This month's issue features polyamines in milk, dairy’s association with improved short-term memory, reducing methane emission from livestock, and how lactation changes mammary fat cells.

**Gut Check: Polyamines in Human Milk Are Essential for Intestinal Maturation**

- Polyamines are amino acid-derived molecules found in all living cells and the milk of all mammals, including humans.
- Human milk polyamine concentration is highest during the first weeks of lactation and varies across mothers.
- Milk polyamines are essential for optimal maturation of the neonatal gut.

Putrescine, spermine, and spermidine may not have the most appetizing names, but these amino acid-derived molecules (called polyamines) are ingredients of all mammal milks. The presence of polyamines in milk is not surprising—putrescine, spermine, and spermidine are manufactured by all mammalian body cells, including mammary tissue. But polyamines are not accidental milk ingredients, passed on simply because they are ubiquitous in mammalian cells. Research from human and non-human animal models demonstrates that optimal nutrient absorption, the composition of the intestinal microbiome, and even food allergy may all depend on a sufficient supply of polyamines during the neonatal period [1–11]. Milk polyamines, although odd in name, are essential for the proper maturation of the gastrointestinal tract in humans and other mammals.

**Making Polyamines**

Amino acids, the building blocks of proteins, are comprised of a carboxyl group and an amine group. Polyamines, as the name suggests, are composed of two or more amine groups. All living cells synthesize polyamines, and mammalian cells make three—putrescine, spermidine, and spermine—by removing the carboxyl groups from the amino acids methionine and ornithine [1].

In mammals, polyamines are involved in numerous functions within cells: they influence cellular growth, cellular differentiation, and the function of cell membranes, and also play a role in protein synthesis by regulating DNA and messenger RNA [1, 2]. Although all cells in a mammal’s body can synthesize polyamines, the importance of a sufficient dietary supply to maintain essential physiological functions indicates that cellular polyamine requirements exceed the body’s manufacturing capabilities [5, 7]. In this sense, polyamines are considered essential nutrients, just like certain amino acids, vitamins, and fatty acids [8].

The body’s polyamine requirements vary over time and are at their highest during growth periods, like infancy, which is characterized by rapid and widespread cellular proliferation [2]. Thus, the infancy period is a time when a sufficient dietary supply of polyamines may be especially critical.

Polyamines are present in all mammalian milks, and although the concentration varies across species (e.g., human milk has higher values than cow milk), all milks peak in polyamine concentration during early lactation [1, 2]. For example, in human milk, polyamines increase in concentration during the first two weeks of lactation, reach their maximum value during the first month, and then decrease [1]. These changes in concentration are believed to be due to the action of the lactation hormone prolactin, which augments mammary gland synthesis of polyamines [2,8].

That milk from cows, rats, pigs, and humans all peak in polyamine concentration at the same stage of lactation indicates an important functional role for these molecules during this period. But if it were simply about growth (making new cells), polyamines would be important throughout lactation. Why are polyamines so important for newborn mammals?

**A Gut Feeling**

Mammals vary in developmental maturity at birth; some are born altricial requiring significant parental investment, whereas others are more developmentally mature, or precocial. One thing they all have in common, however, is the consumption of milk as a first food. During the neonatal period, the mammalian gastrointestinal tract undergoes rapid maturation in preparation for the introduction of non-milk foods. Polyamine ingestion from milk is believed to have an essential role in this accelerated development...
of the small and large intestines.

To understand and identify specific functions of polyamines in mammalian infants, scientists performed experiments on non-human animals that included study groups that did not receive any polyamines—what better way to figure out what something does than to observe what happens when you take it away.

The earliest studies (during the 1990s) focused on rat models and found that rat pups receiving formula supplemented with polyamines (specifically spermine and spermidine) had several physiological differences relating to gut maturation compared with controls that consumed fewer or no polyamines. Key differences included heightened enzymatic activity of the gut (including enzymes responsible for protein digestion) and decreased gut permeability to macromolecules [reviewed in 5].

Taken together, these observations led Dandrifosse and colleagues [5] to propose that polyamines play a role in the development of food allergy. Because food proteins are the source of food allergies, improved protein digestion (via increased protein-digesting enzyme activity) coupled with a less permeable gut, reduces the potential for food antigens to make their way into the bloodstream and to come in contact with the immune system. Infants with more permeable guts due to reduced polyamine intake (particularly spermine) would thus be at a higher risk for developing food allergies [5].

Dandrifosse and colleagues tested their hypothesis in a small sample (n = 45) of human subjects. First, milk samples were collected from mothers and analyzed for polyamine concentration. Five years later, all mothers were contacted and sent a questionnaire requesting information about environmental and food allergies in their child. They found that breastfed children with an allergy at age 5 consumed milk with lower polyamine concentration than those without allergies. They even established what they believed was a “critical value” below which children have an increased risk of allergy (5.02 nmol/ml) [5].

Researchers have long grappled with the question of whether breastfeeding is protective against allergy. Whereas several studies have found decreased risk associated with breastfeeding, many have found the risk factors are identical between the formula- and breastfed infants. This study [5] helps to make sense of those contradictory findings by highlighting the differences in risk associated with breastfeeding alone. Some mothers produce milk with relatively high concentrations of spermine and spermidine with little to no risk of producing allergy, whereas others produce milk with lower concentrations more similar to those found in formula, which has a probability of producing a food allergy that is believed to be closer to 80% [2,5].

Infant formula is made from soy or cow milk, which can explain the lower concentration of polyamines compared with breast milk [9]. But what can explain the variation in breast milk polyamine concentration among human mothers? Several lines of evidence suggest that the polyamine composition of the maternal diet influences milk polyamine concentration. Citrus fruits, such as oranges and grapefruit, are high in putrescine, whereas beans and meat are good sources of spermine and spermidine [8]. However, polyamines are found in so many different types of foods that it is difficult to determine a particular dietary pattern that may result in higher polyamine intake.

Gómez-Gallago et al. [4] found significant differences in the concentration of milk putrescine and spermidine (but not spermine) across four different human populations (Finland, Spain, China, and South Africa), which they attributed to dietary differences across cultures. Atiya Ali et al. demonstrated this relationship with a more detailed study [7], wherein breastfeeding mothers of newborns kept a 3-day food diary. After calculating daily intake of all three polyamines, they found that the concentration of putrescine, spermidine, and spermine in milk were all significantly associated with their concentration in the diet.

In another study, Atiya Ali et al. [8] found that obese mothers produced significantly lower levels (14%) of putrescine and spermidine (but again, not spermine) compared with mothers with a healthy body mass index. Although obesity itself could be a contributing factor to milk polyamine levels, they observed that obese mothers that received nutritional counseling and advice during the study period increased their milk polyamine levels to those matching healthy controls. This finding suggests it is not what the mother has eaten in the past, but what the mother is currently consuming that determines milk polyamine concentration. Except, perhaps, for spermine, the very polyamine implicated in gut permeability.

All three studies [4,7,8] concluded that spermine appears less susceptible to environmental influences. However, interpretations of results are complicated by the fact that polyamines can be interconverted by the infant (putrescine is a precursor to spermine and spermidine, and can be broken down to make either polyamine; spermine and spermidine can also be converted back into putrescine). Thus, the total content of polyamines in milk should be the metric of interest.

**Growing the Gut**

Two recent animal studies have provided more detailed evidence of the potential health outcomes associated with low milk polyamine concentrations [6,10]. Van Wettere et al. [10] were interested in the relationship between milk polyamines and the development of the absorptive, or mucosal, surface of the intestines (that is, the surface where the food meets the intestinal cells). The mucosal surface of the intestines looks a bit like a rollercoaster, with a series of peaks (called villi) and valleys (called crypts). It is along this surface that nutrients (including proteins, fats, carbohydrates, vitamins and minerals) are absorbed into the intestines for eventual transfer into the bloodstream. This roller coaster-like structure is a rather ingenious way of getting more surface area for nutrient absorption; the higher the peaks and the deeper the valleys, the more cells for food to contact for absorption. Van Wettere et al. [10] found that piglets supplemented with spermine every other day over a 10-day period had an increase in the surface area...
of their gastrointestinal tract. And the increase was significant; spermine supplementation was associated with a 41% increase in villus height (the peaks) and a 21% decrease in crypt depth (the valleys) along the small intestine [10]. Importantly, the investigators were able to link the changes in the intestinal surface area with improved growth both during supplementation and after weaning [10]. Higher hills and lower valleys meant improved nutrient absorption, which they argue was critical in helping the piglets maintain optimal growth rates as they transitioned from milk to non-milk foods.

The surface area of the intestines is not the only thing in the gut that milk polyamines help to grow—these molecules are also growth factors for the healthy bacteria that populate the gastrointestinal tract. In a new study, Gómez-Gallago and colleagues [6] found that newborn mice consuming polyamine-supplemented formula had bacterial communities similar to those of mice consuming their mother’s milk, validating their results from a previous study [11]. Because of the strong connection between the development of a healthy gut microbiome and immune function, their new study went one step further and investigated the types of lymphocytes (cells of the immune system) that populated the gut as well as genes related to immune activity within the gut. Again, mice fed the supplemented formula were grouped statistically closer to the suckling mice than those consuming formula without polyamines [6].

It is intriguing to think that human infants would have identical responses to polyamine supplementation as the piglets and mouse pups in the experimental models. Optimal intake levels of polyamines for human infants have not been established. However, both studies [6,10] found significant results using concentrations of polyamines that were lower than those in mouse or pig milk, indicating that human breast milk concentrations could be a helpful signpost for determining an appropriate concentration. Could something as simple as polyamine supplementation in formula (or increased polyamine consumption in the diet of breastfeeding mothers) help resolve health issues associated with food allergy, nutrient absorption, or the intestinal microbiome? Gómez-Gallago et al. [6] suggest this question is important enough to go “one step forward” by reproducing their experiments using human subjects.


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High Dairy Consumption is Associated with Better Short-Term Memory in Men

• A new study investigates the effects of dairy intake on short-term memory, using matched pairs of twins to adjust for genetic and family environmental factors.
• The study found that higher dairy product consumption was significantly associated with better short-term memory in men but not women.
• Short-term memory assessment can be used to screen for mild cognitive impairment and Alzheimer’s disease, and the new study thus suggests that dairy intake could potentially reduce cognitive decline in men independent of genetics and family environment.

Eating dairy products positively influences brain function, with higher dairy intake associated with improved cognitive ability and short-term memory, and reduced cognitive decline and dementia [1-8]. However, previous studies that looked at these associations could not rule out the effects of confounding factors such as genetics and family environment, which are also known to affect cognitive ability and food intake [9,10].
One way to tease apart the effects of dairy from those of genetics and family environment would be to study pairs of twins, and that’s exactly the approach taken by Soshiro Ogata and his colleagues at Osaka University Graduate School of Medicine. In a new study, the researchers investigated the association between dairy product intake and short-term memory, using naturally matched twin pairs to adjust for nearly all genetic and family environmental factors [11].

Ogata and his colleagues collected data from the Osaka University Center for Twin Research, and analyzed 39 Japanese male twin pairs and 139 Japanese female twin pairs [11]. To assess short-term memory, the researchers asked participants to listen to two short stories and immediately recall the details. The researchers assessed participants’ diets using a self-administered diet history questionnaire, and the participants’ dairy sources consisted of low-fat and full-fat milk and yogurt. The researchers found that higher consumption of dairy products was significantly associated with better short-term memory in men, even after adjusting for numerous confounding factors including almost all genetic and family environmental factors. The researchers did not find a similar association in women.

Short-term memory assessment can be used to screen for mild cognitive impairment and Alzheimer’s disease [13]. The results of Ogata et al. [12] thus suggest that eating more dairy products could potentially reduce cognitive decline in men independent of genetics and family environment.

It’s unclear from this study why higher dairy intake was associated with better short-term memory in men and not in women. The results are consistent with a previous longitudinal study that found no significant association between cognitive function and consumption of dairy products in women [14]. The researchers also suggest that the sex differences in the effects of dairy on short-term memory might be due to differences in the age distribution of men and women in the study, or due to previously described seasonal variations in dairy consumption in Japanese women but not men [15].

Future studies are needed to elucidate both the reasons for the observed sex differences and the mechanisms by which dairy consumption affects cognitive function. One potential mechanism involves the effects of increased dairy product consumption on a reduced risk of developing type 2 diabetes and hypertension, which are potential risk factors for cognitive decline [16-18]. Dairy also contains nutritional components such as calcium and vitamin B12 that are known to have some cognitive effects. The authors suggest that follow-up studies could measure the effects of dairy on other cognitive abilities in addition to short-term memory. These studies could also investigate differences between the effects of full-fat and low-fat dairy products on cognitive function, as previous studies indicated differences in their effects [19,20]. By showing that dairy may influence short-term memory regardless of genetic and environmental factors, the current study indicates that looking more deeply at the effects of dairy on cognition is a no-brainer.

Lessening the Gas Leak

- Methane emissions from livestock—in the form of belches and flatulence—compose a non-negligible proportion of greenhouse gas emissions.
- In an experiment, the addition of the methane inhibitor, 3-nitrooxypropanol (3NOP), to animal feed cut dairy cattle’s methane emissions by about 30%, without affecting the animals’ productivity.
- Crucially, the experiment was conducted over a prolonged period under similar conditions to how dairy cattle are normally kept on North American commercial farms.

As ruminants, dairy cattle are home to bacteria that ferment their food, readying it for regurgitation as cud before proper digestion takes place. Methane gas is generated during the fermentation process and eventually makes its way into the atmosphere from either one end of the cow or the other. All of this methane—emanating from cattle all over the world—is a prime target for mitigating greenhouse gas emissions, not least because methane is a far more potent greenhouse gas than carbon dioxide. Although estimates of livestock’s contribution to anthropogenic emissions vary [2], it is widely accepted that emissions from cattle dwarf those from other kinds of livestock [3].

The authors of the recent study set out to evaluate the real-world effectiveness of a compound that they knew had the potential to cut how much methane the average dairy cow releases into the atmosphere. 3NOP was first identified by a computer model in a laboratory in Basel, Switzerland. Researchers there singled it out for further testing because after it was found to bind the active site of a methane-producing enzyme that occurs in one of the most common kinds of ruminant fermentation bacteria, Methanobrevibacter ruminantium. By binding this active site, 3NOP stops the enzyme from working and limits the bacterium’s growth (while leaving unaffected the growth of non-methane-producing bacteria) [4].

Testing the real-world effectiveness required 48 Holsteins. The cows were randomly assigned to either a control group, or to a group that would continually receive low, medium, or high amounts of 3NOP in its food. Many aspects of the cows’ milk production and health were closely monitored, and all of their methane, carbon dioxide and hydrogen emissions were measured regularly throughout the experiment.

Importantly, the Holsteins were kept in conditions similar to those on commercial dairy farms and monitored for a three full months—which is longer than most methane-inhibitor tests of the past. The extended period offers an insight into whether certain key concerns are avoided, such as if the cows eat less over time in the condition where the compound is added to their food; if their milk production tails tailed off; and if methane-producing bacteria can shift their metabolic strategy and adapt to 3NOP. If the latter occurs, methane production may start to rise again after it dips—despite the cows continuing to gobble 3NOP in every meal.

After the three months were up, the Holsteins that ate the most 3NOP consistently emitted the least methane. Indeed, they generated 32% less methane than cows in the control group, which consumed no 3NOP but an otherwise identical diet. The trend in the data showed little sign of abating, indicating that methane-generating bacteria do not readily find a way around the methane inhibitor’s effects. The 3NOP-consuming cows produced more hydrogen, which was to be expected if the fermentation process was
interrupted as the scientists foresaw (because hydrogen is an intermediate in this process). Carbon dioxide emissions did not vary, however, whatever experimental group the cows were in.

The cows’ other vital statistics were encouraging. Eating 3NOP did not curb their appetites; instead, it seems to have helped them put on weight. This is probably because the food energy normally lost as methane became metabolically available to these animals. Additional energy availability may also explain the greater milk protein and lactose yields in the 3NOP-eating cows’ milk, since it could, in part, have been plowed into synthesizing these milk constituents. Overall, the concentration of fat in milk was unaffected by a diet containing 3NOP (even though the relative abundances of particular fats did change).

While further testing is likely required on larger herds and different breeds, 3NOP does appear to generate the kind of methane mitigation that the dairy industry is under pressure to achieve, without impacting how farmers go about their business. This matters in many countries where the industry contributes a sizeable portion of total greenhouse gas emissions, such as New Zealand. But it also comes at an auspicious time in the United States because the U.S. Environmental Protection Agency’s estimates for enteric methane emissions over the country have, in the past few years, come under fire. Satellite data have suggested that the Agency underestimates these emissions by up to 85% [2]. 3NOP could help to reduce the problem.

People obsess about fat. Many have much more fat than they need deposited in various locations in the body and it threatens both health and fashion. Fat has now developed a bad reputation. In times gone by, a bit of extra fat meant a lifesaving energy reserve in times of food scarcity. Indeed, the metabolism we have inherited from our ancestors was originally fine-tuned to suit the feast or famine lifestyle of the past; however, it is not suitable for most people in today’s world where food abundance is the norm. Fat is a simple thing, or so we thought. There have been several surprises of late.

**Fat Tissues Are Not All The Same**

The body of a mammal contains different types of fat, each with specific functions. Scientists have long known that the main functions of fat in animals are the storage of energy in the form of specific fatty acids called triglycerides and the protection of internal organs from blunt force trauma. The fat type that largely characterizes these functions is white fat (adipose) tissue, which is primarily made of fat cells called white adipocytes that are relatively large, and a single large globule of fat typically dominates their internal structure. This is the fat we all know so well.

A second type of fat, brown adipose tissue, was first discovered in hibernating mammals, like bears. Unlike white adipose tissue, brown adipose tissue has the unique property of heat generation, which allows hibernating animals to withstand severe cold. This adipose tissue consists of brown adipocytes with each containing many small fat globules and a large number of mitochondria, the powerhouses of a cell. The latter give this tissue its brown color, and a specific biochemical reaction in them generates heat. Newborn offspring of non-hibernating mammals, including humans, also contain brown adipose tissue that protects them from the cold when they are at their most...
vulnerable. Within a few weeks of birth, however, the brown adipose tissue in most newborn mammals is lost and replaced by white adipose tissue [1].

One of the scientific surprises discovered over the last decade is that adult humans have a small amount of brown adipose tissue in the neck and shoulder region (interscapular region) close to the major blood vessels leading to the brain. Presumably, the primary function of this adipose tissue is to heat blood flowing to the brain when the body is exposed to very cold temperatures. This adipose tissue may also have a role in regulating metabolism [1–3].

Another surprise made only over the last few years was the discovery by scientists of a third type of fat that consists of brown-like adipocytes induced by diet or cold within white adipose tissue [2, 3]. Researchers call this type of fat beige adipose tissue. Even though beige adipocytes look like brown adipocytes, they are different in terms of their cellular origin and function. Scientists from several groups have concluded that beige adipose tissue might help protect people from obesity by increasing the rate of metabolism of fatty acids [3]. Consequently, many scientific groups in both the public domain and private companies are investigating dietary and drug-based strategies that may increase the amount of beige adipose tissue in obese people [3, 4].

The Lactation Cycle Causes Substantial Changes in Mammary Fat Cells

A scientific group led by Saverio Cinti that involved researchers from six universities and three continents recently concluded that adipocytes present in mammary tissue undergo a dramatic change during the lactation cycle [5]. This is the cycle of milk production and cessation occurring in mammary tissue between one pregnancy and the next. During lactation, mammary epithelial cells secrete the various components of milk into mammary tissue alveoli (small sacs found within the mammary gland). When there is no longer any suckling by the young and removal of milk from mammary tissue, the tissue undergoes a dramatic cellular restructuring called involution that stops milk production. Some mammary epithelial cells die but most revert to a quiet state no longer producing milk.

The researchers used the remarkable genetic tools available for studying transgenic mice to visualize the fate of fat cells lying adjacent to the mammary epithelial cells during the lactation cycle. They used light microscopy and the amazing power of transmission electron microscopy to produce exquisite images of these adipose cells during and after lactation. The scientists’ approach allowed determination of the adipocyte type in mammary tissue throughout the lactation cycle.

Cinti [1] showed that during pregnancy and lactation some white adipocytes in the mammary gland converted into milk-secreting epithelia cells, quite a remarkable cellular transformation. This was already known [6]. The surprise was that when they examined mammary tissue during the process of involution they found that some milk secreting epithelial cells changed into brown-like adipocytes, a process called transdifferentiation, whereby a cell of one distinct type converts into another type. It is still unclear whether these cells are brown or beige adipocytes. However, white and beige adipocytes originate from the same cell precursors suggesting that these mammary adipocytes in involuting mammary tissue could be beige adipocytes rather than brown adipocytes. The lactation cycle therefore highlights the striking functional plasticity of adipocytes in mammary tissue. The investigators did not comment on the functions of the brown-like adipocytes in involuting mammary tissue. Clearly, the study is another example highlighting the many lives of fat cells and their underappreciated importance in diverse biological functions.


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