Vitamin D deficiency in UK South Asian Women of Childbearing Age: A Comparative Longitudinal Investigation with UK Caucasian Women

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Mini abstract

This is the first one-year longitudinal study which assesses vitamin D deficiency in young UK dwelling South Asian women. The findings are that vitamin D deficiency is extremely common in this group of women and persists all year around, representing a significant public health concern.

Abstract

Purpose

There is a lack of longitudinal data assessing seasonal variation in vitamin D status in young South Asian women living at Northern Latitudes. Studies of postmenopausal South Asian women suggest a lack of seasonal change in 25-hydroxy vitamin D [25(OH)D] although it is unclear if this is prevalent among premenopausal South Asians. We aimed to evaluate, longitudinally, seasonal changes in 25(OH)D and prevalence of vitamin D deficiency in young UK dwelling South Asian women, as compared with Caucasians. We also aimed to establish the relative contributions of dietary vitamin D and sun exposure in explaining serum 25(OH)D.

Methods

A one-year prospective cohort study, assessing South Asian (n=35) and Caucasian (n=105) premenopausal women living in Surrey, UK (51°N) aged 20-55years. The main outcome measured was serum 25(OH)D concentration. Secondary outcomes were serum parathyroid hormone, self-reported dietary vitamin D intake and UVB exposure by personal dosimetry.

Results

Serum 25(OH)D less than 25 nmol/l was highly prevalent in the South Asians in the winter (81%) and autumn (79.2%). Deficient status (below 50 nmol/l) was common in Caucasian women. Multilevel modelling suggested that, in comparison to sun exposure (1.59; 95% CI 0.83 to 2.35), dietary intake of vitamin D had no impact on 25(OH)D levels (-0.08; 95% CI -1.39 to 1.23).

Conclusions

Year-round vitamin D deficiency was extremely common in the South Asian women. These findings pose great health threats regarding adverse effects of vitamin D deficiency in pregnancy and warrant urgent vitamin D public health policy and action.

Keywords

Seasonal 25-hydroxyvitamin D, vitamin D deficiency, premenopausal women, longitudinal cohort study, UVB exposure, South Asian ethnicity

Introduction

The problem of vitamin D deficiency has been known for many years in relation to bone diseases such as osteomalacia, osteoporosis and rickets. However it is only more recently that vitamin D deficiency has been linked with specific health outcomes, including increased risk of heart disease and cancer, as well as infectious and autoimmune disease[1]. In addition, recent research has highlighted the potential effects of maternal vitamin D deficiency before and during pregnancy, including adverse effects on the health of the mother and the unborn child. Such effects include increased risk of low birth weight[2], caesarean delivery[3], pre-eclampsia[4] [5], infant respiratory infection[6, 7] and maternal gestational diabetes[8]. Of even more concern, such effects have been suggested in the longer term to be of importance to childhood bone health[9, 10], insulin resistance[11], and immune function[12]. At the extreme, there have been clinical cases of hypocalcaemic seizures reported in young infants who had severe vitamin D deficiency at birth[13], most likely due to poor maternal vitamin D status in pregnancy[13, 14]

Although vitamin D deficiency is a widespread and common problem in the UK population [15], it is especially common and severe in UK ethnic minority groups such as South Asian women [16]. These women are veiled for religious and cultural reasons and so have a reduced sun exposure. Indeed, the Department of Health in the UK[17] recommends the use of 10µg vitamin D supplements per day for those with limited sunshine exposure, such as those who are confined indoors or wear concealing clothing. The problem of vitamin D deficiency in South Asian immigrants to the UK, its diagnosis, and clinical consequences has periodically been raised by clinicians [18, 19], suggesting that it is an issue of ongoing concern.

Most published research to date has focused on older South-Asian women living either in the UK or in their country of origin, who are not of child-bearing age. There is a considerable lack of data on pre-menopausal UK South Asian women and no previous published data examining seasonal change in 25(OH)D status in a cohort of women followed over the course of a year. The work reported here adds to existing data by following up the same individuals over a year, rather than assessing different individuals in different seasons. This allows the use of multilevel-modelling to assess the relative contribution of within- and between-subject factors that may influence 25(OH)D status, in addition to the contribution of diet, ethnicity and UVB exposure.

Therefore, in the current study, we followed up a cohort of South Asian and Caucasian premenopausal women over 12 months, assessing seasonal serum 25(OH)D status between summer 2006 and spring 2007, seasonal dietary vitamin D intake and UVB exposure. Based on the findings in the literature base, and knowledge of factors influencing vitamin D status, the a priori hypothesis was that both Asian and Caucasian women would show seasonal change in vitamin D status, but the change would be more pronounced in the Caucasian women. Also, it was hypothesised that the Asian women would have a higher prevalence of vitamin D deficiency in each season.

Methods

Participants

The D-FINES Study: The participants were enrolled in the D-FINES study (Diet, Food Intake, Nutrition and Exposure to the Sun in Southern England) which was a single-centre cohort study conducted at the University of Surrey, Guildford, UK. The study followed up 365 South Asian and Caucasian UK women living in the South of England from June 2006-May 2007. Of these women, 181 were premenopausal and 184 postmenopausal. Data from the postmenopausal Caucasian arm of this study have been published previously[20]. The current analysis focuses solely on the premenopausal South Asian and premenopausal Caucasian women. In accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, ethical reviews were obtained from relevant Research Ethics Committees (National Health Service NHS REC 06/Q1909/1, and University of Surrey EC/2006/19/SBMS). Written, informed consent was obtained from all participants.

Recruitment method: In order to reduce recruitment bias, a random sample of Caucasian participants (likely to fulfil the inclusion criteria) was recruited via the databases of general practitioner (GP) surgeries in Surrey, Hampshire, Berkshire and outer London. This ensured sampling from a variety of areas of differing socio-economic status that reflected the South East of England, in order to reduce risk of bias towards higher socioeconomic status participants. Socioeconomic status was assessed by the Index of Multiple Deprivation (IMD). This was calculated from the Lower Super Output Area rank using the Office for National Statistics (ONS) website[21] and was converted to the corresponding IMD using the Indices of Deprivation for Super Output Areas[22]. South Asian women fulfilling the same inclusion criteria were recruited via local Asian Women's networks.

Exclusion criteria: Potential participants were excluded from the study if they had a diagnosis of any disorder of calcium homeostasis or were currently taking any medication likely to affect bone, calcium or vitamin D metabolism. Women who were currently taking vitamin D supplements or cod liver oil supplements were also excluded or asked to refrain from their use 3 months before and during the 12 months of the study. The participants' first blood samples at the baseline visit were screened for abnormalities in liver, thyroid and renal function. Any women showing an abnormality on any of these tests were excluded and their GP notified of the abnormal result.

Numbers of participants recruited: A total of 181 premenopausal women (135 Caucasians and 46 Asians) were recruited to the study. By the end of the fourth visit, 90 Caucasian women and 35 Asian women had attended the University of Surrey Clinical Investigation Unit on all four occasions. Figure 1 summarises the number of participants recruited and flow of participants through the study.

Protocol

Participants attended the University of Surrey Clinical Investigation Unit four times in the course of the year – in summer, autumn, winter (2006) and spring (2007) for a fasting blood sample. At baseline, they provided lifestyle information by questionnaire on ethnic origin, number of years resident in the south of the UK, education, smoking history, alcohol use and usage of prescribed and over-the-counter medications. Anthropometric information (weight, height) was also obtained and Body Mass Index (BMI) calculated. After each seasonal visit, participants completed four-day diet diaries (as previously validated in the EPIC cohort) and wore two dosimeter badges for one week to estimate weekly UVB exposure (see below for

details of dosimetry). In autumn 2006 and spring 2007, the participants also undertook a Dual X-ray Absorptiometry scan (Hologic) for assessment of body composition (total body fat mass).

Vitamin D and parathyroid hormone status assessment

The laboratory used for the biochemical assay measurements (Vitamin D Research Group, Manchester Royal Infirmary, UK) participates in the Vitamin D quality assurance scheme (DEQAS). Serum 25(OH)D was measured using the manual enzyme immunoassay (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, UK). Manufacturer's reference ranges were 19-58 ng/ml (48-144 nmol/L) but vary with season; sensitivity 2 ng/ml (5 nmol/L); intra- and inter-assay coefficients of variation 6% and 7%, respectively (manufacturer's values). Serum intact parathyroid hormone (PTH) was measured using the OCTEIA immunoenzymometric assay (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, UK). The normal adult reference range is 0.8-3.9 pmol/l; sensitivity 0.06 pmol/l; intra- and inter-assay coefficients of variation 4% and 6%, respectively (manufacturer's values).

UVB dosimetry

UVB dosimetry was undertaken using badges made of polysulphone film. For the purposes of UV measurement, the badges were read at 330 nm on the spectrophotometer (Aquarius, CECIL CE7200) at the University of Surrey (CV <1%) prior to, and after, use. The amount of UV light detected by the badges was translated to standard erythema dose (SED) measurements using the mathematical formula: $SED = 10.7 [\Delta A_{330}] + 14.3 [\Delta A_{330}]^2 - 26.4 [\Delta A_{330}]^3 + 89.1 [\Delta A_{330}]^4$, whereby ΔA_{330} is the change in absorbance of the polysulphone film badge pre- to post-UVB exposure. Subjects were asked to wear one badge on their outer

clothing on weekdays and the other badge at weekends, with their SEDs summed to give an estimate of the weekly SED dose.

Study Power

Power calculations for the Caucasian women were initially structured on the differences in 25(OH)D between women living in Southern England (Surrey) and women living in Northern UK (Aberdeen); re the comparison study of postmenopausal women reported previously by Macdonald et al (2010)[20]. In order to detect a 0.4 SD difference in 25 OHD between Aberdeen and Surrey, a total of 78 subjects were needed at 80% power (108 subjects at 90% power). Therefore, 135 Caucasian premenopausal women (which included an extra 27 premenopausal and postmenopausal subjects to allow for a 25% dropout rate) were recruited. To calculate power for the ethnic difference in 25(OH)D status, the differences in 25(OH)D between Caucasian and Asian women seen in published studies was used. 25(OH)D levels in Muslim Asian populations are known to be in the region of 6.9ng/ml [SD 7.1][23]; hence, in order to detect a 0.8 SD difference in 25(OH)D between Caucasian and South Asian women, a total of 19 premenopausal Asian subjects were needed at 80% power and 26 subjects at 90% power. Therefore, total of 39 Asian premenopausal women were recruited, representing a 45% over recruitment, anticipating the higher dropout rates associated with studies involving ethnic-minority participants. Given the longitudinal nature of the study, these calculations were very conservative to allow for the smaller variance and repeated-measures nature of the longitudinal analysis.

Statistical Analysis

All descriptive (means, standard deviations) and inferential statistics (mixed between-within subjects ANOVA) were run using SPSS 16.0[24]. Inspection of histograms for normality showed a slightly skewed distribution for 25(OH)D. With log transformation unable to normalise the data for all seasonal measurements, data were retained in their non transformed form. As no non-parametric alternative to the mixed between-within subjects ANOVA (that allows longitudinal comparison of the four groups over time) exists, and the ANOVA technique minimizes the risk of a type 1 error by keeping multiple statistical testing to a minimum, this analysis was deemed most appropriate. Non parametric equivalents would have involved multiple testing of the groups in seasons separately, inflating the risk of a type 1 error to unacceptable levels, and not capitalizing on the longitudinal nature of the data. Therefore, non-parametric tests were used only for the cross sectional analyses.

Multilevel modelling (MLM) was used to assess the relative contribution of dietary vitamin D, UVB exposure and ethnicity to 25(OH)D serum levels. Seasonal factors that may influence 25(OH)D include variations in UV exposure due to changes in climatic factors and zenith angle of the sun, as well as individual behaviour (e.g. time spent outdoors, style of dress, sunscreen usage). Also included here are dietary factors that may vary by season due to different preferences for, and availability of, foods (e.g. colder meals in the summer, meals linked to different festivals and holidays) that may influence vitamin D intake. Fixed individual factors that do not vary with season are also likely to affect 25(OH)D status. These include cultural and lifestyle factors such as education, socio-economic status and physiological factors such as and genetic influences. Some fixed individual factors such as gender and ethnicity have a cultural and physiological basis. Multilevel modelling was chosen for the analysis as it enables partitioning of variation in vitamin D status into effects at the seasonal (changing) and individual (fixed) level, and takes advantage of the longitudinal

nature of this dataset where repeated seasonal measurements are clustered in individuals. The MLwiN software (version 2.11)[25] was used for this part of the analysis.

Results

Baseline Participant Characteristics

Table 1 shows the participant characteristics at baseline. For continuous variables, the Mann Whitney U Test showed no significant difference between the Caucasian and Asian groups for body mass index (BMI) ($p=0.413$), total body fat mass ($p=0.196$), or body weight ($p=0.122$). There were significant differences by ethnicity in age ($p=0.002$), index of multiple deprivation ($p=0.024$), serum albumin ($p<0.001$) and height ($p<0.001$). However, the absolute differences were small and were not deemed to be large enough to be of concern physiologically. For nominal variables, chi square tests showed there was a significant difference by ethnicity in the highest level of education ($p=0.007$), % smokers ($p=0.015$) and % alcohol consumers ($p<0.001$). There were no significant differences by ethnicity for previous usage of fish supplements ($p=0.741$) or vitamin supplements ($p=0.897$). However, Caucasian women were significantly more likely to be contraceptive pill users than Asian women ($p=0.015$).

Prevalence of vitamin D deficiency by season and ethnic group

Table 2 shows that more Asian than Caucasian women were classified as having $25(\text{OH})\text{D} < \text{less than } 25 \text{ nmol/l}$ in all seasons. This was highest in both groups in the winter, with 10.0 % of Caucasian and 80.8 % of Asian women having $25(\text{OH})\text{D}$ less than 25nmol/l . There was slight improvement in the summer with 0.8% of Caucasians and 53.5% of Asians having

25(OH)D less than 25 nmol/l. Using the 50nmol/l cut-off point for deficiency, as recently recommended by the new IOM guidelines[26] nearly all Asian women (95-96%) were below 50nmol/l throughout the whole year. Lastly, status below the 75 nmol/l cut off point was universal throughout the year in the Asian group while in the Caucasian group, it ranged from 88.6% in winter to 60.9% in summer. Chi-squared tests showed the above trends to be statistically significant within each season (Summer $X^2=100.1$ $p<0.001$; Autumn $X^2=71.3$, $p<0.001$, Winter $X^2=49.7$, $p<0.001$, Spring $X^2=54.6$, $p<0.001$).

Seasonal change in 25(OH)D

Table 3 and Figure 2 show the raw data for 25(OH)D status. Significant seasonal variation throughout the year can be seen, with highest levels of 25(OH)D in summer, falling in autumn to the lowest level in winter, and rising again in the spring. Indeed, a mixed between-within subjects ANOVA showed a significant main effect of season on 25(OH)D [$p<0.001$], with model estimates showing a change in 25(OH)D from summer to winter for all participants combined from 49.7nmol (95%CI 42.8 to 56.6nmol/l) to 31.8nmol/l (95%CI 27.5 to 36.2nmol/l)

ANOVA also showed that there was a significant between-subjects effect of group [$p<0.001$] with the Asian women having significantly lower 25(OH)D throughout the year. Model estimated showed this as being lower on average than the Caucasian group by -34.8nmol/l (95% CI -45.0 to -24.6nmol/l). There was also a significant interaction between ethnicity and season [$p<0.001$], with Asians showing less seasonal variation than did Caucasian women. Model estimates showed there was a change in Caucasian from 74.3nmol/l (95%CI 68.0 to 80.6nmol/l) in summer to 42.9nmol/l (95%CI 38.9 to 46.8nmol/l)in winter. This was compared to a change of 25.2nmol/l (95%CI 12.9 to 37.4nmol/l) in summer to 20.8nmol/l (95% CI 13.1 to 28.5nmol/l) in winter for the Asians.

UVB exposure

The raw data for mean weekly cumulative UVB exposure (SED) by group can be seen in Table 3. A mixed between-within subjects ANOVA showed that there was a significant main effect of season on UVB exposure [$p < 0.001$]. Indeed, model estimates showed that for all participants together there was a higher weekly UVB exposure in the summer of 5.1 SED (95% CI 2.6 to 7.7 SED) which fell dramatically to its lowest levels in autumn of 0.4 SED (95% CI 0.03 to 0.7 SED) and in winter of 0.4 SED (95% CI 0.1 to 0.7 SED). This then rose again in the spring to 4.3 SED (95% CI 2.5 to 6.0 SED). These results paralleled the changes in serum 25(OH)D concentration described above.

There was a significant between subjects effect of group [$p = 0.021$], with model estimates showing that the Caucasian group had a higher weekly SED by a magnitude of 2.1 SED (95% CI 0.3 to 3.9 SED) over the course of the year than the Asian group. As can be seen in table 3, there was a trend for the summer and spring SED to be higher in the Caucasians than the Asians, however, there was no significant interaction between season and group [$p = 0.284$].

Dietary Vitamin D

Table 3 shows a trend for vitamin D intake to be slightly higher in the Caucasian than the Asian group, although mixed between-within subjects ANOVA showed there was no significant main between-subjects effect of group [$p = 0.427$]. Indeed, model estimates for the yearly average intake were 2.6 $\mu\text{g/d}$ (95% CI 2.2 to 2.9 $\mu\text{g/d}$) for Caucasians and 2.2 $\mu\text{g/d}$ (95% CI 1.5 to 3.0 $\mu\text{g/d}$) for the Asians, with the Asians being lower on average by -0.3 $\mu\text{g/d}$ (95% CI -1.1 to 0.5 $\mu\text{g/d}$).

The ANOVA also showed that there was no significant main effect of season [$p=0.359$] for dietary vitamin D. However, there was a trend for a seasonal change with model estimates for all participants together being 2.7 $\mu\text{g/d}$ (95% CI 2.0 to 3.4 $\mu\text{g/d}$) in summer, 2.3 $\mu\text{g/d}$ (95% CI 1.8 to 2.7 $\mu\text{g/d}$) in autumn, 2.6 $\mu\text{g/d}$ (95% CI 1.9 to 3.2 $\mu\text{g/d}$) in winter and 2.1 $\mu\text{g/d}$ (95% CI 1.5 to 2.6 $\mu\text{g/d}$) in spring.

Last, the ANOVA showed no significant interaction between season and group [, $p=0.396$]. However, there was a non-significant trend for dietary vitamin D intake to be more variable by season in the Caucasian than the Asian group. Indeed, model estimates showed a change from 2.7 $\mu\text{g/d}$ (2.1 to 3.3 $\mu\text{g/d}$) in summer to 2.8 $\mu\text{g/d}$ (2.2 to 3.3 $\mu\text{g/d}$) in winter for the Caucasians, and a change from 2.7 $\mu\text{g/d}$ (1.4 to 4.0 $\mu\text{g/d}$) in summer to 2.3 $\mu\text{g/d}$ (1.2 to 3.5 $\mu\text{g/d}$) in winter for the Asians.

The main sources of vitamin D in the diet (total vitamin D, including D2 and D3, minimum and maximum values across seasons) for South Asians were 21.8% to 33% (flour, grains and starches), 25.6% to 29.9% (meat and meat products), 5.8% to 17.6% (fish and fish products), 11.6% to 19.7% (milk and milk products), and 7.4% to 17.4% (egg and egg products). The main sources for Caucasians were 20 to 30.3% (meat and meat products), 24.2% to 26.6% (flour, grains and starches), 19% to 29.7% (fish and fish products), 10% to 12.9% (milk and milk products), and 4.4% to 9.3% (egg and egg products).

Relative Contribution of Diet and UVB exposure to serum 25(OH)D

Seasonal variables such as vitamin D status, dietary variables and UVB exposure were entered into the multi-level model as continuous variables, with fixed variables (ethnicity) entered as dummy variables (0,1). There were no differences in any of the model parameters when continuous variables were centred or not so they were left uncentred. Any variance left

unexplained by the model parameters was split into unexplained variance at the seasonal (level 1) and individual (level 2) levels. Residual plots were used to plot standardized residuals against normal scores. This checked that distribution of residuals was normal for each model. Random intercept models were used only without random slopes as inspection of spaghetti plots showed only intercepts varied between individuals, not slopes. Random slopes may also not be appropriate due to the small number of measurements (four) for each individual. Dietary vitamin D ($\mu\text{g/d}$), weekly UVB exposure (SED) and ethnicity were retained in the model at all times due to their being of high theoretical interest in this particular study. UVB exposure appeared to show a 'bathtub' shape and a quadratic parameter for UVB was therefore trialled in the model. Other potentially explanatory variables such as calcium intake and body fat mass were trialled in the models but were not found to be statistically significant so were removed.

Table 4 shows the multilevel model constructed by adding the predictor variables dietary vitamin D intake, UV exposure and ethnicity. This full model showed a significant effect of UVB exposure on vitamin D status, with a 1 SED rise in UVB exposure associated with a rise in 25(OH)D by 1.6 nmol/l (95% CI; 0.8 to 2.4). The addition of the quadratic UV parameter was not significant and therefore did not improve the prediction of the model. The parameter for dietary vitamin D was also not significant. Asian ethnicity was associated with a lower 25(OH)D status by -31.4 nmol/l (-95%CI; 40.7 to -22.1). In terms of unexplained variance, 47.5% was at the individual level, whilst 52.5% was for seasons within individuals. Therefore, there was roughly equal unexplained variance at both levels of the model. This means seasonal change within subjects, and between-subjects differences were equally important in explaining 25(OH)D: thus season is as important as fixed individual traits in determining vitamin D status.

Discussion

It has been shown in this study that the vast majority of South Asian premenopausal women in the D-FINES cohort have serum 25(OH)D less than 25 nmol/l, and that many of their Caucasian counterparts also have deficient vitamin D status (less than 50nmol/l). Whilst lower vitamin D status was expected in Asians than in Caucasians, the extent of the deficiency and its consistency across the year was unexpected. The lack of seasonal change in 25(OH)D status in the premenopausal Asian population has not been demonstrated before in the UK and to the authors' knowledge, not elsewhere in the literature. However, these findings mirror previous published results from the postmenopausal Asian subset of this cohort[20]. Serum 25(OH)D less than 25 nmol/l was extremely common in the Asian women, especially in the autumn (79%), winter (81%) and spring (75%) although serum 25(OH)D less than 25nmol/l was also high in summer (54%). The total percentage of Asian women deficient (below 50 nmol/l) was extensive at 95% in summer and 96% in autumn, winter and spring. Vitamin D status less than 75 nmol/l was universal throughout the year in the Asian women. These longitudinal findings support the cross sectional results of Roy et al (2007) who showed that 94% of South Asian women living in the UK had 25(OH)D <37.5 nmol/l and 26% had 25(OH)D <12.5 nmol/l. Our results also support other cross-sectional data for Asian women[27] [28]. Serum 25 (OH)D less than 25 nmol/l was less common in the Caucasian than Asian women, at 1%, 4%, 10%, and 5% in summer, autumn, winter and spring, respectively. This contrasts with findings from our postmenopausal Caucasian subset published previously[20] where no women were less than 25nmol/l in summer and autumn. The total percentage of premenopausal Caucasian women who were deficient (below 50 nmol/l) was also considerable at 20%, 43%, 70% and 52% in summer, autumn, winter and

spring respectively. 25(OH)D less than 75nmol/l was very common at 61%, 79%, 90% and 87% in summer, autumn, winter and spring respectively.

The seasonal variation in 25(OH)D in the Caucasian women was as expected with the highest levels in the summer, falling in the autumn to a minimum in the winter, and rising again in the spring. These longitudinal findings support those of previous cross sectional studies of Caucasian populations in Ireland[29], the UK[15], and the USA[30]. The reasons for the deficient winter 25(OH)D status in some, but not other Caucasians is not clear. However, this could be due to differences in lifestyle (i.e. low sun exposure due to work or leisure patterns) or genetics. .Indeed, it is also likely that sun exposure is an important factor in the 25(OH)D status of Caucasians, and further analysis is required to assess whether the Caucasians in this study with low 25(OH)D also have a low summer sun exposure.

The higher sun exposure registered by the dosimeter badges in summer and spring in the Caucasian than in the Asian group suggests that the Caucasians were outdoors for longer or more often in the summer and spring than the Asians. The low sun exposure of the Asian women is likely a large part of the explanation for lack of seasonal variation of 25(OH)D, and lower overall 25(OH)D and is in agreement with other observational studies that have described sun avoidance in Asian and Middle Eastern women[31, 32] [33]. The wearing of clothing that covers more of the skin by the Asian women is also likely to be a cause of their lower 25(OH)D concentrations, as clothing significantly reduces the skin formation of vitamin D even with a UVB dose as high as 6 Minimal Erythral Doses MEDS[34], with 1MED being defined as the minimum UVB dose required to cause a burn on the skin. Seventy-five percent of the D-FINES Asian women were of Pakistani origin and were partly veiled (showing hands and face only), so had minimal skin exposure to the sun. Overall, a combination of sun avoidance, veiling and also darker skin pigment is likely to be responsible

for the lower 25(OH)D, and a lack of seasonal change in 25(OH)D in the Asian than in the Caucasian women. It is likely that genetic influences also have a role to play in genetic differences in 25(OH)D however, these could not be assessed in the current study.

In terms of diet, both groups had low intakes of Vitamin D, with no significant difference in vitamin D intake between ethnic groups and no significant seasonal change in intakes in either ethnic group. To the authors' knowledge this lack of seasonal change in vitamin D intake has not been reported previously in either ethnic group. We acknowledge that only women who were not on dietary supplements were included in this study, so this excludes the true effect of supplement usage in the UK population. However, only 15% of potential applicants in the D-FINES study were excluded or asked to refrain from taking supplements during the course of the study. This was only in the Caucasian group, as no Asian women applying for the study were taking vitamin D supplements. Therefore it is unlikely that the exclusion of women taking supplements will have significant impact on the results of this study.

Bringing, UVB, diet, and ethnicity together, the full multi-level model showed a significant effect of UVB exposure and ethnicity on vitamin D status. Dietary vitamin D, dietary calcium and total body fat mass were not significant predictors of serum 25(OH)D. Importantly, this suggests that dietary vitamin D has little effect on 25(OH)D even at the reasonably low 25(OH)D concentrations seen in this study. Only UVB exposure and ethnicity were significant predictors of 25(OH)D. Therefore UVB can be deemed a more important contributor to 25(OH)D than dietary intake, and in this dataset dietary vitamin D did not contribute significantly to 25(OH)D status.

The predictors in the model explained the same amount of variance in 25(OH)D at both levels of the model. Therefore, the effects of seasonal within subjects, and between-subjects

differences were equally important in understanding influences on vitamin D status. This means individuals' responses to seasonal change were just as important as fixed individual factors in determining 25(OH)D.

Comparing these results with previous literature, the significance of UVB as a predictor of serum 25(OH)D supports the findings of some previous regression studies [9] [35] but not others [36]. The inability of dietary vitamin D intake to predict serum 25(OH)D in this study supports some but contradicts other regression studies [37] [38] [39]. In contrast to the findings of the current study, Burgaz et al (2007) [40] identified dietary vitamin D as one of the main determinants of 25(OH)D concentration in a multiple linear regression model. Also, Macdonald et al (2008) [41] found that dietary vitamin D was associated with 25(OH)D in winter and spring. However, Burgaz et al's (2007) [40] study was conducted in a Swedish population, where the intake of vitamin D may be much higher, and Macdonald et al's (2008) study [41] was conducted in Scotland where UVB exposure was lower than in the current study. This suggests the discrepancy between these studies may be due to local differences in dietary intake of vitamin D and UVB exposure. Overall, there is contradiction between correlation and regression analyses in the literature as to whether vitamin D intake from food has an influence on vitamin D status. It may only be supplements that have a significant effect on 25(OH)D concentration, a fact supported by a large number of regression studies [40] [38]. The finding in the multilevel modelling analysis that indicates dietary vitamin D as not a significant predictor of 25(OH)D in this group of premenopausal women suggests that diet may not be effective in increasing 25(OH)D concentrations. Indeed, some studies suggest that UVB is the main regulator of cycling of 25(OH)D concentrations [42], and that 80-100% of vitamin D requirements are met by skin production [43]. However, it is possible that the contribution of diet to 25(OH)D is greater when UVB exposure is lower, as discussed above. Further analysis of this dataset could assess whether the contribution of diet to 25(OH)D is

indeed higher in those individuals with lower UVB exposure. It must however be considered that this study was powered statistically to assess differences in 25(OH)D, so may be underpowered for the analysis of diet as a predictor of 25(OH)D. Also, dietary databases give only an estimate of vitamin D intake, and our estimate of vitamin D intake will therefore be influenced by the accuracy of the database used, especially for vitamin D₂. This could be important at the very low levels of intake seen in this study.

The extensive vitamin D deficiency seen throughout the year here in the majority of premenopausal South Asian women is of great concern. Most of these women are of childbearing age and there are known health risks for entering pregnancy with such a high degree of vitamin D deficiency. Vitamin D deficiency in pregnancy has been found to be associated with particularly severe and potentially fatal risks to the child, such as infantile hypocalcaemia[13], possible abnormalities in brain development[44] as well as debilitating musculoskeletal conditions such as infantile rickets[45]. Given that pregnant women are known to have increased requirements for vitamin D compared to our non-pregnant cohort, these results have serious implications for those who do not take vit D supplements.

This study suggests that health providers should urgently consider the screening and treatment of premenopausal women, particularly those at very high risk such as South Asian women. This study showed little influence of dietary vitamin D intake on vitamin D status, at the low levels of intake seen in this study, and compared to the effects of UVB exposure. UVB exposure sufficient to raise 25(OH)D to optimal levels is likely to be an unrealistic aim in this population group. Indeed, although theoretically, women of South Asian origin and Caucasian women may equally be able to produce vitamin D from exposure to UVB radiation, they will need longer to achieve this due to darker skin pigmentation, [46], making it more difficult for them to produce an equal amount of vitamin D to Caucasians in a similar time frame. Supplementation is likely to be the preferred vehicle, as vitamin D intakes are

too low and too few foods contain vitamin D in high enough concentrations to correct such deficiency. Indeed, the Department of Health[17] guideline for 10µg vitamin D supplementation per day for all pregnant and breast-feeding women should be emphasised here.

In terms of strengths and weaknesses, the prospective, repeated-measures design of this data analysis is novel; no previous study has followed up the same cohort of South Asian and Caucasian premenopausal women for a year, obtaining serum 25(OH)D measurements, as well as information on dietary intake of vitamin D, and assessment of UVB exposure in each of the four seasons. This study contributes new, useful information to the literature as no previous studies have assessed premenopausal South Asian women's UVB exposure using dosimeter badges and there are very limited data in premenopausal Caucasian UK women. The assessment of ethnic differences in 25(OH)D was adequately powered (80%) to show a significant difference in 25(OH)D. This study, alongside the data reported previously by Macdonald et al (2010)[20], is novel in highlighting the variation in an individual's 25(OH)D status over the year. Furthermore, it is the only study in premenopausal women to have used a multi-level modelling approach to investigate the interaction between diet and sunlight exposure on vitamin D status in South Asian women and to assess the relative contributions of fixed individual and seasonal within-individual factors.

Due to the nature of these analyses, the mixed between-within subjects ANOVA and the multi level modelling techniques only relied on data from participants who attended on all of the four visits. This could be seen as a potential limitation as it excludes those with who could not attend all the visits. However, it could also be viewed as a strength as it means only subjects with good study compliance were included.. In contrast, the prevalence estimates of deficiency reported were for all participants, so did include those participants who did not

finish the study, or missed one or more visits. There were no significant differences in 25(OH)D, PTH, or vitamin D intake or UVB exposure between both Asian and Caucasian women completing three visits or less and those completing four visits. The exception was a higher BMI in the Asian group in spring only in women who completed the full four visits. Also, the premenopausal Caucasians who completed all four visits had slightly higher UVB exposure in the summer only. These two differences in specific seasons only were unlikely to have significantly influenced the results.

Although there were no differences in the socio-economic status by ethnicity in this cohort, the women came from the South-East of the UK; thus, the results of this study are generalisable to the UK Southern female population. However, it may actually be a slight underestimate for the whole UK female population due to the slightly higher socioeconomic status, and higher ambient UVB exposure seen in the South relative to the rest of the UK. Indeed, the prevalence of vitamin D deficiency is likely to be even higher in the rest of the UK.

To conclude, from this study it is clear that premenopausal Asian women have lower 25(OH)D than their Caucasian counterparts and serum 25(OH)D less than 50nmol/l as well as less than 25nmol/l is very common in Asian women throughout the year. Serum 25(OH)D less than 25nmol/l was less common in Caucasian women but still present, whilst vitamin D status <75nmol/l was very common throughout the year in both groups. Furthermore, vitamin D status remains blunted throughout the year in the Asian women in comparison to the seasonal change in 25(OH)D observed in the Caucasian women. Dietary intake of vitamin D had little impact on 25(OH)D concentration, suggesting that sun exposure was the main source of vitamin D in this group of women, and may be especially influential in Caucasians

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Dedication

This paper is dedicated to Mr John Pheasant, Practice Manager at Thornton Heath Medical Centre, London who helped with the study recruitment and who sadly died in 2008.

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Tables and Figures

Table 1 Baseline characteristics of Caucasian (n=135) and Asian (n=46) premenopausal women living in the South East of England

	Caucasian		Asian	
	Mean	SD	Mean	SD
Age	33.87	5.62	37.91	9.04
Index of Multiple Deprivation	15.87	9.05	13.02	7.78
Body Mass Index	25.3	4.5	25.9	4.6
Total Body Fat Mass (Kg)	23.1	8.1	24.6	7.2
Weight (Kg)	69.20	12.43	65.80	11.33
Height (m)	1.65	0.06	1.59	0.06
Highest level of Education	%		%	
Secondary school	23	-	28	-
A levels	31		25	
University degree	41		35	
Other qualification	2		3	
Professional Qualification	4		10	
% Smoker	15.8	-	2.2	-
Alcohol consumers (%)	93.9	-	9.1	-
(a) Frequency of consumption per week	2.28	1.62	2.00	1.41
(b) Units of alcohol consumed on each occasion	3.64	2.43	2.50	0.71
Previous use of Fish/Fish oil supplements* (%)	15.0	-	13.0	-

Previous use of vitamin supplements*	31.6	-	32.6	-
Dietary calcium intake (mg/d)	834.4	319.4	735.4	249.3
Serum albumin	45.0	2.4	43.5	2.2
Corrected serum calcium	2.24	0.1	2.24	0.1
Use of oral contraceptives (%)	15.9	-	2.2	-

(%)*= in 12months previous to study

Table 2 Cumulative Frequencies (%) by commonly used serum 25(OH)D (nmol/l) status cut offs for a cohort of pre-menopausal women living in the South East of England

Summer 25(OH)D	<25	<50	<75	Autumn 25(OH)D	<25	<50	<75
Caucasian	0.8	20.3	60.9	Caucasian	4.1	43.3	79.4
Asian	53.5	95.3	100.0	Asian	79.2	95.8	100.0
Winter25(OH)D	<25	<50	<75	Spring25(OH)D	<25	<50	<75
Caucasian	10.0	70.0	90.0	Caucasian	5.1	51.9	88.6
Asian	80.8	96.2	100.0	Asian	75.0	95.8	100.0

Table 3 Serum 25(OH)D, serum PTH, vitamin D intake and UV exposure by group and season for premenopausal women living in the South East of England

		Summer			Autumn			Winter			Spring			ANOVA		
	Group	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	Season (within subjects)	Ethnicity (between subjects)	Season x Ethnic group
Serum 25(OH)D nmol/l	C	128	72.1	26.1	97	59.5	25.6	80	44.5	18	79	53.2	23.9	P<0.001	P<0.001	P<0.001
	A	43	26.2	9.9	24	20.9	11.8	26	19.7	10.6	24	22.1	11.3			
PTH pmol/l	C	128	2.6	1.1	101	2.6	1.3	78	2.6	1.1	78	2.6	1.2	P=0.111	P<0.001	P=0.084
	A	43	3.5	1.7	28	4.3	1.8	22	4.3	2.0	26	4.5	2.4			
Dietary vitamin D2&D3µg/d	C	111	2.4	2.0	96	2.1	1.5	78	2.6	1.8	62	2.5	1.9	P=0.359	P=0.427	P=0.396
	A	21	2.2	1.8	20	2.0	1.4	19	2.0	2.0	15	1.6	1.1			
Weekly UVB exposure (SED)	C	113	6.39	6.87	92	0.64	1.27	73	0.64	0.98	63	5.48	6.51	P<0.001	P=0.021	P=0.284
	A	18	2.00	2.13	21	0.12	0.22	17	0.29	0.32	13	3.15	4.43			

C= Caucasian, A=Asian; SED= standard erythmal dose (the minimum amount of UVB radiation required to produce an erythema (burn) on the skin) Values are means +/-

SD.

Table 4 Summary of regression parameters for multi level model explaining vitamin D status

	Full Two level model		95% Confidence interval	
Parameter	Estimate	Standard error		
Fixed Effects			Upper Limit	Lower Limit
Constant	55.66	2.66	60.87	50.45
Dietary Vitamin D	-0.08	0.67	1.23	-1.39
UV Exposure	1.59	0.39	2.35	0.83
UV Exposure Squared	-0.03	0.01	-0.01	-0.05
Ethnicity (reference= Caucasian)	-31.38	4.74	-22.09	-40.67
Random Effects				
$\sigma^2_{u_j}$	268.35	50.35	367.04	169.66
$\sigma^2_{e_{ij}}$	296.36	28.73	352.67	240.05

$\sigma^2_{u_j}$ =Unexplained level 2 variance-(between individuals)

$\sigma^2_{e_{ij}}$ =Unexplained level 1 variance- (between seasons within individuals)

Fig. 1 The flow of subjects through the study

Fig. 2a Vitamin D status [serum 25(OH)D] by ethnicity and season in a cohort of premenopausal women

Fig. 2b Parathyroid Hormone [PTH] by ethnicity and season in a cohort of pre-menopausal women