

The effects of season and stage of pregnancy on plasma 25-hydroxy-vitamin D concentrations in pregnant women

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Summary

Plasma 25-OHD levels were measured in the same 26 Caucasian women before pregnancy and during the first, second and third trimesters of their pregnancy. This was done to assess whether pregnancy alters vitamin D status in healthy women. During most times of the year there was no association between the 25-OHD levels and the stage of pregnancy, but between January and March there was a progressive fall in 25-OHD levels with each trimester. When 25-OHD levels were related to hours of possible sunlight exposure a negative association between 25-OHD levels and stage of pregnancy was noted only in subjects with the lowest possible exposure to sunlight. These observations suggest that pregnancy has an effect on vitamin D metabolism, but that in healthy Caucasian women these effects only become manifest where there is a low level of exposure to sunlight.

Introduction

It is well recognized that in Asians pregnancy can precipitate vitamin D deficiency and even florid osteomalacia (Felton and Stone, 1966). In the offspring of such women, tetany and rickets may be encountered (Purvis *et al.*, 1973; Roberts, Cohen and Forfar, 1973). Pregnancy probably precipitates deficiency by increasing vitamin D requirements (Purvis *et al.*, 1973). More detailed studies on the effects of pregnancy on vitamin D status have become possible with the advent of accurate methods for assaying vitamin D metabolites.

Such studies have yielded conflicting results. Serial sampling through pregnancy in Caucasians and vegetarian and non-vegetarian Asians showed no decline in plasma 25-hydroxy vitamin D (25-OHD) concentrations during pregnancy (Dent and Gupta, 1975). However, in a cross-sectional study of women studied during February, metabolite levels in

non-white women in the last trimester of pregnancy were significantly lower than those in non-white controls while those of Caucasians remained unchanged (Turton *et al.*, 1977). Interpretation of data on the effects of pregnancy on plasma 25-OHD concentrations is complicated by the fact that concentrations show marked seasonal variation (MacLaughlin *et al.*, 1974). In the present study an attempt has been made to take into account both the stage of pregnancy and seasonal variation to see whether, as pregnancy advances, there is any change in vitamin D status.

Methods

Subjects were obtained by advertising in the local press for women who hoped to become pregnant in the near future. This yielded 26 Caucasian women with a mean age of 28 years ranging between 22 and 41 years. All agreed to give regular blood samples until conception and thereafter during pregnancy and for maternal and cord samples to be taken at delivery.

Plasma was stored at -20°C until analysis. From each of the 26 subjects single samples taken between 0 and 5 weeks before conception, between 0 and 13, between 14 and 27 and between 28 and 40 weeks of pregnancy, and at delivery were selected for 25-OHD measurement. Fifteen of the subjects were asked to record their food intake on a single day before pregnancy and the information used to calculate vitamin D intake. Details on monthly hours of sunshine were obtained from the Southampton Weather Centre.

Plasma 25-OHD assays were based on a method described by Belsey, De Luca and Potts (1974). This was modified in that radio-active tracer was added to the ethanolic plasma extract and not to the plasma itself. Tritiated 25-OHD₃ (11.3 Ci/mmol)

was purchased from the Radiochemical Centre, Amersham.

In validating the method the authors found that for plasma pools containing 30 nmol/l and 115 nmol/l intra-assay coefficients of variation were 2% and 8% respectively. Inter-assay coefficients of variation were 10% and 16%. Extraction of tritiated 25-OHD₃ added to plasma gave a mean recovery of 96%. Concentrations of 25-OHD were then measured in plasma samples to which increasing amounts of 25-OHD₃ had been added. A linear regression was found for estimated 25-OHD against added 25-OHD₃ with a correlation coefficient of 0.98. Cholesterol, cholic acid, cortisol 17 β -oestradiol, oestrone and androsterone had no effect on the assay. At 50% displacement from zero binding, cross reactivities with cholecalciferol (D₃), ergocalciferol (D₂), 25-hydroxyergocalciferol (25-OHD₂), 24,25-dihydroxycholecalciferol (24,25-(OH)₂D₃) and 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃) were 0.6%, 0.3%, 100%, 60% and 0.005% respectively. The technique therefore included all 25-OHD₃ and 25-OHD₂ and varying quantities of 24,25(OH)₂D₃ in estimating '25-OHD'. The advantages of the method were that it was a rapid and reasonably precise means of measuring 25-OHD in large numbers of small samples.

Results

Mean daily intakes of vitamin D in 15 subjects were low at 104 i.u. with a range between 30 and 209 i.u. The distribution for plasma 25-OHD concentrations showed a marked skew to the right. Results were, therefore, expressed as geometric

means and confidence limits, and plotted on logarithmic scales. The geometric mean and 95% confidence limits for plasma 25-OHD concentrations measured on samples taken before conception were 74.3 and 24.6 to 223.9 nmol/l (1 nmol/l \approx 0.4 ng/ml). These showed little change during the 3 trimesters of pregnancy (Fig. 1). Plasma 25-OHD concentrations were related to the time of year the samples were taken. There was marked seasonal variation (Fig. 2). The lowest geometric mean level of 44.8 nmol/l was recorded in April and the maximum one of 114.8 nmol/l recorded in September.

In Figure 3, regression lines are drawn for a

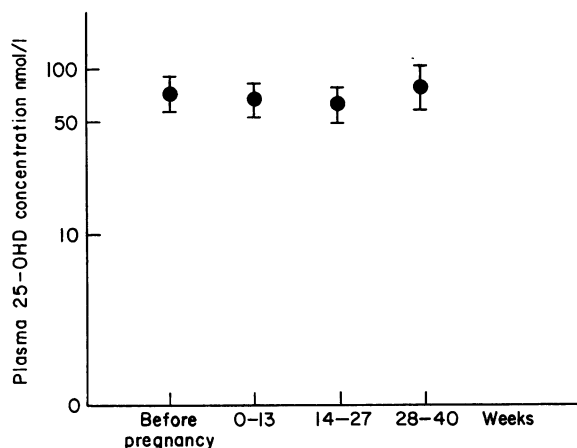


FIG. 1. Geometric means and 95% confidence limits for means of plasma 25-OHD concentrations in 26 women before and during the course of their pregnancies.

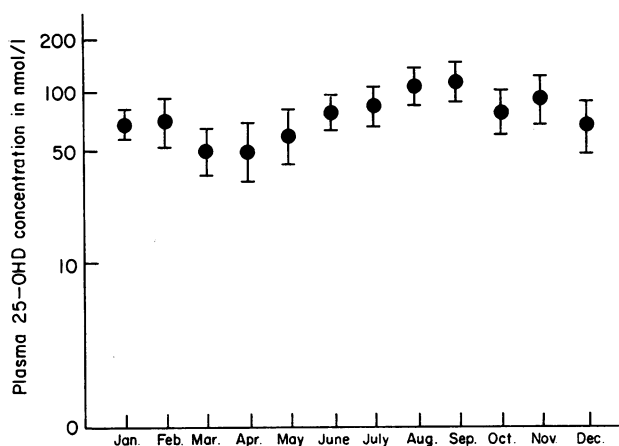


FIG. 2. Seasonal variation in geometric means and 95% confidence limits for means of plasma 25-OHD concentrations in 26 women measured before or during the course of their pregnancy.

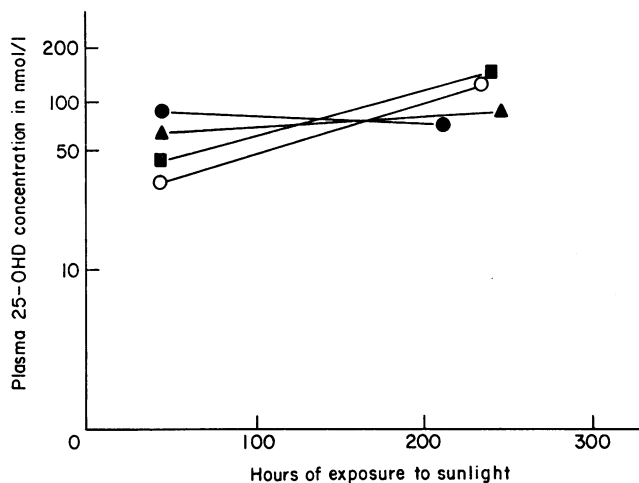


FIG. 3. Regression lines for relationship between plasma 25-OHD concentrations and hours of sunlight exposure recorded during month when sample was taken in women before conception (●—●); in first trimester (▲—▲); in second trimester (■—■); and in third trimester (○—○).

comparison between hours of sunlight over the previous month and plasma 25-OHD concentrations in women before pregnancy, and during the first, second and third trimesters. The coefficients of correlation between hours of sunlight and 25-OHD concentrations at these different stages were -0.090 (n.s.), 0.128 (n.s.), 0.568 ($0.01 > P > 0.001$), and 0.554 ($0.01 > P > 0.001$) respectively. Examination of the separate regression lines reveals that when there had been less than 100 hr of sunlight over the previous month plasma 25-OHD concentrations were highest in women before conception and that there was a progressive fall in concentrations through the 3 trimesters, with lowest concentrations being found in the third trimester. Where there had been more than 150 hr of sunlight over the previous month, this pattern was lost.

The results were next grouped into samples collected from January to March, April to June, July to September and October to December and a cross sectional analysis made of subjects at different stages of their pregnancies during these time intervals (Fig. 4). There was no relationship between the stage of pregnancy and plasma 25-OHD concentrations in samples taken from April to June, July to September or October to December but there was a sharp decline throughout pregnancy in samples taken between January and March, the geometric mean plasma 25-OHD level before pregnancy being 79.4 nmol/l and that in the third trimester being 22.4 nmol/l.

Plasma 25-OHD concentrations in cord blood

were compared with those in maternal blood taken at delivery (Fig. 5). There was a high degree of correlation between cord and maternal levels ($r=0.749$, $P < 0.001$). A regression equation ($y=0.554x+0.848$) suggested that where maternal concentrations were high, cord levels were lower, and where maternal concentrations were low, cord levels were higher.

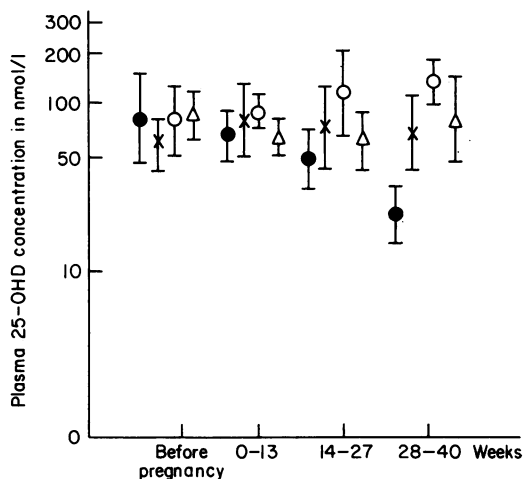


FIG. 4. Geometric means and 95% confidence limits for means of plasma 25-OHD concentrations in women related to stage of pregnancy for samples taken from January to March (●); April to June (×); July to September (○); and October to December (△).

Discussion

The mean intake of vitamin D in the 15 subjects investigated was surprisingly low. This may be due to women underestimating their food intake. A more probable explanation is that food intake plays a relatively small part in vitamin D status in young women.

The study confirms the findings of many other workers that there is a striking seasonal fluctuation in 25-OHD concentrations (Stamp and Round, 1974; MacLaughlin *et al.*, 1974). This is probably due to a variation in sunlight exposure. It was with this in mind that 25-OHD levels were compared with hours of sunlight recorded over the previous month. This effect makes it extremely difficult to assess whether pregnancy has any influence on vitamin D metabolism. It may be the reason why both in this study and that of Dent and Gupta (1975) serial blood samples from the same patients revealed no apparent changes in 25-OHD levels during the course of pregnancy.

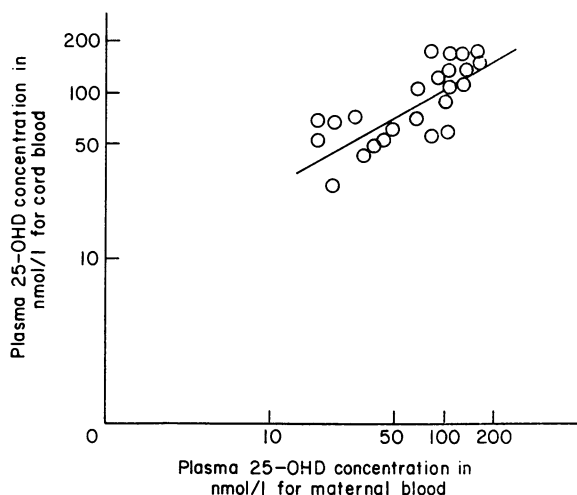


FIG. 5. Relationship between plasma 25-OHD concentrations in maternal and cord blood.

There are several reasons why 25-OHD levels might change in pregnancy. Turton and his colleagues (1977) postulate that pregnancy might affect hepatic 25-hydroxylase activity. Again, changes in parathyroid hormone levels noted during pregnancy might be the cause or result of any change there might be in 25-OHD concentrations (Cushard *et al.*, 1972). Finally, increased metabolic activity in the fetus might be expected to have some effect on maternal vitamin D requirements.

Under most circumstances it seems that the pregnant woman is able to cope with these changes.

Exceptions might be times of the year when there is limited exposure to sunlight. In this study, plasma 25-OHD concentrations taken between the months of January and March were much lower for women in the third trimester of pregnancy than in their non-pregnant counterparts. Throughout the rest of the year, pregnancy appeared to have little effect on plasma 25-OHD concentrations. Evidence in support of this conclusion is the rather complex relationship found between 25-OHD concentrations and hours of possible exposure to sunlight at different stages of pregnancy. Only in the last 2 trimesters of pregnancy was there a positive correlation. Analysis of regression equations associated with these correlations suggests that where there was limited exposure to sunlight there was a progressive fall in 25-OHD concentrations during pregnancy, but with a high degree of exposure to sunlight the association disappeared. A possible explanation for this pattern is that during pregnancy increasing demands are made on vitamin D metabolism, but that with adequate amounts of exposure to sunlight these demands can be met.

The ethnic background of subjects may also be important. Thus, while Turton and his colleagues (1977) found changes in plasma 25-OHD concentrations during pregnancy in non-white women, they had greater difficulty identifying a similar effect in their white counterparts. Differences in dietary habits and exposure to sunlight might account for this discrepancy.

The study confirms the findings of Hillman and Haddad (1974) that there is a close correlation between 25-OHD concentrations in maternal and cord bloods. Of equal interest is that both studies show that where maternal concentrations are normal or high, cord blood levels are lower but that where maternal concentrations are low, cord blood levels are higher. This may be due to variations in maternal 25-OHD carrier protein (Hillman and Haddad, 1974). An alternative explanation might be fetal regulatory mechanism.

This study indicates that under normal circumstances, pregnancy has little effect upon vitamin D metabolism. However, where there is sub-nutrition or limited exposure to sunlight, pregnancy may exacerbate an already parlous situation and produce frank vitamin D deficiency. Even here, however, the fetus may be protected to some extent by the fact that as maternal 25-OHD concentrations fall, the ratio of fetal to maternal 25-OHD concentrations rises in favour of the fetus.

Acknowledgment

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