

## Invited commentary

### Human milk oligosaccharides: 130 reasons to breast-feed

Many nutrition scientists are not yet aware that human milk contains significant quantities of carbohydrates other than lactose. If you've never heard of lacto-*N*-tetraose or sialyl-lactose, you are not alone. The role of oligosaccharides in other fields has attracted widespread excitement but curiously little interest has been shown in the oligosaccharides of human milk. Many of the 130 different human milk oligosaccharides may be important in infant brain development and resistance to infection. Their presence may be one of the reasons that breast-fed infants appear to have higher intelligence quotients and greater resistance to infection than formula-fed infants.

Quantitatively, oligosaccharides are the third largest solute in human milk after lactose and fat. Mature milk contains over 15 g human milk oligosaccharides/l compared with only about 9 g protein/l (Coppa *et al.* 1993). The oligosaccharide content of human milk varies with the duration of lactation, diurnally and with the genetic makeup of the mother (Viverge *et al.* 1990; Coppa *et al.* 1993). Oligosaccharides vary greatly, both within and between individuals, more so than any other component of human milk. Marsupial and monotreme milks are also known to contain significant amounts of oligosaccharides (Messer & Kerry, 1973; Messer & Mossop, 1977) but cows' milk and infant formulas contain very little (Neeser *et al.* 1991). We used to say that the structural complexity of human milk oligosaccharides was unique. However, in this month's issue of the *British Journal of Nutrition*, we learn that elephant milk and human milk have much in common (Kunz *et al.* 1999). It may not be coincidental that elephants secrete large quantities of oligosaccharides containing sialic acid in their milk and are said to have a memory 'that never forgets' (more on this later).

Over the past two decades the chemical structures of human milk oligosaccharides have been studied using NMR spectroscopy and mass spectrometry (Stahl *et al.* 1994). Each individual oligosaccharide is based on a variable combination of glucose, galactose, sialic acid (also known as *N*-acetylneuraminic acid), fucose and/or *N*-acetylglucosamine with many and varied linkages between them, thus accounting for the enormous number of different oligosaccharides in human milk (at least 130, and still counting). Almost all of them have a lactose moiety at their reducing end while fucose and sialic acid (when present) occur at the non-reducing end (Kobata *et al.* 1978).

The large quantity of sialylated oligosaccharides in both human and elephant milk is fascinating. Sialic acid is a nine-C sugar that is a vital structural and functional component of brain gangliosides. It is thought to play an essential role in nerve cell transmission, memory formation and cell-to-cell communication (Rosenberg, 1995). Most

importantly, studies in rat pups indicate that early supplementation with sialic acid improves both brain ganglioside sialic acid and learning ability in well-nourished and malnourished animals, and that these changes persist into adulthood (Morgan & Winick, 1980*a,b*). Human milk contains large numbers of sialylated oligosaccharides that are not found in significant amounts in cows' milk or infant formulas. The immature liver may not be capable of synthesizing all the sialic acid needed by the infant during this peak period of brain growth.

Over the last few years we have explored the hypothesis that sialic acid accretion may be enhanced by breast-feeding. Apart from brain gangliosides, saliva is a rich source of sialic acid and, unlike blood or brain tissue, can be obtained non-invasively from young infants. The high concentration of sialic acid in saliva and other mucins is responsible for their slippery, viscous nature and thus their protective function. In our recent study, breast-fed infants were found to have nearly 50% more total sialic acid in saliva compared with formula-fed infants (Tram *et al.* 1997). It is possible that the sialic acid derived from human milk contributes to higher levels of sialic acid in saliva.

One of the important questions is whether human milk oligosaccharides are digested and absorbed in the small intestine, providing energy and important molecules such as galactose, fucose and sialic acid. The marsupial small-intestinal mucosa has several enzymes that split oligosaccharides into their component monosaccharides. This makes oligosaccharides nutritionally available to the animal in a form that is less osmotically active than if lactose or monosaccharides were the only carbohydrates. Human milk oligosaccharides may be a similar low-osmolar source of energy for the infant. However, the glycosidic linkages between the monosaccharide residues of human milk oligosaccharides are unlikely to be hydrolysed by lactase and other known brush-border disaccharidases. These enzymes have no action on linkages involving fucose, sialic acid or *N*-acetylglucosamine (Rings *et al.* 1994). Furthermore, the brush-border neutral lactase cannot hydrolyse the lactose moiety of trisaccharides or higher oligosaccharides.

Recently, we demonstrated using breath H<sub>2</sub> methodology that human milk oligosaccharides resist digestion in the small intestine of the majority of breast-fed infants and undergo fermentation in the colon (Brand Miller *et al.* 1998). We found that the peak rise and overall production of breath H<sub>2</sub> after a human milk oligosaccharide load was similar to that after an equivalent load of lactulose, in six of eight infants studied. The implication is that human milk oligosaccharides are not digested and absorbed in the small intestine but are readily fermented in the large intestine.

Human milk oligosaccharides, not lactose, may therefore be the normal source of breath  $H_2$  in breast-fed infants. Interestingly, the human milk oligosaccharides challenge did not produce excessive bowel movements or any changes in stool consistency which might normally be associated with non-absorbable oligosaccharides.

If human milk oligosaccharides resist digestion in the small intestine, they become the major source of C and energy for the large-bowel flora. In fact, human milk oligosaccharides containing *N*-acetylglucosamine (the 'bifidus factor') are necessary for the growth of *Bifidobacterium bifidum* in the large bowel. These oligosaccharides form precursors in the biosynthesis of muramic acid, a component of the bacterial cell wall. By the end of the first week of life Bifidobacteria represent 95% of the total bacterial population in the faeces of exclusively breast-fed infants, whereas in formula-fed infants they form less than 70% (Yoshioka *et al.* 1983). By producing lactic acid, this organism decreases the intestinal pH. The acid milieu inhibits the proliferation of many pathogenic microorganisms such as *Shigella* species, *Escherichia coli*, *Streptococcus faecalis* and *Clostridium* species which become predominant after weaning from breast milk (Montreuil, 1994).

Short-chain fatty acids such as acetic, propionic and butyric acids produced by fermentation are absorbed across the large-bowel wall, providing nutrition for the colonocyte and a source of energy to the body. As much as 70% of the energy in the carbohydrate can be 'salvaged' by this mechanism (Cummings, 1983). Thus, most of the energy in the human milk oligosaccharides may eventually become available to the infant.

If human milk oligosaccharides are not digestible in the small intestine, then how might molecules such as sialic acid be able to support brain growth? Sialic acid always occupies the terminal position of oligosaccharides, and the bond may be cleaved even if the remainder of the chain resists digestion. Cleavage of sialic acid is conceivable because sialidase activity in several species is highest during the sucking period and positively correlated to the sialic acid content of the milk (Dickson & Messer, 1978). Rat intestinal cell walls are highly permeable to free sialic acid as well as sialyl-lactose. Radioactively labelled forms of sialic acid and sialyl-lactose were found to be well absorbed (up to 90%) by rat pups, 30% being retained in the body and 3–4% in the brain after 6 h (Witt *et al.* 1999).

It is also possible that smaller oligosaccharides can cross the enterocyte cell wall. In infant marsupials this has been postulated to occur via pinocytosis (Crisp *et al.* 1987). Very small quantities of human milk oligosaccharides have been documented in the urine of breast-fed infants (Crisp *et al.* 1987) but they have also been found in the faeces of full-term and preterm breast-fed infants (Sabharwal *et al.* 1988; Lundblad, 1993). Rudloff *et al.* (1996) showed that in preterm infants, the urine of breast-fed infants contained oligosaccharides, typical of human milk, equivalent to 1% of the daily intake. In contrast, the urine of formula-fed infants contained only minute quantities of these substances. This suggests that intestinal absorption of intact oligosaccharides occurs. The presence of certain oligosaccharides in the urinary tract may be responsible for the lower incidence

of urinary tract infections in breast-fed infants (Coppa *et al.* 1990).

Mounting evidence suggests that human milk oligosaccharides play important anti-infective roles in the intestinal, respiratory and urinary tracts (Newburg, 1999). There is a growing list of pathogens for which a specific oligosaccharide ligand has been described in human milk. The oligosaccharides prevent microbial cells from adhering to the epithelial cell (a prerequisite for infection) because they act as 'decoys', i.e. they are structural analogues of the host receptors. This action, however, depends on the oligosaccharides reaching their target organs (small intestine, respiratory tract, urinary tract) without being broken down. Thus, the ability to resist digestion at least partially in the small intestine may be absolutely critical to their function.

The presence of large quantities of unique oligosaccharides in human milk has implications for the formulation of breast-milk substitutes, especially for those born preterm. Premature infants are increasing in prevalence because of advances in fertility treatment and life-support techniques. They are more vulnerable to infection and cognitive defects than full term infants, brain growth reaches its peak at 26 weeks gestation and rapid growth continues throughout the first weeks of life. Preterm infants fed on human milk in the first month of life have been shown to have a significant advantage in verbal intelligence quotient at 7–8 years of age compared with infants fed on standard infant formulas (Lucas *et al.* 1992). We hypothesize that the sialylated oligosaccharides in human milk may be one of the factors responsible.

In this issue of the *Journal*, Nakhla and colleagues (Nakhla *et al.* 1999) show that preterm human milk is at least as high as full-term milk in terms of quantities of neutral human milk oligosaccharides. Furthermore, there are preliminary indications that concentrations of the acidic (i.e. sialic-acid-containing) oligosaccharides of preterm milk may be even higher than those of full-term milk (Wang *et al.* 1998). Oligosaccharides are another reason why every endeavour should be made to ensure that human infants, especially those born prematurely, receive human milk. Oligosaccharides should be of major interest to those involved in infant nutrition, particularly in improvements in the composition of infant formula. However, their number and complexity ensure that despite advances in knowledge and technology, oligosaccharides will remain 130 reasons to breast-feed.

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