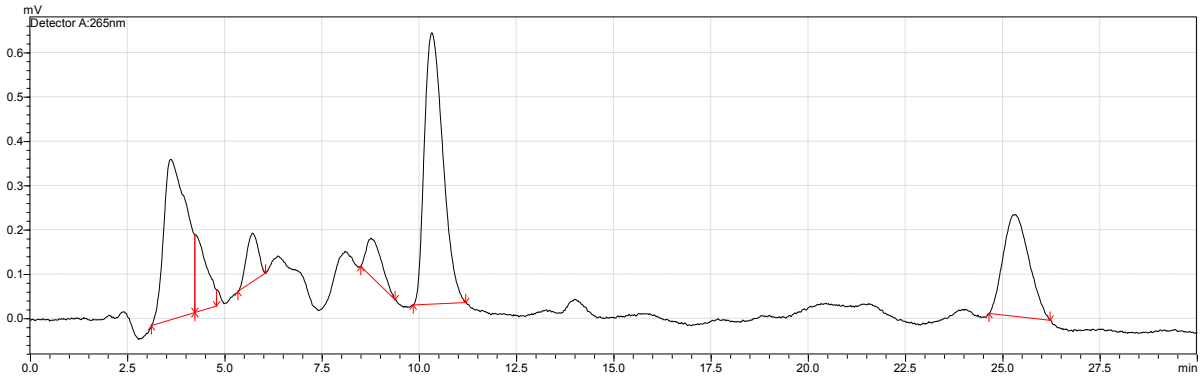

mV
Detector A:265nm
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
min

HPLC Vitamin standard 6/11/12 method 1

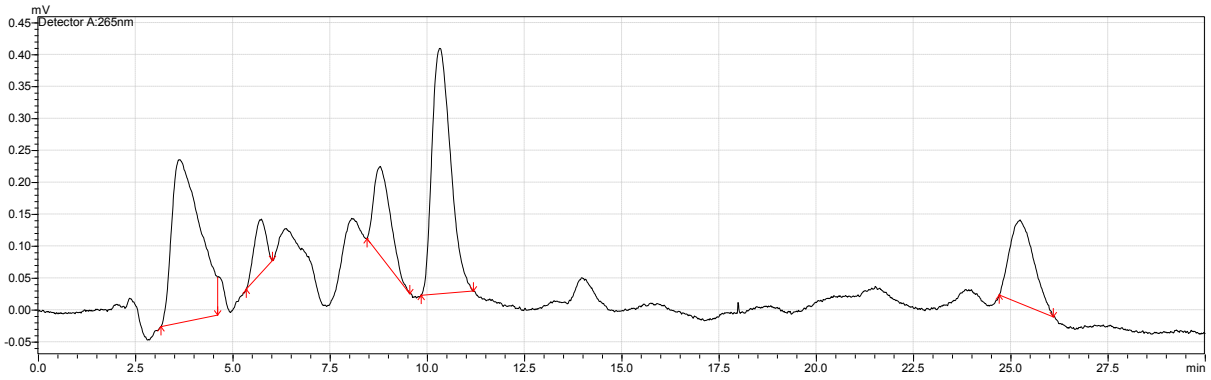
120nml



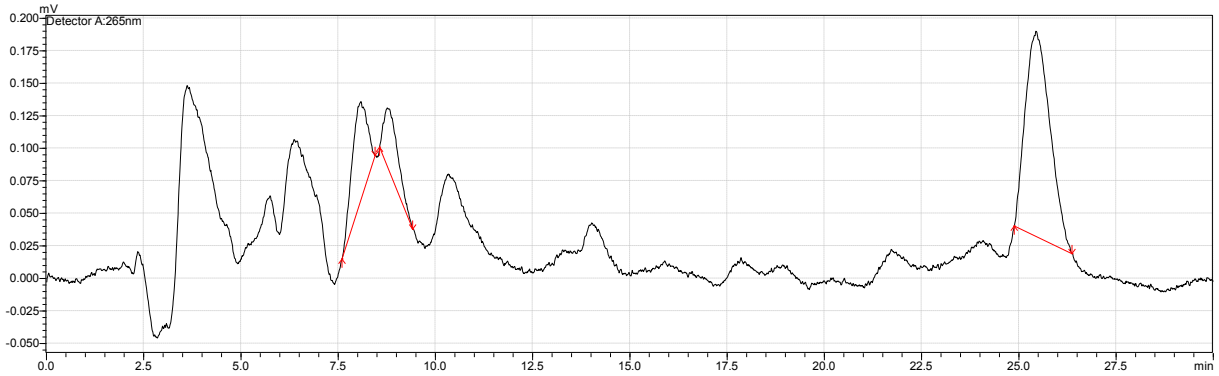
60nml



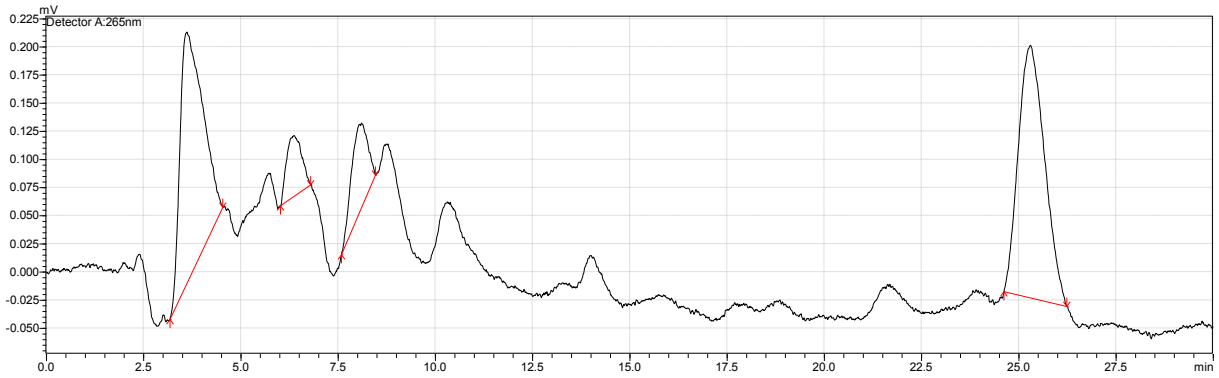
30nml



15nml

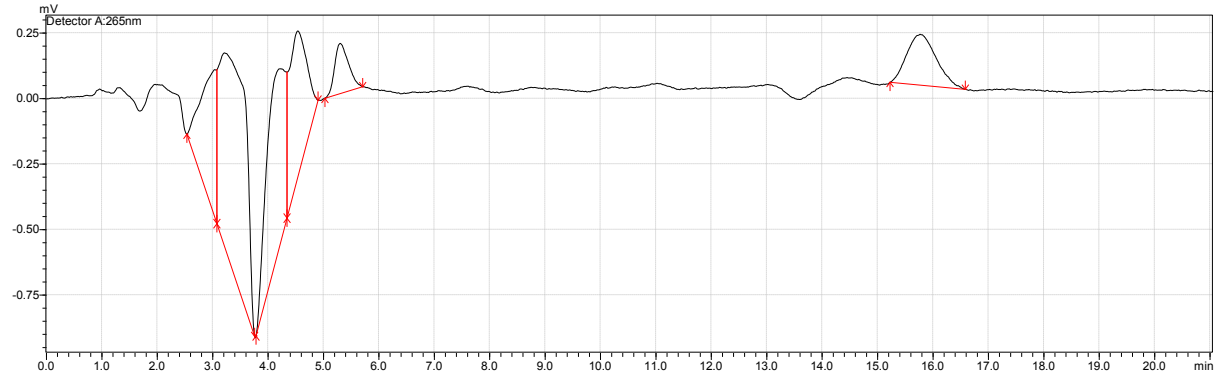

mV
Detector A:265nm
0.200
0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
-0.025
-0.050
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
min

15nml r

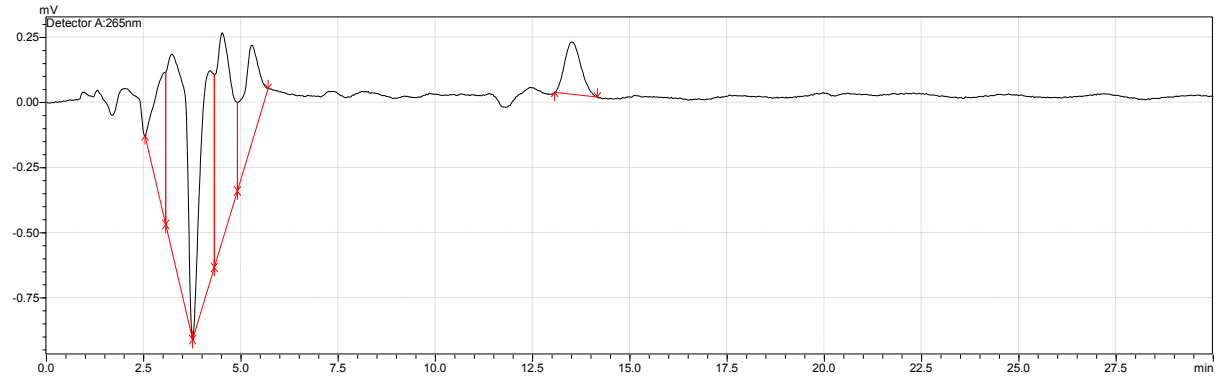

mV
Detector A:265nm
0.225
0.200
0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
-0.025
-0.050
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
min

HPLC Vitamin 10/12/12 method 1

Test 15nml

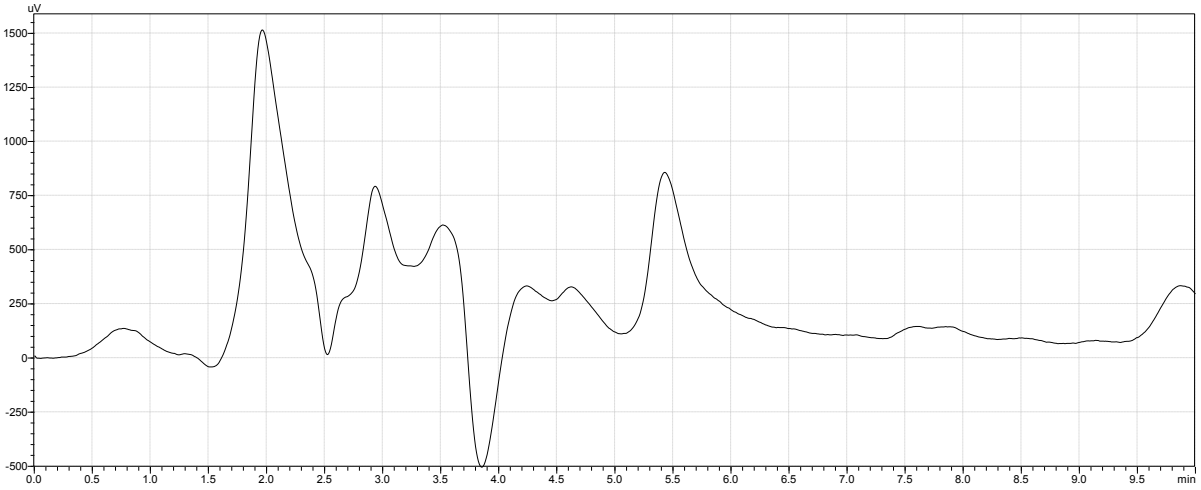


Test2 15nml



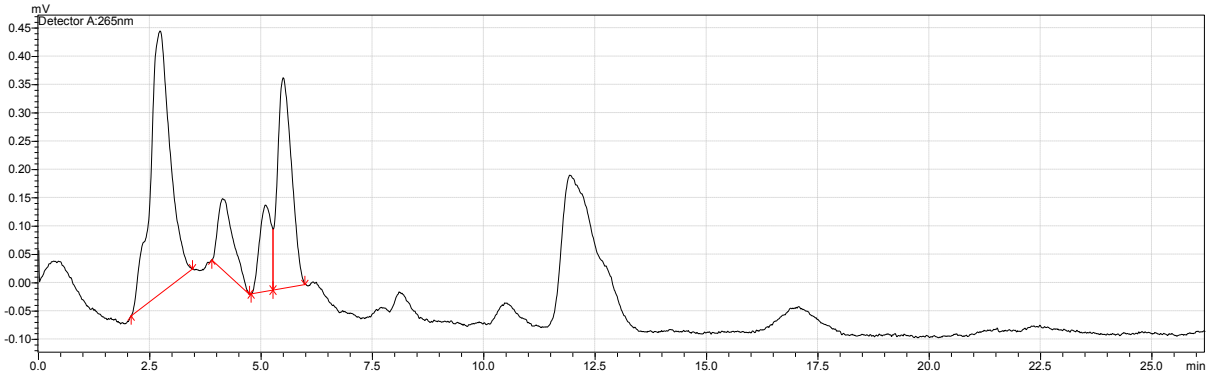
HPLC Vitamin D Standard 11/12/2012 method 1

120 nml

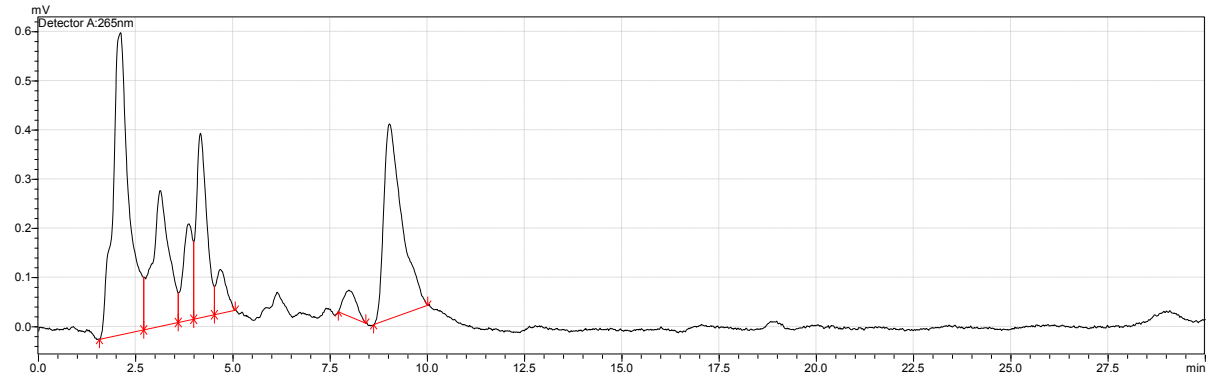


HPLC Vitamin D Standard 14/2/2013 method 1

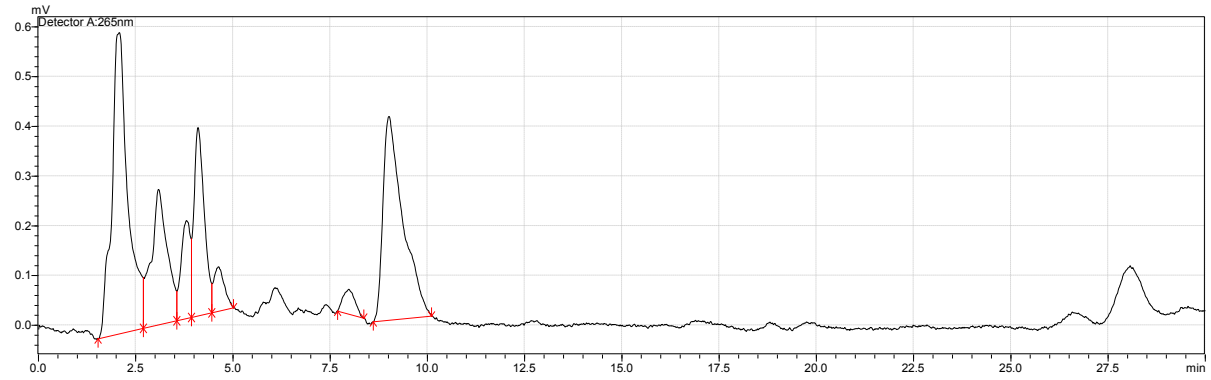
30 nml



15 nml

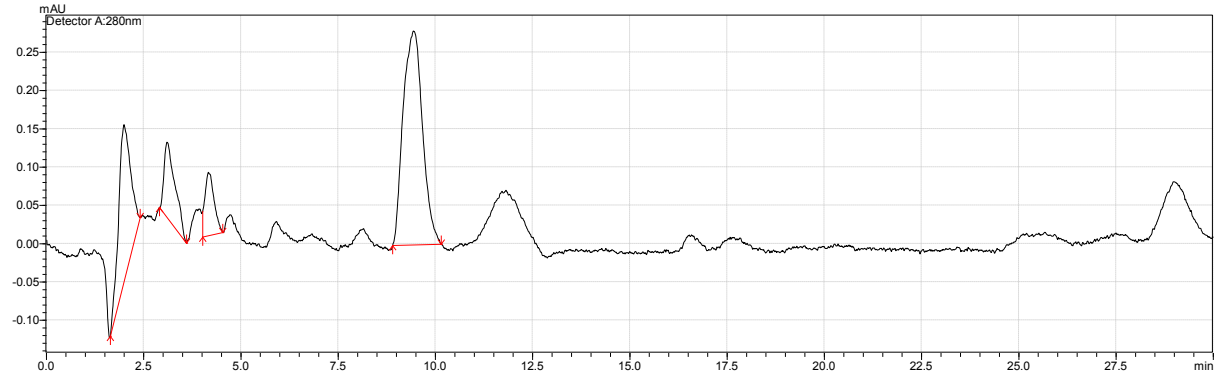


15 nml r

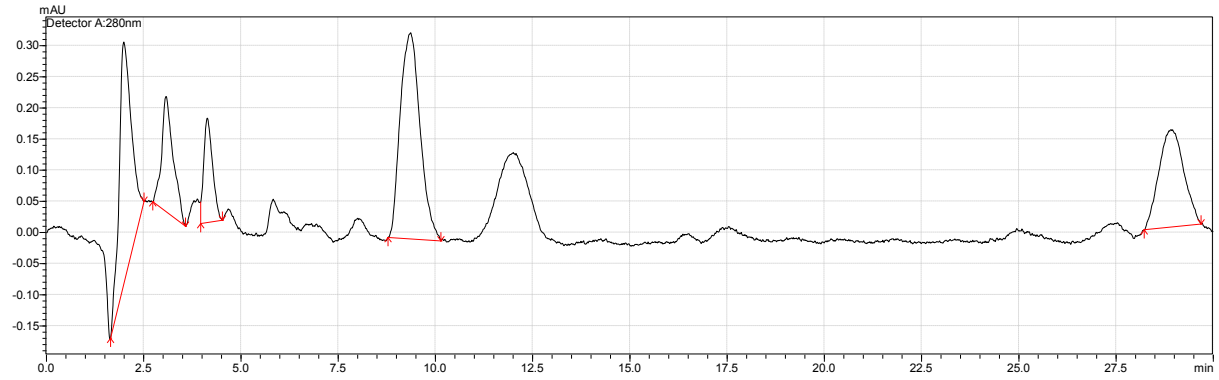


HPLC Vitamin D Standard 15/2/2013 method 1

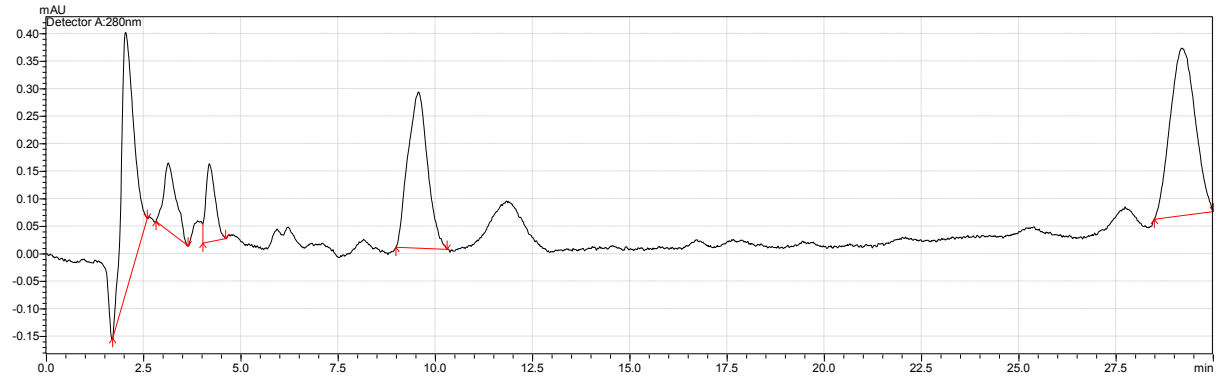
120 nml



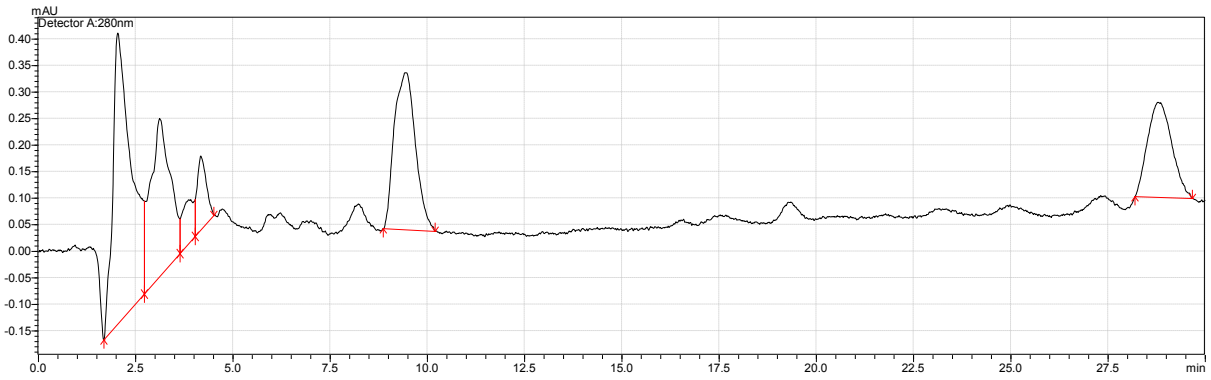
60 nml



30 nml

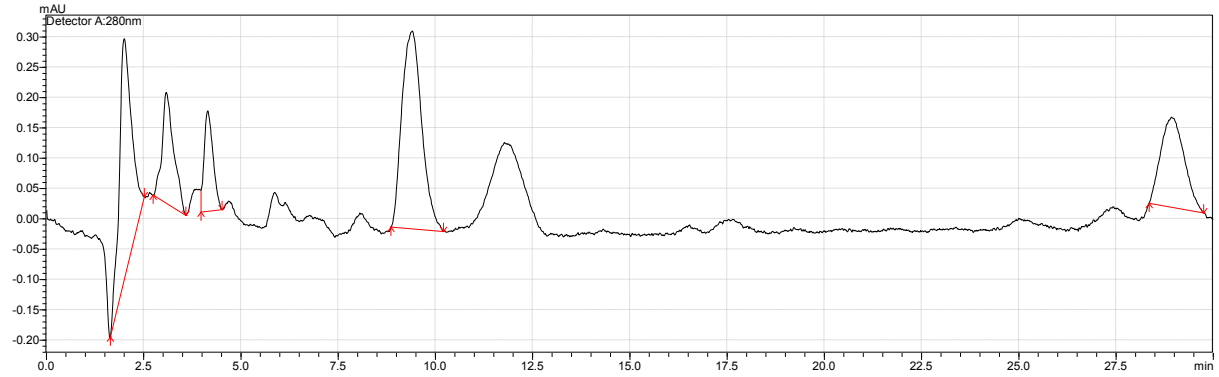


15 nmol

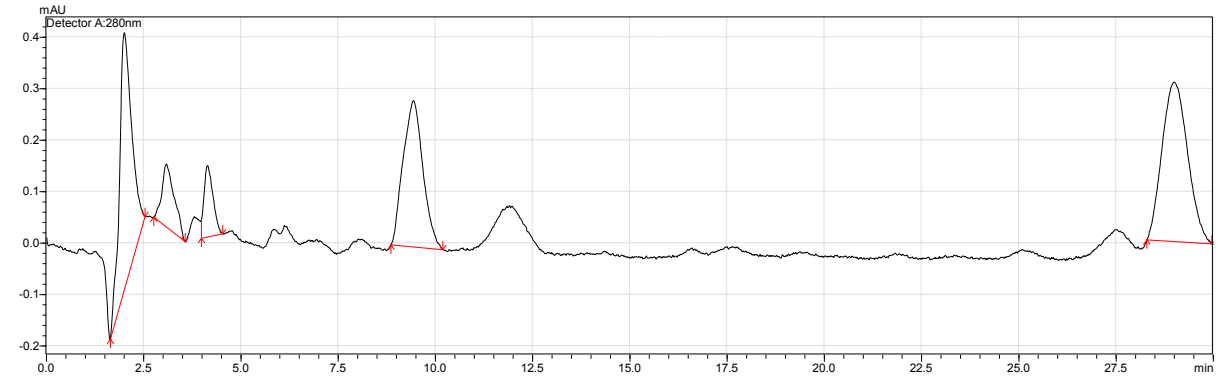

mAU
Detector A:280nm
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
min

HPLC Vitamin D Standard 16/2/2013 method 1

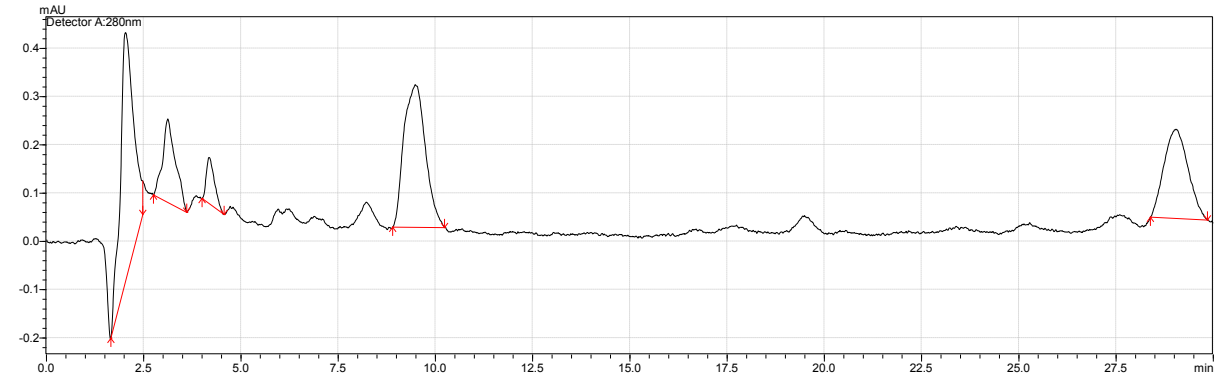
60 nml



30 nml

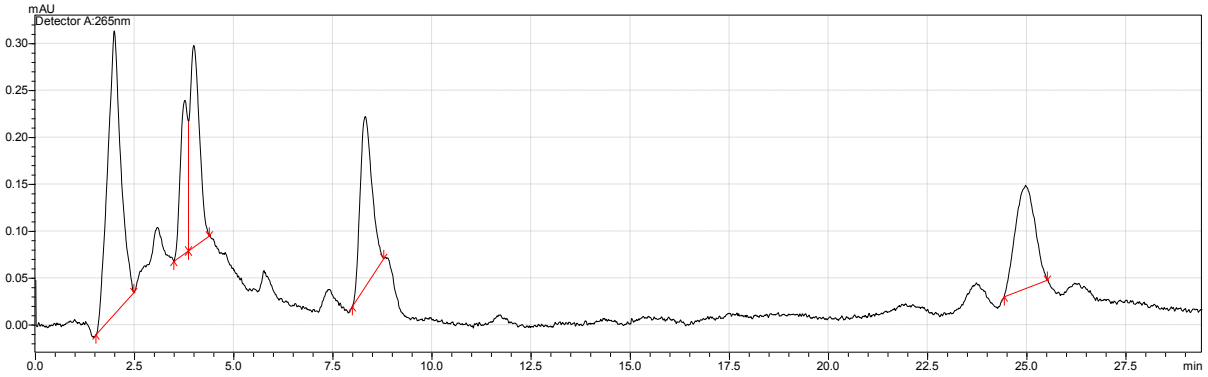


15 nml

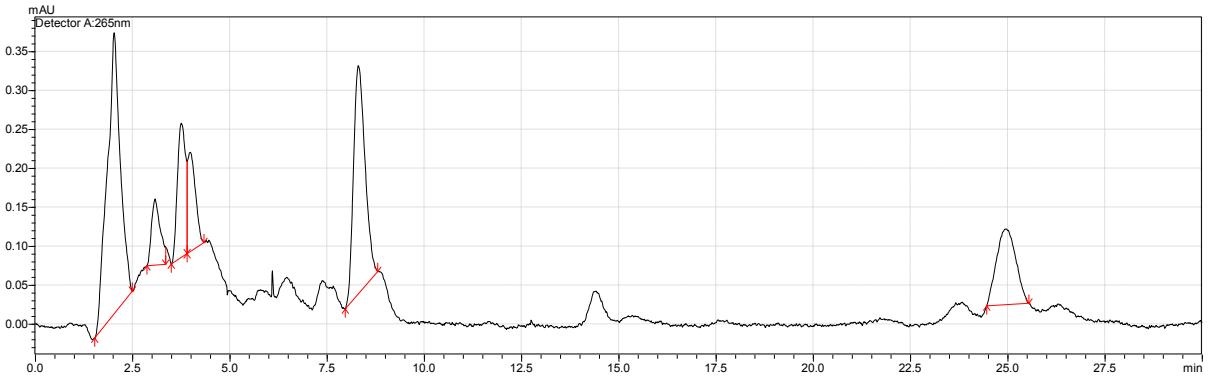


HPLC Vitamin standard 18/3/13 method 1

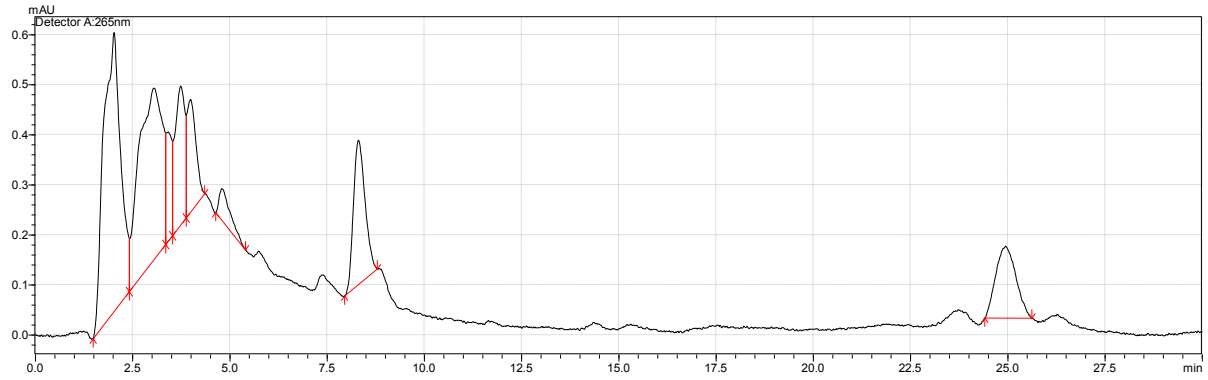
120nml



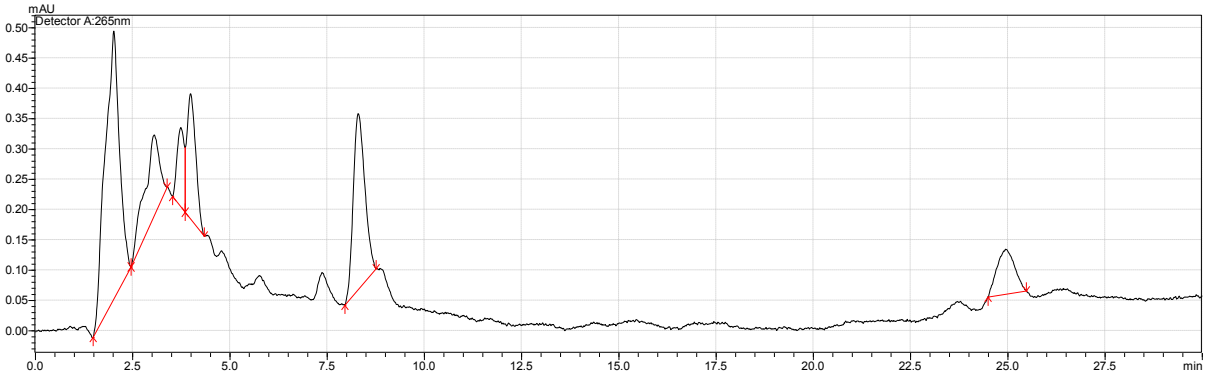
60nml



30nml

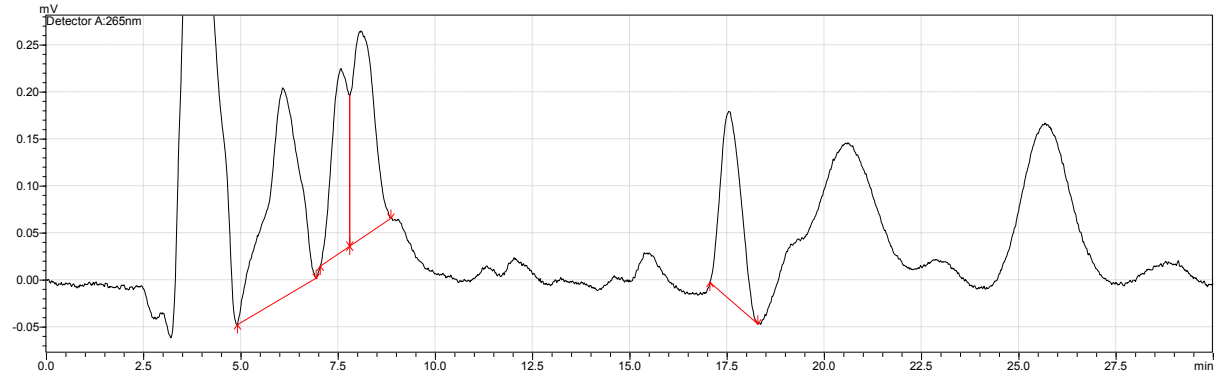


15nml

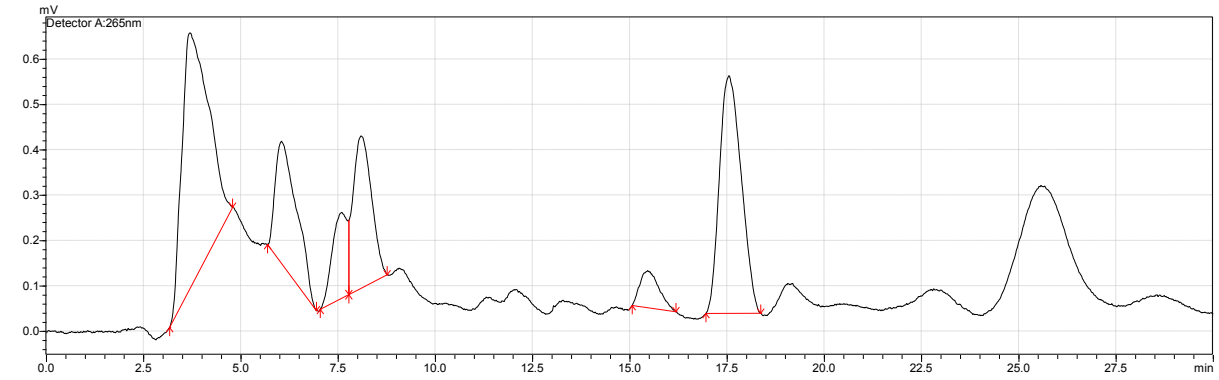

mAU
Detector A:265nm
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
min

HPLC Vitamin D Standard 22/1/2013 method 1

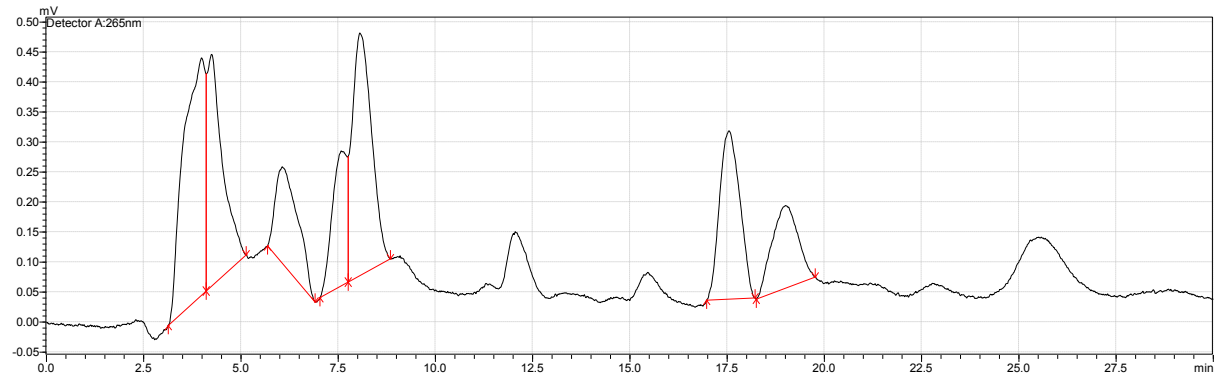
120 nml



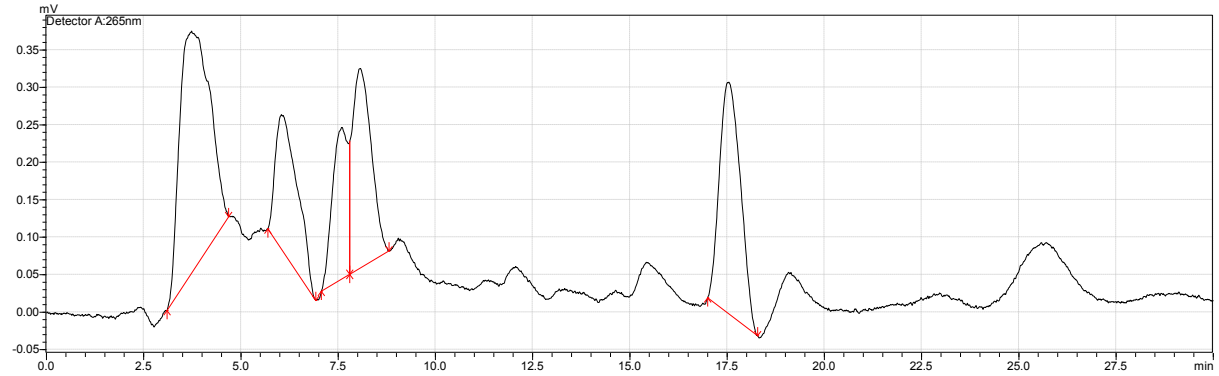
60nml



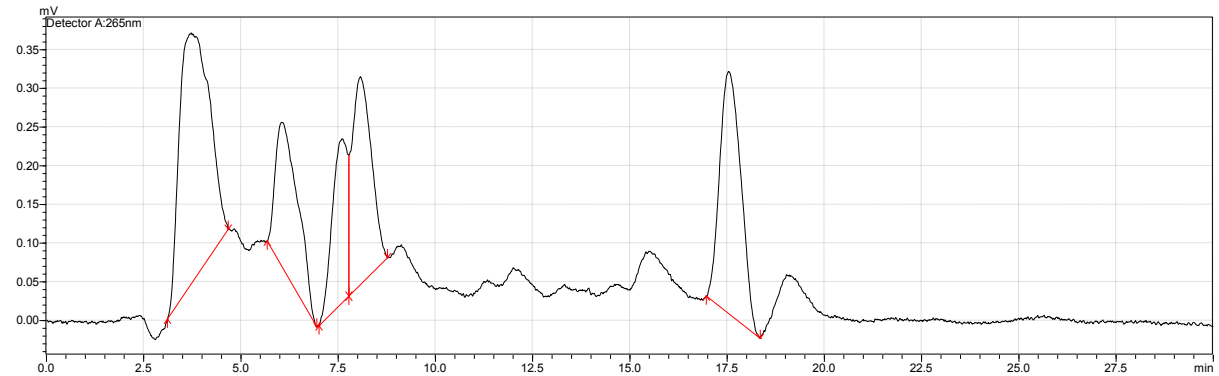
30 nml



15 nml

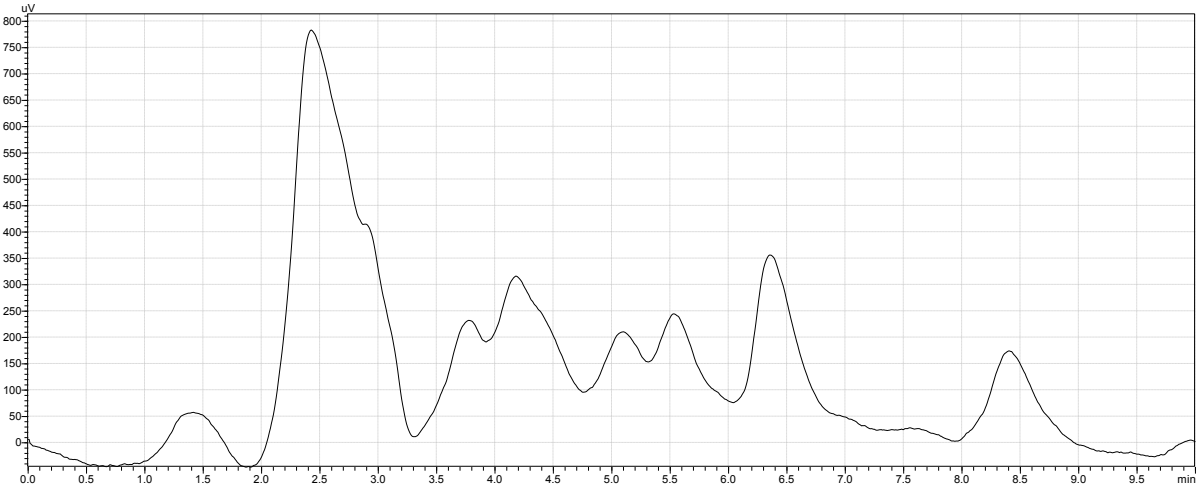


15nml rr

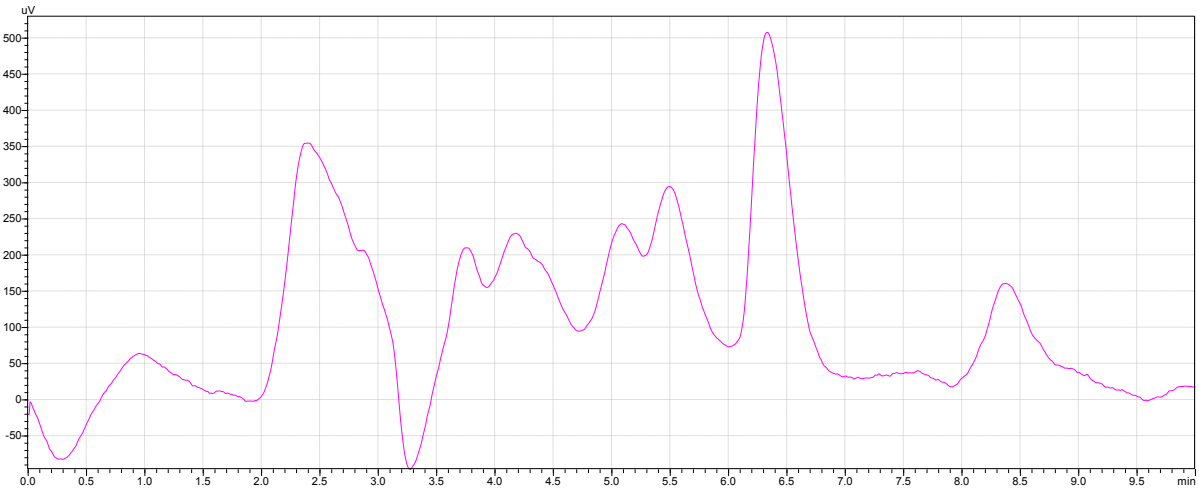


HPLC Vitamin D Standard 7/1/2013 method 2

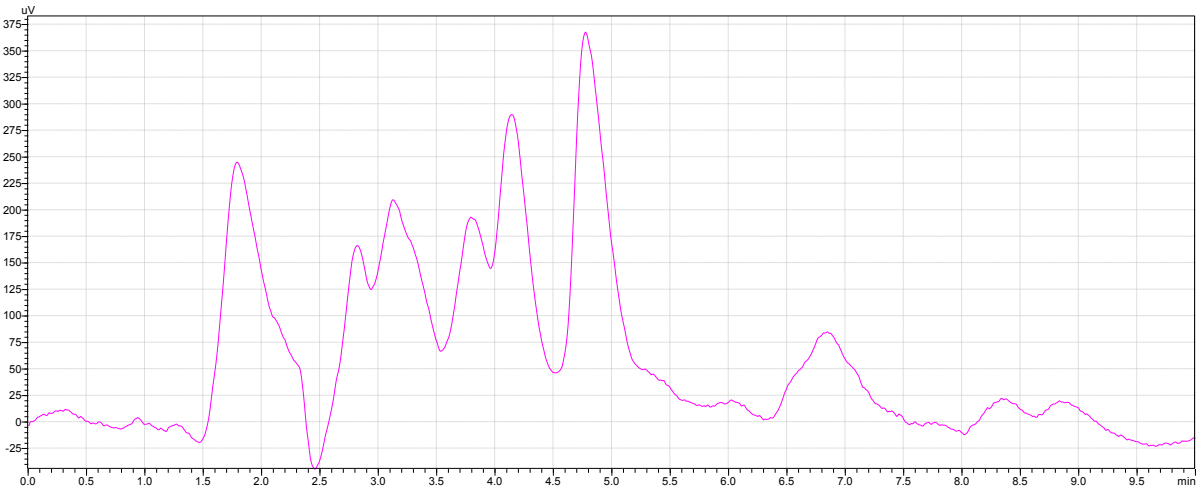
120 nml



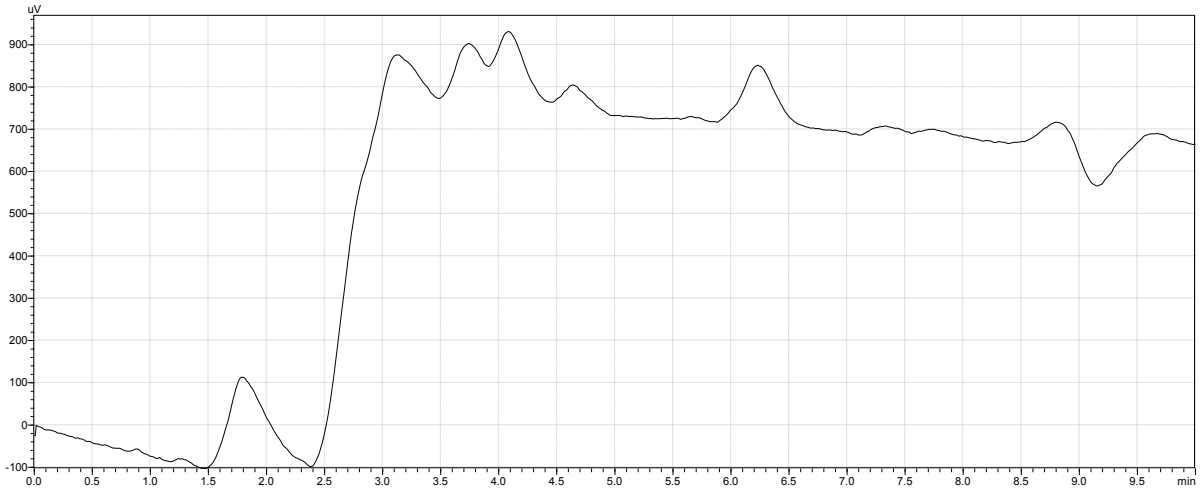
60 nml



30 nml

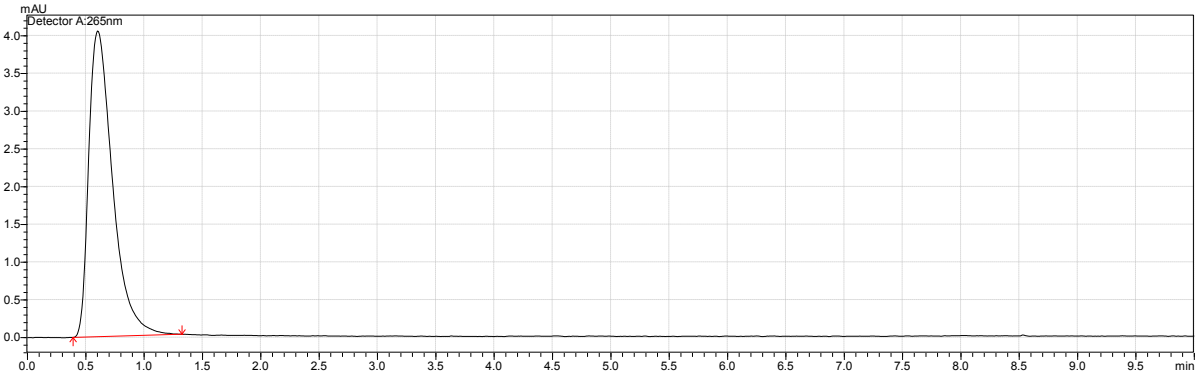

uV
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
-25
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
min

15 nml

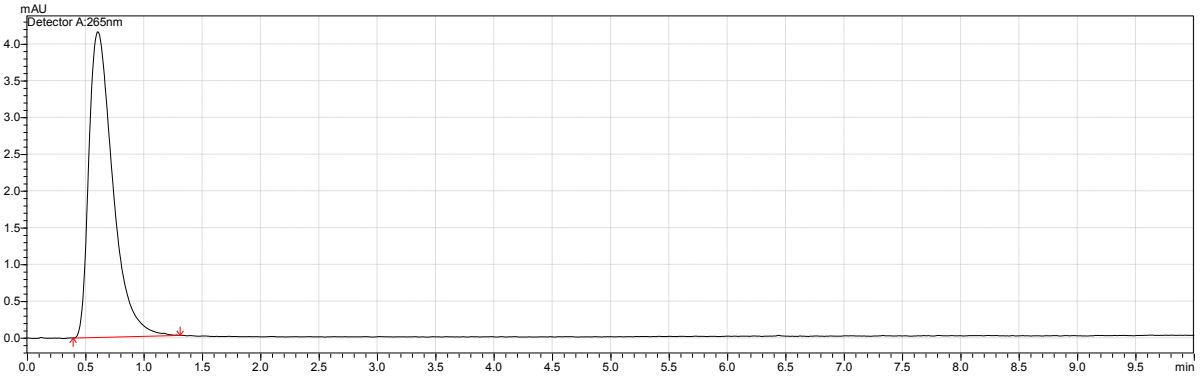

uV
900
800
700
600
500
400
300
200
100
0
-100
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
min

HPLC Vitamin D Standard 25/3/2013 method 2

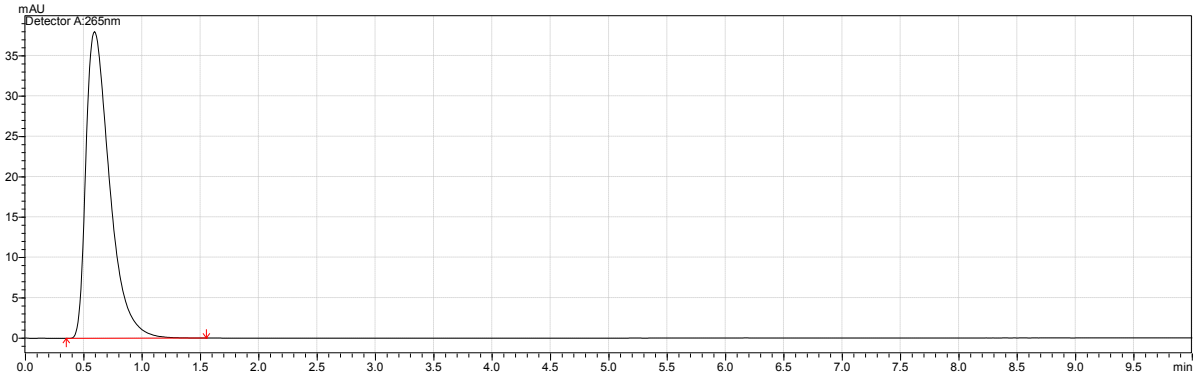
120 nml



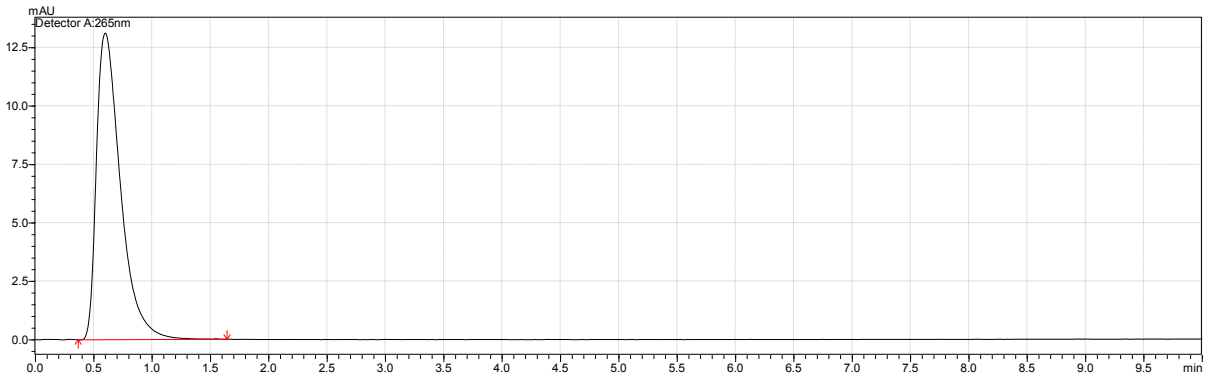
60 nmol



30 nml

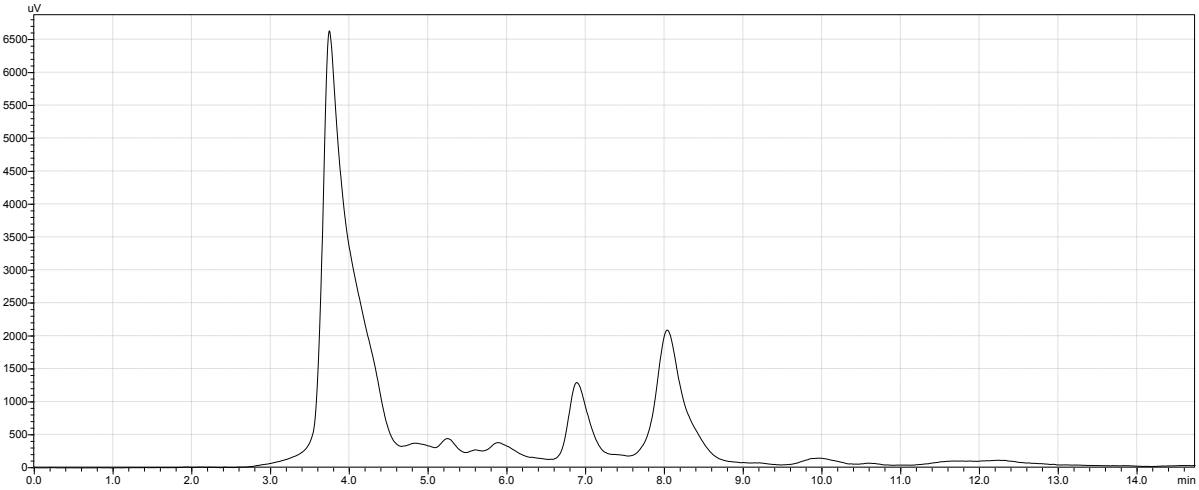


15 nml

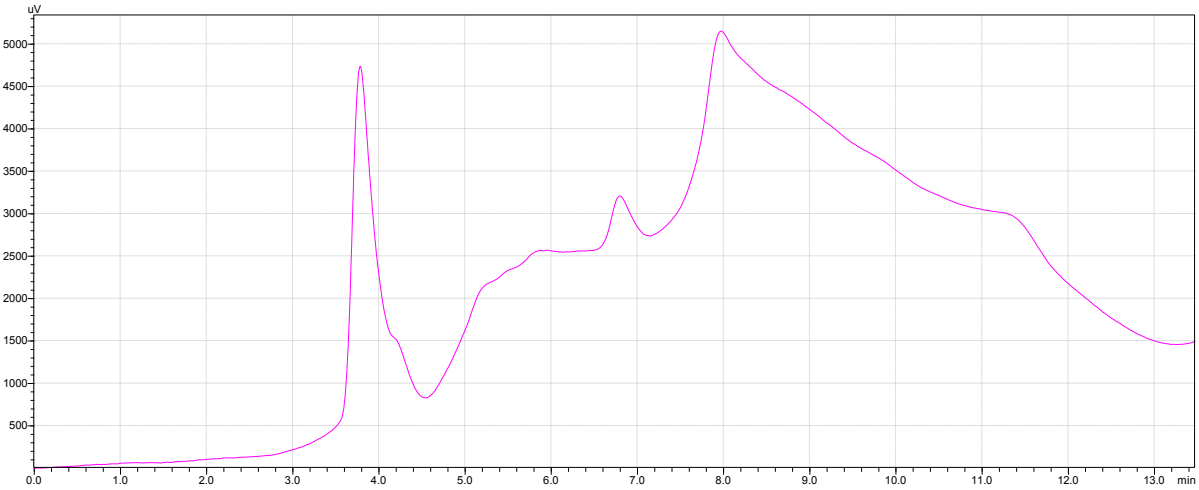

mAU
Detector A:265nm
12.5
10.0
7.5
5.0
2.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
min

HPLC Vitamin tests 27/3/2013 method 2

Test4r

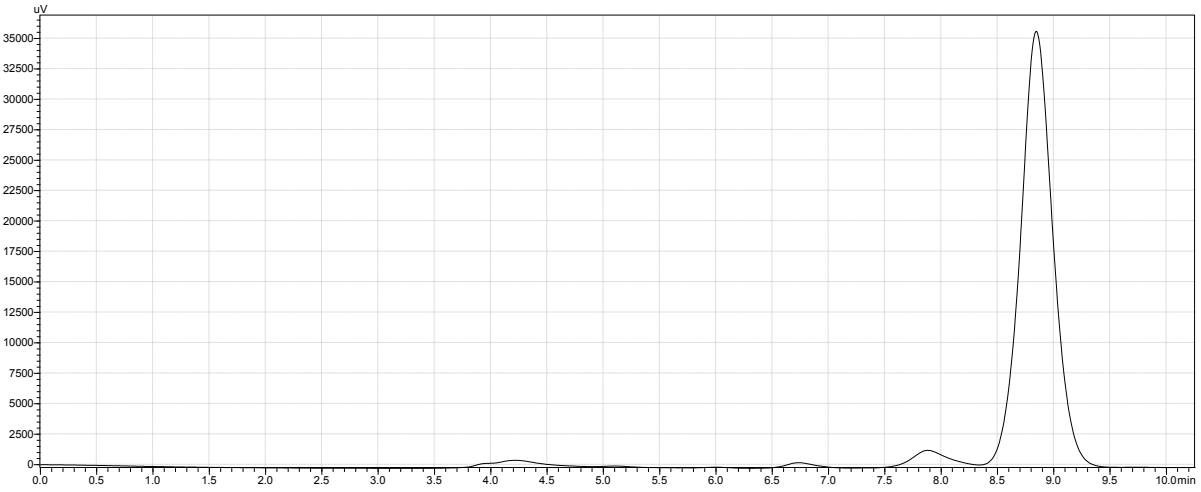


Test 3r

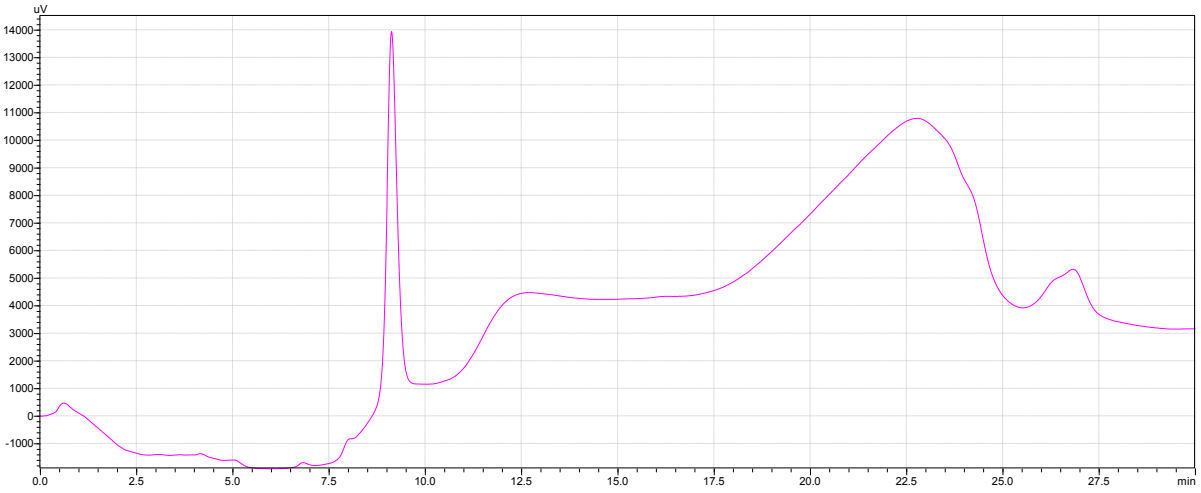


HPLC Vitamin Weili tests 26/3/2013

Test 2


uV
35000
32500
30000
27500
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0min

Test


uV
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
min

12.7 Appendix 7: The obtained results of vitamin D serum level for the KSA group.

Sample ID	Level of vitamin D in serum (ng /ml)
2	8.23
3	6.88
4	16.70
5	3.00
6	3.00
7	3.00
8	3.00
9	3.00
10	3.00
11	3.00
12	7.17
13	3.00
14	29.35
15	3.00
16	16.48
17	4.38
18	11.96
19	8.93
20	6.00
Mean	7.53
SD	6.91
Count	19