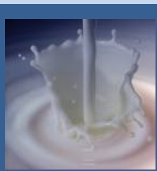
# *SPLASH!* milk science update: August 2013 issue



## *SPLASH!* milk science update
**AUGUST 2013 issue**

This month's issue brings stories of how scientists use teeth to learn about weaning, the power of milk to rehydrate after exercise, the many antibacterial protein fragments in milk, and how mother's milk composition changes with the newborn's weight and gestation time.

We hope you enjoy these fascinating, true tales of milk!

## Weaning in TEETH!

- **When to wean? Humans wean early compared to our ape relatives.**
- **Researchers are using a new technique to measure elements in teeth to track weaning.**
- **The new method has been validated prospectively in humans and in monkeys.**
- **Scientists have even used these new tools to study a fossil Neanderthal tooth.**

Weaning in primates is a fascinating process in which ingestion of mother's milk, as a proportion of daily dietary intake, incrementally declines as the infant ages. From moment to moment, this exquisite negotiation of nipple access between mother and infant can vary in relation to food availability, maternal style, and the compelling power of the infant demand (a.k.a. weaning tantrum). And lots of other factors can influence the weaning process, too.

One of the more remarkable features of human development is that cessation of breastfeeding occurs earlier for us than for our closest ape relatives. This is not just an artifact of a modern industrial world. In 2001, Dan Sellen reviewed >100 non-industrial populations using demographic and ethnographic records. The average age of the introduction of supplemental solids was estimated to be ~5 months (±4 months) and cessation of breastfeeding on average was estimated to be ~30 months (±10 months). Chimpanzees, one of our two closest living relatives, complete weaning ~55+ months (Kappeler & Pereira, 2003).

Skull of a Hom Neanderthalensis (30,000-50,000 years old); American Museum of Natural History

Our accelerated transition to foods other than mother's milk is thought to have emerged in our ancestral history in part due to more cooperative infant care and access to a more nutritious diet (Reiches et al., 2009; Kramer and Ellison, 2010; Kaplan et al., 2000). Shorter lactation periods can translate into shorter interbirth intervals and higher reproductive rates. This has likely played a part in the relatively high lifetime fertility in humans compared to other apes, even before the advent of modern industrial food production. Note the slow reproductive rate of other great apes makes their populations especially vulnerable to deforestation and hunting; they just can't recover faster than we can destroy.

Paleoanthropologists debate exactly when in our evolutionary history accelerated weaning emerged. For many decades, researchers have relied on tooth eruption schedules as a proxy for weaning. Unfortunately, more recent investigations of nursing behavior and tooth eruption in wild-living primates have revealed that these parameters do not necessarily occur together (Smith et al., 2013; Godfrey et al., 2003). Observations of nursing behavior aren't a good proxy for milk transfer either because they tell you nothing about how much milk the infant is actually consuming (Cameron et al., 1999; Cameron, 1998). And toward the end of the weaning process, infants suckle during nighttime co-sleeping (when most primatologists are deservedly recovering from their daytime observations). For living primates, there may be opportunities to measure milk intake from poop samples, but further validation is needed (Reitsema, 2012).

Basically, as anthropologists, we have had a limited number of relatively weak proxies for investigating weaning in the living animals right in front of us, much less going to the fossil record to convincingly deduce when earlier infant weaning evolved in our hominin relatives. Maybe the signatures of early life dietary transitions could be found in teeth?! Many paleoanthropologists began looking for "isotopic biomarkers"--chemical elements laid down as tissue develops--of early life experiences in teeth…but there were some challenges.

## A briefest history of elemental analysis in skeletal material

Back in the 1960s, scientists demonstrated that during the fossilization process, elements were readily exchanged between the dirt/rock and skeletons (e.g., Schroeder, 1969). This is known as diagenesis. And these diagenetic effects can obscure the natural accumulation of elements in tissue during the lifetime (biogenesis). Conventional wisdom (persisting today) was most effectively summarized by Johannes Schroeder: “these elements are not dependable for paleo-environmental analysis.”

Since then, new techniques, controls, and instrumentation have re-opened options for investigating dietary shifts in some particularly well-preserved fossils. We can do fancy-pants things like “high-resolution elemental analysis by laser ablation-inductively coupled plasma-mass spectrometry.” Dr. Louise Humphrey and colleagues used these methods to measure strontium, an alkaline earth metal, in the tooth enamel of humans and baboons to indicate dietary changes (Humphrey et al., 2008a, 2008b). Although they used species-typical estimated averages rather than prospectively following individual mother-infant dyads, these studies importantly opened previously abandoned avenues for studying dietary transitions in teeth.

But strontium seemed to have some minor drawbacks. Kohn and Moses, in an elegant experimental study, revealed that strontium was more susceptible to diagenetic alteration than was barium (2013). This meant that strontium had poor resolution over short time scales and would not be as useful to detect discrete time points for weaning transitions.

## Back to the story…

We wanted to investigate strontium and barium prospectively in human and monkey enamel in relation to early life diet (Austin et al., 2013). In humans, teeth were collected along with daily journals of infant diet--breastmilk, formula, and solids. My long-term monkey milk study at the Comparative Lactation Lab in Human Evolutionary Biology at Harvard University and the California National Primate Research Center provided teeth, milk samples, and behavioral observations of infant suckling and solid food consumption. (Full disclosure, I am not a tooth histologist nor skilled in elemental analysis. I brought the macaque jazz to this cocktail party.)

We found that we could see the period of exclusive breastfeeding and the weaning process--down to nearly the day--by measuring barium concentrations in tooth enamel!

Using sophisticated analytical chemistry and microscopy techniques at the University of Sydney’s Faculty of Dentistry, the Elemental Bio-imaging Facility (University of Technology, Sydney) and the Dental Hard Tissue Laboratory (Harvard University, Cambridge, MA), we were able to track changes in barium concentration in teeth across time. Similar to the growth rings found in trees, teeth follow an incremental growth pattern that creates daily growth lines in enamel and dentine, which can be viewed and counted under the microscope.

When barium appears in the tooth lines, we know the infant has been born and is consuming milk since barium doesn’t appreciably cross the placenta. The barium levels increased toward peak lactation (as milk consumption increases) and then declined with the known introduction of supplemental solid foods. Importantly, barium levels in the monkey mothers’ milk were consistent with barium levels in monkey infants’ tooth enamel.

We then applied this technique to a single Neanderthal tooth found in Belgium dated to the Middle Paleolithic. The specimen was the wonderfully well-preserved Scladina juvenile. Other scientists have successfully recovered proteins and mitochondrial DNA from this individual (Nielson-Marsh et al., 2009; Orlando et al., 2006). Although these well-preserved features argue against diagenetic alteration, just to be sure we further investigated rare earth elements in the enamel. If diagenesis had appreciably altered the elemental structure of the tooth- creating a diagenetic “overprint” if you will--we would expect these rare earth elements to be enriched in the Neanderthal tooth. There was little evidence for diagenetic modification and none in the areas of the tooth in which we sampled barium (discussed *ad nauseum* in the supplemental information that accompanies the main paper)(Austin et al. 2013).

The barium pattern indicated the Neanderthal infant was exclusively breastfed for ~7 months before supplementation with non-milk foods over the following ~7 months. At ~1.2 years of age, there is evidence of an abrupt cessation of weaning. The pattern is the same as found in the macaque separated from its mother during infancy.

The Neanderthal infant survived this early life dietary shift, but the atypical, precipitous decline in barium levels strongly suggests there was some “insult” to the dyad that interrupted the normal, gradual weaning process. Maybe the mother died, became sick, or couldn’t sustain lactation. Maybe she went out foraging while a friend babysat her kid and then there was a flash flood and she was trapped on the other side of the river and in the five days it took the river to subside her milk dried up…there is no way to know. We speculated wildly over e-mail. We were appropriately more circumspect in publication.

## The important take away

This one individual cannot be used to infer species-typical weaning patterns of Neanderthals. The precipitous decrease of barium at 1.2 years of age pretty clearly suggests that the Scladina Neanderthal was abruptly weaned in an atypical fashion. Primates aren't hooded seals. Those guys have four days of mom providing milk and then on day five she abandons the unstable ice pack, returning to the sea from whence she came with hardly a "Go live off your blubber, buddy." Instead, primates grow slowly, developing their big brains and complex social behavior over a long infancy.

But what we can confirm from these human, macaque, and Neanderthal data is the following. We have a really powerful way to investigate the period of exclusive milk feeding and the weaning process with high precision and resolution. For the first time we have a method to look at individual differences in these parameters in primates "collected" by old school naturalists gathering dust in natural history museums or skeletal material recovered at primate field sites. And we can do this in well-preserved fossil hominins...if colleagues are willing to share. That aggregation of data will allow us to establish the range of variation among individuals within species and from that, a better understanding of life history evolution among species.

Austin C, Smith TM, Bradman A, Hinde K, Joannes-Boyau R, Bishop D, Hare DJ et al. (2013) Barium distributions in teeth reveal early life dietary transitions in primates. *Nature* **498**:216-219.

Cameron EZ (1998) Is suckling behaviour a useful predictor of milk intake? A review. *Anim Behav* **56**:521-532.

Cameron EZ, Stafford KJ, Linklater WL, Veltman CJ. (1999) Suckling behaviour does not measure milk intake in horses, *Equus* caballus. *Anim Behav* **57**:673-678.

Godfrey LR, Samonds KE, Jungers WL, Sutherland MR. (2003) "Dental development and primate life histories." in *Primate life histories and socioecology*, edited by Peter Kappeler & Michael Pereira, 177-203. Chicago: The University of Chicago Press, 2003.

Humphrey LT, Dean MC, Jeffries TE, Penn M. (2008b) Unlocking evidence of early diet from tooth enamel. *Proc Natl Acad Sci USA* **105**:6834–6839.

Humphrey LT, Dirks W, Dean MC, Jeffries TE. (2008a) Tracking dietary transitions in weanling baboons (*Papio hamadryas anubis*) using strontium/calcium ratios in enamel. *Folia Primatol (Basel)* **79**:197–212.

Kaplan H, Hill K, Lancaster J, Hurtado AM. (2000) A theory of human life history evolution: diet, intelligence, and longevity. *Evolutionary Anthropology Issues: News and Reviews* **9**: 156-185.

Kramer KL & Ellison PT. (2010) Pooled energy budgets: Resituating human energy-allocation trade-offs. *Evolutionary Anthropology: Issues, News, and Reviews* **19**:136-147.

Nielsen-Marsh CM, Stegemann C, Hoffmann R, Smith T, Feeney R, Toussaint M, Harvati K et al. (2009) Extraction and sequencing of human and Neanderthal mature enamel proteins using MALDI-TOF/TOF MS. *J Archaeol Sci* **36**:1758–1763.

Orlando L, Darlu P, Toussaint M, Bonjean D, Otte M, Hanni C. (2006) Revisiting Neandertal diversity with a 100,000 year old mtDNA sequence. *Curr Biol* **16**:R400–R402.

Reiches MW, Ellison PT, Lipson SF, Sharrock KC, Gardiner E, Duncan LG. (2009) Pooled energy budget and human life history. *Am J Human Biol* **21**:421-429.

Reitsema LJ. (2012) Introducing fecal stable isotope analysis in primate weaning studies. *Am J Primatol* **74**:926-939.

Schroeder JH. (1969) Experimental dissolution of calcium, magnesium, and strontium from recent biogenic carbonates: a model of diagenesis. *Journal of Sedimentary Research* **39**:1057-1073.

Sellen DW (2001) Comparison of infant feeding patterns reported for nonindustrial populations with current recommendations. *J Nutr* **131**: 2707-2715.
Sellen DW (2007) Evolution of infant and young child feeding: implications for contemporary public health. *Annu Rev Nutr* **27**:123-148.

Smith TM, Machanda Z, Bernard AB, Donovan RM, Papakyrikos AM, Muller MN, Wrangham R. (2013) First molar eruption, weaning, and life history in living wild chimpanzees. *Proc Natl Acad Sci USA* **110**:2787-2791.

Contributed by

Prof. Katie Hinde
Department of Human Evolutionary Biology
Harvard University

## Milk Beats Gatorade at Rehydration

- **Milk has a similar electrolyte content and carbohydrate concentration to commercial sports drinks.**
- **The evidence mostly suggests that it is more effective at rehydrating people after vigorous exercise than well-known recovery beverages.**
- **Several theories have been proposed to explain this, among them milk's longer digestion period and its fat and protein content.**

Working out means losing liquid, for some of us more than others. The lost liquid needs to be replaced, particularly if you intend to exercise more on the same day. A whole industry has been built around creating sports drinks to rehydrate athletes—and those of us who can only dream of becoming athletes—quickly and effectively. But for all these branded drinks' isotonic technicalities, hyperactive coloring, and celebrity sponsorship deals, they tend to come second on rehydration tests behind humble, old milk.

There are various reasons why this is the case, starting with the basic fact that milk, too, has an electrolyte content equal (or isotonic) to that of the body and roughly the same concentration of carbs as commercial sports drinks. Beyond that, to understand what's going on first requires understanding how the body responds to strenuous exercise.

To be sure, people sweat to keep cool. But sweating isn't the only reason we are dehydrated after a work out. Intense exercise creates various metabolic waste products, which can only be eliminated in urine. So even when you are quite severely dehydrated after sport, you will need to pee not long into your recovery period. What's more, if in your hurry to rehydrate you drink a lot of water—or another liquid with little solute content—the temporary increase in your blood serum's volume, and concurrent decrease in its osmotic pressure, will trigger excessive urine production. Cue all that marketing about engineered isotonic goodness.

The idea, in short, is to take on board liquid that stays on board. And because of the need to expel metabolic refuse molecules, the evidence suggests that to truly replace all the liquid that is lost through exercise, you need to imbibe a volume of drink equal to about 150% of the body mass that you lost through sweating.

This rule of thumb explains the experimental set up of studies that compare milk's rehydrating capabilities to those of Gatorade and other branded sports drinks. Typically, a group of young and fit volunteers are told to follow a list of behavioral procedures to ensure they arrive at each of the tests, which are usually scheduled a week apart, in the same physiological state on every occasion. On the test days, the volunteers exercise so hard and for so long that they sweat off a percent or two of their body mass. Then they drink 150% of that amount of one of the test liquids—they drink Gatorade on one of the days, chocolate milk another, water on the third test day, and so on. After three or fours hours of rest, the volunteers are often put through their paces again, and closely monitored. If they haven't rehydrated reasonably well after the first bout of physical activity, it should show in their performance.

The downside of these experiments is that they tend to have small sample sizes, which is unsurprising, perhaps, given the nature of the tasks required of them. Nonetheless, the results are similar across the board.

For example, Karp et al. (2006) studied trained endurance cyclists, pushing them to exhaustion during the second exercise period. These poor souls maintained 70% of VO2 max—that is, 70% of their peak aerobic capacity-- until they wilted. This happened after a significantly longer period (40 minutes) when they consumed low-fat chocolate milk during the recovery period that followed their first exercise session, than when they drank Gatorade (27 minutes).

Shirreffs et al. (2007) tried to improve on low-fat milk's rehydration capabilities by dissolving salt in it. Her team recorded their volunteers' urine output in the five hours after exercise. Following an hour or two of recovery, urine production rose when the volunteers drank water or a sports drink (Powerade), leaving them dehydrated despite swallowing the prescribed 150% of the volume they lost through exercise. But when they drank milk, or salty milk, there was no change in their urine production over time, and the volunteers ended up with a net positive fluid balance. The extra salt helped a bit, but wasn't as palatable.

James et al. have instead explored the role of protein in recovery drinks. In an experiment in 2011, they tested not milk per se, but a solution of milk protein and carbohydrate that had exactly the same energy and electrolyte content as a carbohydrate solution. The protein-carbohydrate mixture did a better job at rehydrating the volunteers.

In 2013, this group fiddled with the ratio of milk protein and sugar in the recovery drinks used in their study. They compared a 60 g/liter carb solution, a 40 g/liter carb plus 20 g/liter protein solution, and a high protein option, which contained 20 g/liter carb plus 40 g/liter protein. The worst performer was the carbohydrate-only drink. And the additional protein didn't help with rehydration.

What do these results suggests about the mechanisms at play? One simple idea is that milk – and protein solutions **-** spend longer in the stomach than sugary drinks do, and so the associated water content enters the blood more slowly. This could avert the diuretic effect that a transient fall in blood osmotic pressure causes. Some data collected by Calbet & Maclean (1997) suggests it takes about 14% longer for half of one of the milk drinks in the above experiments to leave the stomach than for half of one of the sports drinks.

More elaborate ideas have been proposed. Exercise depletes muscle glycogen. Karp et al. reckon the cyclists in their study did not have sufficient recovery time to digest the more complex sugars in Gatorade (which include maltodextrin, a long sugar derived from starch), but did have time to break down the sugars in chocolate milk (the monosaccharides glucose and fructose, and the disaccharide, lactose). This, they say, led to more limited glycogen replenishment in the Gatorade trial. They also think chocolate milk may have delayed glycogen depletion during the second cycling session, allowing the cyclists to hang on for longer because, unlike Gatorade, the milk supplied free fatty acids to the cyclists' bloodstreams.

Milk's protein content has a more contentious role in rehydration than its sugar and salt components. Ingesting protein during the couple of hours after exercise is considered by some nutritionists to quicken glycogen synthesis, while others think the data on this is equivocal. There is also data supporting the idea that milk, when used a sports recovery drink, helps with muscle protein synthesis—which makes sense, given that milk contains all nine essential amino acids. Theoretically, this should reduce injury.

What this field really needs are longer data sets in which milk is used day in, day out during athletic training. One study involving soccer players by Gibson et al. (2010) did follow its subjects for a week—and indeed reported less muscle damage among those who drank milk compared to those who stuck to a traditional carbs-only sports drink. But there was no difference in the players' performance, and one week and one soccer team doesn't really do the research question justice.

So here's a challenge for any enterprising sports coaches out there: test milk's capacity to rehydrate your teams on a daily basis for a whole season. You are likely to see fewer injuries, plus better performances on the hottest days and in the longest, most unforgiving matches when winning comes right down to the wire. Plus, milk's probably cheaper than whatever hyperactively colored drink you're currently using.


Gibson S, Saunders MJ, Moran CW, Moore RW, Womack CJ, Todd MK. (2010) Effects of chocolate milk consumption on markers of muscle recovery following soccer training: a randomized cross-over study. *J Int Soc Sports Nutr* **7**:10.

James LJ, Clayton D, Evans GH. (2011) Effect of milk protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat. *Br J Nutr* **105**:393–399.

James LJ, Evans GH, Madin J, Scott D, Stepney M, Harris R, Stone R, Calyton DJ. (2013) Effect of varying the concentrations of carbohydrate and milk protein in rehydration solutions ingested after exercise in the heat. *Br J Nutr* **31**:1-7.

Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, Stager JM. (2006) Choclate milk as a post-exercise recovery aid. *Int J Sport Nutr Exerc Metab* **16**:78-91.

Roy BD. (2008) Milk: the news sports drink? A Review. *J Int Soc Sports Nutr* **5**:15.

Shirreffs SM, Watson P, Maughan RJ. (2007) Milk as an effective post-exercise rehydration drink. *Br J Nutr* **98**:173–180.


Contributed by


Anna Petherick
Professional science writer & editor
www.annapetherick.com

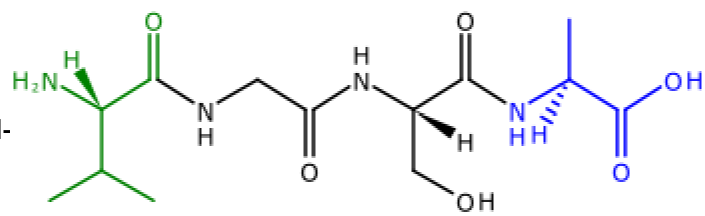
# Milk Peptides Fight Bacteria

- **Human milk contains approximately 1% protein.**
- **Enzymes in milk chop some of these proteins into fragments (peptides).**
- **Specific peptides have evolved to possess biologically active (bioactive) roles.**
- **When analyzing human breast milk, 328 putative bioactive peptides were identified.**
- **From this group of peptides, 41 had anti-bacterial properties.**

Milk is a wonderfully complex fluid that is not only nutritious but is also physiologically proactive. Recently, David Dallas and his colleagues from University of California at Davis used a cutting-edge approach to probe the depths of milk composition. The initial results revealed that human breast milk contains proteins which are digested into peptides, some with anti-bacterial properties (1).

Milk contains proteins, sugars, fats, vitamins, and minerals. The relative amounts of each component vary from species to species. Human milk contains approximately 1% protein with greater than 90% contributed by just a few milk proteins: caseins, lactalbumin, immunoglobulins, lactoferrin, serum albumin, and lysozyme. Milk also contains a mixture of enzymes that can chop these proteins into fragments, which together provide a rich source of biologically active (bioactive) peptides. Yes, milk digests itself!

Bioactive peptides are so called because they can induce a measurable response in the person or animal that consumes them. The bioactives of greatest interest are those that have a clear benefit for the consumer and help stimulate health and well-being. In the case of milk-derived peptides, an area that has attracted a lot of interest are bioactive peptides related to the immune system, which we find in relative abundance in milk.



**Tetrapeptide structural formulae.**

The recent study by Dallas and colleagues (1) focused on identification of peptides that naturally occur in human breast milk, that is, they looked for the peptides that the milk created using its own enzymes. First they adapted a strategy to look for peptides that were hidden beneath the confusion that milk's complexities can present. This is akin to exploring the depths of the ocean to find hidden treasures. The study revealed 328 peptides that were derived mostly from the major proteins. However, it was clear that these peptides were not generated by a random process, rather they were largely produced by one enzyme named plasmin, an enzyme that is well known for its role in the body, but until now, not so much for its role in milk.

When the 328 peptides were compared to databases of known bioactive peptides, 41 (12.5%) were thought to resemble peptides with anti-microbial properties. Anti-microbial properties of milk may benefit the infant that does not yet possess a fully functional immune system to battle pathogens, but there are also potential benefits for the mother to be taken into consideration. Bacterial infections in the ducts of a mammary gland, which we know as mastitis, may block the ducts and lead to painful inflammation and potentially the shutdown of milk production. One way to combat this, or even prevent it from occurring, would be to generate a frontline anti-bacterial defence mechanism in the milk itself.

With mastitis in mind, Dallas tested the mixture of milk peptides he had collected for their capacity to kill mastitis-inducing bacteria. He found that, when concentrated, the mixture was effective against the two types of bacteria tested, but only when concentrated. His work also suggests that specific peptides within the mixture have anti-bacterial activity and that perhaps even individual peptides, at the concentrations found present in milk, may be effective at fighting bacteria. Once again, milk has found a way to deal with the challenges nature has thrown its way. Dallas has just begun to uncover the bioactives that lie in his breast milk samples. Time will tell what other treasures lie beneath the milky waves.

1. Dallas DC, Guerrero A, Khaldi N, Castillo PA, Martin WF, Smilowitz JT, Bevins CL et al. (2013) Extensive in vivo Human Milk Peptidomics Reveals Specific Proteolysis Yielding Protective Antimicrobial Peptides. *J Proteome Res* **12**: 2295-304.

Contributed by

Prof. Peter Williamson
Associate Professor, Physiology and Genomics
Associate Dean of Research
Faculty of Veterinary Research
University of Sydney, Australia

## Mother's Milk Compensates for Smaller Neonates

- **Mothers of premature or small full-term babies produce milk with different fatty acid composition compared to moms of full-size, full-term infants.**
- **Milk for preterm infants is higher in concentration of both medium chain fatty acids and omega-3 long chain polyunsaturated fatty acids, such as DHA.**
- **Milk for babies born small for gestational age have higher concentrations of medium chain fatty acids, which may provide an important energy source.**
- **Milk production appears to be highly responsive to specific needs of the neonate.**

The placenta and the mammary gland may be separate organs, but they are better viewed as part of a coordinated team, charged with transferring nutrients, immune factors, and other bioactive components to the developing offspring. The placenta manages the first 40 weeks, but if the baby is born prematurely (<37 weeks gestation), the mammary gland works overtime to produce milk with higher concentrations of components that should have come from the placenta.

But does the mammary gland always cover for the placenta? Babies born at term, but born small for gestational age (SGA; birth weight<10th percentile), have also missed out on placental nutrients, similar to premature neonates. Do these mothers produce milk that compensates for what their babies may have missed *in utero*? A new study by Bobinski et al. (2013) suggests that milk composition may be strongly shaped by both the gestational age and weight of the neonate.

**Early babies, different milk?**

Differences in milk composition between mothers of premature and term neonates have been investigated for over 30 years and have focused primarily on milk fats (e.g., Bitman et al., 1983; Bokor et al., 2007; Gross et al., 1980; Koletzko et al., 2001). This is because during the last trimester (approximately 26-40 weeks), human fetuses undergo rapid brain growth (called the brain growth spurt) and put on considerable adipose tissue. Of particular interest have been proportions of long chain polyunsaturated fatty acids (LCPUFA) implicated in neural and retinal development, especially docosahexaenoic acid (DHA, an omega-3 fatty acid).

During the last trimester, the placenta transfers higher concentrations of DHA (per kilogram of fetal body weight) than at any other time during gestation. Infants born prematurely are cut off from placental transfer of this essential fatty acid. However, numerous studies have demonstrated higher concentrations of DHA in milk from mothers of premature as compared to mothers of term infants (reviewed in Bokor et al., 2007). In addition, several studies have found higher concentrations of several other types of omega-3 fatty acids (e.g., alpha-linoleic acid, or ALA), which can be used by the infant to synthesize DHA (Bitman et al., 1983; Bokor et al., 2007).

Despite higher postnatal LCPUFA requirements of infants born prematurely, not all investigations have found higher milk LCPUFA in preterm as compared to term milk (e.g., Genzel-Boroviczeny et al., 1997) Interestingly, these studies highlight the higher concentration of medium chain fatty acids (fatty acids with 6-12 carbons) in premature milk, arguing they are easily digestible fats that could serve as an important energy source (Bitman et al., 1983; Genzel-Boroviczeny et al., 1997). Although contradictory in their findings about LCPUFA, taken together these studies all suggest compensatory mechanisms during lactation that are responsive to specific needs of the premature compared to the term neonate.

**Smaller babies, different milk?**

Not only does the mammary gland compensate for placental shortcomings when it comes to the timing of parturition, it also is responsive when babies have had insufficient placental transfer of nutrients throughout gestation. Bobinski et al. (2013) are the first to investigate how pathological processes that occur during pregnancy (i.e., intrauterine growth restriction resulting in SGA neonates) influence the mother’s milk fatty acid composition. To do so, they analyzed transitional milk (days 4-7 of lactation) and mature milk (month two of lactation) from mothers who gave birth to neonates differing in birth weights and gestational age. Specifically, they compared milk from mothers that delivered (1) late preterm (35 – 37 weeks gestation), (2) at term, appropriate weight for gestational age (AGA), and (3) at term, SGA. Both late preterm and SGA neonates have small birth weights, but they do not necessarily have identical nutritional deficits. As a result, the study authors did not predict convergence in milk composition from these two groups but rather hypothesized that both would show important differences from mothers of AGA neonates, differences related to their specific nutritional deficits.

No significant differences were identified in the relative proportion of any of the 34 fatty acids they measured in transitional milk samples among the three groups of mothers. However, mature milk samples collected from late preterm and SGA mothers two months later were significantly higher in medium chain fatty acids compared to milk from AGA mothers. Like previous researchers, Bobinski et al. highlight the importance of medium chain fatty acids such as capric acid (10:0) and lauric acid (12:0) as important energy sources for neonates. In addition to providing calories, these fatty acids require less ATP (adenosine triphosphate) for digestion, thus making more of this energy molecule available for other cellular processes. Increased capric and lauric acid may thus be seen as a compensatory mechanism for both preterm and SGA neonates. Although it may not be filling a nutritional deficit (as was the case for more DHA in preterm neonates), the presence of higher concentrations of these fatty acids in milk lipids may permit the partitioning of digestive energy to cellular proliferation (i.e., body growth) and development.

The authors are not able to explain why this important difference is seen in mature but not transitional milk, as one might predict that such compensatory actions would be critical from the start of lactation. They speculate that it may take longer than the first week of lactation for the mother to modulate the synthesis of milk to the specific needs of her offspring. However, without any milk samples collected between day seven and the second month of lactation, it is not possible to say when these changes between AGA and the other two groups of mothers emerge.

Although primarily focused on medium chain fatty acids, this study did have an interesting finding concerning omega-3 LCPUFA. When looking at changes in proportions from transitional (day 7) to mature milk (month two), milk from mothers delivering preterm decreased in omega-3 LCPUFA (including DHA) while that from AGA and SGA mothers showed no statistically significant changes. This suggests different metabolism of these fatty acids in mothers that deliver preterm compared to term, regardless of offspring body size, and serves to further highlight the potentially intimate relationship between infant condition and milk fatty acid profiles.

## A critical view

The results of Bobinski et al.'s study support previous findings of a link between premature birth and the synthesis of omega-3 LCPUFA, such as DHA, and medium chain fatty acids, but they also provide new data suggesting milk fatty acid composition is modified in mothers that give birth to SGA offspring as well. Milk production, in their view, is not static but rather highly responsive to specific needs of the neonate, most likely facilitated by hormonal communication from the placenta during pregnancy and parturition.

This adaptive explanation is not new, and also is not without its critics. Many have offered nonadaptive explanations for the differences in milk fatty acid concentrations observed in preterm and term milk. For example, Bokor et al. (2007) argue that mothers that deliver prematurely may simply have larger body stores of LCPUFA than term mothers (because they didn't lose them during the last weeks of gestation) and therefore have more available for mobilization during lactation. The mammary gland, in this scenario, is not responding to specific infant needs but rather to maternal physiology.

The differences in medium chain fatty acids are more difficult to explain than LCPUFA, because they can be synthesized by the mammary gland rather than transferred only from adipose stores. However, even the concentration of capric and lauric acid are dependent upon maternal diet, including consumption of saturated fats, unsaturated fats, and even carbohydrates.

An important next step is replicating Bobinski et al.'s results in larger sample sizes (their study had 23 mothers with SGA offspring). Future studies should also control for parity. Bobinski et al. enrolled first-time mothers as well as mothers with up to two previous offspring but failed to control for this potentially confounding variable. Previous research (Finlay et al., 1985) suggests that parity may influence both total milk fat (negative correlation) and the proportion of fatty acids synthesized by the mammary gland (i.e., medium chain fatty acids)(positive correlation). This means that with increased parity, milk is generally lower in fat and that fat contains a higher proportion of medium chain fatty acids. Mammary glands that have previously lactated respond differently to placental hormones and enzymes than do immature mammary glands, and may preferentially secrete medium chain fatty acids over LCPUFA (Finlay et al., 1985). As these are the two groups of fatty acids of interest when looking at milk for preterm and SGA neonates, it certainly is important to consider how this variable may influence study results.

**Formulations of the future**

Although mother's milk is best for the developing infant, many preterm and SGA neonates will require supplementation. A better understanding of the relationship between infant condition and milk synthesis will enable the production of formulas that also help compensate for nutritional deficiencies experienced during gestation. Research of this type is critical as even small dietary changes during the first months postpartum can improve growth and developmental outcomes for these high-risk neonates.


Bokor S, Koletzko B, Decsi T. (2007) Systematic review of fatty acid composition of human milk from mothers of preterm compared to full-term infants. *Ann Nutr Metab* **51**:550-556.

Bitman J, Wood L, Hamosh M, Hamosh P, Mehta NR. (1983) Comparison of the lipid composition of breast milk from mothers of term and preterm infants. *Am J Clin Nutr* **38**:300-312.

Bobinski. R, Mikulaska M, Mojska H, Simon M. (2013) Comparison of the fatty acid compostion of transitional and mature milk of mothers who delivered healthy full-term babies, preterm babies, and full-term small for gestational age infants. *Eur J Clin Nutr* doi:10.1038/ejcn.2013.96.

Finlay DA, Lonnerdal B, Dewey KG, Grivetti LE. (1985) Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. *Am J Clin Nutr* **41**:787-800.

Genzel-Boroviczeny O, Wahle J, Koletzko B. (1997) Fatty acid composition of human milk during the 1st month after term and preterm delivery. *Eur J Pediatr* **156**:142-147.

Gross SJ, David RJ, Bauman L, Tomarelli RM. (1980) Nutritional composition of milk produced by mothers delivering preterm. *J Pediatr* **96**:641-644.

Koletzko B, Agostoni C, Carlson SE, Clandinin T, Hornstra G, Neuringer M, Uauy R et al. (2001) Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal develeopment. *Acta Paediatr* **90**:460-464.



Contributed by

Dr. Lauren Milligan
Research Associate
Smithsonian Institute


## Editorial Staff of "*SPLASH!* milk science update"




***Funding provided by California Dairy Research Foundation and the International Milk Genomics Consortium***