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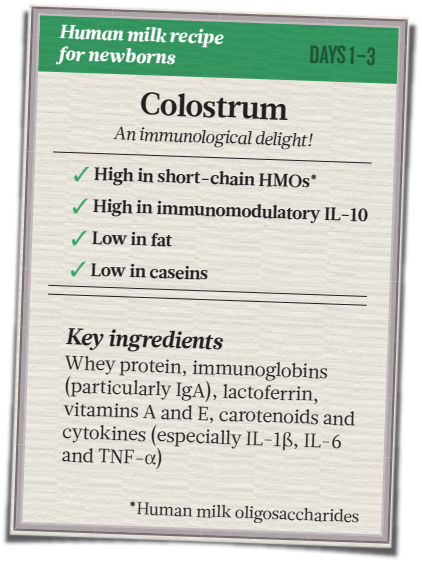

DEVELOPMENT

Mother's milk: A rich opportunity

Research on the contents of milk and how breast-feeding benefits a growing child is surprising scientists.

BY ANNA PETHERICK

Should you ever need to mix formula milk for a tammar wallaby, you will face a complicated recipe. During its 300 days in the pouch between birth and weaning, the baby wallaby, or joey, drinks different milk almost on a weekly basis.

Early on, the joey needs colostrum, which is packed with antibodies. After 60 days, the formula should be rich in asparagine-containing peptides, which are thought to help brain development. Ninety days later, the baby wallaby will need a dose of sulphur-containing amino acids, such as cysteine and methionine, which will cause hair follicles and nails to grow.

For healthy development, the number of calories contained in the milk must also rise, such that the joey is weaned from milk that is four and a half times as energy rich as the liquid it first drank. This compositional sequence appears to be entirely dictated by the mother's body. And, bizarrely, her teats can function independently, with each baby wallaby effectively stuck to one teat for the first 200 days in the pouch. In fact, a joey may have a pouch mate of a different age that feeds on an adjacent teat — and which receives milk of a different composition, appropriate for its age[1].

Unlike a wallaby's, human milk does not change so radically over time because the developmental signals, which wallabies transfer in milk, can be delivered through the human placenta. The major constituents of human milk — the fat, protein and carbohydrate — vary little over the course of lactation. But a closer analysis reveals important time-dependent variation in the complement of bioactive ingredients in human milk — the molecules and cells that have biological functions beyond fuelling metabolism and providing the raw materials for infant growth. Finding what these ingredients are and what they do drives much of today's lactation research.

A MAMMALIAN MIXTURE

Until recently, the study of human lactation was conceived mainly from the perspective of public health. Now the trend is to approach the subject from an evolutionary standpoint. This perspective presumes that an infant should breastfeed as much as possible to maximize its chances of survival, whereas a mother must balance her current metabolic investment in milk production with her potential investment in future offspring.

For example, evolutionary theory suggests that mothers should invest more in feeding sons because a successful son can produce many more offspring than a daughter. Several recent studies support this view by identifying clear differences in the breast milk consumed by males and females. In humans, for example, baby boys receive milk that has substantially more fat and protein than the milk girls get[2].

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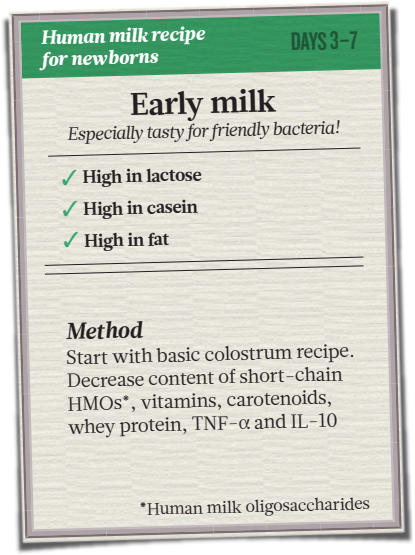
In rhesus macaques, sons drink milk with a higher concentration of cortisol, a hormone that modulates ▶

metabolism, even though their mothers have no more cortisol circulating in their blood than when nursing a daughter. It is unclear whether this cortisol-related sex difference has a function. But there are clues: young male macaques that consume milk containing high levels of the hormone develop bold behaviour, whereas cortisol in milk appears to have no influence on female macaque infants[3]. Whether this has a parallel in humans is yet to be determined.

A second major shift in human lactation research entails the incorporation of new tools to answer traditional questions — such as comparing the effects of breast and formula feeding — and to grapple with evolutionary and functional issues. Human milk is dilute compared to the milk of other placental mammals, but it does contain some surprising ingredients. Advances in high-throughput mass spectrometry, for example, have revealed the existence of more than 200 human milk oligosaccharides (HMOs). Calito Lebrilla, an analytical chemist at the University of California, Davis, has found that mothers seem to produce individual complements of about 100 HMOs — but no one has figured out why different mothers produce different sets of HMOs, or even if it is the same complement of HMOs for each child.

Although they are carbohydrates, HMOs do not appear to nourish infants. Instead they feed certain gut bacteria, giving them a competitive edge over other species. "When a child is born its gut is rapidly populated by pathogenic bacteria," says Lebrilla. "However as the child is fed human milk the population changes to beneficial species." *Bifidobacterium infantis*, which protects against diarrhoea, is particularly efficient at metabolizing the small-mass HMOs that are abundant in early lactation[4]. So breast milk gives *B. infantis* an advantage over other species in establishing a gut population. "The mother is therefore 'selecting' specific bacteria to grow in the infant's gut by her HMOs," says Lebrilla.



Furthermore, some HMOs can inhibit harmful bacteria and viruses directly. For example, certain HMOs block the binding of *Campylobacter jejuni*, the most common cause of bacterial diarrhoea, to intestinal mucosa, and thereby inhibiting pathogenesis[5].

Human milk also delivers some microbes directly to the gut. Breast milk is laced with several species of lactic acid bacteria from the mother's intestine that are thought to travel to her mammary glands inside white blood cells. Most of these species inhibit pathogenic bacteria by secreting hydrogen peroxide and compounds called bacteriocins.

The past decade has seen a large extension in the list of immunological factors detected in human milk. Breast milk was long thought to provide only passive immunity to infants, through maternal antibodies in the form of secretory immunoglobulin A. However, the newly identified crop of immune-regulatory proteins could be prompting and modulating development of the infant's own immune system. Of particular interest are cytokines, which orchestrate the immune system by signalling between its cells.

There is even evidence that breast milk influences gene expression in infant gut cells. In a pilot study, Sharon Donovan, a paediatric nutritionist at the University of Illinois, and Robert Chapkin, a biochemist at Texas A&M University, extracted RNA from exfoliated intestinal cells from several 3-month-old infants. They assessed the statistical difference in RNA expression between breast- and formula-fed infants. Several of the genes that varied were identified as putative master genes, which control the expression of other genes. Most of these genes encode transcription factors associated with angiogenesis and wound repair — including *EPAS1*, a gene that

BRAINY BABIES

Does breast milk make you smarter?

Between late 2002 and the spring of 2005, 13,889 Belarusian children of about six years of age took an IQ test and had their reading and writing skills evaluated by teachers. The mothers of about half of them had been encouraged to breastfeed under a World Health Organization (WHO) programme called the Baby-Friendly Hospital Initiative. As a result, these mothers were seven times more likely to have exclusively breastfed until their child was 3-months old.

Results of this study, called Promotion of Breastfeeding Intervention Trial (PROBIT), showed that the 6-year-olds whose mothers were part of the WHO initiative had better academic ratings from their teachers and IQ scores on average 5.9 points higher[10]. "PROBIT found lots of health benefits in the first year of life," says Michael Kramer, an epidemiologist at McGill University in Montreal, Canada, "but over the longer term the only difference was cognitive ability."

No one is quite sure what causes this intelligence boost. But one 2007 study by

Tests point to higher IQ in breast-fed children.

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Duke University psychologist Avshalom Caspi has identified a candidate: a gene that appears to mediate the effects of human milk on brain development[11]. Caspi and colleagues trawled the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database for genes involved in the metabolism of long-chain polyunsaturated fatty acids. These acids are linked to several aspects of neuron development. Two such fats — docosahexaenoic acid (DHA) and arachidonic acid (AA) — are present in human breast milk, but not in cows' milk or most infant formulas.

The KEGG search identified a gene on chromosome 11, called *FADS2*, which is both regulated by dietary AA and DHA and also encodes an enzyme that catalyses metabolism of these two acids. One specific variant of the *FADS2* gene was present in more than 90% of the cohort in the study. Researchers found that only the breastfed babies who had this specific *FADS2* variant exhibited an IQ advantage. The research implies that fatty acid metabolism could be part of the missing link between breastfeeding and IQ. This *FADS2* variant was estimated to account for a difference of 4.1 IQ points, which goes a long way towards explaining the 5.9 IQ points difference found in the PROBIT trial.

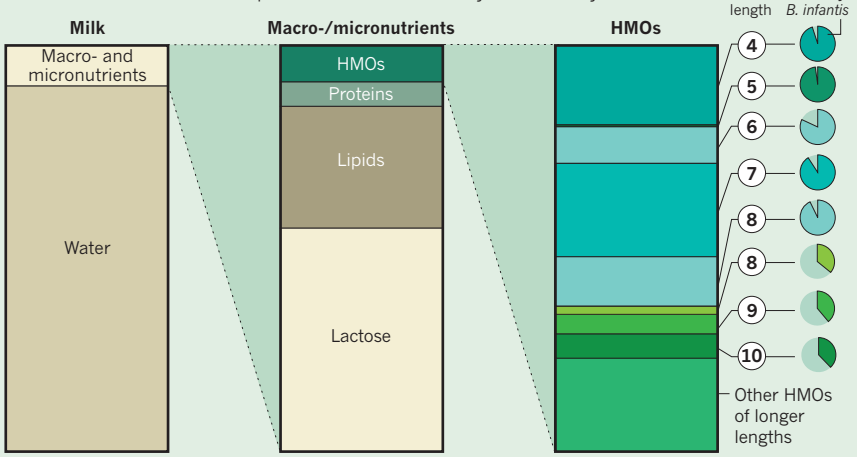
is transcribed three times as much in the gut cells of breastfed infants[6].

Donovan and Chapkin's study is the first evidence that breast milk — rich in natural bacteria — affects infant gene expression, and Donovan cautions about over-interpreting their findings. This is, however, likely to be an expanding area of research as probiotics become more commonly used as ingredients in formula milk. "We have no idea how these are potentially affecting gene expression," says Donovan.

Over the years, the 'breast versus formula' debate has become polarized, and several researchers contacted for this article complained that either breastfeeding advocacy groups or formula companies had exaggerated their findings in the past. Donovan's recent gene expression study was sponsored by a formula milk manufacturer, but she is applying for US National Institutes of Health funding for further studies to avoid the criticism that comes with being commercially funded.

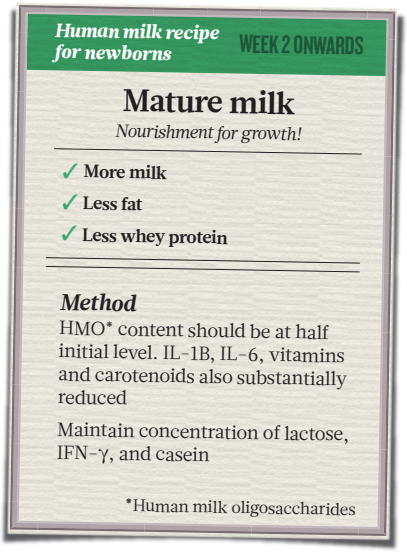
WHAT'S IN HUMAN MILK

Human milk oligosaccharides (HMOs) are food for friendly bacteria like *Bifidobacterium infantis*. Shorter chain HMOs in particular are almost entirely consumed by this microbe.



HEALTH CONTROVERSIES

Researchers have tried to disentangle the effects of feeding an infant formula rather than breast milk. "The vast majority of studies tend to gravitate towards breast milk as better, rather than equal, but the evidence varies in quality," says Jonathan Wells, who studies human ecology at University College London. "Many of the accepted benefits of human milk relate to avoiding pathogens." And while these pathogens might be less dangerous to a baby in a more medically advanced society than in a developing one, breast milk still offers advantages to all infants. Breastfeeding has consistently been found to protect against necrotizing enterocolitis (in which portions of the bowel tissue die) in pre-term infants, and against diarrhoea and ear infections in full-term infants.



Impacts on health in later life stand out less clearly in the data, although associations between formula feeding and type 2 diabetes and inflammatory bowel disease have been observed. Some meta-analyses report that breastfeeding reduces the chance that a child will be obese at school age by about 20%. But these results are not conclusive. The largest breastfeeding trial, Promotion of Breastfeeding Intervention Trial (PROBIT), found no difference in the plumpness of two groups of six-and-a-half-year-old Belarusian children, where one group had been breastfed for much longer before the introduction of formula milk[7]. There was also no difference between these groups in the prevalence of asthma or allergies[8]. PROBIT did, however, show an intriguing link between breastfeeding and intelligence (see Brainy babies, page S6).

The breastfeeding–IQ association had been reported before, but what made PROBIT's results important was the size of its dataset. It is critical to have a very large sample size in order to eliminate confounding factors. Qualities such as obesity and IQ often vary across rich countries in similar patterns to the tendency of mothers to breastfeed. In developed countries, wealthier women are more likely to breast-feed — but they are also generally slimmer, better educated and spend more time talking to their babies.

It might be that certain ingredients in formula milk are responsible for later weight issues. Results from the European Childhood Obesity Project supports the 'early protein hypothesis', which holds that higher levels of protein found in standard infant formulas programme the body to become fatter in later years[9]. The project randomized 1,000 European infants to receive either formula of high-protein concentration (standard formula), formula of low-protein concentration (similar to the protein content of human milk), or breast milk. The result: unlike the high-protein group, the low-protein group grew no tubbier than the breastfed control group.

The diverse ingredients of an infant's first meal have an impact on its development, and no matter how much we tinker with the composition of formula milk it will always lack many of the trace constituents of human milk. As research identifies these substances, it increasingly seems they serve a role beyond direct nutritional benefit: that of communicating information to the infant about the environment and even the social structure around the mother, which affects the richness of her diet and her level of physical activity and therefore also affects her milk.

Wells believes that very young humans should be thought of as having to adapt to the mother's surroundings, rather than to the wider world. Indeed, the fact there are so many bioactive molecules in breast milk means that breastfeeding is an activity that empowers mothers. He adds, "The more we learn about the details of breast milk the more we realize that males have a little chance to influence their offspring by non-genetic pathways. Mothers have a very rich opportunity." ■

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