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Commentary

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The Effects of Different Forms of Lactoferrin on Iron Absorption

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In this month's issue of *The Journal of Nutrition*, Mikulic et al. (1) present data on the effect of lactoferrin (LF) on iron absorption in young infants as "Iron absorption from apo-lactoferrin is greater and that from holo-lactoferrin is similar to that from ferrous sulfate: stable iron isotope studies in Kenyan infants." Because holo-LF does not contain any iron, a more apt title could have been "Added apo-lactoferrin increases iron absorption from ferrous sulfate, and iron absorption from holo-lactoferrin is similar to that from ferrous sulfate in maize porridge consumed by Kenyan infants." In any case, the authors have added to the existing literature examining the potential role of LF in iron homeostasis, a body of literature that has been somewhat confused and contradictory.

LF is a 70-kDa glycoprotein member of the transferrin family. It can bind 2 ferric (Fe^{3+}) ions with very high affinity, and as a result changes from an open apo-LF configuration to a closed holo-LF configuration (2). LF is present in many bodily secretions including saliva and tears, but its highest concentration is in human milk (2) where it can be as high as 7 g/L in colostrum, 5 g/L in early human milk, and 1–2 g/L in mature milk (2, 3). Concentrations of LF are typically far lower in bovine milk (4). Apo-LF and holo-LF have numerous roles in the gastrointestinal tract, including direct antimicrobial effects, as an immunomodulator, a stimulator of cell proliferation, and as a probiotic bifidogenic agent impacting the gut microbiome (2, 4, 5). LF's ability to bind iron and make it unavailable to gut bacteria explains some of its antimicrobial functions, but it also has numerous other antimicrobial actions (2, 4, 5).

Because it is an avid binder of iron, LF's possible role in iron absorption has long been debated. Much of the iron in present in human milk is bound to LF, but because of the relatively high abundance of LF and the low iron content of human milk, as little as 3% of LF can bind iron in vivo (5). LF is susceptible to digestion in the stomach, but some can survive passage through the stomach in adults (6) and even more is likely to do so in infants because of their higher gastric pH (7). The iron-binding, closed, holo-LF form is particularly resistant to gastric digestion (5). In adults, little LF seems to enter the ileum intact (8), but in infants 4–9% of ingested LF can be excreted in the feces (9). A specific LF receptor is present in the small intestine (10) that can bind and internalize both apo-LF and holo-LF, as well as bovine LF (11). This mechanism has been used to explain the high absorption of iron from human milk (7), although clear experimental evidence for this is mixed.

Several studies have examined the absorption of stable isotopes or radioisotopes of iron bound to LF (12, 13). Fairweather-Tait et al. (12) showed that iron-59 bound to LF was as well absorbed by infants from a low-iron formula as was ferric chloride given with ascorbic acid. That study (12) is often interpreted as showing that LF has no effect on iron absorption (1). The truth is more nuanced. Fairweather-Tait et al. compared iron absorption from holo-LF saturated in vitro with an iron isotope with iron absorption from isotopically labeled ferric chloride given with ascorbic acid. Iron absorption was variable, but similar from LF and from ferric chloride (12). This doesn't mean that LF has no effect on nonheme iron absorption, rather it shows that LF iron is as well absorbed as other forms of nonheme iron. To assess the effect of LF on nonheme iron absorption, iron absorption from nonheme iron would need to be measured in the presence and absence of LF, as Mikulic et al. do in the present study (1). In a similar design to that of Fairweather-Tait et al., Lönnerdal and Bryant (13) demonstrated that LF labeled with iron-58 was as well absorbed by adults from rice as iron from ferrous sulfate. Again, this shows that LF-bound iron is as well absorbed as other forms of nonheme iron, but does not address the effect on LF on nonheme iron absorption.

The effects of adding LF to formula or to human milk have also been studied. Addition of LF to formulas with low iron content appears to have little impact on iron status (14), although if formulas have high iron contents then LF may improve growth and iron status (15). Conversely, removal of LF from human milk (with low iron content) may paradoxically *improve*iron absorption (16).

In the face of the uncertainty about LF's role in nonheme iron absorption, Mikulic et al. set out to measure iron absorption in infants from a porridge with or without added bovine LF (1). In 4-mo-old Kenyan infants they measured iron absorption using established stable isotope methods from 3 porridge-based meals. The meals contained similar amounts of iron, but the iron was present in several different forms: as ferrous sulfate, as ferrous sulfate with the addition of iron-free apo-LF, or as extrinsically iron-containing labeled holo-LF. Consistent with

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the isotope studies of Fairweather-Tait et al. and Lönnerdal and Bryant (12, 13) they were able to demonstrate absorption of iron given as LF. The absorption of iron as holo-LF (5.0%) was similar to that from ferrous sulfate (6.3%). Uniquely, they were also able to assess the absorption of ferrous sulfate from the test meal, with or without the addition of iron-free apo-LF. This is the experiment that was missing in previous studies of iron absorption from LF (12, 13). Apo-LF significantly increased the absorption of iron as ferrous sulfate, from 6.3% to 9.8% (1). The results are consistent with apo-LF binding some of the iron in the ferrous sulfate and making it available for absorption by the LF receptor-mediated pathway, in addition to the usual nonheme iron absorptive pathway. However, whether that chelation of labeled iron by apo-LF occurred in the subjects' gut or in the test meal prior to consumption is unclear.

The authors' results are intriguing, but they do not necessarily mean that LF has a significant effect on iron absorption in human milk–fed infants. As noted previously, very little of the LF present in human milk contains iron, and it would seem more likely that LF is acting to sequester iron and prevent its use by gut pathogens, rather than making a significant contribution to iron nutrition of the infant (7). LF content of human milk also falls with advancing lactation (2, 3) although the risk of iron deficiency is increasing.

However, this doesn't mean that the potential role of LF in iron absorption cannot be exploited. The authors suggest one potential area: in infants in developing countries. This is clearly worthy of further examination because such populations are at high risk of both iron deficiency and infectious morbidities, and there is preliminary evidence that LF can be of benefit in both areas. For example, there are data from small studies that an LF-supplemented formula leads to reduced lower respiratory tract infections in healthy infants (17, 18), as well as improved iron status (17) and reduced gastrointestinal illness (19).

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