Transport of the Lipid-Soluble Vitamins A, D, and K by Human Placenta

Edgard E. Delvin

Optimization of transmission of nutrients from, and the return of wastes to, the mother insures maximum fetal growth. Concentration gradient, molecular size, and hydrophobicity of the solutes being transported are critical factors to be considered in placental transfer processes. Membrane permeability to and interaction with the substrate, and the exchange area offered, are also important factors. However, kinetics of events and the preferential equilibrium states are also likely to depend upon the type of placentation. Hence data collected from species such as the ewe, in which placenta is of the epitheliochorial type, must be conspicuously compared with those obtained from other species such as the guinea pig or the human, which have hemochorial placentas. Therefore this chapter will be limited, insofar as possible, to the transport of the lipid-soluble vitamins A, D, and K by human placenta.

VITAMIN A

Vitamin A, which belongs to a class of biologically active isoprenoid polymers, is involved in vision, growth, and reproduction of higher animal species. Figure 1 shows the major forms of retinoids of biological interest. Long-chain retinyl esters from animal tissues and all-trans β-carotene from plant pigments are the major dietary forms of vitamin A. Whereas retinyl esters are hydrolyzed to free retinol in the intestinal lumen (1), β-carotene is absorbed as such, cleaved in the mucosa to yield two retinaldehyde molecules and reduced to retinol (2). The retinol molecules thus formed are then esterified to long-chain fatty acids by a fatty acid-acyl-coenzyme A (CoA) transferase, integrated into chylomicrons and brought to the vascular compartment through the lymphatic system. Once the triglyceride moieties are removed by extrahepatic tissues, chylomicron remnants are removed from the circulation and stored in the liver (90% of the body’s total reserve) from which it is mobilized as the free alcohol and transported by a plasma-specific retinol-binding protein (RBP) (3). This protein interacts stoichiometrically with transthyretin (TTR) insuring a longer circulating and biological half-life for vitamin A (3). In the rat, liver synthesis and
secretion of RBP are subject to feedback regulation, which is dependent upon vita-
mamin A status (4). Catabolism of vitamin A involves an extensive variety of oxidative
and chain cleavage reactions. The resulting products and their conjugates, devoid of
biological activity, are excreted in the feces as glucuronides. Oxidative and chain-
shortened metabolites are ultimately excreted in the urine. For further details on the
metabolism and mode of action of vitamin A, the reader is referred to extensive
reviews (5,6).

Vitamin A nutritional status in pregnancy presents a special interest, as both defi-
ciency and hypervitaminosis may have deleterious effects on fetal development. Data
on vitamin A status and RBP levels, other than those of Howells et al. (7,8), who
have reported on the vitamin A status of preterm infants born small for gestational
age, and on vitamin A nutrition in British and Asian mothers, are scarce. It must be
stressed that most reports on the teratogenicity of vitamin A in humans are retrospec-
tive in nature. For example, Werler et al. (9), from a case-control surveillance study
of drug use in relation to birth defects, evaluated the effect of vitamin A supplementa-
tion on malformations derived from cranial neural crest cells. This epidemiologic
study involving 5267 infants (2658 cases, 2609 controls) revealed no apparent associa-
tion between the use of vitamin A–containing multivitamin supplements and malfor-
mations. The authors, however, qualified their conclusions since they were unable
to obtain the vitamin A dose taken by the mothers. Furthermore, in a recent study,
Mills et al. (10) reported similar maternal retinol levels in 89 pregnancies resulting in neural tube defects (NTD) and 178 control pregnancies identified from the Finnish Registry of Congenital Malformation. Although there are no reported prospective studies on chronic or acute exposure of pregnant women to high vitamin A intake or the analog isotretinoin, spontaneous abortion or major fetal malformation have been occasionally reported in individuals with a chronic excessive intake of vitamin A during the early phase of pregnancy (11,12). In a mouse model, Atkin et al. (13) have shown that treatment of animals with 5000 IU of vitamin A daily between days 11 and 19 of gestation, resulted in reduced weight and length of the long bones of the offspring and excessive calcification throughout the hypertrophic zone of epiphyseal cartilage. In the human, Neel and Alvarez (14) have shown, in an epidemiologic study performed in Guatemala, that there was a significant relationship between the low cord blood vitamin A levels and weight of intrauterine growth-retarded neonates born at term.

There is little information on in vivo vitamin A placental transport. Most data available give the relative concentrations of the retinol transport components in maternal and newborn blood. Selected data are summarized in Table 1. Gebre-Medhin and Vahlquist (15) reported that liver vitamin A concentration increased exponentially in Swedish fetuses during the second and third trimesters of pregnancy. However, this was not the trend observed in samples obtained from Ethiopian fetuses, which showed a lower liver content of vitamin A as pregnancy progressed. In the same study, circulating serum RBP levels in Ethiopian full-term neonates were not statistically different from those of Swedish counterparts. Given that liver is the major storage site, the low fetal liver vitamin A content in Ethiopian subjects at term indicates a depletion of the long-term stores required in the neonatal period. In another study (16), supplementation of low-income pregnant Indian women with 1800 µg of vitamin A/day for more than 12 weeks prior to delivery.

Although there was no correlation between retinol maternal and cord blood retinol levels, there was a positive correlation between RBP saturation, expressed as the retinol/RBP molar ratio, in cord sera and the corresponding index in mothers.

### TABLE 1. Vitamin A and retinol-binding protein levels in maternal and cord blood

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Cord</th>
<th>p value</th>
<th>Corr.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBP</td>
<td>1.51 ± 0.55</td>
<td>0.97 ± 0.29</td>
<td>n.a.</td>
<td>n.a.</td>
<td>15</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.94 ± 0.11</td>
<td>0.47 ± 0.05</td>
<td>*p &lt; 0.01</td>
<td>n.a.</td>
<td>16</td>
</tr>
<tr>
<td>Retinol</td>
<td>1.09 ± 0.12*</td>
<td>0.81 ± 0.11</td>
<td>*p &lt; 0.05</td>
<td>n.a.</td>
<td>16</td>
</tr>
<tr>
<td>Retinol</td>
<td>1.32 ± 0.26</td>
<td>0.52 ± 0.15</td>
<td>*p &lt; 0.001</td>
<td>n.a.</td>
<td>19</td>
</tr>
<tr>
<td>RBP</td>
<td>0.022 ± 0.006</td>
<td>0.010 ± 0.003</td>
<td>*p &lt; 0.001</td>
<td>*p &lt; 0.02</td>
<td>19</td>
</tr>
<tr>
<td>Retinol</td>
<td>1.49 ± 0.39</td>
<td>1.15 ± 0.33</td>
<td>*p &lt; 0.001</td>
<td>n.s.</td>
<td>20</td>
</tr>
<tr>
<td>RBP</td>
<td>3.42 ± 0.85</td>
<td>2.15 ± 0.66</td>
<td>*p &lt; 0.001</td>
<td><em>p &lt; 0.01</em></td>
<td>20</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SE. Units are µmol/liter. The significance of the differences between maternal and cord blood values, calculated from the published individual values when available, were established by the Student's t-test for paired variates. Corr., linear regression analysis was performed when individual data were published; n.a., not available; n.s., not significant.

*Mothers were supplemented with 1800 µg of vitamin A/day for more than 12 weeks prior to delivery.

*Although there was no correlation between retinol maternal and cord blood retinol levels, there was a positive correlation between RBP saturation, expressed as the retinol/RBP molar ratio, in cord sera and the corresponding index in mothers.
μg vitamin A per day for a period of 6 to 12 weeks prevented the decline in maternal levels of vitamin A at the time of delivery at term when compared to a control group. Although levels of vitamin A in cord blood were also significantly increased in the supplemented group, thereby showing the dependence of the fetus upon the maternal stores, they were lower than those of the mother, in agreement with other studies involving vitamin A–replete subjects (17,18). In the above studies, total retinol concentrations were measured. Since circulating vitamin A is bound to RBP and since maternal/cord concentration ratios of this protein and of vitamin A are of the same order of magnitude (19,20), it can be inferred that free vitamin A levels are similar in both pools, providing that maternal and fetal RBP have the same binding characteristics.

Evidence for the transfer of vitamin A to the human fetus is obtained from in vitro studies involving either isolated placental cells or membranes. Törmä and Vahlquist (21) have shown that $^{125}$I-RBP was attached to placental tissue, suggesting that the protein moiety of the complex had been internalized by the cells. They also provided evidence, from kinetic studies, that two types of RBP receptors were present in placenta. The first receives retinol at the surface of the cell, while the second internalizes the retinol-RBP complex. Retinol delivered to placental cells is mostly esterified. High-performance liquid chromatography (HPLC) analysis of placental extracts after incubation with $^3$H-retinol demonstrated that approximately 40% of the intracellular retinol was esterified and that the esters mainly consisted of stearates and palmitates. Because of the model used, the authors could not evaluate whether retinol, either free, esterified or protein-bound, was released to the fetal side. Sivaprasadarao and Findlay (22,23) have reported on the presence of a receptor for RBP on human brush-border membrane of term placenta. The binding of the protein was dependent on both temperature and concentration, showing that the receptors were specific. Carefully studying the uptake mechanisms of retinol by plasma membrane vesicles, these investigators also provided evidence that endocytosis of the retinol-RBP complex is unlikely but that the binding of the RBP to membranes was obligatory for the subsequent delivery of the vitamin. These results confirmed those obtained earlier by Chen and Heller (24) with isolated epithelial cells. Hence the current theory holds that, in the human placenta, the TTR-RBP–vitamin A complex (holo-TTR-RBP) interacts transiently with a membrane receptor and that the apoproteins are returned to the extracellular space while retinol enters the cell, where it is bound to a cellular retinol-binding protein (CRBP), and eventually delivered to the fetal pool where it is associated with a TTR-RBP complex of fetal origin (Fig. 2). Within trophoblasts, retinol may be reversibly esterified to long-chain fatty acids or oxidized to retinoic acid. Underwood (25) has proposed, mostly on the basis of animal studies, that vitamin A transfer from mother to fetus is unrestricted early in pregnancy but that it becomes subject to control once placental and fetal tissues have acquired the capacity of synthesizing receptors and transport binding proteins. Whether, under physiological conditions, retinoic acid crosses the human placental barrier from mother to fetus in late pregnancy is not yet ascertained.
FIG. 2. Proposed transport mechanisms for vitamin A in human placenta.

VITAMIN D

It is well established that vitamin D\textsuperscript{1} is biologically inert and that it must undergo successive hydroxylations to fully express its antirachitic activity. Figure 3 outlines the main steps of the vitamin D activation pathway and gives the range of circulating levels of each of the metabolites. When it is part of the diet, vitamin D appears in the circulation at a peak time of 12 hours after being incorporated into chylomicrons and transported through the lymphatic system\textsuperscript{(26)}. Thus patients with impaired intestinal fat absorption are likely to develop vitamin D deficiency despite an adequate intake of the vitamin. In growing individuals and adults, vitamin D\textsubscript{3} (cholecalciferol) produced in the epidermis\textsuperscript{(27)} is bound to a vitamin D–binding protein (DBP) and translocated to liver where a microsomal cytochrome P-450–dependent hydroxylase yields calcidiol [25(OH)D\textsubscript{3}]\textsuperscript{(28,29)}. In normal conditions, circulating levels of calcidiol are good indices of the vitamin D status of the individuals\textsuperscript{(30)}. Under physiological conditions and in non-pregnant mammals, calcidiol is further metabolized, by the kidney, to 1α,25(OH)\textsubscript{2}D\textsubscript{3} (calcitriol), the hormonal form of vitamin D, and to 24,25(OH)\textsubscript{2}D\textsubscript{3}, believed, at the present time, to be the initial product of the catabolic

\textsuperscript{1} Vitamin D\textsubscript{2} (ergocalciferol) and vitamin D\textsubscript{3} (cholecalciferol) are included under the generic name of vitamin D.
7-dehydrocholesterol $\rightarrow_{hv}$ pre-D$_3$

37 °C $\rightarrow$

D$_3$

$[3 \text{--} 13 \text{ nmol/l}]$

Microsomal Liver D$_3$-25 hydroxylase

Mitochondrial Kidney 25(OH)D$_3$-1α-hydroxylase

Mitochondrial Kidney 25(OH)D$_3$-24R-hydroxylase

25(OH)D$_3$

$[35 \text{--} 100 \text{ nmol/l}]$

1α,25(OH)$_2$D$_3$

$[65 \text{--} 130 \text{ pmol/l}]$

24,25(OH)$_2$D$_3$

$[3 \text{--} 5 \text{ nmol/l}]$

FIG. 3. Main reactions involved in the vitamin D activation pathway.

The enzymes 25(OH)D$_3$-1α-hydroxylase and 25(OH)D$_3$-24R hydroxylase, located in renal mitochondria (32,33) and composed of cytochromes P-450, ferredoxin, and ferredoxin reductase (33,34), are subject to control by extracellular calcium, parathyroid hormone, and phosphate fluxes. For further details the reader is referred to the review by Holick (35).

Placental transfers of vitamin D or its metabolites has mostly been studied indirectly. In studies examining the feto-maternal relationship (Table 2), the cord levels of 25(OH)D, 24,25(OH)$_2$D, and 1α,25(OH)$_2$D are lower than those of the mothers, whether term or preterm infants are involved (36,37). Moreover, venous cord levels of 25(OH)D and 24,25(OH)$_2$D are correlated with those of the mother, thereby implying that these metabolites cross placenta by a passive diffusion. The case of 1α,25(OH)$_2$D is not as clear-cut. Bouillon et al. (38), while agreeing that total cord 1α,25(OH)$_2$D levels are lower than those of the respective mothers, observed a direct relationship between cord and maternal levels. In a preliminary study involving a group of patients with low vitamin D intake we were unable to find such a correlation (36). However, in a subsequent study involving two groups of women, one of which was supplemented with 1000 IU of vitamin D$_3$ per day during the last trimester of pregnancy, we confirmed this relationship in the supplemented group only (39). These results thus suggest that the transfer of 1α,25(OH)$_2$D from mother to fetus depends on the saturation of transport sites on syncytiotrophoblast plasma membranes. This hypothesis is supported by the observation that administration of 1α,25(OH)$_2$D$_3$ in
pharmacological doses to a hypoparathyroid mother resulted in cord blood levels of this metabolite higher than those observed in normal term and preterm infants (40).

The importance of maternal supply to the fetus in terms of vitamin D was substantiated, in the rat, by Clements and Fraser (41) who showed that the in utero supply is the main determinant of the vitamin D status in the early neonatal period. In their elegant experiments, they repleted vitamin D-deprived female rats by injecting either 14C- or 3H-labeled vitamin D3 prior to mating. Animals were then mated to males fed a standard diet. Before suckling, pups from the 3H-labeled mothers were exchanged with those of the 14C-labeled ones. Comparison of the ratio of 14C- to 3H-labeled vitamin D metabolites showed that the vitamin received in utero was the main determinant of the vitamin D status during the first week of life, and that it was gradually replaced by that obtained from milk. We obtained indirect evidence that such a situation prevails in man (39). In this study, pregnant women were randomly assigned to either one of two groups. The first received 1000 IU vitamin D3 per day during the last 3 months of pregnancy, while the second was not supplemented. When, within each group, the newborn serum total and ionized calcium values at 4 days were compared to those at birth, the mean drop in the infants of supplemented mothers was of lesser importance than that observed for the unsupplemented group. Conversely, circulating levels of 1α,25(OH)2D3 increased in infants of the supplemented group only. These results could explain, in part, the association between rickets in infants and maternal vitamin D deficiency (42,43) observed in the Asian population in the United Kingdom and support the provision of maternal vitamin D3 supplementation during the last trimester of pregnancy for groups at risk.

Contribution of the feto-placental unit to vitamin D metabolism should not be disregarded in the frame of placental transfer of vitamin D. Clues for an extrarenal synthesis of 1α,25(OH)2D3 first came from animal studies. It was observed that in bilaterally nephrectomized pregnant rats the circulating levels of 1α,25(OH)2D3 were

<table>
<thead>
<tr>
<th>Table 2. 25 OHD and 1,25(OH)2D levels in maternal and cord blood</th>
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</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>25 OHD</td>
</tr>
<tr>
<td>1.25(OH)2D</td>
</tr>
<tr>
<td>25 OHD</td>
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<tr>
<td>1.25(OH)2D</td>
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<td>25 OHD</td>
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<td>1.25(OH)2D</td>
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<tr>
<td>25 OHD</td>
</tr>
<tr>
<td>25 OHD</td>
</tr>
<tr>
<td>1.25(OH)2D</td>
</tr>
<tr>
<td>1.25(OH)2D</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. 25 OHD: nmol/liter, 1,25(OH)2D: pmol/liter. The significance of the differences between maternal and cord blood values, calculated from the published individual values was established by the Student's t-test for paired variates. Corr., linear regression analysis was performed for correlation studies; n.s., not significant.

* Mothers were supplemented with 1000 IU of vitamin D3/day for the last 3 months of pregnancy.
not completely abolished as they were in their non-pregnant counterparts (44). The hypothesis that rat placenta was the site of the hormone synthesis was supported when placental homogenates were shown to produce 1α,25(OH)₂D₃ (45). Similar results were obtained in human decidua incubated with labeled 25(OH)D₃ (46,47). The synthesis of 1α,25(OH)₂D₃ by the decidua does not exclude the contribution of fetal kidney to the production of this hormone. In fact, rabbit fetal kidney has been shown to hydroxylate 25(OH)D₃ at the 1α-position in vitro (48). Whether this is true for human kidney and whether this reaction has any physiological relevance remains to be shown.

Evidence for 1α,25(OH)₂D production by fetal human kidney has been obtained by measuring cord blood levels of 1α,25(OH)₂D in infants with renal agenesis. In these subjects, levels of the hormone were approximately one-third of those observed for normal infants (49). The residual hormone could reflect either transfer of the hormone from the mother or placentation production. Little other evidence is available for transplacental movement of vitamin D and its metabolites. One report by Ron et al. (50), using the isolated perfused human placental cotyledon, showed relatively low maternal-to-fetal clearance indices for both 25(OH)D₃ and 1α,25(OH)₂D₃. None of the above studies, however, have given clues to the transport mechanisms for vitamin D across the placenta. We have recently observed that DBP concentrations in preterm cord blood were highly related to the levels in the mothers (unpublished observations). This relation was also observed earlier by Bouillon et al. (51,52).

Figure 4 summarizes hypothetical mechanisms involved in the transfer of vitamin D.

**FIG. 4.** Proposed transport mechanisms for vitamin D in human placenta.
and its metabolites through human placenta. In this scheme secosteroids, bound to DBP, are brought to the brush-border membrane of trophoblasts, where they dissociate to allow cellular uptake of the free compound. Once within the cell, vitamin D and its hydroxylated derivatives are captured by cytosolic-binding proteins or receptors and translocated to the basolateral membrane, where complexes are dissociated. The free sterols are released into the fetal circulation where they are again complexed to DBP. This concept is supported by Nestler et al. (53), who, using immunocytochemistry, have shown the presence of DBP at the surface of human cytotrophoblasts. They proposed that since no active synthesis of the protein could be elicited it must have been maternally derived. Since typing of the binding proteins was not performed, they could not rule out the possibility that DBP was synthesized by fetal tissues and then acquired by placental trophoblasts.

VITAMIN K

The generic name of vitamin K includes two distinct natural compounds: vitamin K\_1, from plant and vegetable oils, and vitamin K\_2, of bacterial origin (including that of the intestinal flora). Their molecular structures (Fig. 5) include either an isoprenoid residue followed by a 3-methyl butane trimer (K\_1) or an isoprenoid heptamer (K\_2) side-chain hooked to a 2-methyl 1,4-naphthoquinyl ring. Both vitamins are absorbed by the small intestine with the compulsory assistance of bile acids, incorporated into chylomicrons, and transported through the lymphatic system to the liver. The turnover rate of vitamin K is rapid. Bjornsson et al. (54), using infusion of \(^3\)H-vitamin K\_1 in adult human volunteers, reported an initial circulating residence half-time of 26 minutes, a fractional turnover rate of 0.4 per hour, and a body pool turnover of 2.5 hours. Vitamin K is rapidly metabolized to chain-shortened and oxidized derivatives, which are excreted either free or as sulfo- and gluco-conjugates in urine and feces (55,56). Vitamin K is an essential element for the activation of coagulation factors II, VII, IX, and X. Its action involves the posttranslational \(\gamma\)-carboxylation of glutamic acid, yielding \(\gamma\)-carboxyglutamic acid (GLA)-containing factors having calcium-binding properties and thus permitting their interaction with membrane surfaces.

![Molecular structures of vitamin K\_1 (phyloquinone) and vitamin K\_2 (menaquinone).](image-url)
Although the impact of an inefficient coagulation system is readily perceived in the occurrence of intraventricular hemorrhage in the premature newborn, the underlying mechanisms are still poorly understood. Whether the vitamin is transported through the placenta from mother to fetus or not has practical implications for the treatment of this disease. If vitamin K crosses the placental barrier, prophylactic supplementation of the mother should prove useful. On the other hand, if the vitamin does not reach the fetus, then parenteral administration remains the treatment of choice.

As in the case of vitamins A and D, information on placental transfer of vitamin K derives from clinical studies in which maternal and cord blood levels of the vitamin have been measured. Table 3 summarizes the finding of such investigations. All studies referred to in this text (59–64), except for those of Sann (65) et al. and Greer et al. (66), show a maternal/cord blood concentration gradient of vitamin K ranging between 10 and 20. Moreover when mothers were supplemented prior to delivery, mothers supplemented daily with 20 mg of vitamin K for at least 3 days before sampling. Cord bloods were obtained at term. Mothers received 10 mg of vitamin K intramuscularly 4 days before delivery. If delivery did not occur within 4 days, the dose of vitamin K was repeated. Women who carried their pregnancy beyond 4 days of the second dose received 20 mg of the vitamin orally every day until the end of the 34th week or until delivery, whichever occurred first.

### Table 3. Vitamin K<sub>1</sub> levels in maternal and cord blood

<table>
<thead>
<tr>
<th>Mother</th>
<th>Cord</th>
<th>M/C</th>
<th>p value</th>
<th>Corr.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.022 ± 0.005</td>
<td>11.4</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>59</td>
</tr>
<tr>
<td>0.34 ± 0.01</td>
<td>0.009 ± 0.005</td>
<td>37.8</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>59</td>
</tr>
<tr>
<td>0.50 ± 0.06</td>
<td>&lt;0.02</td>
<td>&gt;25</td>
<td>n.a.</td>
<td>n.a.</td>
<td>60</td>
</tr>
<tr>
<td>0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.030</td>
<td>18.7</td>
<td>n.a.</td>
<td>n.a.</td>
<td>61</td>
</tr>
<tr>
<td>81.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.765</td>
<td>103</td>
<td>n.a.</td>
<td>n.a.</td>
<td>61</td>
</tr>
<tr>
<td>0.40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.210</td>
<td>19.0</td>
<td>n.a.</td>
<td>n.a.</td>
<td>61</td>
</tr>
<tr>
<td>41.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.783</td>
<td>52.6</td>
<td>n.a.</td>
<td>n.a.</td>
<td>61</td>
</tr>
<tr>
<td>1.54 ± 0.33</td>
<td>0.11 ± 0.02</td>
<td>14</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>62</td>
</tr>
<tr>
<td>0.102</td>
<td>0.010</td>
<td>10.2</td>
<td>n.a.</td>
<td>n.a.</td>
<td>63</td>
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<tr>
<td>11.59&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.024</td>
<td>483</td>
<td>n.a.</td>
<td>n.a.</td>
<td>63</td>
</tr>
<tr>
<td>9.03 ± 0.94</td>
<td>10.4 ± 1.0</td>
<td>0.86</td>
<td>n.s.</td>
<td>n.a.</td>
<td>64</td>
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<tr>
<td>82</td>
<td>71</td>
<td>1.15</td>
<td>n.a.</td>
<td>n.a.</td>
<td>64</td>
</tr>
<tr>
<td>9.00</td>
<td>n.d.</td>
<td>—</td>
<td>n.a.</td>
<td>n.a.</td>
<td>64</td>
</tr>
<tr>
<td>0.20 ± 0.02</td>
<td>n.d.</td>
<td>—</td>
<td>n.a.</td>
<td>n.a.</td>
<td>65</td>
</tr>
<tr>
<td>1.70 ± 0.21</td>
<td>1.10 ± 0.12</td>
<td>1.54</td>
<td>&lt;0.02</td>
<td>n.s.</td>
<td>66</td>
</tr>
</tbody>
</table>

M/C, mean mother/cord ratio. Levels are expressed as mean ± SE when available. Units are μmol/liter. The significance of the differences between maternal and cord blood values, calculated from the published individual values when available, were established by the Student's t-test for paired variates. Corr., linear regression analysis were performed when individual data were published; n.s., not significant; n.d., not detectable levels; n.a., data not available.

<sup>a</sup> Mothers were supplemented with 5 mg of vitamin K<sub>1</sub> 0.5 to 4 hours before delivery.

<sup>b</sup> Endogenous levels obtained from maternal and cord blood samples at mid-term pregnancy.

<sup>c</sup> Mothers supplemented daily with 20 mg of vitamin K<sub>1</sub> for at least 3 days before sampling. Cord bloods were mid-term pregnancies.

<sup>d</sup> Endogenous levels obtained from maternal and cord blood samples at term pregnancies.

<sup>e</sup> Mothers supplemented daily with 20 mg of vitamin K<sub>1</sub> for at least 3 days before sampling. Cord bloods were obtained at term.

<sup>f</sup> Mothers received 10 mg of vitamin K<sub>1</sub> intramuscularly 4 days before delivery. If delivery did not occur within 4 days, the dose of vitamin K<sub>1</sub> was repeated. Women who carried their pregnancy beyond 4 days of the second dose received 20 mg of the vitamin orally every day until the end of the 34th week or until delivery, whichever occurred first.
this gradient increased, reaching values as high as 483 (59,61,63). Although cord blood levels of vitamin K₁ increased, they remained in all cases very low, indicating a strong resistance of the placental barrier to the transport of the vitamin. This is further supported by the fact that no correlation between maternal and cord blood vitamin K₁ levels was observed in three studies (59,62,66).

The relatively high cord blood levels of vitamin K₁ reported by Sann et al. (65) and Greer et al. (66) are not understood at the present time, although analytical variance could be involved. How do these results translate into the treatment of neonatal intraventricular hemorrhage of the premature newborn? Most studies show that prophylactic administration of vitamin K₁ to the mothers had no effect on cord blood coagulation factor activities and on the incidence of intraventricular hemorrhage when compared to newborns from unsupplemented mothers. In one study (59), the investigators observed that the protein induced by vitamin K absence (PIVKA-II) was not detectable in any cord blood sample in either group. They concluded that a decrease in the synthesis of precursor proteins rather than vitamin K₁ deficiency was responsible for the hemorrhagic disease and that antenatal supplementation of the mothers was unwarranted.

Kazzi et al. (67) also reported that administration of vitamin K₁ to pregnant women at less than 35 weeks of gestation, for several days prior to delivery, had no beneficial effect on the coagulation factor activity profile or on the incidence or severity of intraventricular hemorrhage of premature infants. Although no measurements of vitamin K levels were reported, cord blood levels of vitamin K₁–dependent coagulation factors were significantly lower than those of the mothers, suggesting a limited synthesis capacity of fetal liver. Moreover, Yang et al. (59) and Mandelbrot et al. (61) observed that, despite an increase in cord blood vitamin K₁ concentration, supplemented fetuses and neonates showed no increase in total or coagulant prothrombin activity. On the other hand, in a study involving 92 patients delivering premature infants of less than 32 weeks of gestation, Morales et al. (68) observed reduced prothrombin and activated partial thromboplastin times, and a reduction in the incidence of intraventricular hemorrhage in newborns from vitamin K₁–supplemented mothers. Unfortunately neither maternal nor cord blood vitamin K₁ levels were measured. Since no final answer has been obtained from the above studies, treatment of neonatal intraventricular hemorrhage remains a subject of debate.

CONCLUSION

As can be appreciated from this brief review, the mechanisms involved in the transplacental movement of lipid-soluble vitamins remain elusive and a number of questions are still unanswered. The role of placenta in the control of the transport and the metabolism of the lipid-soluble vitamins, the kinetics involved in the transfer of these compounds to and from the fetus, and the existence (or absence) of signals from the fetus to modulate the import of the vitamins are all subjects of research that will give a better understanding of fetal nutrition.
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150 PLACENTA TRANSPORT OF VITAMINS A, D, AND K


DISCUSSION

*Dr. Singh:* You mentioned that when toxic amounts of vitamin A are given to mothers there is fetal death and fetal growth retardation. I think it is accepted that very large doses can cause damage to the fetus. But then you said that newborn babies with intrauterine growth retardation (IUGR) have low vitamin A levels. This does not establish a causal relationship. It probably only means that the placental dysfunction that led to IUGR also led to impaired transport of nutrients to the fetus, including vitamin A. You also mentioned a study where optimal doses of vitamin A during pregnancy led to enhanced fetal growth. Was this study done in malnourished or well-nourished mothers?

*Dr. Delvin:* First, with regard to vitamin A toxicity and pregnancy: don't forget that this was a mouse model and extremely large amounts of vitamin A were given. Second, with regard to IUGR and low vitamin A: I agree that other factors have to be considered. My main purpose in pointing out the data was to point out that what is true for the mouse is not necessarily true for the human. We should always apply caution when interpreting data looking at single factors in fetal development. Finally, yes, some of the studies were done in malnourished mothers, who were then repleted with vitamin A.

*Dr. Vidailhet:* How is carotene transported across the placenta?
Dr. Delvin: When we take carotene it is transformed to vitamin A at the gut level. What is transported across the placenta is vitamin A, not carotene.

Dr. Vidailhet: But there is carotene in the plasma. Is there any in cord blood?

Dr. Delvin: I don't believe anybody has looked at this.

Dr. Boyd: I have difficulty with the concept of lipid solubility for this group of compounds. If they are really lipidsoluble and available in solution in plasma, to even a limited extent, then they would cross the cell membrane rather easily. Has anyone looked systematically at water solubility coefficients for these compounds, compared with other lipophilic compounds that are known to cross cell membranes? Do we actually know that they are all lipid soluble?

Dr. Delvin: I think this has been done more by physical chemists than by biochemists or physiologists. My understanding is that when you do extractions they are totally insoluble in water phases. We should not forget, however, that serum of blood plasma is not really water. There are many lipid substances in circulation.

Dr. Boyd: How good is the evidence that there is an element of, say, vitamin D that is free in aqueous solution?

Dr. Delvin: The evidence is quite good. It is mainly derived from the studies of Roger Bouillon (1) and Daniel Bikle (2). Bikle, in particular, has done ultrafiltration studies after adding labeled vitamin D compounds to serum. He observed that the vast majority of the vitamin D was bound, but there was some free vitamin D. The levels of free vitamin were extremely low in comparison to the total values.

Dr. Putet: Were the two groups of women in the vitamin A supplementation study you presented supplemented only with vitamin A? Was there a difference in length of gestation and placental weight?

Dr. Delvin: Yes, they were supplemented only with vitamin A. Nothing else was changed. The supplements were taken at least 1 week before the end of gestation. The babies were born at term. Placental weight was not measured. The only measurements were cord blood and maternal blood vitamin A levels. It was interesting that maternal blood vitamin A levels were not that high, even though the women were supplemented with about 1800 μg of the vitamin. However, the maternal-to-cord blood ratio changed markedly.

Dr. Verellen: With regard to vitamin A supplementation, you showed that levels increased by a factor of 10- to 20-fold in supplemented mothers, but you then stated that the coagulation factor activities were identical. Does this mean that the low levels in the unsupplemented individuals were sufficient to ensure good coagulation?

Dr. Delvin: This is what is thought. The main problem as far as the infant is concerned seems to lie in the maturity of the enzymes that convert the pro-factors to the active factors.

Dr. Von Kries: There was a suggestion that giving vitamin K to mothers prior to delivery reduces the incidence of intracranial hemorrhage. None of the three studies on this topic were definitely conclusive.

Dr. Fukagawa: What about vitamin E?

Dr. Delvin: I don't have much information on vitamin E.

Dr. Orzalesi: Vitamin E crosses the placenta with some difficulty and the gradient between mother and fetus is quite high. This may be related to the transfer of lipids between maternal and fetal blood.

You have examined one compartment, that is, the transfer from mother to fetus. Of course, whatever is transferred to the fetus has to be disposed of. In the case of lipid-soluble vitamins it is possible that they are stored in the tissues and that their removal from blood into stores is an important factor in determining the blood levels.
Dr. Delvin: Binding proteins may be of some help here. A study in Sweden and Ethiopia (3) showed that while vitamin A levels were much the same in neonates in the two countries, there were marked differences in the concentrations of the binding protein, which indicated a relative degree of vitamin deprivation in the Ethiopian infants.

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