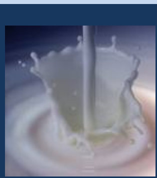
SPLASH! milk science update: October 2012 issue



SPLASH! milk science update
OCTOBER 2012 issue

This month, we learn how milk composition varies when produced for male or female offspring, the risks and benefits of drinking raw milk, how milk digestion begins even before consumption, and, of course, the danger of cow burps!

Enjoy!

Boy milk vs. girl milk

- **Milk reflects the developmental needs of the infant.**
- **If sons and daughters develop differently, we can expect milk to differ, too.**
- **Evolutionary theory predicts sex-biases in maternal investment.**
- **Mothers make different milk for sons and daughters.**

In most sexually reproducing species, one parent ends up doing a majority of the reproductive heavy lifting. And in mammals, it's the ladies. The time and energy costs of pregnancy and lactation are substantial, much more so than the production of sperm. For mammalian young to survive, there are no real short-cuts, so females have made a substantial commitment from conception until the young are independent. And for each conception, most (but not all) mammalian females are investing in the offspring of a single male. In contrast, following copulation, a male's work is largely done. A single male can impregnate many females in a short period of time, becoming very reproductively successful. Prime examples of such mammals are elephant seals, elk, baboons, gorillas, and Genghis Khan. But being the dominant male with a harem of ladies is not for the faint of heart. It requires being big, being strong, and/or being intimidating. Basically, the male needs to be an all-around badass. And for every badass, there are a whole bunch of ninnies, wusses, and pansies that never sire any offspring.

What does this mean for milk? Well, natural selection favors adaptations that increase "fitness" - a term that refers to the number of copies of one's genes passed on to the next generation. And the biggest contributor to fitness is the number of offspring one produces that then survive to reproduce. As a mammalian mother, a daughter is a fairly safe bet; daughters who survive to adulthood are very likely to produce young. In comparison, a son has a greater potential return in number of grand-offspring than does a daughter. But sons are a riskier bet because they have a higher probability of being a zero than a hero. So mammalian mothers hedge their bets and produce both sons and daughters. But in many mammalian species, male infants are born bigger and grow bigger during infancy. Researchers have hypothesized that biases in milk synthesis may contribute to the differences in post-natal growth trajectories between sons and daughters…and data are starting to support, in part, these hypotheses. Even though humans aren't characterized by the uni-male, multi-female, (polygynous) social organization of red deer or hamadryas baboons, signatures of a polygynous mammalian heritage may still be lurking in our milk.

Iberian red deer (*Cervus elaphus hispanicus*)

The first evidence for sex-biased milk was the report of a 5-year study in captive red deer of 91 calves and 60 deer moms (19 deer moms were sampled two seasons, 6 sampled 3 seasons). Landete-Castillejos and colleagues (2005) found that deer moms produced higher milk yield and higher protein concentration (and therefore, higher protein yield) for sons compared to the milk produced for daughters. The mean concentration of fat and lactose was the same for sons and daughters, but because deer moms produced greater volumes for sons, the total fat yield and total lactose yield for sons was greater. However, milk yield and total yield of milk fat, lactose, and protein were positively correlated with birth mass and the latter three correlated with calf growth. This suggests that milk energy transfer is a function of mass and growth rather than specifically sex.

The most suggestive clue of a true sex-bias is the significantly higher protein concentration for sons because it is so critical for gaining lean tissue mass. The higher total fat and sugar yield for sons was a byproduct of greater total yield for the bigger male calves, but the significantly higher concentration of protein for sons reveals that mothers are making *different* milk for sons, not just more milk.

In a follow up study of 46 mom-calf pairs, the same research team reported that mean concentrations of calcium and phosphorus were higher for daughters, but magnesium concentration was higher for sons (Gallego et al., 2009). It should be noted, though, that differences between milk for sons and daughters, although statistically significant, were miniscule and the biological effect in calves is unknown. Taken together, these red deer studies demonstrate there can be differences in milk synthesized for sons and daughters.

Antarctic fur seals (*Arctocephalus gazella*) & California sea lions (*Zalophus californianus*)

Two meta-analyses of wild-living seals and sea lions have addressed sex-biased milk production. Lunn and Arnould (1997) aggregated data from multiple field seasons and found no evidence that seal mothers favored sons or daughters in nursing behavior, foraging effort, or milk energy transfer (as measured through the gold standard of hydrogen isotope dilution). Although male pups weighed more than female pups, their growth trajectories were seemingly parallel. The greatest divergence in growth trajectories occurred after weaning, during juvenility, suggesting that maternal investment during early development was not a major contributor to differences in body size between males and females.

Ono and Boness (1996) also aggregated data from multiple field seasons and found something slightly more exciting in sea lions- milk energy transfer was greater for sons, but only because they were bigger than daughters. The authors concluded that milk production in sea lion mothers was a function of pup mass, not pup sex. Since males were bigger at birth, the mammary gland synthesized milk to sustain that pup mass and growth trajectory. Most interestingly, though, growth in male pups was seemingly enhanced because they had a lower resting metabolic rate than female pups. Whereas female pups burned more energy on body maintenance, male pups got “bonus” energy for growth from their lower resting metabolic rate.

Because both studies used isotopic methods for inferring caloric transfer via milk, we know little about the composition or volume of milk being produced by mothers of sons vs. mothers of daughters. However, the sea lion study demonstrates that mammalian young are not passive recipients of mother’s milk. Physiological processes within the young may differ between the sexes influencing the utilization of ingested milk.

Rhesus monkeys (*Macaca mulatta*)

Sex-biased milk synthesis has been documented in captive rhesus monkeys. At the California National Primate Research Center, mothers in the outdoor breeding colony synthesize different milk for sons and daughters. In the interest of full disclosure, the results reported from rhesus macaques come from my own research program, so two things are obvious: 1) I likely have unconscious biases and 2) it’s the most magnificent research ever.

In rhesus monkeys, the milk produced for sons was significantly higher in percent fat. This effect was primarily due to first-time mothers (known as primiparous). Experienced mothers, known as multiparous mothers, showed much smaller sex-biases. Primiparous macaque mothers that produced a son had milk that was 7.7% fat, whereas mulitparous mothers rearing sons produced milk that was 6.5% fat. In contrast, primiparous and multiparous mothers rearing daughters produced 5.3% and 6.2% milk fat, respectively. Primiparous mothers of sons also had significantly higher protein concentration in milk (2.5% vs. ~2.1% in all other groups) (Hinde, 2007).

Why would primiparous mothers differ from multiparous mothers? Primiparous macaque mothers initiate reproduction while they are still growing, so they are simultaneously paying the energetic costs of growth and reproduction. Additionally, because they are not yet full size, they have fewer fat stores on their body to mobilize for lactation. Lastly, infants must attain a threshold size to survive, so first-born infants are proportionally bigger relative to the mother's size (Hinde, 2007, 2009). This means primiparous mothers have higher operating costs and fewer resources to rear a relatively bigger baby. No easy feat.

Natural selection favors adaptations that maximize lifetime reproductive success, known in evolutionary biology as "life-history theory." Over-investing at the outset of the reproductive career is unlikely to be favored by natural selection. Investing too much on the first reproductive effort can potentially stunt growth, lengthen the inter-birth interval, and reduce survival. All these things are likely to generate a frowny face from natural selection. One solution for primiparous mothers is to cut corners with daughters, because it shouldn't be too costly in terms of the number of grand-offspring she'll produce. And that's what I found, reduced milk energy content, and first-born daughters were quite small.

But the sons of primiparous mothers would have to compete with the strapping lads of multiparous mothers. A subset of primiparous mothers rearing sons cut bait: first-born sons were much more likely to die than expected from the population mortality rates for either male infants or first-born infants. But for the rest of first-born males, primiparous mothers seemingly pumped them full of fat and protein, and at peak lactation they were comparable in body size to the sons of multiparous mothers. To be clear, mothers are not consciously making Sophie's choice investment "decisions." Rather, physiological adaptations sensitive to energy balance and infant characteristics are the underlying mechanisms that contribute to sex-biased milk synthesis.

Humans (*Homo sapiens*)

Sex-biased milk is not just found in other species- humans produce sex biased milk, too! Two preliminary studies indicate there are sex biases in milk synthesis in human mothers. In 2010, Powe et al. reported that among 25 Boston-area women, mothers of sons produced ~25% higher energy density in milk than mothers of daughters. More recently, Fujita and colleagues (2012) revealed sex-biases in the milk fat concentration among 72 women in rural Kenya. On average, mothers of sons produced significantly higher fat concentrations in milk. However, there was an interaction between infant sex and maternal socioeconomic status. Relatively wealthier women- women whose household owned more land and more dairy animals (such as camels, cattle, goats, and sheep)- produced milk with higher fat concentrations for sons. But relatively poorer women produced higher fat concentrations for daughters. Interesting, higher socioeconomic status was associated with lower mean fat concentrations in milk, possibly because such mothers were producing *more* milk, however to date no studies have investigated sex-biased milk volume in humans.

Bank vole (*Myodes glareolus*)

All of the above examples come from studies that typically produce a single young at a time. But in species that produce litters, could there still be sex-biases in milk? In 2009, Koskela and colleagues created all female or all male litters of the same size in bank voles by cross-fostering newborn pups among moms. They found that bank voles rearing litters of all female pups produced significantly more milk than voles rearing all male litters. As a result of more milk, female pups grew more. Unlike the non-human mammals described above in which being bigger is important for male reproductive success, in bank voles being bigger is very important for females. Indeed, adult females are the same size as males-or bigger. The results of this artificial manipulation are quite tantalizing. Importantly, because this was a cross-fostering study, it lets us know that the moms were responding to pup signals after birth! The next question is, what happens in "natural" litters? If you are a female pup born into a mostly male litter, will you get less milk and grow worse than expected? If you are a male pup born into a mostly female litter, will you get more milk and grow better than expected?

Conclusions

The important take home message is that in the deer, voles, monkeys, and humans, the "recipe" for boy milk and girl milk is different. Other parameters, such as infant mass and maternal mass, parity, and access to resources, can also play mediating roles. However, to date, relatively little research effort has been dedicated to investigating sex-biased milk synthesis. Perhaps because the topic sounds dangerously close to "milk is sexist." Understanding how milk varies among mothers, especially in relation to infant characteristics, is critically important for human health. These complexities can be translated into optimal selection of donor milk for at-risk NICU babies and in the modification of commercial infant formulas. Moreover, many, many questions remain. For example, to what extent are other constituents, such as hormones, similar or different in milk produced for sons vs. daughters? How do sons and daughters differentially utilize milk from the mother? What are the signals and mechanisms through which the mammary gland "knows" it's synthesizing milk for a son or a daughter? Clearly this is not an exhaustive list of the potential questions lactation science can tackle, among the many others, but it does highlight exciting potential directions for research in nutrition, evolutionary biology, anthropology, and animal science.

Fujita M, Roth E, Lo YJ, Hurst C, Vollner J, Kendell A. (2012) In poor families, mothers' milk is richer for daughters than sons: A test of Trivers-Willard hypothesis in agropastoral settlements in Northern Kenya. Am J Phys Anthropol. **149**:52-59.

Gallego L, Gómez JA, Landete-Castillejos T, Garcia AJ, Estevez JA, Ceacero F, Piñeiro V, Casabiell X, de la Cruz LF. (2009) Effect of milk minerals on calf gains and sex differences in mineral composition of milk from Iberian red deer (*Cervus elaphus hispanicus*). Reproduction. **138**:859-65.

Hinde K. (2009) Richer milk for sons but more milk for daughters: sex biased investment during lactation varies with maternal life history in rhesus macaques. Am J Hum Biol. **21**:512-519.

Hinde K. (2007) First-time macaque mothers bias milk composition in favor of sons. Curr Biol. **17**:R958-R959.

Koskela E, Mappes T, Niskanen T, Rutkowska J. (2009) Maternal investment in relation to sex ratio and offspring number in a small mammal – a case for Trivers and Willard theory? J Anim Ecol. **78**: 1007–1014.

Landete-Castillejos T, Garcia AJ, López-Serrano FR, Gallego L. (2005) Maternal quality and differences in milk production and composition for male and female Iberian red deer calves (Cervus elaphus hispanicus). Behav Ecol Sociobiol. **57**:267-274.

Lunn NJ, Arnould JPY. (1997) Maternal investment in Antarctic fur seals: evidence for equality in the sexes? Behav Ecol Sociobiol. **40**: 351-362.

Oftedal, O. T. & Iverson, S. J. (1995) Phylogenetic variation in the gross composition of milks. In Handbook of milk composition, (ed. R. G.Jensen), pp. 749–789. Academic Press, New York.

Ono KA & Boness DJ. (1996) Sexual dimorphism in sea lion pups: differential maternal investment, or sex-specific differences in energy allocation? Behav Ecol Sociobiol. **38**:31-41.

Powe CE, Knott CD, Conklin-Brittain N. (2010) Infant sex predicts breast milk energy content. Am J Hum Biol. **22**:50-4.

Contributed by
Prof. Katie Hinde
Human Evolutionary Biology
Harvard University

The evidence around raw milk

- **The CDC estimates the risk of a glass of raw milk causing a disease outbreak is at least 150 times that of a glass of pasteurized milk.**
- **A large cross-sectional study in Europe investigated the effects of raw milk on children's health.**
- **This study found substantial evidence that children who grew up drinking raw milk, instead of boiled milk, were less likely to develop allergies, including asthma and dermatitis, during childhood.**
- **More research is needed to determine the effects of raw and pasteurized milk consumption on allergies, and the mechanisms behind these effects.**

Is unpasteurized (or raw) cows' milk good or bad for you? It's a simple question with vociferous and opposite answers depending on who you ask. Agencies of the United States federal government, such as the Centers for Disease Control and Prevention (CDC), point to numbers that link consumption of the stuff to a heightened risk of tummy bugs and worse. Meanwhile, activists make pasteurization seem outright dangerous and raw milk sound like a wonder food. Is it?

The data suggest that raw milk can cause both trouble and advantage to a human body.

On the one hand (the bad one), milk comes from the end of the cow whence many unhealthy bacteria emanate, and pasteurization serves the purpose of killing those nasty germs. Hygienic milking practices should do away with many of those concerns, but they aren't foolproof: of 4,413 illnesses in the US linked to dairy products between 1993 and 2006 (for which the pasteurization status was known), 60% were due to raw milk or raw cheese [ref. 1]. But, because maybe 1% of the milk consumed in the country is raw, the CDC reckons the risk of a glass of raw milk causing a disease outbreak is at least 150 times that of a glass of pasteurized milk. Moreover, disease outbreaks associated with raw milk were 13 times more likely to land people in the hospital. This is because some involved a particularly nefarious sort of bacterium called E. coli O157:H7, which can lead to kidney damage.

Heidi Kassenborg, of the Minnesota Department of Agriculture, says that raw milk consumption can also cause infection by campylobacter, salmonella, and listeria. She points to a website (realrawmilkfacts.org) with, "Really compelling videos of people who thought they were doing the right thing for their children when they bought raw milk, and are pretty sorry now because they have wound up with a child with a horrible disease."

Now for some good news. To be sure, high heat treatment of milk reduces the odds of a bad belly, but does it also destroy complex proteins and other components that could bolster human health?

Apparently so. The best evidence comes from a large cross-sectional study that began in 2005 and followed school-aged children in rural areas of German-speaking Europe, specifically in Germany, Austria, and Switzerland. The study, called GABRIELA, was led by Erika von Mutius of the University Children's Hospital in Munich, Germany, and involved more than 8,000 kids [ref. 2]. Some lived in quaint villages and typically drank supermarket milk. Others lived on farms and often drank raw milk. The researchers gathered samples of milk from family fridges across the Alps and gave them to their colleagues for blind testing of the fat content, whey proteins, and bacterial count. GABRIELA found substantive evidence that raw milk-consuming farm kids were much less likely to develop allergies including asthma and dermatitis during childhood.

The findings require some clarification in their details. GABRIELA found that kids drinking milk that had received high heat treatment, as usually happens when milk is boiled at home, were more likely to develop allergies than the kids who drank raw milk. But the results for children who drank minimally pasteurized milk – of the sort typically found in US supermarkets, but not necessarily in European ones - were not statistically significant, possibly because the sample size in the study was small. The results were also independent of whether the kids were involved in farming.

You can't yodel with hay fever

The study reports that a category of components in raw milk affords much of this protection. Unlike the better-known casein proteins that compose about four-fifths of the protein content of cow's milk, and which are hard to denature with heat, the activities of most whey proteins (making up about one-fifth) are substantially reduced by high heat treatment. Three whey proteins appear particularly important in stopping farm children from getting allergies: a-lactalbumin, b-lactoglobulin, and another called BSA. The more of these whey proteins found in the Alpine milk, the stronger its preventative properties for asthma.

The study did many things well: it monitored a huge cohort of kids, and it corrected for important confounding factors - for example, the tendency for children with more older siblings to have fewer allergies and for farming families to be bigger. It also double-checked the conclusions by considering farm kids who drank milk boiled at home. (The results were broadly the same as those for the village kids drinking industrially pasteurized milk.) But the researchers openly admit that their culture techniques may not have captured the full diversity of bacteria in milk and that a prospective study would have been better than a cross-sectional one.

"It's also hard to disentangle the effects of the different whey proteins," says Georg Loss, the first author on some of the GABRIELA papers. "It might be that just one [of the three implicated by the study] is really responsible and the others co-vary with it."

Cross-continental effects

The notion that growing up on a farm helps the immune system to develop properly gains credence from a group of rural folk on the other side on the Atlantic, the Amish, a religious sect living as their founders did in the 1850s. In the late 1980s, a physician called Mark Holbriech set up free allergy clinics for the Amish community, who lack health insurance, near his home in Indiana. But almost nobody came. And of those who did show up, allergy was rarely the underlying medical problem.

Holbriech contacted von Mutius after reading her studies on what has become known in the field as 'the farm effect'. The two then embarked on research that revealed just how unruffled the immune systems of Amish children living essentially nineteenth century rural lifestyles really are. "If you go into a school in an urban environment today and do skin or blood testing for allergies, you'll find that about 45-50% of the children will have some evidence of sensitization," says Holbriech. "For the Swiss farm children [in the GABRIELA study], it was about half as likely: 24%. But among Amish it was 7%."

So what is going on? Surely Amish children aren't unknowingly drinking a special kind of raw milk, super-infused with whey proteins? Of course not. But they are doing many other things early in life aside from drinking raw milk that are probably lowering their odds of allergies even more. "Amish children are in the barns from a very early time in their lives. They don't have cars, so they are around horses often," says Holbriech. "The signals the immune system receives may just be more intense in them." Indeed, GABRIELA picked out two other variables - spending time amongst cows and straw - that independently lowered the odds of developing asthma among European farm kids.

In search of mechansims

So is the farm effect largely a case of a much broader theory, known as 'the hygiene hypothesis', whereby early life exposure to general grubbiness lowers the odds of allergies later on? That may be relevant, but GABRIELA found no significant link between the total number of viable bacteria in milk and either asthma or general allergic sensitization (as measured by levels of the antibody, IgE). And the study's implication of specific whey proteins suggests raw milk is actively priming the immune system in some helpful way.

How this priming might happen isn't clear, though. The three whey proteins it highlights doesn't strongly suggest one molecular mechanism over many possible others. The whey proteins could be directly affording protection somehow, or they might influence the gut microbiota of children in a way that predispositions them to developing a healthy immune response over time. "And then there's this idea that maybe for each kind of allergic outcome you have different exposures that are relevant and different pathways," says Loss. "Before the study, lactoferrin was a hot candidate because it has been shown to have some immunomodulatory effects. But, while it did show some reduced risk in GABRIELA, it wasn't significant."

Another large cohort study, called PASTURE, by the same group probes the underlying causal relationships a little deeper [ref. 3]. It points a finger at the innate immune system (which, in turn and unlike GABRIELA, suggests that exposure to diverse germs is important).

PASTURE measured the gene expression of a list of receptors of the innate immune system on the day each baby was born (by sampling umbilical cord blood) and at one year of age (using samples of the babe's own systemic blood). The researchers homed in on this period of life because it is when the innate immune system is known to develop most rapidly and differentially. About half of the babies were born to women who lived and worked on a farm with livestock, and about half to women living nearby and didn't farm.

The blood mRNA showed that neonates of farmers had greater expression levels of innate immune system receptors in general, and notably more for two receptors called Toll-like receptors 7 (TLR7) and 8 (TLR8). Then, after drinking raw milk during much of their first year of life, the farm-bred infants expressed more TLR4, TLR5, and TLR6 receptors. Again, this finding was independent of living on a farm. And once again, babies who drank farm milk boiled at home showed no changes in the expression of these receptor genes compared to babies who drank the supermarket milk in the study.

So what is going on? Specifically, it's hard to say. But immune pathways that are regulated by Toll-like receptors are understood to be important in boosting other regulatory pathways that inhibit allergic responses.

Of course, there are likely to be many mechanisms acting in concert. And GABRIELA didn't consider all of the molecules that might be important [ref. 4], such vitamin A, a metabolite of which is required for the proper development of immune cells called Treg cells. Other work suggests the bovine cytokine IL-10, which is found in cows’ milk, changes the activity of human monocytes and dendritic cells [ref 5].

Is raw milk good or bad for you? It's clear that it can be harmful when contaminated. And there is strong evidence that it benefits young children, but almost no information of substance about adults. To answer the question fully, the world needs studies testing whether large numbers of grown-ups suffering from asthma, hay fever, and similar medical problems see their allergies dampen down after drinking raw milk for a prolonged period. Until that day, the question is still open.

CLARIFICATION: Since its original publication, this article has been updated with the addition of an editor's note (below) and a paragraph (the 7th) that points out differences in the extent to which supermarket milk is typically heat treated, and explains the statistical results in the Gabriela study. Also, the phrase "most whey proteins.. don't survive pasteurization", has been more precisely restated as "activities of most whey proteins... are substantially reduced by high heat treatment".

EDITOR'S NOTE: *While there is now some evidence that uncontaminated raw milk may protect young children from developing allergies and asthma, is that benefit worth the risks? More studies are needed to determine the benefits of raw milk consumption and how those benefits can be retained with milk processing techniques that minimize pathogen risk. Until more is known, the researchers interviewed for this article do not recommend the consumption of raw milk.*

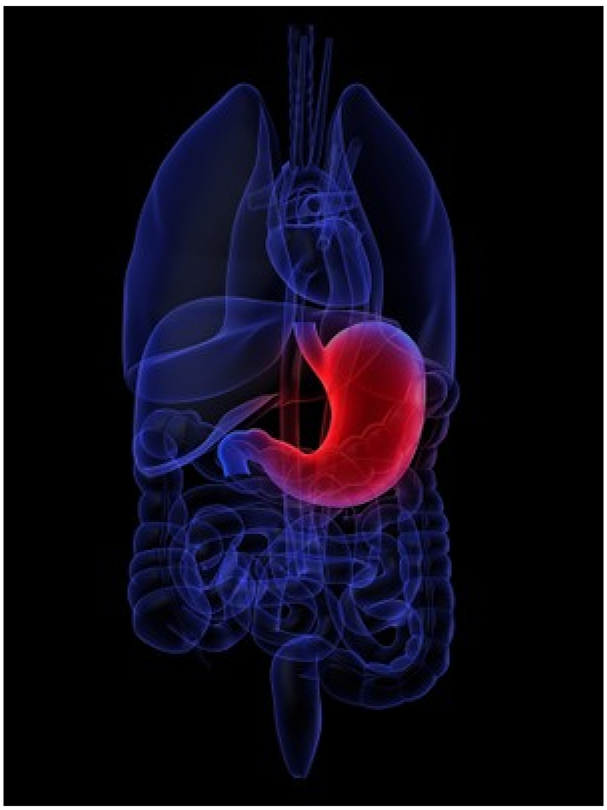
1. Langer AJ, Ayers T, Grass J, Lynch M, Angulo FJ, Mahon BE. (2012) Nonpasteurized dairy products, disease outbreaks, and state laws-United States, 1993-2006. Emerg Infect Dis. 18: 385-391.
2. Loss G, Apprich S, Waser M, Kneifel W, Genuneit J, Buchele G, Weber J et al. (2011) The protective effect of farm milk consumption on childhood asthma and atopy: The Gabriela study. J Allergy Clin Immunol. 128: 766-773.
3. Loss G , Bitter S, Wohlgensinger J, Frei R, Roduit C, Genuneit J, Pekkanen J et al. (2012) Prenatal and early-life exposures alter expression of innate immunity genes: The PASTURE cohort study. J Allergy Clin Immunol. 130: 523-530.
4. van Neerven RJ, Knol EF, Heck JM, Savelkoul HF. (2012) Which factors in raw cow's milk contribute to protection against allergies? J Allergy Clin Immunol. In press.
5. den Hartog G, Savelkoul HF, Schoemaker R, Tijhaar E, Westphal AH, de Ruiter T, van de Weg-Schrijver, van Neerven RJ. (2011) Modulation of human immune responses by bovine interleukin-10. PLoS One. 6:e18188.

Contributed by
Anna Petherick
Professional science writer and editor
www.annapetherick.com

Proteases vs. antiproteases: The battle over milk digestion

- **Milk partially digests its own proteins to release bioactive peptide fragments.**
- **Milk peptides have health-enhancing functions ranging from immune-building to antimicrobial to nutritional.**
- **Supplementation of diets with milk peptides may improve the health of premature infants and other immuno-compromised populations.**

Surprisingly, milk protein digestion begins in the mammary gland, long before the milk is consumed. There, a battle rages between enzymes called proteases, which break down proteins, and antiproteases that act as shields by protecting other proteins from being completely digested. The result of this competition is a delicate balance of intact protein and partially digested protein segments. Research by Ferranti et al. revealed that milk, prior to ingestion, contained over 100 unique milk protein fragments (mostly deriving from the protein family called caseins) [3]. This finding demonstrates that proteases are busy breaking down proteins in the mammary gland.



So what does all of this mean? And what happens to these proteins after milk consumption if they're already partially digested?

In vitro studies trying to replicate milk protein digestion show that milk peptides exert an array of effects, including behavioral, gastrointestinal, hormonal, immunological, neurological, and nutritional [2]. For example, fragments of milk casein aid in absorption of lipids and micronutrients, immune defense, antimicrobial protection, nerve transmission, social behavior modulation, analgesia (pain killers), decreasing diarrheal effects, and stimulating hormonal secretions.

However, all of these functions have only been shown in cell and animal models, and therefore cannot necessarily be extended to humans. Moreover, the peptide fragments tested so far have been artificially created with *in vitro* digestions of human milk proteins, and these fragments are different from those naturally occurring or created during infant digestion. Even the best scientists with advanced technologies cannot hope to accurately replicate the digestion of proteins—the gastrointestinal system is a far too complex environment.

So, though we know milk peptides have a large potential to be bioactive, we are still left with these questions: *What peptides are actually produced and where are they along the digestive tract? How do these peptides actually affect human health?*

Of course, in the gastrointestinal tract, milk proteins are exposed to even more enzymes that produce even more peptide fragments. There is little data on the products of protein digestion in infants. However, it is clear that not all proteins are digested into single amino acids. A small but significant percentage of some milk proteins, including lactoferrin and immunoglobulins, have even been found still intact in infant feces [4]. The annotation of peptides at various sites in digestion is critical for predicting their bioactivities. These fragments are so small and low in abundance, however, that even though their presence was known for decades, their identities could not be determined until recently. Fortunately, advances in mass spectrometry and database search now allow scientists to fully annotate large arrays of liberated peptides for their sequences and precursor proteins during digestion and to predict their function.

But that's not the end of the story!

It has been known for quite some time that a variety of common commensal bacteria in the digestive tract can also help break down dietary protein [5]. Interestingly, even *Bifidobacteria infantis*, which is associated a healthy gut flora and which consumes human milk oligosaccharides, has also been shown to grow well on pre-digested milk lactoferrin and immunoglobulins [6]. However, until now, it was impossible to determine the fragments as they are released. The last obstacle to full characterization of the microbes' effects is the difficulty in collecting the appropriate samples without invasive procedures.

The next step will be to take the peptides shown to exist in intact milk and at various places in the gastrointestinal tract and assay them for an array of bioactivities. Researchers at UC Davis are already exploring many exciting new bioactivities of these peptides.

This work is of critical importance because each year, more than half a million babies (about 1 in 8 deliveries) are born prematurely in the United States. Premature infants are incapable of digesting proteins as well as full term infants. That means premature infants are missing out on an army of protective bioactive peptides. This fact could be partially responsible for their poor health outcomes. Unfortunately for these infants, current neonatal infant care units focus principally on devices to monitor disease progression and little on improving infant nourishment. The development of a formula using an enzymatic pre-digestion of milk could provide exactly the right bioactive peptides from day one to ensure proper bacterial colonization of the premature gut for disease prevention. Besides improving the health of premature infants, bioactive milk peptides could potentially be isolated from bovine milk and employed to enhance health in specific consumer populations, ultimately enabling milk to be even more protective and supportive of human health.

1. Dallas DC, Underwood MA, Zivkovic AM, German JB. (2012) Digestion of Protein in Premature and Term Infants. J Nutr Disorders Ther. 2: 2161-0509.

2. Clare DA & Swaisgood HE. (2000) Bioactive milk peptides: a prospectus. J Dairy Sci. 83: 1187-1195.

3. Ferranti P, Traisci MV, Picariello G, Nasi A, Boschi V, Siervo M, Falconi C et al. (2004) Casein proteolysis in human milk: tracing the pattern of casein breakdown and the formation of potential bioactive peptides. J Dairy Res. 71: 74-87.

4. Davidson L. & Lönnerdal B. (1987) Persistence of human milk proteins in the breast-fed infant. Acta Paediatr. 76: 733-740.

5. Macfarlane G. & Allison C. (1986) Utilisation of protein by human gut bacteria. Fems Microbiology Letters. 38: 19-24.

6. Liepke C, Adermann K, Raida M, Magert HJ, Forssmann WG, Zucht HD. (2002) Human milk provides peptides highly stimulating the growth of bifidobacteria. Eur J of Biochem. 269: 712-718.

Contributed by
Dr. Dave Dallas & Prof. Daniela Barile
Dept. of Foods Science & Technology
University of California, Davis

Do the math - cow burps are profound!

- **Methane is a greenhouse gas with a global warming potential around 25 fold more powerful than carbon dioxide.**
- **Over the course of a year, the methane from one cow's belches is equivalent to the carbon dioxide emission from a small car.**
- **Accurately measuring methane release from cows is important for understand the role of cow erucations in climate change and how we might curtail their effects.**

In some societies, burping in public is considered very bad manners, often evoking a silent, disapproving frown or, if at home, a strong verbal reprimand. Sometimes burping alone gives immense personal satisfaction – a job well done. In more enlightened societies, a guttural burp is the ultimate complement, a mark of appreciation for excellent food. Human burps are, in the grand scheme, socially important, but in reality quite innocuous.

Burping by cows, or to use the more descriptive technical term, eructation (or belching), is a natural sign of good health. However, there is a dark side to cow belching – methane, a potent greenhouse gas.

To put this into perspective, the world's ruminant livestock collectively produce about 100 million tonnes of methane each year, mostly through belching. Non-ruminant species, including people, also emit methane, but only very small amounts in comparison. Hence, cow burps are big contributors to climate change, and they are now at the front line of scientific investigations and government policy.

Efficient management of methane emissions from livestock populations and indicators of the success of intervention programs critically rely on the collection of data, but there is a problem. How do you accurately and cost effectively measure methane emitted in burps by commercial populations of livestock?

Two recent publications by Garnsworthy and colleagues[1] have taken the first practical steps to address this measurement issue in dairy cows. Measurement is all-important. Much heated debate, dare I say hot air, in this politically charged arena occurs in the absence of good comprehensive data sets.

Why do ruminants produce methane?

Methane is a gas produced by specific groups of micro-organisms (largely belonging to the Archaea group) in the rumen of the cow and related species. It is a natural by-product of the fermentation process converting ingested plant material, cellulose, into small molecules that fuel animal growth, milk production, and maintenance of life.

There is also a large amount of energy trapped within methane not used by the cow, and therefore methane emission represents a major energy inefficiency of rumen fermentation. It is estimated the energy associated with methane emission from one cow in one year could power a six-cylinder car for 1000 km. Methane is an energy source not being converted into milk or meat.

Climate change and methane

Methane is a greenhouse gas with a global warming potential about 25 fold more powerful than carbon dioxide. A single cow produces between 80 and 120 kg of methane each year. This is equivalent in impact to the carbon dioxide emission from a small car over one year. Thus, the world's approximate two to three billion ruminant livestock collectively emit very large quantities of methane. Climate scientists conclude this methane is a significant contributor to climate change. About 18% of all greenhouse gas emissions arising from the actions of humans are thought to be due to livestock. Under the terms of the 1997 Kyoto protocol, countries classed as 'Annex 1' are required to submit annual inventories of greenhouse gas emissions and to decrease total emissions by 5.2% of 1990 values before 2012. Measurement of methane is the key to these reports.

Measuring methane

It's technically relatively easy to measure carbon dioxide emissions from cars and factories. Measuring methane emission from cows is much more difficult. Garnsworthy and colleagues have identified the desirable characteristics of an ideal methane measurement. It must be relevant to commercial production systems, inexpensive, easy to implement, accurate, and reproducible and applicable to monitoring individuals within a herd. This is a tall order.

Fundamentally, there are three techniques. The gold standard is the respiration chamber where the quantity and composition of all gases emitted by a single cow are accurately measured in a sealed chamber over a period of two to three days. Unfortunately, this is a very artificial environment for the cow and hence the data may not reflect reality in a herd on pasture. The major limitations of this technique are its expense and impracticality for commercial operations such as a dairy herd.

The second method uses canisters attached around the necks of cows. These continuously sample expired breath, which is then analysed for methane content. The canisters may interfere with cow feeding behaviour. Moreover, the technique also requires the introduction of a chemical bolus into the rumen and considerable animal handling; both interfere with cow behaviour. This method is impractical and likely unacceptable for use within commercial herds.

The third method, and the one exploited by Garnsworthy and colleagues, measures methane emission during the milking of individual cows using methane air analysers installed in the feeding troughs of automatic milking stations. This technique does not disturb cow behaviour or feeding, and it produces considerable data that very clearly demonstrates repetitive belching during milking and feeding. Importantly, the technique was validated by the respiration chamber technique and was shown to be a good indicator of total daily methane emission. The technique is reliable, inexpensive, and potentially easily implemented in a commercial operation. These factors allow collection of considerable data from a large number of animals, thereby increasing the statistical robustness of the data.

Modifying methane emission from the cow

Garnsworthy and colleagues concluded there was considerable individual variability in methane emissions from cows on similar rations. This suggests there may be scope for genetic selection of animals with lower methane emission per litre of milk produced. Recent technological advances in genetics may allow this possibility to become reality in the future.

The availability of good methane measurement technology enables experiments to be performed that optimise feed, animal management, and genetics to minimise methane emissions whilst maintaining milk production. One obvious strategy is to increase fermentation efficiency in the rumen, which has the capacity to both decrease methane emission and make available more energy for milk production. Is this realistic, or just a scientific daydream?

Retrospective calculations made by Chase[2] (Cornell University) indicate there was a remarkable 40% reduction in methane emission per litre of milk produced in the USA from 1944 to 2007. In the same time period, the number of dairy cows decreased by 35% and amazingly, total milk production increased to 159%. Many factors contributed to these figures, but clearly history indicates further improvements in methane emissions from dairy cattle are feasible.

The livestock industry produces milk, meat, fibre, and hides to the world's seven billion people. However, the simple mathematics of multiplying total livestock number by the methane emitted per animal generates a dauntingly large quantity of total emitted methane. Reducing methane emission from livestock is essential to minimise its impact on climate change.

There is realistic opportunity we can achieve this goal. Perhaps the solution is in the words of Dolly Parton - *We cannot direct the wind, but we can adjust the sails.*

1. Garnsworthy PC, Craigon J, Hernandez-Medrano JH, Saunders N. (2012) On-farm methane measurements during milking correlate with total methane production by individual dairy cows. J. Dairy Sci. 95: 3166-3180.

2. Garnsworthy PC, Craigon J, Hernandez-Medrano JH, Saunders N. (2012) Variation among individual dairy cows in methane measurements made on farm during milking. J. Dairy Sci. 95: 3181-3189.

3. Chase (Cornell University). Methane emissions from dairy cattle.
http://www.ag.iastate.edu/wastemgmt/Mitigation_Conference_proceedings/CD_proceedings/Animal_Housing_Diet/Chase-Methane_Emissions.pdf

Contributed by
Dr. Ross Tellam
Senior Principal Research Scientist
CSIRO Livestock Industries
Queensland, Australia

Are you subscribed to our email newsletter?

Sign up for our Email Newsletter