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DETERMINANTS OF VITAMIN D STATUS IN PREGNANT FAIR-SKINNED WOMEN IN SWEDEN

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Abstract

Low maternal vitamin D status during pregnancy may have negative consequences for both mother and child. There are few studies of vitamin D status and its determinants in pregnant women living at northern latitudes. Thus, this study investigates vitamin D status and its determinants during the third trimester of women living in Sweden (latitude 57-58° North). Ninety-five fair-skinned pregnant women had blood taken between gestational weeks 35-37. The study included a 4-day food diary and questionnaires on dietary intake, supplement use, sun exposure, skin type, travels to southern latitudes and measure of BMI. Serum 25-hydroxyvitamin D (25(OH)D) was analysed using chemiluminescence immunoassay. In the third trimester of pregnancy, mean serum concentration of 25(OH)D was 47·4±18·1 (range 10-93) nmol/L. In total, 65% of women had serum 25(OH)D <50 nmol/L and 17% <30 nmol/L. During winter, 85% of the pregnant women had serum 25(OH)D <50 and 28% <30 nmol/L. The main determinants of vitamin D status were: season, use of vitamin D supplements and travels to southern latitudes. Together these explained 51% of the variation in 25(OH)D. In conclusion, during winter the majority of fair-skinned pregnant women had serum 25(OH)D <50 nmol/L in their third trimester and more than every fourth woman <30 nmol/L. Higher vitamin D intake may therefore be needed during winter for fair-skinned pregnant women at northern latitudes to avoid vitamin D deficiency.

Running title: Vitamin D status in pregnancy

Key words: 25-hydroxyvitamin D, pregnancy, dietary intake, sun exposure

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; PTH, Parathyroid hormone; PAL, Physical activity level; FIL, Food intake level

Introduction

Low vitamin D status has been associated not only with suboptimal bone health, but also with higher frequencies of cardiovascular diseases(1), type 1 diabetes(2), cancers(3, 4), infectious diseases(3), multiple sclerosis(3, 5) and psychological conditions such as depression and schizophrenia(6). During pregnancy, vitamin D deficiency also has been associated with maternal health outcomes, e.g. hypertensive disorders(7), gestational diabetes(8, 5), and risk of cesarean section(9). Further, low maternal vitamin D status during pregnancy may impact on fetal imprinting(10), increase risk of low birth weight(11-13) and small-for-gestational age(7, 14), and may also effect the child's bone health(15). Exposure of pregnant women and fetuses to high doses of vitamin D also requires careful attention due to risks of hypercalcemia and hypercalciuria and other possible adverse effects (16).

A major function for vitamin D is its role in calcium- and bone metabolism. During pregnancy the need for calcium is increased due to the requirement of calcium to form the foetal skeleton. This may lead to mobilization of calcium from the maternal skeleton(17). We have shown there is a decrease in whole body bone mineral content (BMC) of about 2% during pregnancy(18). It is not known if these pregnancy-induced skeletal changes are vitamin D dependent. However, women who are pregnant during winter, when the UVB-exposure is low, have higher ultrasound indices of maternal bone loss(19). This may indicate a role for vitamin D. Overall, longitudinal studies suggest that decreases in maternal bone mineral density during reproduction are transient with replenishment of skeletal minerals in the later stages of lactation and after lactation have ceased(20). The role for vitamin D in mineral replenishment process remains to be clarified. Normally, parathyroid hormone (PTH) plays a major role in maintaining the calcium balance, by increasing the calcium release from the skeleton and by increasing calcium reabsorption from the kidneys(21). During pregnancy, however, the role of PTH is unclear and the inverse relationship between serum PTH and serum calcium may not be the same as in non-pregnant adults(22). Also, the inverse relationship between PTH and 25-hydroxyvitamin D (25(OH)D) may be weaker in pregnant women(23, 22).

Vitamin D is partly obtained from the diet and dietary supplements. In Sweden, the major dietary sources of vitamin D are fish, fish containing foods and dairy products (24). Vitamin D is also obtained by cutaneous synthesis induced after ultra violet B (UVB) light exposure(25). Below latitude 35° North, cutaneous vitamin D synthesis is possible all year(25). In the circulation, 25(OH)D is the metabolite, which is measured as a proxy for vitamin D status(26-29). Among non-pregnant women, season(30), skin pigmentation(31), BMI(25, 30) and dietary intake, e.g. fatty fish(32) and vitamin D supplements use(33), have been shown to be associated with serum 25(OH)D. For pregnant women, determinants of 25(OH)D are less understood, but are known to include season and ethnicity(12, 34-37). Lifestyle factors such as sun exposure, supplement use and dietary intake of vitamin D are not well studied. However, a recent Belgian study, reported significant determinants of serum 25(OH)D during pregnancy to include sunscreen use, preference for shadow and holidays to sunny climates, as well as vitamin D supplement use, ethnicity, alcohol use, smoking and education(38).

Studies measuring the vitamin D status of pregnant women in western societies have found mean concentrations of 25(OH)D between 26 and 98 nmol/L(39, 40, 12, 41, 14, 34, 23, 42, 38, 43). Many of these studies were conducted at latitudes where cutaneous production of vitamin D is possible for most of the year. Low serum 25(OH)D in pregnant dark-skinned women have been reported in several studies(44, 45, 41, 23). Less is known about vitamin D status and its determinants in pregnant fair-skinned women living in Sweden or at similar northern latitudes. To our knowledge, only a few studies have been published about vitamin D status of pregnant fair-skinned women living at these latitudes(46, 47, 42, 38, 43), of which only one has reported determinants of vitamin D status thoroughly(38). The aims of the present study were to assess vitamin D status and to evaluate its determining factors during the third trimester of pregnancy in fair-skinned women living in Sweden.

Subjects and methods

Subjects

Women were recruited from July 2008 to July 2011 using posters in maternity health care clinics and in public places in the vicinity of Gothenburg, Sweden, and through advertisement on a webpage addressing pregnant women in western Sweden. In total, 95 pregnant women were recruited. Inclusion criteria were age 25-40 years, pregnancy in gestational week 35-37 when starting the study and to declare oneself as healthy. Exclusion criteria were prescribed medicine intake known to effect calcium and bone metabolism, pregnancy during the last 1·5 years before the start of the present pregnancy, miscarriage after week 12 of pregnancy during the last 1·5 years, breastfeeding during the last year before the start of the present pregnancy, twin pregnancy and development of gestational diabetes or preeclampsia. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Ethics Committee in Gothenburg. Written informed consent was obtained from all women.

Study design

All women visited the Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Sweden when they were between 35-37 weeks pregnant. Venous blood was drawn in the morning after an overnight fast. Body weight in underwear (Tanita, BWB-800MA, Rex Frederiksbergs Vaegtfabrik) and height (standardised wall stadiometer) were measured. Women were asked what their body weight was before they became pregnant. Hence, pre-pregnancy BMI is based on self-reported pre-pregnancy body weight and the height measured at the study visit. Women were also asked questions about their medical history, sun exposure, skin type, dietary intake and physical activity. After birth, women were asked to report day of birth, birth weight, and birth length.

Methods

Sun exposure was estimated using questions compiled by Burgaz et al(32). These included use of sunscreen (always, sometimes or never) and preference for sun or shade when outdoors in summer (always in the sun, both sun and shade or always in the shade). Women were asked if they had used a sunbed during the previous six months. Skin types were defined using the Fitzpatrick scale (I=always burns, never tans, II=usually burns, tans with difficulty, III=sometimes burns mildly, tans gradually, IV=rarely burns, tans easily)(48). Women were asked to estimate the number of hours spent outdoors between 9 am and 6 pm on weekdays, weekends, summer and winter, respectively. Weekdays corresponded to working days and weekends to non-working days. Summer was defined as May-October and winter as November-April. Women were also asked to report travels to southern latitudes during the previous six months. Southern latitude was defined as a location below latitude 35° North where cutaneous synthesis of vitamin D is possible all year round(25).

Dietary intake of vitamin D was estimated using four-day food diaries. Women were asked to record all food and drink consumed as precisely as possible on four consecutive days with at least one non-working day and a preferred start no later than one week after the study visit. Both oral and written information on how to fill in the food diary were given. Women were asked to report the amounts of consumed food items using household measures, weight in grams or using photographs of different portion sizes used in the Swedish portion guide “Matmallen”(49). Women were also asked not to change their diet. Women were contacted if any ambiguities were noted in their food diaries. Dietary intake was calculated using DietistXP, version 3·1 (The National Food Agency food database version 2009-11-10, Kost och näringsdata, Bromma, Sweden). In addition, a short food frequency questionnaire (FFQ) was also used to study the frequency and quantity of foods rich in vitamin D consumed, e.g. fatty fish and dairy products. Specifically, information about the intake of reduced-fat dairy milk and yogurt/sour milk was requested. Details of use, frequency, amount and brand of supplements containing vitamin D were also requested.

Each woman rated her physical activity on a scale between 1 and 10(50). Women were informed that 1 indicated a sedentary lifestyle, 5 a few long walks each week and 10 exercise several times a week. The answer was converted to a physical activity level (PAL), where 1 corresponded to PAL 1·3 and 10 to PAL 2·2, respectively. Each step between them represented a 0·1 increase. In a validation study, PAL assessed using this scale was correlated (r=0·54; p=0·008) with corresponding estimates obtained using criterion methods (i.e. the doubly labelled water method in combination with indirect calorimetry) in 22 healthy Swedish pregnant women (personal communication with Marie Löf). Here, the individual self-estimated PAL was used for validating energy intake from the four-day diary food records and thus for identifying possible under-reporters according to Goldberg et al(51) and Black et al(52). No correction for under-reporting was made. BMR was calculated using FAO/WHO/UNUs´ equation for non-pregnant women(53) and a general increase in BMR of 24% for pregnant women in their third trimester was added as suggested by Butte(54).

Laboratory analyses

Blood samples were protected from UVB light and centrifuged no later than 45 minutes after sampling at 5° Celcius, 3800g, for 9 minutes (Centrifuge CR3i, Jouan Quality System). Serum was then aliquoted and stored at -70° Celcius until analyzed. The analyses of serum concentrations of 25(OH)D and PTH were performed by Central Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden. All samples were analyzed at one time. Analyses of total 25(OH)D, i.e. 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3, were performed in serum with LIASON® 25-hydroxyvitamin D chemiluminescence immunoassay (CLIA) (DiaSorin). Intact PTH was analysed with an immunochemical two step analysis of sandwich type, using chemiluminescence microparticle immunoassay technology (Abbott laboratory diagnostics division). Intra-assay coefficient of variations (CV) were 7·3%, 5·7% and 5·3% for 25(OH)D serum concentrations 22, 50 and 150 nmol/L respectively. For PTH, CVs were 3·7%, 4·5% and 3·5% for PTH serum concentrations 10, 40 and 730 ng/L respectively.

Statistical analyses

Independent sample t-test and ANOVA were used to evaluate difference in mean concentration of 25(OH)D depending on lifestyle and other factors, such as parity, estimates of sun exposure, estimates of vitamin D intake, and PTH. Estimates of sun exposure included season, time spent outdoors, recent travel to southern latitude, skin type, sun preference, sunscreen use, and estimates of vitamin D intake included total intake of vitamin D (from diet and supplements), dietary intake of vitamin D, intake of different food items rich in vitamin D, and vitamin D supplement use. The coefficients of determination for 25(OH)D serum concentrations were calculated using bivariate regression analyses for the following variables: estimates of sun exposure, estimates of vitamin D intake, PAL, body weight and BMI. The variables significant in the bivariate regression analyses were included in the multivariate regression analysis. The effects of interactions between factors on 25(OH)D concentrations, were modelled by the inclusion of combinations of estimates of sun exposure and estimates of vitamin D intake. In the multivariate regression analysis, a variable was considered a confounder if its inclusion in the model caused >10% change in the coefficient of the slope. However, no potential confounders were found**.** The significance level was set to $p<0·05$ (two-sided). All analyses were conducted using SPSS Statistics Softwear, version 19·0, IBM, Somers, NY.

Results

Descriptive characteristics for the 95 women in their third trimester of pregnancy are shown in **Table 1**. All women were fair-skinned and lived in Western Sweden at latitudes 57-58° North. Their mean age was 32·2 years and 14% of the women had BMI ≥ 25 before becoming pregnant. Mean self-reported pre-pregnancy body weight was 64 kg and mean body weight gain until third trimester was 13 kg. Parity ranged from 0-2 and half of the women were nulliparous. All women gave birth to full term healthy babies with mean birth weight 3581 ± 477 g and mean birth length 50 ± 2 cm. Eighty per cent had studied for 3 or more years at university. None of the women were current smokers and only one was using snuff.

The mean serum concentration of 25(OH)D was 47·4 nmol/L ± 18·1 (range 10-93 nmol/L) (**Figure 1**). Concentrations of serum 25(OH)D <30 nmol/L, <50 nmol/L and <75 nmol/L were found in 17%, 65% and 92% of the women, respectively. During winter, 85% of women had serum 25(OH)D <50 nmol/L and 28% had concentrations <30 nmol/L. However, during summer, 41% of women had 25(OH)D concentrations <50 nmol/L and only 2% had <30 nmol/L. Mean serum concentration of 25(OH)D was >50 nmol/L only from June to September (**Figure 2**). Serum concentrations of 25(OH)D were highest during the summer, with the highest mean in August (69 nmol/L). The lowest concentrations were seen in late winter and April had the lowest mean (33 nmol/L). Mean serum concentration was 53% higher in summer compared to winter. The difference in serum 25(OH)D between summer and winter was highly significant (P<0·001).

Mean dietary intake of vitamin D was 6·1 µg/day and mean total intake of vitamin D (from diet and supplements) was 9·3 µg/day. Data from the FFQ on dietary intake of vitamin D rich foods is shown in **Table 2**. No association was seen between serum concentrations of 25(OH)D and dietary vitamin D intake or intake of food rich in vitamin D, such as fatty fish or dairy products. A trend was seen between intake of low fat yogurt and sour milk and serum concentrations of 25(OH)D (P=0·08). More than half of the women (56%) were taking supplements containing vitamin D and for these women mean vitamin D supplement intake was 5·8 µg/day and total vitamin D intake was 12·0 µg/day. Mean serum concentration of 25(OH)D was 46% higher among women taking supplements containing vitamin D, compared to those who did not (P<0·001). A significant association was found between total vitamin D intake (from diet and supplements) and serum concentrations of 25(OH)D (P=0·008). Body weight and BMI were not associated with serum 25(OH)D concentrations.

Mean self-estimated PAL was 1·6 ± 0·19 (range 1·3-2·2). Mean food intake level (FIL: energy intake/BMR) was 1·30 ± 0·21 (range 0·78-1·80). No association was seen between PAL and serum 25(OH)D. When validating energy intake versus PAL, 24% of the subjects were identified as under-reporters.

Sun exposure and other lifestyle variables potentially related to 25(OH)D concentrations are shown in **Table 3.** Women who preferred to stay in the sun when outdoors during summer had 21% higher mean serum concentrations of 25(OH)D compared to women who preferred to stay in the shade or who preferred a mix of sun and shade (P=0·03). The median times spent outdoors during the summer were two hours during weekdays and four hours during weekends. During winter, the median time spent outdoors was one hour and two hours, respectively. Serum 25(OH)D was not associated with the time spent outdoors, either during summer or winter. In addition, no association was seen between serum 25(OH)D and skin type. However, subjects who more frequently used sunscreen tended to have higher serum 25(OH)D (P=0·07). Also, a positive relationship was found between time spent outdoors during the summer and the use of sunscreen (P=0·013 for non-working days and P=0·031 for working days). None of the women had used a sunbed in the last six months.

Eighteen per cent of the women had travelled to southern latitudes during the past six months. Mean serum concentrations of 25(OH)D was 35% higher in this group compared to women who had not travelled to southern latitudes (P=0·001). During the winter, 25% of the women had neither recently travelled to southern latitudes or taken vitamin D supplements. These women had a mean serum 25(OH)D of 30 nmol/L, which was significantly lower than for those who used vitamin D supplements and/or had travelled to southern latitudes (P<0·001).

The mean concentration of PTH was 43·8 ± 15·6 ng/L. A significant inverse association was seen between serum concentrations of 25(OH)D and PTH (P=0·008, r=-0·271) (**Table 5**). Women with serum 25(OH)D <50 nmol/L had significantly higher serum PTH (47·1±16·1 ng/L), compared to women with serum 25(OH)D >50 nmol/L (37·7±12·9 ng/L) (P=0·005). Mean serum PTH was significantly higher during the winter than during the summer (P=0·011).

No association was seen between the maternal serum concentration of 25(OH)D and infant birth weight or birth length. However, a negative relationship was seen between PTH and birth weight (P=0·03) (β=-6·06±2·72). A significant positive association was found between serum 25(OH)D and maternal height (P=0·010), parity (P=0·050) and gender of the baby, where mothers giving birth to boys had higher serum 25(OH)D (P=0·006).

Multivariate regression analyses showed that the major factors determining the concentration of 25(OH)D were season, use of vitamin D supplements and travels to southern latitudes. Together these explained 51% of the variation in serum 25(OH)D (**Table 4**).

Discussion

The strengths of our study are the wide range of possible determinants of vitamin D status studied, including detailed measures of vitamin D intake from diet and supplements separately, several estimates of sun exposure and investigations of seasonal variation in 25(OH)D. Previous studies in pregnant women have found association between serum concentrations of 25(OH)D and season(34-36, 38, 43) ethnicity or skin type(44, 34-36, 38), total vitamin D intake(34), supplement use(41, 36, 38), education(38), smoking and alcohol use (38), and sun exposure(34, 36, 38, 43). None of these studies included, however, measurements of vitamin D intake from diet and supplements separately, and only a Belgian national survey included different estimates of sun exposure (38, 43).

Mean 25(OH)D in our study was lower than means reported previously in fair-skinned pregnant women at similar latitudes e.g. Sweden (47), and Denmark (42), and similar to those reported in Caucasian Belgian pregnant women (38, 43). Despite similar latitudes and ethnicity, differences in vitamin D status between studies may depend on trimester, season of blood sampling, and method used for 25(OH)D analysis. It must be remembered that there is no gold standard for measuring 25(OH)D. The CLIA used in this study has been shown to give lower serum concentrations of 25(OH)D, compared to high-pressure liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry(55).

Sunscreen use tended to be positively associated with serum 25(OH)D. This is probably explained by the positive association also found between sun preference and serum 25(OH)D and the finding that women who spent more time outdoors during the summer were significantly more frequent sunscreen users. Sunscreen use, therefore, seems to rather reflect time spent outdoors in the sun than its inhibiting effect on endogenous vitamin D production. Possibly, a higher number of subjects would give more power to find a significant positive relationship between sunscreen use and serum 25(OH)D, especially since the national Belgian survey also reported that women using sunscreen lotion had a significant lower risk of severe vitamin D deficiency(38).

During winter, UVB mediated production of vitamin D is absent at northern latitudes(25). Accordingly, a majority of the women in our study had serum 25(OH)D <50 nmol/L during this period. Additionally, women who had travelled to southern latitudes below latitude 35° North within the previous six months or preferred to stay in the sun in summertime had significantly higher concentrations of 25(OH)D. This confirms similar findings in the Belgian survey in pregnant women(38). A study of Swedish elderly women also found similar relationships(32).

Both the mean dietary intake of vitamin D (6·1 µg/day) and mean total intake of vitamin D (9·3 µg/day; from diet and supplements) in this study was lower than the recommended daily intake from the Swedish National Food Agency and the Nordic Nutrition Recommendations (10 µg/day for pregnant women)(56, 57) and the Institute of Medicine from 2010 (15 µg/day for adults, including pregnant women)(58). However, mean dietary intake in our study was similar to that reported for women of the same age group of 6·2 µg/day in the new Swedish national survey from 2011 (24). Mean total intake of vitamin D in our study was somewhat higher than that reported for adult women at all ages (mean 7·0 µg/day including supplements)(59). This may partly be explained by the fact that the women in our study were in the third trimester of pregnancy and may have a higher energy intake than non-pregnant and non-lactating women. When adding vitamin D intake from diet and supplements, only 39% of the women in our study had a total vitamin D intake of ≥10 µg/day and only 12% ≥15 µg/day. However, when considering vitamin D intake from diet alone, only 9% had a vitamin D intake of ≥10 µg/day and only 1% ≥15 µg/day.

The significant association found between total intake of vitamin D and serum 25(OH)D, is in agreement with previous studies in pregnancy (60, 34, 36). This association was mainly explained by the strong association between vitamin D supplement intake and 25(OH)D concentrations. A trend was, however, seen between intake of reduced fat yogurt and sour milk and concentrations of 25(OH)D.

Twenty-four percent of the women under-reported energy intake, and may potentially have led to under-reporting of dietary vitamin D intake as well. No attempt was made to correct under-reporting. However, removal of under-reporters from data analyses did not change the significance of the results.

Also when under-reporters were removed from data analyses, no significant association was seen between dietary intake of vitamin D, or food items rich in vitamin D, and serum 25(OH)D. The percentage of women under-reporting energy intake is consistent with results from earlier studies of pregnant women. For example, we have previously shown the percentage of under-reporting among Indonesian women during the third trimester to be 17·6%(61), whereas an Irish study found the proportion of under-reporting in gestational week 14 to be up to 45%(62). Both studies found high BMI and low education to be important predictors for under-reporting(61, 62).

One limitation of our study is that the women included may not be representative for the whole Swedish population of pregnant women. Eighty per cent of the subjects in this study had 3 or more years of education at university level, which is a higher number than among women in the same age group in the general population (37%)(63). Additionally, only 14% had a BMI ≥ 25 before entering pregnancy, compared to 37% of the pregnant women in the same region(64). Thus, the women in this study were leaner, higher educated and, possibly, more health conscious than pregnant women in general. The interpretation of these results needs therefore to be made with some caution. However, despite the high education and normal body weight, the majority of the subjects had concentrations of 25(OH)D <50 nmol/L. Thus, it may be speculated that even lower concentrations of 25(OH)D are expected in the general population of pregnant women living in Sweden, especially in pregnant women with high pigmentation and those wearing concealing clothing. The national Belgian survey showed that vitamin D deficiency was three to six fold higher among women of Asian, African or Hispanic descent as compared to Caucasians(38). More targeted screening surveys on vitamin D status in pregnant women in Sweden and in other countries are needed to confirm this.

At present there are no general recommendations for optimal vitamin D levels. According to the latest guidelines from the Institute of Medicine, serum concentrations of 25(OH)D ≥50 nmol/L are recommended(58). The recommendation is based on the importance of vitamin D for bone health(58). Among non-pregnant adults, a negative relationship is seen between serum PTH and serum 25(OH)D(65-67), and that above 50 nmol/L of serum 25(OH)D, there is no further increase in serum PTH(66). We also found that serum PTH was significantly higher at serum concentrations of 25(OH)D below 50 nmol/L. A weaker association between serum PTH and serum 25(OH)D has, previously been found among pregnant women(40, 68, 23, 22). A seasonal variation in serum PTH, with highest serum concentrations of PTH during winter, was also found. This is in agreement with a Danish study of non-pregnant adults(30). However, whether PTH is a marker for determining adequate vitamin D status during pregnancy needs further evaluation. A specific cut-off for serum 25(OH)D may be needed for pregnant women, due to the plasma volume expansion during pregnancy(69) and possible health benefits of maternal vitamin D status for mother, foetus and child(70). However, more research is needed to evaluate the importance of vitamin D status during pregnancy for such outcomes(70) and to determine what serum levels of 25(OH)D should be considered as appropriate during pregnancy.

There is an ongoing discussion on whether or not to recommend supplementation of vitamin D to all pregnant women(70). It is not clear what dose of vitamin D is required during pregnancy to have an effect on maternal or pediatric outcome. Supplementation with 25 µg/day may be needed to maintain maternal serum concentrations of at least 50 nmol/L(70). This corresponds with the findings of Cashman et al, where a daily intake of 28·0 µg for non-pregnant adults was needed to maintain serum 25(OH)D concentrations >50 nmol/L during winter(33).

In conclusion, during winter the majority of fair-skinned pregnant women had serum 25(OH)D <50 nmol/L in their third trimester, at latitude 57-58º North. In addition, more than every fourth women was vitamin D deficient during winter. The main determinants for vitamin D status were season, use of vitamin D supplements and travels to southern latitudes. Thus, also fair-skinned pregnant women at northern latitudes are at risk of vitamin D deficiency, especially during winter. Higher vitamin D intake may therefore be needed during winter for pregnant women at these latitudes to maintain 25(OH)D serum concentrations >50 nmol/L and to avoid maternal vitamin D deficiency.

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References

1. Giovannucci E, Liu Y, Hollis BW *et al.* (2008) 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* **168**, 1174-1180.
2. Mohr SB, Garland CF, Gorham ED *et al.* (2008) The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia* **51**, 1391-1398.
3. Holick MF (2004) Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* **79**, 362-371.
4. Grant WB (2006) Lower vitamin-D production from solar ultraviolet-B irradiance may explain some differences in cancer survival rates. *J Natl Med Assoc* **98**, 357-364.
5. Lapillonne A (2010) Vitamin D deficiency during pregnancy may impair maternal and fetal outcomes. *Med Hypotheses* **74**, 71-75.
6. Humble MB, Gustafsson S & Bejerot S (2010) Low serum levels of 25-hydroxyvitamin D (25-OHD) among psychiatric out-patients in Sweden: relations with season, age, ethnic origin and psychiatric diagnosis. *J Steroid Biochem Mol Biol* **121**, 467-470.
7. Bodnar LM, Catov JM, Simhan HN *et al.* (2007) Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab* **92**, 3517-3522.
8. Clifton-Bligh RJ, McElduff P & McElduff A (2008) Maternal vitamin D deficiency, ethnicity and gestational diabetes. *Diabet Med* **25**, 678-684.
9. Merewood A, Mehta SD, Chen TC *et al.* (2009) Association between vitamin D deficiency and primary cesarean section. *J Clin Endocrinol Metab* **94**, 940-945.
10. Hollis BW & Wagner CL (2006) Vitamin D deficiency during pregnancy: an ongoing epidemic. *Am J Clin Nutr* **84**, 273.
11. Mannion CA, Gray-Donald K & Koski KG (2006) Association of low intake of milk and vitamin D during pregnancy with decreased birth weight. *CMAJ* **174**, 1273-1277.
12. Bowyer L, Catling-Paull C, Diamond T *et al.* (2009) Vitamin D, PTH and calcium levels in pregnant women and their neonates. *Clin Endocrinol (Oxf)* **70**, 372-377.

13. Viljakainen HT, Saarnio E, Hytinantti T *et al.* (2010) Maternal vitamin D status determines bone variables in the newborn. *J Clin Endocrinol Metab* **95**, 1749-1757.

14. Leffelaar ER, Vrijkotte TG & van Eijsden M (2010) Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. *Br J Nutr* **104**, 108-117.

15. Javaid MK, Crozier SR, Harvey NC *et al.* (2006) Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* **367**, 36-43.

16. Roth DE (2011) Vitamin D supplementation during pregnancy: safety considerations in the design and interpretation of clinical trials. *J Perinatol* **31**, 449-459.

17. Kovacs CS (2001) Calcium and bone metabolism in pregnancy and lactation. *J Clin Endocrinol Metab* **86**, 2344-2348.

18. Olausson H, Laskey MA, Goldberg GR *et al.* (2008) Changes in bone mineral status and bone size during pregnancy and the influences of body weight and calcium intake. *Am J Clin Nutr* **88**, 1032-1039.

19. Javaid MK, Crozier SR, Harvey NC *et al.* (2005) Maternal and seasonal predictors of change in calcaneal quantitative ultrasound during pregnancy. *J Clin Endocrinol Metab* **90**, 5182-5187.

20. Olausson H, Goldberg G, MA L *et al.* (2012) Calcium economy in human pregnancy and lactation. *Nutr Res Rev* **25**, 40-67.

21. Correale M (2012) Parathyroid hormone measurement in chronic kidney disease-an evolving issue for the nephrologist and the clinical laboratorist: minireview. *Immunopharmacol Immunotoxicol*.

22. Wagner CL & Hollis BW (2011) Beyond PTH: assessing vitamin D status during early pregnancy. *Clin Endocrinol (Oxf)* **75**, 285-286.

23. Haddow JE, Neveux LM, Palomaki GE *et al.* (2011) The relationship between PTH and 25-hydroxy vitamin D early in pregnancy. *Clin Endocrinol (Oxf)* **75**, 309-314.

24. Amcoff E, Edberg A, Enghardt Barberi H *et al.* (2012) *Riksmaten 2010-11. Livsmedels- och näringsintag bland vuxna i Sverige. Resultat från matvaneundersökning utförd 2010-11. National Food Agency*. Uppsala.

25. Tsiaras WG & Weinstock MA (2011) Factors influencing vitamin D status. *Acta Derm Venereol* **91**, 115-124.

26. Specker BL (1994) Do North American women need supplemental vitamin D during pregnancy or lactation? *Am J Clin Nutr* **59**, 484S-490S; discussion 490S-491S.

27. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266-281.

28. Borradale D & Kimlin M (2009) Vitamin D in health and disease: an insight into traditional functions and new roles for the 'sunshine vitamin'. *Nutr Res Rev* **22**, 118-136.

29. Grant WB & Boucher BJ (2011) Requirements for Vitamin D across the life span. *Biol Res Nurs* **13**, 120-133.

30. Thuesen B, Husemoen L, Fenger M *et al.* (2012) Determinants of vitamin D status in a general population of Danish adults. *Bone* **50**, 605-610.

31. Nessvi S, Johansson L, Jopson J *et al.* (2011) Association of 25-hydroxyvitamin D3)levels in adult New Zealanders with ethnicity, skin color and self-reported skin sensitivity to sun exposure. *Photochem Photobiol* **87**, 1173-1178.

32. Burgaz A, Akesson A, Oster A *et al.* (2007) Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. *Am J Clin Nutr* **86**, 1399-1404.

33. Cashman KD, Hill TR, Lucey AJ *et al.* (2008) Estimation of the dietary requirement for vitamin D in healthy adults. *Am J Clin Nutr* **88**, 1535-1542.

34. Dror DK, King JC, Durand DJ *et al.* (2011) Association of modifiable and nonmodifiable factors with vitamin D status in pregnant women and neonates in Oakland, CA. *J Am Diet Assoc* **111**, 111-116.

35. Li W, Green TJ, Innis SM *et al.* (2011) Suboptimal vitamin D levels in pregnant women despite supplement use. *Can J Public Health* **102**, 308-312.

36. Perampalam S, Ganda K, Chow KA *et al.* (2011) Vitamin D status and its predictive factors in pregnancy in 2 Australian populations. *Aust N Z J Obstet Gynaecol* **51**, 353-359.

37. Young BE, McNanley TJ, Cooper EM *et al.* (2011) Vitamin D insufficiency is prevalent and vitamin D is inversely associated with PTH and calcitriol in pregnant adolescents. *J Bone Miner Res*.

38. Vandevijvere S, Amsalkhir S, Van Oyen H *et al.* (2012) High prevalence of vitamin d deficiency in pregnant women: a national cross-sectional survey. *PLoS One* **7**, e43868.

39. Salle BL, Delvin EE, Lapillonne A *et al.* (2000) Perinatal metabolism of vitamin D. *Am J Clin Nutr* **71**, 1317S-1324S.

40. Morley R, Carlin JB, Pasco JA *et al.* (2006) Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size. *J Clin Endocrinol Metab* **91**, 906-912.

41. Madar AA, Stene LC & Meyer HE (2009) Vitamin D status among immigrant mothers from Pakistan, Turkey and Somalia and their infants attending child health clinics in Norway. *Br J Nutr* **101**, 1052-1058.

42. Milman N, Hvas A & Berghold T (2012) Vitamin D status during normal pregnancy and postpartum. A longitudinal study in 141 Danish women. *J Perinatal Med* **40**, 57-61.

43. Vercruyssen J, Jacquemyn Y & Ajaji M (2012) Effect of sun exposure and 25-hydroxyvitamin D status among pregnant women in Antwerpen, Belgium. *Int J Gynecol Obstetr* **116**, 78-86.

44. van der Meer IM, Karamali NS, Boeke AJ *et al.* (2006) High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. *Am J Clin Nutr* **84**, 350-353; quiz 468-359.

45. Farrant HJ, Krishnaveni GV, Hill JC *et al.* (2009) Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. *Eur J Clin Nutr* **63**, 646-652.

46. O'Riordan MN, Kiely M, Higgins JR *et al.* (2008) Prevalence of suboptimal vitamin D status during pregnancy. *Ir Med J* **101**, 240, 242-243.

47. Saaf M, Fernell E, Kristiansson F *et al.* (2011) Severe vitamin D deficiency in pregnant women of Somali origin living in Sweden. *Acta Paediatr* **100**, 612-614.

48. Astner S & Anderson RR (2004) Skin phototypes 2003. *J Invest Dermatol* **122**, xxx-xxxi.

49. National Food Agency (1997) *Matmallen*. Uppsala.

50. Bexelius C, Lof M, Sandin S *et al.* (2010) Measures of physical activity using cell phones: validation using criterion methods. *J Med Internet Res* **12**, e2.

51. Goldberg GR, Black AE, Jebb SA *et al.* (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45**, 569-581.

52. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**, 1119-1130.

53. FAO (2001) *Human energy requirements: Report of a Joint FAO/WHO/UNU Expert Consultation. Food and nutrition technicla report series No 1*. Rome.

54. Butte NF & King JC (2005) Energy requirements during pregnancy and lactation. *Public Health Nutr* **8**, 1010-1027.

55. Snellman G, Melhus H, Gedeborg R *et al.* (2010) Determining vitamin D status: a comparison between commercially available assays. *PLoS One* **5**, e11555.

56. Nordic Nutrition Recommendations 2004 - Integrating nutrition and physical activity (2004). Copenhagen: Norden, Nordic Council of Minsiters.

57. National Food Agency (2011) Rekommenderat intag av vitaminer och mineraler. *[cited 20120131]; Available from:*

http://www.slv.se/sv/grupp1/Mat-och-naring/Svenska-narings-rekommendationer/Rekommenderat-intag-av-vitaminer-och-mineraler/.

58. Ross AC TC, Yaktine AL, and Del Valle HB (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Institute of Medicine.

59. Pearson M NC, Ridefelt P *et al*. (2012) Vitamin D status in Swedish adults in Riksmaten 2010. In *Nordic Nutrition Conference*. Reykjavik, Iceland.

60. Holmes VA, Barnes MS, Alexander HD *et al.* (2009) Vitamin D deficiency and insufficiency in pregnant women: a longitudinal study. *Br J Nutr* **102**, 876-881.

61. Winkvist A, Persson V & Hartini TN (2002) Underreporting of energy intake is less common among pregnant women in Indonesia. *Public Health Nutr* **5**, 523-529.

62. McGowan CA & McAuliffe FM (2012) Maternal nutrient intakes and levels of energy underreporting during early pregnancy. *Eur J Clin Nutr*.

63. Statistics Sweden (2011) Befolkningens utbildning. http://www.scb.se/Pages/ProductTables____9575.aspx.

64. The National Board of Health and Welfare (2011) Graviditeter, förlossningar och nyfödda barn *[cited 20120131]; Available from: http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/18267/2011-3-19.pdf*

65. Landin-Wilhelmsen K, Wilhelmsen L, Wilske J *et al.* (1995) Sunlight increases serum 25(OH) vitamin D concentration whereas 1,25(OH)2D3 is unaffected. Results from a general population study in Goteborg, Sweden (The WHO MONICA Project). *Eur J Clin Nutr* **49**, 400-407.

66. Lips P (2004) Which circulating level of 25-hydroxyvitamin D is appropriate? *J Steroid Biochem Mol Biol* **89-90**, 611-614.

67. Holick MF (2006) High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* **81**, 353-373.

68. Hamilton SA, McNeil R, Hollis BW *et al.* (2010) Profound Vitamin D Deficiency in a Diverse Group of Women during Pregnancy Living in a Sun-Rich Environment at Latitude 32 degrees N. *Int J Endocrinol* **2010**, 917428.

69. Faupel-Badger JM, Hsieh CC, Troisi R *et al.* (2007) Plasma volume expansion in pregnancy: implications for biomarkers in population studies. *Cancer Epidemiol Biomarkers Prev* **16**, 1720-1723.

70. De-Regil LM, Palacios C, Ansary A *et al.* (2012) Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev* **2**, CD008873.

Table 1. Characteristics of the 95 pregnant women living in Sweden.

Characteristics	**Mean**	**95% CI**
Age (years)	32·8	32·1-33·5
Gestational week in third trimester	35·7	35·5-35·9
Height (cm)	168·9	167·6-170·2
Weight before pregnancy (kg)	64·3	62·7-65·9
Weight in third trimester (kg)	77·3	75·3-79·3
Weight gain at third trimester (kg)	13	12·1-13·9
BMI before pregnancy (kg/m^2)	22·5	22·0-23·0
Infant birth weight (g)	3581	3485-3677
Infant birth length[†] (cm)	50	49-52
Gestational length[‡] (days)	281	276-286

CI Confidence interval**,** [†]n = 89, [‡]n = 90.

Table 2. Dietary vitamin D intake from four day food record[†] and intake of vitamin D rich foods and supplements from FFQ[‡] (n=95).

Dietary intake	**Mean**	**95% CI**
Dietary vitamin D intake[†] (µg/day)	6·1	5·5-6·7
Fatty fish[‡] (g/week)	110	93-127
Total milk intake[‡] (ml/day)	220	181-259
Low-fat milk[‡] (ml/day)	197	161-233
Total yogurt/sour milk intake[‡] (ml/day)	168	145-190
Low fat yogurt/sour milk[‡] (ml/day)	82	58-106
Spread[‡] (g/day)	18	14-22
Vitamin D supplement intake[‡] (µg/day)	3·3	2·6-4·0
Vitamin D supplement intake[‡§] (µg/day)	5·8	5·0-6·6

[‡] From food frequency questionnaire (FFQ)

CI Confidence interval, [§]Supplement users only, n=53.

Table 3. Lifestyle factors and 25-hydroxyvitamin D (25(OH)D) concentrations of 95 pregnant women during third trimester, living in Sweden.

Life style factors		**n (%)**	**Mean 25(OH)D, nmol/L**
Parity	0	49 (52)	50·8
	1	35 (37)	44·5
	2	11 (12)	41·2
Sunscreen use	Never	4 (4)	30·7
	Sometimes	58 (62)	46·9
	Always	32 (33)	50·8
Preference of sun or shade	Sun	22 (23)	54·8*
	Shade or Sun and shade	72 (76)	45·3
Skin type[†]	I	3 (3)	45·0
	II	18 (19)	43·6
	III	64 (67)	48·1
	IV	10 (11)	50·6
Travels to southern latitudes[§]	Yes	17 (18)	60·2**
	No	78 (82)	44·6
Use of vitamin D supplements	Yes	53 (56)	55·1***
	No	42 (44)	37·7

Mean values were significantly different from the rest of the group: *P<0·05, **P<0·01, ***P<0·001

[†] I=always burns, never tans, II=usually burns, tans with difficulty, III=sometimes burns

mildly, tans gradually, IV=rarely burns, tans easily

[‡] Median hours spent outdoors between 9 am and 6 pm

[§] Travels to latitude 35°N or below, during the last six months prior to the measurements

Table 4. Factors predicting concentrations of 25-hydroxyvitamin D (25(OH)D) of 95 pregnant women, during third trimester, living in Sweden.

	Bivariate model				Multivariate model			
Factors	B	SEM	P	R^2	B	SEM	P	R^2
Season[†]	19.40	3.19	0.000	0.29	16.25	2.76	0.000	0.51
Vitamin D supplement[‡]	17.34	3.31	0.000	0.23	14.06	2.76	0.000	
Travels to southern latitudes[§]	15.57	4.61	0.001	0.11	10.17	3.55	0.005	
Sun preference[∣]	9.50	4.34	0.031	0.05	4.26	3.23	0.190	

Coding for each variable are shown within brackets.

[†] (1) Winter (Nov-April) or (2) Summer (May-Oct)

[‡] (1) No or (2) Yes

[§] Travels to latitude 35°N or below, during the last six months; (1) No or (2) Yes

[∣] (1) Shade or Sun and shade, (2) Sun

Table 5. Parathyroid hormone (PTH) concentrations related to different levels of 25-hydroxyvitamin D (25(OH)D) of 95 pregnant women during third trimester, living in Sweden.

25(OH)D, nmol/L	n (%)	PTH, ng/L	95% CI
<30	16 (17)	49·4	41·1-57·7
30-49·9	46 (48)	46·3	41·6-51·0
≥50	33 (35)	37·7	33·3-42·1
Total	95 (100)	43·8	40·7-46·9