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Dietary iron absorption during early postnatal life

Sheridan L. Helman · Gregory J. Anderson · David M. Frazer

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Abstract Inadequate iron levels during early life can have adverse consequences for the developing infant. Iron deficiency during this critical period of growth can affect brain development and cognitive function, problems that can be lifelong despite subsequent correction of the iron deficit. Therefore, it is critical that the suckling infant has sufficient iron for their developmental needs. Much of the iron used in the immediate post-natal period is stored iron that was acquired from the mother in the final trimester of pregnancy, however, despite having low iron levels, breast milk can also make a significant contribution to infant iron needs. This reflects the ability of the suckling infant to absorb dietary iron far more efficiently than is possible after weaning. The mechanisms underlying this enhanced iron absorption are poorly understood. The iron export protein ferroportin is essential for this process, as it is in adults, however, the role of other molecules normally involved in iron absorption following weaning is less clear. The composition and distribution of iron in breast milk may be important, as could the contribution of more distal parts of the gastrointestinal tract. This review discusses the potential role of each of the above components in intestinal iron absorption during suckling and highlights the need for further research into this important process.

Keywords Breast milk · Iron absorption · Suckling · DMT1 · Hepcidin · Ferroportin

S. L. Helman · G. J. Anderson (✉) · D. M. Frazer
Iron Metabolism Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, QLD 4006, Australia
e-mail: greg.anderson@qimrberghofer.edu.au

S. L. Helman · G. J. Anderson · D. M. Frazer
School of Medicine, The University of Queensland, St Lucia, Australia

G. J. Anderson
School of Chemistry and Molecular Bioscience, The University of Queensland, St Lucia, Australia

D. M. Frazer
School of Biomedical Sciences, The Queensland University of Technology, Gardens Point, Australia

Introduction

Iron is an essential trace element that is vital for human health. The capacity of iron to switch between ferric and ferrous forms makes it crucial for many biochemical functions, such as energy production, DNA synthesis and oxygen transport (Anderson and Frazer 2017). However, iron in excess can catalyse the formation of reactive oxygen species which can damage lipid membranes and other cellular components. As the human body lacks a mechanism for the active excretion of iron, iron levels must be tightly

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regulated at the point of absorption in the small intestine to maintain an adequate supply and prevent tissue iron loading (McCance and Widdowson 1937).

Although iron is necessary at all stages of life, it is during the rapid growth and development that occurs in infancy that iron is of particular importance. Suboptimal iron levels at this time can have severe and sometimes irreversible implications for long term health (Lozoff et al. 2012; Lozoff and Georgieff 2006). Infancy is a critical period for neurological development and iron deficiency at this time can impair cognitive and psychomotor development and interfere with neurotransmitter function (Qasem and Friel 2015). Importantly, the neurological changes caused by iron deficiency during infancy are thought to be irreversible even after subsequent iron repletion (Beard et al. 2003; Tran et al. 2009), with one study finding that infants treated for iron deficiency had impaired cognitive processing, visual-motor and motor skills more than 10 years later (Lozoff et al. 2000). Iron deficiency can also cause growth retardation (lower weight and length for age) in infants and children, although, unlike many neurological effects, this can often be overcome by subsequent iron treatment (Aukett et al. 1986; Chwang et al. 1988; Mamiro et al. 2005). These studies highlight the importance of maintaining adequate iron levels during this crucial stage of development.

Iron balance during suckling

Healthy full-term infants born to mothers with adequate iron stores are endowed with sufficient iron to support their own growth and development for the first 4–6 months of life (Dewey and Chaparro 2007). In fact, such infants can have up to 45% more iron per kg than the average adult male (Rao and Georgieff 2007; Rios et al. 1975). Infant iron levels can be increased further by up to a third by delayed umbilical cord clamping, as blood continues to flow from the placenta to the infant for several minutes following delivery (Dewey and Chaparro 2007). Much of the newborn iron endowment is present as haemoglobin in the bloodstream (60%), with a further 25–30% present as storage iron in the liver (Domellof et al. 2014; Qasem and Friel 2015). Haemoglobin levels at birth are generally higher than at any other time of life as this allows a sufficient oxygen supply to be maintained in the relatively hypoxic environment found in utero (Dewey and Chaparro 2007). Haemoglobin levels decrease from approximately 170 g/L to 120 g/L in first 6 weeks of life, with the iron liberated from the breakdown of haemoglobin, in addition to liver iron stores, providing most of the iron required for growth and development until birth weight is doubled, usually at around 4 to 6 months of age (Domellof et al. 2014). Most of the iron stored in the liver at birth accumulates in the last 8 weeks of pregnancy (Bothwell 2000). For this reason, premature infants are at a much higher risk of developing iron deficiency and require supplementary iron in order to meet their developmental needs.

In addition to the iron endowment at birth, infants also gain iron from their diet, and in the first six months of life this usually consists of either breast milk or infant formula (or both). Human breast milk contains only low levels of iron (approximately 0.35 mg/L) (Lonnerdal 2017). However, the absorption of iron from breast milk is very efficient, with as much as 50% of breast milk iron being taken into the body in human infants (Lonnerdal 2017). In suckling rodents, the absorption of a test dose of iron can be as high as 100% (Anderson et al. 1991; Frazer et al. 2007; Gallagher et al. 1973). The proportion of iron absorbed decreases rapidly to around 10% following weaning in both humans and rodents (Frazer et al. 2007; Gallagher et al. 1973). This decrease occurs despite there being no significant change in iron stores, which are a major regulator of iron absorption in adults, suggesting that changes to iron requirements are not responsible (Anderson et al. 1991). Interestingly, only 10% of the iron in infant formula is absorbed, so infant formulae are generally supplemented with much higher levels of iron than those found in breast milk (Lonnerdal 2017). Whether the high iron absorption during suckling is due to specific components in breast milk or to a characteristic of the suckling intestine, or to a combination of the two, is yet to be determined.

Milk composition and iron absorption

Despite the potential importance of milk components in the absorption of iron by suckling infants, the form of iron in breast milk has not been well characterised. Early studies showed that the distribution of iron between the various milk fractions varied considerably between samples, with 15–46% being associated

Table 1 Concentrations of iron and iron binding proteins in breast milk from different species

	Human milk	Mouse milk	Rat milk
Iron concentration (whole milk)	0.36 μg/mL (Casey et al. 1995)	15 μg/mL (Casey et al. 1995)	3–6 μg/mL (Casey et al. 1995)
Lactoferrin concentration	1 mg/mL (Neville et al. 1998)	0.3 mg/mL (Neville et al. 1998)	–
Major iron binding protein	Lactoferrin (Masson and Heremans 1971)	Transferrin (Ward et al. 2003)	Transferrin (Masson and Heremans 1971)
Maximum possible lactoferrin saturation (%)	14% (Neville et al. 1998)	100% (Neville et al. 1998)	–

with milk fat, 18–56% bound to low molecular weight compounds and the remainder spread between the whey and casein protein fractions (Fransson and Lonnerdal 1980). Approximately 60% of the iron in the fat fraction is thought to be bound to xanthine oxidase, an enzyme that contains eight iron atoms (Fransson and Lonnerdal 1984). While the role of xanthine oxidase in breast milk is unclear, some have speculated that it may serve a nutritional purpose by transferring iron to the neonate (Fransson and Lonnerdal 1980; Keenan and Patton 1995). More recent studies have analysed the whey fraction of human milk and found that greater than three quarters of the iron in this fraction is associated with high molecular weight proteins (M_r above 160,000)(de la Flor St. Remy et al. 2004; Fernández-Sánchez et al. 2012). These corresponded mainly to IgA fractions in colostrum and to IgE and IgG peaks in mature milk although the relevance of this to iron absorption is not known.

The milk component that has received the most attention with respect to iron absorption during suckling is lactoferrin. This glycoprotein (M_r 80,000) is found in high concentrations in human breast milk and shares homology with the serum iron binding protein transferrin (Ward et al. 2003). The high affinity of lactoferrin for iron and the discovery of a highly expressed lactoferrin receptor in the suckling gut (Kawakami and Lonnerdal 1991) have prompted many to propose a role for lactoferrin in neonatal iron uptake (Brock 2012). Indeed, several studies have shown that supplementing pregnant women with iron bound to bovine lactoferrin is at least as effective as ferrous sulfate at treating anaemia, suggesting that it enhances intestinal iron uptake (Paesano et al. 2006). However, the amount of iron given to the lactoferrin treatment groups was far too low to produce any significant change in iron status (approximately 80 μg iron/dose) and it is now thought that the anti-inflammatory characteristics of lactoferrin are largely responsible for the observed increase in haemoglobin levels. In fact, there are several lines of evidence that argue against a direct role of lactoferrin in the efficient absorption of iron during suckling. Firstly, high iron absorption during suckling has been observed in both humans and rodents, yet the predominant iron binding protein in rodent breast milk is transferrin, not lactoferrin (Table 1) (Masson and Heremans 1971; Ward et al. 2003). Iron levels are also much higher in rodent breast milk (Casey et al. 1995). As a result, there is only enough lactoferrin to bind a small proportion of the iron, making it unlikely that lactoferrin mediates the efficient absorption seen. Secondly, studies examining iron absorption in suckling rodents are typically performed in the absence of lactoferrin (Darshan et al. 2011; Frazer et al. 2007; Frazer et al. 2017), demonstrating that lactoferrin is not essential for the efficient absorption of iron at this time. Finally, studies in knockout mice show that pups lacking lactoferrin born to mothers also lacking lactoferrin exhibit normal iron status markers when examined late in the suckling period (Ward et al. 2003). While these arguments do not exclude a role for lactoferrin in iron absorption from human milk, older studies showed that bovine lactoferrin did not enhance iron absorption in human infants (Fairweather-Tait et al. 1987; Schulz-Lell et al. 1991). In addition, a study by Davidsson et al. (1995) found that removing endogenous lactoferrin from human milk increased iron absorption by infants, implying that lactoferrin may actually interfere with iron uptake. Clearly more research is necessary to define the role of lactoferrin

and other milk components in the efficient iron absorption from breast milk.

The adult iron absorption machinery and the suckling intestine

While milk factors may play some role in the efficient absorption of breast milk iron, studies in rodents suggest that inherent characteristics of the suckling intestine are more likely to be responsible, as radiolabelled ferrous iron is highly absorbed in suckling mice and rats in the absence of breast milk (Darshan et al. 2011; Frazer et al. 2007; Gallagher et al. 1973). In addition, the premature induction of intestinal maturation by corticosteroid treatment has been shown to decrease iron absorption in suckling rodents independent of changes in breast milk consumption (Darshan et al. 2011; Gallagher et al. 1973). Precisely what characteristics of the immature intestine contribute to the high absorption of iron from breast milk, and whether this process involves components of the adult iron absorption machinery, remains largely unclear.

Dietary iron absorption in adults is a tightly regulated process which occurs predominantly in the upper small intestine (the duodenum and upper jejunum) (Anderson and Frazer 2017). In contrast to iron uptake during suckling, the molecular basis of adult non-haem iron absorption is relatively well understood (Fig. 1), although the processes involved in the uptake of iron from dietary haem remain unclear. However, as haem iron is normally absent from breast milk (Collard 2009), this aspect of iron absorption will not be discussed further. Dietary non-haem iron is present mainly as ferric iron and must be reduced to ferrous iron in the lumen of the gut prior to absorption, a process carried out by poorly characterised intestinal reductases (McKie et al. 2002). Following this reduction step, ferrous iron is taken up by villus enterocytes, a process mediated by the integral membrane protein divalent metal-ion transporter 1 (DMT1) located on the apical brush border membrane (Gunshin et al. 2005). Once inside the cell, and depending on the body's needs, iron can either be sequestered within the intracellular iron storage protein ferritin, or transported across the basolateral membrane and into the body via the iron export protein ferroportin (Anderson and Frazer 2017), which works in conjunction with the copper-dependent ferroxidase hephaestin (Vulpe et al. 1999). In adults, the basolateral release of iron is controlled at the systemic level by the liver-derived hormone hepcidin (Nemeth et al. 2004). This 25 amino acid peptide is secreted into the circulation and binds to ferroportin on the basolateral membrane of enterocytes, causing the internalisation and subsequent degradation of the protein complex, and thereby reducing dietary iron absorption (Nicolas et al. 2002). The production of hepcidin by the liver is regulated by the body's iron requirements (Anderson and Frazer 2017). An increased need for iron inhibits hepcidin production, allowing ferroportin to be stabilised on the enterocyte basolateral membrane and more iron to be absorbed from the diet. A reduction in iron requirements has the opposite effect, with hepcidin increasing to reduce ferroportin levels and iron absorption.

While brush border DMT1 is essential for adult iron absorption (Fleming et al. 1997; Gunshin et al. 1997), the role of DMT1 in the efficient uptake of dietary iron during suckling is less clear. We have previously shown that *Dmt1* mRNA levels are increased in isolated enterocytes from suckling rats when compared to weaned animals (Frazer et al. 2007). However, a study by Leong et al. (2003), also in rats, showed little change in *Dmt1* message levels throughout this period. The reason for this difference is unclear. Unfortunately, DMT1 protein levels have not been directly compared in the intestine of suckling and weaned infants, making it difficult to discern whether the iron transporter contributes to the high iron absorption from breast milk. In fact, there are conflicting reports in the literature regarding whether DMT1 plays any role at all in dietary iron absorption during suckling. In a study using Belgrade rats, which have a mutation in the *Dmt1* gene that renders the protein product non-functional, Thompson et al. (2007) showed that there was no change in the accumulation of iron from the mother in both wild-type and mutant pups cross fostered onto unaffected mothers. They also showed that, unlike weaned animals, DMT1 was localised to the cytoplasm rather than the brush border during suckling and was not detectable in membrane fractions isolated from suckling enterocytes. This agrees with previously reported results from Lopez et al. (2006) and suggests that DMT1 is not required for the absorption of iron from breast milk. However, a more recent study by

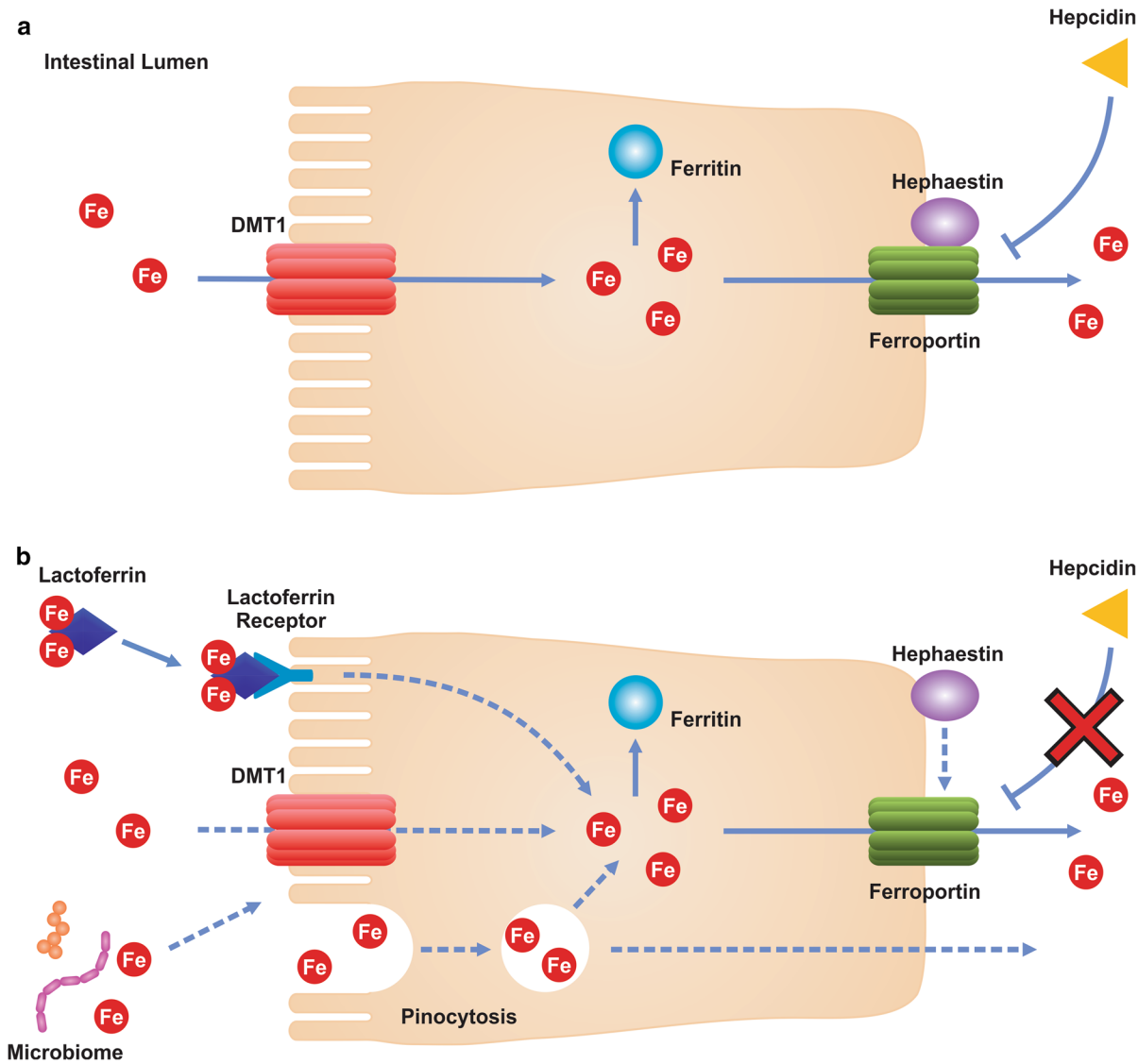

Fig. 1 Mechanisms of dietary non-haem iron absorption in suckling and weaned animals. In the intestine of weaned animals (**a**), dietary iron is transported across the brush border membrane and into the enterocyte by the iron transporter DMT1. Once inside the cell, the iron can either be sequestered within the intracellular iron storage protein ferritin or exported from the cell and into the circulation via the iron export protein ferroportin, a process made more efficient by the ferroxidase hephaestin. Basolateral iron transfer is regulated by the liver-derived peptide hormone hepcidin which binds ferroportin, causing it to be internalised and degraded, thereby inhibiting the efflux of iron from enterocytes. In the suckling infant (**b**), ferroportin remains essential for iron absorption, however, the roles of DMT1 and hephaestin are less clear. Ferroportin is also hyporesponsive to circulating hepcidin. Other pathways that may contribute to iron absorption during suckling include lactoferrin/lactoferrin receptor mediated iron uptake, pinocytosis and the influence of the microbiome. Solid lines represent confirmed pathways. Dashed lines represent proposed pathways that are yet to be confirmed

(Ramakrishnan et al. 2015) found that mouse pups specifically lacking DMT1 in intestine enterocytes developed anaemia by the time of weaning, implying that DMT1 is essential for iron absorption in suckling mice. In neither of these studies was intestinal absorption directly measured, so the precise role of DMT1 in the absorption of iron during suckling requires further investigation.

In contrast to the brush border uptake step, studies using knockout mice have demonstrated a clear role

for ferroportin in the basolateral export of iron from enterocytes during suckling. Initial observations showed that mice with intestine-specific ferroportin deletion became visibly pale prior to weaning, implying that they were developing iron deficiency anaemia due to a reduction in the absorption of iron from breast milk (Donovan et al. 2005). We confirmed this and showed that suckling mice with intestine-specific deletion of ferroportin absorbed 10% of a test dose of iron whereas wild-type littermates absorbed 73% under similar conditions (Frazer et al. 2017). This indicates that the highly efficient absorption of iron during suckling is predominantly dependent on ferroportin-mediated iron transport. Interestingly, we observed no change in ferroportin protein levels in enterocytes isolated from suckling and weaned wild-type mice despite an 11-fold difference in iron absorption (Frazer et al. 2017). This is unexpected as our results clearly showed that ferroportin was responsible for most of the iron absorption that occurred at either developmental time point. While the reason for this is unclear, it suggests that ferroportin-mediated iron transfer can be upregulated independently of ferroportin protein levels in the suckling intestine.

While a role for ferroportin in the absorption of breast milk iron is clear, this is not the case for hephaestin. An early published abstract stated that suckling mice with disrupted hephaestin were able to absorb similar amounts of a test dose of iron as wild-type mice (Bannerman et al. 1973), although this result was never published as a full paper. However, this abstract is supported by a more recent study (Fuqua et al. 2018) showing high absorption of radioactive iron in 11 day old hephaestin knockout mice, suggesting that either a ferroxidase is not necessary for efficient iron absorption during suckling, or that an alternative source of ferroxidase activity is available at this time.

As ferroportin is responsible for the high iron absorption during suckling, it is not surprising that the production of hepcidin, an inhibitor of ferroportin-mediated iron release, is very low at this time, and increases at weaning when absorption decreases to adult levels (Frazer et al. 2007). However, we have demonstrated in both rats and mice that, even if hepcidin levels are increased during suckling, there is little or no decrease in absorption (Darshan et al. 2011; Frazer et al. 2007). This agrees with earlier studies suggesting that increasing iron status in suckling humans and rodents has no effect on iron absorption (Gallagher et al. 1973). While the precise mechanism for this lack of response is unclear, we have shown that an increase in hepcidin expression does not lead to a reduction in ferroportin protein levels in the intestine of suckling mice as it does in adults (Frazer et al. 2017). This is not likely to be due to a modification of the hepcidin peptide that renders it non-functional, as we observed a reduction in serum iron levels in mice overexpressing hepcidin, despite iron absorption remaining high. This suggests that hepcidin was able to restrict iron release from other body tissues in these animals and implies that the lack of hepcidin responsiveness is restricted to the suckling intestine. Interestingly, ferroportin protein isolated from suckling mouse enterocytes has a lower molecular weight than that of weaned animals (Frazer et al. 2017). This raises the possibility that enterocyte ferroportin is modified during suckling to prevent hepcidin binding, allowing the efficient iron absorption that occurs at this time to continue irrespective of any increase in circulating hepcidin.

Why such a mechanism to specifically inhibit the action of hepcidin in the suckling intestine evolved is unclear. The demand for iron is high shortly after birth and so constitutively high dietary absorption may provide a degree of protection against iron deficiency, particularly if hepcidin production increases. Hepcidin expression is most commonly elevated by an increase in iron stores or by infection/inflammation (Nicolas et al. 2002). The former is unlikely, but the latter less so. The increased hepcidin levels associated with inflammation/infection likely evolved as a host defence mechanism to reduce iron release from host cells and thus restrict the iron available to invading pathogens (Ganz and Nemeth 2015). Limiting the action of hepcidin in the intestine during suckling may allow the infant to continue to absorb dietary iron during inflammation so that body iron status is not compromised during this important developmental stage of life.

The role of the distal gastrointestinal tract in iron absorption in early postnatal life

The involvement of specific regions of the gastrointestinal tract may also contribute to the efficient

absorption of dietary iron in suckling mammals. Iron absorption occurs predominantly in the duodenum and upper jejunum of the adult intestine, however, we demonstrated that the distal small intestine and colon can also absorb significant amounts of iron during suckling in rats (Frazer et al. 2007). We also found that the expression of the iron transporters *Dmt1* and *ferroportin* was elevated in the distal gastrointestinal tract at this time. While the amount of iron absorbed in the distal small intestine and colon were not as high as in the duodenum, the greater surface area and generally slower transit time of these regions would significantly increase the amount of iron able to be absorbed. However, we have also shown in suckling mice that 94% of a dose of ferrous sulfate is absorbed in the first half of the small intestine, implying that it is the upper gastrointestinal tract that is responsible for the efficient iron absorption during suckling (Frazer et al. 2017). While these results would appear to be contradictory at first, it is likely that both aspects play a role in suckling iron absorption as low molecular weight iron compounds would be rapidly absorbed in the duodenum, whereas iron bound to proteins and other milk components may require more time to be broken down with the released iron being efficiently absorbed in the distal small intestine and colon.

Other potential factors influencing iron absorption during suckling

There are other characteristics of the suckling intestine, not specific to iron, which may contribute to the efficient absorption from breast milk. One such trait is the capacity of the immature intestine to take up large molecules such as antibodies by pinocytosis (Clark 1959). The pinocytic ability of the intestine decreases with maturity and it has been suggested that the non-specific uptake of iron by this pathway may explain the elevated iron absorption during suckling (Ezekiel 1967). While data from our laboratory has shown that ferroportin is responsible for much of the iron absorption that occurs in the suckling mouse, pups lacking ferroportin in the intestine were still able to absorb approximately 10% of the iron administered (Frazer et al. 2017), and it is possible that non-specific pathways such as pinocytosis are responsible. Another potential factor contributing to the high iron absorption that occurs during suckling is the infant microbiome. A study of Indian children between the ages of one and four showed that supplementation with *Bifidobacterium* species reduced the incidence of iron deficiency, although the mechanism is unclear (Sazawal et al. 2010). As *Bifidobacterium* species are a predominant part of the intestinal microbiome of breast-fed infants (Fanaro et al. 2003), it is possible that these bacteria may somehow enhance iron absorption at this time.

Conclusion

Numerous studies have shown that iron is vital for infant development, with iron deficiency at this time linked to long term cognitive deficits (Lozoff et al. 2012; Lozoff and Georgieff 2006; Qasem and Friel 2015). While the molecules involved in the absorption of dietary iron and its regulation are reasonably well understood in adults, our knowledge of the mechanism responsible for the efficient absorption of dietary iron in suckling mammals remains incomplete. A role for ferroportin has been established (Frazer et al. 2017), at least in rodents, however, few other molecules have been conclusively shown to be involved in iron absorption during suckling. The relevance of rodent studies to the human infant is also unclear, as rodent breast milk contains much higher levels of iron (Casey et al. 1995), which raises the possibility that there might be fundamental differences in the way this iron is absorbed. More studies are required to better understand this important process in human infants, with the knowledge gained likely to have important implications for multiple aspects of infant nutrition including the prevention and treatment of iron deficiency and infant formula development.

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