

## CURRENT TOPIC

## Mineral transport across the placenta

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We here review the transport of three minerals which have in common their role in bone mineralisation, their active transport across the placenta, and the rise in their placental transport rate in late gestation. The growing and differentiating human fetus is entirely dependent on its mother for the supply of nutrients and oxygen and removal of waste products. In mammals, through most of gestation this exchange process takes place across the placenta, a highly complex organ which also has other functions. Despite much research, the physiology of placental exchange and especially its control remains a poorly understood subject.

To follow the literature, it is important to have a basic understanding of the concepts and terminology involved, which in the case of calcium are shown schematically in fig 1.

By the end of normal human pregnancy the fetus acquires approximately 28 g calcium, 16 g phosphorus and 0.7 g magnesium, mostly during the third trimester (fig 2). Calcium, which is not only required for skeletal

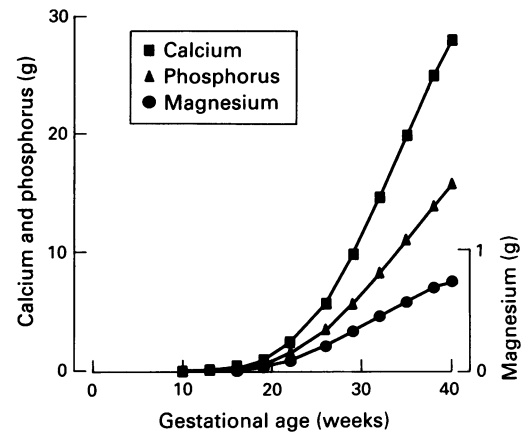


Figure 2 Total fetal content of calcium, phosphorus, and magnesium with increasing gestational age (adapted from Widdowson<sup>1</sup>).

mineralisation but also acts as a regulator for a variety of intracellular functions, has attracted most attention from research workers in this field. It is, therefore, given most emphasis here.

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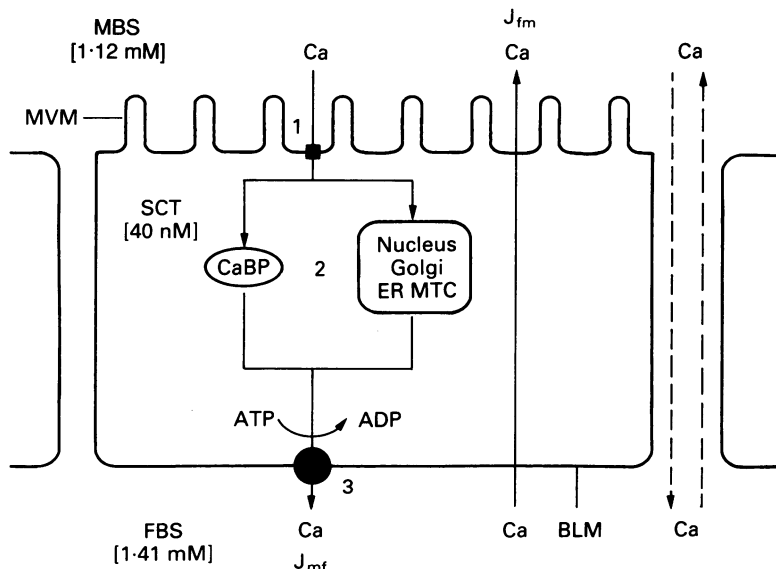


Figure 1 Schematic representation of transplacental movement of calcium. Bidirectional flux is shown with net flux across the cells.  $J_{net}$ —difference between unidirectional maternofetal flux ( $J_{mf}$ ) and unidirectional fetomaternal flux ( $J_{fm}$ ). Movement of calcium may also take place through hypothetical aqueous transplacental channels (which are often called paracellular channels by analogy with other barriers such as capillary endothelium but this term is not strictly correct for the placenta as the trophoblast is a syncytium), shown by the broken arrows. Approximate ionised calcium concentration is shown by [ ]; Ca, calcium; CaBP, calcium binding proteins; MBS, maternal blood space; MVM, microvillous membrane; SCT, syncytiotrophoblast; FBS, fetal blood space; BLM, basolateral membrane; ER, endoplasmic reticulum; MTC, mitochondria; ATP, adenosine triphosphate; ADP, adenosine diphosphate; ■, calcium transport protein; ●, Ca-ATPase; 1, 2, 3: see text. (Fetal capillary wall is not shown.)

### Calcium

Plasma calcium exists in three forms: that which is bound to proteins, mainly albumin (40%), that which forms complexes with ions such as bicarbonate (10%), and that which is 'free' or ionised (50%); ultrafilterable calcium comprises the latter two fractions. In all mammalian species studied so far the concentration of total and ultrafilterable or ionised calcium in fetal plasma is higher than in maternal plasma<sup>2</sup> (table) and, therefore, because net flux occurs against a concentration gradient, active transport mechanisms are likely to be involved. Support for this comes from animal placental perfusion studies<sup>4-6</sup> which have demonstrated maternofetal transfer of calcium against a concentration gradient by a process which is temperature dependent<sup>7</sup> and is inhibited by metabolic poisons such as dinitrophenol and cyanide.<sup>6</sup> Calcium is transported

Human maternal and fetal concentrations of calcium, magnesium, and phosphorus. Values are mean (SD) in mmol/l

	Mother	Fetus
Calcium	2.13 (0.15)	2.65 (0.19)
Ionised calcium	1.12 (0.06)	1.41 (0.09)
Magnesium	0.66 (0.16)	0.71 (0.16)
Phosphorus	1.43 (0.50)	1.92 (0.40)

Adapted from Schaubberger and Pitkin.<sup>3</sup>

across the placenta faster than substances such as mannitol which are likely to cross by passive diffusion alone through the trans-trophoblastic channels illustrated in fig 1.<sup>6</sup> This lends further support to the notion of active transcellular calcium transport.

Calcium transport across the placenta is bidirectional with marked species variation in the relative magnitudes of unidirectional maternofetal ( $J_{mf}$ ) and unidirectional fetomaternal ( $J_{fm}$ ) fluxes which are the determinants of net maternofetal flux ( $J_{net}$ ). Thus in the sheep, placental calcium transport is reported to be highly asymmetric with a  $J_{mf}$  of 212 mg/day/kg fetal weight and a  $J_{fm}$  of 12 mg/day/kg.<sup>8</sup> By contrast, in the rhesus monkey,  $J_{mf}$  and  $J_{fm}$  are said to be similar (390 mg/day/kg fetal weight and 325 mg/day/kg respectively).<sup>8</sup>

#### MECHANISMS OF CALCIUM TRANSPORT

Transcellular placental calcium transport must involve at least three steps (fig 1):

- (1) Influx of calcium ions from maternal plasma across the microvillous (maternal facing) trophoblastic membrane into the trophoblastic cytosol;
- (2) Movement of calcium through the cytosol without causing large fluctuations in the cytosolic ionised calcium concentration;
- (3) Efflux of calcium ions from cytosol across the basolateral (fetal facing) trophoblastic membrane and hence across the fetal capillary and endothelium into fetal plasma.

In other transporting epithelia the intracellular ionised calcium concentration is maintained at a low resting value of  $10^{-7}$ M in comparison to the extracellular concentration of  $10^{-3}$ M.<sup>9</sup> Recently it has been shown that trophoblast cytosolic ionised calcium concentration is  $4.2 \times 10^{-8}$  M.<sup>10</sup> It is thought that calcium enters the trophoblast as a charged ion<sup>11</sup> using a specific carrier in the microvillous membrane,<sup>12 13</sup> probably under the influence of the combined electrical and chemical ionised calcium concentration gradient across the microvillous membrane.

An efficient mechanism for cytosolic compartmentalisation and/or buffering of calcium ions in transit is likely to exist in all calcium transporting epithelia if cell viability is to be preserved. Likely candidates for this process are cytosolic organelles such as mitochondria, Golgi and endoplasmic reticulum,<sup>11 14 15</sup> and calcium binding proteins (CaBP). The latter have been identified in animal<sup>16 17</sup> and human<sup>18</sup> placentas. In the rat, the rapid increase in placental concentration of CaBP and its mRNA during the period of increased rate of fetal growth and calcium accumulation in late gestation suggests that this protein may be involved in placental calcium transport.<sup>16 19</sup> CaBP may enhance the diffusion of calcium through an aqueous compartment,<sup>20</sup> and it has been calculated that the presence of CaBP in enterocytes enhances cytosolic calcium diffusion by some 60 to 70-fold.<sup>21</sup> Thus, CaBP may act as an intracellular shuttle to facilitate

the transport of calcium through trophoblastic cytosol.

It is not known how the efflux of calcium ions out of the cytosol across the basolateral membrane of the placenta occurs. One possibility is that it is translocated by a calcium and magnesium dependent adenosine triphosphatase pump (Ca-ATPase) as this has been identified in animal<sup>22 23</sup> and human<sup>23 24</sup> placental homogenates. Immunohistochemical and fractionation techniques have localised its presence to the basolateral (fetal facing) membrane of the human placenta.<sup>23 25</sup> Among its activators are calmodulin<sup>25</sup> and, in other tissues, acidic phospholipids, long chain polyunsaturated fatty acids, and phosphatidylinositol.<sup>26</sup>

#### CONTROL OF PLACENTAL CALCIUM TRANSPORT

Factors which might effect the net maternofetal transfer of calcium include maternal and fetal placental blood flows, maternal and fetal calcium concentrations, together with the activity of placental transport mechanisms and factors which regulate them. As already mentioned, fetal accumulation of calcium occurs mainly in the last third of pregnancy and, in keeping with this, maternofetal transport of calcium in the rat increases some 70-fold over the last six days of gestation.<sup>27</sup> This is more than can be accounted for by an increase in functioning placental surface area or in blood flow. To account for the total fetal content of calcium in a normal full term infant, it has been calculated that 170 litres of maternal plasma would have to be cleared of its plasma calcium content.<sup>28</sup> With an estimated uterine blood flow of 500 ml/min at term,<sup>29</sup> the rate of calcium delivery to the placenta by the blood stream is unlikely to be directly rate-limiting in its maternofetal transfer. However, we have found that chronic reduction of uterine blood flow in the rat does result in decreased maternofetal transfer of calcium,<sup>30</sup> perhaps through an indirect link such as placental ischaemia resulting in a depletion of the energy supply necessary for active placental calcium transport.

Indirect evidence suggests that the maternal plasma calcium concentration may also influence placental calcium transport. Maternal dietary deficiency leading to a maternal calcium concentration as low as 1.0 mmol/l resulted in the birth of infants with rickets whose plasma calcium concentrations were around 1.6 mmol/l.<sup>31</sup> Poorly treated maternal hypoparathyroidism resulting in maternal hypocalcaemia can lead to transient congenital hyperparathyroidism<sup>32</sup>: it is postulated that under these conditions decreased net placental calcium transfer leads to fetal hypocalcaemia and it is this which in turn leads to fetal hyperparathyroidism. Conversely, transient neonatal hypoparathyroidism has been reported in infants born to hypercalcaemic mothers due to untreated hyperparathyroidism.<sup>33</sup> Thus, in humans, placental calcium transport may be dependent on maternal plasma calcium concentration. In animal studies divergent results have been

obtained, suggesting marked species variation. For example, in the rat, chronic maternal hypocalcaemia induced by maternal vitamin D deficiency and thyroparathyroidectomy did not alter the maternofetal calcium flux compared with sham operated animals.<sup>34</sup>

The placenta has no nerve supply and therefore the changing transport rate during gestation must be either an inbuilt effect of placental growth and differentiation or must result from changes in hormones (or other controlling factors) produced by the mother, fetus, or placenta itself. Calcium regulatory hormones include 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>), parathyroid hormone (PTH), calcitonin, and other peptides secreted by the parathyroid glands, for example, parathyroid hormone related protein (PTHrP).

There are reports of maternal vitamin D deficiency leading to neonatal rickets,<sup>35</sup> suggesting that maternal vitamin D might be necessary for placental calcium transfer. The presence of cytosolic receptors for 1,25(OH)<sub>2</sub>D<sub>3</sub> in human and rat placenta<sup>36</sup> and of CaBP (which is a molecular marker of the action of 1,25(OH)<sub>2</sub>D<sub>3</sub> in tissues such as intestine) in human<sup>18</sup> and rat<sup>16</sup> placenta appears to support the idea. Potential fetal sources of 1,25(OH)<sub>2</sub>D<sub>3</sub>, which may be important if control is from the fetal side, include that transferred from maternal plasma,<sup>37</sup> that synthesised from the fetal kidneys,<sup>38</sup> and that synthesised by the placenta itself.<sup>39</sup>

Direct study of the role of vitamin D on placental calcium transfer has demonstrated some species differences. In sheep, maternal treatment with 1 $\alpha$ -hydroxycholecalciferol<sup>40</sup> or prolactin<sup>41</sup> stimulated maternofetal transfer of calcium — the latter hormone could have exerted its effect on the placenta by increasing maternal 1,25(OH)<sub>2</sub>D<sub>3</sub> concentration. Fetal nephrectomy led to a marked decrease in fetal plasma 1,25(OH)<sub>2</sub>D<sub>3</sub> concentration with reversal of the usual maternofetal calcium gradient which was restored by infusion of 1,25(OH)<sub>2</sub>D<sub>3</sub> into the fetal circulation.<sup>42</sup>

By contrast, in rats, maternal vitamin D deficiency did not affect total fetal calcium content,<sup>43</sup> and there was no effect on the maternofetal calcium gradient after fetal nephrectomy.<sup>44</sup> However, on the fetal side of the placenta, 1,25(OH)<sub>2</sub>D<sub>3</sub> (whether injected subcutaneously into the fetus or perfused into the fetal side of the placental circulation) also had an effect in this species in that it stimulated maternofetal calcium flux across placentas of rat fetuses which had been previously parathyroidectomised by decapitation but not across placentas of intact fetuses.<sup>45</sup> Because PTH is an important trophic stimulator of 1,25(OH)<sub>2</sub>D<sub>3</sub> synthesis, it is likely that the parathyroidectomised fetuses had a lower plasma 1,25(OH)<sub>2</sub>D<sub>3</sub> concentration compared with intact fetuses. This suggests that this hormone only stimulates placental calcium transfer when its plasma concentration is low to start with.<sup>45</sup> As might be expected from the above, PTH (when injected subcutaneously into the fetus but not when perfused on the fetal side of the placental circulation)

stimulated placental calcium transfer across placentas of parathyroidectomised fetuses but not across placentas of intact fetuses.<sup>45</sup>

In 1985 it was reported that the concentration of PTH bioactivity was higher in fetal than maternal plasma whereas the converse was true of the immunoreactivity of PTH.<sup>46</sup> This led to the suggestion that the fetal parathyroids may secrete a second peptide which acts functionally, but not chemically, like PTH. It was later reported that addition of fetal parathyroid gland extract to autologous fetal blood used to perfuse placentas of thyroparathyroidectomised sheep fetuses in situ led to an increase in the rate of calcium accumulation in the fetal blood reservoir.<sup>47</sup> Using a similar experimental model it has been shown that addition of PTHrP (isolated from a human cancer cell line) but not bovine PTH (1–84) or rat PTH (1–34) resulted in an increase in the rate of calcium accumulation in the fetal blood reservoir.<sup>48</sup> These authors suggested that the second peptide produced by fetal parathyroid glands may be similar to the hypercalcaemic PTH-rP implicated in the humoral hypercalcaemia of some solid tumours.<sup>49</sup> However, because of technical difficulties precise quantitative data are difficult to obtain, and the conclusion that this oncofetal protein may have a part to play in the regulation of calcium transport by the placenta remains to be confirmed in other preparations and species. In the rat, two human PTHrP fragments (1–34 and 75–86), when perfused into the fetal circulation of the placenta, could not be demonstrated to have an effect on the maternofetal transfer of calcium.<sup>50</sup>

Calcitonin may also have a part to play in that, in chronic maternal calcitonin deficiency in sheep, induced by thyroidectomy with thyroxine replacement, a significant increase in total fetal calcium content was found, whereas calcitonin replacement led to normalisation of the fetal calcium content.<sup>51</sup>

### Phosphate

During the latter part of gestation, net maternofetal transfer of phosphate is against a concentration gradient (table). Active transport processes are therefore again likely to be involved. In kinetic studies of uptake of phosphate from the maternal or fetal circulations of perfused guinea pig placenta, uptake from the maternal circulation exceeded that from the fetal, implying a net maternofetal flux. Uptake was sodium dependent and was reduced by anoxic conditions or metabolic poisons.<sup>52 53</sup> In vitro studies of transport across human placental microvillous membrane has, interestingly, shown that phosphate transport is reduced by PTH<sup>54</sup> and is modulated by pH, temperature, and sodium and amino acid concentration.<sup>55 56</sup> Apart from this, little is known about the factors which regulate placental phosphate transport and indeed whether phosphate is the molecular form in which phosphorus is transported to the fetus.

### Magnesium

The fetal plasma concentration of total and ultrafilterable magnesium again exceeds that of maternal plasma<sup>57</sup> (table), and a magnesium pump must therefore be postulated. In support of this, in the rat unidirectional maternofetal transfer of magnesium is considerably higher than that of a passive diffusional marker, is reduced by the addition of cyanide, is temperature dependent,<sup>58</sup> and depends on the activity of a sodium/magnesium ion exchanger.<sup>59</sup> Interestingly, amiloride, an inhibitor of other sodium exchangers, has been demonstrated to reduce fetal accumulation of magnesium when injected into pregnant rats.<sup>60</sup>

There is no direct information on the regulation of placental magnesium transport, but an insight can be gained by observing the effects of therapeutic and experimental manipulations of maternal magnesium concentration on fetal magnesium concentration and content. In humans, treatment of pre-eclampsia with intravenous magnesium sulphate led to maternal and fetal hypermagnesaemia,<sup>61</sup> suggesting that either the 'magnesium pump' is not normally saturated and/or increased diffusion across the placenta. Again, there are differences between species: acute maternal hypermagnesaemia in the rat led to only a slight increase in fetal plasma magnesium concentration.<sup>62</sup> Chronic magnesium deficiency in the rat, however, resulted in decreased fetal plasma magnesium concentration and in total fetal magnesium content.<sup>57</sup>

### Conclusion

Placental transport of minerals and its regulation is a highly complex and largely ill understood phenomenon. Much of our knowledge remains fragmentary and there is a great deal that cannot be explained. For example, in relation to the three minerals discussed here, what are the precise placental mechanisms of transfer and are they controlled by maternal, fetal, or intrinsic placental factors or a combination of all three?; how do these factors interact with respect to maternal supply and fetal need?; and what 'switches on' the increased rate of transfer of these minerals in late gestation? These are just a few of the questions which need to be addressed before we achieve a fuller understanding of mineral transport across the placenta.

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