Control of vitamin D metabolism in preterm infants: feto-maternal relationships

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SUMMARY To assess the relationship between maternal and fetal mineral homeostasis, serum calcium, magnesium, inorganic phosphate, parathyroid hormone, and vitamin D metabolite concentrations in venous cord sera from 15 preterm singletons and 3 twin pairs were compared with the levels found in maternal sera. Cord calcium, magnesium, and phosphorus levels were significantly higher than the respective levels in maternal samples. There was a significant relationship between the two compartments for all three analyses. Cord serum 25-hydroxyvitamin D, 24,25-dihydroxy-vitamin D, and 1,25-dihydroxyvitamin D levels were significantly lower than those observed for the mothers. Association of the cord concentration with that of the mothers was observed only for the first two metabolites. There was no relationship between the maternal 1,25-dihydroxyvitamin D levels and gestational age, calcium, magnesium, inorganic phosphate, or 25-hydroxyvitamin D. Cord 1,25-dihydroxyvitamin D correlated significantly only with cord calcium levels. Immuno-reactive parathyroid hormone levels were within normal limits both in cord and maternal samples. Our data suggest that after 31 weeks of gestation: (1) calcium, magnesium, and inorganic phosphate cross the placental barrier against a concentration gradient; (2) the fetus depends on the maternal supply for 25-hydroxyvitamin D and 24,25 dihydroxyvitamin D; (3) the feto-placental unit synthe-sises 1,25-dihydroxyvitamin D according to fetal needs.

Several reports in the last few years have dealt with the feto-maternal relationship of vitamin D* and minerals, particularly in term infants.[1-11] Cord serum 25-OHD levels have been shown to be directly related to the maternal serum concentrations.[1 2 4 5 8] Except for one report,[3] cord levels of 24, 25-$(OH)_2$D were also observed to be correlated to the maternal values.[4 5] Recent studies of serum 1,25-$(OH)_2$D concentration have shown either the absence or the presence of a direct relationship between cord and maternal levels after 35 weeks of gestation.[5 8 9] Studies in which calcium (Ca), inorganic phosphate (Pi), and magnesium (Mg) concentration were considered, all showed that there was a positive feto-maternal gradient[2 3 6-8 10 11] without reaching a consensus on whether a relationship existed between the two pools. The purpose of the present study was

*Vitamin D, 25-hydroxyvitamin D (25-OHD), 24,25-dihydroxyvitamin D [24,25-$(OH)_2$D], and 1α,25-dihydroxy-vitamin D [1,25-$(OH)_2$D] include undistinguishably vitamin D2(ergocalciferol) and vitamin D3 (cholecalciferol) and their corresponding hydroxylated metabolites.

to assess simultaneously the feto-maternal relation-ship of calciferol metabolite concentrations and that of Ca, Pi, Mg, and immunoreactive parathyroid hormone (iPTH) in preterm infants.

Patients and methods

The 18 mothers and their preterm offsprings included in this report were studied at the Neonatal Unit of the Hôpital Edouard Herriot (Lyon, France). All deliveries occurred between May and October and were done by caesarean section. The distribution of infants' gestational ages, as assessed by the Dubowitz scoring system,[12] and birthweights are summarised in Table 1. None was small for gestational age nor did any have a history of maternal diabetes or tox-aemia. The mothers received no vitamin D supple-mentation during pregnancy. The protocol was approved by an *ad hoc* ethics committee for research in human subjects.

Mother and cord venous blood samples were centrifuged within an hour and the serum frozen at

Table 1 *Distribution of fetal age and weight*

Number of infants	Gestational age (weeks)	Birthweight (g)
1	31	1430
7**	32	1180–1350
5*	33	1500–2000
2	34	1720–2100
4	35	1640–2600
2	36	1800–1820
Mean±SD = 33·8±1·9		Mean±SD = 1800±400

In this study 21 preterm infants were studied of which 6 were twins (* = 1 pair of twins). The weights listed are the extremes within each gestational age group.

−20°C until used. Mg and Ca were measured by atomic absorption spectrophotometry[13] and colorimetrically with a Technicon Autoanalyzer (Tarrytown, NJ, USA). Maternal serum albumin was assayed with the bromocresol green binding method[14] using a calibrated human serum as standard. For none of the above mentioned assays, was the interassay variability above 5%. Circulating iPTH was measured using an antiserum reactive to the carboxy-terminal fragment and intact parathyroid hormone.[15] As the ^{125}I-labelled parathyroid hormone was titrated against a standard consisting of a pool of serum from hyperparathyroid patients, only relative units could be used throughout the study. The detection limit for this assay was 25 ml Eq/l with detectable levels in 90% of the infant and adult samples. Serum 25-OHD and 1,25-(OH)$_2$D levels were measured by radioligand assays[16 17] with slight modifications.[18] The circulating 24,25-(OH)$_2$D concentration was also measured by a competitive binding assay[19] after careful purification by high pressure liquid chromatography on a 5 μm particle microporasil column (Beckman Instruments, Palo Alto, CA, USA) equilibrated with 6·5% propan-2-ol/n-hexane as mobile phase. Using sample volumes of 0·75–1·5 ml, the interassay variation coefficients for the three assays were 6, 8, and 10% respectively.

All data are expressed, unless otherwise specified, as mean values±standard error of the mean (SEM). The significance (P values) of the differences in solute mean concentrations between the maternal and cord sera was evaluated by the Student's *t* test for paired variates and their correlation by linear regression analysis.

Results

The biochemical data from the paired maternal and cord sera are shown in Table 2. The mean cord serum total Ca, Pi, and Mg levels were significantly higher (P<0·005) than those of the respective maternal samples. Furthermore, there was a close association between the maternal and cord sera Ca, Mg (P<0·005), and Pi (P<0·01) concentrations. Cord serum iPTH levels were normal (31±4 ml/Eq per l) and no different from those of the mother (30±3 ml/Eq per l). However iPTH could not be detected in 4 cord and 5 non-concordant maternal samples. In these instances the levels were set arbitrarily at 12·5 ml Eq/l (the mid-point between zero and the detection limit). The three vitamin D metabolite concentrations were consistently lower in cord than in the respective maternal samples [25-OHD and 1,25(OH)$_2$D: P<0·0005; 24,25(OH)$_2$D: P<0·01]. While both cord 25-OHD and 24,25 (OH)$_2$D levels are directly correlated to those of the mothers (P<0·0005), those of 1,25(OH)$_2$D are not. Furthermore, no relationship could be elicited between maternal serum 1,25(OH)$_2$D and maternal Ca, Pi, Mg, or 25-OHD. In cord serum, 1,25(OH)$_2$D

Table 2 *Relationship between maternal and cord sera mineral and calciotrophic hormones*

	Total serum calcium (mmol/l)	Magnesium (mmol/l)	Inorganic phosphate (mmol/l)	25-OHD (nmol/l)	24,25-(OH)$_2$D (nmol/l)	1,25(OH)$_2$D (pmol/l)	iPTH (mlEq/l)
Adults	2·20–2·60	0·75–0·95	0·82–1·42	55–75	1·9–3·8	62–86	25–100
Children	2·23–2·47	0·75–0·95	1·10–1·70	32–68	3·6–5·5	79–108	25–100
Maternal (mean±SEM)	1·98±0·07	0·68±0·02	1·06±0·06	45±5	3·0±0·7	142±12	30±4
Cord (mean±SEM)	2·38±0·12	0·72±0·02	1·62±0·07	30±5	1·8±0·4	82±10	31±4
Number of pairs	16	16	16	20	17	20	17
Difference (P value)	<0·005	<0·005	<0·005	<0·005	<0·01	<0·0005	NS
Correlation (r)	0·650	0·609	0·600	0·898	0·766	0·248	—
Significance (P value)	<0·005	<0·005	<0·01	<0·0005	<0·0005	NS	—

Laboratory derived reference values (adults and children) are the 95% confidence limits of the mean for each variable measured. Vitamin D metabolites were obtained from 22 adults (age range 25–40 years) and from 17 children (age range 7–15 years). The significance of the differences in solute mean concentrations between the maternal and various cord sera was calculated by Student's *t* test for paired variates. The correlation factor was obtained by the linear regression analysis.
25-OHD = 25-hydroxyvitamin D; 24,25(OH)$_2$D = 24,25-hydroxyvitamin D; 1,25-(OH)$_2$D = 1,25-dihydroxyvitamin D; iPTH = immunoreactive parathyroid hormone; SEM = standard error of the mean.

levels correlate only with Ca (P<0·025). Due to sample availability, serum albumin could only be measured in maternal samples. The mean concentration (33 ±1 g/l) is statistically lower than that of an age and sex-matched reference group (40 ±1 g/l, P<0·001).

Discussion

It is generally accepted that fetal and perinatal mineral homeostasis depend on endocrine and nutritional factors. In this study on 21 preterm infants and 18 mothers, we focused our attention on the relationship between the feto-placental and maternal mineral and calciotrophic hormone concentrations. Among the studies that have investigated these relationships, only those of Wieland et al.[5] and Pitkin et al.,[10] and now the present one, have considered simultaneously Ca, Pi, and Mg. Our data are basically in agreement with theirs, in that we also found higher concentrations of these three ions in the venous cord blood than in the maternal blood with a consistent correlation between the two compartments. This indicates, as surmised for Ca in human[20] and in other species,[21] that active transport mechanisms are involved in transplacental transfer. Other studies have considered selectively either Ca, Mg, or Pi.[2 3 5 8 11] Among these, there is no consensus on the relationship between maternal and fetal mineral homeostasis although all agree that there is a positive feto-maternal gradient.

The mean concentrations of iPTH in maternal blood was in the normal range with no sample in the hyperparathyroidic range, the maintenance of normal ionized calcium level despite a moderate apparent hypocalcaemia associated with a decrease in serum albumin concentration[22 23] being the likely explanation. These results confirm those reported earlier in term deliveries.[6 10] Levels of iPTH, although below the detection limit in 20% of the cord blood samples, were no different from controls. Furthermore, based on a test of proportion, this percentage was no different from that observed for the reference group (P<0·95). Thus our data do not support the alleged hypoparathyroidism of prematurity.[24]

The 25-OHD and 24,25(OH)$_2$D serum levels of the mothers were slightly below those observed in North America, probably reflecting the lack of vitamin D supplementation of milk in France. The levels of 24,25(OH)$_2$D correlated well with serum 25-OHD in agreement with earlier reports.[4 5]

Maternal serum 1,25(OH)$_2$D levels were moderately increased at the time of delivery as already reported.[25 26] Although the prolactin level has been shown to be high during pregnancy, we[27] and others[28 29] have shown that this pituitary hormone has no effect on vitamin D activation in humans. If different turnover kinetics are not shown to be responsible for the increased serum 1,25(OH)$_2$D levels, stimulating factors other than those classically invoked will have to be investigated.

The cord levels of the three vitamin D metabolites measured were consistently lower than those of the respective mothers, confirming data of others for term[4 9] and preterm[9] infants. Moreover, placental vein 25(OH)$_2$D and 24,25(OH)$_2$D concentrations correlated significantly with those of maternal circulation implying a passive diffusion of these two secosteroids across the placental barrier. Earlier reports with the exception of Hillman et al.[3] have also shown correlation between these two hydroxylated metabolites.[4 5]

Bouillon et al.[9] have shown that while maternal 'total' 1,25-(OH)$_2$D serum levels were higher than those of the respective cord blood samples, the concentrations of the unbound fraction were identical at 35 weeks of gestation. Further, the total and free maternal 1,25-(OH)$_2$D levels correlated with the mixed arterio-venous cord concentrations. However no consensus has yet been reached regarding this relationship.[5–8] As we have found no correlation between the two pools for total levels using venous cord blood, our data would support the hypothesis that, under physiological conditions, there is little maternal-placental crossover. The observed correlation between cord calcium and 1,25-(OH)$_2$D values suggests that the feto-placental unit contributes to the synthesis of 1,25-(OH)$_2$D according to the fetal needs in mineral.[21] However when 1,25-(OH)$_2$D is administered in pharmacological doses to the mother, the high levels of this metabolite, measured in the umbilical vein, suggest some degree of permeability of the placental barrier.[30 31]

In conclusion, our data support the contention that Ca, Mg, and Pi cross the placental barrier against a concentration gradient. They further show that the fetus depends on the maternal pool of 25-OHD and 24,25-(OH)$_2$D. They suggest that the synthesis of 1,25-(OH)$_2$D takes place within the feto-placental unit in response to the fetal needs. However a small contribution of the maternal 1,25-(OH)$_2$D pool to cord circulating levels cannot be excluded.

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