

## Review Article

# Vitamin D and mineral metabolism in normal pregnancy and in the normal fetus

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### Summary

Metabolic bone diseases in neonates are being recognized with increasing frequency but, despite extensive research, the pathophysiology of neonatal osteopenia remains unclear. This review briefly summarizes vitamin D and mineral metabolism in the normal non-pregnant human adult, and then addresses the adaptations that occur during pregnancy and fetal life.

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During the past two decades the physiology of normal vitamin D and mineral metabolism has been studied extensively in mature animals and humans, resulting in a better understanding of metabolic bone diseases like osteoporosis. Moreover, although the precise functions of the various vitamin D metabolites are still controversial, it has become apparent that while the vitamin D family acts predominantly on bone and mineral metabolism it also has effects on other cellular functions including differentiation and immunoregulation. Until recently research in this field has concentrated almost entirely on the adult and older child. Only during the last few years has attention been focused on bone and mineral metabolism during pregnancy, fetal development and in the neonate.

### Vitamin D and mineral metabolism in the normal non-pregnant human adult

The concentrations of calcium and phosphorus in extracellular fluid and plasma are maintained within remarkably narrow limits mainly by the action of parathormone (PTH), vitamin D and calcitonin (CT) on bone, the intestine and kidney. The total body calcium of a normal adult is approximately 1 100 - 1 200 g of which 99% is contained in the skeleton. Its normal concentration in plasma is 2,2 - 2,6 mmol/l, of which about 45% is protein-bound mainly to albumin, 47% is ionized, and the rest exists as ultrafilterable complexes. The ionized calcium fraction is the metabolically active form involved in numerous

physiological functions, e.g. enzyme activation, control of membrane excitation and exocytosis. The recommended daily requirement for calcium is 800 - 1 200 mg.<sup>1,2</sup> The total body phosphorus of a normal adult is about 650 - 850 g, of which 85% is contained in the skeleton. The normal serum phosphate level in the adult is 0,8 - 1,4 mmol/l.

PTH is biosynthesized by the parathyroid glands as the parent hormone pre-pro-PTH, which is cleaved in the gland to pro-PTH and finally to the major secretory form of PTH which consists of 84 amino acids.<sup>2,3</sup> Full biological activity appears to reside in the amino-terminal 1 - 34 peptide, the middle and carboxy-terminal sequence being biologically inert although immunologically highly reactive.<sup>4,5</sup> Production of PTH is stimulated predominantly by a low ionized serum calcium concentration.<sup>1,2</sup> A number of factors, including serum magnesium,<sup>1,2</sup> CT,<sup>2</sup> prostaglandins and steroids as well as a variety of drugs,<sup>6</sup> vitamin D metabolites<sup>2,7-9</sup> and catecholamines,<sup>10</sup> are, however, capable of modulating PTH secretion. PTH acts on renal tubular cells by binding to specific receptors, generating intracellular cyclic adenosine monophosphate, which in turn activates the kinase systems that result in an enhanced fractional reabsorption of calcium from the glomerular filtrate. PTH also stimulates renal 1 $\alpha$ -hydroxylase thereby increasing the production of 1,25-dihydroxyvitamin D, which in turn enhances intestinal absorption of calcium and phosphate.<sup>11</sup> In addition PTH increases renal losses of phosphate and bicarbonate.<sup>2</sup> PTH also acts directly on bone resulting in the mobilization of calcium stores. This action involves both an early mobilization phase independent of new protein synthesis, and a later phase associated with an increased synthesis of bone enzymes, particularly lysosomal enzymes associated with osteoclastic bone resorption.<sup>6</sup> Since bone resorption and bone formation are normally tightly coupled, PTH also stimulates bone formation and plays a vital role in the overall control of bone remodelling.<sup>12</sup>

CT is a 32-amino acid peptide secreted by the parafollicular cells (c-cells) of the thyroid. The exact role of CT in bone and mineral metabolism is still unclear, but the most commonly recognized physiological action of this hormone — a decrease in both serum calcium and phosphate levels — suggests that its primary action involves a reduction in bone resorption.<sup>2</sup> Indeed, CT-induced hypocalcaemia *in vivo* is not dependent on intact renal or intestinal function, and the hormone has been shown to inhibit the effects of various bone resorbing agents *in vitro*.<sup>2</sup>

Vitamin D is essential for the maintenance of mineral and skeletal homeostasis in man.<sup>11,13</sup> Vitamin D is available in two chemical forms, namely vitamin D<sub>2</sub> (ergocalciferol), a plant sterol, and vitamin D<sub>3</sub> (cholecalciferol), which is synthesized in the skin of animals. The chemical structures of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> differ only in the side chains. In humans the metabolism and biological actions of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> are thought to be identical. The human skin contains a high concentration of 7-dehydrocholesterol which upon exposure to ultraviolet radiation is rapidly converted, via

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opening of the  $\beta$ -ring, to previtamin D<sub>3</sub> by a non-enzymatic process which is apparently not under any biological control. Previtamin D<sub>3</sub> is then thermally isomerized to vitamin D<sub>3</sub> over a period of 2 - 3 days.<sup>14</sup>

In the circulation vitamin D is bound to an  $\alpha$ -globulin, vitamin D-binding protein (DBP), and transported to other tissues.<sup>11</sup> It is rapidly cleared by the liver where it is either metabolically inactivated and excreted in bile or hydroxylated to yield 25-hydroxyvitamin D (25-OHD). In temperate climates, under conditions of similar vitamin D intake, the serum 25-OHD in healthy adults is related to the amount of sunshine exposure, and is significantly lower during winter than during summer.<sup>15</sup> In the absence of hepatic disease, serum 25-OHD thus reflects dietary intake and skin synthesis of vitamin D. From the liver 25-OHD is transported by DBP to the kidney where a second hydroxylation reaction occurs at either the 1 $\alpha$ - or the 24 position of the sterol to yield the dihydroxy products 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) or 24,25-dihydroxyvitamin D (24,25(OH)<sub>2</sub>D).<sup>11</sup> In the kidney 24,25(OH)<sub>2</sub>D can be further metabolized to 1,24,25-trihydroxyvitamin D (1,24,25(OH)<sub>3</sub>D) which is less potent than 1,25(OH)<sub>2</sub>D, but more potent than 24,25(OH)<sub>2</sub>D in stimulating bone resorption and intestinal reabsorption of calcium.<sup>2</sup> At present more than 20 other metabolites of vitamin D have been characterized chemically — the exact function of these still remains unclear.<sup>11,16</sup>

Whereas the hepatic 25-hydroxylation appears to be linked to the environmental input of substrate, renal mitochondrial hydroxylation is under the strict control of ionic and hormonal factors. The synthesis of 1,25(OH)<sub>2</sub>D is stimulated by PTH.<sup>17,18</sup> In addition to and independent of PTH, the 1 $\alpha$ -hydroxylase system is also stimulated by hypocalcaemia and phosphate depletion.<sup>19</sup> Conversely, PTH and phosphate deprivation have been shown to suppress 24-hydroxylase activity.<sup>20</sup> In addition to PTH, calcium and phosphate, it has been suggested that a number of other ions and hormones govern the activity of the 1 $\alpha$ -hydroxylase. From present data it is not possible to deduce the relative importance of these factors in the overall regulation of vitamin D metabolism (Fig. 1).<sup>2,12,13,19</sup>

Extrarenal synthesis of these vitamin D metabolites has recently been shown to be a property of bone,<sup>21</sup> placental cells<sup>22,23</sup> and lung macrophages.<sup>24</sup> The physiological significance and the regulatory factors governing the extrarenal production of 1,25(OH)<sub>2</sub>D are not yet known.

The main function of vitamin D and its active metabolites seems to be the maintenance of calcium and phosphate homeostasis and the provision of minerals for bone formation. The exact function(s) of the various metabolites are still uncertain. It has been shown that vitamin D and/or 25-OHD, apart from being mere precursors of 1,25(OH)<sub>2</sub>D, 24,25(OH)<sub>2</sub>D and other metabolites, also have direct influences on intestinal mucosal cell proliferation and on calcium and phosphorus accumulation

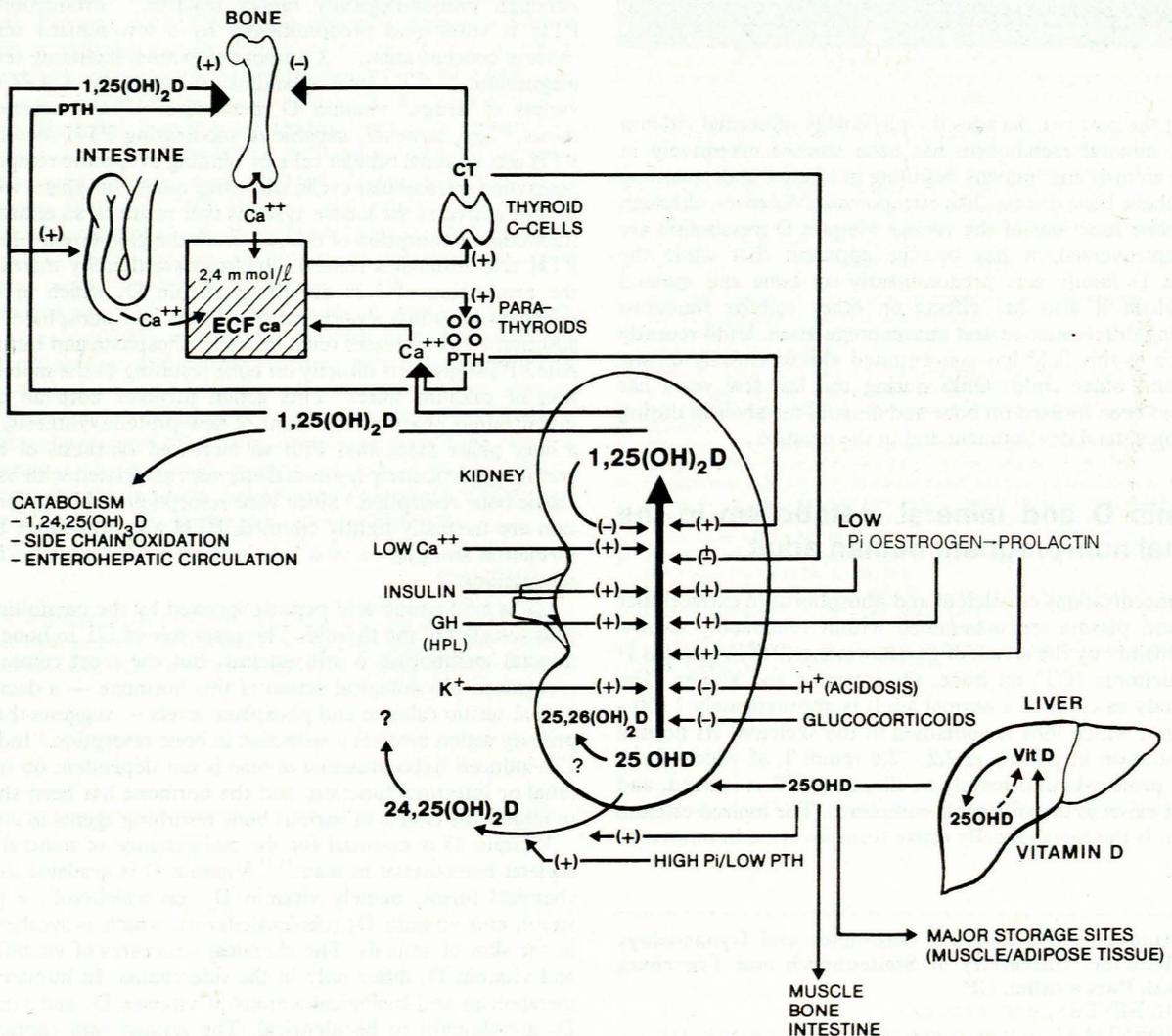


Fig. 1. The metabolism of vitamin D.

in intestinal cells.<sup>25,26</sup> By acting directly on skeletal muscle *in vitro* 25-OHD also accelerates the accumulation of phosphate and the rate of protein synthesis.<sup>27</sup> Regarded as the most potent metabolite of the vitamin D family is 1,25(OH)<sub>2</sub>D which acts predominantly on the intestinal mucosal cells where it stimulates absorption of calcium and phosphorus.<sup>17,28,29</sup> Other target organs include bone, from which it mobilizes calcium and phosphate<sup>17</sup> and appears to be essential for normal mineralization and bone growth,<sup>30,31</sup> and the kidneys where it stimulates calcium reabsorption in the distal tubules.<sup>28</sup> Recent evidence also suggests that this metabolite may be involved in a variety of other biological processes ranging from a suggested regulatory role in the secretion of hormones such as insulin<sup>32</sup> to effects on cellular differentiation and immunoregulation.<sup>33-36</sup> The physiological importance of 24,25(OH)<sub>2</sub>D is still controversial. It has been suggested that 24,25(OH)<sub>2</sub>D has no unique biological actions and merely represents an inactive by-product.<sup>37,38</sup> Recently it has been shown, however, that both 1,25(OH)<sub>2</sub>D and 24,25(OH)<sub>2</sub>D are necessary for normal chick egg hatch-ability<sup>39</sup> and that 24,25(OH)<sub>2</sub>D is needed for optimal bone mineralization in various human and animal experiments.<sup>30,31,40,41</sup> Moreover in animal experiments 24,25(OH)<sub>2</sub>D has been shown to inhibit PTH secretion.<sup>42</sup>

Similarly to other steroid hormones, 1,25(OH)<sub>2</sub>D acts by binding to specific cytoplasmic receptors whereupon it is translocated into the nucleus of the target cell where it induces the synthesis of specific mRNA essential for the synthesis of new proteins mediating the biological actions of the vitamin. Examples of these include the vitamin D-dependent calcium-binding proteins (CaBP) of the gut and bone (bone glaprotein or osteocalcin).<sup>11</sup>

## Maternal vitamin D and mineral metabolism during normal pregnancy

### Requirements

In the human fetus chondrogenesis in the limbs starts at 32 - 34 days of gestation and intramembranous and endochondral ossification at 45 and 56 days' gestation respectively.<sup>43</sup> The skeleton of a normal full-term baby contains 25 - 30 g of calcium and the fetus is totally dependent on the mother for the supply of calcium, phosphate and vitamin D.<sup>44,45</sup> Calcium accretion by the fetus during the last trimester amounts to some 300 mg/d, i.e. 100 mg/kg fetal weight/d.<sup>46,47</sup> Certain maternal alterations are essential in order to supply sufficient minerals to the fetus for normal skeletal mineralization, without concomitant decalcification of the maternal skeleton.

The Joint Committee of the Food and Agricultural Organization of the United Nations and the World Health Organization<sup>46</sup> recommends that maternal calcium intake during the last trimester should be at least 1000 - 1200 mg/d. Limited information is available regarding the requirements for other minerals and vitamin D during pregnancy but one can assume that requirements will be higher than in the non-pregnant woman.

### Biochemical alterations

The total maternal serum calcium level decreases during pregnancy<sup>48-51</sup> and parallels a concomitant decrease in serum albumin level. The physiological hypo-albuminaemia of pregnancy thus appears to be largely if not entirely responsible for this decrease. Studies of the concentration of unbound calcium during pregnancy have produced contradictory results, with either decreased<sup>52</sup> or normal<sup>53</sup> maternal ionic calcium levels being reported. Calcium balance studies have shown increased intestinal absorption and decreased renal loss of calcium during

pregnancy in adolescent girls,<sup>54</sup> but a more recent study showed an increase in fasting urinary calcium to creatinine ratio.<sup>55</sup> Bone turnover is increased during pregnancy but with an adequate diet little bone mineral is apparently lost.<sup>54</sup> Bone mass measurements in pregnant females have yielded contradictory results, some investigators finding no change in bone density<sup>56</sup> and others finding a decrease in trabecular but not in cortical bone.<sup>57</sup>

Most studies have documented lower serum magnesium and phosphorus levels during pregnancy<sup>48,51,53</sup> although normal serum phosphorus concentrations during pregnancy have recently been described.<sup>50</sup> Total serum alkaline phosphatase levels rise throughout pregnancy and are significantly higher at term than in non-pregnant controls.<sup>46,48,50</sup>

At present there is conflicting evidence regarding PTH levels in pregnancy. Initial studies showed an increase in serum PTH levels during the last trimester.<sup>46,53,58</sup> This 'physiological hyperparathyroidism' was assumed to occur in response to the demands of the growing fetal skeleton on maternal calcium stores, but no correlation could be demonstrated between maternal serum calcium and PTH levels in pregnancy.<sup>58</sup> More recent studies, however, could not confirm the pregnancy-associated increase in serum PTH.<sup>49,55,59,60</sup> At present it is thus unclear whether a 'physiological hyperparathyroidism' exists during pregnancy; if so, its biological basis remains equally obscure.

Maternal serum CT levels in pregnancy have been found to be significantly increased in most<sup>60-62</sup> although not all<sup>53</sup> studies. It has been suggested that the raised CT levels may protect the maternal skeleton from excessive resorption due to increased levels of PTH and 1,25(OH)<sub>2</sub>D, yet allow these two calcium mobilizing substances to act on the intestine and kidney to provide the extra calcium needed for fetal skeletal development.<sup>62</sup>

There is a considerable variation in maternal serum 25-OHD that does not bear any relation to the duration of pregnancy. If dietary vitamin D intake and sunlight exposure are taken into account, serum levels of 25-OHD during pregnancy do not seem to differ from that of the non-pregnant state, and show the same seasonal variation.<sup>49,50,59,60,63,64</sup>

Serum levels of 1,25(OH)<sub>2</sub>D in pregnant women are higher than in non-pregnant women.<sup>49,59,60,65,66</sup> The stimulus for the increase in 1,25(OH)<sub>2</sub>D during pregnancy is, however, still unknown. It seems to be unrelated to serum PTH and is noted before any rise in circulatory PTH levels can be demonstrated. It has been postulated that the increase in serum 1,25(OH)<sub>2</sub>D level is secondary to a rise in DBP, similar to that of other steroid hormones in pregnancy, and that the free 1,25(OH)<sub>2</sub>D remains normal until late in pregnancy.<sup>67</sup> Serum 1,25(OH)<sub>2</sub>D levels are high in patients with prolactinomas and acromegaly,<sup>68</sup> and a possible explanation for the increased serum 1,25(OH)<sub>2</sub>D in pregnant women could thus involve the pituitary and/or placental hormones. A direct correlation between the 1,25(OH)<sub>2</sub>D levels in pregnancy and serum prolactin, human placental lactogen, which has growth hormone-like effects, oestrogen and calcium could, however, not be demonstrated, although an inverse relationship was found between serum 1,25(OH)<sub>2</sub>D and serum phosphorus.<sup>49</sup> It has recently been shown that human placental tissue can synthesize 1,25(OH)<sub>2</sub>D *in vitro*.<sup>22,23</sup> The high serum 1,25(OH)<sub>2</sub>D levels in pregnancy may thus be due to placental production and need not involve known regulatory mechanisms. The significance of the high 1,25(OH)<sub>2</sub>D serum values during pregnancy is still undetermined, but almost certainly relates to the increased calcium absorption and stressed calcium homeostatic mechanisms seen in pregnancy.

The concentration of maternal circulating 24,25(OH)<sub>2</sub>D is not altered during pregnancy.<sup>49,60</sup> It has, however, been shown

that human placental cells can synthesize 24,25(OH)<sub>2</sub>D *in vitro*.<sup>23</sup>

## Vitamin D and mineral metabolism in the normal fetus and full-term neonate

Information available on fetal mineral metabolism has been mainly obtained indirectly from animal experiments, examination of amniotic fluid, and extrapolation from studies done after birth or abortion. Very little information can be obtained directly from human fetal experimentation and data are often contradictory and/or difficult to interpret.

### Fetal requirements

The fetus is completely dependent on the mother for its supply of calcium, phosphorus, magnesium and vitamin D. Requirements for calcium, phosphorus and magnesium increase throughout gestation. Most of the calcium is deposited in the fetal skeleton during the latter half of pregnancy and normal fetal skeletal mineralization during the last trimester requires calcium accretion of 140 - 400 mg/d or 100 mg/kg fetal mass/d.<sup>46,47</sup>

### Serum values

Umbilical cord (i.e. fetal) levels of total calcium are significantly higher than maternal total serum calcium levels at delivery.<sup>63,69-71</sup> This increase is entirely due to an increase in the unbound calcium fraction, the protein-bound calcium concentration in maternal and cord blood being identical.<sup>72</sup> After birth the neonate's total serum calcium level decreases for 3 - 4 days and then rises again.<sup>69,73</sup> The significance of this initial decrease in serum calcium level followed by an increase is still unclear. It has been speculated that this 'physiological hypocalcaemia' may be important for the activation of neonatal parathyroid function, while the subsequent rise in serum calcium level may promote active bone mineralization.<sup>74</sup> In the neonatal period the baby's calcium intake and absorption are dependent on the age of the baby, the type of feeding, as well as the availability of vitamin D. Calcium absorption improves as the baby grows older and is much better at 4 - 6 weeks of age than at 5 - 7 days.<sup>75</sup> Cow's milk-based formulas have a much higher calcium content than breast milk.<sup>76</sup> However, calcium absorption from breast milk is more effective than from cow's milk, and milk formulas containing medium chain triglycerides<sup>77</sup> and a low linoleate/stearate ratio similar to that of breast milk improve calcium absorption.<sup>78</sup>

Serum phosphorus levels are higher in cord than in maternal blood.<sup>63,69,71</sup> The serum phosphorus level rises even further after birth,<sup>69,70</sup> but the exact reason for this is still uncertain. It has been postulated that transient neonatal hypoparathyroidism with resultant low urinary phosphate excretion<sup>70</sup> and/or end-organ unresponsiveness to PTH<sup>79</sup> may play a role. Another reason for the high serum phosphorus level may be the high phosphorus content of cow's milk.<sup>76</sup> The significance of this hyperphosphataemia is unclear, but it is most probably important for adequate mineralization of actively growing bone in the infant.<sup>80</sup>

Determinations of PTH in cord blood have yielded contradictory results, probably due to different PTH assays employed. Decreased or undetectable PTH levels were found in studies where c-terminal or intact PTH were measured,<sup>60,70,71,73</sup> while PTH levels in cord serum were similar to that in maternal serum when an n-terminal assay was used.<sup>59</sup> After birth the neonatal parathyroid glands can respond adequately to a hypocalcaemic stimulus and in normal full-term babies the PTH

level rises in response to the lowering of serum calcium after birth.<sup>81</sup> The level of total serum alkaline phosphatase in the normal full-term baby is about 1.5 - 2 times normal adult values.<sup>82</sup>

Cord blood CT levels are significantly higher than circulating maternal CT at birth.<sup>60,61,71,73</sup> Moreover, arterial cord blood CT is higher than the venous blood concentration, suggesting fetal production of this hormone.<sup>61</sup> The CT concentration decreases to normal levels at the age of about 1 month.<sup>61</sup> The high neonatal CT may contribute towards suppression of serum calcium levels after birth.<sup>74</sup> The reason for the raised CT levels in the fetus and neonate is still unknown, although it is possible that CT may have a protective function against excessive bone resorption and may be important for normal skeletal mineralization during a period of active bone growth.<sup>61,74</sup>

The fetus is dependent on maternal supplies of vitamin D. There is a close correlation between maternal and cord blood levels of 25-OHD.<sup>60,71,83,84</sup> The cord blood level of 25-OHD comprises approximately 80% of a normal maternal level, 108% of a low maternal level, and 68% of a high maternal 25-OHD level.<sup>83</sup> The concentration of 25-OH in cord blood is independent of gestational age and birth weight, but shows the same seasonal variation as maternal 25-OHD.<sup>83</sup>

Cord blood levels of 1,25(OH)<sub>2</sub>D seem to be lower than maternal 1,25(OH)<sub>2</sub>D levels at birth, but no clear correlation between cord and maternal levels of this metabolite has been demonstrated.<sup>59,60,66,67,71</sup> Recently it has been shown that the 1,25(OH)<sub>2</sub>D level in the umbilical artery is higher than in the umbilical vein, suggesting that the fetus may participate in the production of 1,25(OH)<sub>2</sub>D.<sup>60,71</sup> The human placenta also contributes towards the synthesis of 1,25(OH)<sub>2</sub>D.<sup>22,23</sup> The explanation for low 1,25(OH)<sub>2</sub>D levels in the fetus in the face of fetal as well as placental production of this hormone is still unclear. It has been speculated that there is no need for high *in utero* concentrations of 1,25(OH)<sub>2</sub>D necessary for duodenal calcium absorption, because the fetus receives its calcium transplacentally.<sup>59</sup> Another possible explanation may involve the lack of potent antenatal stimuli for 1,25(OH)<sub>2</sub>D production due to the high calcium and phosphorus, and low circulating PTH levels in the fetus.<sup>66</sup> The role of gonadal steroids, placental lactogen, CT and prolactin in the regulation of fetal 1,25(OH)<sub>2</sub>D metabolism cannot, however, be disregarded and requires further study. Neonatal serum 1,25(OH)<sub>2</sub>D levels are higher than those of older children and adults,<sup>71,85,86</sup> probably in response to increased PTH secretion caused by the postnatal decrease in serum calcium levels.<sup>70,81</sup> Limited information on perinatal concentrations of 24,25(OH)<sub>2</sub>D is available. The cord blood values of this metabolite seem to be lower than maternal values, but whether there is a direct correlation with maternal 25-OHD levels is still uncertain.<sup>60,71,87</sup>

The exact biological functions of the vitamin D metabolites during fetal life still need clarification. Halloran *et al.*<sup>37</sup> suggest that 24,25(OH)<sub>2</sub>D has no effect on calcium-phosphorus homeostasis during early development, while other workers feel that 24,25(OH)<sub>2</sub>D on its own or in combination with 1,25(OH)<sub>2</sub>D is essential for normal bone mineralization in humans and animals.<sup>30,40,41</sup> Of interest is a study in rats indicating that 24,25(OH)<sub>2</sub>D is essential for epiphyseal bone growth while 1,25(OH)<sub>2</sub>D causes cell proliferation in long-bone diaphyses and intestinal mucosa.<sup>31</sup>

### Placental transfer

It is clear that many substances must be transported transplacentally to maintain adequate fetal mineral and skeletal homeostasis. Calcium ions are transferred across the placenta against a concentration gradient and the concentration of free

unbound calcium in cord blood is higher than in maternal blood.<sup>63,69</sup> It is unclear whether bidirectional fluxes of calcium across the placenta exist, or whether transport is entirely unidirectional from mother to fetus. The exact molecular mechanisms involved in placental calcium transport still need clarification. An intrinsic placental CaBP, dependent on vitamin D has been demonstrated in the rat and mouse.<sup>88,89</sup> At present no placental CaBP has yet been demonstrated in man. CaBPs occur only in the presence of specific receptors for 1,25(OH)<sub>2</sub>D and the human placenta has been shown to contain receptors for 1,25(OH)<sub>2</sub>D.<sup>11</sup> Calcium-dependent adenosine triphosphatase systems have been demonstrated in animal as well as human placental tissue,<sup>90-92</sup> and calcium ions have been localized in placental plasma membrane vesicles, especially the mitochondria within the fetal capillary endothelium. These findings further support the evidence of active placental calcium transport against a concentration gradient.<sup>93,94</sup> It is thus likely that a placental CaBP in man exists which is dependent, at least in part, on the maternal 1,25(OH)<sub>2</sub>D for the active uphill transport of calcium transplacentally to the fetus.

Phosphorus and magnesium also seem to be transported effectively across the placenta,<sup>63,69,84</sup> but the exact molecular mechanisms involved are still unclear. PTH and CT do not seem to cross the placenta.<sup>61,73</sup>

The fetus is totally dependent on maternal supplies of vitamin D but whether it is mainly the parent vitamin or some of its more active metabolites that preferentially cross the placenta is not clear. It has been shown that 1,25(OH)<sub>2</sub>D as well as 24,25(OH)<sub>2</sub>D are synthesized in the human placenta.<sup>22,23</sup> The exact importance of these and other vitamin D metabolites in early fetal development still has to be unravelled. Moreover, since awareness of clinically significant abnormalities of mineral and bone metabolism in the newborn is at present increasing, this complex and fascinating subject deserves much more in-depth study.

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