SPLASH!® milk science update July 2016 Issue

This issue features superior hydration with milk, tolerizing to gut microbes with milk antibodies, whale lactation strategies, and human milk proteomics.

Beverage Hydration Index: Milk Bests Water

- **Researchers in the UK have developed the concept of an index that indicates how effective different beverages are at keeping the body hydrated.**
- **In a trial to calculate a beverage hydration index, full fat and skimmed milk performed better than water at keeping participants hydrated.**
- **The reason for this probably lies with its electrolyte, fat, and protein components, which means that water absorption from milk tends not to prompt sudden reductions in the blood's osmotic pressure.**

What do explorers in the Sahara Desert and cops on a stakeout have in common? They would both benefit from knowing which beverages are best at promoting fluid retention. The explorers can only carry so much liquid. The cops have few opportunities to urinate. They both need to stay hydrated.

This kind of information is exactly what a team of British researchers set out to provide [1]. Led by Ronald Maughan of Loughborough University—an institution that is known for its sports science—the group has proposed an index that describes how well a normally hydrated, healthy person retains the water in a beverage. The idea presumably, is not only to guide the decisions of desert explorers, and police officers hiding in vans—it would also help canoeists, dingy sailors, actors playing roles requiring an all-in-one bodysuit—and anyone trying to break a record for the longest uninterrupted time doing anything.

The researchers present hydration index calculations for thirteen common beverages [1]. To perform these calculations, they first gathered data on how the human body responds to the consumption of those beverages by running a randomized, controlled trial.

Seventy-two young men of roughly the same height and weight were primed for the experiment, by fasting overnight, emptying their bladders upon waking, and then consuming half a liter of still mineral water over a period of fifteen minutes. This ensured they were all equally hydrated at the start of the experiment. After arriving at the lab, the young men were asked to ingest a liter of an assigned beverage over half an hour, and to empty their bladders on the hour, every hour, as the researchers took various measurements.

Every participant followed this routine four times. On one occasion, their assigned beverage was a liter of still water, to serve as a control. On the three other days, they received a different test beverage that was randomly selected from a list that included Powerade, Coca-Cola, Diet Coke, beer, cold and hot black tea, hot coffee, oral rehydration solution, orange juice, and skimmed and full-fat milk.

When it came to keeping people hydrated over time, the results for milk were the most impressive. Overall, the data showed that three of the test beverages were consistently better than water at this. One was obvious—the oral rehydration solution, Dioralyte, which is a medical product designed specifically for that task. But the other two were skimmed and full-fat milk, with the full-fat milk recording a Beverage Hydration Index approximately equal to that of the oral rehydration solution. Even when the index was calculated in such a way as to take into account the exact amount of water in milk—which is arguably required to compare like with like, given that milk contains things other than water, such as fat, protein, and sugars—the benefits of hydrating with milk over water were still highly significant.

Of the other beverages tested, orange juice fared the best. It scored roughly as well as water, and at one time point, it was a slightly better. All the rest of the test beverages, however, scored worse—including the sports drink, Powerade, as well as beer, coffee, tea, Coca-Cola, and Diet Coke.

Why would milk be so good for hydration? For one thing, it doesn't contain diuretic agents like caffeine and alcohol. But what makes it even better at the job than water is probably its blend of electrolytes, plus its small amounts of protein and fat that require breaking down in the gut. The latter fact means that the water in milk entered the blood more slowly than the other drinks did. Which, in turn, means that there is a less dramatic fall in the osmotic pressure of blood. And because a drop in osmotic pressure has a diuretic effect, drinking milk makes one urinate less than drinking water. Other researchers have looked into this effect specifically in the context of replacing fluids lost during exercise [2].

A beverage hydration index might offer a new way to encourage people to think about their hydration levels, and to drink products that are better for their health than beer, coffee, and cola. One could imagine it added to beverage packaging, rather like nutrient content tables are at present. After all, inadequate hydration has been linked to all kinds of bodily disorders—not just to headaches, but to gastrointestinal and circulatory problems, too.

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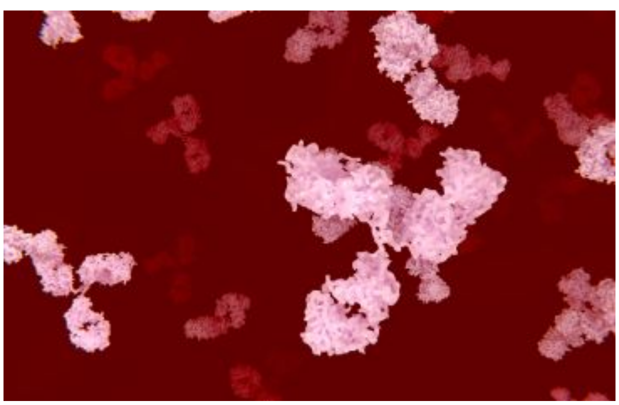
Maternal Milk Antibodies Prepare Newborn Mice to Host Commensal Gut Microbes

- **Mammalian milk is known to contain antibodies, and these are thought to help newborns fight off infections.**
- **A new study finds that milk antibodies are also important in dampening the immune response to commensal gut bacteria in mice.**
- **Mice without milk antibodies from their mothers had increased activation of T cells and were more susceptible to inflammatory responses caused by commensal gut microbes.**
- **Maternal milk antibodies could thus help newborn mice prepare their immune response for commensal gut microbes, many of which they also acquire from their mothers.**
- **Understanding the role of antibodies in human milk could help prevent dysfunctional immune responses to commensals that can lead to chronic inflammation and conditions such as Crohn's disease.**

Our immune system protects us from many harmful microbes, but in doing so it needs to be able to differentiate between friend and foe. Our bodies harbor many beneficial gut bacteria that play important roles in digestion and immunity, and our immune system needs to react differently to these microbes compared with harmful pathogens [1,2].

A new study in mice finds that antibodies present in maternal milk may play an important role in suppressing the immune response to commensal gut microbiota [3]. "It's kind of a cool evolutionary system where the mother will provide her microbiota and then provide these antibodies to instruct the pup's immune system on how to deal with the microbiota," says Meghan Koch, a postdoctoral fellow in Gregory Barton's laboratory at the University of California, Berkeley.



Dysregulated immune responses against gut microbes can lead to chronic inflammation and conditions such as Crohn's disease and ulcerative colitis [4]. "There's definitely a lot of interest in controlling immune responses to the microbiota," says Koch. "You could see that being a potential therapeutic," she says.

Mothers have been previously shown to influence their newborn's immunity by providing antibodies either through milk or through the placenta, and these were mainly thought to help newborns fight off infections [5,6]. Koch discovered that specific types of antibodies in mouse milk—immunoglobulin G2b and G3 (IgG2b and IgG3)—play a role in suppressing the immune response to commensal gut microbes. These antibodies act in conjunction with another type of antibody, called immunoglobulin A (IgA), in maternal milk.

"In particular, the role of IgG in the maternal milk has not really been appreciated," says Koch. She notes that the studies may not translate directly to humans, where IgG is transferred to newborns mostly *in utero* rather than in human milk. If further studies could demonstrate that these maternal antibodies play a similar role in humans, this "could maybe help to improve formula for humans," she says.

Koch et al. [3] started the project with the intention of trying to probe the immune response to microbiota, and decided to do so by looking at antibody responses. "We could easily recover serum antibodies from mice, and we could easily recover microbiota from mice just by taking feces," says Koch. The researchers then looked for serum antibodies that bound to commensal bacteria. "In addition to IgA antibodies which have been previously published, we also found IgG antibodies binding to the microbiota," says Koch. "We were really surprised," she says.

Koch found that IgG2b and IgG3 antibodies bound microbes at a particularly high frequency. "We found that the serum concentrations of these antibodies are really high early in life, around two and three weeks of age, which coincides with the time points that young mice are still with their mothers," says Koch. "That led to our idea that maybe these antibodies are maternally acquired," she says.

The researchers found that the anti-commensal IgG responses were primarily mediated by maternally-derived antibodies. They found that these maternal milk antibodies normally signal the innate immune system rather than T cells of the adaptive immune system. "They were T-independent, and we think somehow they're helping inform appropriate immune responses to the microbiota," says Koch. "The idea was that the mother will provide her young with microbiota, and in addition to providing this microbiota, the mother might also provide some sort of instruction in the form of antibodies of how to appropriately respond to these vast array of diverse microbes that the animal's encountering for the first time," she says.

Without milk antibodies from their mother, newborn mice showed increased effector T cell responses compared with mice exposed to maternal antibodies. Koch notes that despite this increased T cell response, the mice did not get colitis, suggesting that there are other regulatory mechanisms. "I think it really speaks to the fact that there's multiple layers of regulation that are helping control immune responses in the gut and prevent inflammation," says Koch.

The study illustrates the important role maternal milk plays in modulating the infant immune system. Koch also plans to explore the role of maternal milk antibodies in adult mice to see what role they play later in life. "We're trying to work on what are the downstream consequences of lacking maternal antibodies early in life," she says.

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A Whale of a (Milk) Tale

- **Cetaceans are marine mammals that include baleen whales, such as the blue whale, and toothed whales, such as killer whales and dolphins.**
- **Baleen and toothed whales have dramatically different approaches to lactation, with baleen whales producing high quantities of very high-fat milks over a short period of time, and toothed whales producing lower fat milks over a longer period of time.**
- **The slower approach of toothed whales resembles that of primates, reflecting the shared traits of large relative brain size and slow offspring growth and development between these two groups of mammals.**
- **There are likely many exceptions within each group to these broad lactation patterns, but methodological issues that limit lactation studies on whales and dolphins prevent a full understanding of the diversity of lactation strategies within cetaceans.**

How much weight should a female gain during pregnancy? Forty-five thousand kilograms (or nearly 100,000 pounds) may seem an excessive amount, unless of course the question is directed at the blue whale (*Balaenoptera musculus*), the largest animal on the planet. Blue whales, along with other species of baleen whales, are remarkable for their ability to put on massive amounts of blubber during pregnancy. What may be even more remarkable, however, is their ability to quickly transfer that stored energy to their developing calf during lactation, all while consuming little, if any, food. But not all whales share this intense lactation strategy. The toothed whales (the evolutionary "cousins" of baleen whales) are the tortoise to the baleen whales' hare, taking the slow and steady approach to milk production that is reminiscent of humans and other primate species. Why don't the baleen whales pace themselves a bit more? Or, alternatively, why don't the toothed whales pick up some speed? The evolution of two such dramatically different strategies in these closely related marine mammals can only be understood in the context of the species' entire life course. After all, evolutionary success is not about winning a sprint, but successfully playing the long game.

Big gulps and echoes

Cetaceans are an order of mammals divided into two suborders: the baleen whales (Suborder mysticetes) and the toothed whales (Suborder Ondocetes). These two groups diverged approximately 38 million years ago, and the common ancestor had teeth, rather than baleen [1]. Baleen is made out of keratin, the same substance that makes up your fingernails, and baleen whales use it in a unique way to obtain food. Plates of baleen, each fringed by a network of hair-like threads, descend from the upper jawbone like teeth on a comb. Baleen whales open their mouths and take in huge quantities of water along with choice food items—often tiny—swimming in that water. Then, using their tongues, they push the water back out through the baleen like a filter, leaving behind the small fish, plankton, and krill (a small shrimp) that they swallow. One krill may not have many calories, but some estimates suggest a blue whale can eat up to four tons (8,000 lbs) of krill in a single day [2]!

Toothed whales (a group that also includes all dolphins and porpoises) have to work a little harder than simply gulping their food. They are active hunters that seek out and snatch fish, squid, or in the case of some killer whales, large marine mammals. Many toothed whales utilize echolocation to find their prey—like bats, they emit sound waves and use the sound waves that return to determine prey size and location (as well as information about their surrounding environment, such as distance to the ocean floor).

Saving for a milky day

The ways in which baleen and toothed whales make their living are intimately tied to their specific patterns and rate of growth, development, and reproduction. This includes their lactation strategy, which is described by the milk composition, quantity, the frequency of nursing, and the total duration of the lactation period. Baleen whales have developed a lactation strategy that Dr. Olav Oftedal, Emeritus Scientist for the Smithsonian Environmental Research Center and the world's foremost authority on marine mammal lactation, describes as "intensive," whereas that of toothed whales is "extensive" [3]. These terms refer not only to the time investment from the mother, but also to the amounts of nutrients transferred and the source of those nutrients.

Baleen whales travel great distances from their feeding grounds to their breeding grounds. In winter, they mate in warmer waters and then travel to higher latitudes with colder waters to feed and fatten up during the summer. Then, they travel back to warmer waters to give birth and nurse their offspring. By the time mothers and calves return in late spring to colder waters to feed (approximately six to seven months later), the calves have been weaned [3,4]. For mammals of their body size, a lactation period of only six months is extremely short (for comparison, the largest land mammal, the African elephant, weighs 12,000 pounds and can nurse for up to four years). But when the quality and quantity of milk are examined, this "short" nursing period seems unbelievably impressive.

At peak lactation (the period just before offspring begin consuming foods other than milk), baleen whales produce milk with 30–50% fat [3,4]. And they make a lot of it—Oftedal estimates that the blue whale can transfer up to 4,000 megajoules per day, or nearly 1,000,000 kilocalories [3]!

These numbers become all the more extraordinary when you consider that the majority of lactation takes place in warmer waters, which means that the mothers are consuming little, if any food. Energy for lactation in baleen whales comes largely from maternal energy stores of blubber [3]. The body mass of a lactating blue whale has been estimated at 120,000 kilograms (or approximately 265,000 pounds), an increase of 45,000 kilograms over pre-pregnancy body mass. If you are having a hard time picturing just how large 120,000 kilograms actually is, Oftedal provides some perspective: "The body mass of a lactating blue whale is equivalent to 40 lactating elephants, 2000 lactating human females, or 48 million lactating pygmy shrews."

Baleen whales are not the only mammals to fast during lactation; this strategy is also seen in bears and seals. Not surprisingly, these mammals are also large. "Getting big allows you to have options available that are not available to smaller animals," says Oftedal. Namely, bigger mammals can store more energy than smaller mammals, and can hang on to that energy for longer periods of time [5]. But baleen whales and other marine mammals have an additional advantage not available to large terrestrial mammals: buoyancy. "Their aquatic environment allows them to carry much more body mass," explains Oftedal "because the costs of locomotion are much lower."

The extremely intense lactation strategy of baleen whales is thus only possible because of their immense body size and their aquatic environment. But relying exclusively on body stores seems like a demanding strategy—and why all the rush? Why don't baleen whales simply remain in one area with a reliable food source nearby to calve and lactate? According to Oftedal, there are three standard responses provided by marine biologists: seasonal food abundance, thermoregulation, and predator avoidance. Food resources "bloom" in high latitude seas in late spring and summer. But whale calves are born with less body fat than their parents, and the frigid waters would lead to heat loss in neonates. In addition, fewer predators (e.g., killer whales) are present in the sheltered bays and warmer waters favored by lactating mothers, again enhancing offspring survival. From the perspective of evolution, a strategy that is risky or demanding can be maintained by natural selection if it is offset by the benefit of increased reproductive success. Apparently, the opportunity of a seasonally rich food source outweighs the costs and hazards of global travel.

Not fasting, and not too fast

On the other side of that coin lie the potential risks associated with an extended period of lactation. A longer duration of lactation means a longer wait until offspring independence; the more time it takes for the offspring to become independent (and reproductively capable), the greater the risk that they will never make it to independence. Investment in an offspring that never reproduces is, from the perspective of natural selection, equal to never reproducing. So why would toothed whales have evolved such a strategy?

"Large brains, complex interactions, and complex social groups," explains Oftedal. Toothed whales have relatively large brain sizes, which require a long period of development after birth. These larger brain sizes are directly related to their social behavior; toothed whales live in larger social groups than baleen whales and "have much more complex social interactions between group members," says Oftedal. These interactions take time to learn, and these brains take time to grow (for perspective, bottlenose dolphins weighing near 500 kilograms nurse for up to 3 years, perhaps even longer) [3].

There is another order of mammals with large brains, an emphasis on learning, and slow postnatal growth rates—primates. And although they share very little with each other in regard to environment, primates and toothed whales have strikingly similar lactation strategies: longer duration of lactation, no maternal fasting while nursing, and lower daily costs of lactation. "You see convergence between these two taxa because the selective pressures are similar," explains Oftedal.

Milk from toothed whales is not nearly as dilute as primate milk, as whales and dolphins face a thermal challenge not found in terrestrial mammals (they, like baleen whales, have to provide fat and energy for deposition of insulating blubber in their offspring). However, toothed whales do produce milk that is lower in fat and higher in water than their cousins, the baleen whales, demonstrating some convergence with the very distantly related primates [3].

Exceptions to the rule

Although most baleen whales follow the intensive approach and most toothed whales the extensive approach, there are several notable exceptions. And for Oftedal, this is where the research gets really interesting. "There is more diversity than we realize within each type of cetacean," says Oftedal. "There are broad patterns, for sure, but we can not say that all baleen whales or all toothed whales are the same when it comes to lactation."

Take, for example, the bowhead whale. Bowheads are baleen whales, but have a different reproductive strategy than their closest relatives—calves are born in late spring in cold waters and nurse for relatively long periods of time, all while the mothers are consuming food [4]. Why did they diverge? How are they able to get around the constraints of cold water and high predation rates?

There is an interesting exception within the toothed whales as well—the harbor porpoise, for example, is more like a hare than a tortoise. Their lactation is much shorter than other dolphins and porpoises (8 months vs. several years), as is their inter-birth interval (one year) and total lifespan (10 years) [3,4]. When and why did they diverge from other porpoises to follow this more intense life history pattern?

Unfortunately, it may not be possible to ever fully understand the extent of variation within each group nor the reasons for the diversity because the data on whale lactation, particularly milk synthesis, are opportunistic and fragmentary. Most of what we know about the composition of milks comes from samples removed from mammary glands of hunted cetaceans, during the time when such practices were permitted [3]. And unfortunately, additional data points are rare (but see work by West and colleagues with bottlenose dolphins [6]) as there are numerous methodological obstacles in collecting from living whales and dolphins, both in the wild and captivity.

A blessing and a curse

Oftedal ended his publication on lactation in cetaceans by reflecting on the awe-inspiring maternal capabilities of the baleen whales, and it seems a fitting way to end this tale of whale lactation as well [1]. The amount of energy that mothers transfer to offspring during lactation is measured in six digits of kilocalories per day (per day!), and even more mind-blowing is the fact that this energy is derived so predominantly from blubber stores accumulated during pregnancy.

But this remarkable evolutionary adaptation proved to be a curse. Large blubber stores made them highly sought after targets for whalers. Populations of most baleen whales (and the sperm whale, a toothed species) were decimated by nineteenth and twentieth century whale hunters; it is ironic that much of what we know about their lactation is the product of a practice that placed these species in danger of extinction.

Data on milk components of both baleen and toothed whales mainly come from samples removed from mammary glands of hunted whales plus some data on stranded animals [3,4]. Thus, successful protection and conservation of these amazing mammals may limit our understanding of the lactation of many cetacean species, but this is surely a worthwhile tradeoff.

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The Dynamic Human Milk Proteome

- **Human milk proteins are a source of building blocks for growing babies.**
- **Human milk proteins also contribute to the development of a healthy gut, immune system, and brain.**
- **Prior studies have catalogued all proteins present in human milk.**
- **Recent work more accurately compares specific protein abundances at different stages of lactation.**
- **New research shows that protein composition of human milk actually changes with baby's needs.**

Babies change a great deal in six months. Beyond the obvious that they grow bigger, considerable development occurs in all aspects of the infant's physiology and anatomy, especially the brain, gastrointestinal tract, and immune system. New technologies have enabled scientists to discover which proteins are in milk and how they change over time to support this unique developmental period.

Using laboratory instruments for protein analysis, particularly mass spectroscopy, coupled with the available databases, early work showed that there were hundreds of different proteins in human milk and that their amounts changed over time (1–3). More recent catalogues of all the proteins in human milk increased the number of unique proteins beyond 1000 [4,5]. These extensive catalogues were obtained by extensively separating the milk to make sure that the most abundant proteins did not obscure the rare ones. As an analogy, if the same group of people is crammed into an elevator or spread across a basketball court, it is easier to account for everyone on the basketball court.

One drawback of the catalogue studies is that they couldn't be nearly as quantitative about the amount of a particular protein in one milk sample relative to the quantity of that protein in another milk sample. Zhang et al. [6] from Wageningen University in the Netherlands recently addressed this issue by "labeling" proteins in each milk sample before counting them. Again, an analogy is that if two groups of people are to be counted on a basketball court, they are more easily distinguished if they each wear their team's color.

Zhang et al. [6] identified 247 proteins of interest from the human milk of four women. They found that most proteins, approximately 200, were present in each sample and at all time points during the six months. As a result, they refined subsequent analyses to investigate those 200 proteins that were reproducibly detected across human milk from all four mothers.

What do these proteins do? One way of grouping proteins is according to their predicted biological function based on databases of known functions from other experiments. The major functional categories for the 200 proteins identified in human milk serum were immune (26%), transport (21%), and enzyme (14%), with other minor categories complementing the group, including cellular factors, protease inhibitors, signaling proteins, coagulators and protein synthesis components. These categories reinforce the view that human milk is rich in bioactive proteins, and that many of these proteins are related to either passive immunity or immune development of the infant [7,8]. The transport and enzyme categories are most closely linked with development of a fully functional gastrointestinal tract.

How do the levels of these 200 milk proteins change over time? Mother's milk was collected weekly for the first month, then at two-monthly intervals [6]. What became clear from the results was that there was no discernable change in which proteins were present, but there was a marked change in the amount of some proteins. Again, the categories of proteins that changed were immune, transport, and enzyme. The change in concentrations of proteins in the transport category was largely attributed to bile-salt activated protein and lactalbumin, and for the enzyme category, to albumin and fatty-acid binding protein-3.

The number of immune-related proteins and their patterns of changing concentrations were more complex. Generally, molecules associated with passive immunity—immunity provided directly from the mom via the human milk—were at their highest in colostrum or early milk. This is the period when the newborn infant is most susceptible to infection, and when maternal resistance to pathogens can be most effectively transferred. There were over fifty proteins in the immune-related category, but the most significant changes were in immunoglobulins (antibodies), lactoferrin, and a protein called CD14. Immunoglobulins are a family of proteins that specifically recognize bacteria and viruses. Lactoferrin and CD14 are examples of proteins of the innate immune system, so called because this system has an inherent capacity for immediate action against any pathogen. Together, the immune-related proteins provide a front-line defense against infection, and they also actively contribute to development of the neonatal immune system and its complex interactions with the emerging micro-flora of the maturing gut [9–11].

The results of the study by Zhang et al. [6] confirm the dynamic nature of lactation and the composition of human milk. Even though the composition varied widely among mothers, the proteins that were consistently present indicated that human milk continues to have biological activity over six months of lactation in addition to its crucial role in nutrition. Mother's milk changes; infant formulas are static. How much does it matter? And if it does matter, how can infant formulas be improved? How this may be addressed using milk from dairy cows is a key question for the dairy industry and manufacturers of infant formula.

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