Risk factors influencing vitamin D status of ethnic minority adults in Northern England and the efficacy of calcifediol supplementation in 25-hydroxyvitamin D concentrations

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ABSTRACT

Background: Hypovitaminosis D is a major health concern in the UK. Reduced sunshine exposure and limited dietary sources of vitamin D, coupled with other factors lead to increased risk of hypovitaminosis D among the whole population especially amongst ethnic minorities (EMGs) due to their eating habits and high skin pigmentation. Vitamin D_3 supplement is the most common form of vitamin D used today to correct deficiency, however, evidence shows that calcifediol may increase vitamin D levels more rapidly.

Aims: To determine differences in the risk factors for hypovitaminosis D among different EMGs in the UK, and to investigate the efficacy of calcifediol supplementation, and its effect on improving vitamin D levels in deficient adults from EMGs.

Methods: A self-reported questionnaire was completed by 253 participants to assess sun exposure behaviours and lifestyle factors. Food frequency questionnaire (FFQ) was used to estimate vitamin D intake. Overall, seventy-four ethnic minority participants had their vitamin D level measured. 42 of these participants had a vitamin D level ≤30 nmol/l and were subsequently supplemented with 140 µg/week of calcifediol for 5 weeks.

Results: Dietary intake of vitamin D and supplement intake were low among all participants regardless of ethnicity ($P>0.05$). Ethnic differences were found in socioeconomic status, time spent outdoors and sun index ($p<0.001$). Other risk factors included being overweight or obese (64% Arab and 39% Afro/Caribbean); smoking and alcohol intake (13.3% Arab, 45.5% Afro/Caribbean); history of vitamin D deficiency (35% Arab and 24% South Asian). Vitamin D level was low across all of the population studied (29.9 nmol/l), dietary intake of vitamin D (1.26 µg) and total intake of vitamin D (2.83 µg). The median level of 25(OH)D (26.6 nmol/l) significantly increased rapidly to more than ≥75 nmol/l (151.4) in all groups, it declined over the next 5 weeks after supplement termination, while nevertheless remaining above the baseline value (55.9 nmol/l).

Conclusion: Dietary habits and sun exposure behaviours varied between EMGs, but they all were at risk of developing vitamin D deficiency. The calcifediol supplement was found to improve vitamin D status in all the groups and resulted in improved levels even after the five week termination period.

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CONFERENCE PRESENTATION

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25(OH) D	25-hydroxyvitamin D(calcidiol)
25(OH) D_3	25-hydroxyvitamin D_3(calcifediol)
1,25$(OH)_2$D	1,25-dihydroxyvitamin D (calcitriol)
24,25$(OH)_2$D	24,25-dihydroxyvitamin D
7-DHC	7-dehydrocholesterol
AfroC	Afro Caribbean
ANOVA	Analysis of Variance
B	Beta coefficient
BA	Black African
BMD	Bone mineral density
BMI	Body Mass Index
BSA	Body Surface Area
Cauc	Caucasian
CI	Confidence interval
DBS	Dried blood spot
EA	East Asian
EMG	Ethnic minority group
FFQ	Food frequency questionnaire
HPLC	High Performance Liquid Chromatography
IU	International unit
IOM	Institute of Medicine
kg/m^2	Kilograms per square metre
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LIDNS	Low Income Diet and Nutrition Survey
NDNS	National Diet and Nutrition Survey
nmol/L	Nanomoles per litre
ng/ml	Nanograms per millilitre
PTH	Parathyroid hormone
PWF	Portion weight frequency
RNI	Reference nutrient intake
SA	South Asian
SACN	Scientific Advisory Committee on Nutrition
SD	Standard deviation
SPF	Sun Protection Factor
UVB	Ultraviolet B- radiation
µg/d	Micrograms per day
Vitamin D_3	Cholecalciferol
Vitamin D_2	Ergocalciferol
VDBP	Vitamin D-binding protein
VDR	Vitamin D receptor

1. **INTRODUCTION**

1.1 General introduction

Since the discovery of skeletal diseases such as rickets many decades ago, there has been considerable interest in the role played by vitamin D in human health. Findings show that vitamin D deficiency is a significant concern at the population level and that rates of this health risk are increasing worldwide. Therefore, significant attention has been directed to investigating this issue; not only by dietitians, medical practitioners and researchers, but also by organizations and governments. As a result of this interest, the role of the active component, calcitriol ($1,25(OH)_2D_3$), has been well described. Beside its role in controlling calcium homeostasis, where the deficiency causes rickets in children and osteomalacia in adults (Holick and Chen, 2008), it also regulates hormone secretion and is associated with anti-proliferative and immunomodulatory effects, as well as regulating almost 200 genes around the body by binding with vitamin D receptors (VDRs) (Gropper and Smith, 2012; Bikle, 2014). Epidemiological studies have demonstrated that vitamin D insufficiency is related to various adverse health outcomes associated with major diseases such as cancer (Grant, 2016; Jeon and Shin, 2018), immune dysfunction (Cutolo and Otsa, 2008), cardiovascular diseases (Pludowski et al., 2013a), type 1 diabetes (Palermo and Holick, 2017) and hypertension (Vimaleswaran et al., 2014). However, casual relationships between vitamin D and non-skeletal disorders have not been clearly established (Autier et al. 2014).

Globally, hypovitaminosis D in humans is defined as a low concentration of serum 25-hydroxyvitamin D [25(OH)D], meaning a level of ≤25 nmol/L (SACN, 2007; Cancer Research UK, 2010), whereas a level of at least 75 nmol/L is considered optimal (Dawson-Hughes et al., 2005; Holick et al., 2011; Sinha et al., 2013), although the optimal level is still the subject of debate. Symptoms of vitamin D deficiency can be severe, affecting general health and causing fatigue and muscle pain (Pearce and Cheetham, 2010; Holick et al*.,* 2011). However, outward symptoms may not necessarily be apparent to an individual, therefore, vitamin D screening of the whole population should ideally be undertaken periodically.

The principal and richest source of vitamin D in the UK is its cutaneous synthesis by exposing the skin to ultraviolet B (UVB) radiation from sunlight. However, UVB is accessible in sufficient quantities for only part of the year (typically April to September),

due to the relatively high latitude of the UK (Lanham-New et al., 2016; Webb et al., 2018). Vitamin D is also present in some foods, such as egg yolk, oily fish and wild mushrooms, but as foods containing vitamin D are limited, it can be difficult to maintain sufficient dietary intake over the winter months. Individuals can benefit from vitamin D reserves built up during the summertime to sustain a sufficient vitamin D level during the wintertime (Cannell and Hollis, 2008). However, it is now clear that this is not achievable for the entire population, as many individuals have hypovitaminosis D all year round. The National Diet and Nutrition Survey (NDNS) reports that vitamin D deficiency (<25 nmol/L) affects 24% of men and 22% of women year round, while between January and March levels rise to 39% in both sexes (Bates et al., 2014).

According to previous studies of the prevalence of vitamin D deficiency among the British regions, Scotland and the northwest of England have been highlighted as at particular risk (Pal et al., 2003; Hyppönen and Power, 2007; Macdonald et al., 2008; Hirani et al., 2009; Mavroeidi et al., 2010; Zgaga et al., 2011). Vitamin D deficiency/insufficiency is prevalent not only in the UK but worldwide (Hilger et al., 2014; Van Schoor and Lips, 2017). According to Spiro and Buttriss, (2014) vitamin D deficiency has been found in 2-30 % of European adults. In the UK, there is also a high proportion of the ethnic minority population considered at risk of vitamin D deficiency due to environmental and lifestyle factors, as reported in South Asian (Darling et al., 2013), Middle Eastern (Ahmed et al., 2013) and Black African populations (Maxwell et al., 2006).

The Scientific Advisory Committee on Nutrition (SACN) has set a recent recommendation for adults aged ≥18 years to take a dose of 10 micrograms per day (µg/d) (SACN, 2015); this dose can be sufficient to sustain a vitamin D levels of >25 nmol/L, but may not be beneficial in relation to non-skeletal outcomes. Considering the absence of sufficient UVB skin irradiation in wintertime and limited dietary sources rich in vitamin D, it remains problematic to achieve a sufficiently high intake of vitamin D. Nonetheless, vitamin D supplementation is considered an effective way to maintain and correct vitamin D deficiency. Vitamins D_3 and D_2 are common subtypes of vitamin D, which are usually prescribed by clinicians and practitioners in cases of deficient levels of serum 25(OH)D. Several trials have examined which different sources of vitamin D most effectively correct vitamin D deficiency and make it possible to reach sufficient 25(OH)D concentration. Interventions can include oral intake of vitamin D (supplement and food)

(Vieth et al., 2001; Heaney et al., 2003a; Armas et al., 2004; Aloia et al., 2008; Cashman et al., 2008; Gupta et al., 2010; Hashemipour et al., 2012; Gallagher et al., 2014) and exposure to ultraviolet light, whether natural or artificial (Rhodes et al., 2010; Wicherts et al., 2011; Farrar et al., 2013; Lagunova et al., 2013).

1.2 Study rationale

From the literature, it is clear that the rate of vitamin D deficiency/insufficiency is high among the population worldwide. In the UK, and particularly in Manchester, a high percentage of vitamin D deficiency would be expected. As the richest source of vitamin D is cutaneous synthesis, the limited sunlight, when coupled with high skin pigmentation among certain ethnic groups, reduces the ability of individuals to produce vitamin D, particularly placing darker-skinned people at risk of vitamin D deficiency.

This is important from a population-based perspective, as a large number of immigrants from Asia and Africa have settled in Manchester, comprising 33.3% of the total population, and the number is increasing constantly (Manchester City Council, 2015). Coupled with the potential health consequences of vitamin D deficiency and the potential costs associated with these adverse outcomes, a higher priority should be given to prevention.

Owing to the limited number of studies in this field and the lack of ethnic/cultural minorities in the sampled populations, a thorough investigation into the variables known to influence vitamin D status is lacking. In order to inform potentially beneficial interventions to address this problem, there is a need for an in-depth study involving adults at risk of hypovitaminosis D among the main ethnic minority groups (EMGs) living in the UK: Arabs, South Asians, Afro/Caribbeans and East Asians. The research reported in this thesis makes a valuable contribution to exploring differences among ethnic minorities living in Manchester (53°N) who are known to be susceptible to the risk of hypovitaminosis D, by describing differences in vitamin D intake and lifestyle factors. In addition, further research is needed to make sensible recommendations regarding which actions should be adopted or avoided to successfully attain the optimal level of vitamin D for different ethnic groups.

The effect of vitamin D supplementation with cholecalciferol (D_3) or ergocalciferol (D_2) has been assessed in numerous studies, with the aim of improving vitamin D levels in adults (Vieth et al., 2001; Heaney et al., 2003a; Barnes et al., 2006). Recent studies relating to the efficacy of calcifediol have been relatively scant, carried out predominantly on older Caucasians (Cavalli et al., 2009; Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Jetter et al., 2014), with very few studies involving young adults (Barger-Lux et al., 1998; Russo et al., 2011; Shieh et al., 2017). To the best of the author's knowledge, there are no published studies focusing on multi-ethnic minority adults in the UK. Additionally, calcifediol is not currently commercially available in the UK as a nutritional supplement or as a therapeutic drug. Thus, this study will provide an insight into the effect of this supplement for treating hypovitaminosis D, before it becomes commercially available in the UK.

The PhD thesis attempts to:

1. Produce a clear comparison data in diet and lifestyle factors between ethnic minority groups living in the UK, who could be susceptible to the risk of hypovitaminosis D.
2. Emphasis the recommendations regarding lifestyle factors that could improve the vitamin D status for different ethnic minority groups.
3. Provide a new knowledge in vitamin D research regarding the effect of calcifediol supplementation on improving vitamin D level among EMGs in the UK.

1.3 Research aims and objectives

Study 1: Risk factors influencing the vitamin D status of ethnic minority adults living in Manchester.

Aim

- Investigate differences in the risk factors for vitamin D deficiency between ethnic minority groups.

Objectives

- Estimate vitamin D intake for the main ethnic minority groups living in Manchester from dietary sources, supplement use and sun exposure using a questionnaire.

- Evaluate lifestyle factors and sun exposure behaviours that could affect estimated total intake of vitamin D.

Study 2: Association of vitamin D intake and other risk factors with 25(OH)D concentrations in ethnic minority adults living in Manchester.

Aim

- Examine the association between dietary intake, lifestyle factors and 25(OH)D concentrations among ethnic minority adults.

Objectives

- Estimate vitamin D intake for Arab, South Asian and Black African from dietary sources, supplement use and sun exposure using a questionnaire.
- Measure serum 25(OH)D concentrations for ethnic minority participants.
- Determine the diet and lifestyle factors that affect vitamin D status among ethnic minority adults.

Study 3: The efficacy of calcifediol supplementation in increasing 25(OH)D concentration.

Aim

- Investigate the efficacy of calcifediol supplementation, and its effects on improving vitamin D levels in deficient adults from ethnic minority groups.

Objectives

- Investigate the effect of 140 µg/week calcifediol supplementation on vitamin D levels among deficient ethnic minority participants.
- Measure serum 25(OH)D concentrations for ethnic minority participants after five weeks of supplementation.
- Examine the persistence effects of calcifediol after five weeks of termination of supplementation.
- Analysis of serum 25(OH)D concentrations at baseline (pre-intervention), mid-intervention and post-intervention.

1.4 Thesis structure

The remainder of the thesis is structured as follows: Chapter 2 reviews the relevant literature, Chapters 3, 4 and 5 report the conduct and results of studies 1, 2 and 3 respectively, then the work concludes with a general discussion in Chapter 6.

2. LITERATURE REVIEW

2.1 Vitamin D

2.1.1 Vitamin D forms and sources

Vitamin D metabolites are a group of fat-soluble secosteroids consisting of two bioequivalent forms, cholecalciferol (vitamin D_3) and ergocalciferol (Vitamin D_2) (Norman, 2012). The structure of vitamin D is shown in Figure 2.1. Vitamin D_3 can be obtained from sunlight exposure, diet and supplementation, while vitamin D_2 is obtained only from diet or supplementation.

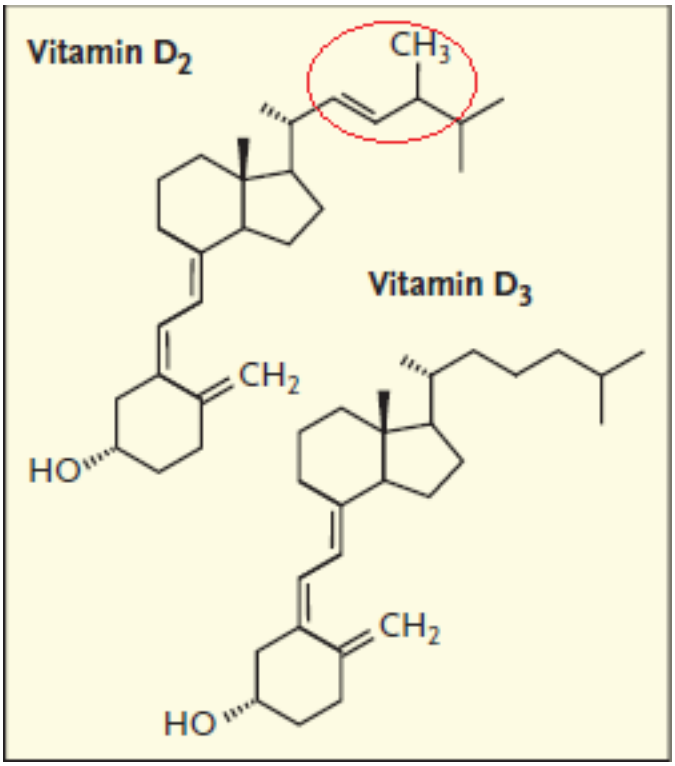

Figure 2.1: The chemical structure of vitamin D_2 and D_3 (Source: Holick, 2007)

2.1.1.1 Cutaneous synthesis

Vitamin D_3 is a hormone that can be produced naturally in the body through exposure of the skin to UVB from either sunlight or artificial light at wavelengths between 290 and 315 nanometres (nm) (Nair and Maseeh, 2012). The effects of ultraviolet radiation differ according to its wavelength: long wavelength UVA can penetrate the dermis (below the surface of the skin), causing tanning and ultimately skin ageing and wrinkling, while medium wavelength UVB can penetrate only the epidermis but burns the surface of the skin and is considered a key factor in skin cancer development (Narayanan et al., 2010). Cutaneous synthesis is the most effective mode of vitamin D production, far exceeding that

which can be obtained from dietary sources (Spiro and Buttriss, 2014). It has been reported that the best time to produce sufficient levels of vitamin D by exposing the body to sunlight is between 10 am and 3 pm in all seasons except winter (Holick et al., 2011). Exposing the whole body to sunlight for one minimal erythemal dose, which is broadly defined as developing a pinkness of skin, produces the equivalent of 250 µg (Macdonald, 2013) to 400 µg of vitamin D_3 (Tsiaras and Weinstock, 2011). The difference in these values may be explained by differential cutaneous synthesis of vitamin D dependent upon extraneous factors, including age and body weight (Tsiaras and Weinstock, 2011). Alternatively, Webb et al. (2018) state that exposure of a quarter of the body (face, arms and hands) to the UK summer sun could produce at least 25 µg of vitamin D_3.

2.1.1.2 Diet and vitamin D fortified food

Vitamin D is also obtained from natural dietary sources in animal-related foods such as egg yolk, oily fish (salmon, mackerel, herring and sardines), liver and cod liver oil (Schmid and Walther, 2013), or from non-animal sources such as wild mushrooms, which are rich in vitamin D_2 (Gropper and Smith, 2012). Because natural foodstuffs containing vitamin D are limited, many countries fortify certain food products with vitamin D_2/D_3 (Calvo and Whiting, 2013). However, fortification practices vary from country to country and may be either optional or mandatory. In the UK, food fortification is governed by strict legislation, limiting its application (Sinha et al., 2013). Only certain foods such as margarine and infant milk are mandatorily fortified with vitamin D (SACN, 2007), with voluntary fortification of products such as soya and breakfast cereals (NHS, 2011). In addition, Jakobsen and Knuthsen (2014) report that vitamin D content may be affected by cooking, depending on the particular foodstuff and the cooking temperature.

2.1.1.3 Supplementation

Vitamin D is available in a range of licensed medicines and food supplements as vitamin D_3 or D_2 and can be administrated either orally or by intramuscular injection. In the UK, most supplemental vitamin D is the D_3 form, provided in doses of 5 to 25 µg without a prescription, and it may be included as an ingredient in multivitamin and calcium supplements (Sinha et al., 2013). Vitamin D deficiency can be also corrected by vitamin D metabolites such as calcifediol (25(OH)D_3), which is available in some European countries but not in the UK. The quantity of vitamin D in either foods or supplements is expressed in

micrograms (μg), or in International Units (IU); 1 μg= 40 IU (Ross et al., 2011). Calcifediol is expressed only in μg when used as a supplement (Brandi and Minisola, 2013; Cianferotti et al., 2015). Vitamin D supplementation will be discussed later in this chapter (Section 2.6).

2.1.2 Vitamin D metabolism

Whilst dietary vitamin D_3/D_2 is initially processed via different metabolic pathways as compared to cutaneously produced vitamin D_3, they both undergo the same pathway at the biological activation phase (Figure 2.2).

Digested vitamin D_2 and D_3 are absorbed by passive diffusion in the small intestine into enterocytes, then incorporated into chylomicrons and carried by the lymphatic system into the blood circulation. From there, the vitamin D is transported by vitamin D binding protein (VDBP) or lipoproteins (chylomicrons) to the liver (DeLuca, 2004). As vitamin D is fat-soluble, excess amounts can be stored in adipose tissue for up to eight weeks and used when required (Tsiaras and Weinstock, 2011).

The process of cutaneous vitamin D synthesis starts in the skin, where UVB radiation is absorbed and converts 7-dehydrocholesterol (7-DHC) into pre-vitamin D_3, which isomerizes under the body's temperature conditions to form vitamin D_3 (Holick, 2008; Tsiaras and Weinstock, 2011). This is diffused into the capillary system by VDBP and then into the liver (Holick, 2008).

The biological activation of vitamin D, irrespective of its source, occurs in the liver. Vitamin D is metabolized to 25-hydroxyvitamin D (25(OH)D) (inactive vitamin D) via the vitamin D-25-hydroxylase enzymes (CYP2R1 and CYP27B1) produced from cytochrome P450 (Gropper and Smith, 2012). 25(OH)D in the bloodstream binds to VDBP and is then transported to the kidneys, where further hydroxylation occurs. It is converted to 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) via the enzyme 24-hydroxylase or to the biologically active form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (calcitriol) by the enzyme 25(OH)D-1α-hydroxylase, such as CYP27A1 (Christakos et al., 2015). In the kidneys, the production of $1,25(OH)_2D$ increases when serum 25(OH)D is low and vice versa (Norman, 2012).

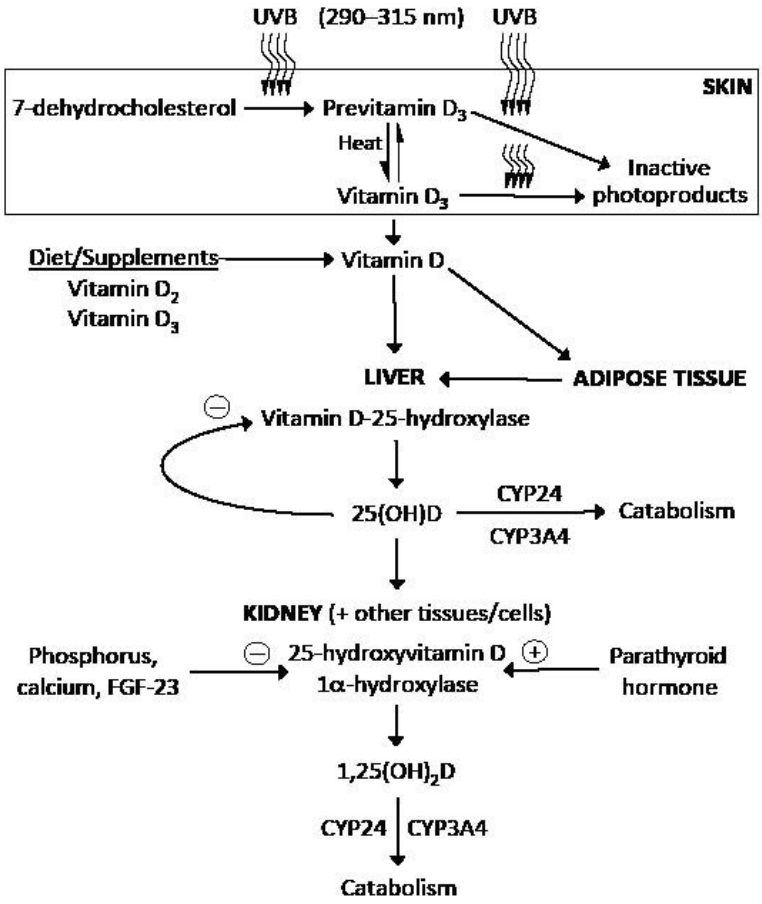

Figure 2.2: Vitamin D metabolism (Source: Tsiaras and Weinstock, 2011)

Previously, it was believed that the second hydroxylation occurs only in the kidneys, but current evidence has shown that $1,25(OH)_2D$ is produced in other tissues such as the prostate and colon (Anderson et al., 2003), as shown in Figure 2.3.

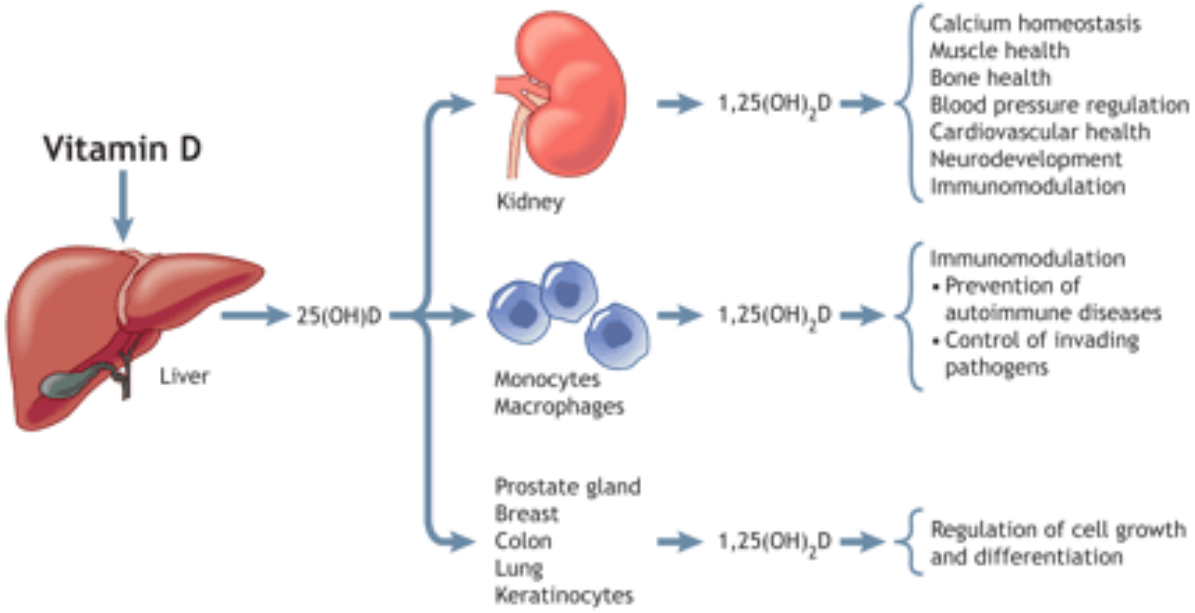

Figure 2.3: Tissues capable of synthesis 1,25(OH)$_2$D (Source: Hollis and Wagner, 2006)

2.1.3 Mechanism of vitamin D action

Vitamin D (1,25(OH)$_2$D) is released into the bloodstream and binds to intracellular vitamin D receptors (VDRs), then acts as transcription factors to modulate gene expression (Holick, 2010; Christakos et al., 2013), eventually triggering a biological reaction. This can either be the stimulation of intestinal absorption of calcium or providing the appropriate balance of elements crucial for bone function and growth (Holick, 2010). The primary role of 1,25(HO)$_2$D is to act with parathyroid hormone (PTH), which is essential for regulating and maintaining blood levels of both calcium and phosphate for bone mineralisation, contraction of muscles and prevention of hypercalcaemia by optimising intestinal calcium absorption (DeLuca, 2004) (Figure 2.3). During hypovitaminosis D, PTH secretion increases, resulting in an increase in the production of 1,25(OH)$_2$D, thus promoting absorption of calcium from bone and potential skeletal disorders (Visser et al., 2003).

2.1.4 Assessment of vitamin D status

2.1.4.1 Biochemical assessment

There is a consensus of opinion that circulating level of serum 25(OH)D is the best indicator of vitamin D status, reflecting the incoming contributions of vitamin D from either diet and/or cutaneous synthesis (DeLuca, 2004; Seamans and Cashman, 2009). The half-life of 25(OH)D, at approximately three weeks (Prentice et al., 2008), is relatively long when

compared to that of 1,25$(OH)_2D$, which is only 4-6 hours (Lips, 2007). Circulating 25(OH)D is measured in nanomoles per litre (nmol/L) or nanograms per millilitre (ng/ml); 1 ng/ml = 2.5 nmol/L.

Although serum or plasma samples are routinely used in clinical practice, dried blood spot (DBS) sampling has more recently been successfully used for the analysis of a range of circulating biological compounds, with valid, reliable and accurate results as compared to serum or plasma (Spooner et al., 2009; Lehmann et al., 2013; Heath et al., 2014). In relation to vitamin D, a number of comparative studies have shown that DBS results were highly correlated with plasma measurements and there were no statistical differences in 25$(OH)D_3$ concentration (Newman et al., 2009; Heath et al., 2014; Shea and Berg, 2017).

Many methods have been used to measure serum 25(OH)D concentrations, including immunoassay and chromatography (Hollis, 2008). Examples of the former are competitive protein binding assays (Preece et al., 1974) and enzyme immunoassay (Hollis, 2008), whereas for the latter, high-performance liquid chromatography (HPLC) has been coupled with a mass spectrometry (MS) methodology (Carter, 2009). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), which is applied in the current study, has been recently proven as superior to other techniques; it can differentiate between 25$(OH)D_3$ and 25$(OH)D_2$ (Hedman et al., 2014), whereas all immunoassays are unable to do so. According to Binkley et al. (2004), measurement of serum 25(OH)D levels often varies from study to study because of the different laboratories and assay methodologies used. Historically, the measurement of serum 25(OH)D faced a major challenge related to the absence of international standards or a common calibrator, leading to difficulties in comparing results from different laboratories and assays (Carter, 2009). More recently, most commercial manufacturers have started to adopt a standard reference, produced by the National Institute of Standards and Technology for use with LC-MS/MS or HPLC methods (Binkley et al., 2014).

2.1.4.2 Nutritional assessment

Dietary assessment is employed to assess the food consumption and nutrient intake of different groups (Coulston et al., 2017). There are several types of assessment to measure nutrient intake, including recalls and records, such as 24-hour dietary recall, dietary history and Food Frequency Questionnaires (FFQs). These have been used in national surveys, as

well as in household and individual analyses (Gibson, 2005). The selection of an appropriate method for any study depends on the type of study and the level of information required (Gibson, 2005; Coulston et al., 2017).

FFQs are the preferred method of assessing dietary intakes in epidemiologic studies (Thompson et al., 1994). They are designed to assess participants' eating patterns and which specific foods they usually consume by asking them to state the frequency of consumption of each food item listed over a specified period (Gibson, 2005). The many advantages of using an FFQ to assess nutritional status include low cost, self-administration and the ability to collect data from a large population sample. These have made this the most commonly used technique among researchers (Barrett-Connor, 1991; Metcalf et al., 2003; Liu et al., 2013). A number of different types of FFQ have been used in epidemiological studies of diet and health, particularly related to disease (Rockett et al., 1995; Kalantar-Zadeh et al., 2002). FFQs have also been used in several studies specifically relating to vitamin D. For example, they were used to explore the relationship between the vitamin D intake and chronic disease by Merlino et al. (2004) and Pittas et al. (2006). Additionally, Maxwell et al. (2006) used an FFQ to study the relationship between dietary vitamin D intake and hypovitaminosis D.

2.1.4.3 Sun exposure assessment

As mentioned above, the richest source of vitamin D is via sunlight exposure. Therefore, when assessing vitamin D intake, it is necessary to estimate how much vitamin D is made from sunlight exposure, alongside the intake of vitamin D from food and supplements. Sun exposure can be estimated by methods such as an electronic UV dosimeter (Thieden et al., 2004), changes of skin colour (Macdonald et al., 2011) and self-report questionnaire (Barger-Lux and Heaney, 2002; Armas et al., 2007; Macdonald et al., 2011; Gould et al., 2015).

Self-report questionnaires have been employed when examining other areas of health, such as ophthalmological and skin cancer studies (Astner and Anderson, 2004; McCarty, 2008; Daniel et al., 2009). However, simply considering a country's latitude does not provide an accurate estimate of UV exposure, nor does it assess an individual's sun exposure behaviour (Millen and Bodnar, 2008). Sunlight exposure questionnaires have tended to be developed ad hoc for individual studies, and as there are no standardised

versions, researchers have typically designed new questionnaires or modified existing ones (McCarty, 2008). Time outdoors, the areas exposed to sunlight, use of sunscreen and sunbathing behaviour are the main factors that should be included in a sun exposure questionnaire (McCarty, 2008; Macdonald et al., 2011; Gould et al., 2015). In light of the various limitations noted above, McCarty (2008) is justified in stating that using questionnaires to assess behaviour or to estimate vitamin D production is not a fully reliable method to measure 25(OH)D levels without additional verification methods.

2.1.5 Classification of vitamin D status

2.1.5.1 Optimal serum 25(OH)D concentrations

Internationally, there remains a considerable debate over the optimal 25(OH)D concentration cut-offs (from deficiency to optimal levels) (Van Schoor and Lips, 2011; Bischoff-Ferrari et al., 2012; Bouillon et al., 2013). Vitamin D deficiency is defined as having a serum 25(OH)D concentration below 25 nmol/L (SACN, 2007; SACN, 2015), relevant to protecting against rickets in children and osteomalacia in adults. Heaney (2004) reports that the range of 25(OH)D insufficiency lies between 30 and 50 nmol/L, which may lead to an increase in adverse health outcomes. However, the Institute of Medicine (IOM) considers the deficient level of 25(OH)D to be <30 nmol/L and it defines a sufficient level as a 25(OH)D concentration of >50 nmol/L (Ross et al., 2011). IOM cut-offs have also been agreed by the National Osteoporosis Society for adoption by UK health practitioners (Francis et al., 2013).

Alternatively, Dawson-Hughes and colleagues (2005) have suggested that the optimal level of vitamin D could be defined as a concentration related to maximum suppression of parathyroid hormone (PTH), to maximal calcium absorption and to elevated bone mineral density (BMD) as well as decreased bone loss. It has been indicated that higher PTH levels (>10-65 pg/mL) are evident when 25(OH)D concentrations decrease to 37.5-50 nmol/L (Lips et al., 1988; Lips et al., 2014) or to 110 nmol/L (Dawson-Hughes et al., 2005). Potential reasons for this wide range of threshold levels are differences in ethnicity, age, calcium intake and the assays of serum 25(OH)D used (Bates et al., 2003; Aloia et al., 2006; Prentice et al., 2008; Gutierrez et al., 2011).

Other functional methods suggested to evaluate the optimal level of vitamin D are calcium absorption and elevated BMD, with numerous studies suggesting that the threshold for

maximal calcium absorption is when serum vitamin D concentrations are at 75 nmol/L (Barger-Lux and Heaney, 2002; Heaney et al., 2003b). However, other studies have found that BMD correlated positively with a serum 25(OH)D concentration above 100 nmol/L (Bischoff-Ferrari et al., 2004). From the preliminary evidence above, it has been suggested by the Endocrine Society Task Force that the desirable level of vitamin D concentration is at least 75 nmol/L (Bischoff-Ferrari et al., 2006; Cashman et al., 2016) when considering other health issues.

2.1.5.2 Toxicity of vitamin D

Hypervitaminosis D is the result of the excessive consumption of vitamin D supplements (Vieth, 2006) or fortified food (Brown et al. 2013), not of excessive exposure to sunlight (De Paula and Rosen, 2012). According to De Paula and Rosen (2012), when pre-vitamin D_3 is produced after excessive exposure to sunlight, the metabolism of vitamin D in the skin will regulate itself and convert the excess to other photoproducts. Vitamin D intoxication symptoms are attributable to hypercalcemia (defined as an excess of calcium level in excess of 10.6 mg/L in the blood). Symptoms include nausea, increased thirst, depression and lethargy (Vieth, 2007). According to Ross et al. (2011), excessive vitamin D levels may be correlated to a range of detrimental outcomes, including cardiovascular disease, fractures and some types of cancers. The toxic level of vitamin D is still widely disputed (Jones, 2008). According to Ross et al. (2011) and Heaney (2005), the safe level of 25(OH)D for children and adults is up to 250 nmol/L, whereas a study in animals showed that hypercalcemia begins to appear when serum 25(OH)D concentration reaches 375-500 nmol/L (Jones, 2008).

2.2 Vitamin D recommendations

Differing recommendations for vitamin D intake have been set in different countries for various life-stage groups. However, Dawson-Hughes et al. (2005) suggested daily doses ranging from 10-40 µg to achieve an optimal vitamin D level.

2.2.1 UK recommendations

The UK Department of Health has suggested Reference Nutrient Intake (RNI) values of vitamin D for infants, adults over 65 years, pregnant women and during lactation (SACN, 2007), but no RNI had been provided for people aged between 4 and 64 years (Cashman et al., 2008; Ashwell et al., 2010) until 2015, when Public Health England's Scientific Advisory

Committee on Nutrition set recommended that they should take a supplement of 10 µg/d (SACN, 2015). Regarding sun exposure, British government policy has focused on reducing the incidence of skin cancer primarily by recommending sunscreen use, with additional advice about covering the body and avoiding outdoor skin exposure around midday (Gillie, 2010). However, to maintain adequate vitamin D production whilst avoiding sunburn, people need a little sun exposure frequently (Sinha et al., 2013).

2.3 Prevalence of vitamin D deficiency among adults

2.3.1 Worldwide prevalence of vitamin D deficiency

Vitamin D deficiency is a major issue worldwide, affecting all age groups in developed and developing countries (Van Schoor and Lips, 2011). Cashman et al. (2016) and Płudowski et al. (2013b) have documented the occurrence of vitamin D deficiency/insufficiency among adults in various countries in Europe. In Denmark, the prevalence of vitamin D deficiency (<25nmol/L) was found to be 13.8% among adults (n= 6784), while 52.2% had an insufficient level <50 nmol/L (Thuesen et al., 2012). A study of 4030 German adults (18 to 79 years old) found that over half of the participants had 25(OH)D concentrations of <50 nmol/L (Hintzpeter et al., 2008). In Finland, there is also a high rate of hypovitaminosis D, affecting 54.8% of Finnish adults (n=328) (Lamberg-Allardt et al., 2001).

The prevalence of vitamin D deficiency and insufficiency is particularly high among ethnic minority groups. A study of pre-menopausal female immigrants in Finland (n= 134; age 20–48 years) reported a mean 25(OH)D of <50 nmol/L amongst in 89.6% of the total sample (Islam et al., 2012). Andersson et al. (2013) conducted a study of immigrant and native Swedish women(n= 61) and found that the mean 25(OH)D level of all participants was <50 nmol/L, with 61% of immigrants having a serum 25(OH)D level below 25 nmol/L. Studies conducted among multi-ethnic adults in Norway reported that serum 25(OH)D levels of <25nmol/L were common (Holvik et al., 2005; Madar et al., 2008; Knutsen et al., 2014). In Denmark, the prevalence of vitamin D deficiency was high amongst Pakistani adults (Andersen et al., 2008). Solar radiation levels are high in many countries in the Middle East, Asia and Africa, yet the highest rates of hypovitaminosis D have been observed among these populations, across different age groups (Bassil et al., 2013; Al-Daghri, 2016). Possible factors explaining these discrepancies are discussed later in this chapter (Section 2.5).

2.3.2 Prevalence of vitamin D deficiency in the UK

As to vitamin D deficiency in the UK, it appears that the prevalence of 25(OH)D concentrations less than 25 nmol/L differs markedly between individuals of different ages, as Figure 2.4 shows (O'Connor and Benelam, 2011).

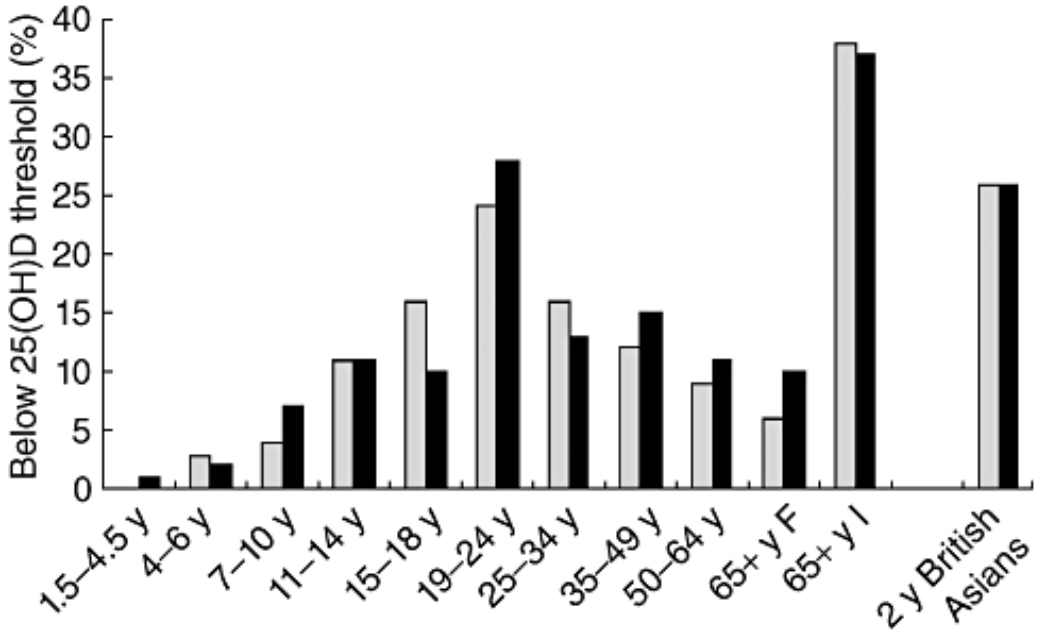

Figure 2.4: Prevalence of vitamin D deficiency [25(OH)D concentration <25 nmol/L] in individuals in the UK (Source: O'Connor and Benelam, 2011)

An NDNS report for the period 2011-12 states that 24% of men and 22% of women aged 19 years and over had serum 25(OH)D concentrations below 25 nmol/L (Bates et al., 2014), while the SACN found that the incidence of rickets was high in specific UK populations (SACN, 2007). Moreover, Blair (2012) notes that approximately half of the UK's Caucasian (Cauc) population and a substantial proportion (90%) of its ethnic minority groups (EMG) are likely to be affected by hypovitaminosis D. In light of these findings, there is concern among nutritionists and medical practitioners, who have suggested a need for regular screenings to detect hypovitaminosis D (Lanham-New et al., 2011; Lanham-New and Wilson, 2016; Pearce and Cheetham, 2010). This concern is also driving researchers to investigate the risk factors and find possible ways to address the issue of vitamin D deficiency (Holick 2004a; Hyppönen and Power 2007; Hirani et al., 2009; Pearce and Cheetham, 2010; Lanham-New et al., 2011; Zgaga et al., 2011; Lanham-New and Wilson 2016). Considering lifestyle as a factor, it has been demonstrated in Britain that working

indoors during daylight hours, coupled with a low dietary intake of vitamin D, obesity and alcohol intake, increases the prevalence of hypovitaminosis D (Hyppönen and Power 2007; Gillie 2010; Zgaga et al., 2011; Rhein, 2014). Table 2.1 summarises the studies showing the prevalence of vitamin D deficiency among the UK population.

Table 2.1: Summary of studies shows prevalence of vitamin D deficiency among adults in the UK

Reference	Study sample	Region	Assay used	Mean Age	25(OH)D nmol/L Mean	Ethnic groups	Results
Zgaga et al. (2011)	2235 females	Scotland	LC-MS/MS	21–82 y	22.5% had ≥50 nmol/L 14% had ≥40 nmol/L 34.5% had <25 nmol/L	Caucasian	A high prevalence of hypovitaminosis D in the sample; high level of vitamin D in some participants who was due to supplement intake.
Pal et al. (2003)	60 males 60 females	Birmingham	RIA	50 years	winter94%,summer 85% had <33 nmol/L	Asian & Non-Asian	The prevalence of hypovitaminosis D was greater in Asians than non-Asians in both summer and winter.
Bunn et al. (2004)	292 Somalis	Liverpool	-	>20 years		Black African	11% and 20% of adults suffering from osteoporosis and osteopaenia respectively. The whole sample had low vitamin D level.
Maxwell et a. (2006)	15 males 45 females Somali	Liverpool	-	42 years	47.8±21.8	Black African	Somalis living in the UK are at higher risk of hypovitaminosis D because of dark skin, limited sun exposure and above all, low vitamin D intake.
Roy et al. (2006)	78 females	Manchester	HPLC[&]	29.2 years	94% had ≤ 37.4 nmol/L	South Asian	Hypovitaminosis is spread among South Asian people and having <37 nmol/L of vitamin D may cause reduced bone mass in this sample.
Ford et al. (2006)	317 white, 125 Afro-C and 251 Asian	Birmingham	NAC and RIA	53 y	Caucasian 23nmol/L Asian 14.2 nmol/L Afro-Caribbean 16 nmol/L	Asian, African and Caucasian	24% of total sample had a deficiency at <25 nmol/l. Ethnic minority groups had a higher deficiency than Caucasian did. 43% of Asian females were deficient, more than Asian males, perhaps due to dress style.
Hypponen & Power (2007)	3725 males 3712 females	Great Britain	ELISA	45 years old	46% and 89% had <40, <75 nmol/L	British (White)	A high prevalence of vitamin D deficiency in the general adult population.
Macdonald et al. (2008)	3113 females	Aberdeen	EIA	54.8 years	59.2 nmol/L in autumn, 49.2 nmol/L in spring	Caucasian	The impact of seasonal variation was slight among sample. The vitamin D intake of Scottish females may prevent hypovitaminosis D.
Smith. (2010)	56 white, 56 SA male and 53 white, 35 SA female	Peterborough	HPLC	43 years	≥70 % from total sample has vitamin D deficiency	Caucasian & South Asian	There was a significant difference between groups in winter and summer. Serum vitamin D decreased from summer to winter. D decreased from summer to winter

Reference	Study sample	Region	Assay used	Mean Age	25(OH)D nmol/L Mean	Ethnic groups	Results
Mavroeidi et al. (2010)	338 female Caucasian, & 138 Caucasian, and 35 Asian female	Aberdeen and Surry	EIA	61.7 y 61.4 y 59.9 y	In winter, Aberdeen had 53.3 nmol/L Caucasian had 60.4 nmol/L and Asian had 25.8 nmol/L. while in winter/spring A had 40.4,43 and 23 nmol/L respectively	Caucasian &Asian	Seasonal variation in 25(OH)D was observed with significantly higher concentration among both groups but it was higher among ethnic group.
Farrar et al. (2011)	12 men 3 women	Mancheste r	HPLC	26 y	27% had <12 nmol/L The rest of them had < 50 nmol/L	South Asians	The current recommendation for sun exposure in the UK is not effective to have at least sufficient vitamin Level
Patel et al. (2013)	1105SA, males,748 BA	Birmingha m	LC-MS^	SA 57.6y BA 61y	75% SA had 29 nmol/L, 48% BA had 29 nmol/L	South Asian and Black African	Vitamin D deficiency was high in total sample but it was higher in SA compered to AC, perhaps due to cultural factors
Darling et al. (2013)	105 Caucasians 35 Asians	Surry	EIA	33.87 y 37.91 y	81 % SA had <25 nmol/L, C had ≤ 50 nmol/L	South Asian and Caucasian	Vitamin D deficiency was high among ethnic groups in Scotland. Caucasians had a sufficient level of vitamin D.

LC-MS/MS: Liquid chromatography–mass spectrometry. ELISA: The enzyme-linked immunosorbent assay. RIA: Radioimmunoassay. EIA: enzyme immunoassay
NAC: Nichols Advantage chemiluminescence. HPLC: High-performance liquid chromatography

Zgaga et al. (2011) assessed the vitamin D status of Cauc females (n=2235, age 21–82 years) living in Scotland. Results showed the prevalence of low vitamin D was high, with 63% of participants having 25(OH)D levels ≤40 nmol/L, while 22.5% had levels of ≥50 nmol/L. The study found that vitamin D supplementation contributed the most to total vitamin D intake and that it was positively correlated with vitamin D status.

Mavroeidi et al. (2010) studied Cauc (n=481) and Asian females (n=35) with a mean age of 61.9 years and found that the mean serum 25(OH)D level of Cauc females was ≥40 nmol/L in summertime and slightly less in wintertime, while more than half of the Asian females had serum levels of 25(OH)D at ≤25 nmol/L in both seasons. Similarly, Mavroeidi et al. (2010) found that low vitamin D was more prevalent in ethnic minority groups than Cauc ($p<0.001$). Possible explanations are discussed in Section 2.5.

Another comparative study by Pal et al. (2003) found that over 80% of South Asians (SA) (n=60) had vitamin D deficiency, while the mean 25(OH)D level among Cauc participants (n=60) was ≥40 nmol/L. Serum 25(OH)D levels differed significantly by sex and ethnicity in this study ($p<0.05$), with lower 25(OH)D levels in SA females compared to the males in both groups. A study carried out in Liverpool by Bunn et al. (2004) showed that a Somali sample (n=292) had vitamin D deficiency, while 20% had osteopaenia or osteoporosis. Similarly, Maxwell et al. (2006) found in a separate study that most of the Somali participants (n=60, mean age 42) had vitamin D deficiency in wintertime, primarily due to a low dietary intake of vitamin D.

A comparative study conducted by Ford et al. (2006) on Cauc, Afro Caribbean (AfroC) and SA (n= 693, mean age 52 years) participants, found that 24% of the total sample had 25(OH)D levels of <25 nmol/l. Ethnic differences were detected in 25(OH)D concentrations, with SA groups having a higher deficiency than the Cauc group. Overall, 43% of female SA participants were vitamin D deficient, significantly more than the males in the same group, an observation that may be partially explained by clothing style and skin coverage. Findings from a study by Smith (2010) on Cauc and SA adults (n=200, mean age 42 years) showed that 70% of SA had vitamin D deficiency. The study found significant seasonal fluctuations in 25(OH)D concentrations, with serum 25(OH)D levels decreasing from summer to winter.

Patel et al. (2013) recently assessed the prevalence of vitamin D deficiency in a multi-ethnic sample, SA (n=1105, mean age 57 years) and Black Afro-Caribbean (n=748, mean age 61 years), finding that 42% of SA had severe vitamin D deficiency, while 48% of AfroC had vitamin D deficiency and only 12% had a severe deficiency. Darling et al. (2013) studied Cauc (n=105, mean age 33.8 years) and SA (n=35, mean age 37.9 years) adults and found that in the winter, a higher percentage of SA (81%) had <25 nmol/L 25(OH)D compared to Cauc women (10%), while in the summertime 60% of Cauc women had ≤ 50 nmol/L 25(OH)D. In a more recent comparative study conducted among Asians, Black and white British living in London (n=222, mean age 72 years), the mean plasma 25(OH)D level of half of the total participants was <25 nmol/L (Jolliffe et al., 2016).

When reviewing these studies, it should be noted that the serum 25(OH)D concentrations were influenced by a range of factors including age, ethnicity, threshold variability considered as a deficient level, season and the method of analysis. Notably, the recommendations of these studies include actions targeting the wider population, rather than narrower at-risk groups such as children and pregnant women (Cashman et al., 2016, Hyppönen and Power, 2007; Gillie, 2010). As to the amount of research into differences in risk factors for vitamin D deficiency between ethnic minority groups, these differences are not as considerable as those observed between Caucs and specific EMGs. Therefore, this research offers an insight into the major factors that could place different EMGs at risk of vitamin D deficiency and provides recommendations on how to avoid these risk factors.

2.3.3 Ethnic minority groups living in Manchester

The definition of an ethnic minority group is ‘a group of people smaller in number than the majority categories and who by their customs, language, race, values, and group interests differ from the majority population’ (Carlson et al., 1984:203). The current ethnic minority population of Manchester is approximately 33.3% of the total population (Manchester City Council, 2015). The largest EMGs in Manchester are of Pakistani and African ethnicity, totalling 9% and 5% of the whole population respectively. Other smaller groups are Chinese (3%), Indian (2%) and Arab (2%) (Jivraj and Simpson, 2015). The African region is geographically defined as comprising Nigeria, Somalia, Zimbabwe, Kenya, Ghana, Uganda and South Africa, while the Arab region comprises Bahrain, Egypt, Iraq, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, Syria, the United Arab

Emirates, Yemen and Palestine. Asia is subdivided into South Asia (Pakistan, Bangladesh and India), East Asia (China, Japan and Korea) and South-East Asia (Malaysia, Indonesia, Philippine and Thailand). While all of these groups live in Manchester, each has a clearly distinct lifestyle, culture, religious practices and dietary habits.

2.3.3.1 Food habits of the diverse ethnic minorities populations

Obeidat (2002:18) states that food habits 'are an aspect of culture in which personal, social and situational factors interplay'. Thus, acculturation and adoption of a Western lifestyle, beside factors such as economic status and the accessibility of the food of the new country, may cause immigrants to change their traditional diets (Gilbert and Khokar, 2008; Wandel et al., 2008). Dietary behaviours vary from one EMG to another, with each of them favouring certain types of traditional food (Leung and Stanner, 2011) that may lack significant levels of vitamin D. Nakamura et al. (2002) analysed the dietary intake of the Japanese population and found that fish was a popular food in Japan. A cross-sectional study by Vyas et al. (2003) which found that meat was the preferred protein source among SA and AfroC groups, with semi-skimmed milk also being favoured by AfroCs (Vyas et al., 2003). Nolan (2007) found that lamb and chicken were widely consumed in the Middle East, as were fermented dairy products such as yoghurt and cheese, while milk-containing desserts were consumed more than milk alone. Margarine and butter were consumed by most Sri Lankans (Wandel et al., 2008). Consumer type can also influence dietary habits; thus, vegans and vegetarians refrain from eating meat and fish, which are considered rich sources of essential vitamins and minerals (Hill et al., 2004).

Specific religious groups including Muslims, Christians, Jews, Hindus, Sikhs and Buddhists often have particular dietary restrictions. For example, Muslims are prohibited from consuming alcohol and pork, while beef is generally forbidden for Hindus (Fam et al., 2004). Alcohol intake is allowed in religious groups such as Sikhs, Buddhists and Christians (Cochrane and Bal, 1990; Michalak et al., 2007), while for Jews, there is no proscription of drinking alcohol, but pork consumption is forbidden (Fam et al., 2004).

2.3.3.2 Prevalence of vitamin D deficiency among ethnic minorities in Manchester

A few studies have provided robust evidence of the prevalence of vitamin D deficiency among different EMG populations, especially SA (Macfarlane et al., 2005; Darling et al.,

2013; Webb et al., 2016). The average Cauc needs approximately 15 minutes' exposure of 35% of the skin to summer sunlight to achieve a sufficient level of 25(OH)D (Rhodes et al., 2014), while ethnic minorities with skin colours V and VI need at least 30 to 40 minutes (Farrar et al. 2013). Roy et al. (2006) studied Pakistani females (n=78, mean age 29 years) and found that 96% of them had 25(OH)D levels of ≤37.4 nmol/L, which was related to the reduction of bone mass at wrist and hip in this sample.

In order to determine whether the UK recommendation of sun exposure is sufficient for Cauc people to attain an adequate 25(OH)D level of ≥50 nmol/L, Rhodes et al. (2010) studied 109 mixed-gender participants (mean age 35 years), finding that their vitamin D level was 37.5 nmol/L at baseline and that after exposing 35% of their skin to the summer sunlight for 13 minutes, it increased to ≥50 nmol/L. Farrar et al. (2011) conducted a similar study but on South Asians (n=15, mean age 26 years), to examine the efficacy of current sun exposure recommendation in achieving sufficient 25(OH)D levels for darker skinned individuals. They found that most participants had 25(OH)D levels of <50 nmol/L and that four had <12.5 nmol/L. Farrar et al. (2011) conclude that current UK sun exposure recommendations are not sufficient for EMGs, especially those having higher levels of skin pigmentation.

A comparative study of 53 patients with a photosensitivity disorder by Rhodes et al. (2014) and a study by Webb et al. (2010) of 109 healthy participants (mean age 44 years) found no significant difference in terms of dietary or supplement intake, while significant seasonal variations in serum 25(OH)D concentrations were observed in both groups ($p=0.01$). Both groups of study participants had a low 25(OH)D levels during the winter months (medians of 31.8 and 45.2 nmol/L respectively), but higher levels at the end of summer (medians of 50 and 67 nmol/L respectively). However, the difference in vitamin D levels in the summer was due to the patient group's higher use of sunscreen at this time of year (Rhodes et al., 2014).

More recently, Hayden et al. (2015) conducted a study using laboratory databases from general medical practitioners in primary care in Manchester (n=11,291 cases) and report that >70% had serum 25(OH)D levels which could potentially increase the risk of skeletal disease. They also found a correlation between vitamin D deficiency and higher levels of social deprivation ($p<0.0001$). Webb et al. (2016) investigated qualitatively the

knowledge and attitudes of people towards sun exposure and vitamin D (n=20 SA and n=6 Cauc). The authors found that the SAs were more knowledgeable about vitamin D than the Cauc group, possibly reflecting that the SAs had experienced vitamin D deficiency.

2.4 Evaluating the health impact of vitamin D deficiency and insufficiency

Vitamin D plays a significant role in maintaining the normal functioning of the human body (Holick, 2004b; Holick, 2011). Serum 25(OH)D level ≤25 nmol/L has metabolic consequences for the skeleton, as it inhibits the accumulation of optimal levels of calcium (Holick, 2010). This results in an increased bone turnover and potentially osteoporosis or osteomalacia in adults (Gillie, 2010), due to a mineralization defect of the collagen matrix, with osteomalacia often characterized by chronic bone pain (SACN, 2007; Messer and Powell, 2009). Several studies have also shown a high incidence of skeletal diseases such as osteomalacia among a number of EMGs (Solanki et al., 1995; Hamson et al., 2003; Roy et al., 2007).

A review of several empirical studies has established a plethora of evidence that the active form of vitamin D 1,25$(OH)_2$D is associated with anti-proliferative as well as immunomodulatory effects. These effects of vitamin D are suggested to improve several serious conditions such as cancer (Jeon and Shin, 2018; Feldman et al., 2014) the risk of breast and prostate cancers is reduced by 30% to 50% when serum 25(OH)D concentrations are at 50 least nmol/L (Ahonen et al., 2000; Holick, 2004; Garland et al., 2007), while the risk of colon cancer decreased by 50% when the 25(OH)D level was above 80 nmol/L (Gorham et al., 2005). Vitamin D insufficiency is also associated with the incidence of cardiovascular disease (Wang et al., 2008; Pludowski et al., 2013a); it has been shown that the risk of CVD among EMGs was approximately 40% higher than that of the white population (Patel et al., 2013), in addition to a higher incidence of hypertension among Black African (BA), as reported by Lane et al. (2001). A systematic review and meta-analysis of 128 studies conducted among multi-ethnic groups showed that the risk of having cardiometabolic disorders decreases by 45% when 25(OH)D level is high (Parker et al., 2010). The influence of vitamin D has also been shown in other diseases including type 1 diabetes, hypertension and rheumatoid arthritis (Vimaleswaran et al., 2014; Palermo and Holick, 2017; Ishikawa et al., 2017).

2.5 Factors which may affect vitamin D status

2.5.1 Low vitamin D intake

The NDNS found that the mean daily dietary intake of vitamin D was 3.9 µg for men and 3.4 µg for women (Bates et al., 2014), i.e., below the current recommendation. Public Health England (2014) published a recent survey about food consumption, showing that most of the UK population has either vitamin D insufficiency or deficiency. Indeed, the dietary intake of vitamin D is much lower in the UK than in other Western nations including the USA and Canada (Calvo and Whiting, 2013). The disparity between the UK and North America has been attributed to the differing extents to which mandatory fortification of foodstuffs occurs in these countries (outlined in Section 2). Clinical nutritional assessments of natural food items suggest that, with the exception of fish and cod liver oil, most natural foodstuffs contain minimal amounts of vitamin D, including meat, eggs and dairy products (Brough et al., 2010; Sinha et al., 2013).

The NDNS showed that only 23% of vitamin D intake was provided by oily fish and 23-35% by consumption of meat products, whereas fat and cereal products contributed 19% and 13-20%, respectively (Bates et al., 2014). Two other national surveys Irish Universities Nutrition Alliance (IUNA) and Irish National Food Consumption have reported that 30%, 12% and 9% respectively of mean dietary intake of vitamin D was obtained from meat, fish and egg product (IUNA, 2011; Hill et al., 2004). Irrespective of these different assessments, Schmid and Walther (2013) state that it is difficult for people to meet their recommended intake of vitamin D through consumption of foodstuffs alone. Therefore, solutions must be found to increase vitamin D intake among the whole population. Vitamin D supplements could be an alternative and effective way to enhance vitamin D status, especially among people who cannot obtain a sufficient level of vitamin D due to a lack of sunlight and natural food sources (Sinha et al., 2013). According to Hyppönen and Power (2007) and Zgaga et al. (2011), higher 25(OH)D levels were positively correlated with supplement intake.

2.5.2 Factors influencing cutaneous synthesis of vitamin D

2.5.2.1 Season and latitude

As the majority of vitamin D is produced in the skin, where UVB is required to initiate the process, seasonal variation in sun strength can have a pronounced effect on vitamin D

status (Bolland et al., 2007), particularly when daylight is limited during the winter as compared to the summer months. In winter, increased cloud cover also affects vitamin D synthesis by reducing the amount of UVB that reaches the earth's surface by around 45% (Sullivan et al., 2003).

Furthermore, latitude has a significant impact on vitamin D levels (Webb, 2006). In countries located below latitude 35°N, the body can produce sufficient vitamin D all year round (Tsiaras and Weinstock, 2011), while at latitudes above 35°N (i.e. much of Europe, including Germany, Italy and the Netherland), skin synthesis of vitamin D tends to be sufficient only in summer time (Lanham-New et al., 2016). Consequently, European citizens are at a higher risk of vitamin D deficiency compared to citizens of countries located nearer the equator (Grimes, 2011).

Seasonal variations are observed in the UK at 55°N (Hyppönen and Power, 2007; Darling et al., 2013), with concentrations of 25(OH)D lower in spring and winter as a result of diminishing UVB photons reaching the earth's surface caused by the increasing solar zenith angle (Levis et al., 2005; Webb, 2006). Figure 2.5 shows a summary of the results obtained.

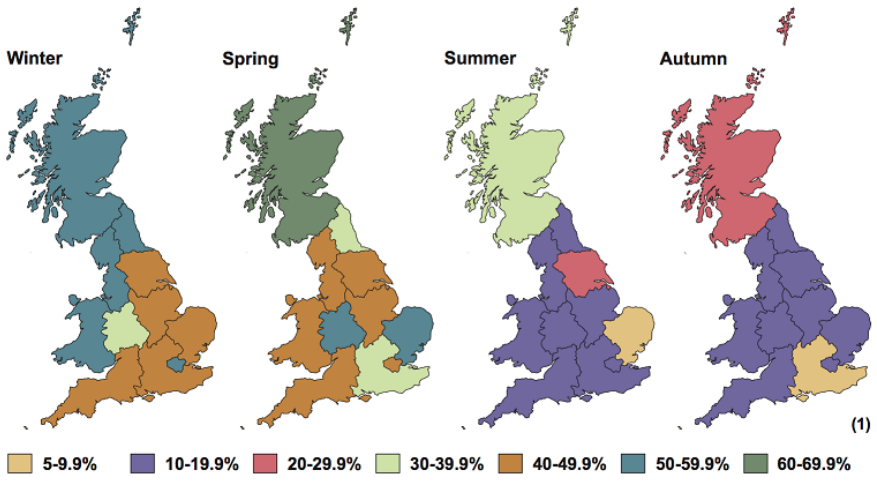

Figure 2.5: Seasonal and geographical variation in the prevalence of 25(OH)D insufficiency (<40 nmol/L) in Great Britain (Source: Hyppönen and Power, 2007)

A study by Pal et al. (2003) found that seasonal timing was statistically correlated to serum 25(OH)D levels, where 94% of the participants (n=120, mean age 50 years) had a

low serum 25(OH)D level (<33 nmol/L) in the wintertime, while 85% had a low serum 25(OH)D level during summer.

A nationwide cohort study of middle-aged British adults (n= 7,437) demonstrated that hypovitaminosis D was more prevalent during the winter and spring months, with 16% of the sample having serum 25(OH)D levels below 24.9 nmol/L, while 46.6% had sufficient levels (>39.9 nmol/L) (Hyppönen and Power, 2007). Similarly, a study carried out by Mavroeidi et al. (2010) on 173 postmenopausal women living in two locations in Surrey found that 17.1% of Asian (n=35) women were deficient in vitamin D in the summertime, rising to 58.1% during the winter. In comparison, none of the Caucs had a 25(OH)D level of less than 19.9 nmol/L. In Aberdeen, the highest rates of hypovitaminosis D were recorded among 25% of Caucs (n=338) during winter and spring, decreasing to 4.2% in the summer. The authors conclude that seasonal variation significantly affects vitamin D status (Mavroeidi et al., 2010).

Conversely, research carried out in Aberdeen by Macdonald et al. (2008) indicated that seasonal changes in vitamin D status were minor among 3,113 Cauc females with a mean age of 54.8 years. In autumn, the serum 25(OH)D concentration was 59.1 nmol/L, while in spring it was 49.1 nmol/L. This stability was attributed to the fact that the participants consumed a diet rich in vitamin D and supplement (Macdonald et al., 2008). This poses a challenge to the vitamin D status of people residing in such areas, particularly if they fail to implement dietary changes to compensate for any deficiency.

2.5.2.2 Skin type and ethnicity

According to Fitzpatrick (1988), human skin colour ranges from the lightest to darkest as presented in .

Table 2.2, dependent on the amount of melanin (Bowyer et al., 2009). It has been found that melanin has an impact on vitamin D synthesis by absorbing UVB, thus reducing the cutaneous synthesis of vitamin D_3 (Kift et al., 2013; Webb et al., 2018). Therefore, whenever the melanin content of the skin is high, exposure time must be increased in order to allow sufficient cutaneous synthesis of previtamin D_3 (Holick, 2004a). Data from the National Health and Nutrition Examination Survey (NHANES) showed that the rate of hypovitaminosis D was higher in Black participants (82.1%) compared to White participants (30%) (Looker et al., 2011).

A meta-analysis conducted by Martin et al. (2016) showed that the rate of hypovitaminosis D was highest among people with darker skin pigmentation. In another study, Chen et al. (2007) measured serum 25(OH)D concentrations in adults with different types of skin as classified by Fitzpatrick (.

Table 2.2), after exposure to a controlled dose of UVB from one type of sunbed over a period of three months. By the end of the study, serum 25(OH)D concentrations had increased dramatically in all skin types. Respective mean increases in 25(OH)D recorded for skin types II, III, IV and V were 210%, 187%, 125% and 40%. The authors conclude that the production of previtamin D_3 in type II skin is five to ten times higher than type V (highly pigmented) skin. In the UK, low vitamin D levels have been seen among EMGs, particularly in those of SA (Shaw and Pal, 2003) and BA origins (Maxwell et al., 2006; Patel et al., 2013). For instance, almost 94% of Pakistani women (n=78), with a mean age of 29.2 years, had serum 25(OH)D levels below 37.1 nmol/L (Roy et al., 2007). Possible explanations for this low level of vitamin D include skin pigmentation and dress style (Brough et al., 2010; De Roos et al., 2012).

A cross-sectional study conducted in 125 SA volunteers with skin type V living in the UK found that in the summer, the participants reached a mean serum 25(OH)D level of 22.4 nmol/L, whilst during wintertime, 40% of them were found to be deficient, with levels as low as 15 nmol/l (Kift et al., 2013). By analysing measured 25(OH)D levels and demographic factors, UV exposure and vitamin D intake for all participants collected by questionnaire, the researchers found that hypovitaminosis D was not due to a single factor, but instead a combination of low dietary vitamin D intake, low sunlight exposure and high skin pigmentation (Kift et al., 2013). Another cross-sectional study, conducted by Maxwell et al. (2006), involving 60 Somali immigrants living in Liverpool, revealed that 36% of the participants were suffering from osteomalacia, with the remaining participants deemed to be at a greater risk of vitamin D deficiency caused by a low vitamin D intake and limited exposure to sunlight. Several comparative studies have shown that the serum 25(OH)D level of BAs was lower than in Caucs, again due to high skin pigmentation (Hall et al., 2010; looker et al., 2002). Consequently, vitamin D intervention may be necessary for certain ethnic minorities in the UK, to assist them in attaining optimal levels of vitamin D.

Table 2.2: Skin type, colour and reaction to sun exposure

Skin type	Skin colour	Skin reaction
I	Pale white; blond or red hair; blue eyes; freckles	Always burns, never tans
II	White; fair; blond or red hair; blue or green eyes	Burns easily
III	Cream white; fair with any hair or eye colour	Mild burn, tans average
IV	Moderate brown; typical Mediterranean skin	Rarely burns, tans easily
V	Dark brown skin types	Very rarely burns
VI	Dark-skinned black	No burn

Source: Bowyer et al. (2009)

The SunSmart campaign promoted by the UK Department of Health has put forward recommendations for the population regarding sunlight exposure (Cancer Research UK, 2010), but may not be appropriate for dark-skinned people, because they will fail to achieve sufficient 25(OH)D levels (Farrar et al., 2011). Furthermore, while the UK National Institute for Health and Care Excellence (NICE) has published health guidance for sun exposure for different population groups (NICE, 2016), it offers no specific advice regarding sun exposure for people with skin type V. Farrar et al. (2011) and Webb et al. (2018) state that people with skin types V or VI need additional exposure to sunlight in the UK (ca. 25 to 40 minutes more) to get the same amount of vitamin D_3 as do people with skin types II or III (Farrar et al., 2011; Webb et al., 2018).

2.5.2.3 Sunscreen

Sunscreen is a physical barrier to both UVA and UVB radiation and therefore affects vitamin D synthesis (Macdonald et al., 2011; Tsiaras and Weinstock, 2011). According to Holick (2004b), sunscreen usage reduces the number of UVB photons that reach 7-DHC in the skin by absorbing or scattering UVB radiation, thus decreasing vitamin D production. Most sunscreens have a stated sun protection factor (SPF), which is for 'measuring the efficacy of sunscreen products in shielding the sun's ultraviolet radiation (UVR) and thereby protecting the skin against sunburn' (Reinau et al., 2015:1). Using SPF 8 to 15 was found to decrease vitamin D production in the skin, but these findings remain contentious. Some studies have found it to be a strong inhibitor, reducing vitamin D

production by more than 95% (Holick, 2004b; Faurschou et al., 2012; Libon et al., 2017), while others have found no significant effect (Kimlin et al., 2007; Linos et al., 2012). Possible explanations for these divergent results are the amount of sunscreen applied (Wolpowitz and Gilchrest, 2006), its thickness (Faurschou et al., 2012) and the level of SPF used (Libon et al., 2017).

Furthermore, individuals using sunscreen may be encouraged to expose themselves to sunlight for longer and more frequently (Linos et al., 2012). In the UK, a campaign established by Cancer Research UK calls for the population to avoid sun exposure and use sunscreen frequently to protect against skin cancer, which in turn can lead to reductions in vitamin D synthesis (Cancer Research UK, 2010). Therefore, studies have attempted to find an appropriate approach to optimize sunscreen use without overly affecting cutaneous vitamin D production (Kockott et al., 2016).

2.5.2.4 Clothing

It has been reported that the production of pre-vitamin D_3 is reduced by clothing (Salih, 2004) and that the specific fabric quality could restrict or block effective skin irradiation of pre-vitamin D (Aguilera et al., 2014). Blocking out sunlight with clothing for religious or cultural reasons is particularly associated with hypovitaminosis D, as reported previously. For example, a cross-sectional study conducted by Glerup et al. (2000) found mean serum 25(OH)D concentrations of 7.1 nmol/L among 60 immigrant women (veiled and ethnic Muslim women), which was found to be significantly lower than in Danish women (n=44, 47.1 nmol/L). Similar results have been shown in a Turkish study (Alagöl et al., 2000), where the mean 25(OH)D concentrations of women who dressed in the traditional Islamic style (covering their whole bodies excluding their hands and faces) were significantly lower (9 nmol/L) as compared to women wearing Western-style clothing (56 nmol/L). A study among immigrants in Sweden (Granlund et al., 2016) showed that wearing long sleeves was associated with a higher likelihood of low vitamin D levels (adjusted odds ratio = 3.15 (95% CI, 11.09–9.12). Individuals living in a cloudy city and choosing a specific dress style for cultural or religious reason could be at a particularly high risk of vitamin D deficiency.

2.5.3 Other factors that influence vitamin D status

2.5.3.1 Obesity

An additional factor that can affect vitamin D levels is obesity (Pourshahidi, 2015), typically defined as a body mass index (BMI) of 30 kg/m^2 and above. It has been shown to be inversely correlated with serum vitamin D concentrations and positively correlated with PTH levels (Wortsman et al., 2000; Macdonald et al., 2008; Rajakumar et al., 2011). Some studies have reported that the association between obesity and vitamin D status is in the opposite direction: It has been posited that cutaneous vitamin D synthesis is greater in obese than in non-obese people due to exposure of a larger skin area to UVB (Verbraecken et al., 2006; Looker et al., 2007). However, the findings of a clinical study by Wortsman et al. (2000) contradict this claim, as there were no differences between 26 non-obese and obese people (mean age 34 years) in terms of vitamin D production after exposure to UVB (38 and 17 nmol/L respectively) or after receiving oral D_2 supplements (5.3 and 3.5 nmol/L respectively). Wortsman et al. (2000) suggest that obesity does not influence vitamin D production directly, but rather the release of vitamin D_3 from the skin into the circulation. A recent study by Turer et al. (2013) supports this hypothesis by showing that the deposition of vitamin D in body fat compartments results in decreased bioavailability of vitamin D_3 from cutaneous and dietary sources. Alternatively, low vitamin D levels among obese people could be linked to behavioural factors, as they may tend to cover themselves more fully, providing a barrier to sunlight and thus reducing the production of vitamin D cutaneously (Pereira-Santos et al., 2015). Kull et al. (2009) found that obese participants who had a BMI over 30 kg/m^2 tended to avoid sunbathing and outdoors activity compared to participants with a BMI below 30 kg/m^2 ($p<0.01$).

Furthermore, BMI and body fat could have a significant impact on identifying the recommended dosage of a vitamin D supplement, as supported by Lee et al. (2009). Obese people may need a higher dose of the supplement than the non-obese to increase vitamin D levels, because of the excessive sequestering and storage of vitamin D in fatty tissue. In fact, those who are obese and living in high-risk regions, such as North-West England, are deemed to be at twice the risk of those living in lower-risk regions of Great Britain (Hyppönen and Power, 2007).

2.5.3.2 Socioeconomic status

Low vitamin D intake may be associated with low economic status (Dealberto, 2006). Numerous studies cite issues such as poor nutrition, poor lifestyle and the inability to afford supplements to treat vitamin D deficiency. According to Brough et al. (2010) and Grimes (2011), a socially deprived population may not be able to afford some basic nutrients or supplements such as vitamin D, which are essential for normal metabolic function. Hirani et al. (2009) conducted a study of vitamin D levels among adults, aged ≥19 years in two British surveys: 1297 people from the NDNS and 792 from the Low Income Diet and Nutrition Survey (LIDNS). They found that the LIDNS sample had a mean 25(OH)D lower than that observed in the NDNS ($p<0.001$). More recently, Hayden et al. (2015) showed that higher social deprivation contributed to an increased proportion of insufficient/deficient people who had 25(OH)D ≤50 nmol/L, while Webster (2013) found in a study that low economic status was only related to severe hypovitaminosis D (25(OH)D <15 nmol/L). However, the latter study focused on EMGs, with a high percentage of these groups living in deprived areas, whereas the former study was sampled the whole population. A more recent study by Léger-Guist'hau et al. (2017) indicated that the risk of severe vitamin D deficiency increased with low socio-economic status.

A report released by the Greater Manchester Poverty Commission (GMPC) in 2002 identified Manchester as one of the most poverty-affected regions in Britain, with approximately 25% of its population living in abject poverty (GMPC, 2012). A few decades ago, there was a five-year campaign aiming to address vitamin D deficiency by giving ethnic Asian schoolchildren a daily dose of vitamin D (2.5 µg), which led to a decrease in the occurrence of rickets (Dunnigan et al., 1985). This effort led to significant improvement in the targeted cities in the north of England. But it is reported that the improvements made almost forty years ago are no longer visible (Roy et al., 2007), which may be due in part to most people not knowing about the Healthy Start programme, which provides a free supplement to pregnant women and children from low-income families to avoid vitamin D deficiency (Blair, 2012). However, low-income EMGs also need such action to avoid hypovitaminosis D.

2.5.3.3 Smoking and alcohol intake

Many studies have shown that alcohol intake is positively correlated with serum vitamin D concentration (Lamberg-Allardt et al., 2001; Ilich et al., 2002; McCullough et al., 2010; Gorter et al., 2016). According to Lee (2012), the effect of alcohol lies in its ability to impede vitamin D metabolism and hinder the conversion of 25(OH)D to 1,25(OH)D. However, the association between vitamin D and alcohol intake is still controversial (Tardelli et al., 2017), as a number of alternative studies show that there is no significant association (Cheng et al., 2014: Rapuri et al., 2000; Egan et al., 2008).

Regarding smoking status, some studies have reported no relationship between smoking and vitamin D level (Arunabh et al., 2003; Cheng et al., 2014), whereas a number of others show a negative association between smoking and serum 25(OH)D concentration among mixed gender adults (Lamberg-Allardt et al., 2001; Jorde et al., 2005; Laaksi et al., 2007; Kassi et al., 2015). However, the exact mechanisms by which the constituents of tobacco smoke affect vitamin D metabolism are still not clear (Shinkov et al., 2015). Additional research is needed to understand the effects of alcohol intake and smoking on serum 25(OH)D levels.

2.6 Supplementation Studies

Many intervention studies have considered the viability of vitamin D supplementation, taking into account factors such as the different forms of the vitamin (D_2 or D_3) used, the dosage levels applied, body fat and the time needed to reach the desired vitamin D level (Zittermann et al., 2014). Supplementation regimes could either maintain a sufficient or optimal level of vitamin D or treat vitamin D deficiency and vitamin D-related diseases (Holick et al., 2011).

2.6.1 Previous supplementation studies using vitamin D_3/D_2

Vitamin D supplementation is an effective strategy to maximize a serum 25(OH)D levels in the absence of sun exposure and limited dietary sources. It has been suggested that people could achieve the optimal level of serum 25(OH)D (75 nmol/L) when they are supplemented with 20-25 µg of vitamin D daily (Dawson-Hughes et al., 2005), although a rival opinion suggests that adults need at least 55 µg/d to reach a desired level of ≥75 nmol/L (Heaney, 2005) and a dose of 100 µg/d may be required for people with baseline levels of 25(OH)D at <45 nmol/L (Talwar et al., 2007). The latter suggestion may be

particularly relevant to dark-skinned people. However, the tolerable upper intake level for oral vitamin D is suggested as 100 µg/d (Ross et al., 2011). A summary of the literature regarding supplementation studies to improve vitamin D status among healthy adults is presented in Table 2.3.

A supplementation study was conducted by Barnes et al. (2006) on 27 healthy mixed-sex adults, with a mean age of 21.6 years, who were divided into a vitamin D_3 group (15 µg/d and 1500 calcium) and a control group (1500 calcium). In the vitamin D treatment group, serum 25(OH)D levels rose from 47.4 to 87.3 nmol/L after eight weeks compared to the control group, which decreased by 7.5 nmol/L. The authors concluded that a 15 µg/d vitamin D_3 supplement increased serum 25(OH)D concentrations by 2.09 nmol/L per microgram of vitamin D_3 taken. A study was conducted on 110 adults (mean age 28 years and baseline 25(OH)D levels of 75 nmol/L), found that a daily supplement of 15 µg of vitamin D_3 reached a mean 25(OH)D levels of 68.9 nmol/L after 22 weeks (Forsythe et al., 2012).

In another intervention study carried out by Talwar et al. (2007) on 208 BA women with a mean baseline 25(OH)D of 46 nmol/L, 104 were given an oral intake of 20 µg/d and 104 women were given matched placebo for two years. The mean serum 25(OH)D reached a level of 71 nmol/L after three months and 65.9 nmol/L after two years, while there was no change in the placebo group. The study continued for an additional year with an increased dose of 50 µg/d and the level of 25(OH)D reached a level of 87 nmol/L, showing that this dose is required for BAs to increase their 25(OH)D to the optimal level >75 nmol/L.

In another study, conducted by Nelson et al. (2009) on 86 women with a mean age of 22.2 years and baseline 25(OH)D level of 62 nmol/L, the participants were given either a dose of 20 µg/d or a matched placebo for a year starting in March. The authors reported that 80% of participants reached the optimal level ≥75 nmol/L by the end of the study, with a mean increase of 35 nmol/L. They conclude that 20 µg/d was sufficient to maintain an optimal level of vitamin D during the winter.

Harris and Dawson-Hughes (2002) examined the effect of age on the response to a supplement in 50 participants (18-35 years and 62-79 years), finding that participants who were supplemented with 20 µg/d for 8 weeks reached mean serum 25(OH)D levels

of 83 nmol/L, while the others remained below the sufficient level. However, there was no evidence of an effect of age on the response to the supplement and serum 25(OH) D was approximately identical in both age groups at the end of the study, having increased by 4.3 and 6.2 nmol/L respectively.

In contrast, the study by Wicherts et al. (2011) found that 20 µg D_3 daily did not reliably increase mean serum 25(OH)D concentrations to 75 nmol/L, as 52% of multi-ethnic participants (n=71) remained below this cut-off at three months, while only 40% of participants reached the cut-off of 50–75 nmol/L at six months. This could be due to inadequate compliance in the sample (Wicherts et al.,2011) or to the influence of high BMI (33% had a BMI ≥30 kg/m^2) on the response to the supplement.

Holick et al. (2008) conducted a study on 68 adults (mean age of 40 years and baseline 25(OH)D level of 49/42 nmol/L), divided into two groups and supplemented with 25 µg of D_3/D_2 for 11 weeks. They found that mean serum 25(OH)D level increased from 49.3 to 72.3 nmol/L for the D_3 group and from a mean of 42 to 67 nmol/L for D_2 group. The authors state that this dose was not effective in correcting the deficiency of vitamin D, although it would be sufficient to sustain an optimal level of vitamin D (≥75 nmol/L). In another intervention, Vieth et al. (2001) found that the serum 25(OH)D levels of healthy adults (n=61, mean age 41 years) who received 25 µg D_3 increased from 43.1 nmol/L to 69.8 nmol/L after 12 weeks, with a 1.02 nmol/L increase per microgram of vitamin D_3 taken. By contrast, in the group administered 100 µg vitamin D_3, serum 25(OH)D levels rose from 37.4 nmol/L to 97.3 nmol/L, with an increase of 0.57 nmol/L per microgram of vitamin D_3 *(*Vieth et al., 2001). Heaney et al. (2003a) agree with the finding of Vieth et al. that either 100 or 125 µg/d of D_3 can increase serum 25(OH)D levels without any adverse effect. The authors conducted a study on 67 healthy adults, with a mean age of 38.7 years who were supplemented with either placebo, 25, 125, or 250 µg/day of vitamin D_3 for five months (Heaney et al., 2003a). They found that serum 25(OH)D levels increased by 12, 92 and 195 nmol/L respectively, while in the placebo group it decreased by 11 nmol/L.

However, another study (Aloia et al., 2008) found that participants whether Caucasian or Black, required at least 100 µg if they had insufficient vitamin D levels (< 50 nmol/L). A recent study conducted among Somali women found that a daily dose of 20 µg of

vitamin D_3 was not sufficient to increase 25(OH)D levels, while a dose of 40 µg increased mean serum 25(OH)D levels from 19 nmol/L to at least 60 nmol/L (Osmancevic et al., 2016).

The above review indicates that numerous studies have been conducted on the effectiveness of a daily dose of vitamin D_3 to increase vitamin D levels to a desirable level. Section 2.6.2, which follows, considers additional factors including safety, efficiency and rapidity in studies of supplementation with another form of vitamin D.

Table 2.3: Supplementation studies to improve vitamin D status amongst healthy adults

Reference	Study participants	Mean age	Vitamin D dose	duration & study design	Assay	Baseline 25(OH)D nmol/L mean	Outcomes
Vieth et al. (2001)	61 male and female	41 years	Oral D_3 25 µg/d 100 µg/d	3 months (RCT)	RIA	40.7 ± 15.4 37.9 ± 13.4	The mean 25(OH)D concentration reached 68.7 nmol/L, 96.4 nmol/L for group of 25 and 100 respectively. Supplementation with 100 µg vitamin D3 is safe and gave a beneficial increase in 25(OH)D levels
Harris and Dawson-Hughes (2002)	25 young adults, 25 older adults (healthy men) in USA	18-35 years and 62-79 years	20 µg/d oral D_3	8 weeks (RCT)	Competitive protein binding.	60 nmol/L	This dose was effective for some participants to reach 83 nmol/L.
Heaney et al. (2003a)	Healthy men 67 in Omaha 41˚N	38.7 years	0, 25, 125 and 250 µg/d Oral vitamin D_3	20 weeks (RCT)	RIA	70.3 ± 19.9 for all groups	The mean 25(OH)D concentration of some participants reached 84 nmol/L at the end of trial with the 25-µg/d dose, while others remained below 75nmol/L. At least 100 µg/day recommended to achieve desirable level.
Barnes et al. (2006)	27 samples in Northern Ireland 55°N	21.6 years	15 µg/d D_3 and 1500 mg 1500 calcium	8 weeks (RDBPC)	ELISA	47.9 ± 16 55.5 ± 18.9	Daily 15 µg increased vitamin D level to mean of 86.5 nmol/L
Talwar et al. (2007)	208 Black women USA 41˚N	59 years	20 and 50 µg/d oral D_3	First stage for 2 years, in third years started taking 50 µg/day	RIA	46.9 ± 20.6	The level of vitamin D could be increased to desired level by consuming 50 µg/d among Black women

Reference	Study participants	Mean age	Vitamin D dose	duration & study design	Assay	Baseline 25(OH)D nmol/L mean	Outcomes
Holick et al., (2008)	68 healthy adults in Boston 42 °N	40 years	25 µg D_3 and D_2, placebo	11 weeks (RDBPC)	LC-MS	D_2 group= 42.3 ± 26.3; D_3 group= 49.0 ± 27.8; Placebo group= 46.5 ± 22.3	Daily 25 µg of D_3/D_2 is effective in sustaining a sufficient level of vitamin D
Nelson et al (2009)	86 women in Main 45°N	22 years	20 µg/d D_3 placebo	12 month (RCT)	RIA	62.1 ± 24.0 61.9 ± 22.6	The mean serum 25(OH)D level of 80% of participants reached ≥75 nmol/L at the end of the study.
Forsythe et al.(2012)	110 sample in Northern Ireland 55°N	28 years	15 µg/d D_3	22 weeks (RDBPC)	ELISA	75.8 (median) 65.7 (median) placebo	Daily 15 µg for 22 weeks was effective for participants to achieve at least >50 nmol/L (mean 68.9)
Osmancevic et al (2016)	114 Somali women in Sweden 55°N	34 years	20, 40 µg/d D_3	12 weeks (RDBPC)	Automated immunoassay	23 (median) 19 (median)	Daily dose of 20 µg increased mean serum 25(OH)D by 15 nmol/L at week 6 and reached 38 nmol/L at week 12. Daily dose of 40 µg increased it to 60.5 nmol/L at week 12

RCT: Randomized control trial, RDBPC: Randomized double blind placebo control

LC-MS/MS: Liquid chromatography–mass spectrometry

ELISA: Enzyme-linked immunosorbent assay

RIA: Radioimmunoassay

2.6.2 Oral vitamin D metabolite (calcifediol)

Although, the two common forms of vitamin D (D_2 and D_3) are mostly employed to treat vitamin D deficiency or insufficiency, other synthetic metabolites such as calcifediol, also referred to calcidiol, can be used as a drug in clinical practice to raise 25(OH)D levels (Cianferotti et al., 2015; Haddad and Stamp, 1974; Haddad and Rojanasathit, 1976). Calcifediol can be taken as a pharmaceutical compound, supplement or ingredient in normal food (Ovesen et al., 2003; Purchas et al., 2007). It has a higher polarity and biological activity than vitamin D itself, which makes it more soluble in the blood, and has a short half-life of 10–13 days (Jean et al., 2008; Bischoff-Ferrari et al., 2012; Cianferotti et al., 2015). As a consequence, it raises 25(OH)D levels more rapidly than vitamin D_3, so that 1 µg of calcifediol administered orally will raise 25(OH)D concentrations by 3–4 nmol/L, compared with a 1 nmol/L increase from vitamin D_3 (Bischoff-Ferrari et al., 2012; Cashman et al., 2012). Recently, concern has been raised over the consequences of taking high doses of vitamin D, including increased risk of falls and fractures (Sanders et al., 2010; Dawson-Hughes and Harris, 2010; Rossini et al., 2012). There is thus a perceived need to avoid high doses of vitamin D (Brandi and Minisola, 2013). A satisfactory solution may be to use calcifediol, as a small dose can treat vitamin D deficiency over a short period.

Section 2.6.2.1, reviews previous studies of the use of calcifediol to treat vitamin D deficiency among healthy people and studies of its use to address deficiency caused by specific medical conditions. A summary of these studies and their key findings is also presented in Table 2.4.

2.6.2.1 Use of calcifediol to treat hypovitaminosis D

Several studies have investigated the effect of calcifediol and have shown its ability to increase serum 25(OH)D level rapidly (Barger-Lux et al., 1998; Shieh et al., 2017; Jetter et al., 2014). Shieh et al. (2017) recently conducted a comparative study on 35 multi-ethnic adults (mean baseline 25(OH)D levels of 40 nmol/L), who were supplemented with either 60 D_3 µg/d (n=16) or 20 µg/d calcifediol (n=19) for 16 weeks. They found that in the calcifediol group, 25(OH)D levels reached 86.1 nmol/L at week 4 and were further

elevated beyond week 16, while in the vitamin D_3 group, 25(OH)D levels remained below 75 nmol/L.

An earlier study by Barger-Lux et al. (1998) assessed the effect of graded oral dosing of calcifediol (10, 20 or 50 µg/d) for 4 weeks and vitamin D_3 (25, 250 or 1250 µg/d) for 8 weeks in 116 healthy men (mean age of 28 years and baseline 25(OH)D levels of 67 nmol/L). The authors report that at week 4 the changes in mean serum 25(OH)D level in the calcifediol group were approximately 40 nmol/L, 75 nmol/L and 204 nmol/L for 10, 20 and 50 µg/d of 25(OH)D_3 respectively, while in the vitamin D_3 group, the respective changes at week 8 were 28.7, 146 and 634 nmol/L for 25, 250 and 1250 µg. The half-life of calcifediol was 19 days (Barger-Lux et al., 1998).

A recent long-term study was conducted by Jetter et al. (2014) to assess the pharmacokinetics of vitamin D_3 vs calcifediol on plasma 25(OH)D levels of 35 postmenopausal women. The participants were divided into seven groups, which were given either a daily oral dose of 20 µg vitamin D_3 or calcifediol, a weekly dose of 140 µg D_3 or calcifediol (both for 15 weeks), or a single dose of 140 µg vitamin D_3 or calcifediol. The plasma 25(OH)D levels of the calcifediol group increased to more than 75 nmol/L at week 4 and levels did not differ significantly between daily and weekly application. In the vitamin D_3 groups, 70% of women who were given the same doses reached 25(OH)D levels of 75 nmol/L at week 15. Finally, it was found that calcifediol was associated with a 2.5-fold increase in plasma 25(OH)D levels compared to vitamin D_3 (Jetter et al., 2014).

Similarly, Cashman et al. (2012) studied 58 adults with a mean age of 57.2 years, to compare the effects of calcifediol and vitamin D_3 in enhancing 25(OH)D levels. These participants were divided into four groups and given a daily dose of either 20 µg vitamin D_3 or calcifediol, 7 µg calcifediol or placebo, for 10 weeks. It was found that mean serum 25(OH)D levels increased from 38.2 nmol/L to 134 nmol/L in the 20 µg calcifediol group and from 42 nmol/L to 70 nmol/L in 7 µg calcifediol group, while in the vitamin D_3 group the increase was from 49 nmol/L to only 69 nmol/L and no change was registered in the placebo group (Cashman et al., 2012). The authors conclude that calcifediol supplementation was about 4.2 to 5 times more efficacious in enhancing serum 25(OH)D levels compared to vitamin D_3 (Cashman et al., 2012). The difference between the Jetter

and Cashman studies in the extent of the superior therapeutic effectiveness of calcifediol over D_3 in raising 25(OH)D levels may due to the assay methods used or to errors in sampling and processing.

2.6.2.2 Use of calcifediol to treat hypovitaminosis D caused by diminished hepatic or renal hydroxylation

Several supplementation trials have been conducted to investigate the efficacy of calcifediol in treating some diseases. A recent study by Bischoff-Ferrari et al. (2012) investigated the effect on postmenopausal women (mean age of 61.5 years and a mean baseline 25(OH)D levels of 34 nmol/L) of a daily dose of 20 µg calcifediol or vitamin D_3 over 4 months. The mean serum 25(OH)D level of the calcifediol group was found to increase to 173.7 nmol/L, compared with 77.5 nmol/L in the vitamin D_3 group. The authors supported the benefit of using calcifediol on systolic blood pressure and as a marker of innate immunity in postmenopausal women, thus implying how the use of calcifediol is likely to boost vitamin D levels.

An intervention study conducted by Jean et al. (2008) found the use of either 10 or 30 µg calcifediol in haemodialysis patients with a mean age of 67 years was effective and safe. Mean baseline serum 25(OH)D levels were 30 nmol/L, increasing to 126 nmol/l after six months of treatment.

In another study, females aged between 24 and 72 years were supplemented with a monthly dose of 500 µg calcifediol for 4 months (Russo et al., 2011). The authors report that with respect to baseline values, there was a highly significant difference in 25(OH)D levels after the first dose, reached a peak at day 3 and stabilised over the rest of the month. Repeated measurements of blood levels at a variety of times were used in this study to assess the pharmacokinetics of the first dosage and showed that subsequent values continued to be significantly higher than baseline ($p<0.001$). At the end of the study, the authors found that all participants had reached serum levels above 75 nmol/L (Russo et al., 2011).

Participants in most studies of the effects of calcifediol in increasing 25(OH)D concentrations have been adults aged from 50 to 70 years, while there are few references in the literature to investigations of the effects of this supplement in younger

adults and there are none on ethnic minority groups in the UK. This was the motivation behind the present study. In the aforementioned literature, no adverse effects were reported in participants who received calcifediol supplements, whether daily, weekly or monthly, in the range of 10 μg/d to 500 μg/month.

Table 2.4: Supplementation studies (calcifediol) to improve vitamin D status among healthy adults

Reference	Study participants	Mean age	Vitamin D dose	Duration & Study design	Assay	Baseline 25(OH)D Nmol/L mean	Outcome
Barger-Lux et al. (1998)	116 healthy men	28 years	10, 20 and 50 µg/d of calcifediol 25, 250 and 1250 µg/d	4 weeks for calcifediol group and 8 weeks for vitamin D	HPLC	67 for calcifediol 8 for vitamin D	Mean serum 25(OH)D level of calcifediol increased rapidly in different doses and reached 40,76.1 and 206.4 nmol/L Mean serum 25(OH)D level of vitamin D level increased to 28, 146 and 643 nmol/L.
Cavalli et al. (2009)	90 Caucasian females	65-75 years	125 µg/week 250 µg/month 500 µg /month with a combination 500 mg calcium	12 week (RCT)	RIA	50.1 51.4 52.0	Mean serum 25(OH)D level of calcifediol increased to 76, 70 and 77 nmol/L respectively.
Jean et al. (2008)	149 haemodialysis patient (mixed gender)	67 years	10 µg/d serum between (50-75 nmol/), 20 serum between 25-50nmol/L)and30 serum ≤ 25nmol/L	6 months	HPLC/MS/MS	30	Daily 10-30 µg effective to achieve ≥75 nmol/L, without any cases of hypercalcemia calcifediol effective to correct vitamin D deficiency in haemodialysis patients
Russo et a. (2011)	18 females	24-72 years	500 µg/month	4 months	RIA	45	Monthly dose of 500 µg calcifediol increased serum 25(OH)D significantly. Serum 25(OH)D declined after peak but remained over 75nmol/L until the next dose
Bischoff-Ferrari et al. (2012)	20 healthy postmenopausal women	61.5 years	Daily 20, weekly 140 µg vitamin D_3 and daily 20, weekly 140 µg calcifediol	12 weeks (RCT)	HPLC/MS/MS	33	1- Mean 25(OH)D levels increased to 77.5 nmol/L with a slow increase in the vitamin D_3 group. Women on had a 2.8-fold improvement compared with vitamin D_3

Reference	Study participants	Mean age	Vitamin D dose	Duration & Study design	Assay	Baseline 25(OH)D Nmol/L mean	Outcome
							2- There were no cases of hypercalcemia at any time point. 3- Significant benefit on lower extremity function, systolic blood pressure, and innate immune response.
Cashman et al. (2012)	56 heathy males/females	57.2 years	Daily 20 µg vitamin D_3 daily7, 20µg calcifediol and placebo	10 weeks (RDBPC)	ELISA	49.7 D_3 group 42.5 for 7 calcifediol 38.2 for 20 calcifediol	Mean 25(OH)D levels increased rapidly in both groups of calcifediol more than in vitamin D_3
Jetter et al. (2014)	35 healthy females	60 years	Daily 20, weekly 140 µg vitamin D_3 and daily 20, weekly 140 µg calcifediol	15 weeks (RCT)	HPLC/MS/MS	20 D_3= 30 20 calcifediol= 32 140 D_3=40 140 calcifediol= 28	Calcifediol is about 2–3 times more potent in increasing plasma 25(OH)D_3 level than vitamin D3. Plasma 25(OH)D_3 concentrations of ≥75 nmol/l were reached at week 5 while only a few participants reached this cut off at week 15
Shieh et al. (2017)	35 multi-ethnic adults	≥18 years	60 µg/d D_3 20 µg/d Calcifediol	16 weeks (RCT)	CI	40	25(OH)D level of all participants supplemented by calcifediol was increased to ≥ 75nmol/L at week 4 while it was reached 75 nmol/L at the end of study

RCT: Randomized control trial, RDBPC: Randomized double blind placebo control

CI: Chemiluminescence Immunoassay, ELISA: The enzyme-linked immunosorbent assay

RIA: Radioimmunoassay, HPLC: High Performance Liquid Chromatography, HPLC/MS/MS: Liquid chromatography–mass spectrometry

2.7 Summary

This chapter has reviewed the relevant literature on hypovitaminosis D, beginning with the stages of vitamin D metabolism in the human body, from its production through to its biological actions. A great proportion of the review evaluated the prevalence of vitamin D deficiency in the UK, factors exacerbating the problems of vitamin D deficiency, the health outcomes of deficiency and recommended levels. The review also explored supplementation studies conducted on adults using different types of vitamin D for different durations.

Despite the growing literature in the area of vitamin D deficiency, there is still sparse information in the context of EMGs living in Manchester. A few studies suggest that this population needs a stronger focus on addressing the risk factors for vitamin D deficiency and establishing beneficial vitamin D sources to enhance public health outcomes. It is apparent that various studies have established the need for multiple interventions, ranging from dietary to medical, to tackle an apparently growing issue of vitamin D deficiency. In order to increase the efficacy of these interventions, there is a significant need for education among the population on the importance of adopting a healthy diet and lifestyle (De Roos et al., 2012; Farrar et al., 2011).

The next chapter considers EMGs in the UK and the risk factors influencing their vitamin D status.

3. RISK FACTORS INFLUENCING VITAMIN D STATUS OF ETHNIC MINORITY ADULTS LIVING IN NORTHERN ENGLAND

3.1 Introduction

Over the years, a great number of immigrants from Asia and Africa have settled in the UK (Patel et al., 2013). According to the 2011 census, the ethnic minority population of Britain has experienced an increase since 1991; in Manchester, the site of this study, ethnic minorities now make up 33% of the population, as mentioned in Section 2.7.3 (Jivraj and Simpson, 2015). Recently, there has been increasing interest in the vitamin D status of the UK population, especially among ethnic minority groups. Various studies have shown that hypovitaminosis D is a serious issue among these communities throughout the UK due to several factors, including low sun exposure and skin pigmentation, which would affect the vitamin D status of those living in Manchester (Shaw and Pal, 2002; Ford et al., 2006; Farrar et al., 2011). These studies have concentrated on sun exposure behaviour and vitamin D intake in specific ethnic groups. Therefore, this chapter considers risk factors applicable to the different EMGs living in Manchester which may affect their vitamin D status.

3.1.1 Aim

- Investigate differences in the risk factors for vitamin D deficiency between ethnic minority groups.

3.1.2 Objectives

- Estimate vitamin D intake for the main ethnic minority groups living in Manchester from dietary sources, supplement use and sun exposure using a questionnaire.
- Evaluate lifestyle factors and sun exposure behaviours that could affect estimated total intake of vitamin D.

3.1.3 Research hypothesis

Lifestyle factors and vitamin D intake differ among ethnic minority adults living in the UK.

3.2 Methodology

3.2.1 Ethical considerations

Ethical approval was obtained through the Manchester Metropolitan University Research process for all phases of the study. Participants were provided with a participation information sheet (Appendix 1) and signed informed consent was obtained (Appendix 2). Each participant could withdraw at any time from this research, as participation was voluntary. Participants' contact numbers and email addresses were kept in anticipation of their willingness to take part in the next stage of the study in accordance with the Data Protection Act, 1998.

3.2.2 Sample size consideration

Although it has been claimed that a larger sample size gives a narrower confidence interval (Cottrell and McKenzie, 2010), Guthrie (2010) states that a sample size of 384 is sufficient for a research survey in a given population of one million or greater. The current study targets the ethnic minority population of Manchester, which is stated to be 33% of the total population (530,000) of the city (Manchester City Council, 2015).

3.2.3 Study population and recruitment

Study recruitment took place in Manchester, located in the North West of England (53°N) and was performed between September and November 2015. The electronic version of the questionnaire was created using the website Google Docs (docs.google.com). It was then distributed via different forms of social media, including targeting relevant communities on Facebook, Twitter and Instagram. In addition, a paper version was distributed at specific places typically frequented by ethnic minority groups, e.g. towns, neighbourhoods, markets and religious centres.

The eligibility criteria were:

- Resident in Manchester;
- Aged 18 to 50-years;
- From an ethnic minority background (Arab, South Asian, Black African/Caribbean (AfroC) and East Asian).

3.2.4 Study protocol

The design of the observational study is presented in Figure 3.1 as a flowchart:

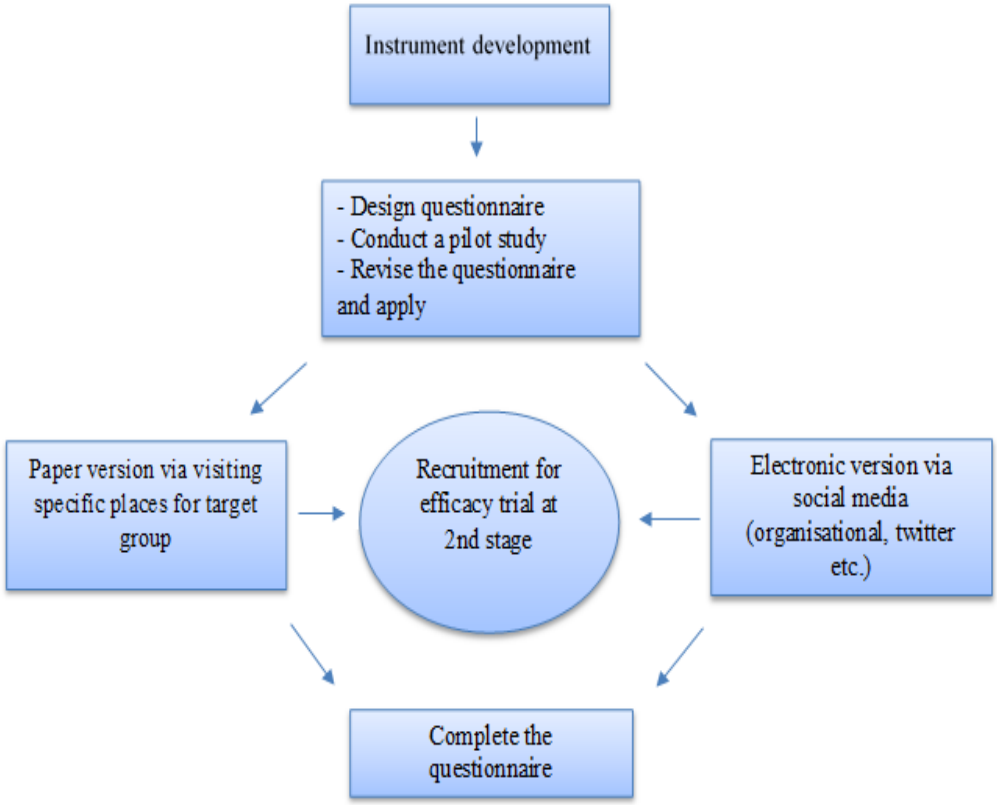

Figure 3.1: Flowchart of observational study

3.2.4.1 Questionnaire design

The questionnaire gathered the following information: age, sex, ethnicity, place of birth, and length of residence in the UK, along with data relating to socioeconomic factors such as income, religion, education level and occupation (Appendix 4). Religion was classified as Muslim, Christian, Sikh, Hindu, Jewish and other. Occupational status was classified as employed, unemployed and student. Generally, the questionnaire was designed to determine vitamin D status by estimating dietary vitamin D intake and to assess other lifestyle and demographic factors hypothesised to affect vitamin D status among ethnic minority groups. The questions were predominantly closed-ended to simplify coding and analysis (Obeidat, 2002). They were piloted among 13 postgraduate students and staff members at Manchester Metropolitan University and the University of Manchester to determine whether the questions were clear and understandable, and whether the response options were appropriate. The questionnaire consisted of three sections: sun exposure assessment, health factors and vitamin D intake.

3.2.4.1.1 Sun exposure assessment

A sun exposure assessment is a self-reported questionnaire used for determining sun exposure behaviour by collecting data about the participants' usual daily routines that lead to sun exposure (Appendix 4). The questions regarding usual sun exposure were based on those from previous studies (Gandini et al., 2005; Hanwell et al., 2010; Cargill et al., 2013). Participants were asked about the time spent outdoors on a typical weekday and on weekends, between 7 am and 5 pm, to estimate the amount of sun exposure. Participants indicated exposure using these frequency options: '<15 mins', '15 mins' '15–30 mins', '30–45 mins' and '45–60 mins'. Subsequent questions were concerned with skin colour and sun-protection behaviour. The description of skin colour was based on the Fitzpatrick Scale, as described in (Section 2.9.2.2) (light, white–fair, medium cream white, olive, brown skin and black skin). It has been noted that the Fitzpatrick Scale has a high correlation with self-reported skin colour ($r = –0.63$, $p < 0.001$), which in turn was found to be highly correlated with the skin-reflection spectrophotometer ($r = –0.70$, $p < 0.001$) (Daniel et al., 2009). Sun-protective behaviour, including the areas of the body exposed to the sun (face, hands, full arms, half arms, full legs and half legs), the use of sunscreen with a specific SPF (Cargill et al., 2013) and the use of a sunbed were also documented (Humayun et al., 2012; Gandini et al., 2005).

3.2.4.1.2 Health factors

In this section, the participants answered questions about their health, including current health conditions that can bring about hypovitaminosis D, a history of hypovitaminosis D, smoking status and alcohol intake. Female participants were also asked to report if they were pregnant or breastfeeding.

3.2.4.1.3 Dietary intake of vitamin D

FFQ is used to assess individuals' habitual diets, detailing which specific foods they usually consume over a specific period (Dehghan et al., 2013; Coulston et al., 2017). This study used modified version of an existing validated FFQ designed to assess vitamin D intake (Taylor et al., 2009). It was composed of questions about consumption of 22 high-vitamin-D-containing food items, including oily fish, dairy and meat, and how often they were consumed over a period of three months on a scale of six levels from never to twice a day. Participants were asked to complete the FFQ themselves by indicating the frequency of food consumption of each food item listed. An open-ended question regarding consumption of particular breakfast cereals was included to capture the vitamin D contribution from what are often fortified products that differ markedly despite being categorised as the same product. The participants were asked to report their brands in order to obtain the exact amount of vitamin D consumed. Additional information was obtained to distinguish consumer type (vegetarian or vegan) and the volume of milk consumed. They were also asked about supplement intake and the brands and dosages of any supplements taken.

3.2.4.1.4 Anthropometric data

Participants were asked to state their body weight in kilograms (kg) and height in centimetres (cm) to facilitate the calculation of BMI. According to the World Health Organisation (2010), BMI is calculated by dividing body weight in kilogrammes by height in metres squared (kg/m^2). The categories of BMI are: <18.5 kg/m^2 (underweight), 18.5–24.9 kg/m^2 (normal weight), 25 –29.9 kg/m^2 (overweight) and ≥30 kg/m^2 (obese).

3.2.5 Data analysis

3.2.5.1 Sun exposure calculation

To calculate time outdoors, data were converted into time values by calculating the average figure for each of the four categories (<15 min, 15–30 min, 30–45 min and 45–60min), yielding respective values of 7.5, 22.5, 37.5 and 52.5 minutes. Hence, the total time spent outdoors for each participant in minutes per day can be estimated by adding up the daily time of sun exposure during weekdays and weekends (Glanz et al., 2010). As Farrar et al. (2011) note, UV radiation levels vary from hour to hour in a single day and the highest UV level for vitamin D production occurs at midday. Thus, the exposure time to sunlight in a single day was divided into two periods: off-peak time represented the times of when vitamin D production is low, whereas peak time was when vitamin D production is at its highest. To calculate these figures, each time group was created by adding up the time of exposure to sunlight reported by participants:

Equation 1:

$$\textbf{Peak time} = \textbf{reported time of } (\textbf{10 am} - \textbf{11 am}) + (\textbf{11am} - \textbf{12 pm}) + (\textbf{12 pm} - \textbf{1 pm}) + (\textbf{1pm} - \textbf{2 pm})$$

Equation 2:

$$\textbf{Off} - \textbf{peak time} = \textbf{reported time of } (\textbf{7 am} - \textbf{8 am}) + (\textbf{8} - \textbf{9 am}) + (\textbf{9am} - \textbf{10am}) + (\textbf{2pm} - \textbf{3pm}) + (\textbf{3pm} - \textbf{4pm})$$

A method established by Knaysi et al. (1968) called the 'rule of nine' was used to estimate the Body Surface Area (BSA) (Table 3.1). This formula is frequently used in adults suffering burns to estimate the proportion of body burned; the major body segments are divided into multiples of 9%, except for the genital area, which is allotted 1%. In this study, four categories were adopted to estimate usual skin exposure: head, face, legs and hands. Some changes have thus been made to suit the nature of the study (Table 3.2)

Table 3.1: Original 'Rule of Nine'

Body segment (%)						
Arms	legs	Anterior trunk	Posterior trunk	Head	Perineum	Total
0.18	0.36	0.18	0.18	0.09	0.01	1

Source: Barger-Lux and Heaney (2002).

Table 3.2: Adapted 'Rule of Nine'

Body segment (%)						
Arms			**Legs**		**Head**	**Face**
Hands	Half arms	Full arms	Half legs	Full legs		
0.04	0.09	0.18	0.18	0.36	0.09	0.05

Adapted from Barger-Lux and Heaney (2002), with changes have made in the categories to suit the current study

The sun exposure index is an equation generated by Barger-Lux and Heaney (2002) that combines exposure time and the total area of skin exposure in that time in one measure, which can be used to compare participants. It is calculated as shown in Equation 3.

Equation 3:

$$\textbf{Sun index} = \textbf{hours of sun exposure per week} \times \textbf{fraction of BSA exposed to sunlight}$$

3.2.5.2 Vitamin D intake calculation

The vitamin D contents of various foods was obtained from McCance and Widdowson's The Composition of Foods according to the Food Standards Agency (2014) Table 3.3. The proportional weight in grams assigned to each frequency (PWF) was as follows: never = 0, one to two times a month = 0.067, once a week = 0.15, two to three times a week = 0.43, once a day = 1 and twice a day = 2 (Ireland et al., 1994; Willett, 2013).

The average dietary intake of vitamin D was calculated as shown in Equation 4:

Equation 4:

$$\textbf{Vitamin D intake} = \textbf{PWF} \times \textbf{nutrient content}$$

The total vitamin D content of all foods was added together to obtain each participant's total vitamin D intake (Food Standards Agency, 2014; Willett, 2013).

The dietary recommendation for vitamin D is 5 µg/day (WHO and FAO, 2004). Therefore, vitamin D intake was classified by three cut-off points: less than 5 µg represented low

intake, 5–10 µg a medium intake, and over 10 µg a high intake (Zagag et al., 2011). To calculate the proportion of vitamin D contributed by each food group, the 22 foods were categorised into five groups according to the similarity of their nutrient composition (Cotton et al., 2004; Margetts and Nelson, 1997) then the percentage contribution was calculated according to equation 5.

Equation 5:

$$\text{Contribution of vitamin D proportion} = \sum \text{vitamin D contributed by each food group} / \text{total vitamin D from all food groups}$$

Table 3.3: Vitamin D content of food items

Food	gram	Vitamin D (µg)
Fresh mackerel (cooked)	80	8.0
Fresh tuna (cooked)	85	4.2
Fresh sardines (cooked)	85	10.5
Fresh salmon (cooked)	122	11
Canned tuna	45	1.6
Canned sardine	109	10
Canned salmon	109	9
Herring	85	13
Beef	85	0.1
Pork and Ham	85	0.8
Beef liver	85	0.3
Pork liver	85	0.3
Egg	60	1.8
Cream cheese	28	0.2
Hard cheese	28	0.2
Milk	207	2.2
Semi-skim Milk	207	0.15
Margarine	12	1.2
Butter	12	0.1
Sun dried mushroom	28	0.7
Cod liver oil	12	25.0
Breakfast cereal	60	2.2

Source: Food Standards Agency (2014)

3.2.6 Statistical analysis

Data analysis was performed using SPSS software, version 21 (SPSS Inc., Chicago, IL, USA). The data were transferred from the questionnaires to an Excel spreadsheet, then from Excel to SPSS for analysis. Prior to commencement of statistical analysis, the data were checked for inputting errors and histograms and box plots were used to identify outliers. Wherever an outlier was detected, the value was excluded from the analysis. By using

descriptive statistics, the results of categorical variables were given as percentages and frequencies. As for the continuous variables, normally distributed data were reported as mean values accompanied by the standard deviation (SD), with a median value given for skewed data (Kirkwood and Sterne, 2010). Normality of data was checked statistically by using frequency histograms, and appropriate transformations such as square root were used to normalise the data. A value of $p<0.05$ was considered statistically significant.

To explore the difference between ethnicity and other continuous variables, including BMI and vitamin D intake, the one-way ANOVA test was used, along with a post hoc test when significant differences were detected (Kirkwood and Sterne, 2010). However, the data for usual sun exposure was found to be skewed and were therefore analysed using the Kruskal–Wallis H test. For nominal data, the associations between each categorical variable were explored using the chi-square statistic. Additionally, a multiple linear regression model was used after checking its assumptions (Draper and Smith, 2014) to explore which factors were considered as predictors for vitamin D intake from the following independent variables: ethnic origin, income, sex, age and education. In this case, some dummy variables were created from the nominal predictor to fit this test (McDonald, 2009).

3.3 Results

3.3.1 Recruitment of participants

A total of 400 questionnaires were distributed and 268 individuals from different ethnic minorities agreed to participate in the study, then completed and returned the questionnaire, either via electronic survey or on paper. Of these responses, 15 were excluded because nine respondents were aged >50 years and six others resided outside Manchester. Participants included Arabs (Iraqi, Egyptian, Lebanese, Jordanian, Syrian, Palestinian, Yemeni, Saudi Arabian, Kuwaiti and Omani), Africans (Nigerian, Somali, Kenyan, Tanzanian, Zimbabwean and South African), Black Caribbeans (Jamaican), South Asians (Indian, Pakistani and Bangladeshi), East Asians (Chinese and Japanese) and Southeast Asians (Malaysian, Thai and Indonesian). Figure 3.2 is a flowchart of participants.

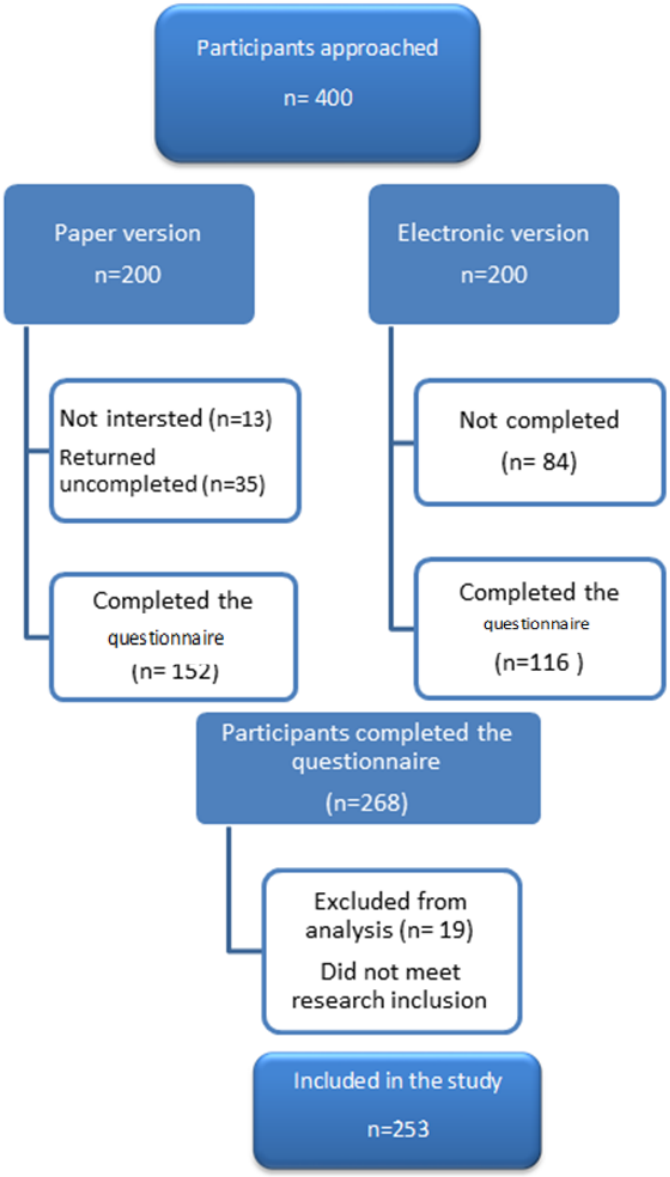

Figure 3.2: Flowchart of study participants

3.3.2 Participants' demographic characteristics

A total of 253 respondents completed either the electronic or the paper questionnaire. Slightly more than half of the sample (53.4%) comprised females. The distribution by sex, age, ethnicity, income status, education level, occupation status and location is presented in Table 3.4. The largest ethnic group was the Arabs. Three-quarters of respondents were Muslims and 16% Christians; the remaining participants were classified as 'other' because there were very few responses in any other religious category. Nearly half of the participants had lived in the UK for more than five years and

only 14% had done so for less than a year. The ages of respondents ranged from 18 to 49 years, with nearly half aged under thirty, and almost as many between 30 and 39 years. A total of 28% of participants reported an income of less than £5,200 annually, while a few reported an income of over £26,000. The majority had received a higher education, over half were employed and only 11% were unemployed.

Table 3.4: Demographic characteristics and health factors for study sample (n = 253)

Variable	*n* (%) or mean ± SD
Number	
Males	118 (47.6)
Females	135 (53.4)
Age	
18–29	112 (44.3)
30–39	101 (39.9)
40–49	40 (15.8)
Anthropometric data	
Weight	71.58 ± 14.95
Height	165.86 ± 10.09
BMI	25.89 ± 4.25
Country of Birth	
Gulf countries[$]	71 (28.1)
The Levant[^]	11 (4.3)
North Africa	23 (9.1)
Other Afro-countries	61 (24.1)
South Asia	34 (12.4)
East Asia	18 (7.1)
Ethnicity	
Arab	105 (41.5)
Afro/Caribbean[**]	66 (26.1)
South Asian[***]	61 (24.1)
East Asian [****]	21 (8.3)
Religion	
Muslim	189 (74.7)
Christian	41 (16.2)
Other[^^]	23 (9.0)
Length of resident	
<1 year	37 (14.3)
1–5 years	95 (36.7)
>5 years	127 (49.0)
Education level	
College/diploma	37 (14.6)
Bachelors	118 (46.6)
Postgraduates	98 (38.7)
Occupation	
Employed	131 (51.8)
Unemployed	28 (11.1)

Variable	*n* (%) or mean ± SD
Student	94 (37.2)
Occupation description	
Mainly indoors	158 (62.5)
Half indoor/outdoors	75 (29.6)
Mainly outdoors	20 (7.9)
Income (£)	
>5,200	73 (28.9)
5,200 to 10,399	46 (18.2)
10,400 to 25,999	71 (28.1)
≥26,000	63 (24.9)
Smoking status	
No	236 (93.3)
Current	17 (6.7)
Every day	13 (5.1)
Occasionally	4 (1.6)
Alcohol intake	
No	203 (80.2)
Yes	50 (19.8)
3 days and below	40 (80.0)
More than 3 days	10 (20.0)
Reported health conditions£	29 (11.5)
History of vitamin D deficiency	68 (26.9)
Supplement intake^	58 (22.9)
Type of consumer (vegetarians)	
Yes	14 (5.5)
No	239 (94.5)
Consumed fortified food	
Yes	61 (24.1)
No	50 (19.8)
Sometimes	92 (36.4)
Don't know	50 (19.8)

^From Lebanon, Jordan, Syria and Palestine. **Nigerian, Somalian, Kenyan, Tanzanian, Zimbabwean, South African and Jamaican.

***From India, Pakistan and Bangladesh. ****Chinese, Malaysian, Thai and Japanese. $From Saudi Arabia, Kuwait, Qatar, Oman and the United Arab Emirates. ^^Sikh, Buddhist and no religion.

£Chronic diseases (diabetes mellitus, heart disease, asthma, dermatomyositis and kidney disease) or skeletal diseases (osteoporosis and ankylosing spondylitis).

^Multivitamins.

Figure 3.3 shows that fewer than half of participants fell into the range of healthy weight, while a third were overweight and 21% were obese (BMI≥30 kg/m^2).

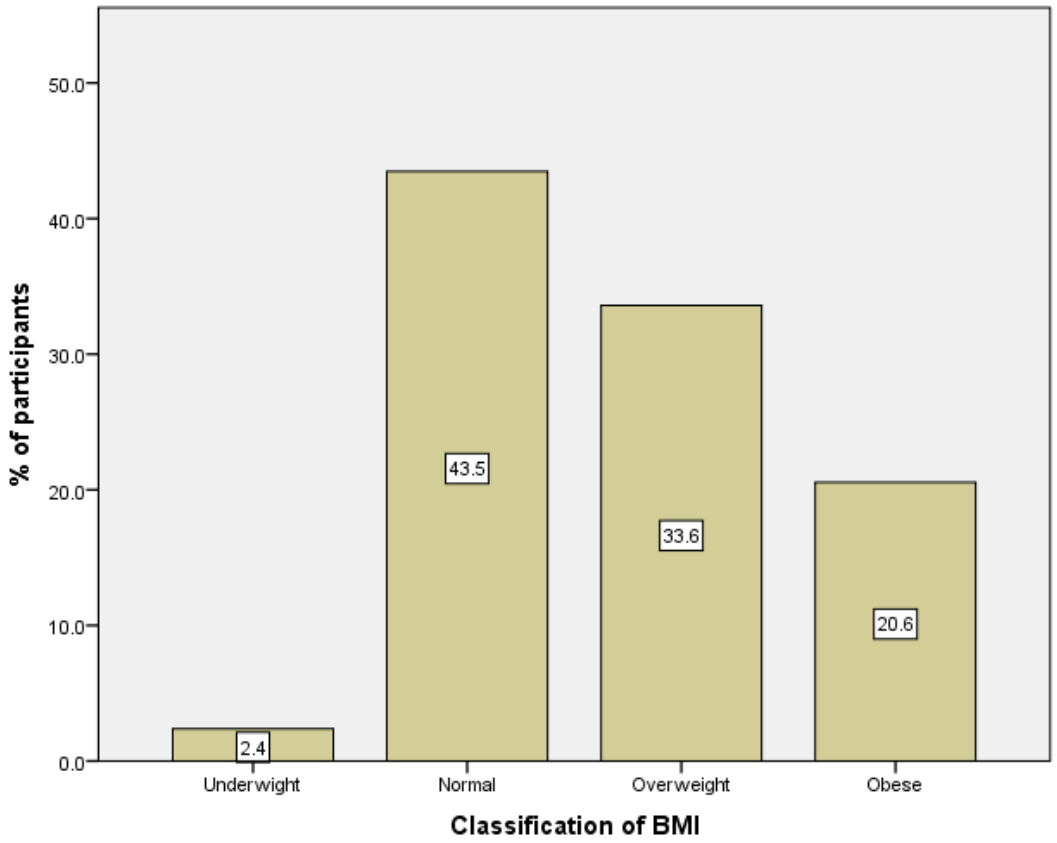

Figure 3.3: Percentage of participants by BMI (n = 253)

Table 3.4 shows that very few participants were smokers and that most were non-alcohol drinkers; fewer than 20% reported alcohol intake, with one in five of these drinking on more than three per week. Only a few participants reported suffering from health conditions, but over a quarter of the sample had experienced vitamin D deficiency. Supplements were taken by a little less than a quarter of participants and a similar proportion reported regular consumption of fortified food. Only 14 of the total sample reported themselves as vegetarians.

3.3.3 The association of variables with sex

3.3.3.1 Socioeconomic and health factors

Table 3.5 illustrates how education level, occupation, income, and age groups significantly differed by sex (p<0.05). Over half of females held a bachelor degree, while 45% of males held a postgraduate degree. Table 3.5 demonstrates that there was a significant association (p=0.04) between smoking status and sex, with more males than females being smoker, but no significant difference between the sexes on intake of either alcohol or supplements. There were also significant differences (p<0.001) between the sexes in reported health conditions and in the incidence of vitamin D deficiency, with more females being affected by hypovitaminosis D and reporting health conditions.

Table 3.5: Socioeconomic and health factors according to sex

Variable	Male *n* = 118	Female *n* = 135	*p* value[^]
	n (%)		
Age			
18–29	38 (32.2)	74 (54.8)	
30–39	52 (44.1)	49 (36.3)	<0.001
40–49	28 (23.7)	12 (8.9)	
Education level			
College/diploma	19 (16.1)	18 (13.6)	
Bachelor	45 (38.1)	73 (54.1)	0.03
Postgraduate	53 (45.8)	44 (36.6)	
Income status (£)			
<5,200	24 (20.3)	48 (35.6)	
5,200–10,399	28 (23.7)	21 (15.6)	0.02
10,400–25,999	32 (27.1)	39 (28.9)	
≥26,000	34 (28.8)	27 (20.0)	
Occupation			
Employed	74 (62.7)	56 (41.5)	
Student	36 (30.5)	20 (14.8)	<0.001
Unemployed	8 (6.8)	59 (43.7)	
Smoking status			
Current	12 (10.2)	5 (3.7)	0.04
Every day	11 (91.7)	2 (40.0)	
Occasionally	1 (8.3)	3 (60.0)	
Alcohol intake			
Yes	26 (22.0)	24 (17.8)	0.43
3 days and below	23 (88.5)	17 (70.8)	
More than 3 days	3 (11.5)	7 (29.2)	
Health conditions[£]	6 (5.1)	23 (17.0)	<0.001
History of vitamin D deficiency	23 (19.5)	45 (33.3)	0.01
Supplement intake[^]	22 (18.6)	36 (26.7)	0.13

[^]Chi-square test. [£]Chronic diseases (diabetes, heart disease, asthma, dermatomyositis and kidney disease) or skeletal diseases (osteoporosis and ankylosing spondylitis). [^]Multivitamins.

BMI was found to significantly differ by sex (p<0.001), with male participants having a higher BMI than females. As shown in Figure 3.4, the BMI of approximately half the females was in the healthy category, whilst many males were obese than females, although the proportion of those classified as overweight differed very little between the sexes.

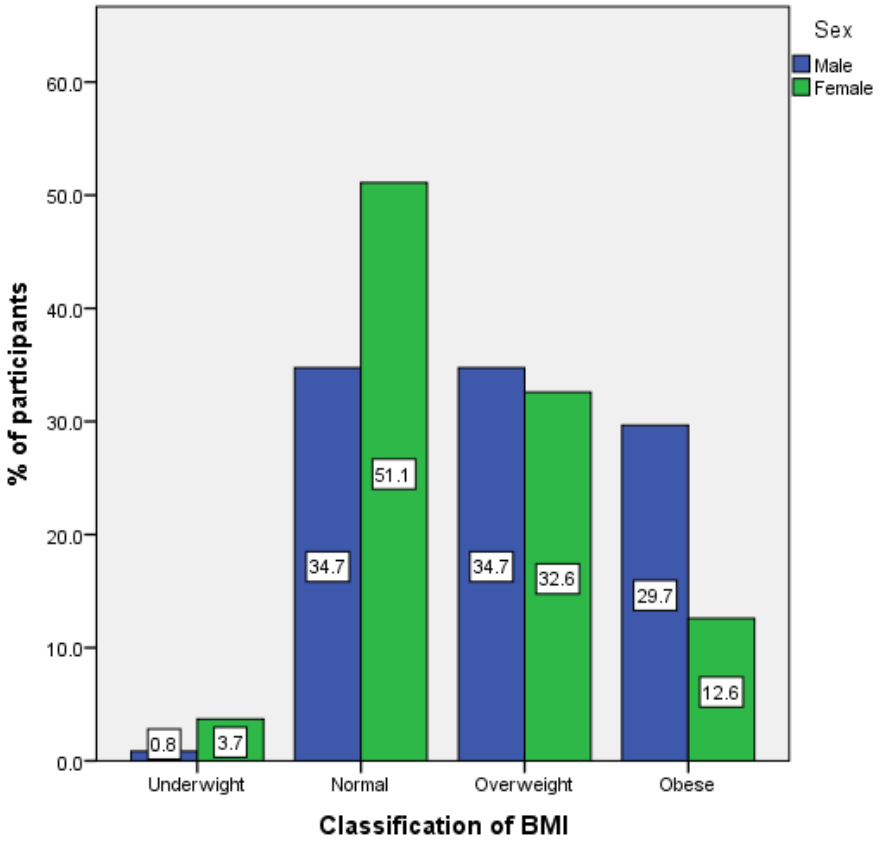

Figure 3.4: Distribution of participants by body mass index (BMI) and sex (n=253)

3.3.4 The association of variables with age

3.3.4.1 Health and lifestyle factors

Table 3.6 demonstrates that only alcohol intake, supplement intake and BMI were significantly (p<0.001) influenced by age. The highest percentage of alcohol drinkers was among participants aged 40–49 years, while those aged 30–39 years were the greatest users of supplements. Kruskal-Wallis H test showed that there was a significant difference in BMI status between the different age groups (p<0.001), with a mean rank BMI of 103.30 for those aged 18–29 years, 138.50 for the 30–39 group and 164.34 for the 40–49 age group.

Table 3.6: Health and lifestyle factors according to age

Variable	18–29 years *n* = 112	30–39 years *n* = 101	40–49 years *n* = 40	*p* value[$]
	n (%)			
Smoking status	6 (5.4)	9 (8.9)	2 (5.0)	0.46
Alcohol intake	17 (15.2)	18 (17.2)	15 (37.5)	<0.001
Health conditions[*]	8 (7.1)	16 (15.8)	5 (12.5)	0.16
History of vitamin D deficiency	28 (25.0)	33 (32.7)	7 (17.5)	0.16
Supplement intake[^]	15 (13.4)	29 (28.7)	14 (35.0)	<0.001
BMI (kg/m^2)[**]	23.3 (5.5)	26.4 (5.0)	29.4 (7.8)	<0.001***

[*]Chronic diseases (diabetes, heart disease, asthma, dermatomyositis and kidney disease) or skeletal diseases (osteoporosis and ankylosing spondylitis).
[^]Multivitamins.
[$]Chi-square test
*** Kruskal-Wallis H test
[**]Median (interquartile range).

3.3.5 Distribution of characteristics by ethnicity

3.3.5.1 Demographic characteristics

Table 3.7 shows that there were more female than male respondents in each ethnic group, except for the Arab grouping. More than half the SA group were in the age range 18–29 years, while an equal percentage of Arabs fell in the 18–29 and 30–39 age ranges. The data also show that approximately half of the Arabs held postgraduate degrees, a high proportion compared with other ethnicities. The highest percentage of people who had not attended university was among AfroC respondents. Education level, occupation, sex and religion differed significantly across ethnic groups (p<0.001), but no statistical differences were found between age groups, income status or occupation location across ethnicities (p = 0.10, 0.44 and 0.19, respectively).

Table 3.7: Demographic characteristics of participants according to ethnicity

Variable	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbean *n* = 66	East Asian *n* = 21	*p* value[$]
Sex					
Male	61 (58.1)	21(34.4)	31 (47.0)	5 (23.8)	
Female	44 (41.9)	40 (65.6)	35 (53.0)	16 (76.2)	<0.001
Age (year)					
18–29	45 (42.9)	37 (60.7)	21 (31.8)	9 (42.9)	
30–39	45 (42.9)	16 (26.2)	34 (42.9)	6 (42.9)	0.10
40–49	15 (14.3)	8 (13.1)	11 (42.9)	6 (42.9)	
Religion					
Muslim	105 (100.0)	49 (80.3)	28 (42.4)	7 (33.3)	
Christian	0 (0)	2 (3.3)	37 (56.2)	2 (4.9)	<0.001
Other*	0 (0)	10 (16.4)	1 (1.5)	12 (57.1)	
Education level					
College/diploma	14 (13.3)	4 (6.6)	15 (22.7)	4 (19.0)	
Bachelor	38 (36.2)	40 (65.6)	29 (43.9)	11 (52.4)	<0.001
Postgraduate	53 (50.5)	17 (27.9)	22 (33.3)	6 (28.6)	
Income status (£)					
<5,200	29 (27.0)	23 (37.7)	12 (18.2)	8 (38.1)	
5,200–10,399	20 (19.0)	7 (11.5)	17 (25.8)	5 (23.8)	0.44
10,400–25,999	27 (25.7)	17 (27.9)	22 (33.3)	5 (23.8)	
≥26,000	29 (27.6)	14 (23.0)	15 (22.7)	3 (14.3)	
Occupation					
Employed	50 (47.6)	29 (47.5)	44 (67.7)	7 (33.3)	
Unemployed	9 (8.6)	11 (18.0)	3 (4.6)	5 (23.8)	<0.001
Student	46 (43.8)	21 (34.4)	18 (27.7)	9 (42.9)	
Occupation location					
Mainly indoors	64 (61.0)	41 (67.2)	38 (58.5)	15 (71.4)	
Half indoor/outdoor	36 (34.3)	16 (26.2)	17 (26.2)	6 (28.6)	0.19
Mainly outdoors	5 (4.8)	4 (6.6)	10 (15.4)	0 (0.0)	

[$]Chi-square test to examine the association between ethnicity and socioeconomic factors, * ^^Sikh, Buddhist and no religion. n (%)

3.3.5.2 Health and lifestyle factors

Table 3.8 illustrates how smoking status, alcohol intake and history of vitamin D deficiency differed significantly (p<0.001) across the ethnic groups studied. The Arab group had the highest percentage of smokers, whilst the highest percentage alcohol intake was reported in the AfroC group. By contrast, no significant differences were found between ethnic groups in reported health conditions or supplement intakes.

Table 3.8: Health and lifestyle factors of respondents according to ethnicity

Variable	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbean *n* = 66	East Asian *n* = 21	*p* value[$]
	n (%)				
BMI (kg/m$^{2)}$^^	26.9 (6.62)	23.7 (5.03)	25.7 (5.58)	24.4 (6.09)	0.02
Smoking status	14 (13.3)	2 (3.3)	1 (1.5)	0 (0)	<0.001
Alcohol intake	10 (9.5)	2 (3.3)	30 (45.5)	8 (38.1)	<0.001
History of vitamin D deficiency	35 (33.3)	24 (39.3)	7 (10.6)	2 (9.5)	<0.001
Health conditions* **	6 (11.4)	2 (8.2)	6 (9.1)	6 (28.6)	0.10
Supplement intake^	21 (20.0)	14 (23.0)	19 (28.8)	4 (19.0)	0.58

^^Median (interquartile range) and Kruskal–Wallis H Test was used.

***Chronic diseases (diabetes, heart disease, asthma, dermatomyositis and kidney disease) or skeletal diseases (osteoporosis and ankylosing spondylitis).

^Multivitamins.

[$]Chi-square test.

The Kruskal-Wallis H test showed that there was a statistically significant difference between ethnic groups in BMI (p=0.02). Post hoc comparison demonstrated that this difference was only between the Arab and SA groups (p<0.001): the median BMI of the Arabs, 141.28, was higher than among SA participants, 109.14.

3.3.6 Sun exposure assessment

The distribution of skin colour according to ethnicity is shown in Figure 3.5. The majority of East Asians had medium skin, while almost the whole AfroC group had black skin (VI).

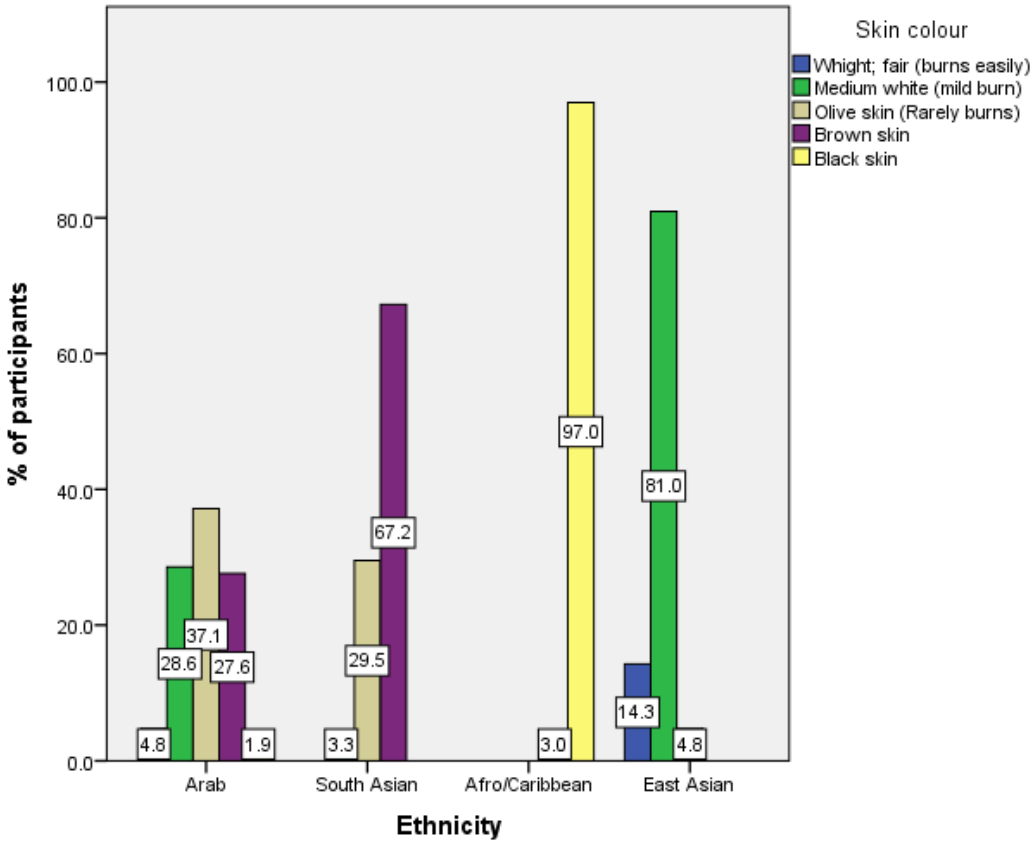

Figure 3.5: Percentage of participants by skin colour and ethnicity (n=253)

Average time spent outdoors ranged from 30 to 420 minutes per day for the month prior to completing the questionnaire. The mean (SD) sunlight exposure of the whole sample was 159 (82.8) minutes per day.

Figure 3.6 shows that there was a significant difference (p<0.001) between time spent outdoors and different ethnicities. The post hoc statistics indicate that the time spent outdoors was lower amongst Arabs than the SA (p=0.03), AfroC (p=0.009) and EA (p<0.001) groups.

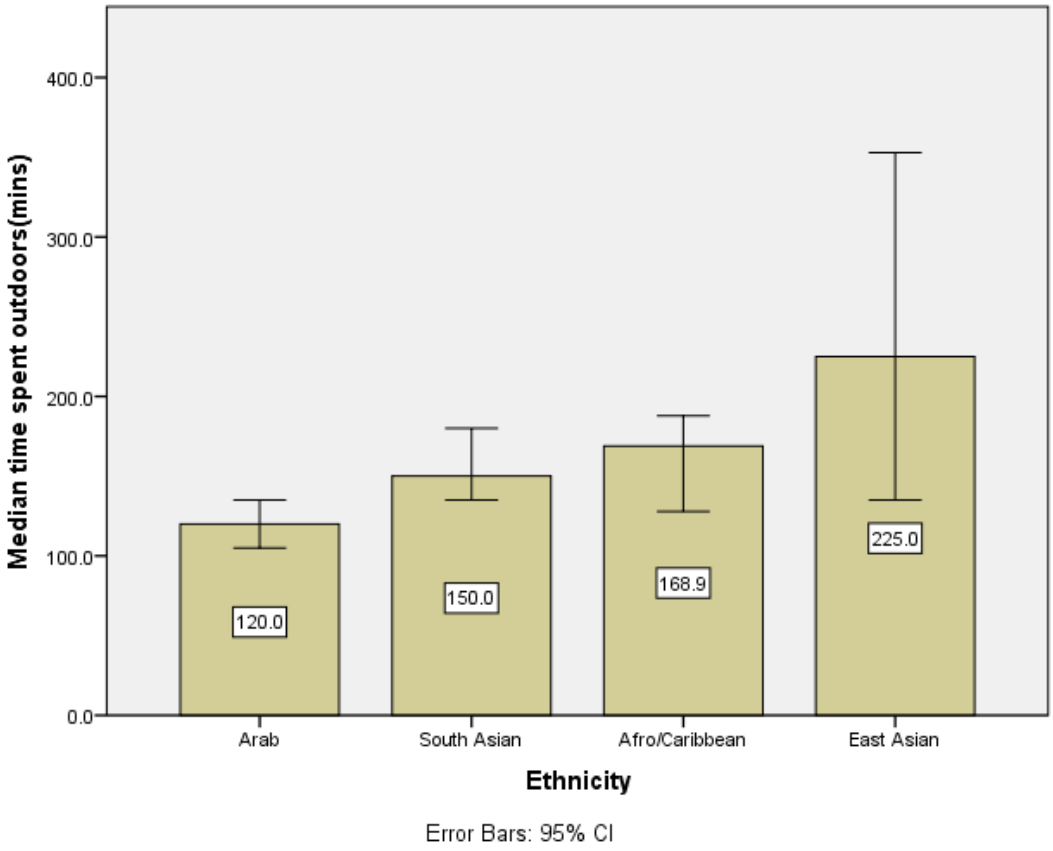

Figure 3.6: Distribution of median time spent outdoors by ethnicity. Error bars represent 95% confidant interval

Figure 3.7, shows that the group with the largest area of skin exposure was EA, followed by AfroC, while a high percentage of Arabs exposed only face and hands ($p<0.001$).

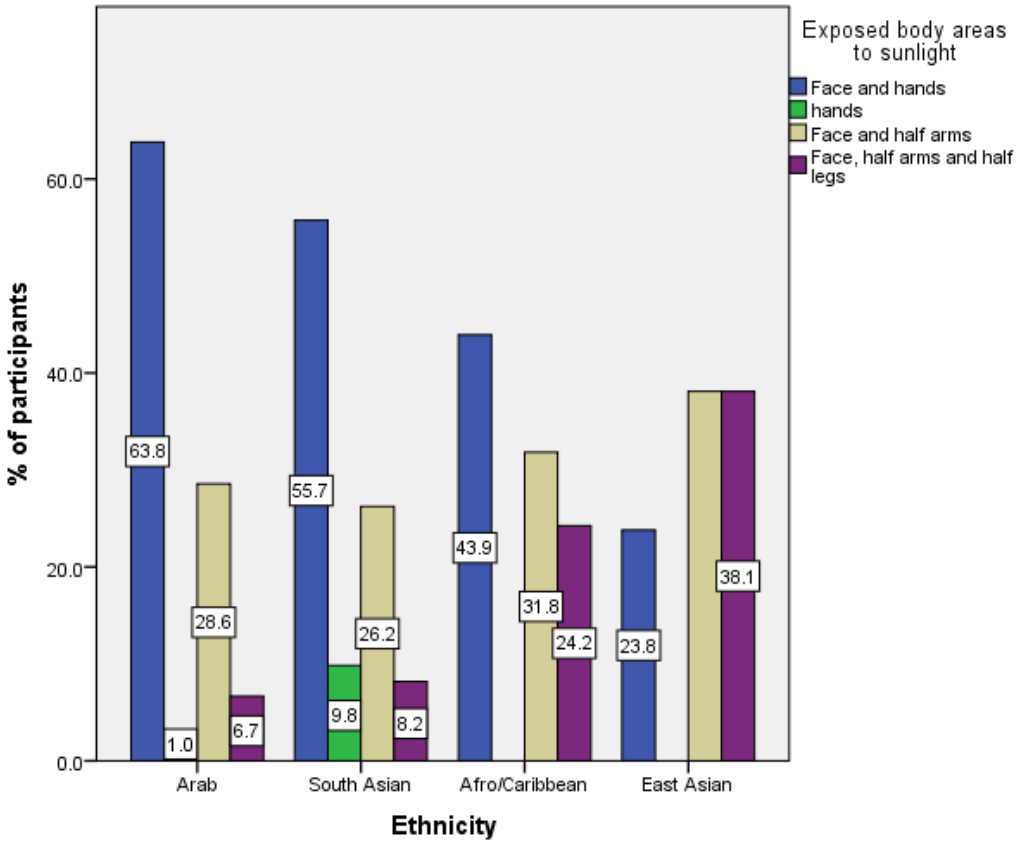

Figure 3.7: Percentage of participants by body area exposed to sunlight and ethnicity (n=253)

To analyse the usual sun exposure relating to vitamin D production, the time was divided into two periods: peak time and off-peak time (See section 3.2.5.1). The various factors that generally affect the amount of vitamin D produced from sun exposure include area exposed, sunscreen used and time of day (peak/off-peak), all of which were associated with differences in vitamin D production among the study sample (Table 3.9).

Table 3.9: Comparison of usual sun exposure among ethnicities

Sun exposure	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbean *n* = 66	East Asian[a] *n* = 21	Total sample *n* = 253	*p* value[3]
	Median (Interquartile range)					
Sun exposure during peak time (hours per day)	1. 0 (0.75)	1.2 (0.25)	1.4 (1.16)	2.0 (1.31)	1.25 (1.13)	<0.001
Sun exposure during off peak time (hours per day)	0.8 (0.75)	1.2 (1.0)	1.2 (0.74)	1.3 (2.4)	1.0 (0.81)	<0.001
Fraction of average peak time of BSA	0.10 (0.11)	0.11 (0.16)	0.15 (0.15)	0.24 (0.13)	0.13 (0.14)	<0.001
Fraction of average off peak BSA	0.9 (0.08)	0.14 (0.16)	0.17 (0.16)	0.35 (0.44)	0.12 (0.12)	<0.001
The total sun index (hours/week)^	0.12 (0.22)	0.19 (0.39)	0.27 (0.56)	0.77 (0.89)	0.20 (0.40)	<0.001
Using sunscreen (%)						
Yes	26 (24.8)	16 (26.2)	12 (18.3)	5 (23.8)	59 (23.3)	0.70**
No	79 (75.2)	45 (73.8)	54 (81.8)	16 (76.2)	194 (76.7)	

[a]Malaysian, Chinese, Thai, Japanese, and Indonesian.

^The total sun index was calculated by multiplying the average of sun exposure per week by a fraction of BSA exposed to sunlight.

[3]Kruksal–Wallis H test. **Chi-square test. BSA: body surface area.

Ethnic differences were found in sun exposure time (p≤0.001). The post hoc statistics indicate that peak time sun exposure was higher among EAs than Arabs, while off-peak exposure was higher in the SA than Arab populations. The post hoc tests also demonstrate that BSA, both peak and off-peak, was higher in EAs than in Arabs, while the total sun index was higher among EAs than the other groups. Table 3.9 also demonstrates that sunscreen use did not differ significantly across ethnicities (*p*=0.70).

3.3.7 Dietary intake of vitamin D

The information in Table 3.10 to Table 3.13 and Figure 3.8 to Figure 3.11 and Figure 3.10 were obtained from the FFQ. These show the differences between ethnicities in the frequency of the food items consumed, beginning in Table 3.10 with fresh fish.

Table 3.10: Frequency of consumption of fresh fish according to ethnicity

Food	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbean *n* = 66	East Asian *n* = 21	*p* value*
	n (%)				
Fresh mackerel					
Never	98 (93.3)	46 (75.4)	55 (83.3)	16 (76.2)	
1–3 times a month	6 (5.7)	15 (24.6)	7 (10.6)	3 (14.3)	<0.001
Once or more a week$	1 (1.0)	0 (0.0)	4 (6.0)	2 (9.5)	
Fresh tuna					
Never	67 (63.8)	35 (57.4)	41 (62.1)	15 (71.4)	
1–3 times a month	27 (25.7)	19 (31.1)	19 (28.8)	2 (29.5)	0.50
Once or more a week$	11 (10.5)	7 (11.4)	6 (9.0)	4 (19.1)	
Fresh sardine					
Never	66 (62.9)	41 (67.2)	35 (53.0)	11 (52.4)	
1–3 times a month	31 (29.5)	13 (21.3)	23 (34.8)	10 (47.6)	0.18
Once or more a week$	8 (7.7)	7 (11.5)	8 (12.1)	0 (0.0)	
Fresh salmon					
Never	85 (81.0)	46 (75.4)	45 (68.2)	16 (76.2)	
1–3 times a month	19 (18.1)	12 (19.7)	18 (27.3)	4 (19.0)	0.36
Once or more a week$	1 (10.0)	3 (4.9)	3 (4.5)	1 (4.8)	

$Several categories were collapsed into 'once or more a week' because there were few responses.
*Chi square test.

Table 3.10 shows that fresh mackerel was not a preferred food for most of the participants, with the highest percentage being recorded by SAs, who consumed it one

to three times monthly (p<0.001). For the consumption of fresh tuna, sardine and salmon, there were no significant differences across ethnicities (p>0.05).

Table 3.11: Frequency of consumption of canned fish according to ethnicity

Food	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbean *n* = 66	East Asian *n* = 21	*p* value*
	n (%)				
Canned tuna					
Never	32 (30.5)	13 (21.3)	30 (45.5)	8 (38.1)	
1–3 times a month	35 (33.3)	32 (52.5)	16 (24.2)	5 (23.8)	<0.001
2-3 times a week	22 (10.5)	13 (21.3)	10 (15.2)	3 (14.3)	
Once a day	16 (15.3)	3 (4.9)	10 (14.1)	5 (23.8)	
Canned sardine					
Never	69 (65.7)	40 (65.6)	53 (53.0)	15 (71.4)	
1–3 times a month	26 (24.8)	12 (19.7)	8 (12.1)	4 (19.0)	0.36
Once or more a week$	10 (20.7)	9 (14.7)	5 (7.6)	2 (9.5)	
Canned salmon					
Never	82 (81.0)	41 (75.4)	60 (90.9)	15 (71.4)	
1–3 times a month	18 (18.1)	13 (21.3)	4 (6.1)	5 (23.8)	0.36
Once or more a week$	5 (4.8)	7 (11.5)	2 (3.0)	1 (4.8)	
Herring					
Never	82 (78.1)	51 (83.6)	63 (95.5)	17 (81.0)	
1–3 times a month	21 (20.0)	7 (11.5)	2 (3.0)	2 (9.5)	<0.001
Once or more a week$	2 (1.9)	3 (4.9)	2 (1.5)	2 (9.5)	

$Several categories were collapsed into 'once or more a week' because there were few responses.
*Chi square test.

Table 3.11 shows the group consuming the highest amount of canned tuna (2–3 times a week) was SA, while the Arabs consumed it once or more a month (p<0.001). However, most participants from all groups never consumed canned sardine (p=0.36). Most participants did not consume canned salmon either, but amongst those who did, SAs were the most prevalent and tended to consume it weekly. The consumption of herring was significantly (p<0.001) different across ethnicities, with Arabs consuming it once or more a month and more often overall than other ethnic groups.

Table 3.12: Frequency of consumption of meat according to ethnicity

Food	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbean *n* = 66	East Asian *n* = 21	*p* value*
	n (%)				
Beef					
Never	22 (21.0)	19 (31.1)	14 (21.2)	8 (38.1)	
1–3 times a month	26 (24.8)	13 (21.3)	16 (24.2)	2 (9.5)	<0.001
Once or more a week[$]	57 (54.3)	29 (47.5)	36 (54.5)	11 (52.4)	
Beef liver					
Never	80(76.2)	47 (77.0)	47 (71.2)	20 (95.2)	
1–3 times a month	17(16.2)	9 (14.8)	10 (15.2)	1 (4.8)	0.42
Once or more a week[$]	8 (7.6)	5 (8.2)	9 (13.6)	0 (0.0)	
Pork/ham					
Never	105 (100.0)	50 (82.0)	41 (62.1)	12 (52.4)	
1–3 times a month	0 (0.0)	2 (3.3)	12 (18.2)	1 (4.8)	<0.001
Once or more a week[$]	0 (0.0)	9 (14 .8)	13 (19.7)	8 (38.1)	
Pork liver					
Never	105 (100.0)	53 (86.9)	60 (90.9)	20 (95.2)	
1–3 times a month	0 (0.0)	6 (9.8)	4 (6.1)	1 (4.8)	<0.001
Once or more a week[$]	0 (0.0)	2 (3.3)	2 (3.0)	0 (0.0)	

[$]Several categories were collapsed into 'once or more a week', as they contained few responses.
*Chi square test.

Table 3.12 shows Arabs and AfroCs having the highest consumption of beef, whilst 38.1% of EA never ate it ($p < 0.001$). The highest recorded consumption of pork/ham was by EAs, while Arabs did not consume pork or its derivatives at all ($p<0.001$).

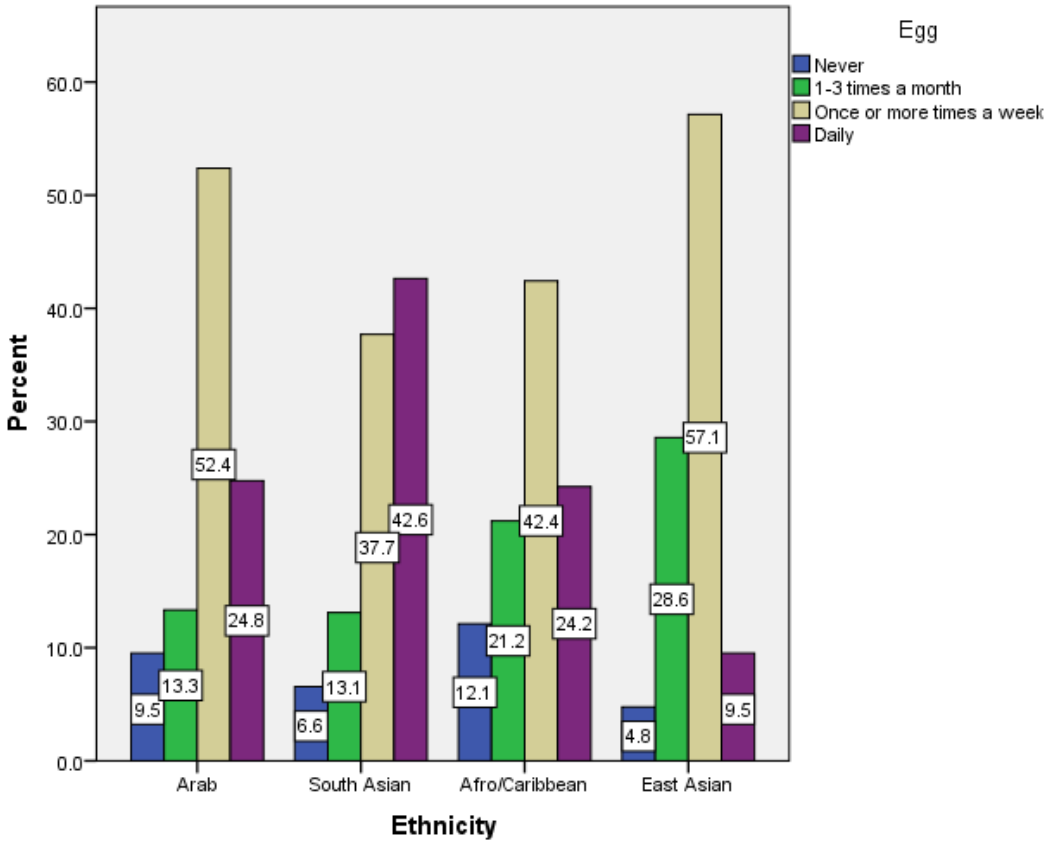

Figure 3.8: Frequency of consumption of whole egg according to ethnicity (n=253)

Figure 3.8 shows that there was no difference between ethnic groups in terms of whole egg consumption. EAs had the highest consumption of egg, one to three times a week, while 42.6% of SAs consumed it daily.

Table 3.13: Frequency of consumption of dairy products according to ethnicity

Food	Arab *n* = 105	South Asian *n* = 61	Afro/Caribbea n *n* = 66	East Asian *n* = 21	*p* value^
	n (%)				
Yogurt					
Never	24 (22.9)	6 (9.8)	14 (21.1)	2 (9.5)	
1–3 times a month	20 (19.0)	22 (36.1)	21 (31.1)	3 (14.3)	0.06
2–3 times a week	43 (40.9)	19 (31.1)	23 (34.9)	12 (57.2)	
Once a day	18 (17.1)	14 (23.1)	8 (12.1)	4 (19.1)	
Cream cheese					
Never	19 (18.1)	13 (21.3)	31 (15.2)	3 (4.8)	
1–3 times a month	20 (19.0)	13 (21.3)	11(16.7)	4 (19.0)	<0.001
2–3 times a week	44 (41.9)	22 (36.0)	14 (21.3)	13 (61.9)	
Once a day	22 (21.0)	13 (21.3)	10 (15.2)	1 (4.8)	
Hard cheese					
Never	35 (33.3)	13 (21.3)	26 (39.4)	10 (47.6)	
1–3 times a month	20 (19.0)	11 (18.6)	17 (25.8)	3 (14.3)	0.24
2–3 times a week	33 (31.4)	20 (41.0)	14 (21.5)	5 (23.8)	
Once or more a day^	17 (19.7)	12 (19.7)	9 (13.6)	3 (14.3)	
Whole milk					
Never	39 (37.1)	16 (26.2)	28 (42.4)	10 (47.6)	
1–3 times a month	12 (11.4)	6 (9.8)	7 (10.6)	5 (23.8)	0.12
2–3 times a week	22 (20.9)	12 (19.6)	8 (13.6)	4 (14.3)	
Once or more a day^	32 (30.4)	27 (44.3)	23 (34.9)	2 (9.5)	
Semi-skimmed milk					
Never	63 (60.0)	30 (49.2)	28 (42.2)	10 (47.6)	
1–3 times a month	9 (8.6)	5 (8.2)	18 (27.3)	4 (19.0)	
2–3 times a week	8 (7.6)	11 (18.1)	11 (16.6)	1 (4.8)	0.006
Once or more a day^	25 (23.8)	15 (24.8)	9 (13.6)	6 (28.6)	

^Several categories were collapsed into once or more a day, due to containing few responses.
*Chi square test.

For the dairy group, there were no ethnic differences in the consumption of yogurt, hard cheese or whole milk ($p > 0.05$). As shown in Table 3.13, there was significant difference in the consumption of semi-skimmed milk and creamy cheese across ethnicities ($p < 0.001$). The Arab group consumed the most of both items daily.

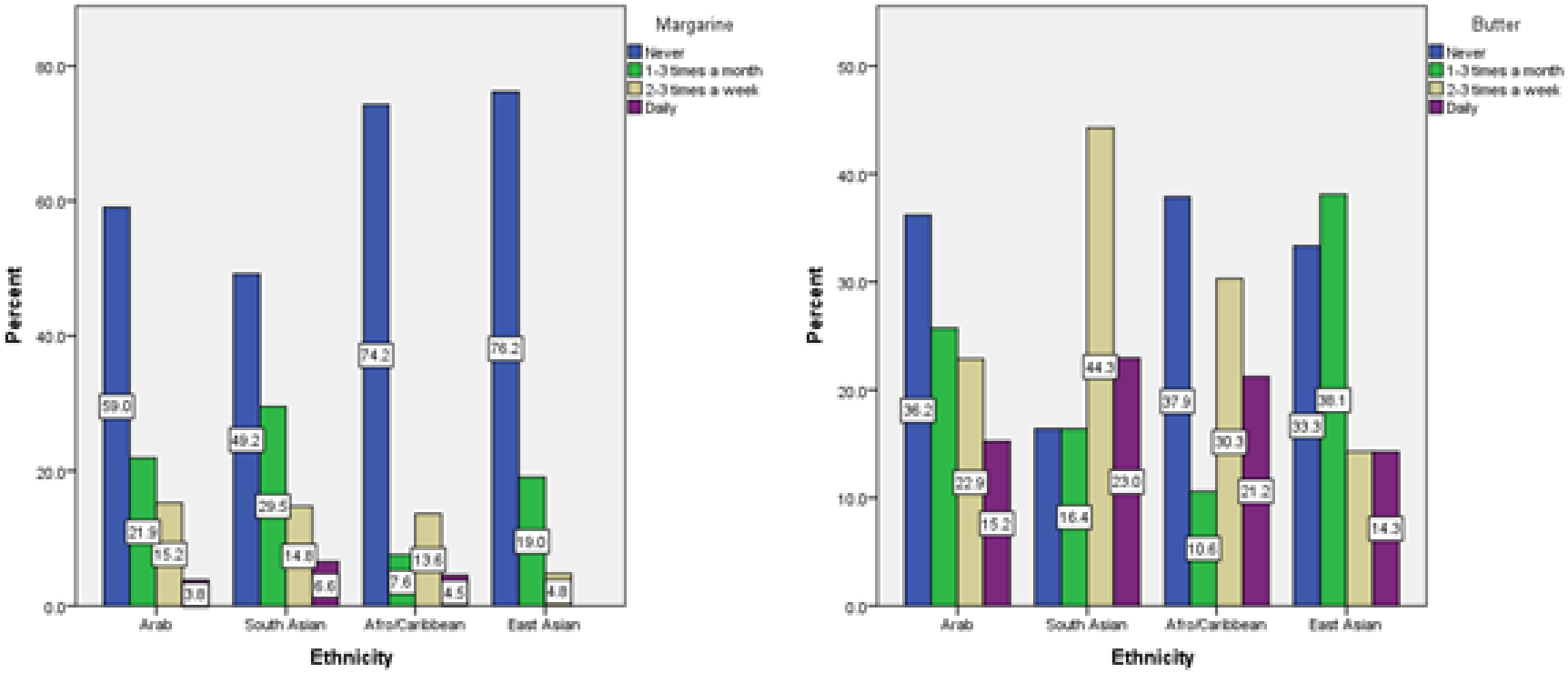

Figure 3.9: Frequency of consumption of fat products according to ethnicity (n=253)

Figure 3.9 illustrates that a high percentage of Arabs had never eaten butter, while more SAs consumed it two to three times a week than in any other group (p<0.001). Margarine was not favoured, as more than half of the participants never ate it (p=0.05).

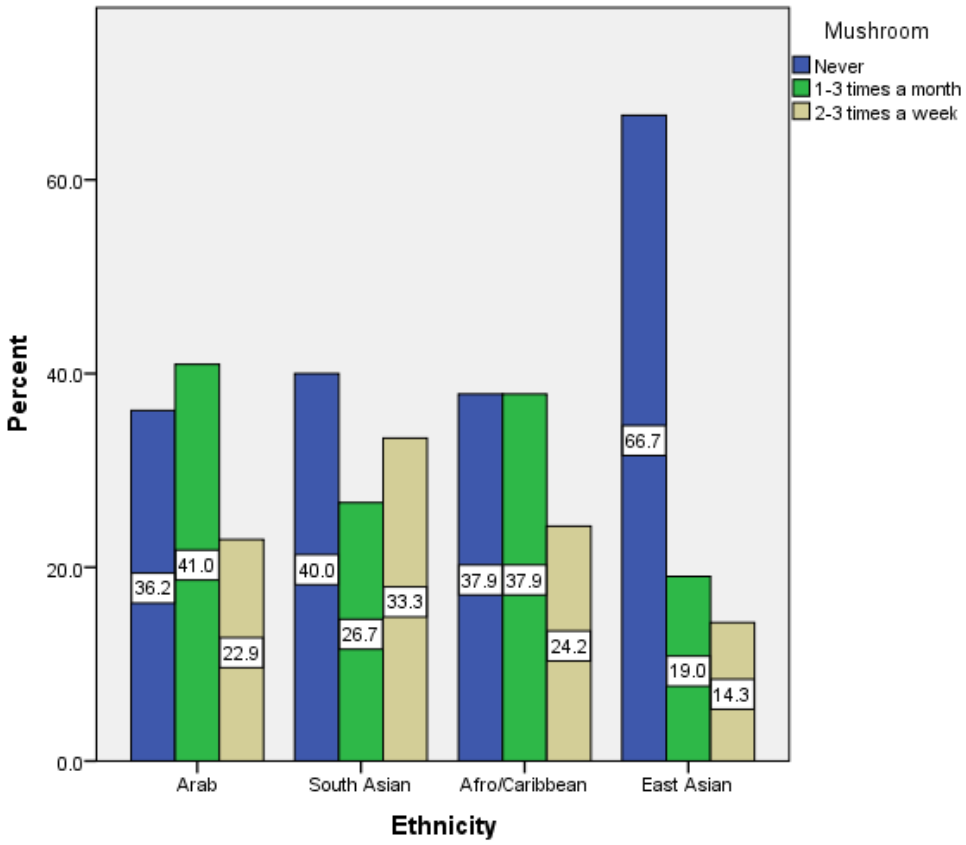

Figure 3.10: Frequency of consumption of mushroom according to ethnicity (n=253)

The consumption of mushrooms was relatively low (Figure 3.10), as less than half of each ethnic group reported an intake of two to three times a week (p = 0.56).

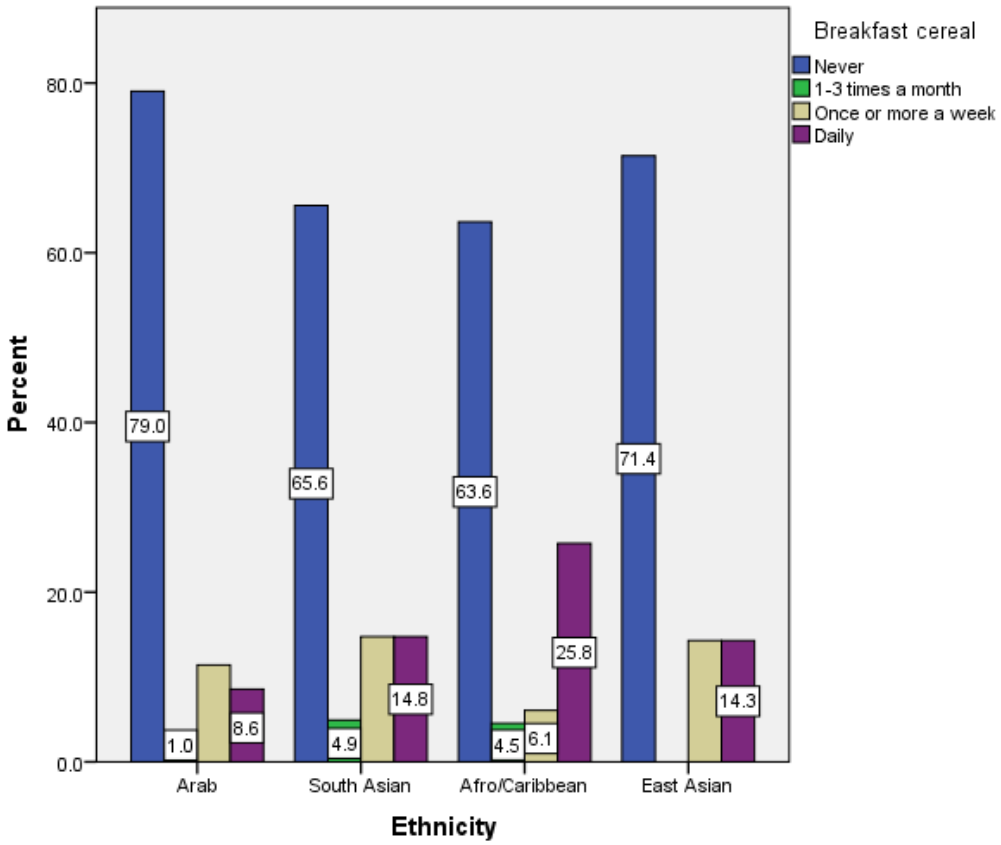

Figure 3.11: Frequency of consumption of breakfast cereal according to ethnicity (n=253)

Figure 3.11 shows that the highest percentage of participants who eat cereal daily were AfroC, followed by SA and EA. There was no significant difference (p=0.06) in the consumption of breakfast cereal by the ethnic groups studied.

3.3.7.1 The estimation of oral intake of vitamin D

The estimated mean dietary intake of vitamin D for all participants was 2.98 µg/day, which is lower than the recommended value of 5 µg/d (WHO and FAO, 2004). The classification of total vitamin D intake among the sample is shown in Figure 3.12. Most participants had a low intake of vitamin D (<5 µg/d) and only 17% had a medium intake (5-10 µg/d).

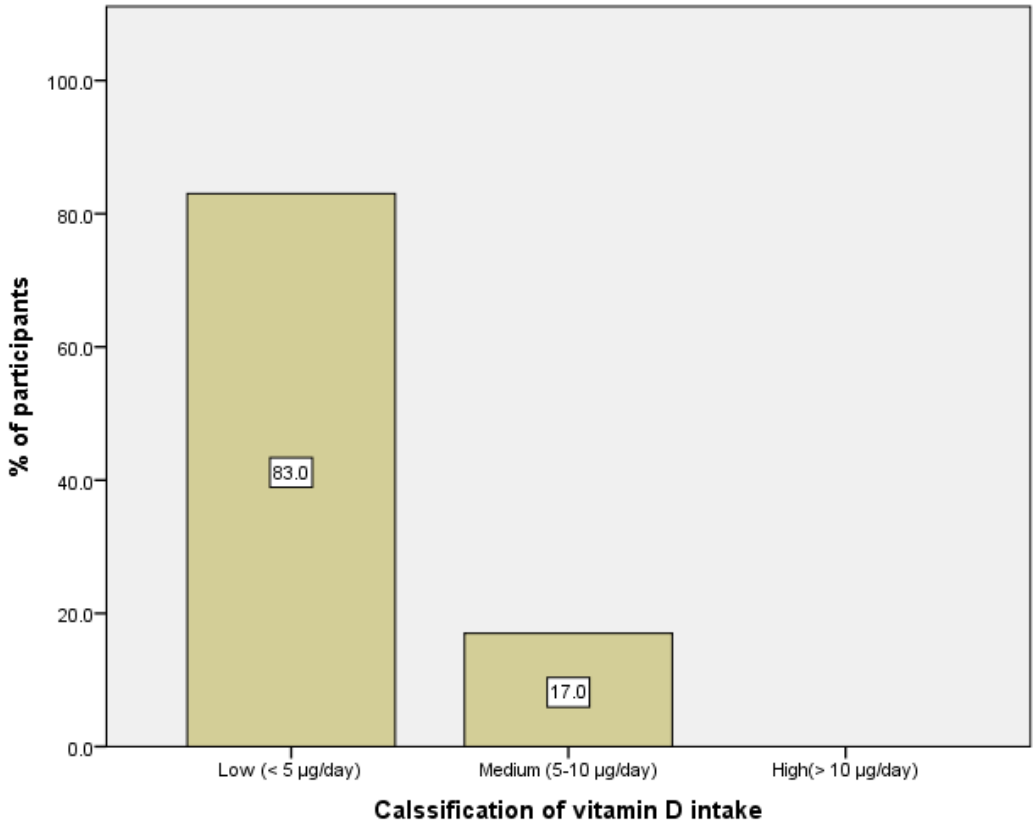

Figure 3.12: Percentage of participants by classification of dietary intake of vitamin D (n=253)

Table 3.14: Comparison of vitamin D intake by ethnicity

	Arab	South Asian	Afro/Caribbean	East Asian	Total sample	*p* value[&]
Dietary intake of vitamin D (µg/day)[*]	2.55 (2.4)	3.07 (1.7)	2.62 (3.1)	2.44 (2.1)	2.27 (2.4)	0.28
Vitamin D supplement (%)	23 (21.9)	8 (13.1)	13 (19.7)	3 (19.4)	49 (19.4)	0.52 ^^
Total vitamin D intake (µg/day)[**]	3.81 (3.3)	3.43 (3.3)	3.93 (4.0)	2.72 (2.8)	3.66 (3.5)	0.51

[*]Data expressed as median and interquartile range.

^^Chi-square was used.

[&]Kruskal-Wallis H test was conducted to determine the differences between dietary intake of vitamin D and total oral intake of vitamin D among different ethnicities.

**Diet + supplement.

The Kruskal-Wallis test (p>0.05) carried out on the data shown in Table 3.14, revealed no association between ethnicity and vitamin D intake. Additionally, a chi-square test indicated that there were no differences in taking vitamin D supplements between different ethnicities (p>0.05). The total intake of vitamin D from food and supplements for each individual was estimated using Equation 6.

Equation 6:

$$\textbf{Total vitamin D intake} = \sum \textbf{dietary intake of vitamin D} + \textbf{supplement intake}$$

The median total of vitamin D for the whole sample was 3.66 µg/d, as presented in Table 3.14. The Kruskal-Wallis test showed no differences between the total intakes of vitamin D across differing ethnicities ($p > 0.05$).

Table 3.15: Mean contributions of different food products

Food (µg /day)	%
Fish group	39
Egg	35
Dairy food	21
Meat	4
Mushroom	1

The contribution to vitamin D intake of each food group is shown in Table 3.15. Fish was the richest dietary source of vitamin D among the study group.

3.3.7.2 The total intake of Vitamin D

The total vitamin D intake of each individual from the three sources, i.e. dietary, supplement and sun, was estimated by summing the results of Equation 6 and Equation 3. The median total intake of vitamin D for the whole sample was 4.30 µg/d. As shown in Table 3.16, many important factors that may affect vitamin D intake were included in the multiple regression model. Every one year increase in age was associated with a 0.17 µg decrease in total vitamin D intake (B = – 0.17, 95% CI: –0.35, 0.00, p=0.05), while no other factors significantly affected the total vitamin D intake.

Table 3.16: Multiple regression model of total vitamin D intake from diet, sun exposure and supplements

Total vitamin D intake	Coefficient (B)	95% CI	*p*-value
Sex			
Male	Reference		
Female	–0.06	–0.22, 0.10	0.46
Age			
18–29 years	Reference		
30–39 years	–0.17	–0.35, 0.00	0.05
40–49 years	0.01	–0.22, 0.25	0.87
Ethnicity			
Arab	Reference		
South Asian	0.14	–0.05, 0.34	0.16
Afro/Caribbean	0.15	–0.03, 0.34	0.11
East Asian	0.01	–0.27, 0.31	0.90
Occupation			
Employed	Reference		
Student	–0.00	–0.18, 0.18	0.97
Unemployed	0.01	–0.24, 0.27	0.90
Education level			
College/diploma	Reference		
Bachelor	–0.19	–0.43, 0.03	0.09
Postgraduate	0.11	–0.13, 0.36	0.35
Income, £			
<5,200	Reference		
5,200–10,399	–0.02	–0.25, 0.20	0.81
10,400–25,999	–0.05	–0.27, 0.17	0.63
≥26,000	–0.10	–0.33, 0.13	0.39
Occupation location			
Mainly indoors	Reference		
Half indoor/outdoor	–0.03	–0.19, 0.13	0.70
Mainly outdoors	0.02	–0.26, 0.31	0.88

CI: confidence interval.

The total vitamin D intake from diet and sun only is shown in **Error! Not a valid bookmark self-reference.**. Educational level showed a significant effect on vitamin D intake. Compared with participants who did not attend university, those with a postgraduate degree had higher levels of vitamin D intake (B = 0.23, 95% CI: 0.03, 0.43, p = 0.02). Ethnicity was another significant predictor of vitamin D intake: SA and AfroC had higher vitamin D intake compared with Arabs (B=0.24, 95% CI: 0.07, 0.40, p=0.00), (B=0.15, 95% CI: 0.00, 0.31, p=0.05) respectively.

Table 3.17: Multiple regression model of total vitamin D intake from diet and sun exposure

Total vitamin D intake	Coefficient (B)	95% CI	*p* value
Sex			
Male	Reference		
Female	–0.07	–0.20, 0.05	0.28
Age			
18- 29y	Reference		
30-39y	0.02	–0.11, 0.17	0.70
40-49y	0.00	–0.19, 0.27	1.00
Ethnicity			
Arab	Reference		
South Asian	0.24	0.07, 0.40	<0.001
Afro/Caribbean	0.15	0.00, 0.31	0.05
East Asian	0.17	–0.05, 0.41	0.13
Occupation			
Employed	Reference		
Student	0.00	–0.15, 0.14	0.93
Unemployed	0.07	–0.14, 0.28	0.51
Education level			
College/diploma	Reference		
Bachelor	0.11	–0.07, 0.30	0.24
Postgraduate	0.23	0.03, 0.43	0.02
Income, £			
<5,200	Reference		
5,200–10,399	0.03	–0.15, 0.21	0.74
10,400–25,999	–0.02	–0.20, 0.15	0.79
≥26,000	–0.05	–0.24, 0.13	0.55
Occupation location			
Mainly indoors	Reference		
Half indoor/outdoor	0.03	–0.10, 0.16	0.66
Mainly outdoors	–0.09	–0.32, 0.13	0.42

CI: confidence interval.

3.4 Discussion

The purposes of this research were to examine the factors that influence vitamin D status amongst EMGs in the city of Manchester and to investigate the differences in the risk factors of hypovitaminosis D across EMGs. A total of 253 adults, consisting of Arabs, South Asians, Afro/Caribbeans and East Asians, completed the study. The rate of response was low among the target samples, which was also reported in previous studies conducted on the same population demographic (Ford et al., 2006; Roy et al., 2007; Ahmed et al., 2013).

3.4.1 Demographic Factors

It has been found that vitamin D intake is related to demographic factors such as education levels, ethnicity, occupation, income and age group (Holvik et al., 2005; Moore et al., 2005; Ahmed et al., 2013; Moore et al., 2014). Understanding ethnic differences in these factors could play an important role in investigating which EMGs are at higher risk of developing hypovitaminosis D. In the current study, ethnic difference in dietary vitamin D intake was not statistically significant ($p=0.28$). While this is consistent with previous reports in adults (Hall et al., 2010; Goodman et al., 2016), significant ethnic differences have been observed in several other studies (Calvo et al., 2004; Moore et al., 2005; Rees et al., 2005). Nonetheless, the dietary intake of vitamin D was higher among SAs than the other groups, as SAs have the highest consumption of oily fish, which is considered to be the richest source of vitamin D (Van Der Meer et al., 2008). This result is also in line with those of previous studies (Gozdzik et al., 2008; Hall et al., 2010). A variety of lifestyle behaviours, such as sunscreen use and clothing choices, could result in the ethnic differences in sun exposure (Hall et al., 2010), while other lifestyle factors not analysed in this research, such as physical activity, may contribute to the increased cutaneous synthesis of vitamin D (Lee and Im, 2010). In the current study, as in another previous study, dietary intake of vitamin D and supplements did not differ between the sexes (Holivk et al., 2005).

Beside ethnicity, educational levels and age had significant correlations with vitamin D intake in the current study; the higher the educational level of an individual, the higher the mean score for vitamin D intake, as it was in those aged 30–39. These findings, which are in line with previous studies (Ahmed et al., 2013; Holvik et al., 2005; Moore et al.,

2014; Moore et al., 2005), suggest therefore, that lower education levels and age ≥ 40 increase the risk of hypovitaminosis D (Chowbey and Harrop, 2016). This association may be explained by the greater tendency of older adults to maintain a traditional diet (Kumar et al., 2004; Gilbert and Khokar, 2008) and by the physiological changes of ageing that can impair appetite, hence reducing total food intake (Pilgrim et al., 2015).

In addition, it has been suggested that income could affect food choices: low income may make EMGs more likely to consume poor-quality food (Gilbert and Khokar, 2008). Accordingly, there have been reports that income influences dietary intake of vitamin D (Hayden et al., 2015); and a more recent study by Léger-Guist'hau et al. (2017) showed that the high cost of vitamin-D-enriched foods, such as fish and dairy or fortified food, as well as supplements, could lead to dietary deficiency. In the current study, there was no statistically significant association ($p>0.05$); however, a higher income level did correlate with a rise in the total dietary intake.

3.4.2 Health and lifestyle factors

Data from a meta-analysis show that obesity is an epidemic issue prevalent in EMGs in the UK and Europe and that it has a significant effect on vitamin D levels (Pereira-Santos et al., 2015). Regardless of the biological effect of obesity on vitamin D, as mentioned Section 2.9.3.1, it has been hypothesised that avoiding outdoor activities and covering the body because of low confidence and body dissatisfaction will tend to reduce exposure to the sun, thus, reducing cutaneous vitamin D synthesis (Yates and Aruguete, 2004; Pereira-Santos et al., 2015). Findings from the current study show a statistically significant difference ($p<0.001$) in BMI among ethnicities; in particular, the prevalence of overweight was highest in the Arab group; a similar result has been found in the literature (Ahmed et al., 2013; Musaiger, 2011). Additionally, data from a Health Survey for England showed that it was likely for EAs and SAs to be in the range of normal weight (Leung and Stanner, 2011); similar findings have also been reported for SAs (Darling et al., 2013). The findings contradict the data from the Department of Health, which show a high prevalence of obesity was among AfroCs and SAs, particularly in women (Gilbert and Khokar, 2008). However, it has been suggested that the increased level of people overweight and obesity among EMGs may be due to lower levels of physical activity (Koshoedo et al., 2009).

Furthermore, current findings showed that the prevalence of obesity was higher among males and among older participants. This result may be explained by the impacts of quality of life and reduced physical activity (Chodzko-Zajko et al., 2009). It is in line with data from the Health Survey for England (2013), which found that the prevalence of obesity and overweight among adults was 62.1% and that men are more likely to be obese. In addition, ethnic differences have been clearly identified, with SAs and Arabs experiencing vitamin D deficiency more frequently than AfroCs and EAs.

It has been separately suggested that smoking and alcohol intake affect vitamin D metabolism (Lamberg-Allardt et al., 2001; Lee, 2012; Shinkov et al., 2015). In the current study, there was a significant difference (p=0.04) between smoking status and gender, with more male smokers than females, while the highest proportion of smokers was in the Arab sample. This result is likely to be related to the fact that smoking by females is less socially acceptable (Islam and Johnson 2003; Thom, 2003). Alcohol intake was the highest amongst the middle-aged participants, which is consistent with previous studies (Lamberg-Allardt et al., 2001). It was also higher among AfroC than any other group, most of whom were Muslims, which is again in line with previous studies (Food Standards Agency, 2007; Michalak et al., 2007; Roy et al., 2007). Specifically, this can be explained by religious factors, as mentioned in Section 2.9.3.3.

3.4.1 Sun exposure assessment

Sun exposure is considered to be the richest source of vitamin D; indeed, about 90% of vitamin D can be synthesised cutaneously by exposing the skin to UVB radiation (Holick, 2004a), which is more than can be obtained from the diet (O'Connor and Benelam, 2011). Therefore, it was important to assess the sun exposure behaviour of the participants to identify habits which might negatively affect vitamin D levels. As no typical or authenticated questionnaire was available (McCarty, 2008), the current study used questions designed according to the following requirements expressed in previous studies (Macdonald et al., 2008; McCarty, 2008; Gould et al., 2015): the questionnaire should include items on time outdoors, skin area exposed to sunlight, the use of sunscreen and sunbathing. The literature also indicates that the skin's capacity to produce vitamin D is affected by the degree of pigmentation (Kift et al., 2013). Hence, identification of skin colour was important for the current study, which found that all

participants had dark skin, except the EAs and some Arabs, which suggests that the AfroC and SA groups might be at risk of inadequate vitamin D synthesis. According to reports in the literature, a high level of melanin in the skin blocks UVB rays from entering (Correia et al., 2014; Akhtar, 2016), to the extent that during winter, the level of vitamin D synthesis may fall to zero (Macdonald et al., 2011).

Low UVB exposure may not be caused by deliberate avoidance of sunlight but could be the unintentional consequence of practices such as the wearing of particular clothing for cultural or religious reasons (Meltzer, 2007; Brough et al., 2010; Macdonald et al., 2011; De Roos et al., 2012; Darling et al., 2013; Webb et al., 2016) or of a person's type of occupation; for example, office workers and students tend to receive relatively little exposure to sunlight (Hyppönen and Power, 2007; Sowah et al., 2017). Alternatively, however, avoidance of sunlight can be intentional: to avoid damaging solar radiation by staying in the shade or by using sunscreen to help maintain skin tone and beauty (Nesby-O'Dell et al., 2002; Thieden et al., 2005; Kift et al., 2013). Although most Arabs participating in the current study had relatively light skin pigmentation, they were still at risk of developing vitamin D deficiency, as they used sunscreen and avoided sun exposure more than the other groups; this result is in line with previous findings (Ahmed et al., 2013).

The current study also showed that the area of uncovered skin differed significantly between ethnicities: a high proportion of Arabs, SAs and AfroCs exposed only their faces and hands to sunlight, thus limiting the benefit of exposure to UVB. This finding is consistent with previous studies in the UK (Pal et al., 2003; Ford et al., 2006; Maxwell et al., 2006; Macdonald et al., 2011; Patel et al., 2013), as well as those conducted in sunnier countries (Bassil et al., 2013; Al-Daghri, 2016). According to Farrar et al. (2011), the current recommendations are not suitable for darker-skinned groups to produce sufficient levels of vitamin D. In particular, the authors suggest that EMGs should expose 35% of the skin surface area to the midday sunlight on a cloudless day for more than half an hour, three times a week (Farrar et al., 2011). Furthermore, time spent outdoors was low among all participants, regardless of ethnicity, which is in line with studies conducted on SAs (Kift et al., 2013), BAs (Nesby-O'Dell et al., 2002; Skull et al., 2003), Middle Eastern populations (Ahmed et al., 2013), a multi-ethnic sample (Van der Meer et al., 2008) and

EA women (Brock et al., 2013; Jang et al., 2013). However, the amount of vitamin D found by this study to be produced cutaneously differed slightly from the findings of other researchers, which can be attributed to differences in the techniques used to calculate sun exposure.

3.4.2 Dietary intake of vitamin D

The estimation of dietary intake of vitamin D was performed using an FFQ, a method which is commonly used in epidemiologic studies (Thompson et al., 1994) and has been employed in many studies of vitamin D status (Maxwell et al., 2006; Madar et al., 2008; Taylor et al., 2009). Other nutritional assessment tools employed in similar studies, depending on the aim of the study, include 7-day diaries (Farrar et al., 2011) and 24-hour recall (Moore et al., 2014); however, these methods do not reflect dietary habits, although they are useful to validate FFQ data (Willett, 2013).

Data from the NDNS and LIDNS in the UK have shown a low average vitamin D intake, at 2.8 μg/d (Mavroeidi et al., 2010; Whitton et al., 2011), while data from the third National Health and Nutrition Examination Survey showed that vitamin D intake was low among ethnic minority adults (Looker et al., 2002), as well as for African-American women in wintertime (Nesby-O'Dell et al., 2002). In the current study, all EMGs had vitamin D intake below the RNI recommendation, suggesting that all EMGs living in Manchester are at risk of vitamin D deficiency, which is consistent with a number of previous studies of ethnic adults in the UK (Wilkinson et al., 2000; Maxwell et al., 2006; Ahmed et al., 2013; Darling et al., 2013; Farrar et al., 2013; Kift et al., 2013; Patel et al., 2013). It should be noted that these other studies did not compare different EMGs; rather, each study focused on one or two groups only. To the researcher's best knowledge, this is the first study to assess the ethnic differences in vitamin D intake and other risk factors in healthy adults from all of the main EMGs in the UK.

Furthermore, the results are in accordance with those of studies among multi-ethnic groups living in other countries, which indicates that vitamin D is low globally (Holvik et al., 2005; Madar et al., 2008; Islam et al., 2012). However, the estimated vitamin D intake (although still below RNI) was somewhat higher for EMGs living in the USA (Moore et al., 2005), Sweden (Andersson et al., 2013) and Canada (Gozdzik et al., 2008) compared to

the UK studies. This may be attributed to the abundance of fortified foods (Calvo et al., 2004), which suggests that fortified products contribute approximately 60% towards dietary intake of vitamin D (Calvo and Whiting, 2013), while in the UK, strict legislation governs food fortification (Sinha et al., 2013), as mentioned in Section 2.1.1.

Despite oily fish being the richest source of vitamin D available in the UK (Lanham-New et al., 2011), it makes only a relatively small contribution to the UK diet, due to its low consumption by the population. Results data the NDNS indicate that the contribution of fish is 21% and 30% of the dietary intake of vitamin D for male and female adults respectively (Weichselbaum et al., 2013). Bates et al. (2014) report the mean intake of oily fish by UK adults at only 54 g per week, which is below the recommended intake of at least one 140 g portion per week (Weichselbaum et al., 2013). Indeed, fish consumption was low among the participants in the current study, which is consistent with the literature (Maxwell et al., 2006; Ahmed et al., 2013; Andersson et al., 2013). Similarly, oily fish intake was low among the ethnic minority population living in Oslo, despite oily fish being popular in Norwegian cuisine (Holvik et al., 2005; Madar et al., 2008). Andersson et al. (2013) consider it possible that these residents do not eat enough vitamin-D-rich food because they adhere to the traditional dietary customs of their ethnic groups; however, according to Cashman and Kiely, (2016), people cannot achieve the RNI of 10 µg/d even by following modern Western-style diets, which include few rich natural sources of vitamin D.

The intake of egg was low in the present study, consistent with research findings that egg consumption is very infrequent among AfroCs (Maxwell et al., 2006) and Arabs (Ahmed et al., 2013). There is no doubt that frequent intake of eggs can be an effective source of vitamin D, as there are 1.8 µg in an egg yolk.

It should be noted that there are many influences on dietary habits, including religion, lifestyle and environmental factors (Gilbert and Khokar, 2008; Ngo et al., 2014). For instance, although meat does not contain much vitamin D, it can be an effective dietary source if it is consumed frequently, but religion may forbid some people from consuming some types of meat (Higgins, 2008), as mentioned in Section 2.3.3.1. Data from the current study demonstrate that people of all ethnicities commonly consumed beef but that 31% of SAs never consumed it because they were Hindus. The majority of

participants never ate pork, probably because of their religious beliefs. These findings are comparable with those reported by Ahmed et al. (2013), Andersson et al. (2013), Nolan (2007) and Wandel et al. (2008). The highest intake of dairy products was among Arabs and SAs, which agrees with the findings of Nolan (2007) and Mellin-Olsen et al. (2005).

Although it has been reported that dietary supplements are an excellent source of vitamin D whose use can provide normal vitamin D levels in the absence of other sources (Sinha et al., 2013), the consumption of vitamin D supplements was low among participants in this study. These results are in agreement with studies of SAs (Rees et al., 2005), Black Africans and EAs (Holvik et al., 2005; Brock et al., 2013). However, supplement intake was low among EMGs living in Scandinavian countries including Norway, despite supplement intake being common among the population (Calvo et al., 2005). Cost and lack of awareness are possible reasons for not taking supplements.

The regression coefficient results in Table 3.17 indicate that ethnicity was a determinant of total intake of vitamin D and it was therefore not surprising that most EMGs had insufficient or deficient vitamin D intake, considering that consistent with previous research (Salamoun et al., 2005), both sun exposure and consumption of supplements and foods containing vitamin D were low. Therefore, members of EMGs need to obtain the maximum benefit from summer sunlight by regularly exposing at least a quarter of their bodies to sunlight, particularly around midday. Those who have type V or VI skin should use a sunbed once a month or follow the UK Department of Health recommendation by consuming one portion of oily fish a week.

3.5 Conclusion

The study has shown lifestyle differences between ethnic groups living in Manchester which may affect their ability to achieve the recommended intake of vitamin D. The main common cause of vitamin D deficiency amongst EMGs was found to be sun exposure behaviour, as sunlight is the main source of vitamin D. The study has shown that the highest sunlight exposure at peak time was among the EA group, while members of other groups were less exposed to sunlight. Regardless of environmental factors, the reasons for not benefiting adequately from sunlight are related to cultural and religious factors,

as most Arabs tend to avoid sunlight by using sunscreen. They also share with many SAs and AfroCs the wearing of clothing which limits sun exposure by following traditional Islamic dress styles. Type of occupation could be another a major factor limiting sunlight exposure by keeping people indoors at midday.

Another common cause of vitamin D deficiency among EMGs is their dietary habits and infrequent use of supplementation. Generally, dietary intake was low among all groups, even the South Asians, who had the highest intakes of dietary vitamin D. However, this study provides evidence that the higher the education level of individuals, the higher their vitamin D intake. It is therefore recommended that healthcare policies and prevention campaigns focus on the at-risk population identified here and that preventive interventions be implemented to help them improve their health by increasing their vitamin D intake.

4. ASSOCIATION OF VITAMIN D INTAKE AND OTHER RISK FACTORS WITH 25(OH)D CONCENTRATIONS

4.1 Introduction

The effects of vitamin D deficiency on bone health are well known (SACN, 2016; Shina et al., 2013). Epidemiological and experimental data indicate that hypovitaminosis D may increase the risk of several non-skeletal diseases, including some cancers and type 1 diabetes (Holick, 2004b; SACN 2016). Serum 25(OH)D concentration is considered a reliable indicator of vitamin D status, but the deficiency cut-off level is still under debate, the highest cut-off in use being ≤30 nmol/L (Sinha et al., 2013). Hypovitaminosis D has been shown to be common in the general population in the UK due to reduced sun exposure and low dietary vitamin D intake (SACN, 2016). However, it is markedly more common among EMGs (Roy et al., 2006; Webb et al., 2016). Other factors which can affect cutaneous synthesis of vitamin D include the amount of melanin in the skin (Webb et al., 2018); the ability to synthesise vitamin D_3, which decreases with rising age (Holick, 2004b); BMI (low vitamin D has been associated with higher BMI) (Vanlint, 2013; Pourshahidi, 2015; Wortsman et al., 2000); sun avoidance for cultural or safety reasons (Holick, 2004a; Holick, 2007); health conditions such as kidney or liver failure; and the use of any medication which can interfere with vitamin D metabolism (Holick, 2007).

Chapter 3 reported a study in which the dietary intake of vitamin D and length of time spent outdoors were assessed using a questionnaire and which compared the responses of four ethnic minority groups: Arabs, South Asians, Afro/Caribbeans and East Asians. Relevant factors including demographic characteristics, health factors and sun exposure behaviour were examined to assess their influence on vitamin D intake. The study reported in this chapter measured the vitamin D level in participants' blood to investigate the associations of vitamin D intake and other risk factors with 25(OH) D concentrations in ethnic minority adults.

4.1.1 Aim

- Examine the association between dietary intake, lifestyle factors and 25(OH)D concentrations among ethnic minority adults.

4.1.2 Objectives

- Estimate vitamin D intake for Arab, South Asian and Black African from dietary sources, supplement use and sun exposure using a questionnaire.

- Measure serum 25(OH)D concentrations for ethnic minority participants.
- Determine the diet and lifestyle factors that affect vitamin D status among ethnic minority adults.

4.1.3 Research hypothesis

There is an association between vitamin D status and vitamin D intake or lifestyle factors among members of ethnic minority groups.

4.2 Methodology

4.2.1 Study population and recruitment

Potential volunteers from EMGs were recruited in Manchester (Figure 4.1) between October and December 2016, using the same method as that detailed in Chapter 3 (see Section 3.2.3). Additional recruitment was done by using posters containing summary information about the research and the objectives of the study along with an email address for further information (Appendix 5). The posters were displayed in different places, such as universities, markets and places of worship, as well as being distributed via email and posted online at on the ethnic community pages of various social media platforms. The inclusion criteria were the same as those listed in Chapter 3, Section 3.2.2.

4.2.2 Confidentiality

All information provided by the participants was kept confidential and used for the purposes of this study only. The data were collected and stored in accordance with the Data Protection Act, 1998.

4.2.3 Study protocol

A flowchart of the recruitment and study protocol is presented in Figure 4.1

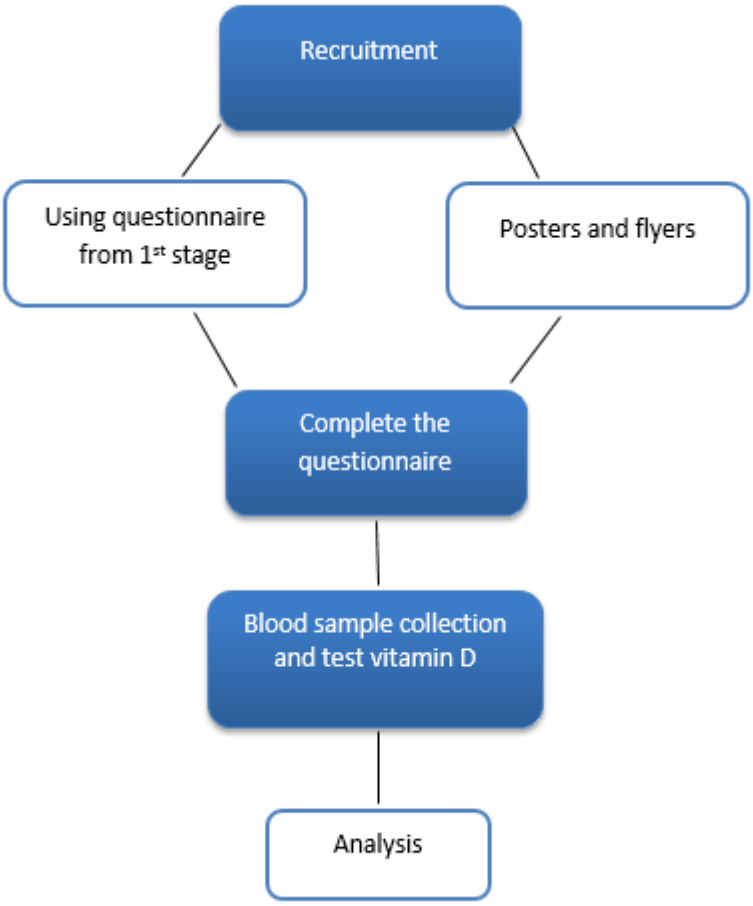

Figure 4.1: Recruitment and study protocol flowchart

4.2.3.1 Questionnaire design

Participants completed a food frequency questionnaire (FFQ), a sun exposure assessment and a questionnaire on lifestyle and health factors (see Section 3.2.4).

4.2.3.2 Blood collection

The dried blood spot technique was used to measure 25(OH)D, this having been shown by Shea and Berg, (2017) to be a valid and reliable method. The results of their test of the correlation between blood and plasma values are shown graphically in Figure 4.2. Each participant's finger was pricked with a lancet and four blood drops placed on the test card provided as part of the vitamin D test kit supplied by Sandwell and Birmingham Hospitals NHS trust. Total 25(OH)D concentrations were subsequently determined by LC/MS/MS. A 3 mm punched section was taken from each dried blood spot and the 25(OH)D was extracted with 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) and then the

sample was injected into LC/MS analyser (Waters i-Class UPLC and Waters Xevo TQ-S Triple Quad Mass Spectrometer. Multiple reaction monitoring (MRM) was used to analyse the samples for 25-(OH)D_2 and 25-(OH)D_3 and then the total 25-(OH)D was reported.

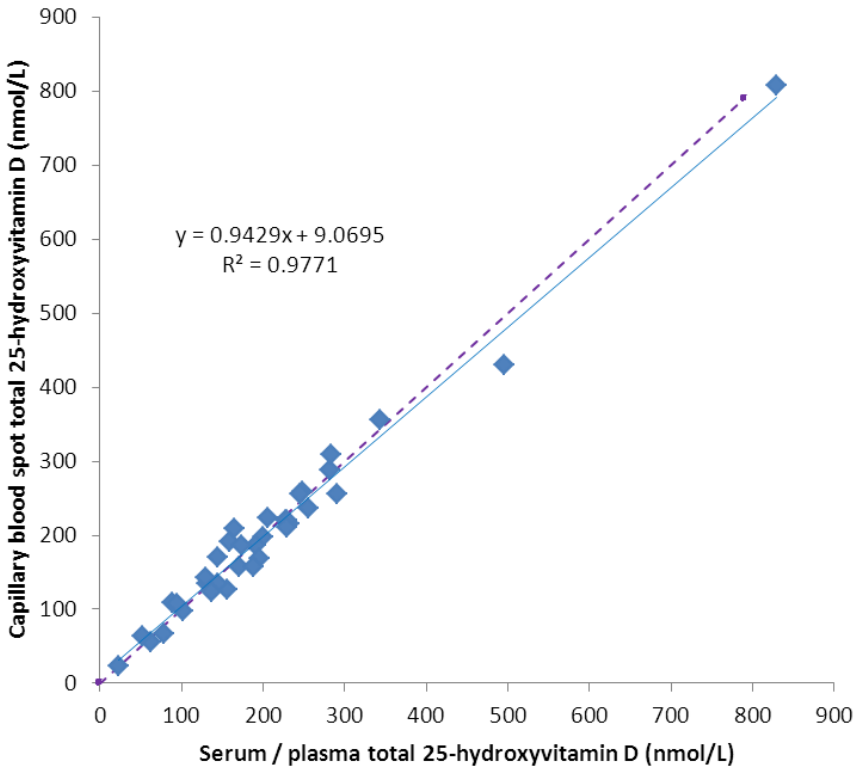

Figure 4.2: Relationship between paired plasma and blood spot samples for measurement of 25-hydroxyvitamin D (Source: Shea and Berg, 2017)

The cut-off values used, shown in Table 4.1, were in line with the reference range of the laboratory providing the tests, the Institute of Medicine and the National Osteoporosis Society (Ross et al., 2011; Francis et al., 2013).

Table 4.1: References of vitamin D

Total vitamin D reference (nmol/L)	Vitamin D status
<15	Severe deficiency
15–30	Deficiency
30.1–50	Insufficiency
>50	Adequate

Source: SWBH NHS Trust.

4.2.3.3 Anthropometric measurements

Participants' height and weight were measured without shoes or heavy clothing. Height in centimetres was measured using a calibrated stadiometer (Model 2251821009, Seca, Hamburg, Germany) while an electronic standing calibrated scale (DP2400 BMI Indicator, Marsden, UK) was used to measure participants' weight in kilograms. Body fat was estimated using the BMI equation as detailed in Chapter 3, Section 3.2.4.5.

4.2.4 Statistical analysis

Data analysis was performed using SPSS software, version 21 (SPSS Inc., Chicago, IL, USA). Vitamin D status was stratified into the four categories listed in Table 4.1. Descriptive analysis was used to analyse the socio-demographic, diet and health factors relating to vitamin D. The distribution of data was checked using a histogram and Q–Q plot. The differences and association between ethnicity and the selected factors (continuous variables) were examined by one-way ANOVA if the data were normally distributed. In cases where the data were skewed, the Kruskal-Wallis H test was used to analyse them. The chi-square test was performed to detect the differences and associations between ethnicity and selected factors (categorical variables). Two-way ANOVA was performed to measure the effect of two categorical variables on a dependent variable (McDonald, 2009).

4.3 Results

4.3.1 Recruitment of participants

A total of 213 participants were approached, of whom 87 were recruited from the first stage. Of the 213, 63 did not respond, 26 others did not complete the questionnaire and 22 participants withdrew. A total of 102 participants met the inclusion criteria (section 3.2.2) and were recruited, but 28 of these were excluded from the study because they refused to a give blood sample. Therefore, 74 participants had their vitamin D levels tested and completed the study.

Figure 4.3 is a flow diagram of recruitment and selection

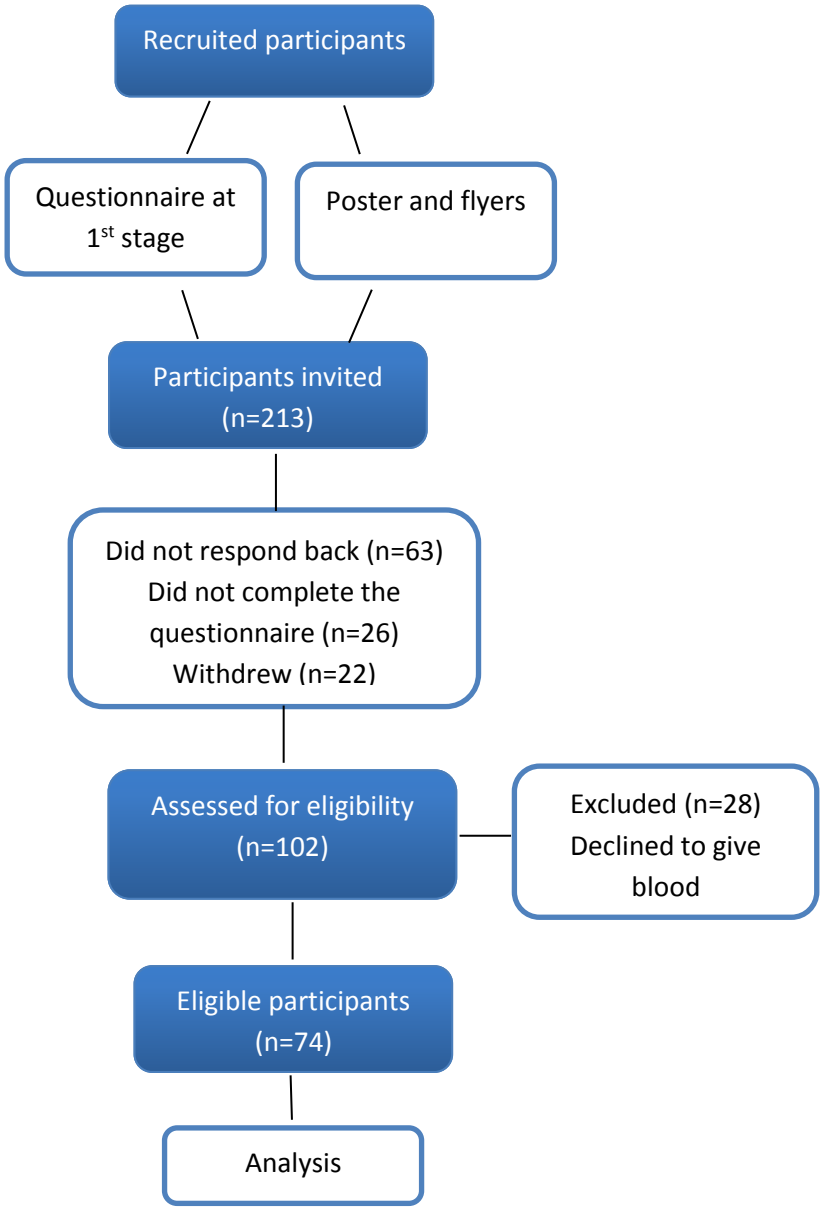

Figure 4.3: Flow diagram of recruitment and selection of study participants

4.3.2 Participants' demographic characteristics

Table 4.2 illustrates the main demographic characteristics of the sample. Among the 74 who completed the study, there were more males than females. The study sample comprised 37.8% Arab, 35.1% South Asian and 27.0% Black African participants, with a mean age of 29.8 years. The majority defined themselves as Muslim. More than half of participants had lived in Manchester for more than five years and only 12.2% had been resident in the city for less than a year.

Table 4.2: Demographic characteristics of sample (n = 74)

Variables	*n* (%)
Age[a]	29.9± 8.3
Sex	
Male	39 (52.7)
Female	35 (47.3)
Country of Birth	
Gulf countries[$]	20 (27.0)
Syria	3 (4.1)
South Asia[^]	15 (20.3)
Africa	19 (25.7)
Other[&]	17 (23.0)
Ethnicity	
Arab[*]	28 (37.8)
South Asian	26 (35.1)
Black African	20 (27.0)
Religion	
Muslim	57 (77.0)
Christian	11 (14.9)
Hindu	6 (8.1)
Length of residence in UK	
<1 year	9 (12.2)
1–5 years	22 (29.7)
>5 years	43 (58.1)

[a] Mean± Standard Deviation.

[$] Saudi Arabia, Kuwait, United Emirates. ^India, Pakistan and Sir Lanka.

[&]France, England and Poland. *Saudi Arabia, Kuwait, United Emirates, Syrian, Algeria and Egypt.

4.3.3 Socioeconomic factors according to ethnicity

Table 4.3 shows that the median age of the total sample was 28.5 years and that the median age of the Arabs was slightly higher than the other groups. As to education level,

a higher percentage of the Arabs held postgraduate-level qualifications compared with other ethnicities, while the BA group had a higher percentage of members who were educated to college/diploma level. The Arabs reported the highest income, while the ethnicity with the highest percentage of members reporting an income of less than £5,200 was the BA group. More than half of all participants were students and more than a third were in employment. The highest percentage of employed participants was seen among the Arabs, while 15% of BAs were unemployed. The majority of participants reported that they typically spent much of their time indoors during the day; this may be related to their majority status as students. A quarter of the Arab group reported working half indoors and half outdoors, while only 15% of SAs were mainly based outdoors. Table 4.3 shows that there was no association between ethnicity and the socioeconomic factors studied ($p>0.05$), except for religion ($p=0.009$).

Table 4.3: Socioeconomic factors according to ethnicity (n=74)

Variable	Arab (*n* = 28)	South Asian (*n* = 26)	Black African (*n* = 20)	Total sample (*n* = 74)	*p* value[$]
	n (%)				
Age[&]	30.0 (10.7)	28.0 (6.25)	27.5 (16.2)	28.5 (10.7)	0.64[^]
Religion					
Muslim	27 (96.4)	19 (73.1)	11 (55.0)	57 (77.0)	
Christian	1 (3.6)	1 (3.8)	9 (45.0)	11 (14.9)	<0.001
Hindu	0 (0)	6 (23.1)	0 (0)	6 (8.1)	
Education level					
College/diploma	0 (0.0)	3 (11.5)	5 (25.0)	8 (10.8)	
Bachelor	13 (46.4)	11 (42.3)	9 (45.0)	33 (44.6)	0.07
Postgraduate	15 (53.6)	12 (46.2)	6 (30.0)	33 (44.6)	
Income status (£)					
<5,200	6 (21.4)	11 (42.3)	9 (45.0)	26 (35.1)	
5,200–10,399	10 (35.7)	10 (38.5)	8 (40.0)	28 (37.8)	0.15
10,400–20,000	12 (42.9)	5 (19.2)	3 (15.0)	20 (27.0)	
Occupation					
Employed	12 (42.9)	9 (34.6)	6 (30.0)	27 (36.5)	
Unemployed	0 (0.0)	3 (11.5)	3 (15.0)	6 (8.1)	0.31
Student	16 (57.1)	14 (53.8)	11 (55.0)	41 (55.4)	
Occupation location					
Mainly indoors	26 (71.4)	17 (65.4)	16 (58.5)	53 (71.6)	
Half indoor/outdoor	7 (25.0)	5 (19.2)	4 (20.0)	16 (21.6)	0.39
Mainly outdoors	1 (3.6)	4 (15.4)	0 (0.0)	5 (6.8)	

[&]Median interquartile range (IQR). [$]Chi square test was used to examine the association between ethnicity and socioeconomic factors.

^Kruskal–Wallis H test was used to find the difference between ethnicity and age.

4.3.4 Distribution of health and lifestyle factors according to ethnicity

The median BMI by ethnicity is shown in Figure 4.4. The highest median weight was 79.5 kg among the Arabs, with the median weight of SAs and BAs being similar. The median BMI of the Arabs was 26.4 kg/m^2, which is defined as overweight, while the other groups were in the normal range. However, all three median values were close to the cut-off value of 25 kg/m^2 and the Kruskal-Wallis test revealed no significant differences in BMI associated with ethnicity ($p>0.05$).

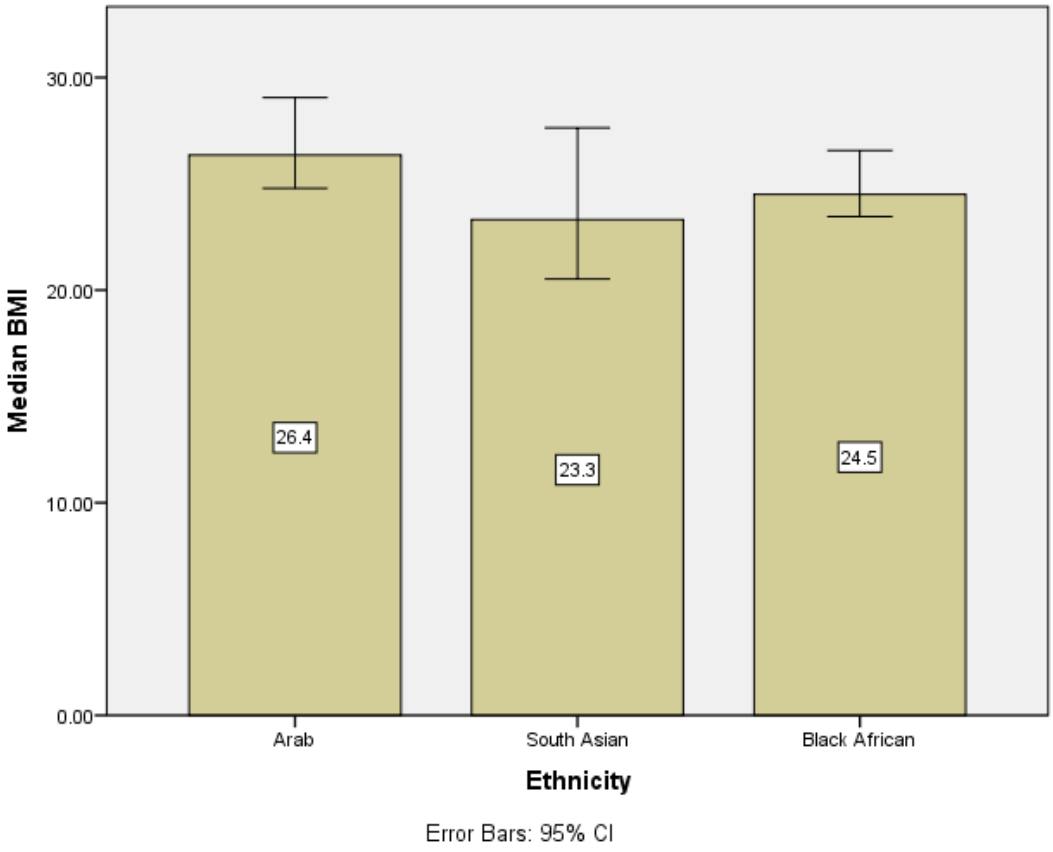

Figure 4.4: Distribution of BMI (median) by ethnicity. Error bars represent 95% confident interval

The incidence of smoking, alcohol intake, and history of vitamin D deficiency according to ethnicity are presented in Table 4.4. Only 17.6% of participants were smokers and 20.3% alcohol drinkers. The highest percentage of alcohol drinkers was among the BA population, while only a few Arabs reported any alcohol intake. Looking at history of vitamin D deficiency, only 31% of the whole sample had experienced of this. The highest

percentage was among the SA at 42.3%. Significant ethnic differences were not found between the factors shown in Table 4.4, except in alcohol intake, which was significantly different across ethnicities (p=0.002). Table 4.4 shows that approximately the same number of participants consumed fortified food in each ethnicity.

Table 4.4: Health and lifestyle factors according to ethnicity

Variable	**Arab *n* = 28**	**South Asian *n* = 26**	**Black African *n* = 20**	**Total sample *n* = 74**	***p* value^**
	n (%)				
Smoking status					
Yes	5 (17.9)	4 (15.4)	4 (20.0)	13 (17.6)	0.93
No	23 (82.1)	22 (84.6)	16 (80.0)	61 (82.4)	
Alcohol intake					
No	27 (71.6)	21 (80.8)	11 (55.0)	59 (79.7)	
Yes	1 (3.6)	5 (19.2)	9 (45.0)	15 (20.3)	0.002
History of vitamin D deficiency					
Yes	6 (21.4)	11 (42.3)	6 (30.0)	23 (31.1)	0.25
No	22 (78.6)	15 (57.7)	14 (70.0)	51 (68.9)	
Consumption of fortified food					
Yes	4 (14.3)	4 (15.4)	5 (25.0)	13 (17.6)	
No	5 (17.9)	11 (42.3)	8 (33.3)	24 (32.4)	0.32
Sometimes	12 (42.9)	6 (23.1)	4 (18.2)	22 (29.7)	
Don’t know	7 (25.0)	5 (19.2)	3 (15.0)	15 (20.3)	

^Chi-square test was used to examine the association between ethnicity and health factors.

4.3.5 Ethnic differences in sun exposure behaviour

The incidence of self-reported skin colour by ethnicity are shown in Figure 4.5. More than half of the Arabs said that they had medium skin colour and a third had olive skin, whilst, half of SA participants had brown skin.

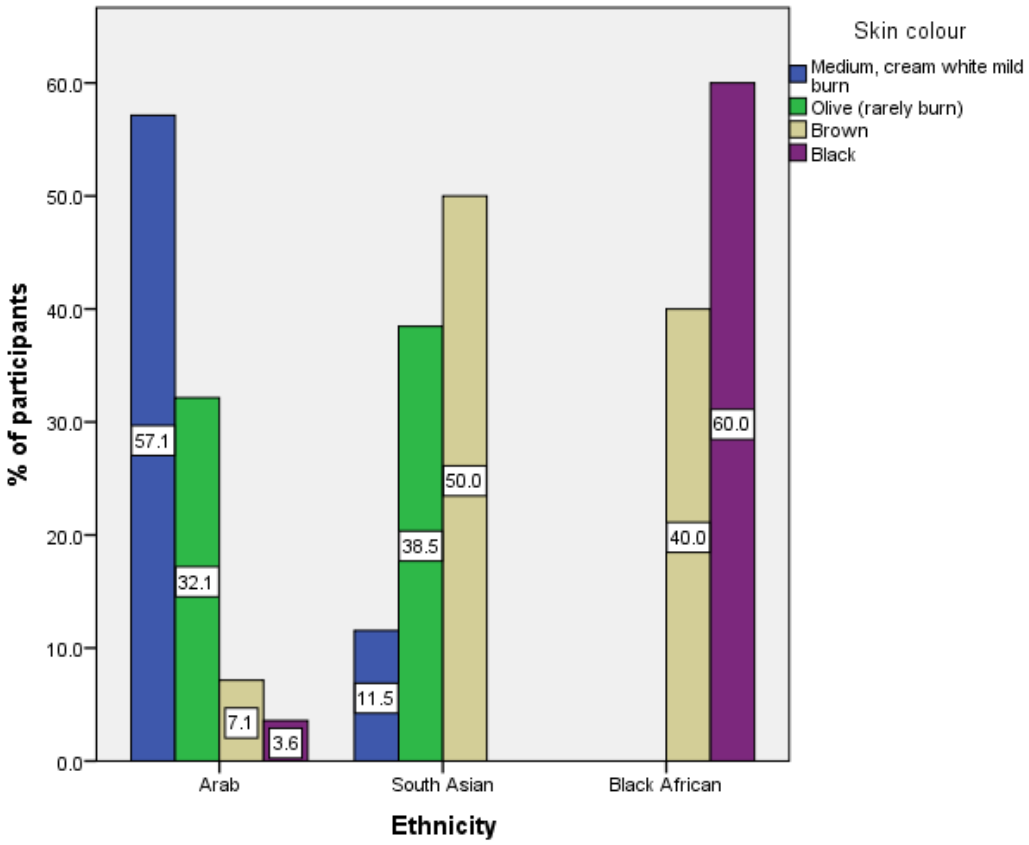

Figure 4.5: Percentage of participants by self-reported skin colours and ethnicity (n=74)

The average time spent outdoors for all participants was 102 min/d, ranging from 15 to 232.5 minutes per day for the month before completing the questionnaire.

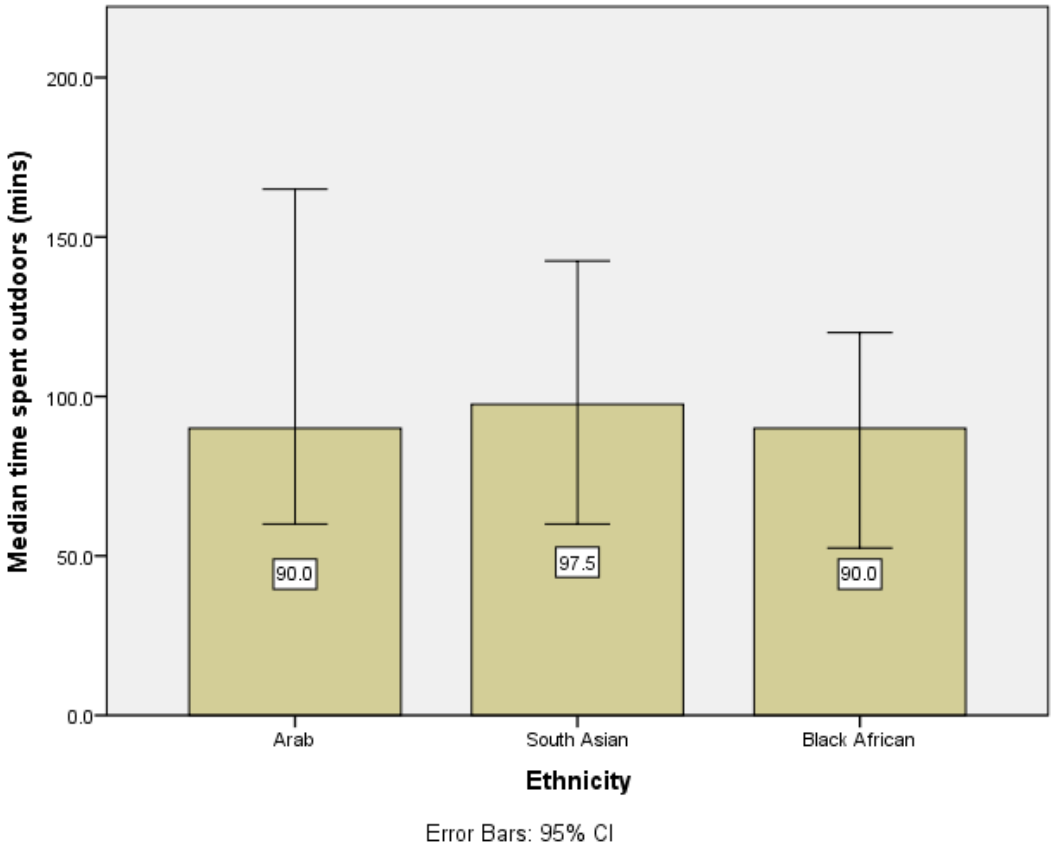

Figure 4.6: Distribution of median time spent outdoors by ethnicity. Error bars represent 95% confident interval

The median time spent outdoors for each ethnic group is shown in Figure 4.6. The daily time outdoors for the SA group was 97 minutes, while for both Arabs and BAs it was 90 minutes. Application of the Kruskal-Wallis H test showed that there was no significant difference between the ethnicities in time spent outdoors (p=0.88).

Table 4.5 shows that no statistically significant ethnic differences were detected in sun index or sunscreen use. In terms of exposed body area, the results of the chi-squared test reveal a significant difference between ethnic groups in body area exposed to sunlight (p=0.002). The highest percentage of people who exposed only face and hands to the sun was among the Arabs (46.4%), whereas the highest percentage of those who exposed their face, full arms and full legs was in the BA group (30.3%).

Table 4.5: Sun exposure behaviour and protection according to ethnicity

Variable	Arab *n* = 28	South Asian *n* = 26	Black African *n* = 20	Total sample *n* = 74	*p* value[&]
The total sun index (hours/week)[$]					
Median (IQR)	0.27 (0.31)	0.30 (0.35)	0.33 (0.57)	0.28(0.34)	0.49[*]
Sunscreen use *n* (%)					
Yes	5 (17.9)	5 (19.2)	5 (25.0)	15 (20.3)	0.87
SPF 15	2 (40.0)	2 (40.0)	2 (40.0)	6 (8.1)	
SPF 45	2 (40.0)	1 (20.0)	1 (20.0)	4 (5.4)	
SPF 50	1 (20.0)	2 (40.0)	2 (40.0)	5 (6.8)	
Exposed area to the sun *n* (%)					
Face and hands	13 (46.4)	9 (34.6)	2 (10.0)	24 (32.4)	
Face and half arms	15 (53.6)	16 (61.5)	12 (60.0)	43 (58.1)	0.002
Face, full arms and full legs	0 (0.0)	1 (3.8)	6 (30.3)	7 (9.5)	

[$]Sun index = hours of sun exposure per week × fraction of BSA exposed to sun light.

[&]Chi-square test was used to examine the association between ethnicity and sun behaviour exposer.

[*]Kruskal–Wallis H test.

BSA: body surface area; IQR: interquartile range; SPF: sun protection factor.

4.3.6 Ethnic differences in vitamin D intake and vitamin D status

4.3.6.1 Dietary intake of vitamin D

Table 4.6 illustrates the findings that the majority of the participants in each group never consumed fresh mackerel and that only a few Arabs consumed it at least once a week. A similar percentage in each ethnic group ate no fresh tuna, while three quarters of Arabs consumed canned tuna at least once a month, as did more than half of SAs and BAs. Very few participants of any ethnic background ate sardines, either canned or fresh. Approximately half of Arabs consumed fresh salmon at least once a month, followed by BAs, while no more than 15% of any ethnic group reported eating canned salmon. Herring had low consumption rates, with only one member of each group consuming it weekly. Overall, ethnicity was not found to be significant in the consumption of fish (p>0.05).

Table 4.6: Frequency of consumption of fish group according to ethnicity

Food	Arab *n* = 28	South Asian *n* = 26	Black African *n* = 20	*p* value[$]
	n (%)			
Fresh mackerel				
Never	24 (85.7)	24 (92.3)	17 (85.0)	
1–3 times a month	3 (10.7)	2 (7.7)	3 (15.0)	0.92
Once or more a week[$]	1 (3.6)	0 (0.0)	9 (0.0)	
Fresh tuna				
Never	17 (60.7)	18 (62.2)	12 (60.0)	
1–3 times a month	7 (25.0)	6 (23.1)	5 (25.0)	0.93
Once or more a week[$]	4 (14.3)	2 (7.7)	3 (15.0)	
Canned tuna				
Never	7 (25.0)	11 (42.3)	9 (45.0)	
1–3 times a month	13 (46.4)	11 (42.3)	7 (35.0)	0.54
Once or more a week[$]	8(28.6)	4 (15.4)	4 (20.0)	
Fresh sardine				
Never	22 (78.6)	21 (80.8)	18 (90.0)	
1–3 times a month	3 (10.7)	1 (3.8)	1 (5.0)	0.72
Once or more a week[$]	2 (10.7)	4 (15.4)	1 (5.0)	
Canned sardine				
Never	24 (85.7)	22 (84.6)	17 (85.0)	
1–3 times a month	4 (14.3)	1 (3.8)	2 (10.0)	0.31
Once or more a week[$]	(0.0)	3 (11.5)	1 (5.0)	
Fresh salmon				
Never	10 (35.7)	14 (53.8)	9 (45.0)	
1–3 times a month	13 (46.4)	9 (34.6)	8 (40.0)	0.78
Once or more a week[$]	5 (17.9)	3 (11.5)	3 (15.0)	
Canned salmon				
Never	24 (85.7)	22 (84.6)	17 (85.0)	
1–3 times a month	4 (14.3)	2 (7.7)	3 (15.0)	0.50
Once or more a week[$]	0(0.0)	2 (7.7)	0 (0.0)	
Herring				
Never	25 (89.3)	13 (88.5)	19 (95.0)	
1–3 times a month	2 (7.1)	2 (7.7)	0 (0.0)	0.87
Once or more a week[$]	1 (3.6)	1 (3.8)	1 (5.0)	

[$] Some categories which had very few responses were collapsed into once or more a week.

Table 4.7 shows the frequency of the consumption of meat among by the ethnic groups. Beef was the most popular meat in each ethnic group, the majority of whom ate it more than once a week. The majority of participants never ate pork, but a fifth of SAs and of BAs did so daily. None of the Arabs and only two members of each of the other groups consumed any pork liver at all, while a quarter of participants of SA and BA origin had lamb's liver once or more a week. Overall, ethnic differences in the meat consumption of the groups studied were found to be statistically non-significant ($p>0.05$).

Table 4.7: Frequency of consumption of meat groups according to ethnicity

Food	Arab *n* = 28	South Asian *n* = 26	Black African *n* = 20	*p* value^
	n (%)			
Beef				
Never	8 (28.6)	7 (26.4)	6 (30.0)	
1–3 times a month	7 (25.0)	8 (30.8)	6 (30.0)	0.98
Once or more a week[$]	13 (46.4)	11 (42.3)	8 (40.0)	
Pork				
Never	27 (96.4)	21 (80.8)	15 (75.0)	
1–3 times a month	(0.0)	0 (0.0)	1 (5.0)	0.09
Once or more a week[$]	1 (3.6)	5 (19.2)	4 (20.0)	
Lamb/beef liver				
Never	12 (42.9)	10 (38.5)	7 (35.0)	
1–3 times a month	11 (39.3)	9 (34.6)	8 (40.0)	0.94
Once or more a week[$]	5 (17.9)	7 (26.9)	5 (25.0)	
Pork liver				
Never	28 (100.0)	24 (92.3)	18 (90.0)	
1–3 times a month	0 (0.0)	1 (3.8)	1 (5.0)	0.51
Once or more a week[$]	0 (0.0)	1(3.8)	1 (5.0)	

[$]Some categories having very few responses were collapsed into once or more a week. ^Chi-square test and Fisher's exact test.

Table 4.8 shows that a quarter of SAs consumed eggs daily, more than any other group, while more than half of participants in all ethnicities reported eating eggs at least once a week. Ethnicity was not found to be a significant factor in egg consumption (p>0.05).

Table 4.8: Frequency of consumption of eggs according to ethnicity

Food	Arab *n* = 28	South Asian *n* = 26	Black African *n* = 20	*p* value^
	n (%)			
Egg				
Never	0 (28.6)	2 (7.7)	2 (30.0)	
1–3 times a month	8 (25.0)	2 (7.7)	3 (30.0)	0.18
Once or more a week[$]	16 (46.4)	16 (61.5)	14 (70.0)	
Once a day	4 (14.3)	6 (23.1)	1 (5.0)	

[$] Some categories were collapsed into once or more a week; because of that, they contained a few number of responses. ^Chi-square test.

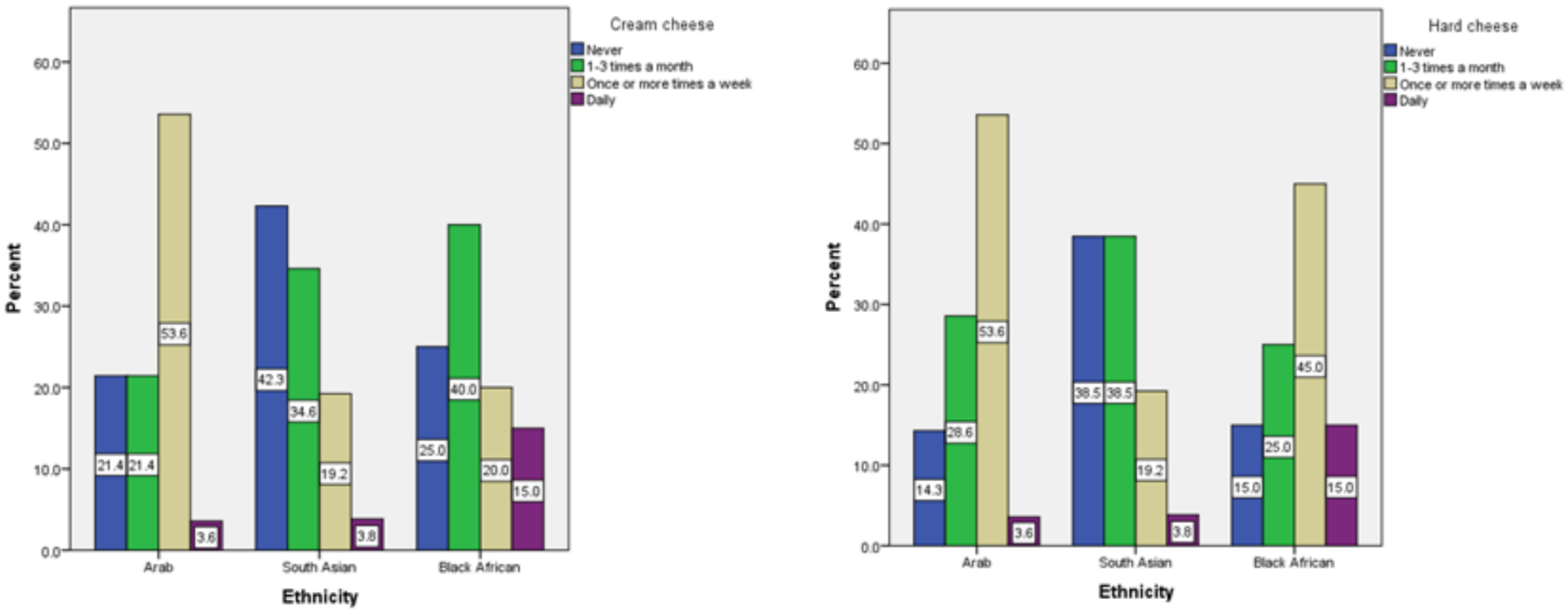

Figure 4.7: Frequency of consumption of cheese according to ethnicity

Similarly Figure 4.7 indicates no significant difference (p>0.05) between ethnic groups in their consumption of either cream cheese or hard cheese. More than half of Arabs consumed both types once and more a week, while 15% of BAs consumed them daily and around 40% of SAs consumed none of each type of cheese.

Table 4.9 lists results for the dairy group. The highest percentage of participants who reported never consuming yogurt was among the SAs, followed by the Arabs (50.0%), while 65% of BAs consumed it at least once a week and a few participants from each ethnicity reported daily consumption. More than half of Arabs and of SAs drank whole milk more than once a week, while consumption was slightly lower among BAs and the semi-skimmed alternative was avoided by at least half of every group.

Table 4.9: Frequency of consumption of dairy products according to ethnicity

Food	**Arab *n* = 28**	**South Asian *n* = 26**	**Black African *n* = 20**	***p* value[$]**
	n (%)			
Yogurt				
Never	14 (50.0)	16 (61.5)	7 (35.0)	
Once a week	5 (17.9)	6 (23.1)	6 (30.0)	0.41
2–3 times a week	6 (21.4)	2 (7.7)	6 (30.0)	
Once a day	3 (10.7)	2 (7.7)	1 (5.0)	
Whole milk				
Never	7 (25.0)	9 (34.6)	9 (45.0)	
Once week	2 (7.1)	4 (15.4)	3 (15.0)	0.55
2–3 times a week	8 (28.6)	6 (23.1)	2 (10.0)	
Once a day	11 (39.3)	7 (26.9)	6 (30.0)	
Semi-skimmed milk				
Never	14 (50.0)	17 (65.4)	10 (50.0)	
1–3 times a week	8 (28.6)	7 (26.9)	6 (30.0)	0.61
Once a day	6 (21.4)	2 (7.7)	4 (20.0)	

[$] Fisher's exact test.

Figure 4.8 shows that the majority of participants from each ethnic group consumed butter one or more times a month, while 39% of Arabs and 27% of SAs consumed it at least once a week. On the other hand, margarine was not favoured, as more than half of participants did not eat it at all (p>0.05).

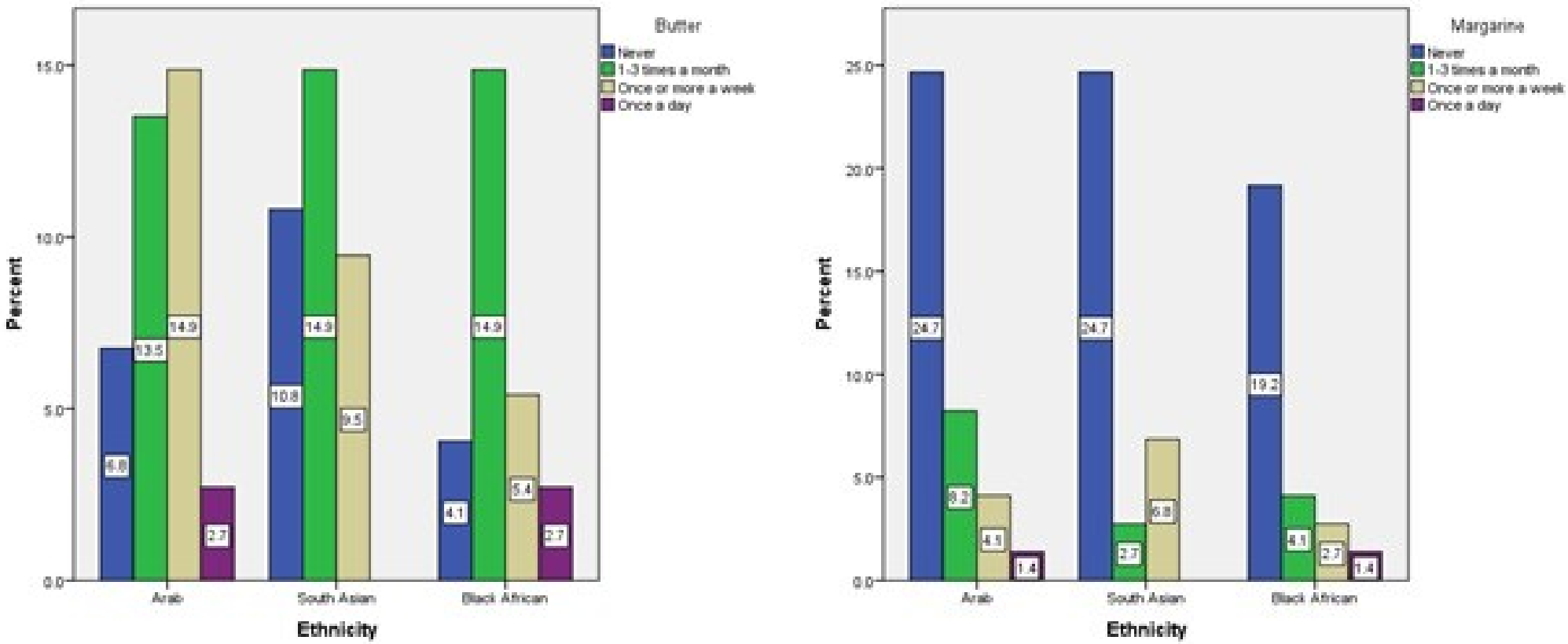

Figure 4.8: Frequency of consumption of fat products according to ethnicity

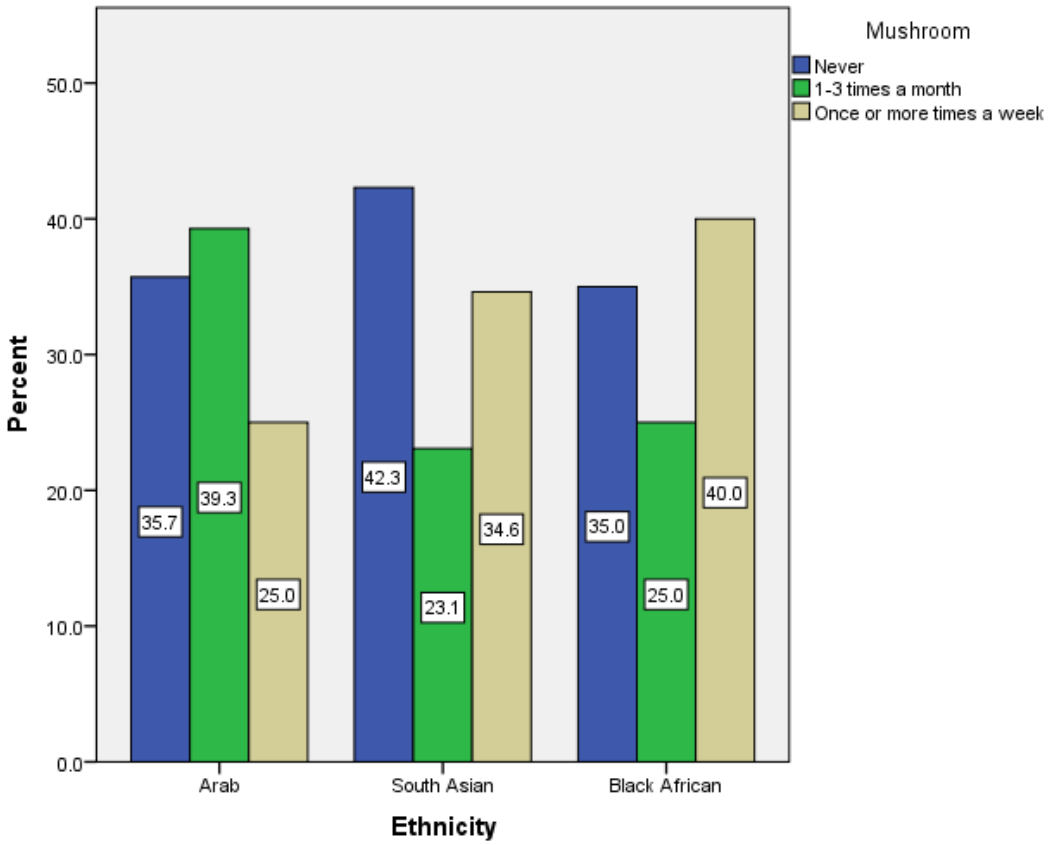

Figure 4.9: Frequency of consumption of mushrooms according to ethnicity

The consumption of mushrooms is shown in Figure 4.9. The largest percentage consuming them daily was 40% among BAs, followed by 34% of SAs (p=0.41).

As to breakfast cereals, Figure 4.10 shows that daily consumption was low in all groups, although two thirds of BAs, half of Arabs and around 40% of SAs ate cereals at least twice weekly. There was, however, no significant difference (p=0.67) in the consumption of breakfast cereal among the ethnic groups studied.

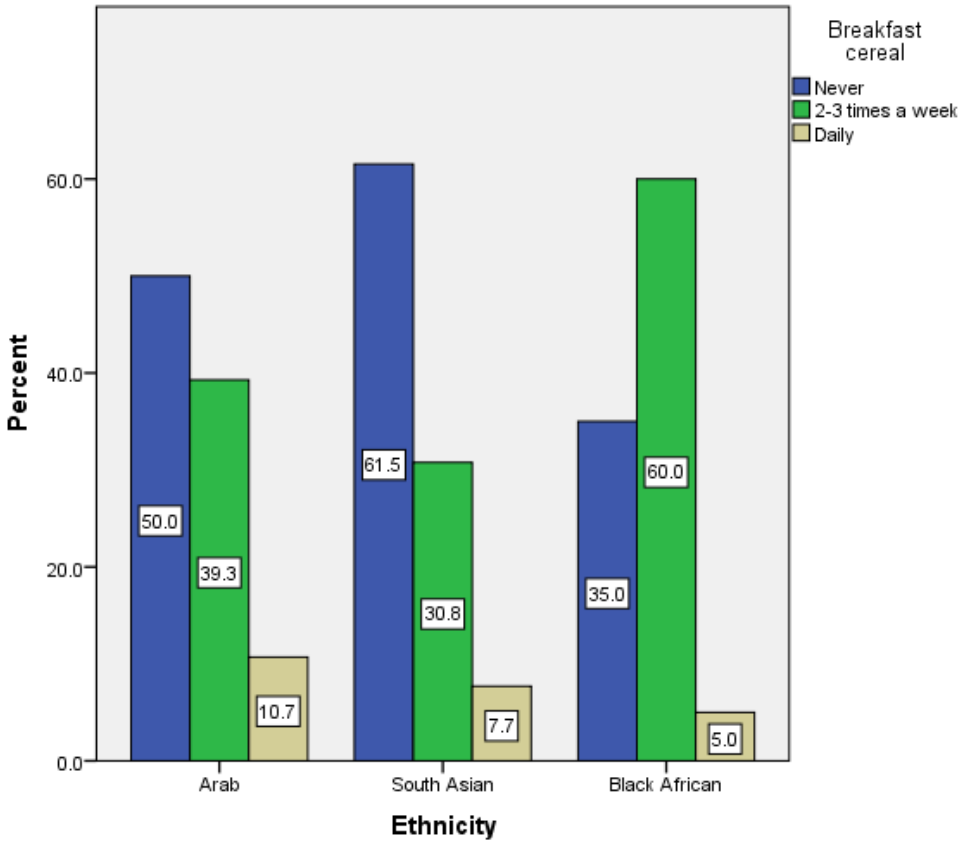

Figure 4.10: Frequency of consumption of breakfast cereal according to ethnicity

4.3.6.2 Estimates of vitamin D intake by ethnicity

Vitamin D intake from different sources including diet, sun and the use of supplements is presented in Table 4.10. The estimated mean value of dietary intake of vitamin D for all of the participants was 1.35 µg/day, considerably lower than the recommended value of 5 µg/day (WHO and FAO, 2004). The intake of vitamin D supplements was reported by less than a fifth of the total sample and by only 15% of BAs. Conversely, the highest dietary intake of vitamin D was among the BAs, at 2.31 µg/day, followed by SAs (1.75 µg/day). To examine the total intake of vitamin D (dietary, supplemental and sun), Equation 3 (Chapter 3, Section 3.2.5.2) was used. The highest total intake, which was among the BAs, at 2.76 µg/d is considerably below the RNI for vitamin D intake (10 µg/d) (SACN, 2015). None of these factors differed significantly by ethnicity ($p>0.05$).

Table 4.10: Estimates of vitamin D intake by ethnicity

Food	Arab n=28	South Asian n=26	Black African n=20	Total n=74	P value&
Vitamin D supplement (%)					
Yes	6 (21.4)	5 (19.2)	3 (15.0)	14 (18.9)	0.93^
No	22 (78.6)	21 (80.8)	17 (85.0)	60 (81.1)	
Average dietary intake of vitamin D (μg/day)[a]	1.56 (1.2)	1.75 (0.93)	2.31 (1.4)	1.26 (1.3)	0.20
Total oral vitamin D intake (μg/day)*[a]	2.31 (1.5)	1.98 (0.9)	2.49 (1.3)	2.20 (1.6)	0.43
Total vitamin D intake (μg/day)[a]**	2.39 (1.8)	2.24 (0.9)	2.76 (1.6)	2.38 (1.37)	0.19

[a] Data presented as median (IQR).
*Sum of vitamin D intake and supplement intake.
**Sum of vitamin D from diet, supplement and sunlight.
& Kruskal-Wallis H test was used to determine ethnic difference in vitamin D intake.
^Chi-square test.

4.3.6.3 Vitamin D status

The median level of vitamin D for all participants was 29.9 nmol/L, with a range of 11.8 to 112 nmol/L. The classification of vitamin D levels was based on the laboratory cut-off values as presented in Table 4.1. Figure 4.11 shows that the median level of serum 25(OH)D was approximately the same for the Arabs (29.7, range 19 to 80 nmol/L) and BAs (29.95, range 11.8 to 91 nmol/L) and lowest in the SAs (28.70, range 15.6 to 78.6 nmol). The Kruskal-Wallis H test showed that there was no statistical significance between ethnic groups in vitamin D status (p=0.43).

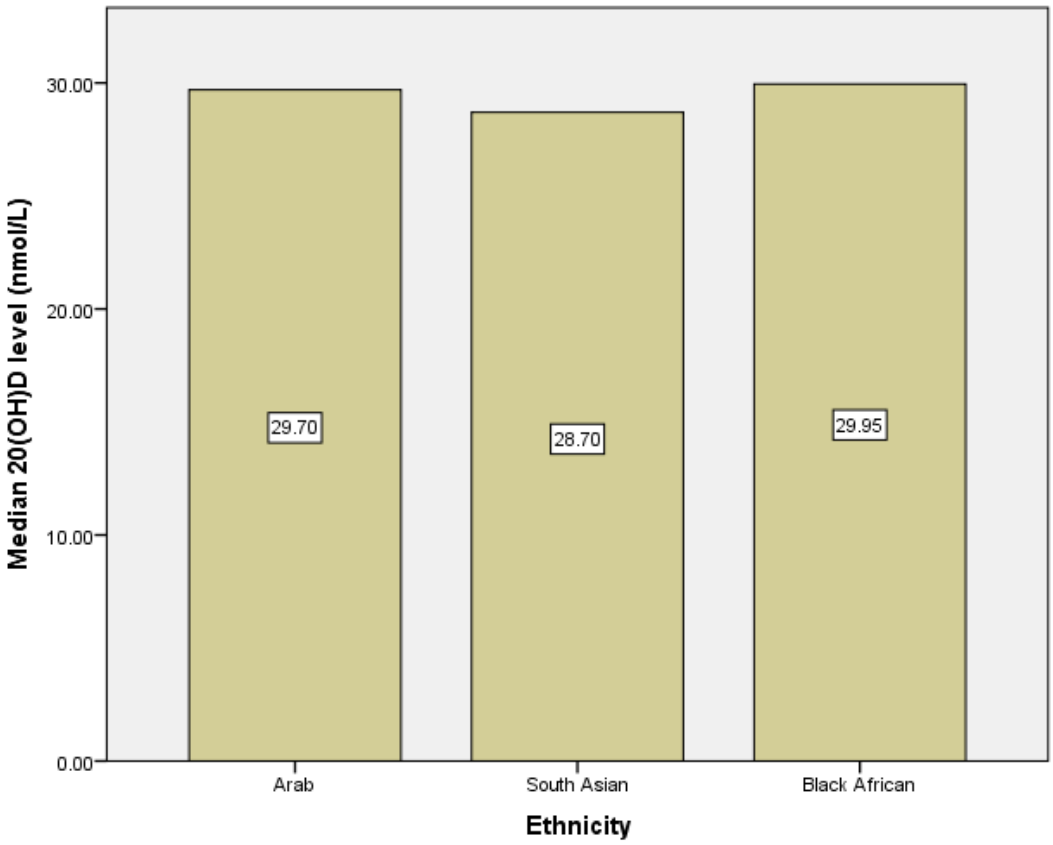

Figure 4.11: Median 25(OH)D levels (nmol/L) according to ethnicity.

The classification of vitamin D status for the whole population studied is shown in Figure 4.12. Little more than a quarter of the total sample had above the 50 nmol/L level which is deemed to be adequate, while more than half had a vitamin D deficiency and 4% had a severe deficiency.

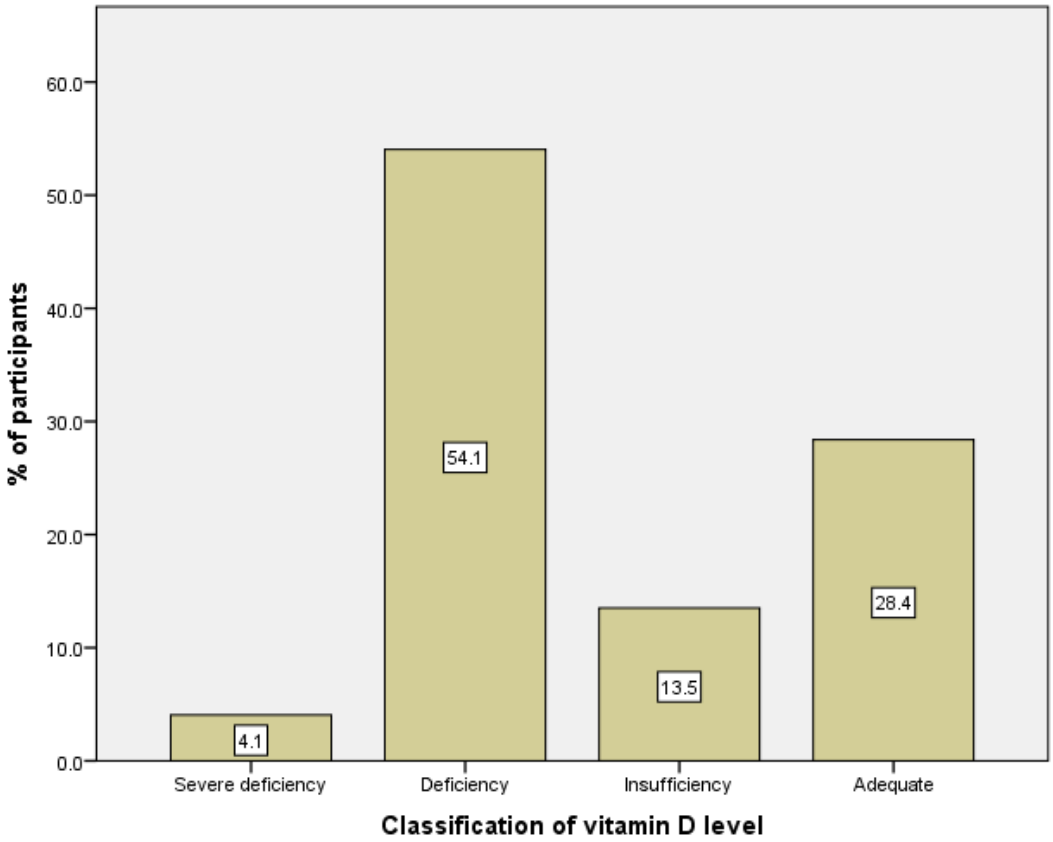

Figure 4.12: Percentage of participants by the classification of vitamin D levels (n=74)

The classification of vitamin D levels by ethnicity is presented in Figure 4.13. A few BAs and SAs had a severe deficiency, while an approximately equal percentage of SAs and Arabs had a deficient level of vitamin D. There was no significant variation according to ethnicity (p=0.58).

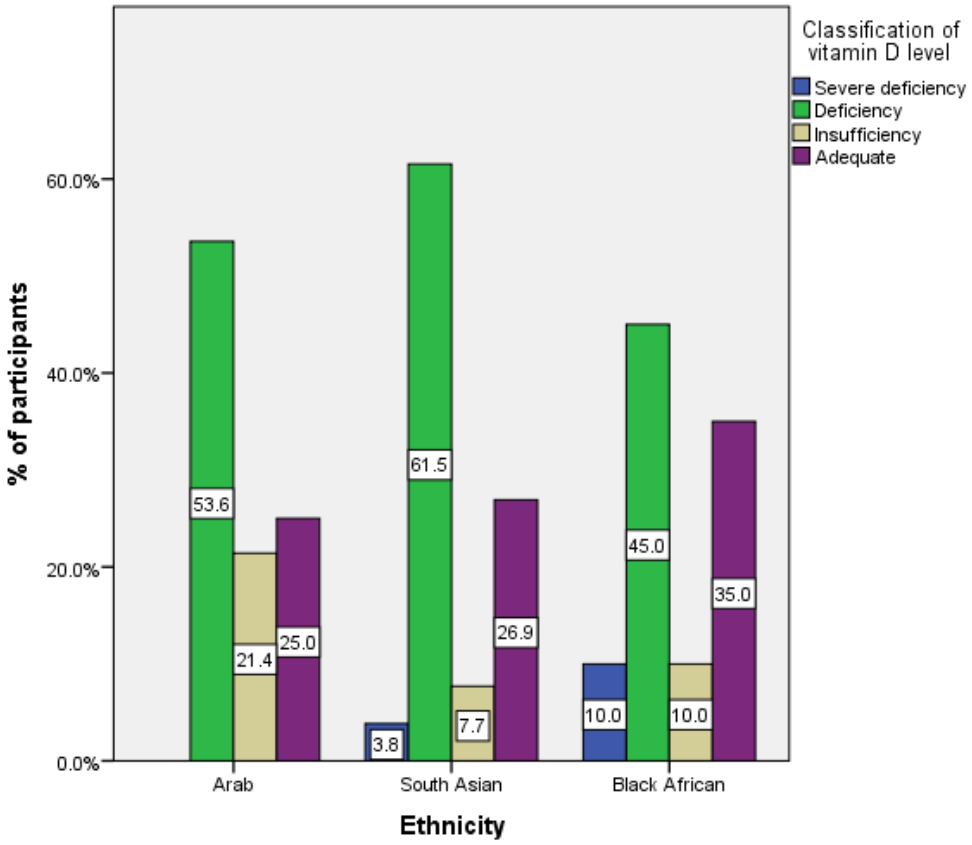

Figure 4.13 Percentage of participants by classification of vitamin D levels and ethnicity (n=74)

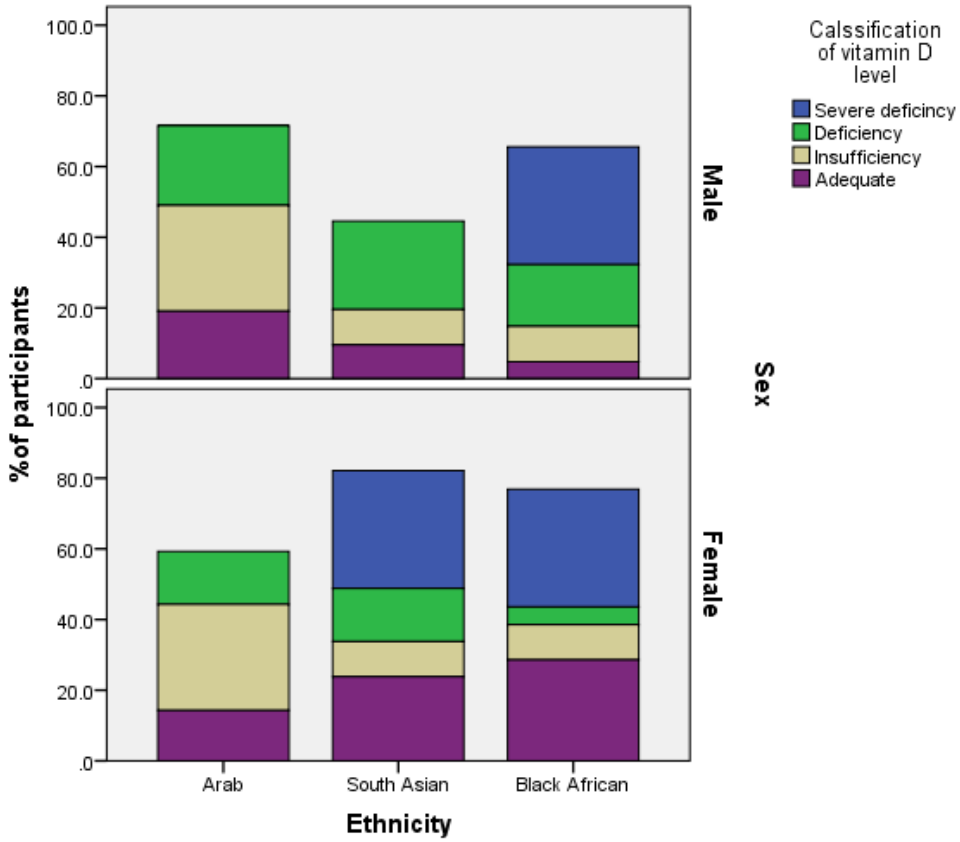

Figure 4.14: Percentage of participants by classification of vitamin D level, sex and ethnicity

Figure 4.14 shows that among the SAs and Arabs, the prevalence of low 25(OH)D concentration was higher in men than women, while among the BAs, more women than men had an adequate level of 25(OH)D concentration. A two-way ANOVA conducted to examine the effects of ethnicity and sex on 25(OH)D level found no statistically significant associations (p=0.31).

Figure 4.15 shows 25(OH)D level against education level ethnic grouping. Again, a two-way ANOVA was used and found no statistically significant effect of ethnicity and education on 25(OH)D level (p=0.52).

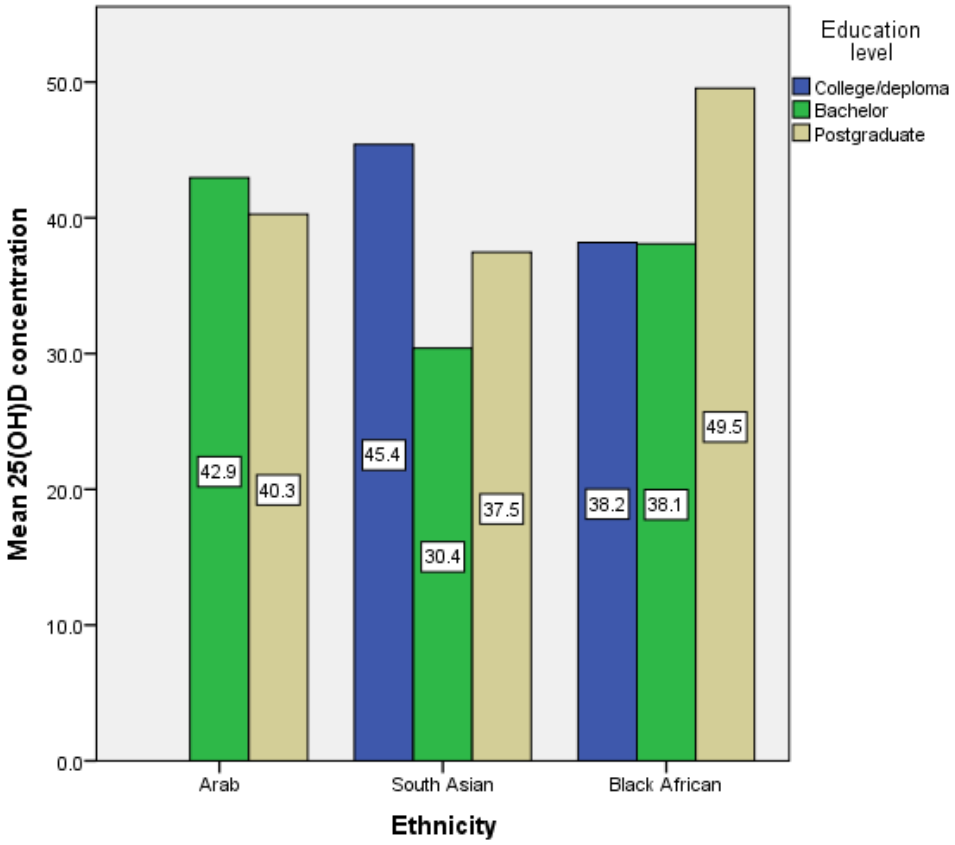

Figure 4.15: Distribution of mean 25(OH)D concentration by education and ethnicity

Figure 4.16 shows that a high level of vitamin D was associated with taking vitamin D supplements and that this association was approximately equally strong among all ethnic groups. When a two-way ANOVA was conducted to examine the effect of ethnicity and supplement intake on serum 25(OH)D level, it found a significant difference at for all supplement intake ($p<0.001$) but no significant main effect for ethnicity ($p=0.52$). There was no significant interaction between the effects of ethnicity and supplement intake on serum 25(OH)D level ($p=0.77$).

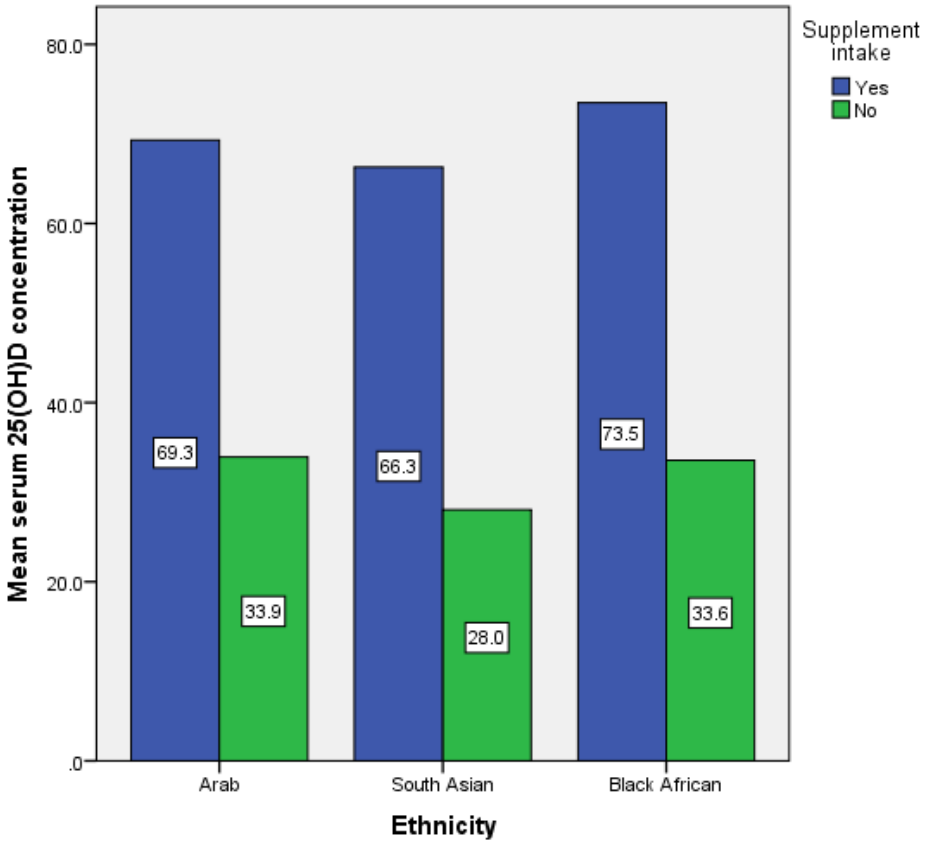

Figure 4.16: Distribution of mean 25(OH)D concentration by supplement intake and ethnicity

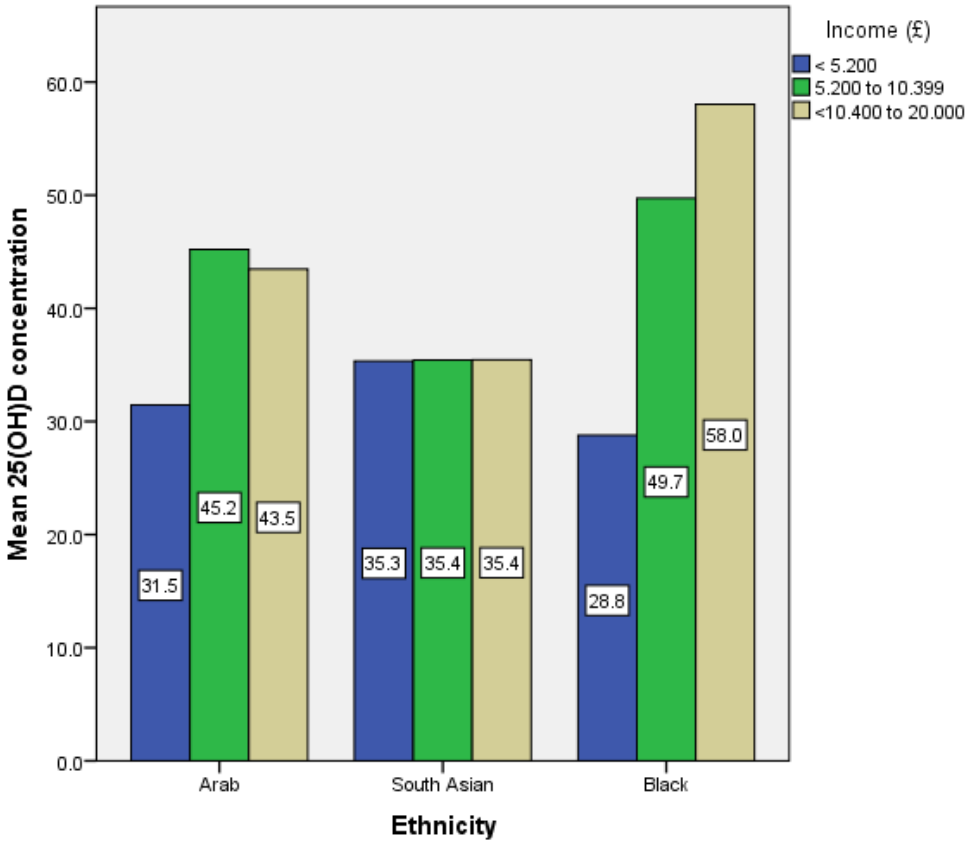

Figure 4.17: Distribution of mean 25(OH)D concentration by income and ethnicity

Figure 4.17 indicates that the mean level of vitamin D was greater in BAs who had a high income (over £10,400), while the Arab and SA participants with low income had a mean level of vitamin at 31 nmol/L and 35 nmol/L respectively. There was a significant main effect for income only (p=0.03). However, there was no statistically significant interaction between the effects of ethnicity and income on 25(OH)D concentrations (p=0.64).

4.3.6.4 Effects of different characteristics on 25(OH)D concentration

Multiple linear regression analyses were conducted to examine the relationships of predictors with 25(OH)D concentration and the results are tabulated in Table 4.11. After adjustment for sex, age, BMI, ethnicity, occupation and educational level, income status was seen to have a statistically significance association with 25(OH)D concentration. Participants who had an annual income of £5.200–£10.399 had higher levels of 25(OH)D than those receiving <£2,500 (B=0.135, 95% CI: 0.017, 0.252, p<0.001). Additionally, time

spent outdoors (B=0.002, 95% CI: 0.00, 0.00, p<0.03), supplement intake (B=0.855, 95% CI: 0.107, 0.636, p<0.001) and dietary intake of vitamin D (B=0.238, 95% CI: 0.115, 0.361, p<0.001) showed significant associations with 25(OH)D concentration.

Table 4.11: Effects of different characteristics on 25(OH)D concentration using multiple linear regression model

Variables	Coefficient (B)	95% CI	*p* value
Sex			
Female	Reference		
Male	0.04	−0.05, 0.15	0.35
Age	0.00	−0.00, 0.00	0.99
BMI(kg/m2)	0.00	−0.01, 0.01	0.69
Time spent outdoors	0.002	0.00, 0.00	0.03
Ethnicity			
Arab	Reference		
South Asian	−0.081	−0.20, 0.03	0.18
Black African	−0.016	−0.14, 0.11	0.80
Occupation			
Unemployed	Reference		
Student	−0.087	−0.282, 0.10	0.38
Employed	−0.002	−0.112, 0.10	0.97
Education level			
College/diploma	Reference		
Bachelor	−0.04	−0.22, 0.13	0.60
Postgraduate	0.00	−0.18, 0.17	0.96
Exposed area to the sun			
Face and hands	Reference		
Face and half arms	0.014	−0.04, 0.07	0.61
Face, full arms and full legs	0.046	−0.02, 0.11	0.17
Smoking (yes)	0.06	−0.04, 0.17	0.24
Alcohol consumption (yes)	0.09	−0.03, 0.22	0.14
Income status			
<5,200	Reference		
5,200–10,399	0.13	0.01, 0.25	0.02
10,400–20,000	0.12	0.00, 0.25	0.05
Supplement intake (yes)	−0.85	−0.107, −0.63	<0.001
Vitamin D intake (µg)	0.23	0.115, 0.361	<0.001

B: beta; CI: confidence interval; BMI: body mass index.

4.4 Discussion

The aim of the work reported in this chapter was to determine whether there was an association between lifestyle factors and levels of 25(OH)D among healthy ethnic minority adults living in Manchester. Seventy-four participants were categorised into three ethnic groups: Arab, South Asian and Black African.

4.4.1 Socioeconomic status and lifestyle factors

It is indicated in the literature that socioeconomic status has a significant impact on a person's vitamin D status (Dealberto, 2006). Lack of awareness and knowledge of vitamin D can lead to increased vitamin D deficiency, as can the inability to afford enough vitamin D fortified food or supplements to maintain a sufficient level of 25(OH)D (Brough et al., 2010). Therefore, it was important for this study to detect ethnic differences in socioeconomic status and examine the association between these factors and vitamin D level.

It has been reported that low socioeconomic status was related to the incidence of severe vitamin D deficiency among EMGs and obese adults (Webster, 2013; Léger-Guist'hau et al., 2017), whereas a large epidemiologic study showed an association between higher socioeconomic status and improved diet and health status (Darmon and Drewnowski, 2008). This was attributed to greater awareness and adoption of healthy dietary choices among the better-educated populations (Attorp et al., 2014) and to the ability to afford the higher cost of low-energy diets with higher nutritional quality (Maillot et al., 2007), as well as taking vitamin D-enriched food and supplements (Léger-Guist'hau et al., 2017).

Results of the current study identified no significant difference ($p>0.05$) in socioeconomic status variables, including income, education status and occupation, between the EMGs sampled. This result may be explained by the fact that merging ethnic categories in one group does not reflect actual socioeconomic status gradients between ethnicities. In spite of this, it was found that Arabs tended to report higher incomes than the other groups and have higher levels of education, with more than half of Arabs in the study having a postgraduate degree. It should be noted that this finding may not reflect rates in the UK population at large, as most Arab participants recruited had come to the UK to study.

The present findings point to the possibility that higher educational levels and socioeconomic status could, to some extent, account for Arab participants having the lowest percentage of vitamin D deficiency; these findings are in agreement with a previous study carried out by Ahmed et al. (2013). It would be interesting to study this aspect further on a larger cohort to see whether such a correlation exists among the minorities studied in Manchester.

It has been reported in the literature that a higher BMI, having been associated with lower vitamin D levels due to the deposition of vitamin D in body fat compartments, results in decreased bioavailability of vitamin D_3 (Vanlint, 2013; Wakayo et al., 2016). Thus, it was important to consider differences in BMI between ethnicities, but the current study found none of these to be significant, although the median BMI of Arab participants (26.3 kg/m^2) is defined as overweight, while the median BMIs of other ethnicities were within normal range. This indicates, interestingly, that the diet of Arab participants, despite their higher socioeconomic status, may not be any healthier than that of the other two groups in this study. The correlation between 25(OH)D levels and BMI was not statistically significant in this study, as the measured BMI levels showed insufficient variation; however, this subject warrants further examination.

Among lifestyle factors studied, alcohol intake varied significantly ($p=0.002$) based on ethnicity, but smoking status did not; the prevalence of drinking behaviour was higher in BAs (45%) than in the other two ethnic groups. These results are in line with a previous study carried out by Bayley and Hurcombe (2011), which suggested that this could be due to religious considerations, since alcohol intake is prohibited among Muslims (Michalak et al., 2007). Although smoking reportedly affects vitamin D metabolism (Lamberg-Allardt et al., 2001; Cheng et al., 2014; Kassi et al., 2015), this study found no significant correlation, which is in line with previous research (Rapuri et al., 2000; Arunabh et al., 2003; Lee, 2012). The role of alcohol in vitamin D levels is still not clear (Touvier, et al., 2015), but it has been hypothesised that alcohol intake results in the suppression of PTH secretion; PTH is responsible for decreasing the conversion of 25(OH)D to 1,25(OH)D_2, so high levels of unconverted serum 25(OH)D would be expected with moderate alcohol intake (Tardelli et al., 2017). Unlike previous studies (Grimes et al., 2011; Léger-Guist'hau et al., 2017), this study did not show socioeconomic status and health to be predictive of 25(OH)D concentration, which is likely to be because of the

relatively small size of the cohort studied. However, income was found to be a significant predictor of 25(OH)D concentration, which is consistent with a previous study (Webster, 2013).

4.4.2 Sun exposure behaviour and protection

Cutaneous production is a rich source of vitamin D, but is markedly reduced in the UK, which is situated at a high latitude where a large amount of solar radiation is lost in the atmosphere (Webb and Engelsen, 2008; Webb, 2006). In fact, it has been suggested that sufficient vitamin D levels may not be achieved even in the summertime at UK latitudes (Rhodes et al., 2010), particularly among people with highly pigmented skin (Farrar et al., 2011; Martin et al., 2016). Although the capacity to produce vitamin D cutaneously is similar across ethnicities (Holick, 2004a; Roy et al., 2007), longer exposure time may be needed for EMGs than for Caucasians because of their darker skin pigmentation (Farrar et al., 2013; Holick, 2004a).

Self-reported sun exposure behaviours was used to determine potential risk factors affecting vitamin D synthesis. The study found that time spent outdoors was ~90 minutes/d amongst EMGs, showing no significant difference (p = 0.88), which is in line with a study conducted on a multi-ethnic population (Van der Meer et al., 2008). Time spent outdoors was higher in the current study than that reported by SAs in previous studies (Hamson et al., 2003; Kift et al., 2013). As this study found that over half of the participants in all EMGs were students, it is not surprising that the majority, irrespective of ethnicity, reported spending most of their time indoors, particularly at midday, when the outdoor UV radiation levels are highest and more vitamin D is synthesised (Farrar et al., 2011). Therefore, it is reasonable to question whether the results concerning time spent outdoors can be generalised to the whole population in the UK. It has been shown in previous studies that lack of sun exposure also existed among outdoor workers from EMGs (Nesby-O'Dell et al., 2002; Skull et al., 2003; Kift et al., 2013). This suggests that the avoidance of sun exposure may be a problem that exists more widely among EMGs.

It is worth noting, however, that the average amount of time spent outdoors has been found to be lower among Cauc: A study by Kift et al. (2013) found that Caucs spent 63 minutes per day outdoors on average, while SAs spent 69 minutes. A similar result is reported by Webb et al. (2010), who found that Caucs spent an average of 70 minutes

per day outdoors. Regardless of shorter sun exposure times, 25(OH)D levels are expected to be higher in Caucs than in EMGs, because the former need less exposure time to attain a sufficient level (Holick, 2004a). This suggests that time spent outdoors does not necessary reflect the actual effective exposure due to the effect of clothing. Nevertheless, Caucs require exposure of only a quarter of the body to sunlight for 10 minutes at midday without applying sunscreen (Rhodes et al., 2010). Time spent outdoors was significantly associated (p=0.03) with vitamin D status in the present study, as has been reported previously by some researchers (Kim et al., 2012; Darling et al., 2013), but not by others (Madar et al., 2008; Van der Meer et al., 2008). There could be several explanations, including the differences in calculation and the effects of clothing and skin pigmentation.

Furthermore, it has been indicated that the amount of vitamin D produced by the skin is dependent on how much of the body surface area is exposed to sunlight (Macdonald 2013); therefore, covering the skin with clothing can reduce vitamin D synthesis (Rhodes et al., 2014; Webb et al., 2014). In the current study, the exposed body area differed significantly (p=0.002) between ethnicities, since skin exposure was markedly higher among BAs than any of the other groups studied, which is in line with previous studies (Patel et al., 2013; Van der Meer et al., 2008). This result may be explained by the fact that a majority of Arab and SA participants were Muslim and thus followed Islamic dress codes.

Although BAs tend toward greater body exposure, they have been shown to require longer exposure times to achieve adequate 25(OH)D levels (Farrar et al., 2013); this is consistent with the findings of earlier studies (Brough et al., 2010; De Roos et al., 2012). This study found no significant difference between Arabs and SAs in extent of body exposure, but the latter group was predominantly darker in skin colour; the Arab group had the lowest incidence of vitamin D deficiency, which may be partly attributable to this group being lightest in skin colour.

It is possible that a combination of environmental factors and lifestyle behaviours, including dress style and the use of sun-blocking cosmetics, contributes to decreased vitamin D production among EMGs and particularly SAs, who have a history of disproportionate vitamin D deficiency; although not statistically shown in this study, it

has been indicated that the use of sunscreen and the wearing of clothing that covers large areas of the body are predictors of vitamin D deficiency (Gillie, 2010; Holick, 2004b).

Latitude is well known as the major determinant of vitamin D status. Previous studies have been conducted on EMGs and Caucs in the UK and Europe to observe how high latitude affects vitamin D synthesis (Hyppönen and Power, 2007), with results suggesting that lifestyle must be adjusted to take advantage of sunlight during summer in order to achieve sufficient vitamin D levels. Kift et al. (2013) suggest that EMGs can maintain sufficient 25(OH)D levels through the winter by attaining 25(OH)D levels of at least 89 nmol/L by late summer.

4.4.3 Dietary intake of vitamin D

As mentioned earlier, few foods are naturally rich in vitamin D (Sinha et al., 2013). It was not unexpected that the dietary intake of vitamin D reported in this study was below the recommended level of 5µg/d (WHO and FAO, 2004) for all ethnicities, as it would be difficult to achieve sufficient vitamin D levels from food alone. This finding is consistent with British studies of SA (Darling et al., 2013; Kift et al., 2013; Webb et al., 2010), BA (Maxwell et al., 2006; Patel et al., 2013), and Middle Eastern (Ahmed et al., 2013) populations. Ethnic differences were not found to be significant in this study, which is consistent with two previous studies (Hall et al., 2010; Goodman et al., 2016).

Fish consumption was low among all participants, regardless of ethnicity. Although it has been recommended by Public Health England (2014) that one portion of oily fish be consumed per week, British studies have found fish consumption to be low among Caucs (Bates et al., 2012; Weichselbaum et al., 2013) as well as EMGs (Ahmed et al., 2013; Andersson et al., 2013; Lanham-New et al., 2011; Maxwell et al., 2006). Despite fish consumption being high in northern European countries such as Norway, EMGs there are reported to have the lowest intake of fish (Holvik et al., 2005; Madar et al., 2008). This suggests that immigrants often do not adopt the consumption of traditional foods of the host country.

Consumption of other dietary sources of vitamin D was relatively low among all group of participants, as reported in Chapter 3, Section 3.3.7 and discussed Section 3.4.3. The British Nutrition Foundation (2014) has recommended that an individual consume two to three 200 ml portions of milk, 150 g of yoghurt and 30 g of cheese per day. As dairy

products contain only small quantities of vitamin D, many countries tend to fortify them with vitamin D.

In this study, fewer than 20% of participants reported consuming fortified foods, while half of participants reported 'sometimes' doing so or stated their consumption as 'unknown', perhaps because the availability of foods fortified with vitamin D is limited in the UK market (NHS, 2011; O'Connor and Benelam, 2011). However, it is also possible that these participants were not aware of the types of vitamin D-fortified foods available, such as breakfast cereal and margarine, and thus had never sought them, or that they had indeed consumed such foods but were unaware that they were fortified. Data from the 2008–2009 National Diet and Nutrition Survey (NDNS) indicates that the dietary intake of vitamin D whether from natural or fortified foods, is low across the UK population (Whitton et al., 2011). Participants in the present study reported low consumption of vitamin D supplements, with fewer than 20% saying that they consumed them. Similar intakes have been reported in previous studies of SAs (Rees et al., 2005; Darling et al., 2013; Kift et al., 2013) and BAs (Holvik et al., 2005), as well as a studies involving Arab, BA and SA groups (Van der Meer et al., 2008). This study showed that the total daily intake of dietary vitamin D and vitamin D supplements, which did not differ significantly across ethnic groups, was a strong determinant of 25(OH)D concentration, agreeing with earlier published studies (Holvik et al., 2005; Nesby-O'Dell et al., 2002). In the absence of sun and natural food, taking vitamin D supplements is considered an effective way to maintain sufficient vitamin D level, especially during winter (Macdonald, 2013).

4.4.4 Assessment of vitamin D status

Levels of 25(OH)D were measured to determine vitamin D status and to identify vitamin D-related factors among EMGs. Assessment of 25(OH)D levels was performed by subjecting dry blood spot samples to LC-MS/MS analysis. The DBS method has been employed in some recent vitamin D studies and has been reported to be a reliable and valid alternative method for measuring plasma/serum vitamin D (Heath et al., 2014; Shea and Berg, 2017).

There has been considerable debate over the cut-off level of 25(OH)D to define hypovitaminosis D. Symptoms of skeletal disease (rickets and musculoskeletal pain)

typically occur in adults and children with levels of serum 25(OH)D below 30 nmol/L (Sinha et al., 2013), but some research has placed the threshold at below 25 nmol/L (Heaney, 2004). It has been suggested that every individual should have a vitamin D level of at least 75 nmol/L to ensure the beneficial effects of vitamin D on bone and muscle metabolism (Cashman, 2016), while 75–100 nmol/L is ideal when considering other health issues (Binkley et al., 2010; Holick and Chen, 2008; Sinha et al., 2013). In this study, the prevalence of vitamin D deficiency was generally high among all EMGs (≤30 nmol/L), and could relate to adverse clinical effects (IOM, 2011; Sinha et al., 2013). Skeletal disease has been reported among a number of EMGs (Hamson et al., 2003; Roy et al., 2007; Solanki et al., 1995), while Patel et al. (2013) estimate the risk of cardiovascular disease in BAs and SAs with low 25(OH)D to be 40% higher than in Caucs.

This study's finding of low 25(OH)D among SAs (28.7nmol/L) is in accordance with other British studies. Darling et al. (2013) found that 25(OH)D levels in SA participants averaged 20 nmol/L, lower than those in Caucasians (59.9 nmol/L). Farrar et al. (2011) report SAs as having an average 25(OH)D level of 16.2 nmol/L, while a study by Kift et al.(2013) found that 25(OH)D levels among SAs was 24.9 nmol/L. Levels of 25(OH)D reported by previous studies were lower than those in this study. There are multiple possible explanations for this inconsistency. For example, Darling's study focused on females only, and dress style may have affected the result; Farrar's study was conducted in the winter, when exposure to the sun is less frequent. This study's findings are in accordance with studies conducted on BAs (Bunn et al., 2004; Maxwell et al., 2006) and Arabs (Ahmed et al., 2013). Furthermore, this study accords with research showing vitamin D deficiency to be prevalent among minorities in other parts of Europe (Meyer et al., 2004; Madar et al., 2008; Eggemoen et al., 2013; Holvik et al., 2005; Cashman et al., 2016; Demeke et al., 2015; Van der Meer et al., 2006).

Although no significant ethnic differences in 25(OH)D concentrations were found in this study, it is worth noting that SAs had the lowest median 25(OH)D levels. This is consistent with the findings of Ford et al. (2006), Lowe et al. (2010) and Patel et al. (2013), who found SAs to have the lowest median 25(OH)D levels of the groups studied, and is possibly due to the effect of genetic variation in VDBP.

It has been found that vitamin D levels differ between sexes (Holvik et al., 2005; Zadshir et al., 2005; Webb et al., 2010); in particular, males have been shown to have higher serum 25(OH)D levels than females. A possible explanation for this is that many women habitually wear clothing that covers most of the body, increasing the risk of vitamin D deficiency. Other studies found no significant difference (Hagenau et al., 2009; Van der Meer et al., 2008) and, interestingly, neither did this study. Within ethnicities, severe deficiency was higher among SA females than males, in line with an earlier study by Ford et al. (2006) this is likely to be due to the dress style of SAs (Roy et al., 2007). It has been found that more BA females than males have adequate vitamin D levels (Patel et al., 2013). In contrast, Pal et al. (2003) found no statistical differences in EMGs' vitamin D levels based on sex, which is in agreement with the results of the present study. This may be due to a lack of adequate sunlight in Manchester, where even a high degree of body surface area exposure may not allow for enough vitamin D. The observation that vitamin D deficiency occurs more frequently in SA and BA males than in SA and BA females warrants further study.

While latitude and season have also previously been found to be predictors of vitamin D status (Tsiaras and Weinstock, 2011), participants were recruited for the current study in autumn between October and December, hence, the only significant predictors were income status, dietary intake of vitamin D and supplement intake. According to Webb et al. (2016), Vitamin D cannot be produced between October and March, so it has been suggested that individuals can meet winter requirements of 25(OH)D through vitamin D stores in the body acquired during the previous summer (Macdonald et al., 2008). Kift et al. (2013) report that EMGs could maintain sufficient 25(OH)D levels during the winter months by having 25(OH)D levels of at least 89 nmol/L at the end of summer. It would appear from the current result that the Manchester EMGs did not derive enough benefit from the summer sun to maintain sufficient 25(OH)D levels during winter, meaning that vitamin D supplementation was required. These findings are in line with previous multi-ethnic studies (Holvik et al., 2005; Nesby-O'Dell et al., 2002); it would be likely that people in Manchester, especially members of ethnic minority groups, have insufficient levels of vitamin D, due to their low consumption of dietary vitamin D and to not taking supplements (Holick, 2006).

4.5 Conclusion

The link between the lack of sun exposure due to skin colour and cultural factors has been shown previously among minorities not originating from the northern hemisphere (Mishal 2001; Madar et al., 2008; Reed et al., 2007). While the association is important, it is appreciated that challenging such behaviours as mode of dress would be difficult, if not impossible, in the majority of those communities (Reed et al., 2007). The low intake of fortified foods and supplements, coupled with low income, could account for the low intake reported in this study. Thus, low levels of education and income influenced vitamin D intake, which would affect vitamin D status (Chowbey and Harrop, 2016). It is recommended that preventative and health education programmes be introduced, targeting the population identified as at risk. Farrar et al. (2011) rightly assert that the sunlight exposure and vitamin D intake recommendations need to be reviewed in light of evidence that a higher level of melanin reduces the skin's ability to synthesise this vitamin. Kim et al. (2012) propose the introduction of 'specific vitamin D educational programmes that can target certain groups according to beliefs about vitamin D and demographic characteristics'.

5. THE EFFICACY OF CALCIFEDIOL SUPPLEMENTATION IN INCREASING 25(OH)D CONCENTRATIONS

5.1 Introduction

The prevalence of vitamin D deficiency among EMGs has been demonstrated in the work reported Chapter 4 and in previous studies cited in this thesis (Roy et al., 2006; Farrar et al., 2011; Webb et al., 2016). Vitamin D supplementation has been suggested to be an effective option for maintaining vitamin D at an optimal level (Dawson-Hughes et al., 2005; Wicherts et al., 2011; Sinha et al., 2013).

There is a consensus among researchers that the level of 25(OH)D should be at least 75 nmol/L (Dawson-Hughes et al., 2005; Holick et al., 2011) and that maintaining this optimum level is important not only for bone health but also to prevent non-skeletal disease (Holick et al., 2011). However, many people, especially those who have high skin pigmentation, do not reach this level (Farrar et al., 2011; Buttriss, 2015; NICE, 2016). Recently, a new recommended intake (RNI) of 10 µg/d has been set for adults (SACN, 2015). However, it has been reported that a dose of 10 or 20 µg/d may result in a level of only 50 nmol/L and that a daily dose of 40 to 100 µg may be needed to reach the target of 75 nmol/L (Bischoff-Ferrari et al., 2010). There is much evidence that a daily intake of 20 µg of D_3 taken over 2-3 months could help to achieve a 25(OH)D level of 75 nmol/L (Harris et al., 2002; Nelson et al., 2009; Gallagher et al., 2012).

Alternatively, the optimal level of vitamin D could be achieved in a short duration by taking a single dose of 2500 µg vitamin D_3 (Bischoff-Ferrari et al., 2012) or 175–250 µg/day (Pludowski et al., 2013b), but concerns have been raised that the use of these mega doses of vitamin D to correct hypovitaminosis D may lead to toxicity (Brandi and Minisola, 2013). Given these concerns and the difficulty of attaining an optimal 25(OH)D level and eliminating the symptoms of hypovitaminosis D with a small dose over a short period (Bischoff-Ferrari et al., 2006; Wicherts et al., 2011; Cashman et al., 2016), an alternative strategy of using calcifediol would be beneficial.

Researchers have recently examined the effect of using calcifediol to correct vitamin D deficiency among older adults (Russo et al., 2011; Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Jetter et al., 2014), although published research is still limited on multi-ethnic young adults. Therefore, the present efficacy trial was performed to investigate the effect of calcifediol in enhancing 25(OH)D levels and treating vitamin D deficiency among EMGs (since deficiency is more common among this population) living in Manchester

over a short period. This study is the first of its kind to be conducted in the UK, as calcifediol is not readily available here, so that it could be considered as another option for treatment of hypovitaminosis D in the UK.

This chapter presents the method, results and analysis of the calcifediol efficacy study, whose aims and objectives were as follows.

5.1.1 Aim

- Investigate the efficacy of calcifediol supplementation, and its effects on improving vitamin D levels in deficient adults from ethnic minority groups.

5.1.2 Objectives

- Investigate the effect of 140 µg/week calcifediol supplementation on vitamin D levels among deficient ethnic minority participants.
- Measure serum 25(OH)D concentrations for ethnic minority participants after five weeks of supplementation.
- Examine the persistence effects of calcifediol after five weeks of termination of supplementation.
- Analysis of serum 25(OH)D concentrations at baseline (pre-intervention), mid-intervention and post-intervention.

5.1.3 Research hypothesis

Supplementation with 140 µg calcifediol, per week, will result in a rapid improvement in the vitamin D status of deficient ethnic minority adults.

5.2 Methodology

5.2.1 Sample size considerations

Sample size was determined using Minitab software, version 16 (Minitab Inc., State College, PA, USA). The calculation of the optimal sample size for the efficacy study are based on three major principles: sample number, based on earlier approved published work; the availability of participants for recruitment, including ethical considerations; and the statistical boundaries. The sample size was estimated using the power size method with an alpha of 0.05, a power of 80% and predicted standard deviations (SDs) used in previous studies (Barger-Lux et al., 1998; Jetter et al., 2014). The predicted SDs in these studies were 13.2 ng/ml and 19.9 ng/ml, respectively. According to the calculation using the predicted data, the minimal sample size is between five and nine participants for each group; however, a higher sample size than the calculated values would help to achieve more precise results and cover any participant withdrawals or exclusions during the study. A minimum sample size of 45 participants, anticipating a 25% dropout rate, is a number typically used in the literature (Jetter et al., 2014; Cashman et al., 2012; Cavalli et al., 2009).

5.2.2 Study population

Potential volunteers were recruited in Manchester between July 2016 and September 2016 by using the questionnaire and posters described in Chapter 3, Section 3.2.3, and chapter 4, Section 4.2.1. The inclusion criteria for participation are outlined in Chapter 3, Section 3.2.2. Participants who had chronic disease were pregnant or lactating, were vegan or vegetarian, or were using medication that could interfere with vitamin D metabolism (e.g. phenobarbital and phenytoin) were excluded from the study. Additionally, participants were excluded if they were taking a vitamin D supplement or experienced significant sun exposure (via a trip to sunny countries/visits to a tanning salon) during the study, or were unable/unwilling to return for follow-up.

5.2.3 Study protocol

An outline of the study protocol is shown in Figure 5.1. In the initial phase, interested individuals attended a first meeting with the researcher to learn more about the study, to read the information sheet, and to have their eligibility assessed using a brief self-

administered questionnaire, covering health, lifestyle and vitamin D intake prior to study enrolment (Appendix 4). Other participants received the information sheet via email and agreed to participate in the study by completing the questionnaire.

The efficacy trial was conducted from October 2016 for 10 weeks.

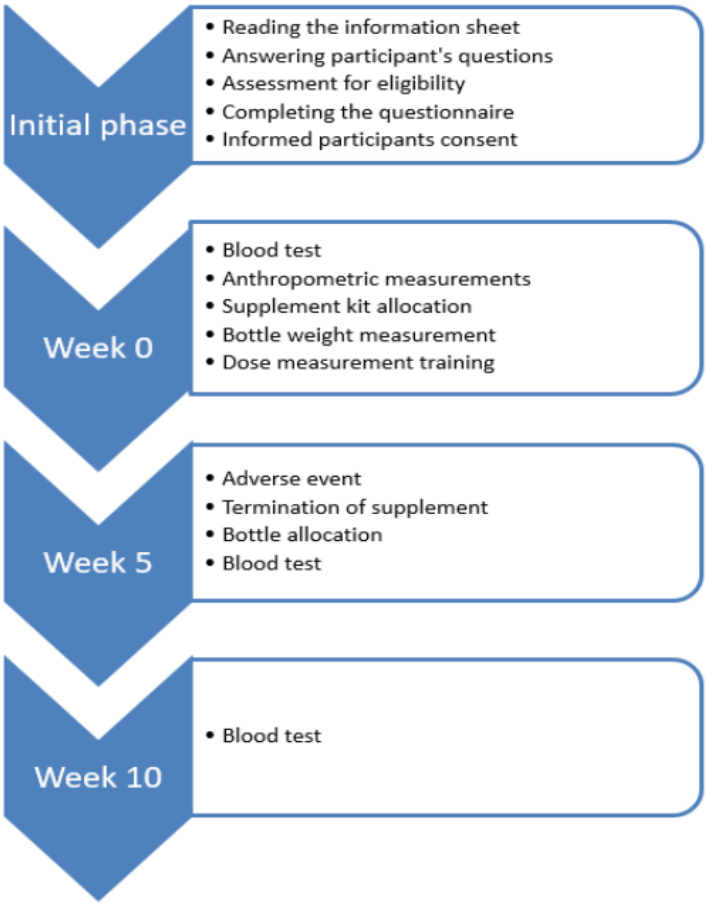

Figure 5.1: Flow diagram of efficacy study protocol

As shown in Figure 5.1, eligible participants attended three times over the 10-week study period: at baseline (week 0), then at week 5 and at week 10. Study visits took place at a physiology lab based at Manchester Metropolitan University. At week 0, the anthropometric measurements of participants were taken (Section 5.2.3.4) and blood samples were collected (see section 5.2.3.3) to confirm vitamin D levels ≤30 nmol/L (10 ng/ml). In addition, the participants met with the researcher for supplement allocation

and to be trained to measure the exact dose (Section 5.2.3.2). They were advised to follow their usual diet intake, to avoid any food-containing vitamin D or fortified food during the study and to contact the researcher with any queries. A blood sample was collected during the second visit, at the midpoint of the trial period (week 5) to detect any improvement in vitamin D levels and to ensure safety. Participants were instructed to stop the calcifediol and not to take any other supplement or vitamin D-fortified food for a minimum period of five weeks. Once the trial was nearing completion, they received an email or text message for a follow-up examination appointment. At this follow-up visit (week 10), the final blood samples were taken from participants to detect supplement persistence (Appendix 7).

5.2.3.1 Calcifediol supplementation

As shown in Figure 5.2, each participant received a kit containing the supplement, an instruction leaflet and an oral syringe. The supplement was given as a liquid in a bottle of 10 ml of calcifediol (Bruno Farmaceutici S.p.A., Rome, Italy), each drop containing 5 µg of 25(OH)D_3 as indicated by the supplier. Participants were asked to take 28 drops (140 µg) per week for five weeks. To avoid counting errors that might occur while taking the drops, participants were instructed to administer it using a 1 ml oral syringe (28 drops = 0.93 ml) (Figure 5.3) (Sobhani et al., 2008; Elliott et al., 2014). According to Jetter et al. (2014) and Bischoff-Ferrari et al. (2012), serum 25(OH)D concentrations did not significantly differ according to administration frequency (weekly or daily). To minimize error, the instruction sheet provided information on dose frequency, length of treatment, storage and instructions for dose measurement and consumption (Appendix 6). Furthermore, participants took the first dose in front of the researcher, demonstrating that they were clear as to how they should measure the exact dose. In the period between weeks 5 and 10, participants were asked to stop taking the supplement and to avoid taking any supplement containing vitamin D or vitamin D-fortified food.

Figure 5.2: The supplementation kit

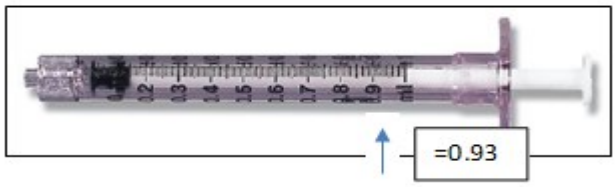

Figure 5.3: The oral syringe used for taking the supplement

5.2.3.2 Adherence monitoring

In order to maximize adherence (their commitment to taking the supplement), participants were given a table in the leaflet to record the dose taken and were asked to bring it at follow-up stage (Appendix 6). Apart from recording dose times, participants were asked to bring the supplement bottle on follow-up visits in order that the researcher could assess their adherence (Williams et al., 2013) by measuring the bottle's pre- and post-trial weight using an analytical balance (AS X2 Analytical Balance, Radwag, Radom, Poland). During the trial, participants received reminders to take the supplement via text messages and phone calls from the researcher; this method has been shown to improve medication adherence by 17.8% (Strandbygaard et al., 2010).

5.2.3.3 Safety

A weekly dose of 140 µg of calcifediol is based on published findings on its efficacy and safety among elderly populations (Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Jetter et al., 2014) and adults (Barger-Lux et al., 1998; Russo et al., 2011). Participants were asked to contact the researcher if they encountered any problems during the study such as weakness, fatigue, anorexia, nausea, vomiting, or constipation.

5.2.3.4 Blood collection

Blood samples were collected three times, at baseline, mid-study and at the end of the study using a Vitamin D blood spot test (Chapter 4, Section 4.2.3.2). Vitamin D deficiency was defined as having a vitamin D concentration at 30 nmol/L or below.

5.2.3.5 Anthropometric measurements

Height and weight were measured without shoes or heavy clothing on the date of the first visit using the methods described in Chapter 4, Section 4.2.2.3.

5.2.4 Statistical analysis

The data were analysed using the SPSS software package, version 21 (SPSS Inc., Chicago, IL, USA). Descriptive analysis was used to examine the socio-demographic data for different ethnicities and biochemical measurements. The results were given as a median and interquartile range (IQR) for skewed data, and mean and SD for normally distributed data. The data were first checked for normality and were found to be skewed, so could not be normalised using a transformation; hence a non-parametric test was used to analyse the result. The Wilcoxon signed-rank test was used to assess the change in serum 25(OH)D concentrations (pre–post supplement) within all samples and for subgroups. The Kruskal-Wallis H test was used to assess the differences between EMGs. Additionally, the Friedman test was performed to analyse the changes in serum 25(OH)D between baseline, week 5 and week 10 within all participants (pre–mid–post). It is worth noting that not all participants were included in the inferential statistics, because two had dropped out at the mid-point and another at the final test stage.

Correlation tests were run to determine the relationship of some variables such as BMI and age with serum 25(OH)D level at mid-point. Multiple linear regression analysis, using as dependent variables 25(OH)D concentration at week 5 and week 10, was performed to determine the significance of several explanatory variables including age, sex, BMI, baseline 25(OH)D, smoking, dietary intake of vitamin D and sun index.

5.3 Results

5.3.1 Recruitment of participants

A total of 102 participants were recruited for the study. Twenty-eight of these were excluded from the study due to pregnancy, having chronic disease or for taking vitamin D supplement. Only 74 participants met the primary inclusion criteria and were subsequently tested to determine their vitamin D status. Forty-two participants who had vitamin D level ≤ 30 nmol/L were deemed eligible, subsequently started on the trial in October 2016 and were given the calcifediol supplement. Two participants were excluded from the study after the baseline test because they were unwilling to comply and one other participant failed to respond to follow-up for the final test. Thus, as. Figure 5.4 shows, a total of 39 sets of data were subjected to analysis.

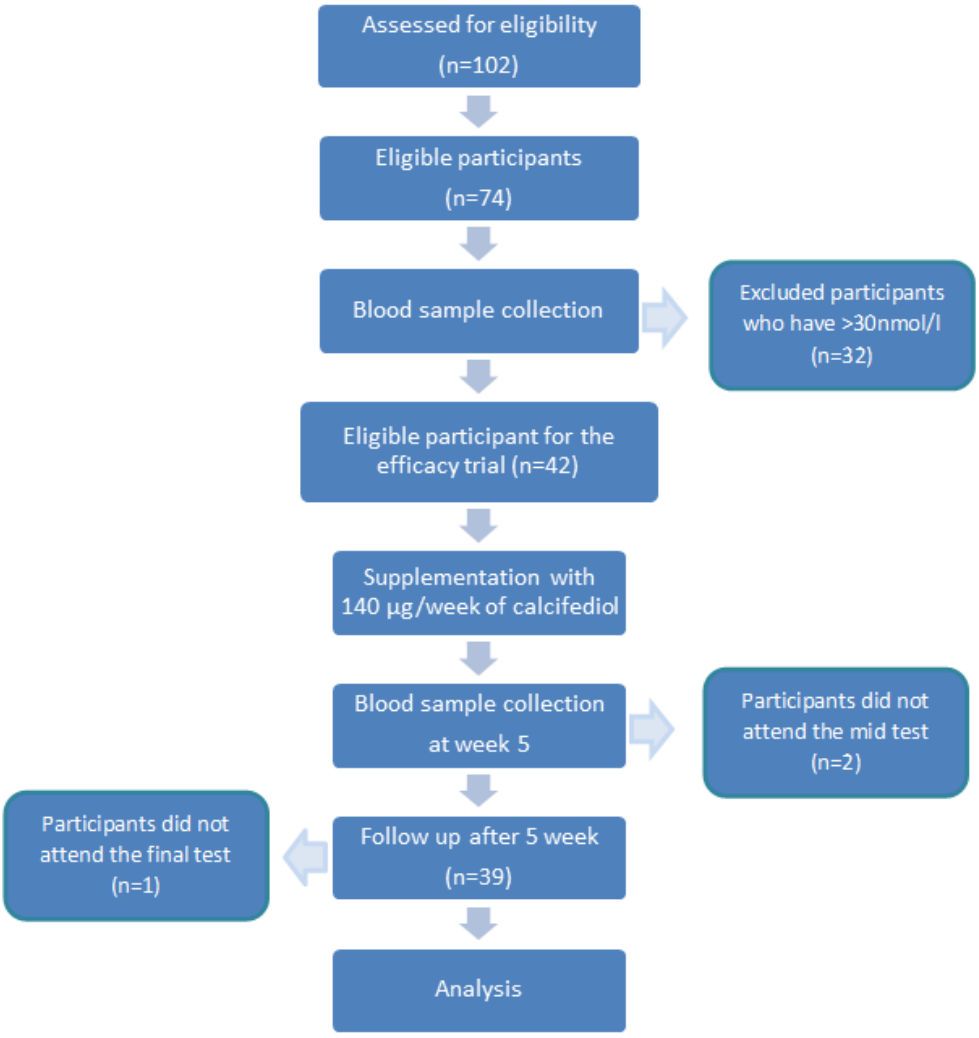

Figure 5.4: Flow diagram of study participants

5.3.2 Participants' demographic, health and vitamin D intake characteristics

The socio-demographic, anthropometric and dietary data are summarised in Table 5.1. The participants were divided into three groups according to their ethnicity: Arab, South Asian and Black African. Sixty-two percent of participants were male and the mean age of the sample was 30.4 years (range 18 to 48 years). Looking at socioeconomic status in Table 5.1, more than half of participants were students and almost a third were employed. The highest percentage of employed participants was found in the Arab group (60%). A little less than half of respondents received an average income of £5,200 to £10,399, while only a fifth received in excess of £21,000. None of the demographic characteristics differed significantly between the ethnicities (p>0.05).

Table 5.1: Baseline characteristics of the total sample according to ethnicity (n=42)

Variable	Arab n=15	South Asian n=15	Black African n=12	Total sample n=42	P-value[$]
	n (%)				
Age (years)*	32.2 ± 7.4	29.3 ± 5.4	29.2 ± 9.7	30.3 ± 7.5	0.68
BMI(kg/m^2)*	28.0 ± 3.3	24.2 ± 5.2	26.1 ± 4.0	26.4 ± 4.4	0.68
Sex					
Male	10 (66.7)	8 (53.3)	8 (66.7)	26 (61.9)	0.92
Female	5 (33.3)	7 (46.7)	4 (33.3)	16 (38.1)	
Education level					
College/diploma	4 (26.7)	4 (26.7)	5 (41.7)	13 (31.0)	
Bachelor	3 (20.0)	6 (40.0)	4 (33.3)	13 (31.0)	0.59
Postgraduate	8 (53.3)	5 (33.3)	3 (25.0)	33 (38.1)	
Annual income (£)					
<5,200	4 (26.7)	7 (46.7)	8 (66.7)	19 (45.2)	
5,200–10,399	6 (40.0)	5 (33.3)	3 (25.0)	14 (33.3)	0.36
10,400–20,000	5 (33.3)	3 (20.0)	1 (8.3)	9 (21.4)	
Occupation					
Employed	6 (40.0)	4 (26.7)	3 (25.0)	13 (31.0)	
Unemployed	0 (0)	2 (13.3)	2 (16.7)	4 (9.5)	0.71
Student	9 (60.0)	9 (60.0)	7 (58.3)	25 (59.5)	
History of vitamin D deficiency					
Yes	2 (13.3)	5 (33.3)	3 (25.0)	10 (23.8)	0.44
No	13 (86.7)	10 (66.7)	9 (75.0)	32 (76.2)	
Smoking					
Yes	3 (20.0)	3 (20.0)	3 (25.0)	9 (21.9)	1.00

Variable	Arab n=15	South Asian n=15	Black African n=12	Total sample n=42	P-value[$]
	n (%)				
No	12 (80.0)	12 (80.0)	9 (75.0)	33 (78.6.3)	
Alcohol intake					
Yes	0 (0.0)	1 (6.7)	3 (25.0)	4 (9.5)	0.19
No	15 (100.0)	14 (93.3.0)	9 (75.0)	38 (90.5)	
Dietary intake of vitamin D (µg/d)**					
Total intake	1.41 (1.01)	1.36 (1.01)	1.42 (1.26)	1.41 (1.03)	0.87
Oily fish intake	0.95 (0.57)	0.95 (1.56)	0.77 (0.46)	0.95 (0.59)	0.83
Meat intake	0.03 (0.02)	0.7 (0.11)	0.05 (0.12)	0.04 (0.05)	0.21
Egg intake	0.26 (0.17)	0.18 (0.17)	0.09 (0.46)	0.15 (0.19)	0.81
Dairy intake	0.16 (0.20)	0.15 (0.19)	0.10 (0.24)	0.15 (0.21)	0.83
Time spent outdoors (minutes)**	86.2 (116.25)	101.2 (91.8)	101.2 (86.2)	97.5 (93.7)	0.89
Sun index (hours/week)**	0.24 (0.05)	0.31 (0.31)	0.32 (0.52)	0.27 (0.35)	0.77

*Mean ± SD. **Median interquartile range (IQR). [$]Chi-square test was used to examine the association between ethnicity and socioeconomic factors.

[$]Kruskal–Wallis H test was used to find the difference between ethnicity and age, BMI, dietary intake and sun exposure.

Health information is also summarised in Table 5.1. All participants stated that they had no health problems. Three-quarters had not experienced deficiency and the remaining 24% had all had a diagnosis of vitamin D deficiency at some time. The mean BMI was 26.4 kg/m^2, which was classified as overweight, with most of these being from the Arab and BA groups. The dietary intake of vitamin D was not significantly different across ethnicities (p=0.87). Oily fish was the highest contributing dietary source of vitamin D among all ethnicities, followed by egg products, while meat intake was the lowest contributing food group, with a mean of just 0.08 µg/d. The median time spent outdoors for all participants was 94 minutes and the shortest time spent outdoors was reported by the Arab group, with a median value of 86.2 minutes.

5.3.3 The effect of using calcifediol supplement to increase vitamin D level

The changes in 25(OH)D concentration between pre- and post-supplementation for all participants are shown in Figure 5.5. The median level of 25(OH)D increased from 26.6

nmol/L to 151.4 nmol/L, with no adverse effect reported by the participants. The Wilcoxon signed-rank test was performed and the results reveal a steep and highly significant ($p<0.001$) increase in vitamin D level after supplementation. Changes in median 25(OH)D concentrations (week 5 minus pre-test) according to ethnicity are compared in Table 5.2.

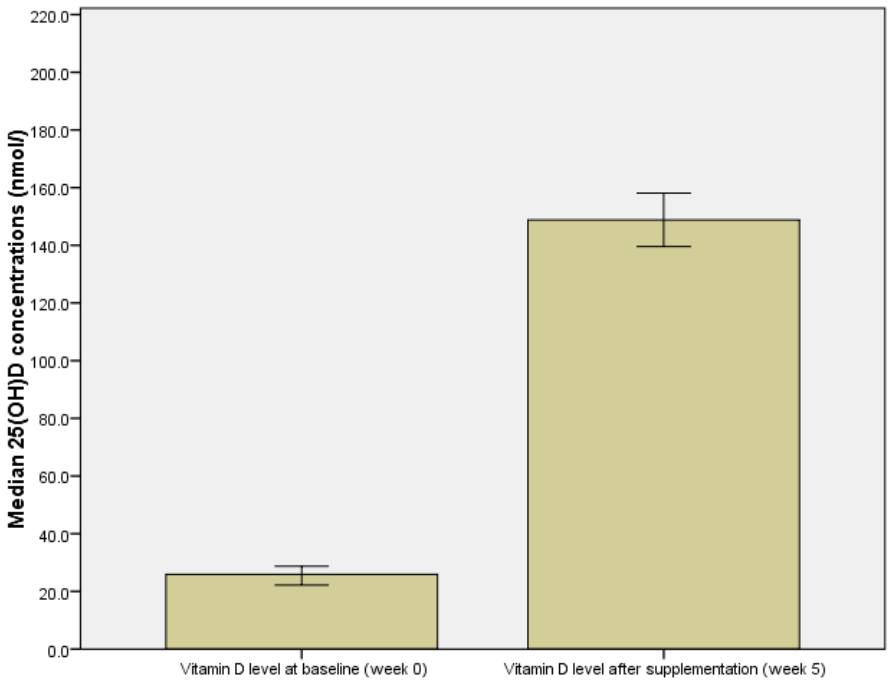

Figure 5.5: Changes in 25(OH)D levels for all participants from week 0 to week 5. Error bars represent 95% confidence interval (n=40)

Table 5.2: Difference 25(OH)D concentration before and after 5 weeks of receiving 140 µg calcifediol by ethnicity (n=40)

Ethnicity	Baseline week 0 (nmol/L)	Week 5 (nmol/L)	Change**	*p* value*
	Median (IQR)			
Arab(n=14)	27.7 (4.9)	135.3 (65.1)	108.7 (62.7)	<0.001
South Asian (n=14)	19.8 (13.1)	171.4 (35.4)	140.1 (45.2)	<0.001
Black African (n=12)	26.6 (11.7)	140.1 (37.0)	110.0 (31.4)	<0.001

*Wilcoxon signed rank test.

**Week 5 minus pre-test.

IQR: interquartile range.

From Table 5.2, the key facts seem to be that all ethnicities showed a marked increase after supplementation and that this effect was most prominent in the SA group.

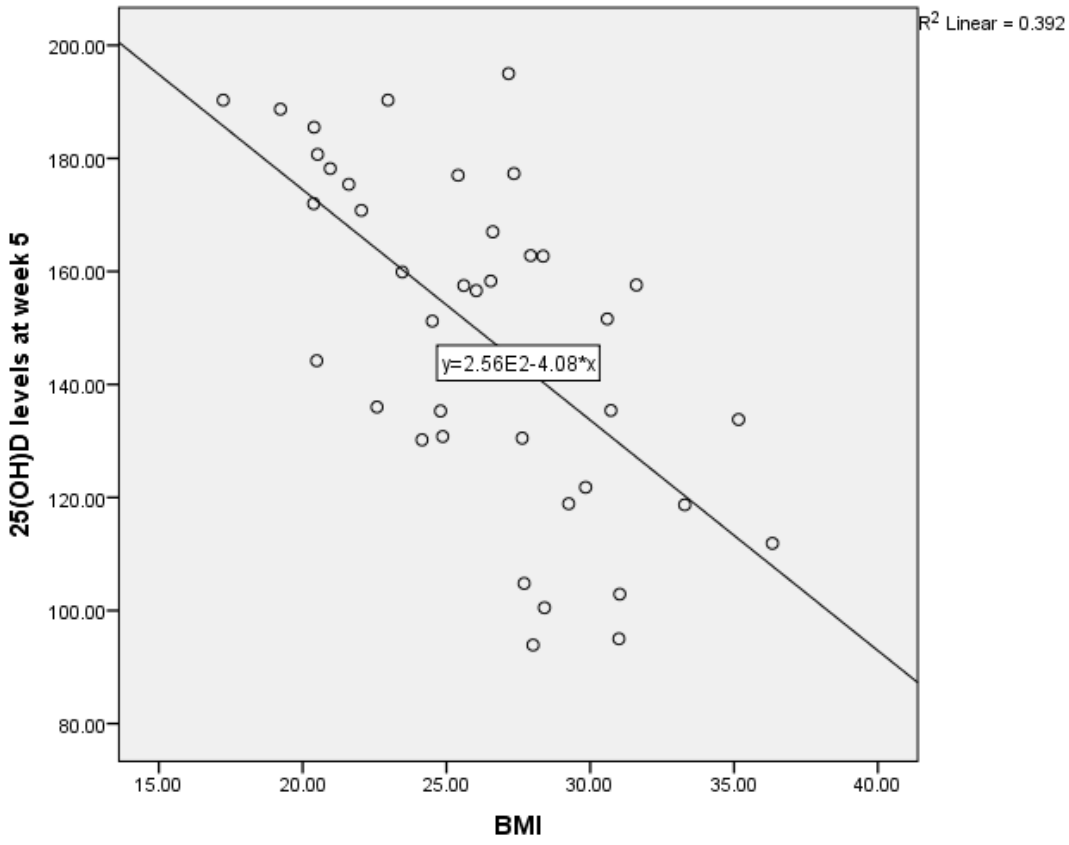

Figure 5.6: Correlation between BMI and 25(OH)D levels at week 5

Pearson correlation was used to determine the strength of the relationship of some variables such as BMI and age with 25(OH)D levels at mid-point. Figure 5.6 shows that

there was a strong inverse correlation between BMI and 25(OH)D level, which was statistically significant (r=–0.63, p<0.0001). Conversely, neither age nor sex was found to have a significant correlation with 25(OH)D level at mid-point (p>0.05).

5.3.4 Factors that may affect 25(OH)D levels at week 5

BMI, age, sex, dietary intake of vitamin D, smoking and baseline 25(OH)D level are all considered factors that may affect a participant's 25(OH)D levels at week 5. Multiple linear regression was performed and only BMI showed a statistically significant association with 25(OH)D level at week 5. Each 1 kg/m^2 increases in BMI, it was associated with 3.89 nmol/L decrease in 25(OH)D level at week 5 (B=-3.89, 95% CI: -4.80, 1.97, p=0.00). (

Table 5.3).

Table 5.3: Multiple regression model of 25(OH)D level at week 5

Variable	Coefficient (B)	95% CI^	*p*-value
Sex			
Male	Reference		
Female	8.88	–8.76, 26.53	0.31
Age	0.73	-0.29, 1.76	0.15
Sun index	19.4	-23.78, 62.7	0.36
BMI(kg/m^2)	- 3.89	-5.80, -1.97	<0.001
Dietary intake	-1.44	-11.4, 8.59	0.36
Baseline 25(OH)D(nmol/L)	.23	-1.37, 1.85	0.67
Ethnicity			
Arab	Reference		
South Asian	16.40	-3.23, 36.04	0.09
Black African	- 5.32	-24.16, 13.51	0.56
Smoking			
Yes	Reference		
No	16.01	–0.15, 0.14	0.15

^CI: confidence interval.

5.3.5 Persistence of vitamin D insufficiency after calcifediol supplement

As shown in Figure 5.7, median 25(OH)D concentrations decreased from 151.4 nmol/L at week 5 to 55.9 nmol/L at week 10 (p<0.001).

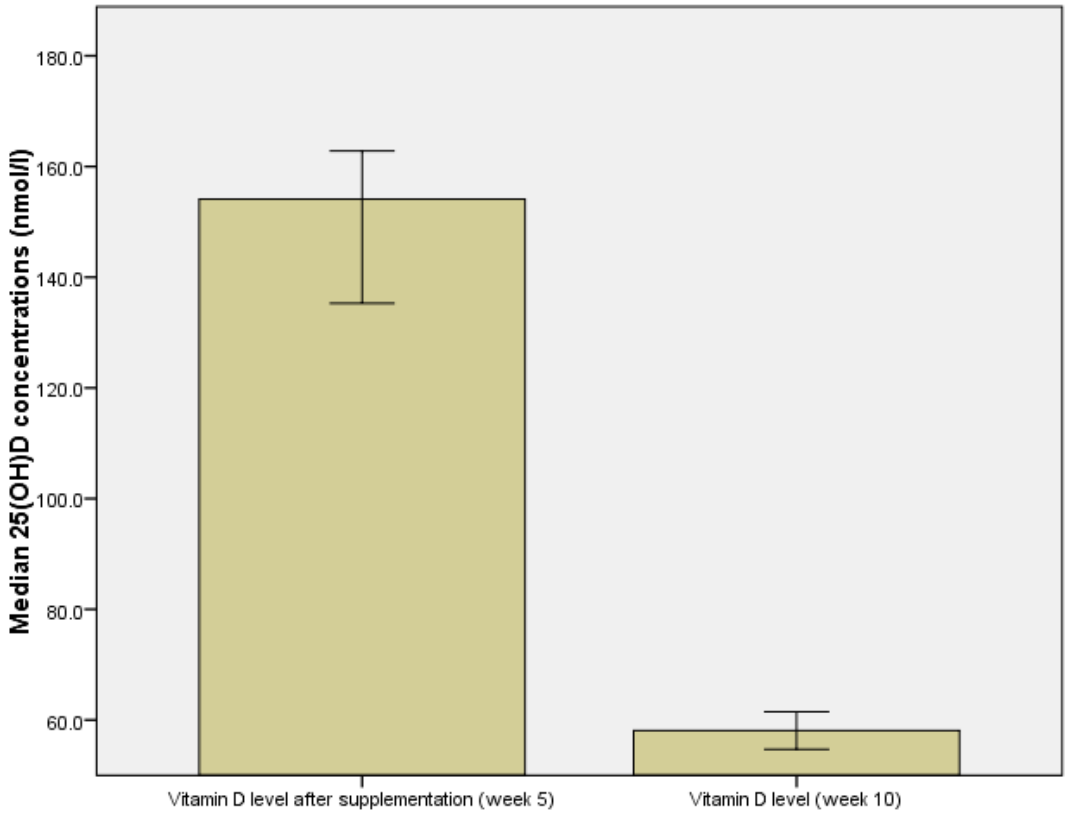

Figure 5.7: Persistence of vitamin D insufficiency after termination of calcifediol supplement. Error bars represent 95% confidence interval (n=40)

The changes in median 25(OH)D concentration (week 5 minus persistence-test) in the Arabs, SA and BA groups are compared in

Table 5.4, the level of 25(OH)D decreased over 5 weeks for all ethnic groups, but the median reduction was higher in the SA group than in either of other groups.

Table 5.4: Difference in 25(OH)D concentrations between weeks 5 and 10 by ethnicity

Ethnicity	Week 5(nmol/L)	Week 10 (nmol/L)	Change**	*p* value*
	Median (IQR)			
Arab (n=14)	135.3 (65.1)	58.8 (22.3)	83.1 (54.5)	<0.001
South Asian(14)	171.4 (35.4)	57.1 (15.9)	108.0 (32.7)	<0.001
Black African (n=11)	140.1 (37.0)	51.3 (10.6)	82.9 (22.0)	<0.001

*Wilcoxon signed-rank test.**Week 5 minus week 6. IQR: interquartile range.

Multiple linear regression was applied to determine which factors predicted the post-5-weeks change in 25(OH)D level and the results are presented in Table 5.5. The level of 25(OH)D at week 5 showed the following significant association: For every 1 nmol/L increment in 25(OH)D level at week 5, the persistence of vitamin D rose by .18 nmol/L (B = 0.18, 95% CI: 0.01, 0.35, *p* = 0.03).

Table 5.5: Multiple linear regression model of persistence of vitamin D

Variable	Coefficient(B)	95% CI^	*p*-value
Sex			
Male	Reference		
Female	-5.02	–12.82, 1.87	0.14
Age(year)	- 0.10	-0.58, 0.38	0.67
BMI(kg/m^2)	-0 .02	-1.13, 1.07	0.96
Dietary intake	2.40	-1.85, 6.80	0.25
25(OH)D at week 5	0.18	0.01, 0.35	0.03
Ethnicity			
Arab	Reference		
South Asian	- 5.13	-14.02, 3.75	0.24
Black African	- 5.38	-13.74, 2.98	0.19
Smoking			
Yes	Reference		
No	1.48	–7.43, 10.41	0.34

^CI: confidence interval.

5.3.6 Differences in 25(OH)D concentrations over three time points

Figure 5.8, shows that the median 25(OH)D value for all participants at baseline was 25.1 nmol/L (range 11.8–30.0 nmol/L). This increased to 151.4 nmol/L (range 93.9 to 195.0 nmol/L) after supplementation for five weeks, then dropped once supplementation was stopped to 55.9 nmol/L (range 27.7 to 96.1 nmol/L). The Friedman test showed that there was a significant difference ($p<0.01$) in vitamin D level over the three time points.

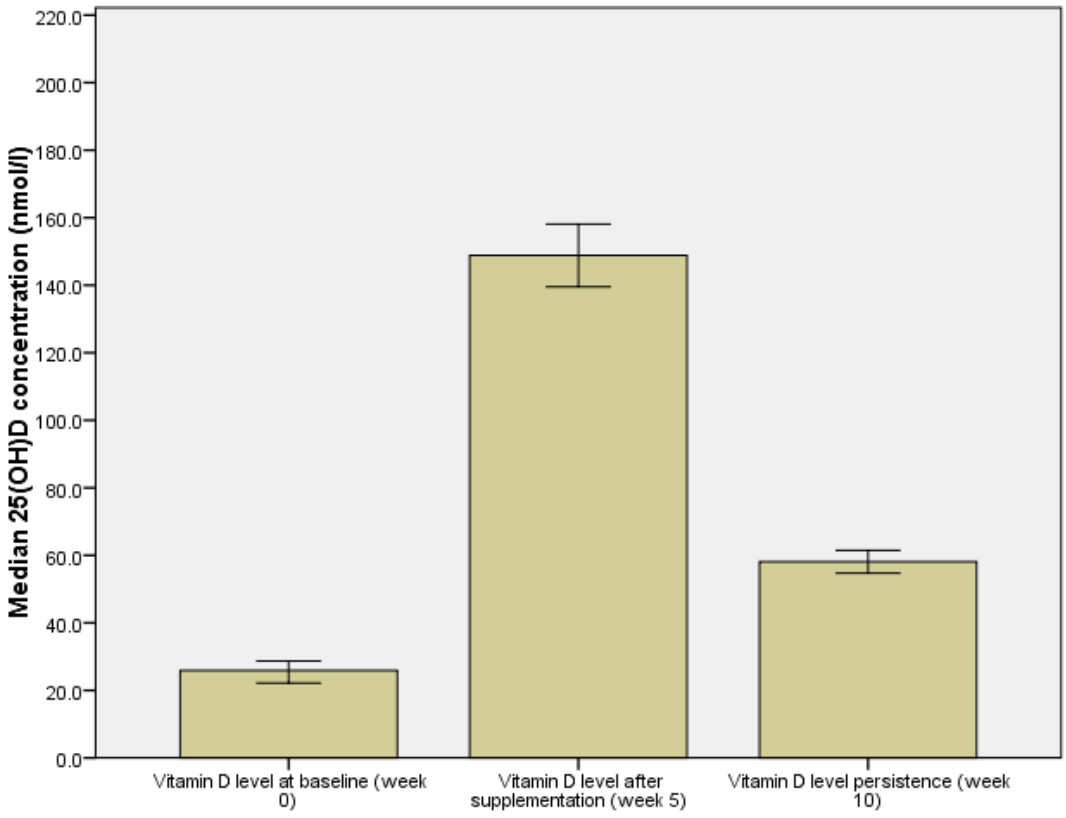

Figure 5.8: Difference in 25(OH)D concentrations over three time points for participants receiving 140 µg calcifediol. The error bars are the 95% confidence interval

5.3.7 25(OH)D levels over different times by ethnicity

From Table 5.6 it can be seen that there was no statistical difference in baseline 25(OH)D level between the different ethnicities ($p>0.05$), while there was a significant difference ($p=0.01$) in serum 25(OH)D level at mid-point. A post hoc comparison showed that the median 25(OH)D was higher in SAs than the other groups, whereas there was no

significant difference between Arabs and BAs (p>0.05). No significant differences were observed in the median serum 25(OH)D level at the final test.

Furthermore, looking at the changes, which were detected between different time points in Table 5.6, the median change in 25(OH)D level between baseline and mid-point was higher among SAs than in the other groups (p=0.02). The changes from mid-point to the final week also differed significantly (p=0.01), the largest changes again being in the SA group.

Table 5.6: Comparison between 25(OH)D concentration at different time points by ethnicity

Variable (nmol/L)	Arab	South Asian	Black African	Total sample	*p*-value*	*Post hoc*[a]	*p* value****
	Median (IQR)						
Baseline vitamin D test*	27.7(4.9)	19.8(13.1)	26.6 (11.7)	26.6(10.2)	0.42	Arab–SA	0.10
						BA–SA	0.67
						Arab–BA	0.94
Mid-point vitamin D test**	135.3 (65.1)	171.4(35.4)	140.1 (37.0)	151.4 (50.6)	0.01	Arab–SA	0.01
						BA–SA	<0.001
						Arab–BA	1.00
Final vitamin D test***	58.8 (22.3)	57.1(15.9)	51.3 (10.6)	55.9(17.3)	0.50	Arab–SA	0.47
						BA–SA	0.19
						Arab–BA	0.47
Changes (baseline–mid-point)	108.7 (62.7)	140.1 (45.2)	110.0 (31.4)	127.0(40.4)	0.02	Arab–SA	0.02
						BA–SA	0.01
						Arab–BA	> 0.05
Changes (mid-point–final)	83.1 (54.5)	108.0 (32.7)	82.9 (22.0)	88.9(33.3)	0.01	Arab–SA	0.02
						BA–SA	<0.001
						Arab–BA	1.00

*N= 42. **N= 40. ***N= 39. ^The Friedman test was used to determine the difference over repeated measures ($p<0.01$).
****The Kruskal–Wallis test was used to determine if the observed difference were statistically significant between ethnicity and time points.
[a]Dunn's nonparametric comparison 25(OH)D. IQR: interquartile range.

5.3.8 Adherence

According to then information that they gave via the recording table (Appendix 6), all participants achieved 100% compliance in consuming the stated doses. Therefore, there was no significant difference in adherence across the ethnicities (p>0.05).

5.4 Discussion

5.4.1 Participants' demographic, health and vitamin D intake characteristics

There were forty-two participants from the Arab, SA and BA ethnic groups, with a mean age of ~30 years. Ethnic differences in the socioeconomic status, health and vitamin D intake were not statistically significant (p>0.05) in the present study. By contrast, as discussed in Chapter 4, a number of studies have found significant differences in these factors among different ethnicities (Meyer et al., 2004; Holvik et al., 2005; Moore et al., 2005; Madar et al., 2008; Patel et al., 2013). The current study also found no statistically significant ethnic differences in baseline 25(OH)D levels (p=0.42), which is consistent with previous studies (Ford et al., 2006; Lowe et al., 2010; Patel et al., 2013) as well as with data from the study reported in Chapter 4. Nevertheless, the median baseline 25(OH)D level was lower in the SA group (19 nmol/L) than among the Arab (27.7 nmol/L) and BA (26.6 nmol/L) participants. These results are likely to be related to the variants of the VDBP gene (Gozdzik et al., 2011).

5.4.2 The effect of using calcifediol supplement to increase vitamin D level

The aim of this study was to investigate the efficacy of calcifediol supplementation, in order to improve vitamin D levels in deficient adults from ethnic minority groups. This study found that weekly supplementation with calcifediol was associated with significantly (p<0.001) higher 25(OH)D levels by week 5 in all EMGs, reaching a level of ≥75 nmol/L. The supplementation of 140 µg calcifediol for five weeks was thus effective and was also safe, as no adverse effects were reported.

To the researcher's knowledge, this is the first study to examine the efficacy of calcifediol in enhancing 25(OH)D levels among EMGs living in the UK. A study in the USA by Shieh et al. (2017), which was published when the present study was close to completion, had only 19 participants (half as many as this study), in four ethnic groups: Caucasian, African American, Asian American and Hispanic/Latino. Other studies of Caucasian participants in Europe and the USA have also had relatively small samples (Barger-Lux et al., 1998; Cashman et al., 2012; Bischoff-Ferrari Et al., 2012; Jetter et al., 2014). The current study can thus be considered the largest to examine the efficacy of calcifediol to increase the level of vitamin D.

Given the existence of differences in various aspects of study design such as demographic characteristics and inter-laboratory techniques, including the type of assays used and the cut-off values used to define 25(OH)D deficiency (Binkley et al., 2004; Jones et al., 2012; Phinney et al., 2011), any comparison of the results of the current study with those of previous studies needs to be undertaken cautiously.

Comparing this study's findings with those of the abovementioned research examining healthy multi-ethnic adults (Shieh et al., 2017), the two studies used the same dose of 20 µg/d (140 µg/week) of calcifediol and both found that 25(OH)D levels increased rapidly as a result. However, in the Shieh et al. study, participants' mean serum 25(OH)D level rose to a mean of 86.2 nmol/L at week 4, which was lower than the 125.3 nmol/L level recorded in the present study. Barger-Lux et al. (1998), who assessed the changes in serum 25(OH)D levels of healthy men after consuming 20 µg/d calcifediol for four weeks, reported that the mean change in serum 25(OH)D levels was approximately 76 nmol/L, whereas the change in 25(OH)D level in the current study was 101 nmol/L at week 4.

Despite the difference between these studies in the sex distribution of the samples, there is no indication in the results of earlier studies that sex affects circulating 25(OH) levels in response to supplementation (Trang et al., 1998; Cashman et al., 2012; Shieh et al., 2017) and the current study also found this (p=0.31). Therefore, the differences in the results could be attributed to the baseline 25(OH)D level, which was lower (26 nmol/L) in the current study than in the Shieh et al. study (40.4 nmol/L), as well as in the Barger-Lux et al. study (67 nmol/L). As demonstrated by Mazahery and Hurst (2015), the response to supplementation is stronger when baseline 25(OH)D levels are low than when they are high, suggesting that 'hepatic 25-hydroxylation is a saturable process' (Barger-Lux et al., 1998). Both studies also concluded that calcifediol was more effective than vitamin D_3 in enhancing 25(OH)D levels, although they used a larger dose than in calcifediol (Barger-Lux et al., 1998; Shieh et al., 2017).

As reported in earlier studies, no significant difference in the extent of the increase in 25(OH)D level was observed when the regimen of administration was varied between daily and weekly (Bischoff-Ferrari et al., 2012; Jetter et al., 2014). Jetter et al. (2014) found that supplementation by a daily 20 µg dose or 140 µg weekly resulted in a rapidly

increased plasma 25(OH)D level to at least 154.7 nmol/L at week 5. Another clinical trial reported that mean plasma 25(OH)D level increased from 30.6 nmol/L to 112.3 nmol/L at week 5 and continued to increase until it reached 175 nmol/L after 16 weeks (Bischoff-Ferrari et al., 2012).These results are consistent with those of the current study, regardless of ethnicity and age, which show that a weekly dose of 140 µg calcifediol was quickly effective in shifting 25(OH)D levels into an optimal level of vitamin D.

Additionally, beside the benefit of calcifediol for treating vitamin D deficiency, Bischoff-Ferrari et al. showed that there was a significant therapeutic benefit of calcifediol on blood pressure and lower extremity function, compared to vitamin D_3. This result supports the advantages of using calcifediol over vitamin D_3. In addition, both studies found that in groups supplemented with the same dose of vitamin D_3, serum 25(OH)D levels reached 75 nmol/L after nine weeks (Jetter et al., 2014) and that only half of participants remained below this cut-off. This confirms that calcifediol is more potent in enhancing 25(OH)D level than vitamin D_3.

A study conducted by Cashman et al. (2012), similar in design and sample to that of Bischoff-Ferrari et al., found that mean serum 25(OH)D levels increased rapidly from 38.2 to 98.1 nmol/L at week 5, rising further to 134.6 nmol/L at week 10. Despite the levels of 25(OH)D reaching over 75 nmol/L in the studies both of Cashman et al. (2012) and of Bischoff-Ferrari et al. (2012), the increase was less than in the current study, which may be due to differences in methodology. In a more recent study by Navarro-Valverde et al. (2016), it was shown that the serum 25(OH)D level of 40 women (baseline 25(OH)D level of 37nmol/L) who received a supplement of 20 µg/day reached a mean value of 161 nmol/L over six months.

It should be noted that most previous studies were conducted in the summertime, meaning that sunlight could have affected their results (Bischoff-Ferrari et al., 2012; Navarro-Valverde et al., 2016; Jetter et al., 2014). An exception is the study by Cashman et al. (2012), which was carried out in wintertime to avoid the influence of vitamin D synthesis; they also gave one group a placebo and found that there was no change in serum 25(OH)D level. Similarly, the current study was conducted in wintertime, so summer sunlight did not affect the 25(OH)D level, and participants were instructed to avoid consuming any vitamin D-fortified food.

In addition, a study conducted by Russo et al. (2011) on 18 females showed that three days of oral administration of 500 µg/month of calcifediol raised plasma 25(OH)D levels from a base level of 45 nmol/L to 124.8 nmol/L, after which it declined slowly for the rest of the month. Some concern has been expressed that monthly administration of a large dose of calcifediol this could lead to an absorption problem by increasing the level of $1,25(OH)_2D_3$, as reported by Brandi and Minisola (2013:5), who refer to 'high calcitriol levels with consequent increase in osteoclast activity and activation of the bone 24,25-hydroxylase that would reduce the local calcidiol pool'. However, Russo et al. (2011) reported no adverse effect being detected during course of four months.

The present study found ethnic differences in post-supplementation 25(OH)D levels, which were higher in South Asians compared to the other groups, which is inconsistent with the study of Shieh et al. (2017). A possible explanation for this is that there were ethnic differences in baseline 25(OH)D, with the lowest value being found among South Asians, resulting in a higher subsequent increase in 25(OH)D level.

In spite of significant increases in level of 25(OH)D in the previous trials, none of these reported the incidence of hypercalcemia; urinary and serum calcium levels were stable during the studies (Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Jetter et al., 2014; Russo et al., 2011; Shieh et al., 2017). This suggests that calcifediol is safe and that the risk of toxicity is low.

5.4.3 Factors affecting levels of 25(OH)D at week 5

It has been indicated that the 25(OH)D level at week 5 is affected by several factors such as BMI, baseline 25(OH)D level, age and ethnicity (Aloia et al., 2008; Mazahery and von Hurst, 2015). In the current study, sex and age were not found to have any significant effect on 25(OH)D, these results being consistent with those of previous studies (Harris and Dawson-Hughes, 2002; Aloia et al., 2008; Talwar et al., 2007; Fu et al., 2009; Cashman et al., 2012; Shieh et al., 2017). However, Zittermann et al. (2014) report that there was a 3.7% variation in 25(OH)D levels which can be explained by age.

The current study found that the levels of 25(OH)D at week 5 were inversely associated with BMI, which is in line with the literature (Barger-Lux et al., 1998; Gallagher et al., 2012; Nelson et al., 2009). This association is attributed to the biological pathway by

which vitamin D is stored in adipose tissue for later use (Mazahery and von Hurst, 2015); Wortsman et al. (2000) report that the greater the amount of adipose tissue, the more vitamin D is likely to be trapped. It has also been reported that response to supplementation is about 30% lower in obese subjects (Heaney et al., 2003a; Zittermann et al., 2014). In common with the other studies (Holvik et al., 2007; Aloia et al., 2008; Shieh et al., 2017), ethnicity was not a significant factor affecting 25(OH)D levels.

5.4.4 Persistence of vitamin D insufficiency among ethnicities

It has been reported that once vitamin D is ingested, some is converted to 25(OH)D, while the remainder is stored in fat and released into the circulation over time (Heaney et al., 2008). Therefore, a vitamin D supplement could sustain levels in the blood circulation for an extended period. Calcifediol is easily absorbed by the gut and is cleared speedily from the blood, having a high affinity to VDBP and high polarity. These properties make it a highly bioavailable compound; with a decreased risk of toxicity, so it is less likely less to be stored in the fatty tissue. However, there is no evidence to support this (Brandi and Minisola, 2013).

Supplementation trials using vitamin D_3 have found that the levels of 25(OH)D reached a plateau after 6-8 weeks (Harris and Dawson-Hughes, 2002) with no further increases after week 5 (Cashman et al., 2012). In the calcifediol study by Bischoff-Ferrari et al. (2012), it seems that 25(OH)D levels reached equilibrium by week 11, remained stable for a few weeks and then started to increase.

In studies using vitamin D_3, 25(OH)D levels fell on cessation of supplementation by week 5–6 to below 75 nmol/L for a high dose of 1250 µg (Armas et al., 2004; Heany et al., 2011) and for a dose of 100 µg (Tjellesen et al., 1986). Gupta et al. (2017) showed that an oral dose of 1500 µg of D_3 for 6 weeks resulted in a decline in serum levels to 40 nmol/L at week 12. The findings of the current study show that after supplementation ceased at week 5, the level of 25(OH)D declined over the next 5 weeks, while nevertheless remaining above the baseline value. This result is in agreement with those obtained by Russo et al. (2011), who found that 25(OH)D levels increased rapidly and reached a peak at day 3 of consuming a monthly dose of 500 µg, then declined during the rest of the month but remained higher than the basal value. In addition, Cashman et al. (2012)

report that in participants receiving 20 µg/d of D_3, 25(OH)D level reached a plateau at week 5, while in the group on 20 µg/d of calcifediol, levels increased further until week 10. This suggests that calcifediol is more effective not only raising 25(OH)D levels but also at sustaining a sufficient level.

After addressing their vitamin D deficiency, people can maintain sufficient 25(OH)D levels by changing their lifestyle and their dietary intake of D_3 and by increasing their sunlight exposure time without any barriers that may block vitamin D synthesis. They should increase their dietary intake of vitamin D, especially by consuming a weekly portion of oily fish as per the UK recommendation, but this recommendation may not be sufficient for EMGs (Farrar et al., 2011; Webb et al., 2018). Therefore, low doses of supplement would be another option. According to Kennel et al. (2010), a 15-20 µg dose may be required after addressing the initial deficiency for an extended period. However, Balvers et al. (2015) have argued that due to the 3–4-week half-life of 25(OH)D (Holick 2004a; Sinha et al., 2013), a single high annual dose of vitamin D_3 will not be enough to sustain sufficient levels throughout the year. DeLuca et al. (2014) support this conclusion and recommend frequent dosing.

5.5 Conclusion

The results of the current study demonstrate the therapeutic advantage of using oral calcifediol in raising 25(OH)D level to ≥ 75 nmol/L in ethnic minority adults living in Manchester. The results also show the effectiveness of calcifediol to enhance 25(OH)D levels without any obvious detrimental effects, such as symptoms of hypercalcaemia. As reported in the literature, calcifediol is more potent than vitamin D_3 in significantly raising serum 25(OH)D levels, which provides evidence that it could be employed as a satisfactory option for treating and preventing vitamin D deficiency. It is recommended that the calcifediol supplement be made available on the UK market.

6. GENERAL DISCUSSION

6.1 Introduction

This final chapter of the thesis summarises the findings of the study and its three component trials, considers its original contribution, reviews its strengths and limitations, then details its practical implications.

6.2 Summary of findings

The literature review highlighted the issue of the prevalence of vitamin D deficiency in the UK and showed how specific environmental and lifestyle factors increase the risk of developing vitamin D deficiency among the population, with particular reference to ethnic minority groups. In spite of intensive research into vitamin D, there is still debate as to the optimal 25(OH)D level that the body needs to ensure related health benefits, as there is regarding the optimal behaviour to benefit from sun exposure, given that the current recommendations are not sufficient for people with highly pigmented skin. Although a dose of 10 µg/d vitamin D has been recommended for adult (SACN, 2015), it would be difficult to achieve this through dietary sources alone. In the UK, few foods contain sufficient levels of vitamin D; and food fortification, which could provide an effective means of correcting vitamin D deficiency, is restricted by the government.

Additionally, most of the previous trials conducted on elderly Caucasians and a few in among young adults have shown that calcifediol is more effective in raising serum 25(OH)D levels than vitamin D_3. However, no supplementation trials have been conducted in the UK using calcifediol. Therefore, this research was conducted to identify ethnic differences in lifestyle factors, vitamin D intake and sun exposure behaviours among ethnic minority adults living in Manchester to determine the group(s) who may be most prone to developing vitamin D deficiency. Further, an efficacy trial was then performed to examine the effect of calcifediol in enhancing serum 25(OH)D levels among multi-ethnic adults in the UK.

6.2.1 Study 1: Risk factors influencing vitamin D status of ethnic minority adults living in Manchester

The first aim was addressed by using a questionnaire comprising of three sections on sun exposure assessment, health factors and vitamin D intake. A total of 253 respondents, consisting of Arabs, South Asians, Afro/Caribbeans and East Asians completed the

questionnaire. The findings revealed that there were significant differences between these EMGs in some of the demographic and lifestyle factors. Notably, religion was identified as a fundamental factor that could affect vitamin D intake by restricting dietary choices and encouraging dress styles that can block UVB radiation. All of the Arab participants and half of SAs identified as Muslims. Multiple regression analysis revealed that education level and economic status were significant predictors of vitamin D intake, with higher levels of education and income associated with increased vitamin D intake. However, the only ethnic difference in these factors detected in this study was in education level: the AfroC group had the largest percentage of member who had obtained only a diploma or college education, followed by the EA group. Notably, the sample size was relatively small in the latter group.

Statistically significant differences were also found in smoking and alcohol intake by ethnic group, with the highest percentage of smokers in the Arab group and the highest percentage of alcohol intake in the AfroC group. More SAs had a history of vitamin D deficiency compared to the other groups studied. However, the reason could be a lack of vitamin D screening among the other groups, as it has been reported that symptoms of vitamin D deficiency can be unclear (Holick et al., 2011; Pearce and Cheetham, 2010). The current study has also shown that a lack of sun exposure among Arabs could be a cause of vitamin D deficiency, as this ethnic group spent the longest time indoors during the day, used sunscreen the most and limited most the body area exposed to sunlight. This finding is consistent with a previous study (Ahmed et al., 2013). Thus, the EA group may be at a lower risk than other EMGs due to their sun exposure behaviour.

6.2.2 Study 2: Association of vitamin D intake and other risk factors with 25(OH)D concentrations in ethnic minority adults living in Manchester

In the second study, the associations of various risk factors with 25(OH)D levels were examined in order to identify potential explanations of low vitamin D status among ethnic minority groups. Ethnicity made no significant difference to lifestyle, health factors or sun exposure in the study sample, except in relation to religion, alcohol intake and sun-exposed skin area, as shown in Study 1. However, these factors were found to differ between ethnic groups in previous studies, as discussed in chapter 4. In agreement with previous studies, the dietary intake of vitamin D reported here was low among all

participants, as were the levels of 25(OH)D with a median of 29.9 nmol/L. This evidence suggests that research should cover all EMGs rather than focusing solely on SAs as a group at risk of vitamin D deficiency/insufficiency. Severe deficiency of vitamin D was found in BA and SA women, while vitamin D deficiency was higher in men than women in the Arab and SA groups. Multiple regression analysis revealed that time spent outdoors, income status, dietary intake of vitamin D and supplement intake were reliable predictors of vitamin D status.

This research indicates that the current recommendations are not sufficient to achieve optimal vitamin D levels among EMGs. In addition, there is unfamiliarity with the current vitamin D recommendations and the use of fortified food, exacerbating the prevalence of vitamin D deficiency/insufficiency. This study has revealed significant trends in vitamin D intake among EMGs that could potentially help people to improve their vitamin D status. It is recommended that members of EMGs in the UK modify their sun exposure behaviour during the summer months as much as possible to increase their capacity for cutaneous synthesis of vitamin D. In general, Webb et al. (2018) found that dark-skinned people could sustain a sufficient level of vitamin D throughout the winter months by daily exposure of 35% of their skin area to the summer midday sun for at least 40 minutes.

6.2.3 Study 3: The efficacy of calcifediol supplementation in increasing 25-hydroxyvitamin D concentration

Chapter 5 reported an investigation of the effect of five weeks of supplementation with calcifediol, a type of vitamin D unused in the UK, to increase 25(OH)D level among ethnic minority adults living in Manchester. The results of this investigation show that regardless of ethnicity, a weekly dose of 140 µg of calcifediol rapidly increased the 25(OH)D concentration of participants to more than 75 nmol/L in only five weeks. This is the first trial to examine the efficacy of calcifediol in enhancing 25(OH)D levels among EMGs living in the UK and provides incisive proof that calcifediol supplementation is effective in correcting vitamin D deficiency over a short period. Multiple regression analysis revealed that of the variables measured, only BMI showed a statistically significant association with 25(OH) D level at week 5.

Although a daily dose of 10 µg vitamin D should theoretically increase 25(OH)D concentrations to the optimal level, several studies carried out on ethnic groups have

reported that supplementation with 15-20 μg/d vitamin D was not sufficient to increase or maintain an adequate concentration in the absence of sun exposure and with limited natural dietary sources (Osmancevic et al., 2016; Shieh et al., 2017; Talwar et al., 2007). Therefore, it is apparent that relying on the RNI recommendation is insufficient to reach a desirable level of vitamin D and to avoid negative health outcomes related to vitamin D insufficiency; instead, it should be considered as a daily recommendation for sustaining 25(OH)D level throughout the year. In addition, 25(OH)D concentration decreased slightly in five weeks and reached a sufficient level of vitamin D with a median 55 nmol/L, while a multiple regression test showed that only the level of vitamin D at week 5 had an impact on the persistence of vitamin D.

At present, calcifediol is not licensed as a source of vitamin D in the UK; however, this study has shown that it is effective in increasing the 25(OH)D level and indicates that it is more effective than the existing D_3/D_2 supplements, as it results in increased 25(OH)D once an oral dose is consumed. Although no incidence of hypercalcemia was reported in the previous studies, it is worth mentioning that caution should be exercised when considering the dose and duration of supplementation, as calcifediol was found to produce a significant enhancement in 25(OH)D level.

6.3 Research contribution

This research determined differences in the influencing factors that could lead to vitamin D deficiency between the main EMGs and based on the findings an appropriate recommendations have been developed to improve the vitamin D status among those groups.

Most research to date has tended to focus on community-dwelling South Asians in Manchester, rather than other ethnic minority groups, whose numbers have increased markedly in recent years and who could be at high risk of vitamin D deficiency. The current research contributes to the existing literature and adds to vitamin D research by concentrating on the differences in vitamin D-influencing factors between EMGs, including time of the day, age, skin colour, BMI, clothing, sunscreen, health condition and socioeconomic factors. Current findings would help to set an official and proper recommendation for those communities as the current recommendations are not

sufficient to assure an adequate level of vitamin D. Factors that were not studied in this research such as physical activity, seasonal and genetic variance should be taken into consideration when setting recommendations.

Different habits and behaviour were reported between EMGs in the current research. Although Arabs have light skin they may not benefit from this because they tended to avoid sunlight by spending less time outdoors and used sunscreen. Whilst, BAs tend toward greater body exposure, they have been shown to require longer exposure times due to the high skin pigmentation. In addition, one of the most significant findings to emerge from this study was that the dietary intake of vitamin D from supplementation was lower than the recommended intake among all groups, as well as the level of vitamin D among participants regardless of ethnicity. Although the current study did not detect significant differences in some factors, which is most likely due to the small sample size, it showed that all EMGs could be susceptible to vitamin D deficiency, that there is, therefore, a need for attention and effective intervention.

This research also examined the efficacy of calcifediol on vitamin D status among EMGs. There is no doubt, however, that vitamin D3 lead to an increase in serum 25OHD concentration. But according to this research, calcifediol resulted in a greater increase in 25(OH)D over a short period, where the results showed that regardless of ethnicity, a weekly dose of 140 µg of calcifediol rapidly increased the 25(OH)D concentration of participants to more than 75 nmol/L in only five weeks, with no adverse effects reported. This study provides evidence that it could be employed as a satisfactory option for treating and preventing vitamin D deficiency. However, a large, controlled randomised trial is needed to control for confounding variables in order to determine the true factors affecting response to calcifediol including genetic variants.

6.4 Strengths of the study

A major strength of the present study is that it is to the best of the researcher's knowledge the first to compare the lifestyle factors, vitamin D intake and sun exposure behaviour of ethnic minority adults living in Manchester. Not only were sun exposure habits assessed, but the sun index was also calculated, to estimate the amount of vitamin

D produced by cutaneous synthesis. This is also the first trial of the use of a calcifediol supplement to correct vitamin D deficiency among multi-ethnic groups living in the UK.

6.5 Limitations and suggestions for future studies

Although the researcher has done a great effort to attain its aims. However, this study has several limitations, which could potentially be addressed in future studies by considering some ideas developed by the researcher.

For study 1 and 2, the most important of these limitations was the small sample size, a consequence of the restricted time and budget available to the researcher, which may have preclude the detection of differences between EMGs in the operation of risk factors. Although the researcher explored all possible ways to increase the number of participants through a considerable connection with their communities and places of assembly and worship, such as mosques and churches, the rate of response was low amongst the target sample. The requirement to use English language was a barrier to many potential participants who did not read or write the language and were therefore unable to complete the questionnaire. Future studies conducted on multi-ethnic or immigrant populations should use data-gathering tools (e.g. questionnaires or food diaries) translated into the different languages spoken by the target groups or should employ the services of interpreters to maximise the rate of participation.

Another limitation in this study was that the researcher used existing valid questionnaires from other studies rather than to create new questionnaires. If time were not a limiting factor, it would have been useful to create questionnaires especially for the current sample and determine reliability and validity. In addition, data from self-reported questionnaire may have been affected by social desirability bias such as self-reported BMI, whereby participants would have failed to respond truthfully. The type of BMI measurement is often costly, particularly in population research that requires large samples. Future research that would use self-reported BMI, it could be minimise the bias by developing correction methods and compare it to population-based datasets, which contain both measured and self-reported values of BMI for the same individuals..

There are also some limitations in the efficacy trial (study 3) primarily reflecting limited budget and time constraints:

- Although the researcher did her best to recruit a large number of participants, the recruitment was difficult among EMGs because many of their member did not speak English or were unaware of consent forms and the nature of the study (three visits for blood tests). This was reflected in the sample size of the group that contributed to this study. Having interpreters would have been beneficial in increasing the number of participants.
- The fact that this study did not use a control group may have compromised its ability to detect actual changes after supplementation. Double-blind randomised control trials should be conducted to control for confounding variables in order to determine the true factors, particularly genetic ones, affecting the response to calcifediol supplementation. In addition, as this study shows that 140 µg/week (20 µg/d) of calcifediol increased 25(OH) levels to ≥75 nmol/l, it would be interesting to see whether the RNI of 10 µg/d (SACN, 2015) calcifediol is sufficient to achieve a desirable level of vitamin D among different ethnic groups.
- The researcher faced a problem in finding a supplier for calcifediol as it is not available on the market in the UK with only a few companies providing calcifediol in liquid form. Therefore, the researcher was restricted to using the liquid form instead of tablets, making it more problematic for participants to administer and for the researcher to assess compliance. The researcher put into place strategies to minimise any error that could occur by using a liquid supplement, i.e., using a syringe to help to measure the dose more precisely than by using a more inaccurate dropper, which might have resulted in some of the dose being lost. Participants were trained to measure the required dose and they took the first dose in front of the researcher. The use of a weekly regimen was chosen to help maximise compliance. Finally, to monitor overall compliance the supplement bottle was accurately weighted pre-trial and post-trial.
- Calcium levels were not measured during the trial to check for hypercalcaemia, therefore, participants were asked to contact the researcher if they were having any problems during the study. It would be beneficial to measure calcium levels as well as 25(OH)D concentration, thus obtaining an accurate indication of vitamin D status while monitoring toxicity (Kennel et al., 2010).

6.6 Implication of findings

This section considers the implications of the study and its findings for the research community, for health professionals, for policymakers, for public health and for individuals.

6.6.1 Research community

There has been a research focus on the vitamin D status of members of the South Asian community in the UK, as it has been considered the EMG at particular risk of vitamin D insufficiency/deficiency, especially in Manchester. The findings of the current study show that other EMGs are just as vulnerable to vitamin D deficiency. This study needs to be replicated using a large sample with equal group sizes to increase statistical power, as well as exploring seasonal differences in dietary intake of vitamin D and sun exposure behaviour by repeating the assessment throughout the year. Further studies are also needed to focus on the social and cultural barriers to seeking health among this at-risk population, in order to develop effective policy interventions and awareness campaigns. Further research into the perceptions of EMGs in the UK of the importance and benefits of vitamin D should be conducted in order to address their concerns and the barriers to increasing their vitamin D intake.

Most supplementation trials have used vitamin D_3, treating it as the only form of vitamin D that can be used to address vitamin D deficiency, which may have led to the potential value of other forms of supplementation being ignored. As the present study has highlighted the efficacy in human use of calcifediol, a form which is not available everywhere, research interest should now be focused on using it, with the potential to enhance its availability.

6.6.2 Health professionals

Although a daily intake of 10 µg of vitamin D is recommended for adults, this study raises serious concern about the execution of this guideline, as it appears to leave a large number of ethnic minority adults deficient in vitamin D. It is unclear whether this is due to inadequate screening for deficiencies by health workers, or to the existence of barriers to vitamin D intake within the communities in question. In 2012, the UK government's Chief Medical Officer wrote that the duty of practitioners and health workers was to raise

awareness of vitamin D among ethnic minority groups and to highlight the recommended vitamin D intake (Davies et al., 2012).

This first efficacy trial conducted to date in the UK has shown conclusively that calcifediol is effective in improving vitamin D status, raising 25(OH)D levels to an optimal level and maintaining them. In the clinical setting, cholecalciferol may be preferential in treating vitamin D deficiency. It is, however, important to note that the small dose of calcifediol did increase 25(OH)D levels, but to a greater extent than vitamin D_3 and within a shorter time frame (according to the literature). When addressing hypovitaminosis D, a high dose of vitamin D_3 would be required if the goal were to achieve ≥25 nmol/L within a short time, while smaller doses of calcifediol are required to achieve a similar effect and then for maintenance, which may result in better adherence to the supplement.

6.6.3 Policy makers

This study has provided evidence that there are few foods containing vitamin D which members of EMGs in Manchester tend to consume and that their sun exposure is restricted by environmental and lifestyle factors. These findings suggest that strong action is needed to establish dietary compensation for these limitations, to produce sufficient vitamin D and to prevent hypovitaminosis D, by fortification of foods commonly consumed within multi-ethnic groups, in order to help them reach at least the recommended value of 10 µg/d and to sustain adequate 25(OH)D levels.

Calcifediol should be approved for use as a nutritional supplement to correct vitamin D deficiency, particularly given that a small dose of calcifediol increases the level of vitamin D to the optimal level at lower cost than vitamin D_3, as a bottle of calcifediol costs £4.37, while a 12-week course of vitamin D_3 costs £50 (NICE 2014).

6.6.4 Public health

The diseases resulting from vitamin D deficiency have worsened among ethnic minorities and the high cost of treatment has become an escalating problem in public health. This could be avoided by the following steps:

- Advantage should be taken of modern technologies, in two ways in particular. First, social media should be used to reach a large number of people with

awareness messages about the beneficial effects of exposure to the summer sun. Current mobile applications should also be used to track and calculate the amount of vitamin D from food and sun exposure.

- Comprehensive education and awareness campaigns should be launched to challenge false assumptions among ethnic groups and educate them about the benefits of normal vitamin D levels, as well as providing information on the symptoms of vitamin D deficiency and its prevention. In addition, sources of false information about the risk of exposure to sunlight should be blocked.
- Dietitians or health practitioners should make regular visits to places typically frequented by EMGs to educate people about the importance of vitamin D and the risks associated with its deficiency.
- A vitamin D screening programme should be established, involving in particular the periodic testing of the level of vitamin D among at-risk groups and people who have a history of vitamin D deficiency.

6.6.5 Individuals

Individuals should spend a daily minimum of 25 minutes outdoors while exposing 35% of the body area in the case of men and wearing non-restrictive dress in the case of women, particularly at midday, without sunscreen or facial products that may have a high SPF rating (Farrar et al., 2011; Webb et al., 2018). Muslim women who are restricted in their dress style may achieve a sufficient vitamin D level from sunlight by exposing their face and hands for 25 minutes daily during summertime (i.e. between June and August) without sunscreen. However, those with skin types V and VI will need to expose their face and hands for more than 25 minutes daily in the same conditions.

Although foods containing vitamin D are limited, members of EMGs should make some changes to their diet by increasing the intake of food rich in vitamin D, such as fatty fish. According to Nakamura et al. (2000), frequent consumption of fish can be an effective means of preventing vitamin D deficiency. However, it is probable that fish is not a preferred food for many people, among whom vitamin D supplementation may be a more effective way of addressing this issue.

6.7 Conclusion

In conclusion, the present study has shown that members of EMGs living in Manchester are at high risk of developing vitamin D deficiency. Effective interventions and strategies need to be established to address this problem, which may augment the risk of developing other diseases (Solanki et al., 1995; Hamson et al., 2003; Roy et al., 2007).

The study also found that 25(OH)D levels increased significantly following five weeks of supplementation with calcifediol, regardless of ethnicity. It thus adds to the growing body of evidence supporting the use of calcifediol not only to correct vitamin D status over a short period of supplementation, but to achieve this with a relatively small dose.

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Appendix 1: Ethical Approval

FACULTY OF HOLLINGS

Manchester Metropolitan University

M E M O R A N D U M

TO Mona Almujaydil

FROM Megan Schofield

DATE 4 April 2016

SUBJECT Application for Ethical Approval **(HOLL151605)**

On 4 April 2016 the Head of Ethics for Hollings considered your application for Ethical Approval (HOLL151602) entitled "Risk factors influencing vitamin D status of ethnic adults living in the UK and the efficacy of calcifediol supplementation on 25-hydroxyvitamin D concentrations". The application has been granted Favourable Opinion and you may now commence the project.

MMU requires that you report any Adverse Event during this study immediately to the Head of Ethics (Dr Ajay Patel) and the Administrator (Megan Schofield). Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence. Please notify Professor Craig Banks of any issues relating to this.

If you make any changes to the approved protocol these must be approved by the Faculty Head of Ethics. If amendments are required you should complete the MMU Request for Amendment form (found on the Graduate School website) and submit it to the Administrator.

Regards

Megan Schofield
Research Degrees Group Officer

Appendix 2: Participants information sheet

Participant Information Sheet

Study title: Risk factors influencing vitamin D status of ethnic minority adults living in the UK and the efficacy of calcifediol supplementation on 25-hydroxyvitamin D concentrations.

Background:

Vitamin D is essential for bone health and plays a role in maintaining the level of calcium and phosphorus for mineralisation. Therefore, a lack of vitamin D causes rickets in children and osteoporosis in adults. It is also associated with an increased risk of non-musculoskeletal conditions such as cancers, autoimmune and infectious diseases. Vitamin D precursors are found in a few foods, including fatty fish, fish liver oil, and egg yolks. The level of vitamin D produced in the body by the skin is much higher (90%) than that obtained from the diet. Vitamin D deficiency is a pervasive problem in the world, It is estimated that a billion people in the world suffering from vitamin D deficiency or insufficiency. The UK statistics note that an approximately half of the UK's population who are of white Caucasians and a substantial proportion (90%) of its ethnic minority populations are also likely to be affected by hypovitaminosis. For instance, the percentage of ethnic minorities who are living in Manchester are approximately 33.3% of the total population (Manchester city council, 2015). Reduced sunshine exposure and few sources of vitamin D in Manchester could increase hypovitaminosis D especially among those groups due to their eating habits and high skin pigmentation.

I would like to invite you to take part in a research study. Before you decide, you need to read and understand why the research is being done and what it would involve for you. Please take time to read the following information carefully:

What is the purpose of this study?

The purpose of this study is to determine which ethnic minorities who are living in Manchester are at a higher risk of vitamin D deficiency by detecting the main risk factors that effect on their vitamin status, and to determine the efficacy of vitamin D supplement to increase vitamin D level.

Who has approved this project?

The Research Ethics Committee of Manchester Metropolitan University has reviewed the project and given its ethical approval.

Why have I been invited?

We have invited healthy volunteers from different ethnic minority groups who wish to measure their vitamin D level and to treat the deficiency if it is detected by following an intervention with calcifediol supplement for 10 weeks.

Do I have to take part?

Your participation is voluntary. If you agree to take apart in this study, we will ask you to sign a consent sheet. In addition, you can withdraw at any time without having to give an explanation.

What will happen to me if I take part?

You will be asked to complete a short questionnaire to assess lifestyle, health factors and vitamin D intake. Blood spot will be taken on two separate occasions, one at the beginning to check the vitamin level, and one at the end of study by a trained phlebotomist. You will have to come to our Physiology Laboratory in order for us to be able to take a blood sample at MMU.

Will taking part be of any benefit to me?

By taking a part in this study, you will be told about your current vitamin D status and whether you need to increase vitamin D level by following an intervention, which is taking drops of calcifediol supplements once a week for 10 weeks. Supplementation of calcifediol is safe and simple. It used to treat vitamin D deficiency. After this period you well be told if you increased your vitamin D level. After completion of study, you will be given a compensation for your participation. In addition, the information you provide can contribute to the future development of the treatment for hypovitaminosis D and to know the efficacy of the supplement that used.

Are there disadvantages to taking part?

We do not expect anyone to suffer any harm or injury as a result of participating in this project. 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 time and place convenient for you.

*** The study will be carried out in accordance with:**

1. Human Tissue Act (2004), that covers the use of tissue for a number of which include research, clinical diagnosis and teaching.

2. Declaration of Helsinki guidelines, which is a statement of ethical principles to provide guidance to participants in medical research involving human subjects.

What if I have any concerns?

If you have any complaint or think of questions about the study please feel free to contact the research team using the contact details below.

Will my data be confidential?

All the information you give us will be confidential and used for the purposes of this study only. The data will be collected and stored in accordance with the Data Protection Act 1998 and will be disposed of in accordance of Human Tissue Act (2004).

What will happen to the results of the research study?

If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used.

*** If you have any further question please do not hesitate to contact the research student of the project.**

• Researcher: Mona Sulaiman

• Email: 13161463@stu.mmu.ac.uk

• Direct of studies: Dr Andrew Plunkett.

Appendix 3: Consent forms to participate in research project

Consent form to participate in research project

Manchester Metropolitan University

Study title: Risk factors influencing vitamin D status of ethnic adults living in the UK and the efficacy of calcifediol supplementation on 25-hydroxyvitamin D concentrations.

Name of Researcher: Mona Almujaydil

Please initial box

1. I confirm that I have read and understood the information sheet dated …. for the above project and have had the opportunity to ask questions about the research procedure. ☐

2. I understand that my participation in this research project is to provide blood sample on three separate occasions. ☐

3. I have been informed that the blood sample will be drawn by putting a lancet into a finger. ☐

4. I have been informed that my blood sample will be kept and used for testing until study activities are completed. At that time, it will be disposed of. ☐

5. I understand that I can withdraw my consent for use of my blood in this research at any time. ☐

I have read the above information and have been given an opportunity to ask questions. I agree to provide blood for this study, and I have been given a copy of this signed consent form for my own records.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Consent form to participate in research project

Manchester Metropolitan University

Title of Project: Risk factors influencing vitamin D status of ethnic adults living in the UK and the efficacy of calcifediol supplementation on 25-hydroxyvitamin D concentrations

Name of Researcher: Mona Almujaydil

Please initial all boxes

1. I confirm that I have read and understood the information sheet dated .....for the above study and have had the opportunity to ask questions about the research procedure. ☐
2. I confirm that I am eligible (no chronic disease or using vitamin D supplements (3 months prior to or during the trail), not pregnant or breast feeding and not using of medication that could interfere with vitamin D metabolism such as Phenobarbital and Dilantin (phenytoin). ☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason to the named researcher. ☐
4. I understand that my responses will be used for analysis for this research project. ☐
5. I understand that my responses will remain anonymous. ☐
6. I agree to take part in the above research project. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix 4: Questionnaire

QUESTIONNAIRE

Name: …….

Contact number: ……

Email: ……..

PART 1: PERSONAL INFORMATION

1) **What is your gender?**

☐ Male ☐ Female

2) **Your Age:**

☐ Under 18

☐18 to 29 years

☐ 30-39 years

☐ 40-49 years

☐ > 50

3) **Your weight**: …….**kg**

4) **Your height:** ……..**cm**

5) **Country of Birth:** ……..……

6) **How long have you been in the UK?**

☐ Less than one year

☐ 1- 5 years

☐ More than 5 years

7) **What is your race/ethnicity?**

☐White/ Caucasian ☐ Black African ☐ Caribbean

☐ Arab ☐ South Asian (Indian, Pakistan and Bangladesh)

☐ Chinese ☐Other Asian Background

8) What is your religion?

☐ Muslim ☐ Christian

☐ Jewish ☐ Other

9) Socioeconomic Status:

9.1 Income position: (per year)

☐ Less than £5,200 ☐ £26,000 to £36,399

☐ £5,200 to £10,399 ☐ £36,400 to £49,399

☐ £10.500 to £20.000 ☐£62,400 to £77,999

9.2 Education level:

☐ Did not complete primary school

☐ Primary school

☐ Secondary school

☐ Diploma

☐ Bachelor's degree

☐ Postgraduate degree

9.3 Occupation:

☐ Employed

☐ Unemployed

☐ Student

9.4 Which best describes your occupation?

☐ Mainly indoors

☐ Half indoor and half outdoor

☐ Mainly outdoors

PART 2: SUN EXPOSURE

The following questions ask about your skin and how it reacts to the sun

10) How would you describe your skin colour?

☐ Pale white; blond or red hair; blue eyes; freckles (Always burns, never tans)

☐ White; fair; blond or red hair; blue or green eyes (Burns easily)

☐ Cream white; fair with any hair or eye colour (Mild burn, tans average)

☐ Moderate brown; typical Mediterranean skin (Rarely burns, tans easily)

☐ Dark brown, Arab and Asian skin types (Very rarely burns)

☐ Dark-skinned black (No burn)

11) What areas of your body are often exposed to sunlight? (Tick more than one)

☐Face ☐ Hands

☐Full arms ☐ Half arms

☐Full legs ☐ Half legs

☐Other …….

12) Have you been sunbathing or used a sunbed in the last month?

☐Yes ☐No (go to question 15)

13) How many times did you do that per month?

……..

14) How long did it take for each session?

……

15) Do you use sunscreen?

☐Yes ☐No (go to question 17)

16) What is the sun protection factor (SPF) number of the sunscreen that you use must often?

☐ 8 ☐ 45

☐15 ☐ Other …….

17) How often are you exposed directly to the sun (not through glass or window) in typical weekdays? (Monday- Friday)

Please tick only one per line

The time	< 15 minutes	15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
7-8 am	☐	☐	☐	☐	☐
8-9 am	☐	☐	☐	☐	☐
9-10 am	☐	☐	☐	☐	☐
10-11 am	☐	☐	☐	☐	☐
11-12 am	☐	☐	☐	☐	☐
12-1 pm	☐	☐	☐	☐	☐
1-2 pm	☐	☐	☐	☐	☐
2-3 pm	☐	☐	☐	☐	☐
3-4 pm	☐	☐	☐	☐	☐
4-5 pm	☐	☐	☐	☐	☐

18) How often are you exposed directly to the sun (not through glass or window) in typical weekends? (Saturday-Sunday)

Please tick only one per line

The time	< 15 minutes	15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
7-8 am	☐	☐	☐	☐	☐
8-9 am	☐	☐	☐	☐	☐
9-10 am	☐	☐	☐	☐	☐
10-11 am	☐	☐	☐	☐	☐
11-12 am	☐	☐	☐	☐	☐
12-1 pm	☐	☐	☐	☐	☐
1-2 pm	☐	☐	☐	☐	☐
2-3 pm	☐	☐	☐	☐	☐
3-4 pm	☐	☐	☐	☐	☐
4-5 pm	☐	☐	☐	☐	☐

PART 3: HEALTH INFORMATION

19) Do you suffer from any disease?

☐ Yes ☐ No

If yes, do you suffer from any of the following?

☐ Osteoporosis ☐ Parathyroid

☐ Liver disease ☐ Kidney disease

☐ Heart disease ☐ High blood pressure

☐ Diabetes ☐ Cancer

☐ Other ……

If you are a female: please answer questions number 20 and 21, otherwise move to question number 22

20) Are you currently pregnant?

☐ Yes

☐ No

21) Are you currently breastfeeding?

☐ Yes ☐ No

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

☐ Yes ☐ No

23) Smoking status?

☐ Current

☐ Former

☐ Never (go to question 25)

24) How would you describe yourself?

☐ I smoke every day ☐ I smoke occasionally, but not every day

25) Do you drink alcohol?

☐ Yes
☐ No (go to question 28)

26) On how many days during a typical week did you usually drink alcohol, on average?

Number of days……

27) On the days that you drank alcohol, how many drinks did you have on average?

☐ Half pint/glass of beer, lager, stout or cider

☐ Single measure of spirits (e.g. whiskey, rum, vodka, gin)

☐ Single glass of wine, sherry, port

☐ Premixed drinks (e.g. Twodogs, Bacardi Breezer, Hooch)

Number of drinks …….

PART 4: FOOD AND DIETARY ASSESSMENT

Food frequency questionnaire

28) Please put a tick (√) in the box to indicate how often ,on average ,you have eaten these food

FOOD	AVERUGE USE						
	Once a day	Twice a day	Once a week	2-3 times a week	1-3 times a month	Less than once a month	Never
Fresh mackerel cooked	☐	☐	☐	☐	☐	☐	☐
Fresh tuna cooked	☐	☐	☐	☐	☐	☐	☐
Fresh sardines cooked	☐	☐	☐	☐	☐	☐	☐
Fresh salmon cooked	☐	☐	☐	☐	☐	☐	☐
Canned tuna	☐	☐	☐	☐	☐	☐	☐
Canned sardine	☐	☐	☐	☐	☐	☐	☐
Canned salmon	☐	☐	☐	☐	☐	☐	☐
Herring	☐	☐	☐	☐	☐	☐	☐
Beef	☐	☐	☐	☐	☐	☐	☐
Pork and Ham	☐	☐	☐	☐	☐	☐	☐
Beef liver	☐	☐	☐	☐	☐	☐	☐
Pork liver	☐	☐	☐	☐	☐	☐	☐
Egg (Whole)	☐	☐	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐	☐	☐
Cream cheese	☐	☐	☐	☐	☐	☐	☐
Hard cheese (e.g cheddar, Swiss)	☐	☐	☐	☐	☐	☐	☐
Butter	☐	☐	☐	☐	☐	☐	☐
Milk (Whole)	☐	☐	☐	☐	☐	☐	☐
Semi-skim Milk	☐	☐	☐	☐	☐	☐	☐

Margarine	☐	☐	☐	☐	☐	☐	☐
Sun dried mushroom	☐	☐	☐	☐	☐	☐	☐

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

☐ Yes ☐No

30) How much milk do you drink each day, including with tea, coffee and cereal etc.?

☐ None ☐ 1 pint (568ml or 2 cups)

☐ 250 ml (1 cup or mug) ☐ More than 1 pint

31) Do you usually eat breakfast cereal?

☐ Yes ☐No (go to question 32)

If yes, which brand and types of breakfast cereal, did you usually eat?

Brand e.g. Kellogg's	Type e.g. cornflakes

32) Do you use any products fortified with vitamin D such as margarine, orange juice and breakfast cereal?

☐ Yes ☐ Sometimes

☐ No ☐ Don't know

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

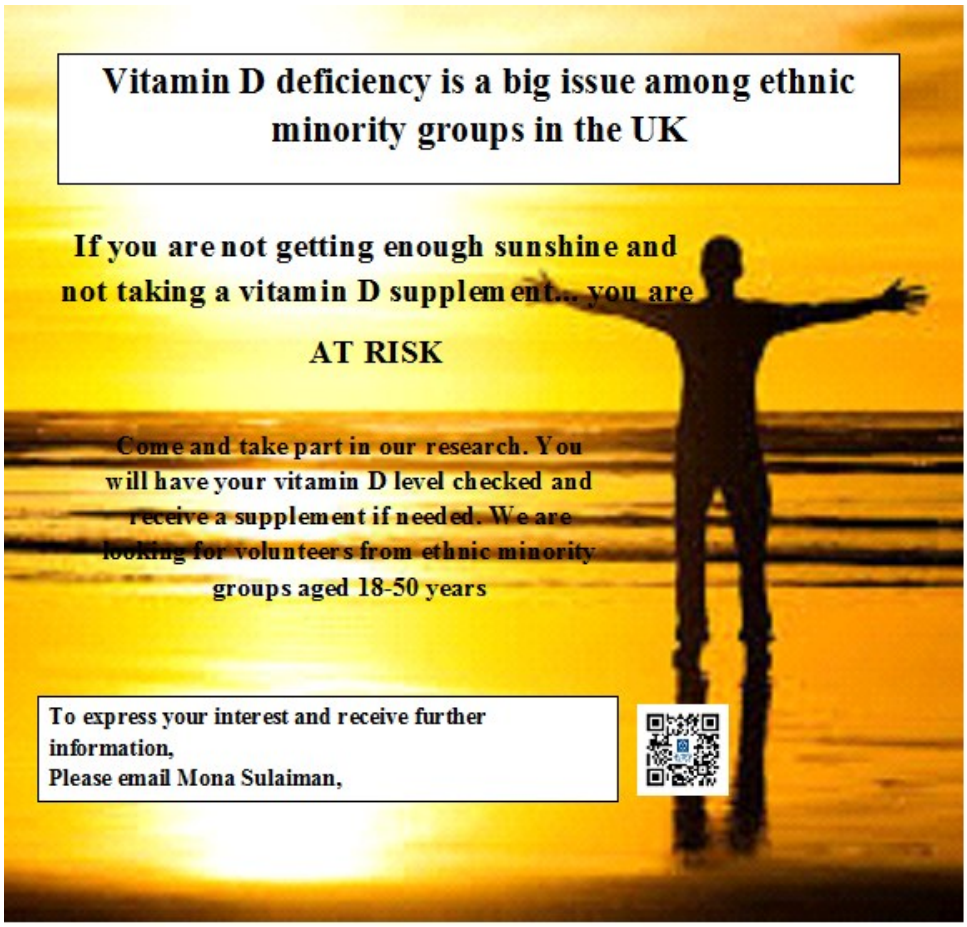
☐ Yes ☐ No

If yes, please complete the table below:

Name and brand	Dose please state number of pills or capsules or teaspoons consumed	How often do you take these?		
		Daily	Weekly	Monthly

Thank you very much for completing this questionnaire.

Appendix 5: Poster



Appendix 6: Leaflet

Mona Almajaydi
Hollings Faculty,
Manchester Metropolitan University
M156 BG
Email Address
Manchester.vitamind@gmail.com

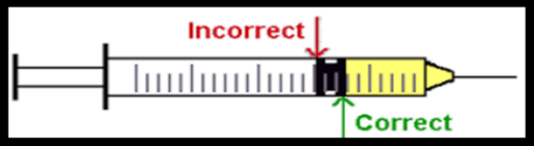
Intervention Trial

Instruction for taking the supplement

Recording Doses Taken

Number of week	Date	Tick if you have had it
Week 1		
Week 2		
Week 3		
Week 4		
Week 5		
Week 6		
Week 7		
Week 8		
Week 9		
Week 10		

Storage condition



Appendix 7: Checklist for the procedures

	Visit 1					Visit 2			Visit 3	Visit 4
ID code	**Date**	**Consent forms for research trial & provide blood sample**	**Anthropometric measurements**		**Blood sample (week0)**	**Intervention**			**Blood sample (week 5)**	**Blood sample (week 10)**
			Height (cm)	Weight (kg)		**allocation**	**pre**	**post**		