Calcium, Phosphorus, and Vitamin D in Human Milk

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Human milk is considered an optimal source of calcium and phosphorus for the feeding of normal term infants because of its satisfactory calcium/phosphorus ratio and low phosphorus concentration, and because it is an adequate source of antirachitic activity (1). Indeed, many studies have suggested that human milk protects the full-term neonate and the infant against the development of infantile rickets.

In premature infants, on the other hand, the adequacy of the mineral and vitamin D content of human milk is in doubt, since rickets and osteomalacia are often reported in such infants, particularly when fed human milk (2–8), which is now a widely encouraged practice. This article reports (a) the calcium, phosphorus, and vitamin D content of native human milk; and (b) the absorption of calcium and phosphorus in the full-term neonate and the premature infant.

CALCIUM AND PHOSPHORUS CONTENT OF HUMAN MILK

Mature milk contains 27 to 32 mg/dl calcium and 14 to 15 mg/dl phosphorus (Table 1) (9). Human milk from mothers who deliver prematurely has the same content of calcium and phosphorus (and also magnesium), but a slightly higher ionized calcium than term milk, which, it has been speculated, may increase the efficiency of calcium absorption. However, the total calcium content of human milk is low for a premature infant when compared to the amount needed to match the intrauterine accretion rate of about 120 to 130 mg/kg/day.
TABLE 1. *Milk composition and retention of intake in full-term newborns*

<table>
<thead>
<tr>
<th>Element</th>
<th>Mature human milk (mg/100 ml)</th>
<th>Retention (% of intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>27–32</td>
<td>62–64</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>14–15</td>
<td>87–90</td>
</tr>
<tr>
<td>Magnesium</td>
<td>3.5–4.5</td>
<td>50</td>
</tr>
</tbody>
</table>

**CALCIUM AND PHOSPHORUS BALANCE IN THE FULL-TERM NEWBORN**

The calculated increment in body content of calcium between birth and 4 months is about 155 mg/day. This value agrees with the observed retention of calcium reported by Fomon et al. (1963) from metabolic balance studies in full-term infants fed human milk. The mean retention of calcium in balance studies reported in the literature during the first 4 months averages 25 to 28 mg/kg/day. Calcium in human milk is relatively well absorbed due to many factors, but chiefly to the high content of lactose and good fat digestibility (1,10-13).

The calculated increment in body content of phosphorus has been estimated to be 75 to 79 mg/day. Metabolic balance studies with infants fed human milk show that phosphorus is well absorbed (~90%) and that phosphorus retention averages 12 to 13 mg/kg/day during the first 4 months of life (1).

Retention of calcium and phosphorus (expressed per unit body weight per day) is greater in infants receiving a high intake of these minerals (infants fed formulas or whole cows' milk) than in infants receiving low intakes (human milk). Nevertheless, feeding human milk, which provides low mineral intake, results in retention of calcium and phosphorus that is approximately equal to the estimated requirements (1).

Several studies have demonstrated the relation of fat absorption to calcium absorption in newborn infants receiving various types of feed (14–20). There is a positive correlation between excretion of fat and excretion of calcium, probably due to the fecal excretion of calcium soaps when calcium intakes are high.

It is also known that steatorrhea may be accompanied by increased fecal calcium excretion (21–24). Hanna et al. (25) demonstrated that the fatty acid composition of the diet affects calcium absorption and that there is a correlation between calcium and palmitic and stearic acid in the stools. The absorption of these fatty acids is higher in infants fed human milk than those fed formula. Human milk is rich in lipase activity, and this might explain
the better absorption of fat in infants fed human milk than in those fed formula.

CALCIUM AND PHOSPHORUS BALANCE IN PRETERM INFANTS

The amount of calcium that can be accumulated by a preterm infant is in doubt. Low positive calcium balance and signs of defective skeletal mineralization have been reported in preterm infants fed milks with a wide range of calcium content (26). A number of factors, such as inappropriate calcium/phosphorus ratio in the milk, poor absorption of lipids, increased fecal loss of endogenous calcium, abnormalities of metabolism of vitamin D, influence of postnatal age, and the amount of calcium intake, have all been implicated in the inadequate calcium absorption process in such infants (26).

We have shown that in premature infants, there is an appropriate secretion of parathyroid hormone (iPTH) in response to the hypocalcemic stimulus of the first week of life, and this increase in serum iPTH appears immediately after birth (27). Later, elevated iPTH levels are observed up to the third week of life, probably due to the high calcium requirement of the premature infant (28).

We have also demonstrated that preterm infants of more than 28 weeks of gestation can also absorb and hydroxylate vitamin D in the liver and kidney, and that there is a highly significant positive correlation between the levels of 25-OHD and 1,25(OH)₂D during the first week of life. This suggests that the concentration of substrate is a rate-limiting factor in the synthesis of the renal vitamin D metabolite in vitamin-D-depleted premature infants or hypocalcemic infants (29,30).

Table 2 shows the results of calcium, phosphorus, and fat balance studies in preterm infants fed banked human milk and given either no vitamin D supplementation or 30 μg (1,200 IU) vitamin D/day. The intake of calcium, phosphorus, and fat was identical in both groups. With no vitamin D supplementation, the mean calcium absorption was 49%. When vitamin D was given, calcium absorption reached 70%, but at the same time urinary excretion of calcium doubled to 21 mg/kg/day. Calcium retention was apparently little influenced by vitamin D and was about 20 mg/kg/day, with or without vitamin D supplements. Phosphorus was comparably well absorbed. There was little or no urinary excretion of phosphorus, and retention was about 25 mg/kg/day. The increased calciuria and low urinary excretion of phosphorus may be explained by an insufficient dietary supply of phosphorus, which prevents calcium from being sufficiently retained. This is further evidence in support of the well-established fact that human milk is deficient in phosphorus and should be supplemented. Furthermore, it is interesting to note that preterm infants can respond to a low phosphorus intake in exactly the same way as adults, with hypophosphatemia, hypophosphaturia,
TABLE 2. Results of metabolic balance studies in two groups of premature fed banked pasteurized human milk according to vitamin D intake

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D&lt;sub&gt;3&lt;/sub&gt;</th>
<th>No supplementation (n = 20)</th>
<th>30 µg/day (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Calcium (mg/kg/day)</td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>55 ± 9</td>
<td>63 ± 6</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>28 ± 9</td>
<td>19 ± 7</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>10 ± 5</td>
<td>21 ± 9</td>
<td></td>
</tr>
<tr>
<td>Retention</td>
<td>17 ± 9</td>
<td>23 ± 11</td>
<td></td>
</tr>
<tr>
<td>Net absorption (%)</td>
<td>49 ± 11</td>
<td>70 ± 11</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>29 ± 4</td>
<td>30 ± 3</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>1 ± 1</td>
<td>Trace</td>
<td></td>
</tr>
<tr>
<td>Retention</td>
<td>25 ± 5</td>
<td>26 ± 4</td>
<td></td>
</tr>
<tr>
<td>Net absorption (%)</td>
<td>90 ± 5</td>
<td>87 ± 4</td>
<td></td>
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</tbody>
</table>

* Mean ± SD.

hypercalcemia, and in the most severe cases, rickets (31,32). This deficiency of phosphorus is accentuated if human milk is enriched with calcium or with protein (33). We have clearly demonstrated that if the low phosphorus content of human milk is supplemented, calcium retention improves significantly to 35 to 40 mg/kg/day. This is nevertheless far below calcium retention in utero (34).

Many reports in the literature have shown that vitamin D and its metabolites are present in low concentration in human milk (50-60 IU/liter). The infants in our study were given 1,200 IU vitamin D/day because our previous studies have shown that the vitamin D requirement of preterm infants is relatively high due to the high rate of growth and poor vitamin D status at birth, particularly in France and other European countries. French breast-fed preterm infants given 1,200 IU/day vitamin D have normal 25-OH vitamin D levels during the first months of life (30). These data support our previous study showing both that vitamin D is metabolized to 1,25(OH)<sub>2</sub>D and that the gut response to 1,25(OH)<sub>2</sub>D is operative in preterm infants (35).

Figure 1 demonstrates the effect of phosphorus and calcium supplementation of human milk in comparison to human milk plus vitamin D and human milk plus phosphorus alone. Calcium retention only increased in the groups supplemented with phosphorus alone or with phosphorus and calcium together. Fecal loss of phosphorus was always low in all groups, and phosphorus was always well absorbed. Phosphaturia appeared only in groups
FIG. 1. Calcium (Ca) and phosphorus (P) intake; excretion and retention in preterm babies fed human milk with different supplementation; (D) vitamin D.

receiving human milk supplemented with phosphorus. Net phosphorus retention was significantly higher in the two supplemented groups.

Phosphorus supplementation is necessary in human milk, but calcium supplements may also be advocated; it is also clear that if this is done, supplementation with phosphorus must be increased. In our view the amount of added calcium should not exceed 27 to 30 mg/dl to avoid elevating the osmolality of the milk. This will double the intake of calcium in premature infants fed on breast milk (34). Further data from metabolic balance studies will be required before a higher level of calcium supplementation can be recommended.
TABLE 3. Calcium, phosphorus, and fat metabolic balance in premature infants fed pooled human milk at two different postnatal ages

<table>
<thead>
<tr>
<th></th>
<th>Study I (n = 6; 21–27 days)</th>
<th>Study II (n = 8; 44–45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcium (mg/kg/day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>49 ± 5</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>Feces</td>
<td>17 ± 8</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Urine</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Retention</td>
<td>30 ± 5</td>
<td>35 ± 9</td>
</tr>
<tr>
<td>Absorption (%)</td>
<td>66 ± 14</td>
<td>82 ± 9*</td>
</tr>
<tr>
<td><strong>Phosphorus (mg/kg/day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>44 ± 7</td>
<td>53 ± 15</td>
</tr>
<tr>
<td>Feces</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Urine</td>
<td>14 ± 5</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>Retention</td>
<td>27 ± 11</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>Absorption (%)</td>
<td>93 ± 3</td>
<td>96 ± 2</td>
</tr>
<tr>
<td><strong>Fat (g/kg/day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>5.30 ± 0.69</td>
<td>5.74 ± 0.70</td>
</tr>
<tr>
<td>Feces</td>
<td>1.50 ± 0.42</td>
<td>0.51 ± 0.23</td>
</tr>
<tr>
<td>Absorbed</td>
<td>3.80 ± 0.54</td>
<td>5.23 ± 0.70b</td>
</tr>
<tr>
<td>Absorption (%)</td>
<td>72 ± 6</td>
<td>91 ± 4b</td>
</tr>
</tbody>
</table>

* Mean ± SD.

The influence of postnatal age on calcium absorption and retention and on fat absorption in premature infants is shown in Table 3; premature infants fed on human milk enriched with phosphorus (9 mg/dl) and supplemented with 1,200 IU/day vitamin D were studied at age 21 to 26 days and later at 40 to 50 days. The improvement of fat absorption during this period was associated with increased calcium absorption but had less effect on calcium retention, due to the low calcium intake with human milk.

VITAMIN D

Native human milk has long been considered an adequate source of antirachitic activity, even before the discovery of vitamin D and its metabolites (36). Antirachitic potential was originally assessed solely by biological assay techniques and calorimetric analyses (36–38). In 1969, Sahashi et al. (39) reported the existence of vitamin D sulfate in human milk; the levels of antirachitic activity found by this method were between 500 and 900 IU/liter. These authors estimated that the antirachitic activity of vitamin D sulfate is equivalent to that of vitamin D.
TABLE 4. Vitamin D metabolite concentration in human milk (ng/liter)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Greer et al. (47)</th>
<th>Hollis et al. (42)</th>
<th>Reeve et al. (46)</th>
<th>Hollis et al. (41)</th>
<th>Hollis et al. (41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergocalciferol</td>
<td>40</td>
<td>41</td>
<td>51</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Cholecalciferol</td>
<td>ND (&lt;500)</td>
<td>311</td>
<td>ND (&lt;20)</td>
<td>101</td>
<td>131</td>
</tr>
<tr>
<td>25(OH)D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>163</td>
<td>151</td>
<td>203</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)&lt;sub&gt;2&lt;/sub&gt;D</td>
<td>ND (&lt;3)</td>
<td>5–2</td>
<td>ND (&lt;0–6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(ND) not determined; (UV) ultraviolet irradiation.

The development of ligand binding assays for vitamin D, 25(OH)D, 24,25(OH)D, and 1,25(OH)D allows analyses of vitamin D and its metabolites to be made in mature milk. Table 4 summarizes the results in the literature. The amount of vitamin D found in mature human milk by several authors does not exceed 30 to 60 IU/liter vitamin D<sub>3</sub> and 25(OH)D, and the amount of 1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25(OH)<sub>2</sub>D is very low (40).

Hollis et al. (41–43) published the first direct assessment of the effects on mature milk of supplementing the intake of vitamin D in the mother. They showed that increased maternal vitamin D intake will increase the antirachitic properties of the milk, and thus the milk from a mother who is vitamin D deficient would be low in antirachitic sterols. It is the maternal blood levels of the various antirachitic sterols that determine the amounts of these sterols that gain access to the milk. For example, Hollis et al. (41) reported that in a lactating mother receiving 50 μg vitamin D<sub>2</sub>/day for 14 days, there was a rise in plasma vitamin D<sub>2</sub> levels, with a sharp concurrent rise in vitamin D in her milk. In contrast, levels of 25(OH)D remained stable; but the effects of this supplementation are very short (not more than 2–3 days).

Does mature milk supply the required amount of antirachitic activity? The American Association of Pediatrics recommends that a neonate receives 400 IU vitamin D/day regardless of size, growth rate, and mineral intake. Human milk contains about 30 IU antirachitic activity/liter, mainly as vitamin D<sub>3</sub> and 25(OH)D, which have been shown to be 8 to 13 times more active than the parent vitamin. It is thus apparent that mature milk will not supply the neonate with 400 IU vitamin D/day (44–48).

The need for supplementation of breast-fed infants with vitamin D is currently a matter of controversy, however. Birbeck and Scott (49) in New Zealand reported adequate serum concentrations of 25(OH)D in a group of breast-fed infants who were not receiving vitamin D supplements, although
the cord level of 25(OH)D was high in this group. In the United States, Roberts (50) found that unsupplemented infants had a bone mineral content similar to a group of infants with supplementation or a group fed with supplemented formula. On the other hand, Greer et al. (51) reported that bone mineral content was lower in breast-fed infants without supplementation than in those supplemented with vitamin D. Rothberg et al. (52) have also shown that supplementation of mothers with 500 or 1,000 IU vitamin D raised the 25(OH)D plasma levels in breast-fed infants significantly in comparison with infants of unsupplemented mothers.

More recently, Ala-houlala (53) has shown that breast-feeding, with or without maternal supplementation of 1,000 IU/day vitamin D, results in very low 25(OH)D levels in the infant during the winter, which can be reversed by a supplement of 400 IU/day given to the infant. In summer, the infant 25(OH)D plasma concentration is normal.

The question arises as to how the vitamin requirements of term infants fed human milk are met. It is likely that vitamin D and 25(OH)D acquired transplacentally during the last trimester of gestation furnish the main vitamin D requirements of the infant in the first months of life. During summer, the vitamin D pool of the mother and her newborn is high and maintains a normal level of 25(OH)D in the infant for several months. Since determination of the serum levels of 25(OH)D in the pregnant women is not a routine procedure, and it is therefore not possible to assess the maternal vitamin D pool and to identify vitamin-D-deficient mothers, it is advisable during winter to provide 400 IU day/vitamin D to breast-fed infants. Furthermore, in countries where dairy products are not fortified with vitamin D, supplements should be provided all year round.

REFERENCES

CALCIUM, PHOSPHORUS, AND VITAMIN D


DISCUSSION

Dr. Kostalos: What do you think about babies between, say, 1,300 g and 2 kg? Do you think they need supplementation with calcium and phosphorus and vitamin D?

Dr. Salle: I did not say any infant needed supplementation with calcium. What they need is phosphorus supplements. I only discussed the question of calcium supplementation of human milk because it is advised by Tsang and others. We studied it to see whether it works; to show that if you increase the calcium content of human milk, the calcium is absorbed and retained, which it is. However, I do not advise putting calcium in human milk. I only advise the addition of phosphate, which is very important. About the vitamin D requirement, it seems to me that over about
2 kg there is no problem with calcium absorption or with the metabolism of vitamin D, so you do not need to give more than the usual 400 IU/day.

Dr. Kostalos: Do we really need to give extra vitamin D to babies over 1,500 g, even 400 IU?

Dr. Salle: It rather depends on the neonatologist. Some, for example, Reginald Tsang, like to give 400 IU to all babies less than 1,500 g. In Europe, however, the situation is different from North America and many other places, because in some European countries, like Belgium, France, and Spain, it is forbidden by law to add vitamin D to milk, and that includes infant formulas. In France, particularly, the mother may often have a very low plasma level of 25-OH vitamin D, and if she wants to feed her baby, his vitamin D intake will also be very low. This is the reason why we advise an increase in vitamin D supplementation and give 1,500 IU/day. As you can see from our data, you cannot cause intoxication; the 25(OH)D levels are all normal.

Dr. Marini: In relation to calcium and phosphorus supplements, we have to make a big distinction between formula and human milk. The level of calcium in human milk is very low for the needs of a preterm infant. If you only give phosphate, you will stimulate the bone matrix, but there will not be enough calcium for adequate calcification to occur in the matrix, and you will cause an increase in iPTH.

Dr. Salle: I do not agree with you about the role of phosphate in causing increased iPTH secretion. That has not been well demonstrated.

Dr. Lucas: We have found that babies who develop metabolic bone disease in the newborn period really have quite a long-term deficit in linear growth, and therefore asymptomatic rickets is more of a problem than people have imagined. If you just supplement human milk with phosphorus, you will produce a dramatic increase in calcium retention but to nowhere near the same degree as would have occurred in utero, which means that the infant is drawing on bone reserves of calcium throughout the whole neonatal period and will leave hospital with poorly mineralized bones. My feeling is that one should add calcium as well as phosphorus to human milk. You have tried this in your own department, Dr. Salle. What advice do you have for adding calcium and phosphorus to human milk without causing precipitation?

Dr. Salle: We have examined this. We supplemented human milk with 27 mg elemental calcium on its own, and when we analyzed it 1 day later, we found the expected increase in calcium concentration in the milk and the phosphate had not changed. We then supplemented with phosphorus alone, 25 or 50 mg/100 ml, and again found the expected concentration of phosphate in the milk 24 hr later, with no change in calcium, although the pH and osmolality changed. Finally, we tried adding both calcium and phosphorus together. If you add calcium first and then phosphorus, there is heavy precipitation of calcium phosphate, and the phosphate content of the milk does not change. If you add the phosphate first, and then 3 min later the calcium, subsequent analysis shows good agreement with the theoretical concentrations of calcium and phosphate that should now be in the milk. This was the reason for my question this morning. If you add the phosphate salt first, it could be absorbed on to the fat globules and thus escape precipitation with calcium.

Dr. Lucas: The calcium-phosphorus solubility product is very temperature dependent, and when a nasogastric tube passes into an incubator and heats up, you get precipitation. It is quite possible to give what looks like an entirely stable solution of calcium and phosphorus, only to have it precipitate within inches of the baby as it passes down the tube. It is an important practical point that solubility testing should be done at 37°C and not at room temperature.

Dr. Gaull: A few years ago there were some studies described at a Ross Laboratories Meeting that highlighted this problem of precipitation. From a technical point of view, do you consider this problem to be solved now, Dr. Guesry?
Dr. Guesry: We do not think this problem has been solved. It is quite impossible to add calcium and phosphorus at the same time to human milk. Our human milk fortifier, which is mainly designed for low-birth-weight feeding, contains phosphorus but no calcium, since we feel that it is more important to supplement low-birth-weight infants with phosphorus.

Dr. Ogra: Do you think the ratio of ionized versus protein-bound calcium may be important in determining whether it is deposited in bone?

Dr. Salle: No. I do not think so, because ionized calcium is normal in premature babies. If you look at all the different parameters of calcium metabolism in the premature baby during the first month of life, the most striking thing is the high level of iPTH. It is so high that it explains the high level of 1,25(OH)₂D₃ and probably explains the rickets and demineralization of bone, at least in part, because iPTH acts on the osteoclast first and not on the osteoblast.

Dr. Pemberton: I wonder about the relevance of this supplementation to the metabolic bone disease that we see in very low birthweight babies, which seems to mirror the intensity of illness and complications rather than the nutritional status. Do the results of these balance studies and supplementations really affect our management of this asymptomatic process that we see radiologically as a passing phase in many tiny babies?

Dr. Pemberton: Later on I mean. I am referring to the X-rays at 2 to 3 months, when you see the characteristic translucent humeri just as a passing trend, following chronic lung disease and other problems. Does your supplementation actually make the bone more radio-opaque at this time?

Dr. Gaull: Would making the bones more radio-opaque be an improvement?

Dr. Pemberton: Maybe it would be more relevant than the results of calcium and phosphorus balances. It is the outcome that we are interested in clinically rather than the trends. I do not know whether radiolucent bones are good or bad, but if we are pursuing physiologic ends, then I think we would like preterm bones at 3 months to look like the bones of a mature infant of similar postconceptual age.

Dr. Gaull: Dr. Räähä, do you have a final comment on you own current practices, since I know you favor human milk feeding of very immature infants?

Dr. Räähä: In my department we do not supplement human milk with calcium or phosphorus in very low birthweight infants, but we give separate supplements of both minerals to all infants below 1,200 g, because the babies who develop bone disease really only come into this weight category. I think if we were to supplement all babies below 1,500 g, we might overdo it.