Milk Fat Globule Membrane Reduces Weight Gain in Mice

- Milk fat occurs in a highly structured arrangement, with globules composed of different types of fat molecules in the center and in the surrounding membrane.
- The watery part of butter—butter serum—is a rich source of the membrane component of milk fat globules.
- Scientists who recently fed butter serum to mice found that mice who consumed significant amounts of this substance gained strangely little weight for the energy they consumed.

The fat component of milk is not sludgy and unstructured, as most people imagine. Rather, it is a complex mixture of different kinds of lipid molecules organized into membrane-bound bubbles called milk fat globules. Common fats, or triglycerols, occur in the middle of these globules. Fats that are known to have various regulatory functions, such as sphingolipids, phospholipids and glycolipids, are found in the surrounding membrane. Because these membranes have been found to have positive effects on human physiology beyond raw energy provision, scientists have gathered evidence that consuming milk fats—that is, whole globules, core and membrane combined—can be on-balance health promoting. Now researchers based mainly in Lyon, France, led by Marie-Caroline Michalski, have shown that when healthy mice consume butter serum, which is rich in milk fat globule membrane, on top of a high-fat diet, the mice gain less weight than when they eat a high-fat diet lacking this addition. Surprisingly, consuming the high-butter serum diet also led the mice to gain less weight than when they consumed less energy in the form of a low-fat diet.

Research into the effects of milk fat globules has uncovered a range of health-promoting effects. For example, SPLASH! reported in March of this year that Thomas Douéllou, who also works in Lyon, and his colleagues, discovered that milk fat globules bind to different strains of pathogenic Escherichia coli in such a way that they reduce the potential for bacterial infection [2]. In Douéllou and colleagues’ experiment, the intestines of mice fed E. coli-contaminated cheese containing a lot of milk fat globule membrane had fewer signs of bacterial colonization than did those of mice fed similarly contaminated cheese with lower levels of milk fat globule membrane. In separate work looking at human infants, researchers have associated the consumption of milk fat globule membranes with lower infection rates and better cognitive performance [3].

Michalski and her colleagues fed the mice in their experiment butter serum, which is the watery part of butter [4]. “If you take butter and put it in a frying pan, the first thing that happens is you bubble off the water—that’s butter serum,” explains Bob Ward, an associate professor at Utah State University, who spent his sabbatical year working with the Lyon-based research group behind the recent findings. “It’s basically a component of butter that has been separated from the triglycerides, so it’s a particularly rich source of milk fat globule membrane.”

The experiment involved four groups of 15 mice each. For eight weeks the mice received different diets, while their body weight and food intake were monitored. The reference group received a low-fat diet of vegetable matter, known to scientists who work with mice as “chow.” A high-fat control group ate chow mixed with palm oil. Two treatment groups also got the chow and palm oil diet, however, their food either contained an addition of a small amount (1.9%), or a more significant amount (3.8%), of butter serum. “One of the points they make in this paper is that they are working with chow [as opposed to completely
New Milk Biomarkers Predict Preterm Infant Growth

- Novel methods of milk analysis, including milk’s metabolome, lipidome, and glycome, are providing a more detailed and complex picture of human milk composition.
- Using an “omics” approach, a team of French researchers recently published the most comprehensive picture to date of preterm (27–34 weeks gestation) human milk composition.
• Optimal preterm growth from birth to hospital discharge was associated with milk ingredients that improved fat digestion, enhanced energy metabolism, and increased protein synthesis in muscles.
• The team identified a small set of milk biomarkers, including the amino acid arginine, with a strong ability to predict infant weight gain from birth to hospital discharge.
• A larger study is required to validate the predictive ability of these biomarkers.

A current trend in the marketing of healthy foods and drinks is highlighting a product’s short ingredient list; the less “stuff” in a food, the healthier it must be, right? This may be true for energy bars or fruit juices, but when it comes to human milk, a long list of ingredients is precisely what makes it optimal for infant health. Over the last decade, as the health food aisle has increased in so-called simple and clean foods, human milk’s ingredient list just keeps getting more complicated. Innovations in analytical tools have led to more in-depth studies detailing the specific types of fats, amino acids, sugars, and other metabolites present in human milk. Creating milk “-omes”—specifically, the milk metabolome, glycome, and lipidome—complicates human milk research in the best possible way, opening the door to identifying specific milk components that influence infant growth and development.

The most recent application of milk “-omics” comes from a team of French researchers investigating milk composition and growth in preterm infants [1]. Specifically, they wanted to find out if there was a milk phenotype—or signature list of ingredients—that was associated with faster growth rates among infants born between 27 and 34 weeks gestational age.

Preterm infant growth patterns are variable, even among those infants receiving protein-fortified human milk, indicating that factors other than total protein or calories may influence weight gain [Using an “-omics” approach offered the potential to identify metabolites or other bioactive compounds in human milk associated with optimal early growth among preterm infants.

The study population of 26 infants was drawn from a larger prospective study of 138 infants, each of whom received their mother’s breast milk for a minimum of 28 days. Each infant was ranked based on their change of weight Z-score, a statistical tool that represents an infant’s weight change relative to the mean weight change across the study population. Out of this initial group of 138 infants, the 11 infants with the highest weight change Z-scores made up the faster growth group and the 15 infants with the lowest weight change Z-scores were the slower growth group. Each infant had three associated milk samples; mothers provided 24-hour breast milk samples (manually collected) during the second, third, and fourth week post-partum, each sample considered representative of that week of lactation.

Now comes the complicated part of the study design—comparing the data on milk composition between the two groups. When comparing classic milk constituents like protein, fat, lactose, and calcium, there might be only a handful of data points for each milk sample. In contrast, data generated by analyzing each sample’s metabolome (all of the small molecules in milk), lipidome (all of the fats in milk), glycome (all of the sugars in milk), and free-amino acid profiles results in thousands of data points. To help separate the wheat from the chaff, the team utilized statistical methods that selected the particular constituents that created a clear separation between the faster and slower growth groups, all while controlling for potentially confounding variables (e.g., maternal age, infant birth weight).

Some of the significant differences between the faster and slower growth groups were easy to predict. For example, branch-chained amino acids (BCAA)—which play a role in muscle growth by signaling protein synthesis—were significantly higher in milk from the faster growth group compared with the slower growth group. But many of the differences were less obvious. The faster growth milks had lower concentrations of glycine and taurine, amino acids involved in bile acid conjugation. On its own, this result may not directly relate to growth. But the team also found the faster growth group had higher milk fat content. They suggest that these combined findings indicate enhanced bile acid conjugation in the faster growth milk
group; less glycine and taurine was present in the free form because these amino acids were instead used to convert bile acids to salts. Such an increase in bile acid conjugation would make milk fats more soluble and easier to digest, which could be especially important for infants with immature guts [1].

BCAA aren’t the only component influencing muscle protein synthesis in faster growing infants. Higher concentrations of the amino acid arginine are an additional molecular signal for initiating protein synthesis in muscle tissue. Moreover, the researchers propose that arginine also confers protective benefits to the mucosal lining of the infant’s intestines. Higher concentrations of this amino acid could play a role in the possible prevention of necrotizing enterocolitis, a common disease among premature infants and a major cause of morbidity and mortality in the NICU [1].

Arginine was actually one of a handful of milk ingredients that demonstrated a strong ability to predict weight gain from birth until discharge across the study population [1]. That is, the concentration of arginine in milk could reliably tell researchers how much weight an infant would gain during their stay in the NICU. Other significant predictive biomarkers included tyrosine (a precursor of thyroxine, a hormone involved in energy metabolism), beta-hydroxybutyrate (a ketone body implicated in protein translation and the modulation of inflammation), niacinamide (a form of niacin, a B3 vitamin that influences fatty acid metabolism and energy production), choline (which enhances the liver’s production of glycogen, a backup energy source), and lacto-N-fucopentaose I (a human milk oligosaccharide that supports the growth of a healthy gut microbiome) [1].

Looking at only milk macronutrients would have led to the conclusion that optimal preterm growth was the result of higher energy milk. But the findings from this study’s comprehensive “-omics” approach—the most comprehensive picture of preterm milk to date—suggests that total milk energy is only one part of a much more complicated and fascinating story: milk producing faster growth had energy provided in a more digestible form for naïve digestive tracts, higher concentrations of amino acids that promoted protein synthesis and muscle growth, and bioactive molecules that influenced energy homeostasis and intestinal health [1].

The study authors readily admit that, because of the small sample size, their findings are best framed as testable hypotheses about milk composition and preterm infant growth. But if these results are validated in a larger study, they offer the potential of using human milk to improve the growth and long-term outcomes of premature infants.


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**Genetically Engineered Yeast Make Low-Calorie Sugar from Lactose**

- **Tagatose** is a low-calorie alternative to sucrose and high-fructose corn syrup, which tastes similar to sucrose.
- **Tagatose’s commercial use in the food industry has been limited by the high costs of industrial production.**
- **A group of researchers recently reported that they had genetically engineered yeast to create a cheap and scalable method of tagatose production that uses lactose as its main input.**

High-fructose corn syrup has a bad reputation. It is a common ingredient in all kinds of processed food, from cookies to bread and salad dressing, primarily because, unlike sucrose, it is predictably cheap over time [1]. As with other sugars with a high glycemic index, its consumption is associated with an increased risk of metabolic disorders, such as type 2 diabetes. For a long time, nutritional scientists have known about healthier sugars with the prerequisite sweetness to replace high-fructose corn syrup, however, the production costs of these alternatives have not been competitive. Now, thanks to the efforts of an international group of researchers, this may change. The group has genetically engineered yeast to turn...
lactose into tagatose—a sugar with about half the calories of table sugar, and that tastes like the real thing.

Specifically, tagatose has 92% of the sweetness of sucrose. Its current means of industrial production has received “generally recognized as safe” status from the United States Food and Drug Administration [2]. This method involves an enzyme-catalyzed isomerization reaction. The more tagatose you wish to produce, the more enzyme is needed, which is one reason the costs of industrial production have been so high. The main reason, however, is that only a very impure product can be manufactured in this way. At 30°C, the isomerization reaction yields a mere 30% tagatose to 70% galactose—the raw ingredient from which it is made—because a thermodynamic equilibrium is set up between the galactose substrate and tagatose product that is hard to nudge towards a greater proportion of product. Purifying this mixture requires substantial money and effort.

What if these purification costs could be essentially eliminated, and if there were no need to add enzyme in proportion to the scale of the operation? This is what the research team, led by Yong-Su Jin of the University of Illinois at Urbana-Champaign, have achieved. They have come up with a “carbon-partition strategy.” This begins with normal laboratory yeast—a workhorse of geneticists. The researchers tweaked its genetics so as to divert lactose consumption away from the pre-existing metabolic pathway, and towards alternative routes. The resulting yeast can absorb lactose, break it up into glucose and galactose, and then use this glucose to fuel the conversion of galactose into tagatose, as well as to power cellular replication—which makes for a self-sustaining and scalable process. Doing things this way generates a fermentation broth that is 90% pure. The last little bit of galactose in the mixture can be mopped up by adding standard yeast. The work is published in a recent issue of the journal, Nature Communications [3].

The researchers had to overcome a series of genetic challenges to create the tagatose-producing yeast. The first step was to delete a gene called GAL1. This gene encodes the enzyme that normally begins galactose breakdown by phosphorylating the sugar. The team then introduced new genes into their chosen “EJ2g” strain of yeast using plasmids. They added a xylose reductase (XR), which turns galactose into galactitol, and a fungal gene called galactitol-2-dehydrogenase (GDH), to take the galactitol produced by XR and create tagatose. Then the challenge was to optimize the tagatose yield, which they did by adjusting the expression levels of XR and GDH, aiming for a fermentation broth as rich as possible in tagatose.

The authors hope to commercialize their findings, having already been granted a patent. Three of them, including Yong-Su Jin, have a financial interest in a company called Sugarlogix, Inc., a start-up based in Berkeley, California, that aims to create, in a commercial setting, sugars that promote the growth of “good bacteria” in the human gut, such as 2′-fucosyllactose, which occurs in breastmilk. Presumably tagatose will soon be added to the company’s repertoire. Aside from the low cost of the production method, the main ingredient—lactose—should be cheap to acquire, since it is a waste product as part of whey in the making of cheese and Greek yogurt.

Other innovators have tried over the years to figure out a cheap way of making tagatose[4], without great success. But by tearing up the old recipe and starting afresh, these researchers appear to have found a low-cost and scalable method—and, if development continues to go well, they may provide cookie factories everywhere with a financial incentive to make healthier biscuits.

Dairy-Containing Supplement Reduces Rates of Stunting in Babies Born in Resource-Poor Communities

- Babies born in resource-poor communities have a high risk of stunting at birth, leading to growth failure, impaired brain function, and higher rates of mortality.
- Supplementing mothers’ diets from the early stages of pregnancy through birth with a dairy-containing, macro- and micronutrient supplement significantly decreases rates of infant stunting, regardless of maternal body mass index.
- Beginning maternal supplementation prior to conception, versus late in the first trimester, does not appear to confer additional benefits in terms of infant size.

Babies born in resource-poor rural and semi-rural communities have a high risk of stunting, that is, being born short for their gestational age. Rates as high as 60% have been documented in one indigenous population in Guatemala [1]. Stunting at birth predicts increased infant and child mortality as well as ongoing growth retardation. Growth retardation in turn carries a higher risk for impaired brain function and loss of economic productivity. In female infants, growth failure presents a greater reproductive risk for themselves and their eventual children due to intrauterine growth restriction [2]. The issue is primarily a consequence of inadequate nutrition, and is considered to be a major public health challenge in developing nations. However, nutrition interventions carried out during the infant and toddler stages have had only limited success in either treating or preventing growth failure. Those aimed at maternal nutrition during pregnancy, primarily focusing on micronutrients, have produced positive, albeit modest, effects on newborn size. Interventions prior to conception have not been well studied in humans, but animal studies have shown promise [3].

A new study published in *The American Journal of Clinical Nutrition* aimed to test the effect of an inexpensive, dairy-containing supplement on newborn length and overall birth size when given either prior to conception or during pregnancy [4]. This study is unusual in that it looked at the effect of supplementing both macro- and micronutrient intake. The supplement is described as “a multi-micronutrient fortified lipid-based supplement composed of dried skimmed milk, soybean and peanut extract, sugar, maltodextrin stabilizers, and emulsifiers.” Each package weighs less than an ounce and provides vitamins, minerals, protein, and fats that may be missing in the women’s diets. It is blended into a peanut butter-like consistency and can be eaten alone or mixed with other foods [5]. The paste is also water-free and shelf-stable for up to 18 months in hot, humid environments and is acceptable to vegetarian populations. The supplement is a modified form of Nutributter, a formulation created at UC Davis for use by children at risk of malnutrition. Nutributter was inspired by the success of the original therapeutic nutrient paste, Plumpy’nut, a French concoction that consists of blended peanuts, skimmed milk powder, oil, sugar, and vitamins, and that has proven highly successful at treating acute malnutrition in famine emergencies. The paste formulation is key, because previous treatments relied upon mixing with local water, which in many cases was a source of pathogens.

The study consisted of three experimental groups: one in which the intervention was given starting at least three months prior to pregnancy, one in which the intervention began in the late first trimester, and a control group that received no intervention. In both treatment groups, the supplement continued through birth. A second fat- and protein-containing supplement was also given to women in the treatment groups with a low BMI, as well as those who failed to gain enough weight during the second or third trimesters of pregnancy. The study took place across rural and semi-rural locations in four developing
countries: the Democratic Republic of Congo, Guatemala, India, and Pakistan. All participants were between the ages of 16 and 35, and had given birth fewer than six times. Women with iron deficiencies were treated prior to acceptance in the study, and no one was excluded based on height, weight, or body mass index. The mothers also varied in diet, culture, socioeconomic status, and level of education. Following a live birth, newborns were measured within 48 hours for length, weight, and head circumference.

Over 2400 newborns were measured across the study. The researchers found a positive effect on both length and weight between the treatment and control groups, leading to a decrease in stunting of more than a third and a 22% lower incidence of infants that were small for their gestational age, a result that occurred despite maternal weight gain during pregnancy that was low relative to international recommendations. Surprisingly, researchers found no difference in outcome between the infants whose mothers had begun intervention prior to conception and those who had begun late in the first trimester.

"In settings with high rates of poor growth in children's height, including stunting, we found that improving mothers' nutrition either before they became pregnant or by the end of the first trimester of pregnancy resulted in their babies being longer and having better birth weights," explains Dr. Nancy Krebs, a professor of pediatrics at the University of Colorado School of Medicine and one of the study’s co-authors. "Since women often do not recognize that they are pregnant until after the first trimester, these results support strategies to improve women's nutrition before they conceive.”

A weakness of the study was the absence of gestational age data for one of the four study populations, the Democratic Republic of Congo, meaning those measurements could not be corrected for gestational age, which may have made improvements there less pronounced. It’s possible, the authors point out, that the differences in outcome between the two treatment groups won’t be seen until the next generation of children born to the female babies included in this study. In multiple studies, the effects of malnutrition take several generations to “wash out,” and a review of 14 studies on intergenerational associations in birthweight found that for each 100 g increase in maternal birthweight, there was a further 10–29 g increase in offspring birthweight [6]. There was also evidence that these increases are largely independent of the postnatal growth of the parents, underscoring the importance of prenatal nutrition to later generations.


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