This month’s issue features premature babies, soy milk, food allergies, and metabolics and yogurt.

**Very Small Babies Benefit from Either Human or Cow-Based Milk Fortifiers**

- Premature babies with very low birth weights face many challenges to their survival and health.
- Human milk alone does not adequately support the growth of very low-birth-weight babies.
- A recent clinical trial suggests there is benefit to very low-birth-weight babies fed human milk supplemented with fortifiers based on either human or cow milk.

Life’s tough for very small premature babies. They face a daunting list of potential complications that could challenge their present and future health, and sometimes their survival [1-4]. Their families dread hearing the foreboding medical word “complication.” Babies weighing less than 1.5 kg (3 lb 4 oz) are classified by the World Health Organization as very low-birth-weight (VLBW) babies [5, 6]. To put that into perspective, the average birth weight in the USA is 3.3 kg (7 lb 4 oz) with 95% of babies ranging between a petite 2.5 kg (5 lb 8 oz) and the king or queen of the nursery at 5 kg (11 lb). Amazingly, many VLBW babies overcome their early life challenges with help from intensive neonatal medical care and go on to face the more important challenges within a child’s life, like learning to swim and passing grammar tests at school. One factor contributing to the health of VLBW babies is the use of milk-based nutritional fortifiers. But which fortifier is best?

Deborah O’Conner and twelve colleagues compared the supplementation of human milk with human milk-based fortifier or cow milk-based fortifier in a large group of VLBW babies [7]. This was the first clinical trial to investigate the relative merits of these fortifiers when added to mother’s milk topped up with donated human milk. The investigators are based in several Canadian institutions, although the extensive list of people in the acknowledgments highlights the broader North American scale of the investigation and its logistical complexity. The results of the investigation were recently published in the *American Journal of Clinical Nutrition* [7].

**Very Low-Birth-Weight Babies Require Intensive Care**

The main causes of very low birth weight in babies are premature birth and problems with the placenta, birth defects, or maternal health [8]. Some VLBW babies born prematurely have the expected maturity corresponding to their gestational age (time in pregnancy), others are not at their expected level of maturity. Thus, in addition to birth weight, doctors also rely on measures of baby development to assess the health risks of a VLBW baby and to tailor an intensive health-care treatment [9]. VLBW babies without early life complications often have remarkable “catch up” growth that eventually leads to a more normal growth pattern occurring in childhood [10]; resilience in biology is amazing. However, scientists report that these individuals may have greater risks of metabolic diseases, like obesity, but later in life [10].

For VLBW babies, it is all about putting on weight as soon as possible; time is not on their side. Scientists have demonstrated a strong correspondence between birth weight and the chance of survival [11]; the impersonal mortality-birth weight graph summarizing millions of births is precipitous and ominous. The survival rate of VLBW babies at one year of age in the U.S.A. in 2012 was about 100 fold less than normal birth weight babies [5], but this number is likely inflated somewhat by a population of extremely low-birth-weight babies unfortunately with very poor prospects. Increasing the weight of VLBW babies by intensive medical care and tailored nutrition helps them avoid complications and increases their survival chances.
Although mother’s milk is superbly formulated to meet the demands of the average baby, it alone is insufficient for the specific needs of a VLBW baby, which is often vulnerable to under-nutrition [7, 8]. Neonatal health-care specialists recommend that VLBW babies need additional nutritional support in the form of milk-based fortifiers to supplement mother’s milk or donated human milk [8]. The combined nutrition is typically fed through a tube directly into the stomach of the baby. Medical authorities now recommend that this approach to the feeding of VLBW babies is the best [7, 8]. Investigators involved in several older clinical trials demonstrated that human milk supplemented with a milk-based fortifier compared with human milk containing either no fortifier or supplementary formula milk increases growth of the VLBW baby and enhances its tolerance to the feeding tube [7, 12-15]. In addition, these investigators concluded that maternal milk containing fortifier reduces the risk of necrotizing enterocolitis (NEC), a life-threatening condition where parts of the bowel die for unknown reasons in premature babies [7,12-15]. The investigators inferred that optimized growth of the VLBW baby may also decrease the risks of other complications.

The fortifiers are commercially-produced milk products containing concentrated milk protein and added carbohydrates, fats, calcium, trace minerals, and vitamins from various sources. Manufacturers of fortifiers often use protein components in cow’s milk due to its markedly lower cost and greater availability compared with donated human milk. Human milk-based fortifiers have only recently become available and have not been as extensively tested for benefits to babies compared with cow milk-based fortifiers [14, 15]. Health authorities usually make the assumption that the much more expensive human milk-based fortifiers must be better for VLBW babies than fortifiers made from cow’s milk. This assumption may lead to greater demand for the human product and a potential strain on the limited supply of donated human milk. However, this basic assumption has not been tested in a rigorous clinical trial, until recently.

A Clinical Trial Tells All

O’Conner and colleagues used a randomized and blind clinical trial to assess whether human milk-based fortifiers were better than cow milk-based fortifiers when administered to VLBW babies with mother’s milk topped up with human donor milk [7]. A randomized and blind trial is considered by scientists as the gold standard method to decide whether one clinical treatment is better than another. The investigators primarily measured babies’ growth, feeding tolerance, health complications, and gut inflammation. They also monitored the incidences of infection, NEC, lung disease, and eye damage. The investigators excluded babies from entering the trial if they had chromosomal or innate abnormalities affecting their growth or they did not meet specific feeding criteria. The exclusion of some babies was important so that informative results could be obtained from the trial. There were about 64 VLBW babies in each of two groups receiving mother’s milk topped up with donated and pasteurized human milk supplemented with either human or cow milk-based fortifiers. The average birth weight of all babies in the trial was a meager 888 grams (2 lb) and they were born on average about 10 weeks prematurely. The babies were randomly allocated to each treatment group to remove known and confounding biases from the analysis. The clinical trial was blinded, meaning that the staff administering the fortified milk did not know what treatment was being given to each baby during the trial, which continued from birth to 12 weeks of age. A few babies graduated early out of the trial because of their good general health.

When O’Conner and colleagues analyzed the extensive trial results they found no statistically significant differences between the two treatments for all the major baby measurements [7]. This is an important finding as the investigators concluded that VLBW babies can benefit from either the markedly less expensive and more widely available cow milk-based fortifier or the more expensive human milk-based fortifier. The use of the cow milk-based fortifier may reduce the strain on the limited supply of donated human milk. The investigators stated that they would need to follow-up on a slightly greater incidence of eye damage to some babies in the group receiving the cow milk-based fortifier.

Investigators who undertook related but different clinical trials in the past often concluded that a diet exclusively containing human milk and human fortifier is more helpful to VLBW babies than one using human milk supplemented with cow milk protein [16-18]. However, O’Conner and colleagues noted substantial differences in the designs of these clinical trials, their research aims, and the nature of the cow milk supplementation; they concluded that these other clinical trials were not comparable to their trial [7].
Implications

Medical authorities report that VLBW babies occur at a frequency of only about one in a hundred births, although the impact of these births within hospitals is very high [6,8]. Milk fortifiers that improve VLBW baby growth and health may help free much-needed pediatric hospital space and expertise. Moreover, health workers can either use the widely available cow milk-based fortifiers or the more expensive human milk-based fortifiers and thereby help restrain the soaring costs of intensive care for VLBW babies.

VLBW babies are tiny, incredibly vulnerable, and often clinically unstable. Even using the best intensive medical care and the best nutritional supplementation in the clinical trial undertaken by O'Conner and colleagues, tragically a few babies died in both treatment groups and about half of the babies in both groups developed a major complication including infections, NEC, eye damage, and brain injury [7]. The risks of complications in both groups were presumably reduced by the nutritional fortifiers used in the trial, however the overall risk of adverse complications was still substantial. On the more positive side, many of the babies without complications in this trial will likely live a full and productive life, unlike what might have occurred if they had been born only a couple of decades ago. This is a remarkable story about how milk fortifiers and modern intensive medical care can change some lives for the better.


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What’s in the Dairy Case? Soy Milk: The Original Alternative Milk

- Soy milk contains high-quality protein and is a good dietary source of isoflavones, plant compounds similar in structure to human estrogen.
- Naturally occurring ingredients from soy can interfere with the body’s ability to absorb iron, calcium, and iodine.
- Soy milk may be the most nutritionally similar plant-based milk alternative to cow milk, but it should not be used as a nutritional substitute for cow milk by consumers that can tolerate dairy.

Humans domesticated soybeans over 10,000 years ago—roughly the same time that cattle were domesticated—so it should be no surprise that soy “milk” is the original plant-based milk alternative [1]. The first soy milk, believed to have originated in China over 2,000 years ago, was a byproduct from the tofu-making process and was much more like bean-flavored water than the “milk” you find in grocery stores today. Thanks to thickeners and emulsifiers (as well as many other technological advancements that remove components associated with the “beany” flavor), soymilk has become one of
the most widely consumed plant-based milk alternatives in the U.S. and across the globe [1,2].

Many consumers select soymilk because of the unique health benefits provided from soybeans, including high protein and high isoflavone (phytoestrogen) content. Indeed, across all plant-based milk alternatives, soymilk is the only one to match cow milk in protein content. But consumers may be surprised to find out that many of soy’s natural components actually act to limit the body’s ability to absorb several essential nutrients [2]. Moreover, the very agents used to transform it from bean water to milk may actually have negative impacts on health [2]. When considering soymilk as a nutritional substitute for cow milk, it is important to consider the content and availability of all nutrients. Soymilk has most certainly come a long way in terms of taste and texture over the last 2,000 years, but a bean is simply unable to match a cow when it comes to the business of making milk.

Quantity, Quality, and Digestibility

With a diet highly dependent on soybeans and a dearth of dairy cattle, it is no surprise that early Chinese cultures manufactured soy milk. But what’s the appeal for modern-day humans? Compared with other plants used to produce milk alternatives (e.g., almonds, rice, coconut), soybean proteins provide all nine of the essential amino acids (that is, the amino acids the human body cannot make on its own) and are therefore considered a complete protein [1-4]. Whey protein, one of the two classes of proteins in mammal milk, also contains all essential amino acids, making milk a complete protein source as well [4]. Indeed, both whey and soy receive a score of 1.0 (the highest score on the scale) on the digestible indispensable amino acid scale (DIAAS), a measure of protein quality [4].

Soy milk is the only plant-based milk alternative to match cow milk’s protein content; both provide 8 grams of protein per cup [1, 4]. And yet despite these similarities, they are not interchangeable sources of protein. In addition to essential amino acids, milk protein includes biologically active components, including immune factors and peptides. Although their intended consumer was a baby cow, many of milk’s bioactive ingredients have demonstrated benefits to human consumers as well, including improving glucose metabolism, controlling appetite, and building muscle.

The casein component of milk protein also gives milk an edge over soy and all other plant-based milks. Milk’s casein proteins are delivered as micelles, small spheres containing both water-loving and water-phobic proteins. Once these micelles reach the stomach, digestive enzymes snip away at the water-loving proteins, transforming the soluble micelle into an insoluble curd. Milk may have started out as a liquid, but casein protein digestion effectively transforms it into a solid meal. Increased digestive time offers many advantages, including increased satiety, slower release and digestion of amino acids, and improved absorption of casein-bound calcium.

Plant Hormones?

Mammal milks contain maternally-derived hormones, such as cortisol, adiponectin, and estrogen, which influence infant growth and development. Soymilk does not have cortisol or adiponectin, but it actually contains plant-derived compounds that are molecularly similar to estrogen. The structural similarity allows these plant compounds, called isoflavones, to mimic the effects of estrogen (hence, their classification as phytoestrogens). But is this a good thing for consumers?

First off, it is important to point out that the estrogenic effect of isoflavones is considerably weaker than the estrogen our own bodies manufacture; a cup of soymilk is not the hormonal equivalent of a birth control pill. Secondly, isoflavones have both estrogen-agonist and estrogen-antagonist activities [5]. Sometimes, isoflavones bind to a cell’s estrogen receptor and elicit a cellular response similar to that of estrogen. However, it is also possible that they bind to the estrogen receptor and nothing happens. But when an actual (human-made) estrogen molecule comes along looking to bind, the receptor is now blocked; by stealing its binding site, the isoflavone has kept estrogen from acting on that cell. Depending on which physiological system you are interested in, both actions can have health benefits or risks. Potential benefits under investigation for post-menopausal women include reduced bone loss and lower rates of some cancers [3,5-7]. For children, men, and pre-menopausal women, the health benefits of
isoflavones are less well understood, and they could potentially act as endocrine disruptors.

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**Anti-nutrients**

Bean water may offer a lot of protein and isoflavones, but those are not the only cow milk ingredients consumers are looking for in a milk-alternative beverage. Cow milk and dairy foods are the best dietary sources of calcium in U.S. diets because of their high calcium concentration and bioavailability. In order to compete as a milk-alternative, soy and other plant-based milks are fortified with calcium. Indeed, many even boast that they offer more calcium than cow milk. Unfortunately, consumers may not be getting as much calcium out of their cup of soymilk as the carton advertises. Soybeans contain a compound called phytic acid, which binds to minerals, including calcium, and prevents their absorption. Phytic acid also binds to iron. So even though soymilk beats cow milk when it comes to iron content [1,2,8], the presence of this anti-nutrient in the soymilk may significantly reduce the amount of iron that actually makes its way from the digestive tract to the circulation [1,2,4,8].

Individuals that switch from cow to soy milk may also find themselves deficient in iodine. Dairy foods are the best dietary source of iodine; soy, almond, and rice milks contain only trace amounts. Moreover, natural ingredients in soybeans may interfere with the thyroid’s ability to absorb iodine, which could act to further increase the risk of iodine deficiency.

**Bean Vs. Cow**

Soy milk’s high protein content, healthy polyunsaturated fats, and lack of cholesterol make it an appealing alternative to cow milk. This appeal increases when considering the possibility that isoflavones may provide unique health benefits to post-menopausal women, such as reduced risk of bone loss or breast cancer. But consuming soymilk because it is a healthy beverage and using it to replace cow milk and other dairy in the diet are two very different nutritional strategies. Compared with nut, rice, and coconut milk, soymilk offers the closest nutritional profile to cow milk (which may explain why it has been used as an alternate protein source for infant formula for over 40 years). Those that select soy because of cow milk protein allergy or lactose intolerance, or a commitment to animal-free diets may want to make sure that they are getting essential nutrients, such as calcium, iron, and iodine, from other dietary sources.


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### Unique Sugars in Human Milk Cut Infant Food Allergies

- Breastfeeding is usually linked to a lower probability of an infant developing food allergies.
- Variation in the results of multiple breastfeeding and allergy studies may be due to the differing sugar profiles of the milks produced by different women.
- A recent study shows that these profiles are an important influence on the risk of a one-year-old infant developing food sensitization.

Often, studies that investigate a possible association between breastfeeding and the development of
allergies find one, but not every time. For many researchers this would simply suggest random variation in the world. However, for Kozeta Miliku, of the University of Manitoba, Canada, and her colleagues, the variation has sparked a new avenue of research. They have discovered that instead of being random, the suite of mid-sized sugar molecules that are present in an individual mother’s milk contribute to the probability of her infant having food allergies at the age of one [1]. Because the list and amount of these sugars varies from mother to mother, so does the extent to which human milk protects infants from developing allergies.

The unique sugar molecules in question are known as human milk oligosaccharides (HMOs). Curiously, these sugars make up a substantial portion of the total solids in milk, which implies a metabolic cost to the mother’s body to produce them. Yet infants are unable to digest them. Together, these facts suggest that HMOs must have been maintained in human evolutionary history because they conferred survival advantages to infants other than direct nourishment. The past decade or so has seen an uptick in research on these sugars. Investigators have shown, among other things, that HMOs prevent germs from finding a foothold on the gut wall, which is the first step to them entering human cells, and that they promote the bacterial colonization of the gut towards “good,” as opposed to pathogenic, bacteria.

Researchers have also found that the HMO profile of two different mothers’ milks can be quite different. Estimates of the total number of HMOs vary, and are typically in the range of 150 to 200. But the group of HMOs that are the most prominent in any individual woman’s milk is influenced by her health and the stage of lactation, as well as by her genetic ancestry. For example, a study published in 2017 found that one particular HMO called 2’-fucosyllactose was much more common in the milks of breastfeeding women living in the outskirts of Lima, Peru, and in Southern California than it was in the milks of women from rural Gambia [2].

The new evidence that HMO profiles of milk can influence allergy development in infants comes from a cohort known as CHILD (Canadian Healthy Infant Longitudinal Development cohort), which includes 421 pairs of a mother and her infant. Miliku and her colleagues took samples of milk from the mothers enrolled in this study, and examined the levels of 19 different kinds of HMOs [1]. Specifically, the team used a technique called Projection on Latent Structures-Discriminant Analysis, which can work out the absolute amount of each HMO in each sample. They then followed the mother-infant pairs until the infants turned one year old. At that stage, 59 of the infants (or 14% of the total) were sensitive to at least one food allergen.

After crunching some statistics, the researchers concluded that only 10 out the 19 HMOs that they measured were important in predicting food sensitization among the year-old infants. In some cases, the influence was not slight. The Projection on Latent Structures-Discriminant Analysis enabled the researchers to work out a ranking of the HMOs according to their association with food sensitization, and from this they created “discriminant scores” for each mother’s milk, depending on the milk’s HMO profile. Infants whose mothers produced milk in the top 20% of the discriminant scores had a 90% lower risk of developing food allergies by their first birthday than infants whose mothers’ milks scored in the lowest 20%.

This is not the first examination of HMOs and food allergies. However, it is the first to assess the contribution of a reasonably long list of HMOs, and to draw a conclusion that the overall profile is what matters. A few other studies have probed the influence of individual HMOs, and concluded that the sugar 2’-fucosyllactose is important for this outcome in humans [3] and in rodents [4]. Although this sugar is on Miliku and coauthors’ list of 10 relevant HMOs for infant food allergy risk, it was not a significant contributor to this risk on its own.

These conclusions suggest that attempts to supplement infant formula with just one or two HMOs should really seek to cast the net much more widely. But it’s also early days to reach firm conclusions. The mechanisms by which HMOs could reduce the odds of food allergies are largely speculative, and based on related roles that science perhaps has not fully uncovered. It is a fertile area for future research.

How Milk’s Metabolic Footprint Changes After Fermentation Into Yogurt

- Fermented dairy products such as yogurt have been associated with several beneficial health effects, but the underlying mechanisms are still unclear.
- A new study characterized the metabolic footprint of milk and yogurt, as well as the changes to the human serum metabolome after either yogurt or milk intake.
- The study finds that the milk metabolome becomes more complex during fermentation, and human metabolic pathways related to amino acids, indole derivatives, and bile acids are modulated by yogurt consumption.

Fermentation is an age-old practice used to make foods last longer and easier to digest. About 40–80 pounds of fermented dairy products are consumed per person each year in Western countries, of which yogurt constitutes about 40% [1,2].

Yogurt consumption is known to have several beneficial effects, including modulating the immune system, lowering circulating cholesterol, and improving many gastrointestinal conditions such as lactose intolerance, constipation, and inflammatory bowel disease [3–5]. However, the mechanisms underlying these beneficial effects are still unclear.

In a new study, Grégory Pimentel of Agroscope and his colleagues set out to explore the mechanisms underlying the health benefits of yogurt [6]. The researchers used mass spectrometry-based methods to characterize the metabolic footprint of both milk and yogurt. They also assessed how acute or short-term intake of milk and yogurt by healthy adults influenced the assortment of small molecule metabolites, known as the metabolome, present in their blood. They found that the milk metabolome becomes more complex during fermentation into yogurt, and metabolic pathways related to amino acids, bile acids, and a class of biologically important molecules known as indole derivatives are modulated by yogurt intake.

Fermentation is known to cause major changes to a food’s composition and nutritional value. Lactic acid bacteria, which play a particularly important role in the fermentation of milk to yogurt, may contribute to the reported health benefits of yogurt both through the release of bioactive metabolites and the modulation of the host intestinal microbiota [7–10].

Few studies have used metabolomics—or the study of the metabolites present in a cell or substance—to investigate fermented dairy intake [11–15]. Metabolomics studies of the milk fermentation process have identified free amino acids, peptides, and volatile compounds as some of the metabolites released in yogurt, cheese, and various fermented milks [16–19].

The new study evaluated the metabolic response of 14 healthy men to the ingestion of probiotic yogurt or nonfermented milk [10]. The nonfermented milk control was chemically acidified to endow it with a similar color, texture, and pH as mild, semiliquid yogurt. Participants ingested a single 800-gram dose of either milk or yogurt within 15 minutes, and their serum—the clear part of the blood left after removal of blood cells and clotting factors—was then sampled up to 6 hours later to evaluate the effects of acute intake on the metabolites present. To evaluate the effect of short-term intake on the serum metabolome, participants consumed 400 grams of either milk or yogurt daily during a two-week test phase, and serum samples were taken from fasting participants at the end of the two weeks.

The researchers found that milk and yogurt share a lot of their metabolites, but their analysis identified clear differences between the two metabolomes. Yogurt had a higher number of metabolites compared...
with milk, which wasn’t a surprise as the process of fermentation is expected to release new metabolites. Acute intake of either milk or yogurt resulted in two different metabolic profiles. Two weeks of daily milk or yogurt intake also resulted in two different metabolic profiles, although the differences were less marked than after acute intake. The researchers conclude that there are clear differences in the effects of milk and yogurt on the serum metabolome after both acute and short-term intake. The researchers also noticed that about a third of all the metabolite changes observed after two weeks were already present within 6 hours of milk or yogurt intake. They suggest that identifying the metabolic changes that occur after acute intake could help predict the changes that are likely to occur after chronic intake.

Yogurt intake was characterized by higher concentrations of seven free amino acids. However, the differences in free amino acids between milk and yogurt intake were limited in intensity and in time. They returned to baseline values within four to six hours, and were no longer different in fasting serum after two weeks of daily milk or yogurt intake.

Yogurt intake also reduced concentrations of five bile acids, and these were some of the major differentiators between the effects of milk and yogurt intake. However, their presence appeared to be short-lived, as there was no difference in fasting serum after two weeks of daily intake. Bile acids are known to be influenced by the intestinal microbiota, suggesting that differences in the effects of yogurt or milk on intestinal microbiota could explain differences in the concentrations of bile acids [20].

Yogurt intake modulated four additional compounds: indole-3-lactic acid (ILA), indole-3-acetaldehyde (IAAld), indole-3-acetic acid (IAA), and 3-indole propionic acid (IPA). These compounds are part of a biologically and pharmacologically important class of molecules called indole derivatives. ILA and IAAld clearly differentiated yogurt intake from milk intake, and were present at a higher concentration in yogurt compared with milk as well as at a higher concentration in serum after yogurt intake. IPA and IAA were not detected in either milk or yogurt but were present in serum. Indole compounds are known to be produced by microbial metabolism, and the researchers suggest that their presence in serum could originate from intestinal microbiota.

Indole derivatives appear to play a role in the functioning of the gut epithelial barrier and in gut inflammatory homeostasis. IAA and IAAld are ligands for the aryl hydrocarbon receptor (AhR), and they may influence the gut through the AhR signaling pathway [21–24]. Interestingly, the researchers found that the gene coding for AhR was one of the top genes that significantly changed after yogurt intake [25]. The researchers suggest that follow-up studies could investigate the metabolites identified in greater detail. For instance, studies could examine whether the modulation of bile acids or the AhR signaling pathway contribute to the beneficial effects of yogurt. Identifying the molecular targets modulated by these metabolites could also help elucidate the underlying mechanisms of these beneficial effects.

The researchers conclude that milk and yogurt each has an identifiably different metabolic footprint, and milk and yogurt intake have clearly different effects on both the acute and short-term serum metabolome. The researchers also found that metabolic pathways related to amino acids, indole derivatives, and bile acids are modulated in healthy men by the intake of yogurt. This indicates that fermented dairy products may help regulate metabolic activity, and the researchers suggest that more studies of the beneficial health effects of fermented dairy products are needed.


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