Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

Applying **MFGM** and **Human Milk Science** to Improve Nutrition for Infants and Toddlers

Presented by
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Applying MFGM and Human Milk Science to Improve Nutrition for Infants and Toddlers

Target Audience

This activity was developed for physicians, pediatric nurse practitioners, nurses, registered dietitians, and other health care professionals who have an interest in newborns, infants and toddlers.

Learning Objectives

At the conclusion of this activity, participants should be better able to:

- Describe how dairy processing advances have isolated MFGM for supplementation to infant formula
- Examine how purified MFGM from cow's milk added to formula is similar in composition and bioactivity to human milk

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Consultant	Mead Johnson Nutrition-scientific area: Dairy Science

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Additional content planners

Marion Groetch, MS, RDN (registered dietitian planner)

Speakers Bureau	Abbott, Mead Johnson Nutrition, Nutricia North America-scientific area: Food Allergy

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The following have no significant relationship to disclose:

Victoria Anderson (medical writer)
Eugene Cullen, MD (peer reviewer)
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This activity is supported by an educational grant from Mead Johnson Nutrition.

This activity is an online enduring material. Successful completion is achieved by reading and/or viewing the material, reflecting on its implications in your practice, and completing the assessment component.

The estimated time to complete the activity is 1.0 hour.

This activity was originally released on June 30, 2018 and is eligible for credit through June 29, 2020.

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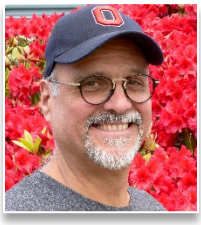
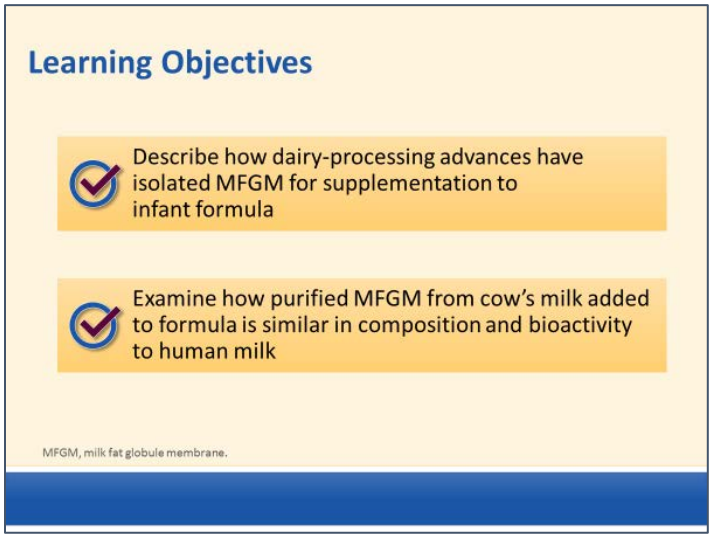
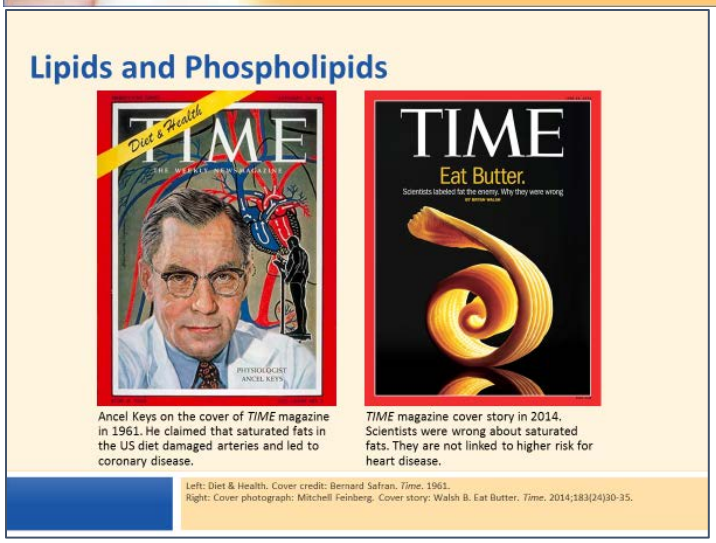


Dr. Rafael Jiménez-Flores: I always start defining the learning objectives to my students, and in this case, my audience.

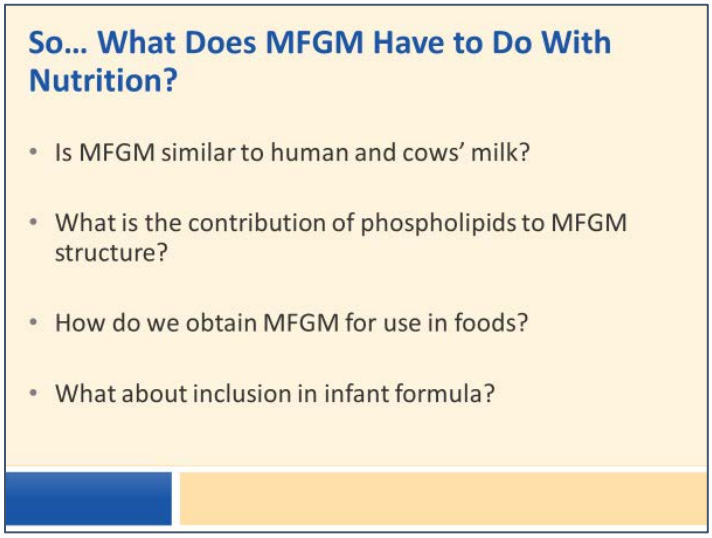


The first learning objective is going to describe how dairy processing has succeeded in isolating this fraction from milk, and how this is important for supplementation to infant formula. We are going to see how the bioactivity is reflected once the component is added to the infant formula.

So, first of all, let's find out how we find ourselves in the research world, where we are, and what advances we have. According to a survey on dairy processing, we have several underutilized dairy components, such as lactose, the minerals that are in milk, but, more important for the talk today is that the milk fat and phospholipids is an area of very active research and a resurgence in terms of importance in nutrition. So, we really need to know more, as scientists, from the biology and biochemistry, about the milk fat globule membrane. Where does it come from, and what is its structure?

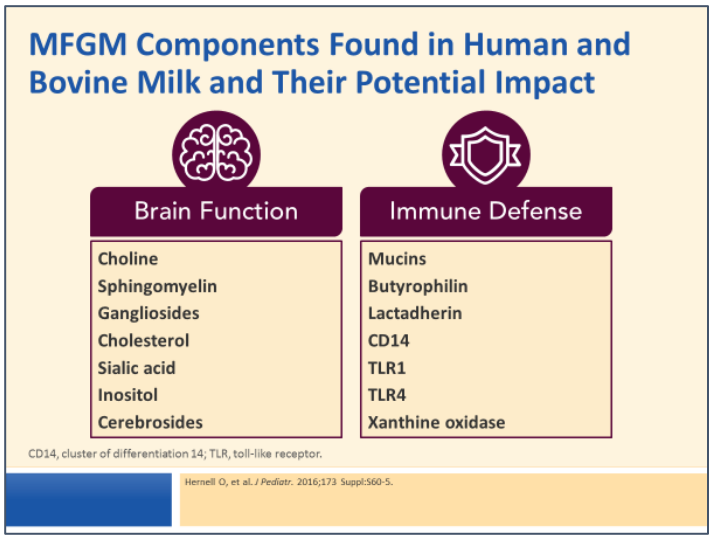


Just as background on the social media and what is out there… there may be still some remnants of maligning the fats from milk or animal origin. The vast majority of our research now, and supported by many studies, really has shown that it's not fair to demonize, specifically, dairy fat. And that has opened the gates for renewed information, renewed research on this area.

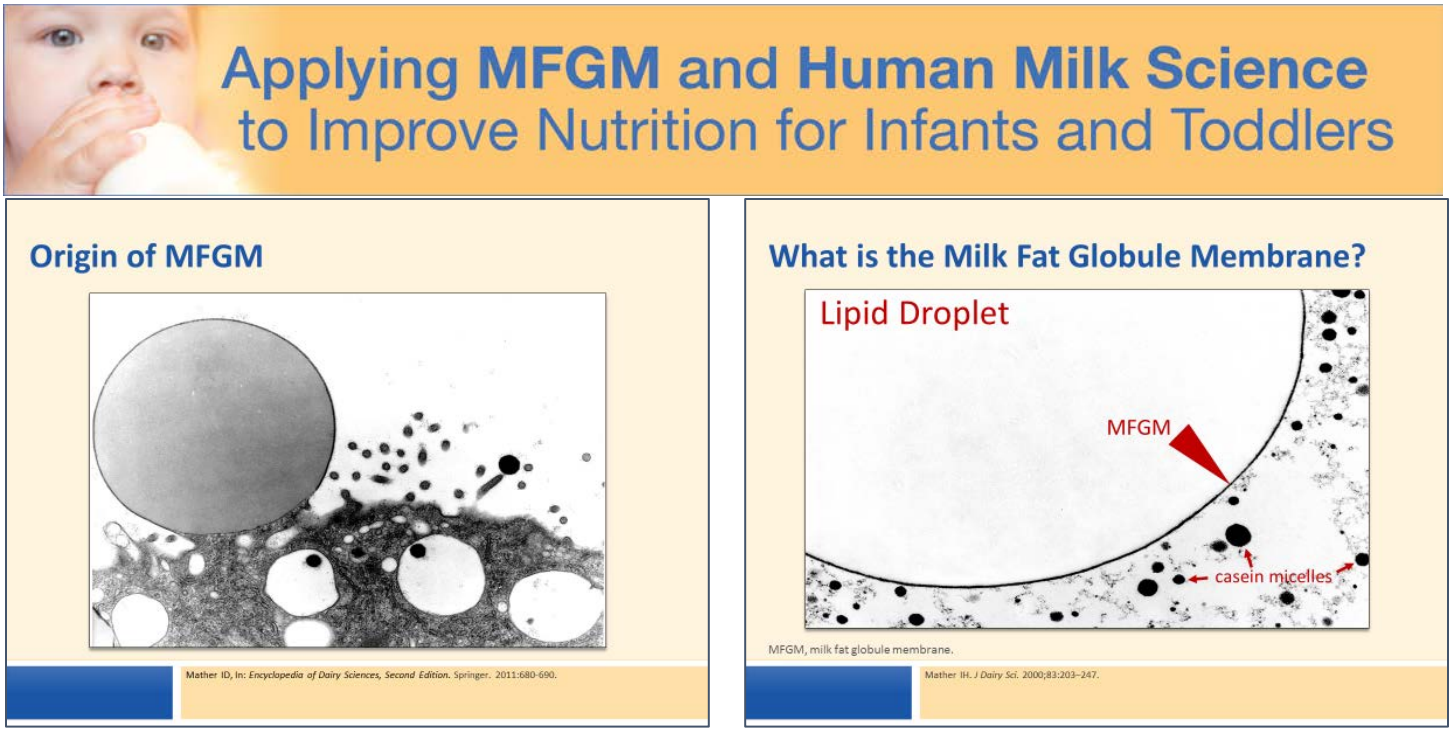


And you may—as an audience—be asking, well, what does the milk fat globule membrane have to do with everything—nutrition and especially infant nutrition? Some of the questions from my students and the audiences that I talk to is: Is milk fat globule membrane similar in human and cow's milk? What are the contributions of the phospholipids to structure? Does structure have any importance? How do we obtain this ingredient and how about inclusion in the formula?

So, we'll take it by parts.



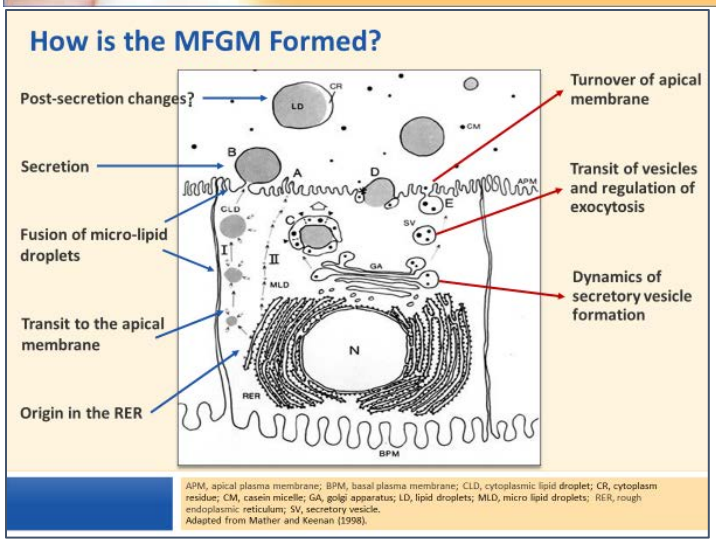
Let's just start by the components. I would say they're not major nutritional components like protein, water, [and] sugars. But definitely, in the milk fraction component of the milk fat globule, we have components that are very important, and they have been studied in many other fields on brain function, like cholines, sphingomyelin, gangliosides, which were initially isolated from ganglia, cholesterol, sialic acid, and some other very specific lipids that are glycosulated lipids, called cerebrosides. All of these are found in the milk fat globule membrane. On the immune defense system, we have mucins, butyrophilin, lactadherin, [and] something very interesting, toll-like receptors, which are indicators and signals about pathogens in our diet. All of this is condensed in what I consider to be one of the more interesting frontiers in dairy science, and especially milk chemistry.

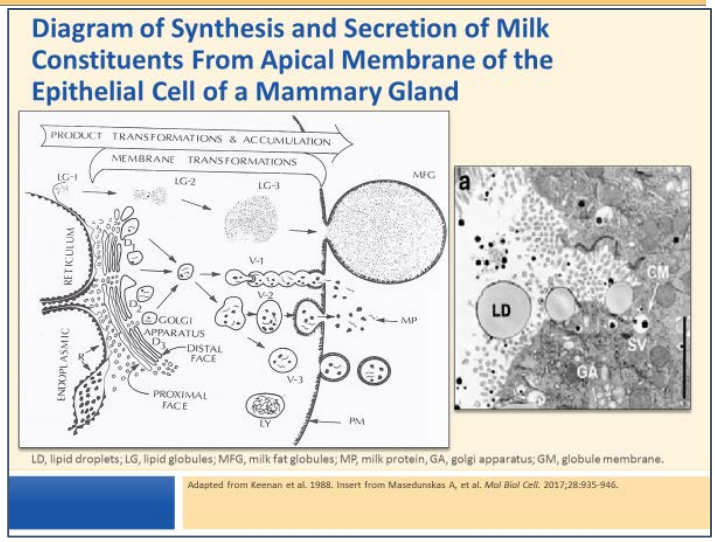


What you should be looking at right now, is an electron micrograph of a fat globule being secreted into the milk lumen by a mouse milk secretor cell. These cells are classified as epithelial cells. They are all in the mammary glands of *all* mammals. Anything that produces milk has these cells, and they are very well-conserved, as we will see later on. You will see that big gray sphere is composed almost exclusively of triglycerides. So, that's what would be the butter fat. And you will notice it is surrounded with [a] perfect margin of a very thin line. That thin line is the membrane, which at that point is 3 layers. Because, if you look at the part that is touching the cell on the bottom of that sphere, you'll notice a very thin line. That's an initial monolayer of phospholipids that then pushes through and gets secreted—or students in good science would say, it's microencapsulated into the milk.

The next picture shows us with even more detail, how thin this membrane is, and, hence, why it is so challenging to study, because it really… although we can see the effects as we pour cream in our coffee and it does not leak the way it would if we added, let's say, mayonnaise or something that is not encapsulated by a membrane like that. We can see that the dimensions are exquisitely small. You can see by comparison, we cannot even look at them under a normal microscope, the casein micelles. And, yet, that membrane is even smaller.

Let's explore what happens at the biological point of view on [the] secretion of milk.

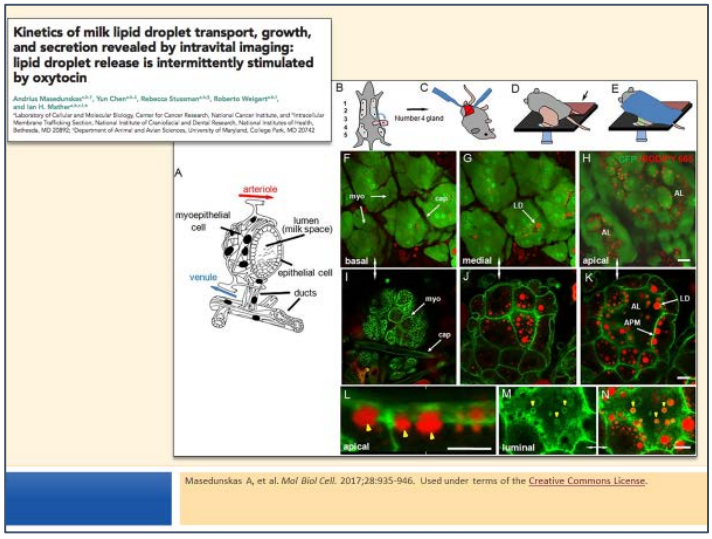




I apologize if the audience is already milk secretion experts. This cartoon depicts with the endonuclease, the golgi apparatus [GA] right in front of it, where a lot of activity is done to synthesize proteins, as we can see. And that 'E' is a vesicle; it's a secretory vesicle. But on your left-hand side, you'll see how the microlipid droplets grow from very minuscule droplets that keep aggregating (like the drops of fat on your chicken broth), until they reach to a larger size. And by the time that they get to the apical membrane, which you can see where the upper part is, where we have the milk and inside the cell, that fat needs to be extruded through the membrane, which engulfs it perfectly, and then we have our little droplets of cream in the milk.

I always put these post-secretional changes with a question mark, because it's my belief that Mother Nature really didn't intend milk to be bottled or put into a piece of cheese, or anything like that. And it is up to us, food technologists, to know and to mimic whatever happens with that membrane later on.

Now this process has been long studied and is more complex than what I'm describing, because there's many other pathways of secretory vesicles that we're just continuing to understand. As you see, [in] the electron-micrograph on your right-hand side, the lipid droplet is really surrounded by a lot of the other smaller vesicles that are present in the milk of all mammals.

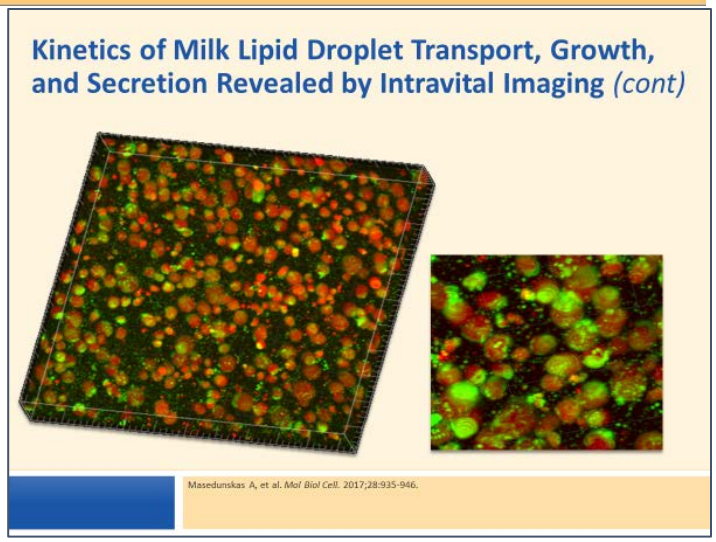


That has been very recently reviewed and beautifully explained by Dr. Ian Mather and his team. By using a technique—and if you ever have a chance to see this paper,[1] I really recommend it, it's on intravital imaging —and this is done with a small rodent,

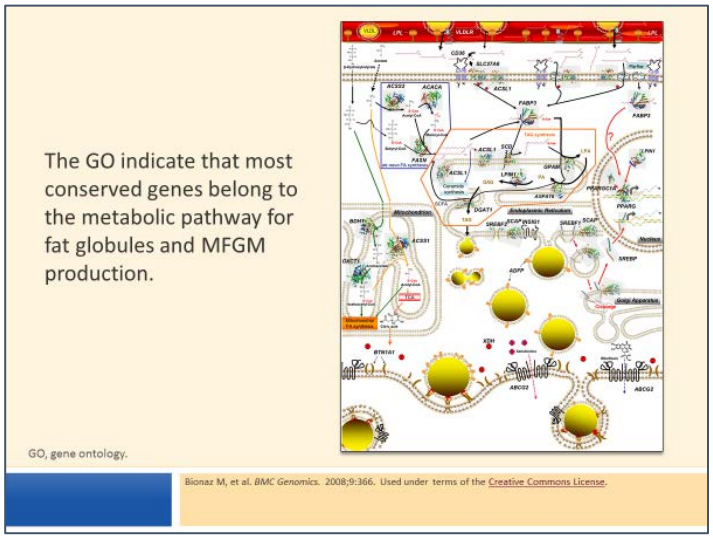
a rat, in which the live rat is surgically intervened to expose the mammary gland onto the confocal microscope. This is very important, because the mouse, as you can see on the top right, is kept alive. That is a blanket to keep the mouse as comfortable as it is with an exposed mammary gland to the microscope. And then the microscope takes a bunch of pictures in 3 dimensional.

So, first I want to show you the cartoon of the alveoli, of the milk secretion alveoli, on your left-hand side, and see the similarity in your panels J and K on these fluorescent pictures. The red dots are fat globules that are inside the cell. In your panel M, you can see—and this is the newest contribution by this group—that the fat globules are there ready to go, but they're not secreted into the lumen until there is a very specific signal, or oxytocin. Once oxytocin is injected to the mouse—that is comfortably laying on top of the microscope—there is a rain, literally a rain of lipid droplets that get secreted into the milk. So, hopefully, at one point we will have this in a movie or you can go and see this paper by Masedunskas and it is fantastic as added information to have that.

Just so that we emphasize this point, this a very, very well-conserved mechanism across the mammalian world.



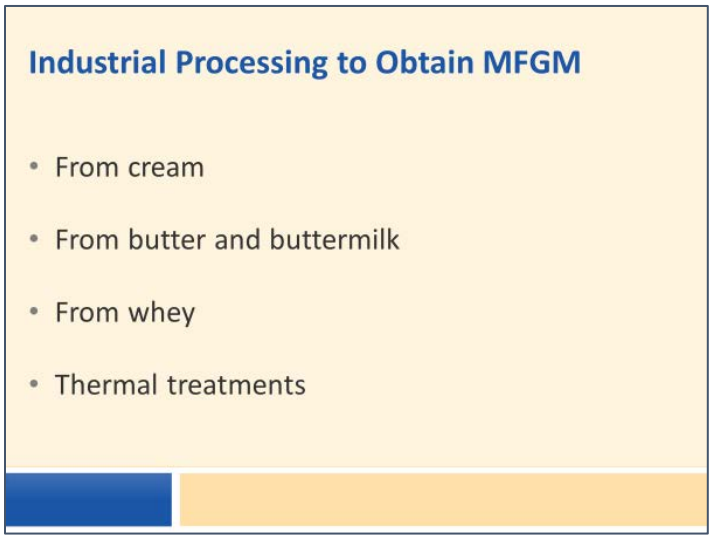
And what is very impressive is the amount of fat globules we have, especially early lactation, we have smaller globules. If you were to add all the surface of those little spheres you could, with a glass of milk that has all [of these] globules in there, cover a surface that is similar to a tennis court—that's what physical chemists tell me. I've done some calculations and my goodness, it is incredible what happens. And that would be on normal milk.



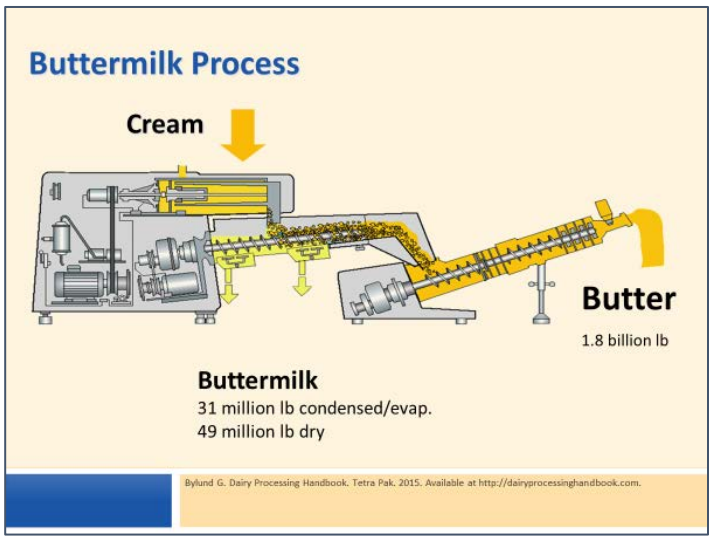
But there's something else that is very important to this discussion, at least to the biological part of the discussion, and

that is that back there in 2008, my friends Massimo Bionaz and Juan Loor,[2] published the whole metabolic pathway of all the enzymes that are needed to secrete the fat, the milk. In this case, it goes upside down, or to the other pictures, because the milk is at the bottom of the slide, and you can see where the fat little globules get zipped by the membrane.

But the important part about this slide is that it gives us, for the first time, a whole list of enzymes that we could see and compare between humans, cows, goats, donkeys, horses. And it was amazing to see that those enzymes that are involved in this very process that you're looking [at], are 99% conserved. Which means that the gene ontology indicate that these are some of the most conserved genes in metabolic pathways, which to any biologists (understanding of life) tells us that this is a process that is very important, according to nature, because it hasn't been modified.

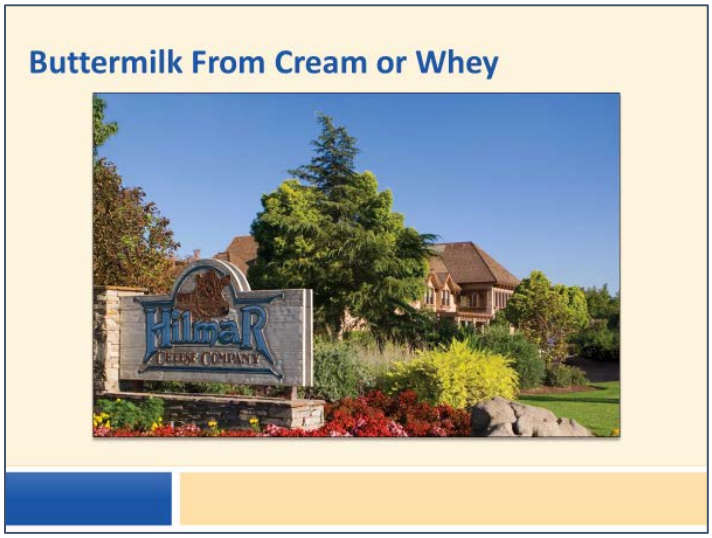


All right, now that we have seen a little bit about the milk fat globule membrane and where it comes from, I'm hoping that my audience is starting to have a new appreciation for what we have in milk, and not just that we ask for a nonfat milk in our coffee with foam or without foam. **We are going to show you some of the industrial processing steps we have to isolate this particular component.** We can isolate it from cream, from butter or buttermilk, from whey. And our main focus is to keep in check the thermal treatments that we do to our milk because heat, as we know, changes the conformation of proteins, and the protein and lipid interaction.

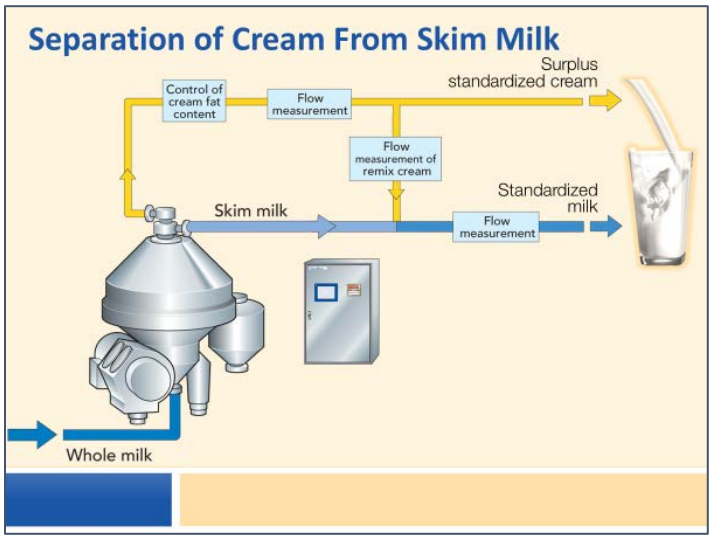


Here is a diagram of the modern butter churn. When I say butter churn, most of the people that read history, think of a nice wooden bucket with a stick made in a dairy and is shaken. This is far from that. What you see is this butter churn gets fed the cream on the top. Then it has a very fast churner that is the sign to break the milk fat globule membrane, and thus the triglycerides get separated from the serum. As you see by the screw mechanism, we squeeze out the serum that we call buttermilk. Coincidentally, buttermilk, even though the name has butter in it, has less fat than your regular skim milk. And, then of course, butter has 40% fat. And in this nation, we produce vast amounts of buttermilk. But it's not the buttermilk you buy in the supermarket. That is just fermented skimmed milk. Right now, the bulk of our buttermilk goes to big dairy

plants, and it gets dried into powder. If you ask me where is it used, it is used mostly in bakeries and ice cream, where the properties of the phospholipids are great as emulsifiers.



The way it all starts? Well, if you're a researcher like me or you want to start getting your milk fat globule membrane for your infant formula, you identify a very good producing dairy farm. You get the milk.



The milk then is processed through a machine that is called a cream separator, because it gives us the skimmed milk, as is shown here by the blue line on the bottom, and the cream. Now, a well-known trick of the dairy processors is that we separate skim and cream, and then we remix it so that we can give you 1% fat milk, 2%, 3%, or as it's whole milk, when I talk [about] how much fat is there in whole milk? It sounds like a lot of fat. It's only 3.5% fat. We could sell a product that is 96.5% fat-free, but dairy farmers decide[d] not to go that way.

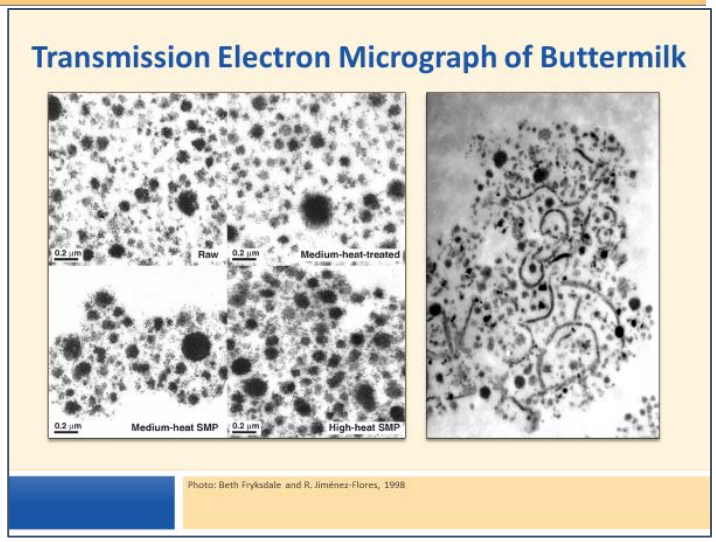
And right there in the glass, I'm trying to depict that's where we get the cream. And that cream is a raw material for making butter. I'm going to exemplify how we get milk fat globule membrane from butter.

The main reason—and in this picture, you'll see me lecturing [to] my students here at The Ohio State University—those that take dairy processing get to work with an automation machine that is a continuous butter churn. As long as you feed cream to this machine, it gives you butter. You can see it in the bucket, right there, the butter. Or, you see the red arrow that is pointed to the left, points to a tank, where that tank was added for our research, and it collects all the buttermilk. How does the buttermilk look? Well, just like skimmed milk, right there on

your right-hand side in the bottom panel, you see the appearance of real buttermilk.

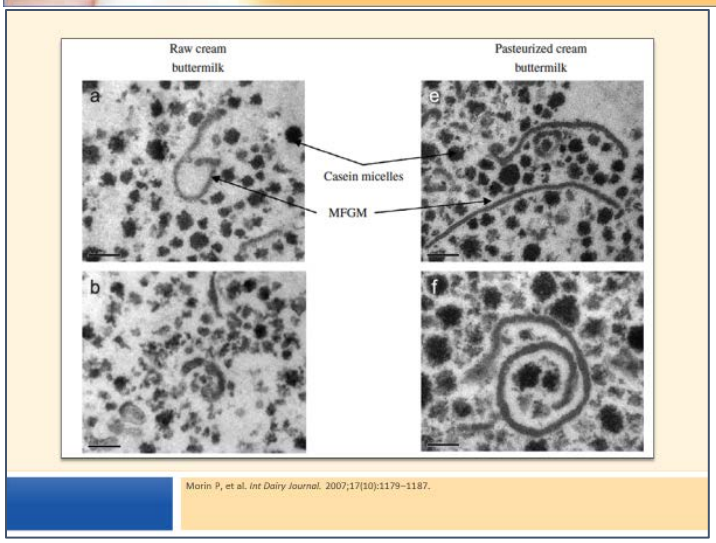
Well, like any industry, what do we do? We can research this as such: liquid. Hopefully, we'll get some advances, and I'm told that in Europe and Ireland, they're starting to have drinks, like it used to be 50 years ago, of real buttermilk. Maybe somebody will commercialize it.

For the moment, what we do industrially is pass that buttermilk—after some concentration—through a spray dryer. Here's an example of a hydroplants spray dryer. The ones in industry are 4 or 5 stories tall. This is not even one—it's about 10 to 12 feet tall. And we can get powders. And those powders are a very interesting characteristic when they have a milk fat globule.



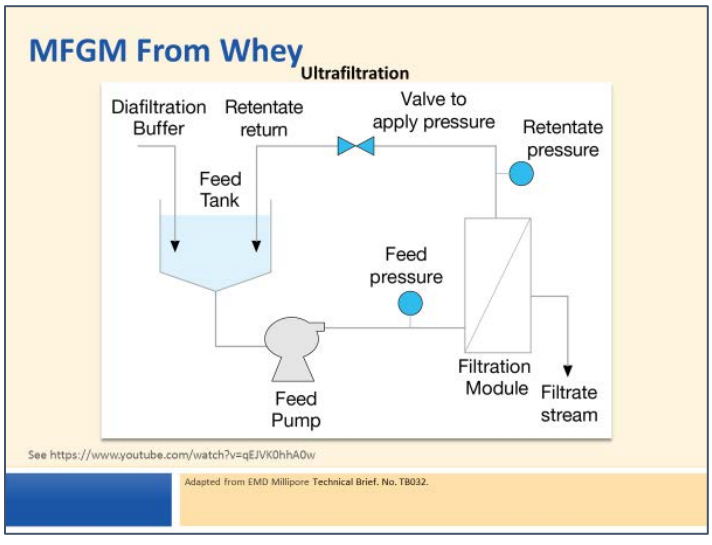
The next slide, this slide shows us a transmission electron micrograph. On your left-hand side is just skimmed milk, treated with different heat treatments, but that's just how the electron microscope looks at the casein micelles that are in skimmed milk. So, there's no cream there, no fat.

And the buttermilk, although you know I told you that it has no fat, it does have those phospholipids in a very peculiar form. If you notice, on your right-hand-side picture, there's a lot of wormy-like [images] there, like little serpentine figures. That happens to be the original, very thin membrane that is rolled over—like if you were to roll over a carpet where your kids have been playing with their Legos and their toys and everything. So [in this example], the carpet happens to be the membrane, and the Legos and the toys are the tropines that are inherited to the milk from the mother.



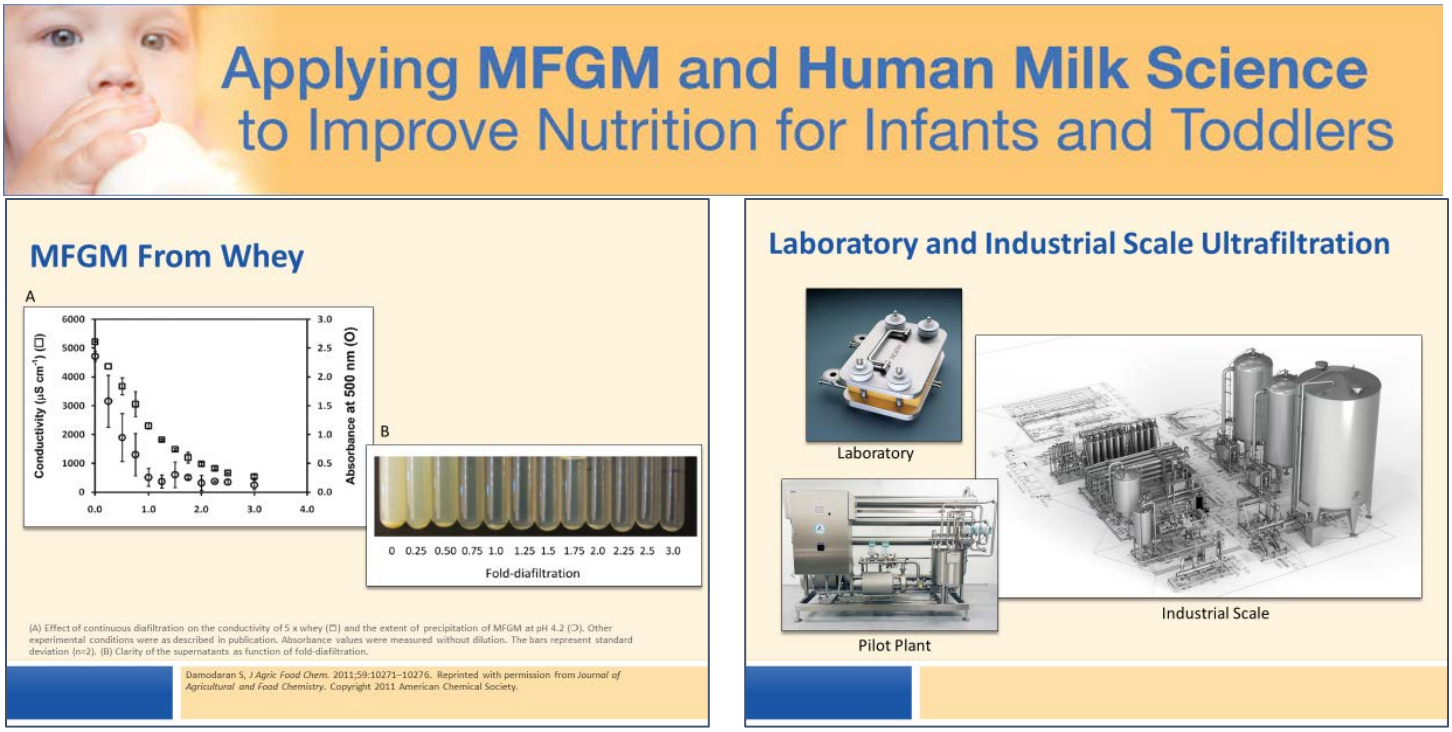
So that's why we think that there's something very interesting about these little wormy things, because we do believe, and we have seen some evidence that these are means of communicating nutritional signals to the baby, and in case of the industrial buttermilk, they're all encased in this component.

Well, that's all fine and dandy, but how are we going to separate that for our use, say, in infant formula?



As you can see, this is not a normal thing that would happen in regular processing. And since we industrialize our milk, this is not a normal part of our diet; [al]though, we use an advance [system] called ultra-filtration. Whether this can be done from whey or from buttermilk, the principal is the same. You'll see here on your left-hand side, it says Feed Tank. You put the butter, the buttermilk, or the whey [in], and you have a pump that goes through what we call the filtration module. So, imagine that diagonal line on that little rectangle is the membrane we use. It's very special. Now we're talking about the milk fat globule membrane, and this is a ceramic membrane; it's a man-made membrane. What it is, is the filter, for all practical purposes. So, if you follow the flow directly out of the feed pump, it goes there, and the liquid will find that filter. And that filter is very special. The low molecular weight components are going to go out through the filtrate screen, we call it a permeate or filtrate. And the rest is going to be concentrated. And it keeps being concentrated, the more loops that we go through. And that is very important, because according to some of the processes that we have, we can get rid of some of the proteins, certainly the calcium and the lactose, and concentrate [on] our milk fat globule membrane.

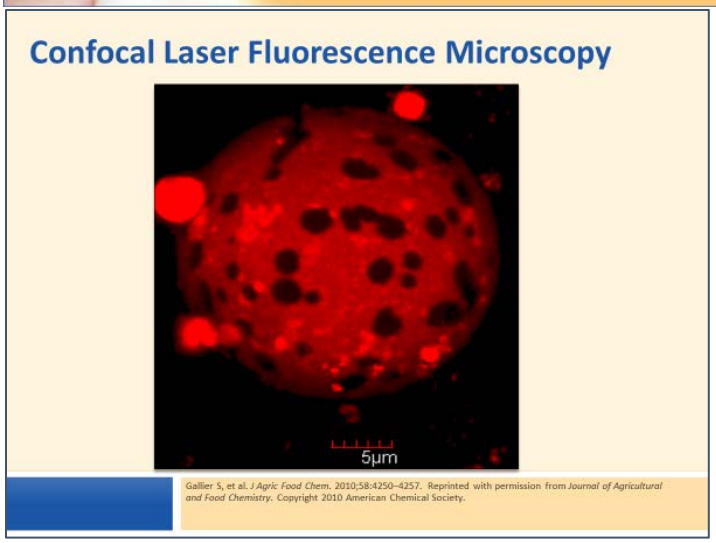
For those of you who want to [know] how efficient this is, I can tell you, to make 1 kg of the milk fat globule membrane ingredient, you need close to 200 kg of whey, of milk, or any other component. As in this case—and this is a very interesting part of something that is happening in our research field right now—of any filter, it can get clogged. It can really hamper our economy and our efficiency of this process. It turns out that **what clogs this membrane happens to be material very rich in membrane.**

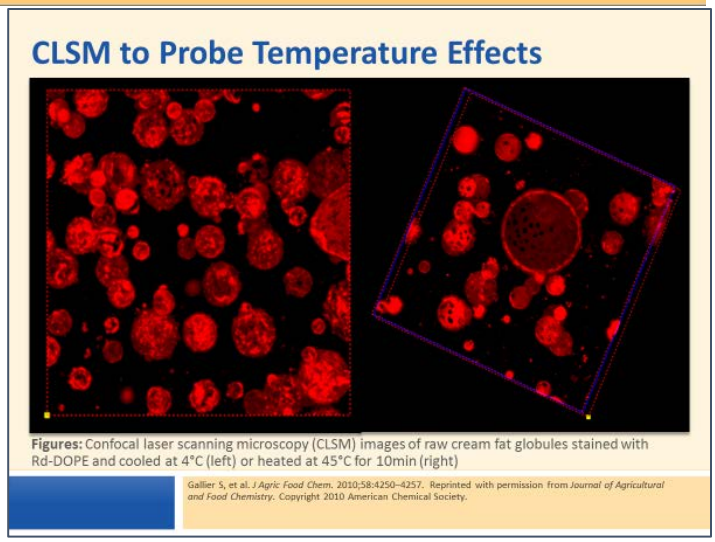


So, this slide is a clear example that [what's] one man's waste, is another man's treasure. Dr. Damodaran, from the University of Wisconsin, spent a lot of time trying to better prepare the whey, to isolate the whey proteins that we use so much these days, in whey protein concentrates, whey protein isolates. Because he identified, that if you see those test tubes, have like a little sandy residue in the bottom, and that's what was clogging the membrane. So, he devised a way of getting rid of it. And when we analyze that, it is very rich material for the milk fat globule membrane and the phospholipids I've been talking to you about.

So, this is just an illustration that you can have this membrane system for the laboratory or pilot plant, which is what I have here at The Ohio State University. But it has been scaled up to [an] industrial scale to produce whey, whey protein isolate, and whey protein concentrates, which is, by the way, one of the major economic impacts that we have had in our dairy industry. We use that in many foods. We export it, and it's right now, I would say a byproduct of cheese, but I think right now there's more value in these fractions than there are even in cheese.

But let me just go back for a minute about the importance of the structure of these components that we have been describing: **how do we isolate them from milk? What do they do?**

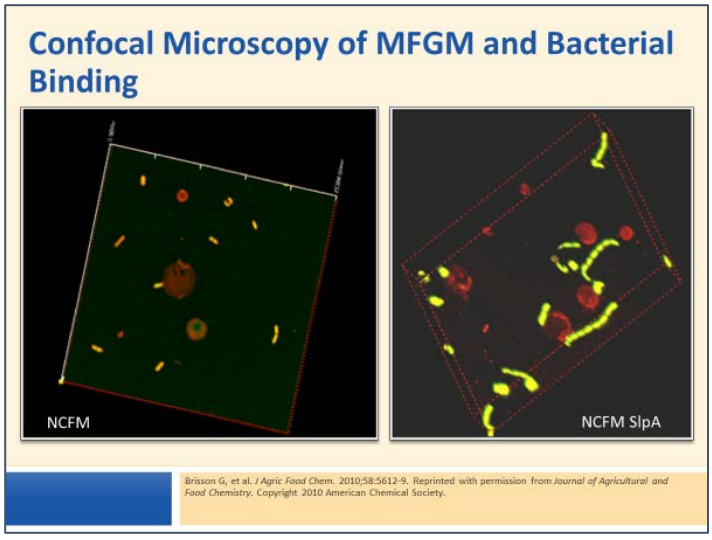




Well, I'm going to show you a picture that a former student of mine, Sophie Gallier, very artfully did, of a droplet of fat in milk. And with a fluorescent microscope, she showed that there are these black spots, which happen to be what physical chemists call, liquid ordered domains of phospholipids. These are so organized, so well structured, that they don't allow the fluorescent pigment to go in there—and that's why we see them as black spots.

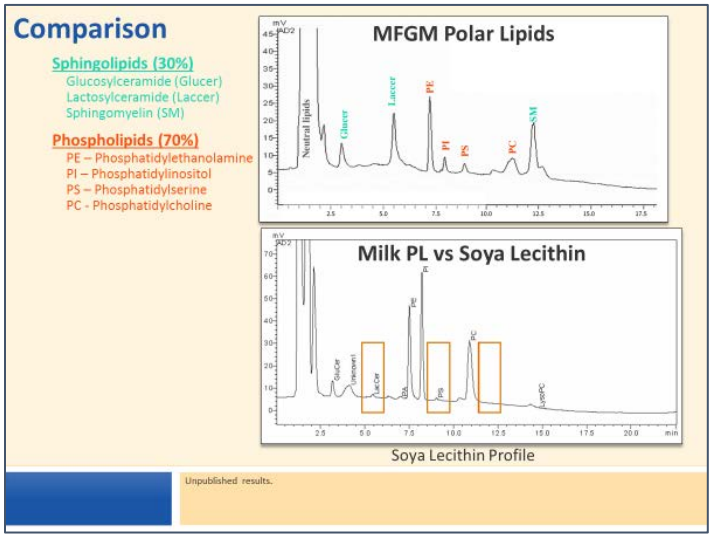
The lesson that we got is that it complicates our studies, because clearly the surface of the fat globules, for that matter, the surface of the epithelial cells producing milk, are not homogeneous. And that has deep implications in chemical reactions, biochemistry, and being very interested in processing, I can show you in this next picture…

…that even mild thermal treatments, like on your left, is cooling it to 40 degrees, which is how we refrigerate milk. You can see how that surface looks: very rough, very rugged. And, if we heat it up to only 45 degrees centigrade—which is a very hot day in California, for example—the surface is going to take a completely different morphology: very smooth, and we see very well-defined domains of these little black spots.

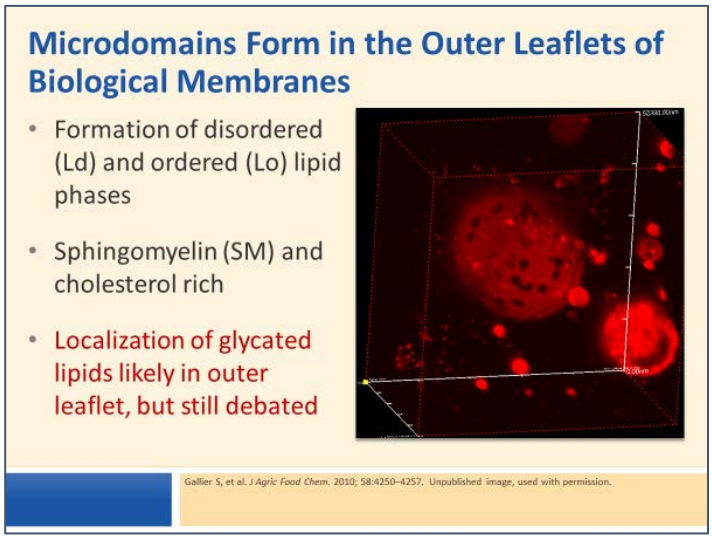


Furthermore, now that we are very interested in how the microbiome gets affected by our food, let me tell you, it is not just that we have the sugars or the little saccharides or not,

whatever, that they're feeding, we have seen that the bacteria, by very specifically—depending on the kind of bacteria to the surface—and what we know now is that the interaction is mutual. Not only [do] the bacteria bind to the fat to cause some (probably chemical) very important changes in digestion. But because of that interaction, the milk fat globule can also be thought [of] as changing the composition of the bacteria. Because the bacteria need to secrete other enzymes in order to get food from the fat.



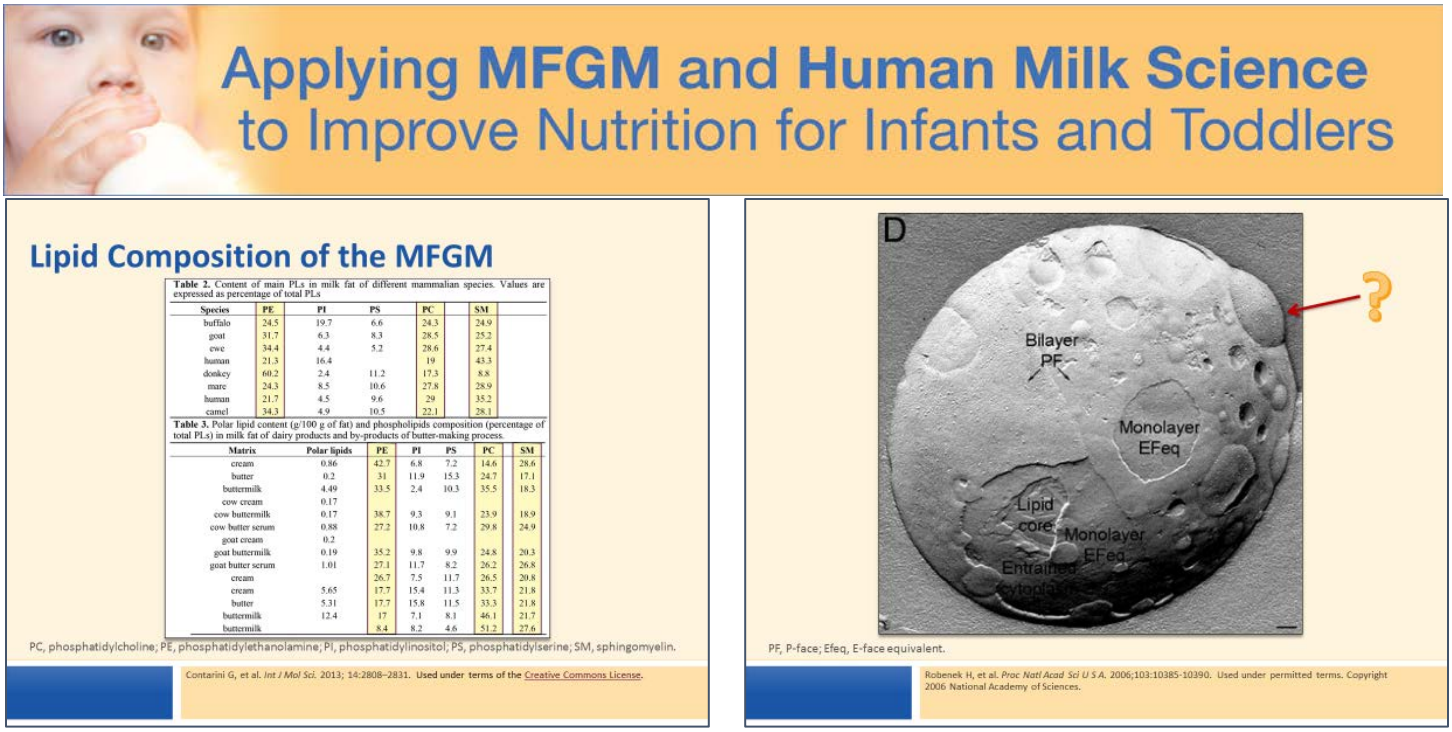
When we thought about this… I'm showing you a liquid chromatography of the comparison of the phospholipids from milk (on the top) and from soy or, for that matter, any other plant, phospholipid component, which are called lecithin. Up here, you would see that—to me, most important—there's sphingomyelin, that you see that peak. It's completely absent where it should be if you compare them in soy. If you ask me, that stands to reason, because there's no real compelling law that says a soybean needs to produce a component that is very good for brains or growth on the intestine, since the beans really are not designed to be eaten. I've looked, and [the] soybean does not have 2 very important things: the soybean does not have a brain or a mammary gland. And hence, they don't need to synthesize those components.



So, we know that there's microdomains forming the outer leaflets of these membranes, and they have an organization. What that means exactly is still a question, but it is a very scientific question, and we start by describing it.

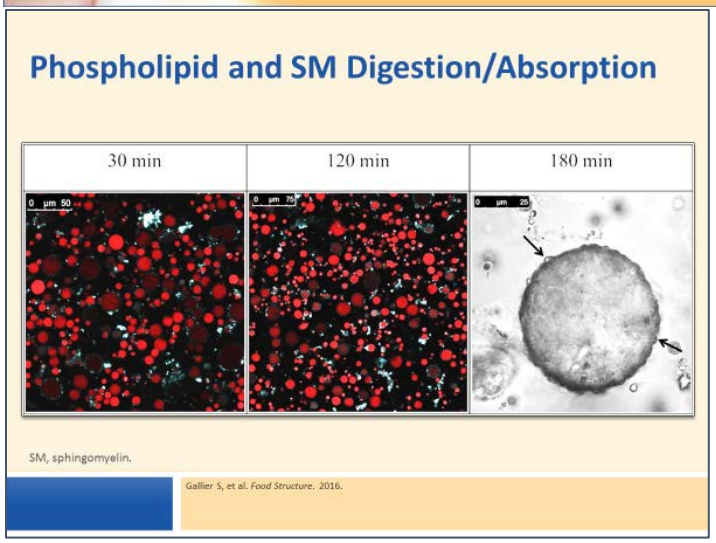
So, where's the sphingomyelin? Where's the cholesterol? And it turns out that these 2 components—sphingomyelin and cholesterol—play a very important role in what I call the health of the membrane. Now, where are those glycosalated lipids, like the gangliosides, where are they located, and what role is there—is what several of my students are now studying.

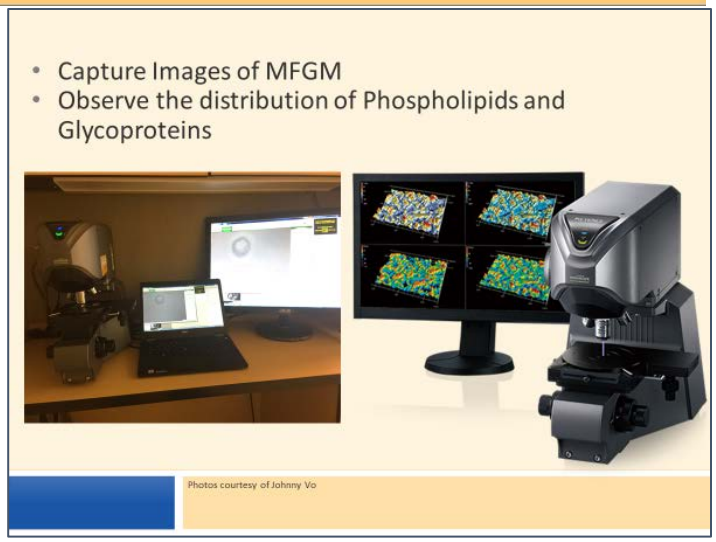
But here's an observation. It turns out that if we change the proportions of the phospholipids, we don't get those little black spots. So, that tells me that it is important.



And very recently, Dr. Contarini and her group published a review[3] in which they compare many publications of milks from, as you can see, buffalo, goat, ewe, human, donkey, mare, camel. And what is remarkable is that the ratios of phosphatidylethanolamine, phosphatidylcholine, and the sphingomyelin—highlighted in yellow in the upper part—always, more or less, compose one-third, one-third, and one-third of all the phospholipids present in the milk of all of these mammals. And when we obey this rule in making model fat globules, they do present these microdomains that I was showing in the previous slide. So, these proportions are very important for the normal function of the membrane. And just the lower part is the same phospholipids. And it pretty much does not matter what we do—cow butter, serum, or cream, or buttermilk—our best efforts to disrupt that, these 3 families of phospholipids remain attached together.

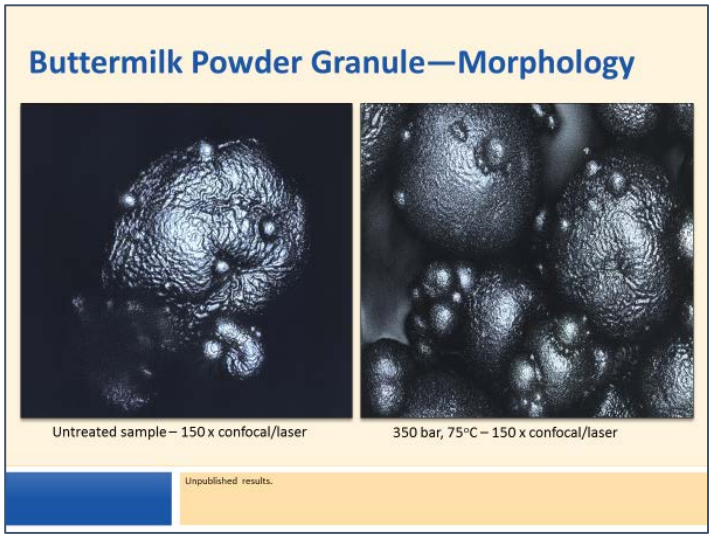
Now comes a personal view of what is the importance of this membrane and its structure. In 2006, PNAS, had a paper by Robenek[4] in which he wanted to see how deep one of these proteins that are in the milk fat globule membrane could get. Did it get all the way to the lipid core? They are very elegantly shown here on this pre-structure scanning electron micrograph. You can see clearly that, yeah, there's no question there are 3 layers: a monolayer and, then on top, a bilayer. But, what I want to call to your attention is that he also published, with no explanation, these little bubbles that were present there. And really, they're not in the size or morphology of the domains that I was showing you in the previous confocal microscope.





So, for many years we were wondering what might that be? And it wasn't until 11 years later or so, and my thanks to Sophie Gallier, now working in New Zealand—she had to go all the way to New Zealand to work with a friend of mine, Dr. Harjinder Singh. They did a study of this phospholipid membrane in vitro digestion. And the surprise to me was that, you see there [at] 180 minutes, this is a picture of a fat globule normal in cream, and you can see those little [kind of] blisters on the surface of the fat. Which denotes that a membrane has not been digested, but at the same time, the triglycerides inside that sphere are being digested. And so that is really telling us—and this is for future studies and how we probably will design the formula or even the drinks of the future—on how we really should digest the fats more slowly or in a very regulated way. So, that's a lesson for all the nutritionists out there. I think that this is very important.

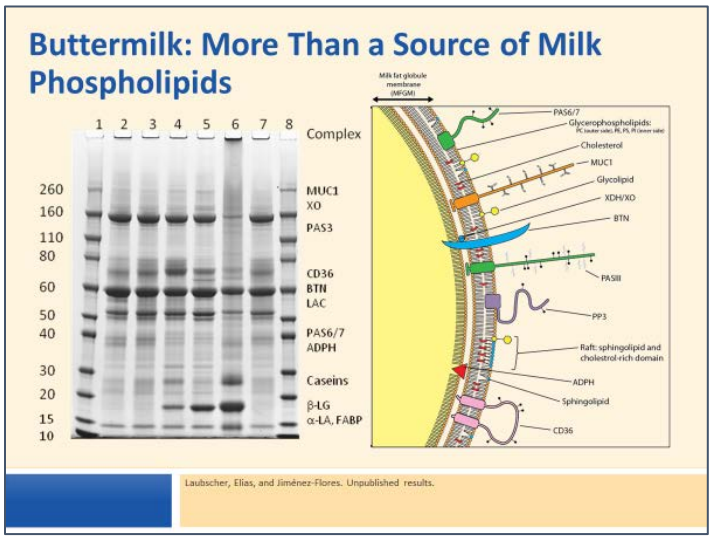
More technologically, we really want to know, what is happening to our powder ingredient? What do we do? And that powder ingredient, believe me, we analyze in many ways. On the list is something that is like the… this is a laser 3-dimensional microscope.



And what you see there is a little droplet of powder. This is not of the same scale I was showing. This is just that minute granule of powder, and on your left-hand side is how it comes, with a lot of the triglycerides on the surface. And we've been testing

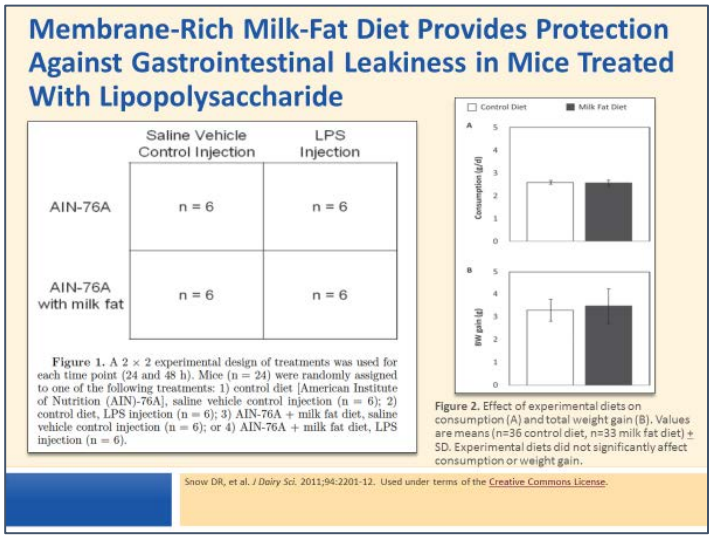
them with some… we've been processing them with some new process, which is called supercritical fluid extraction (SFE).

We use the CO_2 at very high pressure and temperature. Well, not very high, a couple of atmospheres. And that makes it work as a solvent. Interestingly enough, it just dissolves the triglycerides, leaving intact some of the other proteins. And it is a process much milder than some of those thermal processes we make.



On this slide, you will see on your left-hand side are very nice… (You know, when my students run gels that look like that, I think they're ready to graduate.) These are [sodium dodecyl sulfate] SDS protein gels, and in lane number 1, you have your molecular weight standards. Lane number 2 is perfectly preserved, raw milk, and number 3 is refrigerated milk. And 4, 5, and 6 are increased heat intensity treatment of the cream that we used to prepare these proteins.

Well, I draw your attention to the bottom of this [slide], and you'll see how the heat treatment increases the band that is shown, at beta-lactoglobulin height, which is 18,000 molecular weight band. You'll see that this low band—and we're showing 3 here—increased. Yet, the number 7 is what we get from the treatment with the supercritical, which does not seem to even compare to a mild pasteurization process, as number 4. So, that is a very hopeful thing, we probably are on the verge of developing very exciting technology that will preserve right now all our [hopefully] bioactivity as it comes from milk.

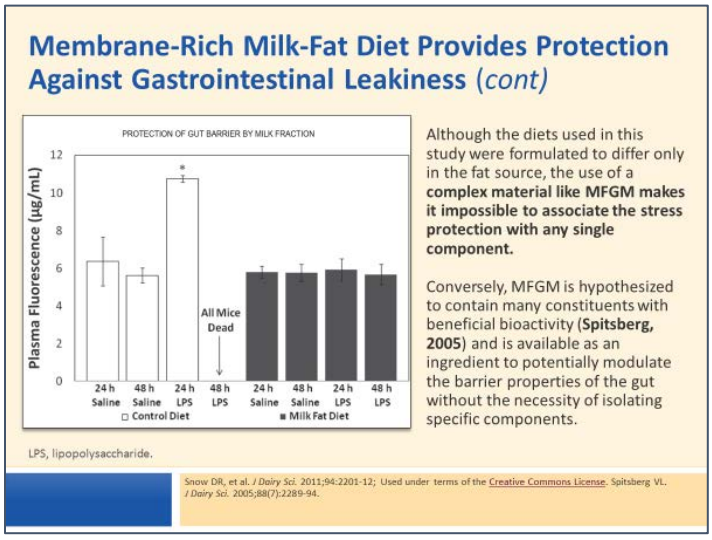


Let me change gears here a little bit and start talking about our evidence for the biological activity of these components that I've been describing. Now, a little while ago, in 2011, with my friend Bob Ward, he designed this experiment,[5] and we were just supplying the milk fat globule membrane material.

So, if I draw your attention to the squares here on your left-hand side: There were 12 mice that were fed a normal diet. Some of them had saline injection, and 6 of them… we challenged them with lipopolysaccharide, which is a toxic component that causes inflammation and all kinds of toxic problems, especially if you have a subject that has a leaky gut. But knowing that that's what we wanted to do, we have exactly the same mouse, same strain, and we gave him exactly the same feed, except we added the milk fat globule membrane. Six of

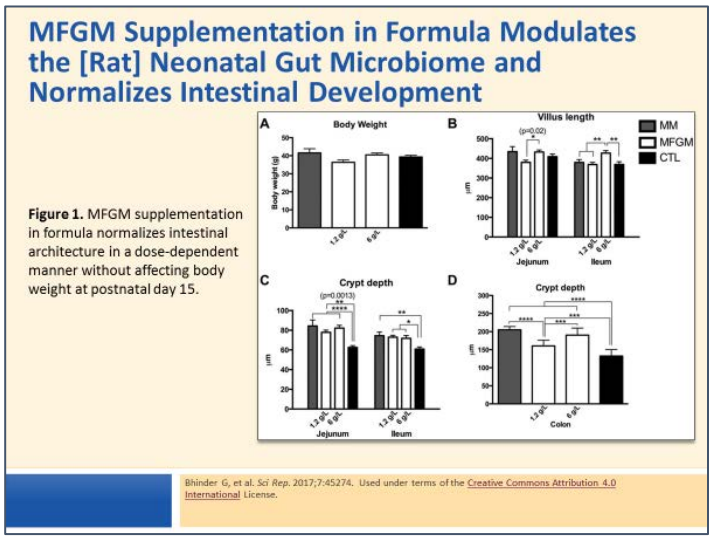
them, we gave them an injection of just saline to see if there was any response to that, and we challenged them just as the normal fed ones with lipopolysaccharide [LPS].

The nutritionists in the group were looking at the consumption of feed and the body weight gain, [so there] was no significant difference.

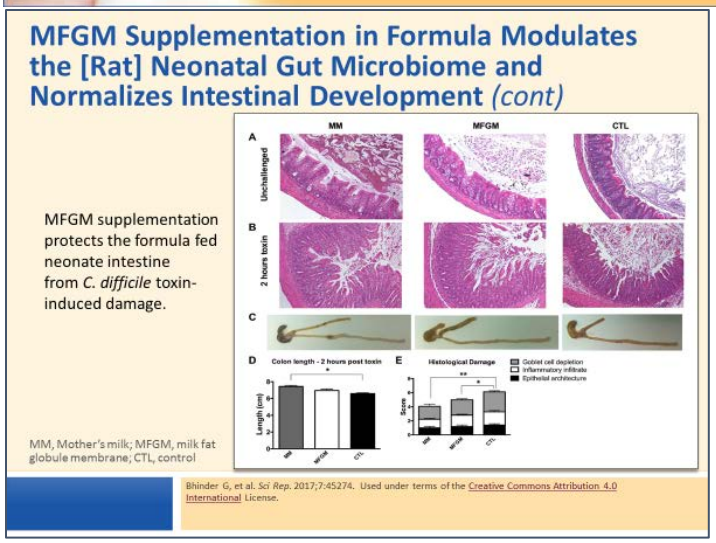


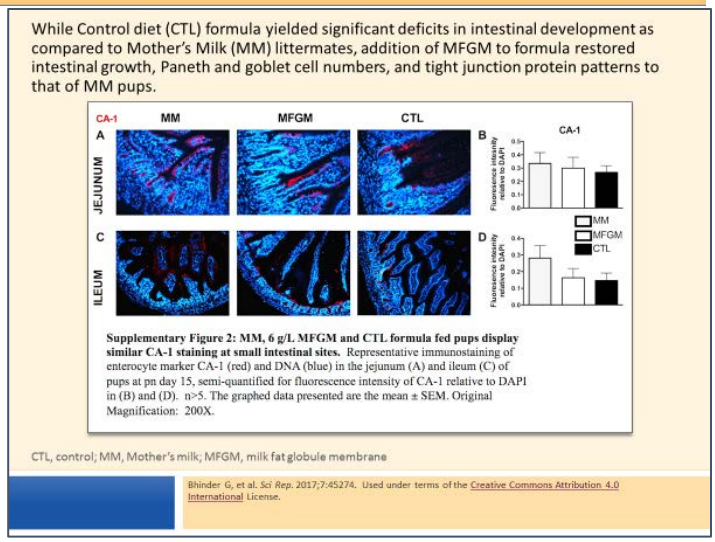
Where there were very important differences was— and I love the statistic of this experiment—the first 2 squares on the graph here show what happened after 24 hours or 48 hours of the saline injection to the mice [with] normal diets. And, as you can see, well, they fell [something], but there is no significant difference on their plasma fluorescence. However, the next square you see, the plasma fluorescence of the normally fed mice when challenged with LPS went through the roof. And unfortunately, at 48 hours, all 6 of these mice, were dead. Deader than a doornail. Did you see the dark squares where these mice that were fed the milk fat globule membrane, where they were [given an] injection of saline, were healthiest, there was absolutely no difference in the plasma fluorescence, and they all were alive and well.

So, that is a great result in my book, and no need of statisticians or anything like that. But the reductionist mind of scientists, wanting to see what is the one component that is doing this. And [in] this I have been very involved; this is complex material and makes it impossible to associate the stress protection with any one single component. I may even add, I think, that we have to have the right composition, the right ratio of the components, to achieve these results.



Let me move to another example. The next one is how these components modify the tissue inside the intestines of these mice. The body weight, the villus length, and everything were the same. And what we have is the group—the gray squares denote the mother's milk. That's pups, the little mice, that were fed with the milk of the mother, and then, the control is just those that were fed similar [to an] infant formula, and the white blocks in the middle were 2 doses of the milk fat globule membrane. As you can see, the body weight, the villus length, did change a little bit. But where there was a dramatic difference is in this picture that I'm going to show you.

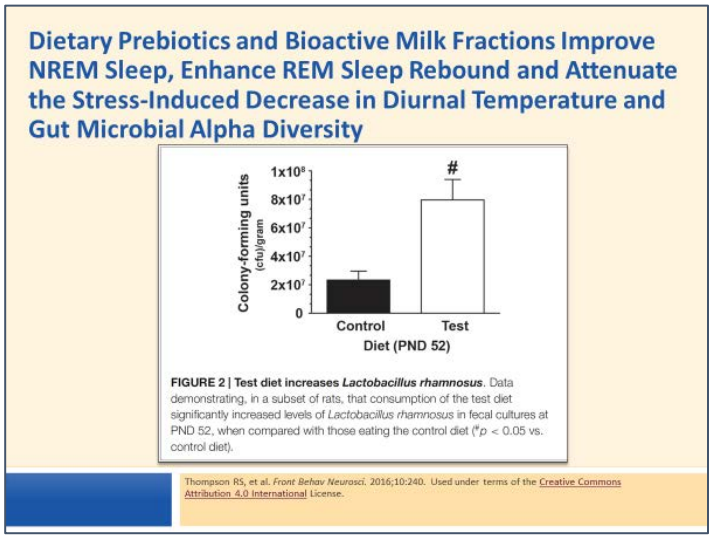




So, if you go to [the one to] the left end of the squares, you can see the mother-milk fed on challenged villi of the intestine of these pups; and 2 hours after the toxin, mother's milk gave perfect protection. There didn't seem to be much degeneration of the tissues. But in the control, which was the infant formula by itself—and it was mostly with soy components—you can see that there's a dramatic decrease in the villi of the intestinal lining of these animals when they were fed exactly the same formula. But with other milk fat globule membrane, well you can see, especially in the middle picture, that the tissue resembled much more… the one of the tissue of the pups fed their mother's milk.

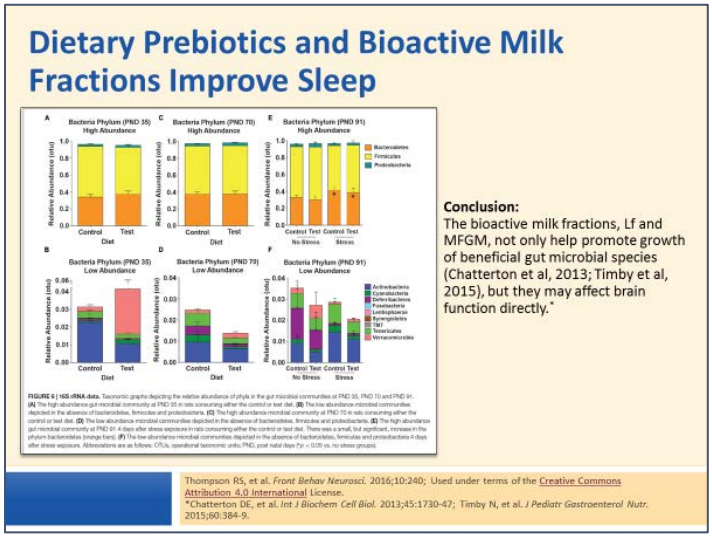
Something like that was also observed when these same tissues were looked [at] under blue fluorescence, in which the intensity of the fluorescence of the mother's milk was compared to the fluorescence of the control.

You can see that data on the bars all the way to your right. And you can see that the mother's milk and the formula with milk fat globule membrane are much more similar than those in the control.

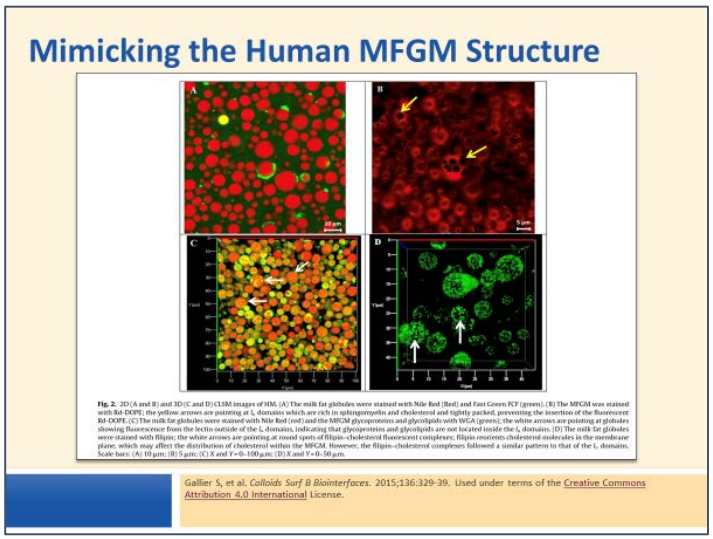


That is very exciting, and I picked just this one paper to show something that I want to really stress the point on the biological activity. We all know that any... I have a daughter that is specializing in cognitive science. She has a PhD… But all the cognitive tests have to do with teaching something, and then seeing how rapidly somebody [learns] or not. And this paper by Thompson published in 2016,[6] really didn't have to look at that. It only looked at the non-REM sleep, which this REM sleep is just the normal response of our central nervous system to something as normal as sleep.

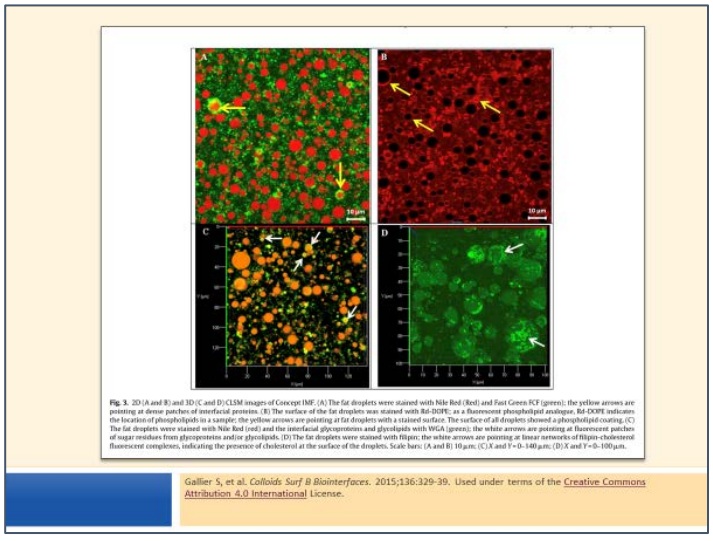
And here's something very interesting that they did: They analyzed, not only that, but **they analyzed the microflora, the microbiome** on these control mice and the test mice. And you can see that **once they had milk fat globule membrane, the colony-forming unit was enhanced** a lot.



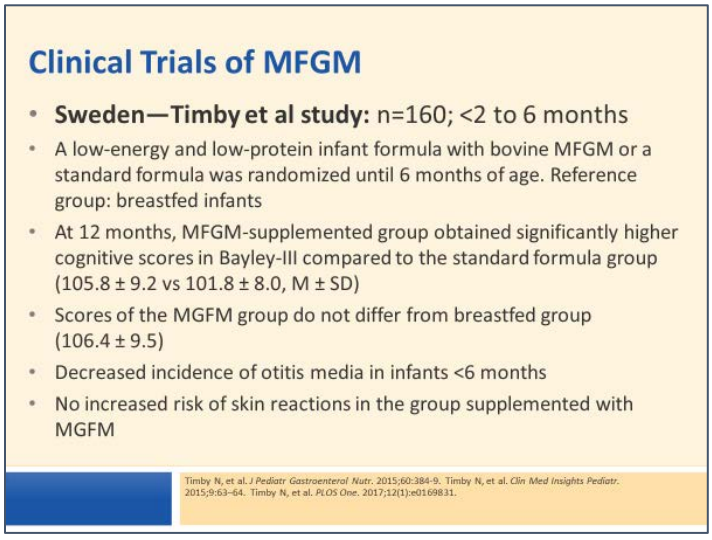
Well, so was their microbiome. There were very interesting changes in the populations on the microbiome, as you can see [there]. And I'm not going to go into the detail, except for highlight[ing] the conclusion of the scientists, in which they showed us that the **bioactive milk fractions—lactoferrin and milk fat globule membrane—not only helped promote the growth of the beneficial gut microbial species, but they may affect the brain function** directly. That is something I really need to stress for this audience.



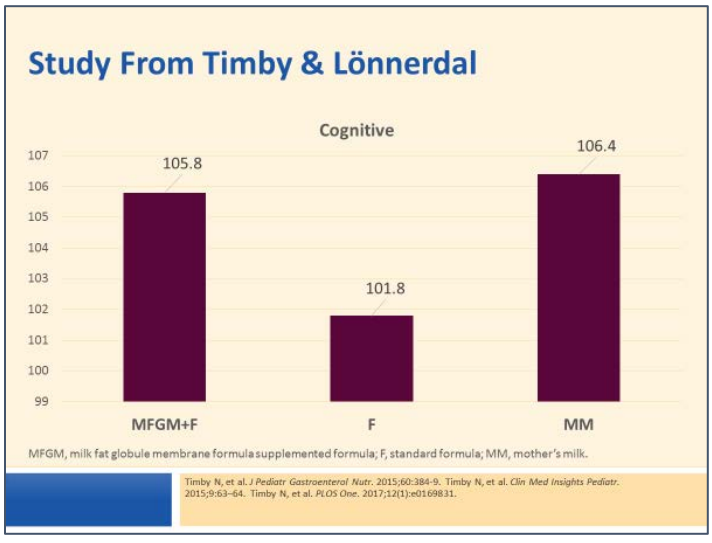
Effects that have happened… the industry, is looking at the same pictures that my student, Sophie Gallier, and they say, how can we humanize this? So, there's been some efforts. And I'm going to go back to this slide in which you can see the domains in your upper right-hand, and then you can see the distribution of proteins in the lower one, on the right-hand side.



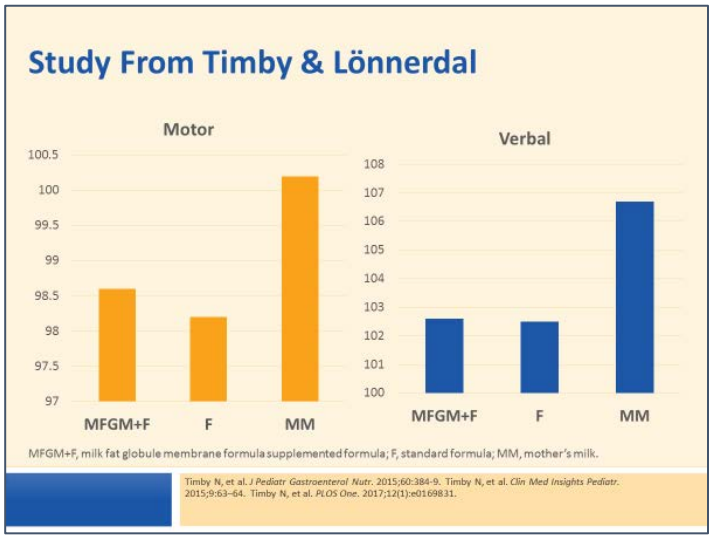
And then by doing some process that they don't disclose, you can see that this is bovine milk looking a lot like human [milk], still not perfect, but this is very important.



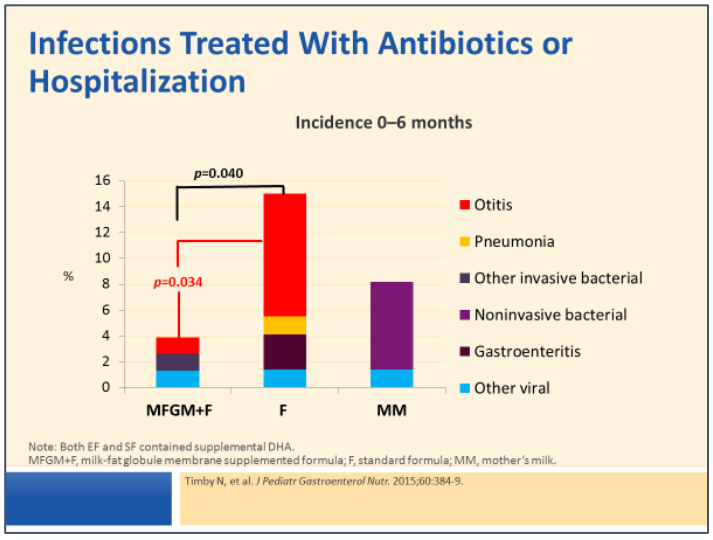
Now, rapidly, I want to move to the clinical trials, the study of Timby[7] in which they used 160 children, focused on cognitive ability. This, all of this you can read, increased incidence of otitis media and everything, what are the results of this.



Let me just show you very rapidly the result. On the very far end, on your right-hand side, mother's milk, responds 106.4 on their scale. Formula fed, response only 101.8, but exactly the same formula, added with milk fat globule membrane. Those babies responded, and their score was 105.8. Very similar to mother's milk. And this was only in the cognitive test.

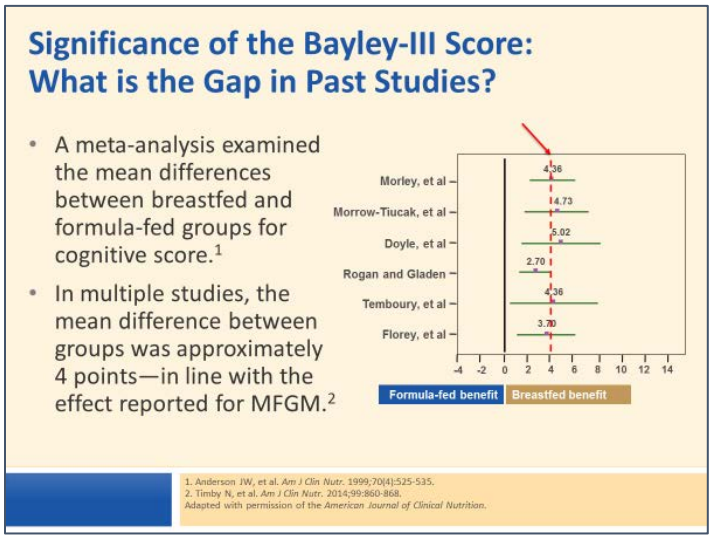


The motor or verbal skills did not change.



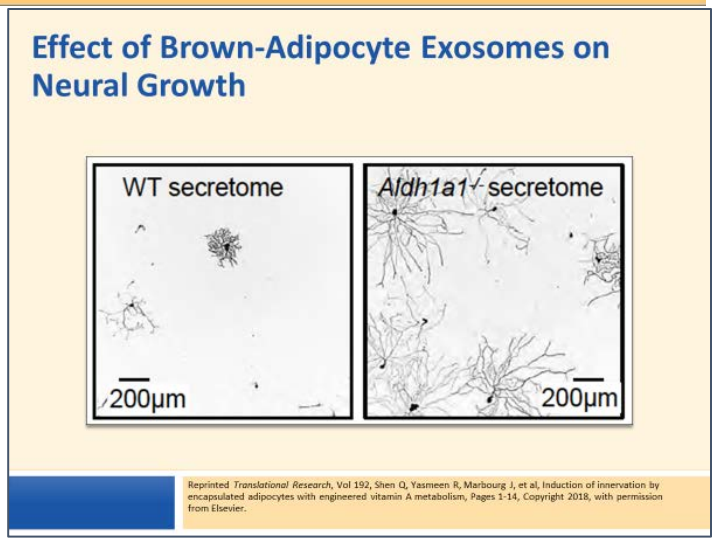
But for my pediatrician friends, there was a very significant reduction on otitis media—and those ear infections are really a plague for formula-fed babies, and that got reduced

tremendously. Nothing to do with some noninvasive bacterial infections, which you give to everybody.



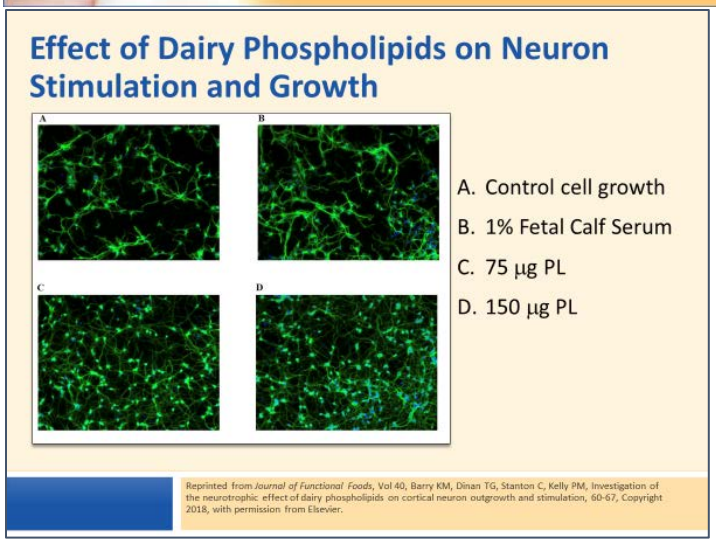
The significance of this finding was—and I call your attention to the results of the meta-analysis of all of these papers, in which on your left-hand side were formula-fed benefits, and on your right-hand side the breastfed benefits. Of course, they're not even touching the line at zero, which means that there [are] very significant difference between formula fed benefit and breast fed. But if we were to increase, and we do, with the addition of milk fat globule membrane, that score [went] all the way to 4. Well, you see that the comparison would be a very, very important one.

Now, you may be asking, well this is fine and dandy, but how much do we need? And really, the amount of milk fat globule membrane that we need to affect the change is not that drastic.

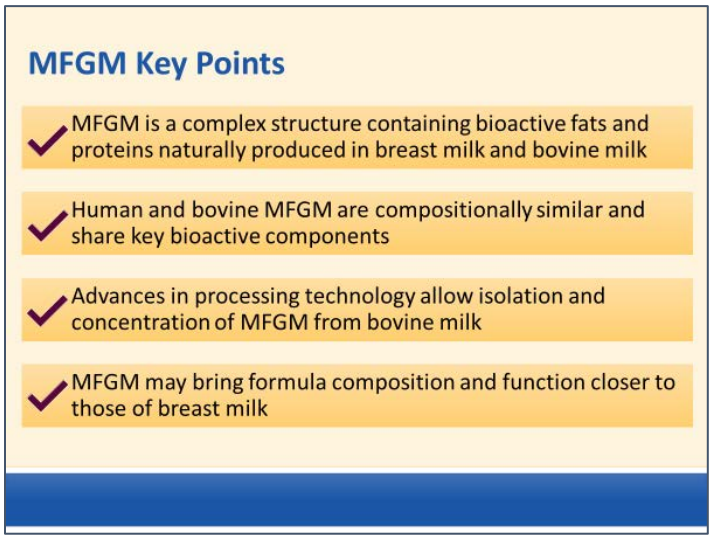


Let me give you an example of what… this was an observation here at Ohio State. I have a colleague that is looking at adipocytes. And we saw that these adipocytes secrete membrane material that is very similar to the one on the milk fat globule. What you see is the effect that these phospholipids have on neural cell culture. These are neurons in vitro, so they're cultured.

So, the panel on your left-hand side is just with a wild-type cell. And the one on the right hand, in which we see a dramatic increase of cells, you see this growth, but these are only with the secretome of the adipocytes that are very similar to milk. Very interesting that nature would have adipocytes that are having milk-like phospholipids.



So, of course we wanted to link to that, but as luck would have it, I got beat by some of my colleagues in Ireland. They published this paper,[8] and I am summarizing the results here. Panel A is a controlled neuronal cell growth; B is with the most advantageous and cell culture, know[ing] that 1% fetal calf serum promotes the myelination and the growth of neurons, and you can see clearly how they are. But guess what? 75 µg per liter or 150 µg, you can see a tremendous change in growth. And this is a direct effect that we have even in cell culture.



I think I have shown some compelling evidence that as we process our dairy products, we can really modify the bioactivity of our infant formula. So, I will leave you with that.

Some of your key points. The milk fat globule membrane is a complex structure that contains bioactive fats and proteins naturally produced in breast milk. The human and bovine milk fat globule are compositionally similar, and share key bioactive components. And, as we can see, they do have an effect nutritionally on brain and cognitive development. Now, the advances in processing technology are allowing us to isolate these very components from bovine milk so that we can add them into formula. And, this formula is being shown that it functions closer to breast milk.

I think the future is bright and as Sun Tzu said, the opportunities multiply as they are seized.

And I'm going to leave you with an image of me, very happy, working with my butter churn. I think it's now time to see if I can answer some questions. Thank you very much.

Question & Answer

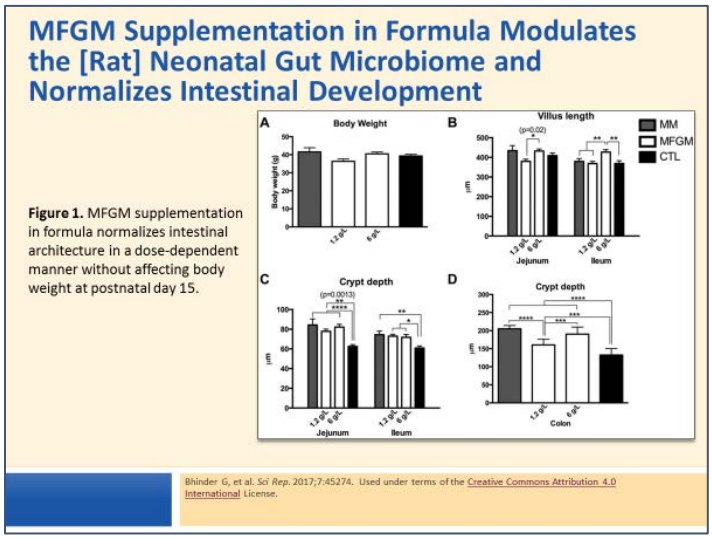
Is the milk fat globule membrane structure derived from cow's milk similar to the milk fat globule membrane structure in human milk?

Dr. Jiménez-Flores: Yes, the milk fat globule components are 1:1. Now, whether that is presented in the formula at exactly the same structure as with fresh milk, either from the cow or the mother, that's another issue. But we can draw a perfect parallel on the composition and the ratio of the components that are there. We have proteins, as important as I was talking about—lactoferrin, mucins—and they all form an integral part of the milk fat globule membrane, which is formed by phospholipids: the phosphatidylcholine, ethanolamine, and the sphingomyelin. And those are very similar and very synthesized in mammary glands in very similar ways in any [of the] mammals.

Is the bioactivity of milk fat globule membrane derived from cow's milk unaffected during processing and is there an assay to verify activity?

Okay, the second part first. Unfortunately, we don't have exactly a biological activity that tells us, well, this preparation really helps cell growth, or neuronal growth, or not. Hopefully, we're working towards that end. But, we know that it's very important to have all of the components present, that means all of the phospholipid components, including sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine in the same proportions. And that we can measure very accurately with techniques of [high-pressure liquid chromatography] HPLC, etc. As a matter of fact, I think that it should be used nowadays, because I'm not in the business, but it is, I would say, the perfect quality control for the ingredient that we add to the

formula to make sure that all the components are there, present, and at the same proportions.

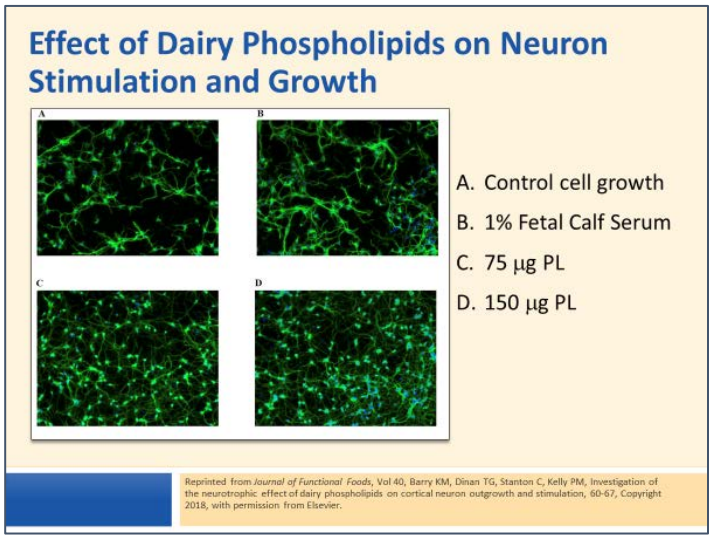


Is the bioactivity of the milk fat globule membrane derived from cow's milk and added to infant formula the same as the milk fat globule membrane bioactivity in human milk?

The answer to that question I think I showed is, mostly, yes. We can recover effects in the intestine that you can see using mother's milk as the control, our golden standard, and then comparing it to the formula without the milk fat globule membrane, the comparisons were always very bad. The moment we added this component in a small concentration—we don't need a tremendous amount, but we need to have it in a significant amount in mg per L—we saw the effect. There was an effect not only on the cognitive, but we saw the effect on the tissue development on the pups. We have seen the effect on the infection, the otitis media, for example. Although that experiment also included something that is present in the milk fat globule membrane, and protein called lactoferrin. So, you may split hairs, and ask what would be the effect with just lactoferrin? And while I don't doubt there would be an effect, probably measurable, the important thing is that there is a synergistic effect when that lactoferrin is in the context of the milk fat globule membrane components.

How much milk fat globule membrane is in formula and how does this amount compare to the literature?

Well, the amount that is being added to the formula is such that it reflects in the amount that was used in the review of the experiments… the ones that I have discussed and the ones that have been studied. There is also an important part that beyond a certain amount—I'm going to change the slide, in which we have 1.2 g per L or 6 g per L on the preparation that has a fraction of those 1.2 g is the phospholipid. But as you can see, there was not that big of a difference going from 1.2 to 6…



…the same thing that we saw for the neuronal development that, while there is 75 µg per L or 150 µg per L… there is one data missing there.... But when they went to 300 µg per L, there was no further increase or change. So, how are we going to justify that… is [first] we're going to see what was done on these experimental trials and limit that amount to the infant formula.

How much phospholipid is in milk fat globule membrane?

I would say that if you isolate just the membrane it represents about 60%. The other 40% to 45% are the proteins present there. The membrane material is almost exclusive[ly] phospholipid and protein. Now, as I showed in my picture, if you isolate the cream, the amount of phospholipids in cream only represents say a tenth of a percent, maybe two-tenths of a percent of the total lipid present in there. That's why it's important that the technology that we develop can concentrate this component to transfer it from cow's milk into our infant formula.

What is the most efficient way to derive milk fat globule membrane from bovine milk at this time? And through buttermilk or whey?

Right now, because of all the advances that we have had with whey processing, I have to say that the ingredient most likely to be utilized immediately is from whey. And it obeys also that the milk used in cheese making is really not thermally abused that much, because everybody knows if we overheat our milk to make cheese, we don't end up with a good cheese. So, we like the fact that it is going to be treated nicely and reduce all fetal microbial contamination, but it is also at the same time not thermally abused. And, I think that's going to be the first commercial way. The second one will be buttermilk, once we get over the hurdle of the thermal treatment to cream for butter because it's certainly much more abundant in buttermilk than it is in the whey fractions that have been used up to now.

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