

Original articles**Maternal compared with infant vitamin D supplementation**

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SUMMARY Vitamin D metabolites were studied in mother-infant pairs at delivery and eight and 15 weeks after that to evaluate the possibility of vitamin D supplementation of infant through the mother. Healthy mothers (n=49) delivering in January received daily either 2000 IU (group 1), 1000 IU (group 2), or no (group 3) vitamin D. Their infants were exclusively breast fed, and those in group 3 received 400 IU of vitamin D a day. After eight weeks of lactation the infantile vitamin D concentrations were similar in groups 1 and 3 but significantly lower in group 2. The serum 24,25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations were also lower in group 2. The mean mineral, parathyroid hormone, and alkaline phosphatase values showed no intergroup differences at any point. No infants showed any clinical or biochemical signs of rickets, and their growth was equal. In conclusion, a daily postpartum maternal supplementation with 2000 IU of vitamin D, but not with 1000 IU, seems to normalise the vitamin D metabolites of breast fed infants in winter. Maternal safety with such supplementation over prolonged periods, however, should be examined.

It has been widely discussed whether breast milk is sufficient to prevent rickets in infants. Several studies have shown that vitamin D supplementation is a necessity for breast fed infants, especially during winter in northern Europe.^{1 2} Asian infants in Britain are also at risk of developing rickets.^{3 4} Because the poor vitamin D stores of the mother may further impair vitamin D state in the infant,⁵ it is important to know whether rickets can be prevented in breast fed infants by supplementation of the mother, especially as some mothers, such as vegetarians, are not willing to give their infants any pharmaceutical medicine.

According to our earlier study, however, normal infant concentrations of vitamin D metabolites could not be reached by giving 1000 IU of vitamin D to the mother.² The present study was designed, therefore, to analyse the effects of a larger maternal vitamin D supplementation on the serum vitamin D metabolite concentrations and the mineral metabolism of breast fed infants during winter and spring.

Patients and methods

Study groups. The study was conducted at the maternity wards and outpatient clinic of the

department of pediatrics of the University Central Hospital of Tampere (latitude 61°N). Healthy, well nourished mothers delivering in January 1984 were divided in succession into three groups according to vitamin D supplementation as follows:

- Group 1: mothers (n=17) given 2000 IU of vitamin D₃ a day, infants not supplemented.
- Group 2: mothers (n=16) given 1000 IU of vitamin D₃ a day, infants not supplemented.
- Group 3: mothers (n=16) not supplemented, breast fed infants given 400 IU of vitamin D₂ a day.

The serum samples of the mother-infant pairs were collected at delivery and eight and 15 weeks later under exclusive breast feeding, when the infants were also examined. Venous blood samples were collected from the umbilical cord at delivery and within two hours of delivery from the mothers. During pregnancy, 33 mothers had no vitamin D supplementation (group A), eight mothers received 500 IU a day of vitamin D during the second trimester of pregnancy (group B), and eight mothers 500 IU a day throughout the pregnancy (group C). The mothers from these three groups supplemented

during pregnancy were distributed into the three groups supplemented after delivery as follows: group 1=11:4:2 (A:B:C, respectively), group 2=9:4:3, and group 3=13:0:3. The mothers received detailed information on the study and gave their written consent.

Sample analysis. The blood samples were collected without anticoagulant and centrifuged immediately after clotting; the serum samples were stored at -70°C until analysed. The vitamin D metabolites 25-hydroxyvitamin D (25-OHD), 24,25-dihydroxyvitamin D ($24,25(\text{OH})_2\text{D}$), and $1\alpha,25$ -dihydroxyvitamin D ($1\alpha,25(\text{OH})_2\text{D}$) in all the serum samples (0, 8, and 15 weeks) of each mother-infant pair (2×3) were analysed in the same assay. The other serum variables calcium, albumin, inorganic phosphorus, parathyroid hormone, and alkaline phosphatase were measured after each control—that is, at delivery and eight and 15 weeks thereafter. Heparinised, anaerobic infantile blood samples for ionised calcium were analysed immediately after collection.

Serum vitamin D metabolites were analysed from 1–2 ml samples, to which tritiated vitamin D_3 derivatives had been added to monitor recovery. The samples were deproteinised and prepurified, using the acetonitrile- C_{18} Sep-Pak procedure of Turnbull *et al.*,⁶ followed by further purification and separation of the metabolites by high performance liquid chromatography. A Waters Z-module equipped with a Resolve silicic acid cartridge eluted with hexane-isopropanol-methanol (90–10–1) was used. 25-OHD and $24,25(\text{OH})_2\text{D}$ were quantitated by competitive protein binding assay, employing serum from a pregnant woman diluted 1/20 000 in barbital-acetate buffer, pH 8.6, and [^3H] 25-OHD. Non-radioactive 25-OHD served as standard. $1\alpha,25(\text{OH})_2\text{D}$ was analysed by the radioreceptor assay of Reinhardt *et al.*⁷ For the 25-OHD and $24,25(\text{OH})_2\text{D}$ assay, the intra-assay ($n=10$) variation coefficients were 11.6% and 12.5% and the interassay ($n=6$) variations 10.8% and 14.5%, respectively; for the $1\alpha,25(\text{OH})_2\text{D}$ assay the intra-assay variation coefficient was 15.7%, whereas the interassay variation was 11.1%.

Serum calcium concentrations were assayed by atomic absorption spectrophotometry. Plasma ionised calcium and pH were measured by ion selective electrodes (ICA-1 Radiometer A/S, Copenhagen, Denmark). Serum albumin concentration was determined by the bromocresol purple technique, using a Hitachi 705 analyser, inorganic phosphorus by the phosphomolybdate method, serum parathyroid hormone by midregion-(44–68)-specific radioimmunoassay with commercial reagents from

Immuno Nuclear Corporation (Stillwater, Minnesota, United States), and alkaline phosphatase activity with a system Olli 3000 analyser with Oriola reagents (Helsinki, Finland), made to correspond to the Scandinavian recommendation.

Statistical analysis. Statistical analysis was performed using two tailed paired *t* test for intragroup differences and Student's *t* test for intergroup differences and for the correlation coefficient.

Results

Serum concentrations of vitamin D metabolites in the mother-infant pairs are presented in Figure 1,

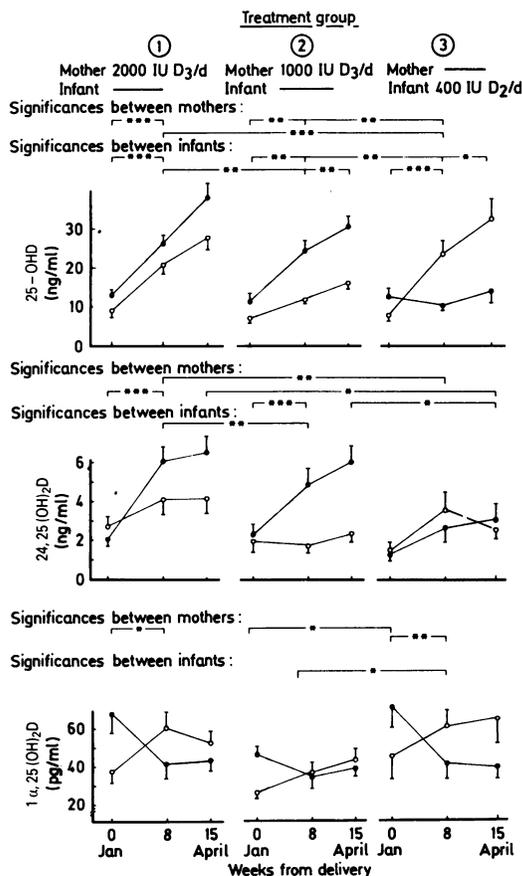


Fig. 1 Serum vitamin D metabolite concentrations (mean (SEM)) in winter of mothers (●) and infants (○) in different groups with vitamin D supplementation at delivery and 15 weeks later.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$. A detailed table of the concentrations of the vitamin D metabolites can be obtained at request from the author.

Conversions: traditional to SI units - 25-OHD: 1 ng/ml=2.5 nmol/l; $24,25(\text{OH})_2\text{D}$: 1 ng/ml=2.4 nmol/l; $1\alpha,25(\text{OH})_2\text{D}$: 1 pg/ml=2.4 pmol/l.

and the corresponding serum minerals (calcium, ionised calcium, and inorganic phosphorus), albumin, parathyroid hormone, and alkaline phosphatase in Figure 2.

Maternal data. At delivery, the concentrations of 25-OHD (mean (SEM)) were significantly lower ($p < 0.01$) in mothers not receiving vitamin D during pregnancy than in those receiving 500 IU of vitamin D a day throughout pregnancy (10.1 (1.0) ng/ml (25.3 nmol/l), $n=31$, and 23.0 (4.5) ng/ml, $n=8$, respectively). Maternal supplementation during middle pregnancy only did not influence the concentrations of 25-OHD (10.7 (1.8) ng/ml, $n=8$) at

delivery. There was a close association between the corresponding 25-OHD concentrations in maternal and cord serum samples ($r=0.8023$, $p < 0.001$, $n=47$), but there was no correlation for 24,25(OH)₂D ($r=0.1725$, NS) and only a slight positive correlation for 1 α ,25(OH)₂D ($r=0.3328$, $p < 0.025$).

As expected, the postpartum maternal vitamin D supplementation increased the serum 25-OHD and 24,25(OH)₂D concentrations of mothers significantly (Fig. 1) during the follow up of 15 weeks. During the first eight weeks maternal 1 α ,25(OH)₂D concentrations in all groups decreased to a constant concentration, where they stayed thereafter.

There were no significant intergroup differences in maternal calcium, inorganic phosphorus, albumin, alkaline phosphatase, and parathyroid hormone values (Fig. 2) at any of the study points.

Infant data. At birth, the serum concentrations of all three vitamin D metabolites were comparable in all groups. The mean (SEM) concentrations of 25-OHD were lower in cord serum samples (8.5(0.9) ng/ml) than in maternal serum samples (11.9(1.1) ng/ml). There was no difference between the umbilical and maternal serum concentrations of 24,25(OH)₂D (2.1(0.3) ng/ml (5(0.8) nmol/l) and 1.9(0.3) ng/ml, respectively). The umbilical serum 1 α ,25(OH)₂D concentrations (35(4.4) pg/ml (84 (10.6) pmol/l)) at delivery were 56% of the corresponding maternal concentrations (63(5.5) pg/ml) ($p < 0.001$, $n=49$).

At 8 weeks of age the 25-OHD concentrations of the infants in groups 1 and 3 were similar, but in group 2 they were significantly lower ($p < 0.01$), and in three infants 25-OHD concentrations were at or below the so called risk limit for rickets (5 ng/ml).⁸ In group 2 the 24,25(OH)₂D and 1 α ,25(OH)₂D concentrations of infants were significantly ($p < 0.01$) lower than in group 1 and the 1 α ,25(OH)₂D concentrations significantly ($p < 0.025$) lower than in group 3.

At 15 weeks of age, the infant 25-OHD concentrations were significantly ($p < 0.01$) lower in group 2 than in groups 1 and 3, which were similar. The intergroup differences found at 8 weeks of age for 24,25(OH)₂D and 1 α ,25(OH)₂D were absent at 15 weeks of age.

The intergroup differences in weight and height between the infants were not significant. At 8 weeks of age, however, two of the three infants in group 2 with low serum 25-OHD concentrations had a low weight gain (500 g and 1160 g) compared with the mean (SD) of the other infants in this group (1540 (383) g).

There were no significant intergroup differences in the infantile serum mineral concentrations (cal-

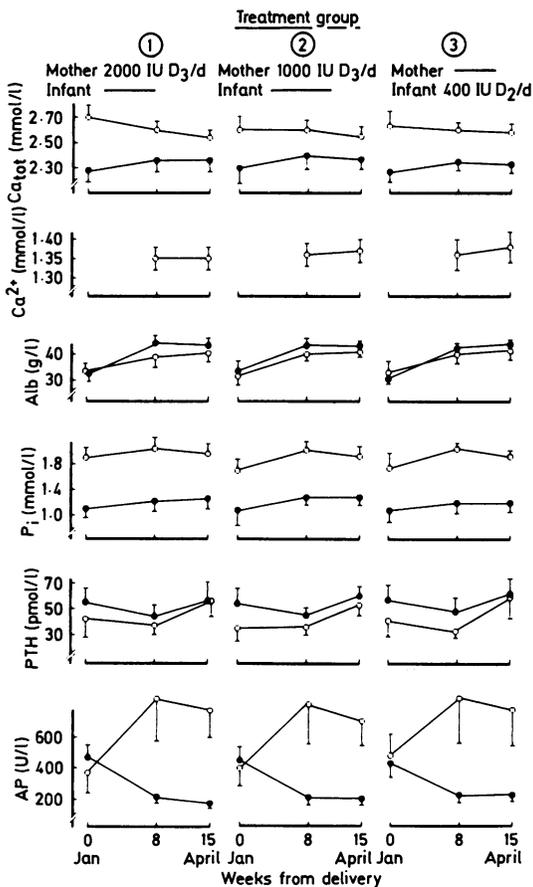


Fig. 2. Maternal (●) and infantile (○) values (mean (SD)) for serum total calcium (Ca_{tot}), ionised calcium (Ca^{2+}), albumin (Alb), inorganic phosphorus (P_i), parathyroid hormone (PTH), and alkaline phosphatase activity (AP) in different winter groups with vitamin D supplementation at delivery and eight and 15 weeks later. There were no significant intergroup differences at any study point.

cium, ionised calcium and inorganic phosphorus), nor in the albumin or parathyroid hormone concentrations and alkaline phosphatase activities (Fig. 2) between the study points, and none of the infants had any clinical signs of rickets.

Discussion

The purpose of the present study was to evaluate the possibility of vitamin D supplementation of infant through the mother. During the last decade, breast feeding has become increasingly popular in many western countries.⁹ It has been suggested that nutritional rickets¹⁰ or low 25-OHD concentrations^{2 11} are associated with unsupplemented breast feeding. Even intrauterine vitamin D deficiency may give rise to poor growth in infancy unless supplements are given.¹² Prenatal and post-natal vitamin D supplementation is advised for Asian infants living in Britain.¹³ Greer *et al.*, in a double blind prospective study, have reported decreased bone mineral content of infants receiving breast milk alone,¹⁴ but in a simultaneous larger, but not blind, study Roberts *et al* could not confirm these findings.¹⁵ Another study has suggested that human milk alone may provide sufficient dietary vitamin D for the needs of term infants under optimal social and environmental circumstances.¹⁶ Two of the three above studies^{14 15} were performed at moderate latitudes, and all three studies consist of mixed summer-winter populations, while at the latitude of Tampere the amount of sunlight very much depends on the season.¹⁷

According to the present data, it seems that the milk of supplemented mothers alone may provide the infant with sufficient vitamin D if the dose given to the mother is large enough. When the mother received 2000 IU of vitamin D a day the 25-OHD concentrations of the infants nearly equalled those of the infants receiving 400 IU of vitamin D directly. The dose of 1000 IU of vitamin D daily given to the mother is not high enough, which agrees with the result of our earlier study.²

A similar tendency in results, showing a transfer of 25-OHD (or vitamin D) from the mother with 2100 IU, but not with 900 IU of vitamin D daily, to the breast fed infant has recently been described by other Finnish investigators.¹⁸ In this report, however, the season was not controlled. In a Norwegian study the daily dose of 400 IU of vitamin D to mothers during lactation had no apparent effect on the serum 25-OHD concentration of breast fed infants.¹¹ On the other hand, a double blind South African study showed a clear effect on infant 25-OHD concentrations by even 500 IU of daily vitamin D supplementation through the mother.¹⁹

In our earlier study the results were similar.² The breast fed infant of mothers receiving 1000 IU of vitamin D₂ a day still had, in winter, however, lower 25-OHD concentrations (5.6(3.7) ng/ml) than in the present study ($p < 0.005$) at the same stage (eight weeks) of lactation, which raises the question whether the vitamins D₂ and D₃ are metabolised differently in man. This has also been suggested in some recent studies.^{20 21} The present study used vitamin D₃ instead of vitamin D₂ for technical reasons and was not designed to compare the two forms of vitamin D.

During eight weeks, 1 α ,25(OH)₂D increased in all infant groups to a concentration similar to that described earlier,²² but the slowest rate of increase was in group 2. As there were no disturbances in the infant mineral metabolism in group 2 (Fig. 2), the 1 α ,25(OH)₂D concentrations can be considered adequate, despite low 25-OHD concentrations in this group. The infantile 24,25(OH)₂D concentrations were significantly lower in group 2 than in group 1, which may reflect a reduction in the availability of 25-OHD.

It is unclear whether low 25-OHD concentrations are disadvantageous to growing infants. In 1936 Stearns *et al* reported more rapid growth in infants given 340-400 units of vitamin D daily than in those given 60-135 units.²³ In the present study the differences in infantile supplementation routes did not influence the weight or height gains of an infant up until 15 weeks. The low weight gain in two infants in group 2 may be associated with low 25-OHD concentrations, although this finding cannot be considered significant. It must be remembered that rickets occurs much later than 15 weeks, and normal mineral metabolism and growth at eight and 15 weeks may be of little reassurance.

This study shows once again that the cord blood 25-OHD concentration correlates well with maternal concentrations.^{24 25} The concentration of 25-OHD in the maternal circulation seems to be the major factor governing neonatal concentrations. Some of the mothers were supplemented throughout pregnancy with vitamin D, which was clearly reflected in their serum 25-OHD concentrations at term. Their infants also had higher cord serum 25-OHD than those of the unsupplemented mothers. The maternal supplementation during pregnancy did not create any intergroup differences at term as the mothers were evenly distributed among the three groups.

During eight week lactation, maternal 1 α ,25(OH)₂D decreased to normal adult concentrations and remained quite constant thereafter (Fig. 1), which has also been previously shown in man.²⁶ The normal parathyroid hormone concentrations

also confirmed the above results. The rise in maternal 24,25(OH)₂D was concomitant with that of 25-OHD (Fig. 1), which has not been reported in an earlier study with a similar supplementation and a long study period.

In conclusion, the vitamin D supplementation of 400 IU/day to breast fed infants is adequate and the most secure way of preventing rickets during winter on northern latitudes. Breast milk does not have enough antirachitic activity by itself or when the mothers are supplemented with 1000 IU/day. A sufficient supply of vitamin D to the breast fed infant is achieved only by increasing the maternal supplementation up to 2000 IU/day. As such a dose is far higher than the daily dietary allowance recommended for lactating mothers²⁷ its safety over prolonged periods is not known and should be examined.

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