



## **Individualized Fortification of Breast Milk for Preterm Infants**

Miami Neonatology 2018 – 42nd Annual International Conference

### Overview

Mother's breast milk is notably considered the gold standard for infants' nutrition. However, **Christoph Fusch, MD, PhD**, discusses evidence that reveals the macronutrient composition from breast milk is variable and may not provide adequate nutrients for the essential growth and neurodevelopment needed in preterm infants. The challenge with enteral nutrition is determining how to achieve a healthy balance between appropriate nutritional intake while also reducing the risk of sepsis and necrotizing enterocolitis (NEC) in preterm infants in order to achieve good growth and developmental outcomes. Dr. Fusch reviews the benefits and draw backs of mother's milk, donor milk, and fortified infant formula in premature infants, as well as evaluating the optimum composition of infant milk fortification.

### Content Areas

- Discern enteral nutrition needs of preterm infants
- Evaluate postnatal growth in preterm infants
- Recognize that neurodevelopmental outcome relies on nutrient intake of sufficient protein, calories, and minerals
- Understand carbohydrate-to-fat ratio that influences the rate and quality of growth in preterm infants
- Select the right infant formula for optimum composition of fortification

### Target Audience

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

### Learning Objectives

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

- Assess the benefits and optimum compositions of fortified human milk and formula in preterm infant enteral nutrition
- Develop individualized, evidence-based nutritional strategy for preterm infants to include the protective effect of breast milk, and provide appropriate growth and neurodevelopment rates

### Faculty

#### **Christoph Fusch, MD, PhD**

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Chief, Department of Pediatrics  
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Christoph Fusch, MD, PhD

*Research Support* Prolacta Bioscience – clinical area: nutrition and growth

*Consultant* Baxter – clinical area: nutrition and growth  
Hamilton Medical – clinical area: ventilation and respiratory support

*Speakers Bureau* Nestlé - clinical area: nutrition and growth  
Milupa (Danone) - clinical area: nutrition and growth  
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Prolacta Bioscience – clinical area: nutrition and growth

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Victoria Anderson (medical writer)

Heather Marie Jimenez, FNP (nurse reviewer)

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This activity is supported by an independent educational grant from **Mead Johnson Nutrition**.

This activity is an online enduring material. Successful completion is achieved by reading and/or viewing the materials, 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 released on March 15, 2019 and is eligible for credit through March 15, 2021.

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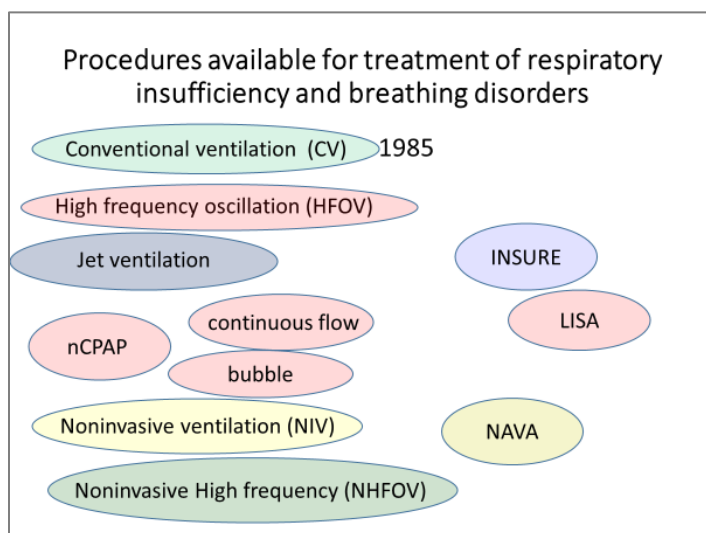
*Editor's Note: This is a transcript of the live presentation from Miami Neonatology on November 14, 2018.*



**Dr. Cristoph Fusch:** This [Slide 1] shows you procedures available for the treatment of respiratory insufficiency and breathing disorders. In 1985, we had conventional ventilation, which we were using in babies. Then we got an armada of new procedures— [some of which] you see here. There's lots of research on this. When should we use what? We have heard a few things about nasal CPAP, LISA, and so on. [Is this] the wrong talk? No, it is not.

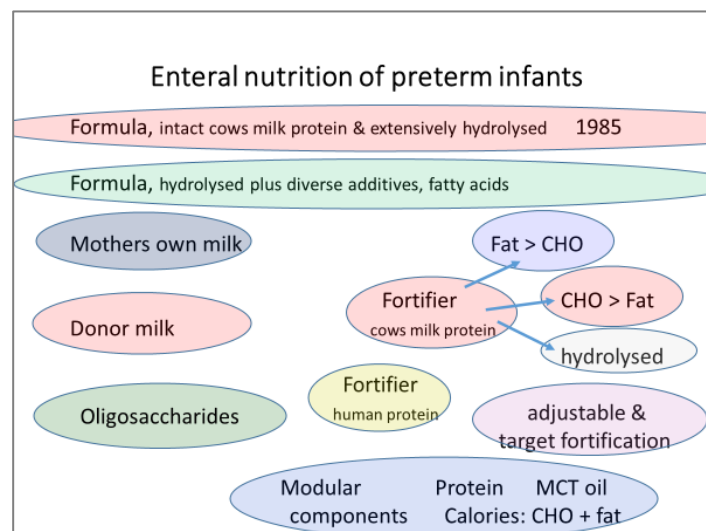
[formula]. That was all we had. Rarely was someone feeding human milk to preterm babies.

Now, 30–33 years later, it looks like with ventilation, we have lots of new products, lots of new strategies. We need to work with these new products and do [more] research, to see which [will] grow babies best. There is also, maybe, a generational issue, moving away from ventilator lung towards growth. Both need to be done to get the right product: a good baby, at the end.



Slide 1

Enteral nutrition of preterm infants, [in] 1985, we had formula, [which included] intact cow's milk protein, and maybe extensively hydrolyzed

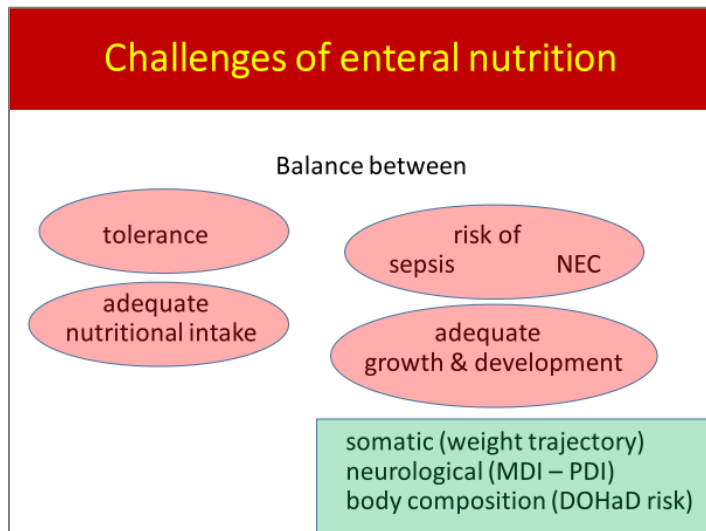


Slide 2

Challenges of enteral nutrition are to achieve a balance between—when we implement it—tolerance and adequate nutritional intake, thereby reducing the risk of sepsis and necrotizing

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enterocolitis (NEC), and still achieving, at the end, good growth and developmental outcomes.



Slide 3

I would define this as somatic, like weight trajectories; neurological, with [mental development index] MDI, [physical development index] PDI; but also body composition, because it's not only growth, but weight gain, that matters. We need to gain weight with[in] the right body compartments to reduce the risk for DOHaD, which is developmental origins of health and disease. This affects typically men my age, earlier or later, and to reduce this also in preemie babies, also for female preemie babies.

This is a comparison of different nutritional regimes that have different effects on outcome categories, and it's all summarized here [Slide 4]. So, I can stop the talk here, but I won't.

### Comparison of different nutritional regimes: differential effects on outcome categories

Formula	NEC/ Sepsis/Death	Growth	Neuro- development
	-	+	+/-
<b>HM</b>			
MOM (native)	+	--	-
Donor (native)	+	--	-
HM + HMF (bovine)	+/-	+	+
Donor + HMF (bovine)	+/-	+/-	+/-
HM + HMF (human)	+	+	(+)
HM + TF	+/-	++	?
Formula + HMO	+	+	?

Slide 4

Apparently, neurodevelopmental outcome has to do with growth and with feeding, with nutrient intake. This study [Slide 5] from Bonne E. Stephens, MD, et al,<sup>1</sup> which is very frequently quoted, showed that early nutritional intake increases the MDI if you manage to bring the kcal up. Each kcal that you achieve during the first week of life increases your MDI by 0.46, and each g protein/kg/d will increase your MDI by 8.2. That is significant. Others have shown in studies, as well, that nutrition matters because it affects brain growth. And, with a bigger brain, at the end, you have better capacity, and fortunately it is currently as easy as that.

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**Growth spurt: brain**

— N=124 ELBW infants  
— Mean birth weight 787 ±133 g  
— Mean gestational age was 25.9 ±1.6 weeks

**Neurodevelopment of ELBW infants correlates with the nutritional intake**

**TABLE 3 Regression Analysis Week-1 Energy Intake and 18-month MDI**

Variable	b (SE)	P	Partial R <sup>2</sup>
Birth weight	0.03 (0.01)	.0244	0.06
Male gender	-8.23 (2.90)	.0055	0.09
IWH	-1.51 (6.15)	.8063	0.01
CLD	0.38 (3.14)	.9046	0.00
NEC	-1.99 (3.94)	.6143	0.01
Maternal education, college graduate	4.77 (2.90)	.1036	0.01
Energy, kcal/kg/day	0.46 (0.18)	.0134	0.05

RP = 0.23. Adjusted RP = 0.18. b (SE) indicates effect size (SE).

**TABLE 4 Regression Analysis Week 1 Protein Intake and 18-month MDI**

Variable	b (SE)	P	Partial R <sup>2</sup>
Birth weight	0.03 (0.01)	.0227	0.06
Male gender	-8.72 (2.90)	.0033	0.09
IWH	-2.41 (6.14)	.6948	0.01
CLD	-0.06 (3.14)	.9838	0.00
NEC	-3.17 (3.93)	.4210	0.01
Maternal education, college graduate	4.32 (2.90)	.1397	0.01
Protein, g/kg/day	8.21 (3.67)	.0274	0.04

RP = 0.22. Adjusted RP = 0.17. b (SE) = effect size (SE).

— Each **kcal/kg/d** increases **MDI by 0.46-points**  
*Stephens BE et al. Pediatrics 2009*

— Each **g protein/kg/d** increases **Bayley MDI by 8.2-points**

at admission in 33%, and at discharge, in 63%. So, you see here again [top left], growth curves like this.<sup>2</sup>

**Extra-uterine growth restriction at discharge observed in 58% of VLBW infants fed predominantly standard fortified breast milk (Henriksen, Corvaglia)**

Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation  
Christine Henriksen<sup>1</sup>, Ana C. Weeseberg<sup>2</sup>, Asbjørn Rønnestad<sup>3</sup>, Britt Nakstad<sup>4</sup>, Mads B. Vainio<sup>5</sup>, Christian A. Damm<sup>6</sup> and Per O. Jensen<sup>2\*</sup>  
*British Journal of Nutrition* (2009), **102**, 1179–1186

127 VLBW infants part of an interventional trial assessing PUFA supplementation on neuro-outcome

All infants on fortified BM, either own mothers (76%) or donor milk (24%), fortified once  
EI > 120 ml/kg/d

Extra-uterine growth restriction is common in VLBW infants fed fortified human milk. Recommended energy intakes for growing preterm infants were not met in the present study. We observed a significant positive association between energy intake and reduced risk of growth restriction at discharge. The present study shows that preterm infants have

	All (n 127)		Growth restricted at discharge (n 72)		Adequate weight at discharge (n 55)		P
	Mean	SD	Mean	SD	Mean	SD	
Maternal characteristics							
Age (years)	31	78	31	82	31	72	0.62
Non-smokers (%)							0.21
Infant characteristics							
SGA at birth (%)	33		63		11		<0.001
Birth weight (g)	1056	285	1055	296	1082	271	0.59
Initial weight loss (g)	169	200	233	184	110	175	0.73
Time to regain birth weight (d)	9.8	4	10.2	4	9.5	4	0.37
Gestational age at birth (weeks)	28.8	2.7	29.6	3	27.8	2	<0.001
Length at birth (cm)*	35.3	4.2	34.9	4.5	35.9	3.5	0.29
Head circumference at birth (cm)	26.5	2.5	26.5	2.8	26.4	2.2	0.78
Weight at discharge (g)	2883	656	2417	601	3055	541	<0.001
Length at discharge (cm)*	44.6	4.6	44.1	4.8	45.5	4.4	0.19
Head circumference at discharge (cm)	34.4	2.6	34.0	2.7	34.9	2.4	0.10

Slide 7

We are currently working on individualized trajectories, putting a few physiological observations together, creating individualized trajectories for babies to come out of this “no man’s land” here, ‘How to know to grow,’ but I can’t elucidate on that due to time constrictions. I will go now to the few different products we have to [help] achieve postnatal growth. On one hand, we can use formula in babies. The good thing on formula is it’s balanced and a constant composition of macronutrient. It’s easily available and costs are relatively low.

Slide 5

How do babies grow? This is a slide [Slide 6] from Richard Ehrenkranz, MD, to whom I would like to devote this lecture. It shows these are the reference curves (intrauterine), and we frequently achieve growth curves like this.<sup>1</sup> So, this is a severe risk of postnatal growth restriction due to cumulative energy deficits. This is maybe not the way babies should grow in the next 20 years.

**Postnatal Growth Restriction and Cumulative Energy Deficits**  
**A Universal Problem in VLBW infants?**

Ehrenkranz RA, *Pediatrics* 1999;104: 280-9.      Cooke R J, *Arch Dis Child Fetal Neonatal Ed* 2000;83(3):F215-8.  
Embleton N E, *Pediatrics* 2001;107(2):270-3.      Cooke R J, *Arch Dis Child Fetal Neonatal Ed* 2004;89(5):F428-30.

Slide 6

In this study, you see here [Slide 7] was done in [very low birth weight] VLBW babies due to another reason that they were SGA, small for gestation age,



## Individualized Fortification of Breast Milk for Preterm Infants

Formula	
+	-
<ul style="list-style-type: none"> <li>Balanced and constant composition of macronutrients (MN)</li> <li>Easily available</li> <li>Costs</li> </ul>	<p>Most of the naturally occurring ingredients (enzymes, hormones, growth factors) &amp; cellular components are missing</p> <p>Cows milk protein/NEC</p> <p>Contains no oligosaccharides</p> <p>Microbiome</p> <p>Oxidative stress^^</p>

Slide 8

On the negative side [Slide 8, right], most of the natural occurring ingredients (ie, enzymes, hormones, growth factors and cellular components), are missing. And, it's based on cow's milk protein and has an association with the risk of NEC. It doesn't contain oligosaccharides. We have heard a little bit about microbiomes and how important oligosaccharides are to introduce, or to establish the right microbiome that might be protective also for NEC. Formula also causes more oxidative stress than human milk.

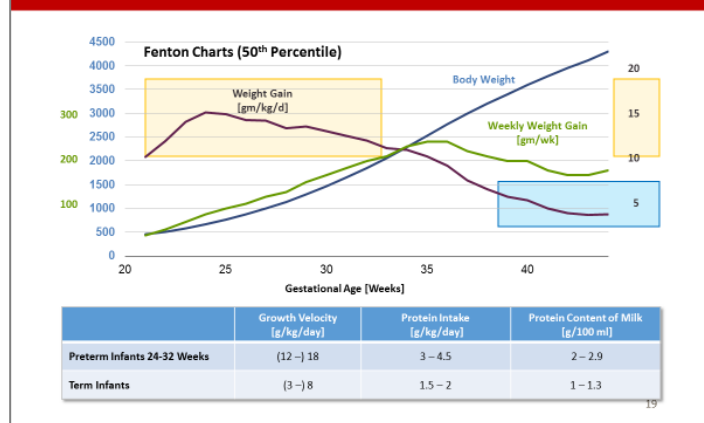
Mother's own milk contains many natural substances; [it] contains these oligosaccharides, which is good for the microbiome. The human proteins lead to a better tolerance, lower sepsis, and lower NEC rates; therefore, also to less catheter days and sepsis. Costs are still relatively low. The product itself is for free, but the handling and the storage might cost some money. However, **macronutrient composition is very variable in mother's own milk and is not balanced; and macronutrient content is generally too low for preterm infants.**

Mothers own milk	
+	-
<ul style="list-style-type: none"> <li>Containing many natural substances &amp; cells</li> <li>Oligosaccharides</li> <li>Microbiome</li> <li>Human proteins tolerance/sepsis/NEC (association cath days and sepsis)</li> <li>Costs</li> </ul>	<p>MN composition variable not balanced</p> <p>MN content generally too low for preterm infants</p>

Slide 9

You see here [Slide 10], growth curves, gestational age here, weight, weight per week, and weight in g/kg/d. And you see, here, that term babies have a growth rate of about 5 g/kg/d [shaded blue], whereas preterm babies have 15-17 to 20 g/kg/d [shaded yellow]. So, much higher.

**Fetal Weight Gain varies during the last trimester of gestation  
Breast milk composition meets the growth needs of term, but not that of preterm infants**



Slide 10

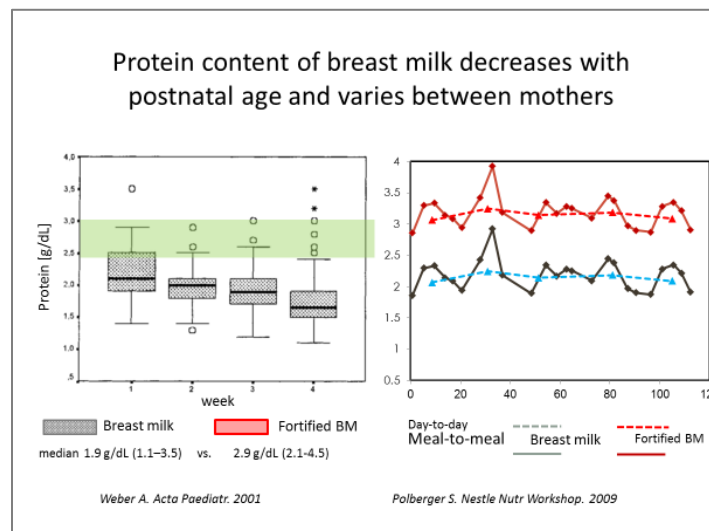
Protein intake and growth velocity are very tightly correlated (I will come to that a little later). If you want to achieve 3-8 g/kg/d of growth velocity, then you need to have a protein intake of 1.5-2 g/kg/d. If you're assuming an intake of 150 ml/d, then the

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protein content of that milk that you are feeding to the baby would need to have 1–1.3 g/dL of protein, and that is exactly the composition of breast milk.

However, **preterm babies with a higher growth velocity need a much higher protein intake, up to 3 g/100 ml, which is basically not available in human milk.**

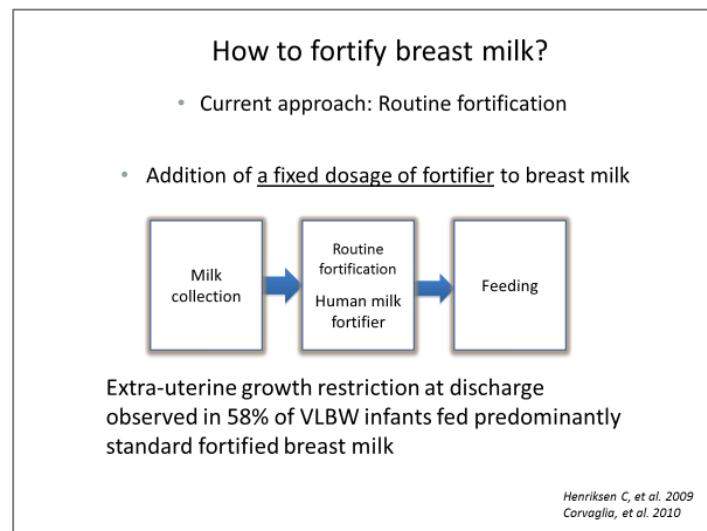
What you see here [Slide 11] is data on the composition of breast milk, protein content in weeks of lactation; so, the week 1, 2, 3, 4 after birth. You see on one hand that the protein content decreases during the first few weeks, and there's a huge inter-individual—but also intra-individual—variation, but most concerning is the inter-individual variation.<sup>3</sup> The green shaded areas are the ESPGHAN [European Society for Pediatric Gastroenterology Hepatology and Nutrition] recommendations to make preterm babies grow the right way.



Slide 11

So, how do we get out of this? Everybody does it. We are doing routine fortification using human milk fortifiers. Basically, this work has been done in the '80s and '90s. The products have developed since then but have not improved a lot. They are basically based on a standard—assuming a standard composition of breast milk. If you have this

standard composition of breast milk, then everything is fine. But if you don't, then things are not so good any longer for the preterm babies.



Slide 12

How are these fortifiers [composed]? What are their properties? They increase nutrient intake, because they add extra protein, extra calories and minerals. They add about 1–1.1 gm protein/dL, and they add about 14–18 kcal/dL. But, they are based on cow's milk protein; therefore, they reduce the NEC-protective effect of breast milk. The optimum composition of non-protein calories, fat vs carbohydrates, is unknown (I'll come to that later). There are concerns about osmolality, which I think are very weak because the basis of osmolality recommendation is extremely weak. The variability of the macronutrient composition is still present. The big question is, is it adequate for all? Because we are assuming the standard composition of breast milk.

## Individualized Fortification of Breast Milk for Preterm Infants

### Fortifier (cow's milk), standard

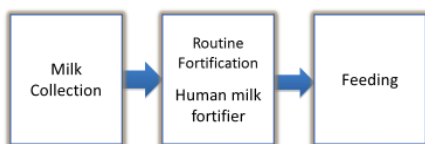
- |   |   |
|---|---|
| <p><b>+</b></p> <ul style="list-style-type: none"> <li>Increases nutrient intake protein, calories, minerals</li> <li>adds about 1 -1.1 gm protein/dL</li> <li>adds about 14 -18 kcal/dL</li> </ul> | <p><b>-</b></p> <ul style="list-style-type: none"> <li>cow's milk protein reduces NEC-protective effect of breast milk</li> <li>optimum composition of non-protein calories (F : CHO) unknown</li> <li>osmolality ^^</li> <li>Variability of MN composition still present</li> <li>adequate growth for all? assumption of standard composition of all BM</li> </ul> |
|---|---|

Slide 13

If we look at growth under human milk fortifiers, we still see that 58% of the VLBW infants fed predominantly standard fortified breast milk do not grow well.<sup>4,5</sup>

### How to fortify breast milk?

- Current approach: Routine fortification
- Addition of a fixed dosage of fortifier to breast milk



Extra-uterine growth restriction at discharge observed in 58% of VLBW infants fed predominantly standard fortified breast milk

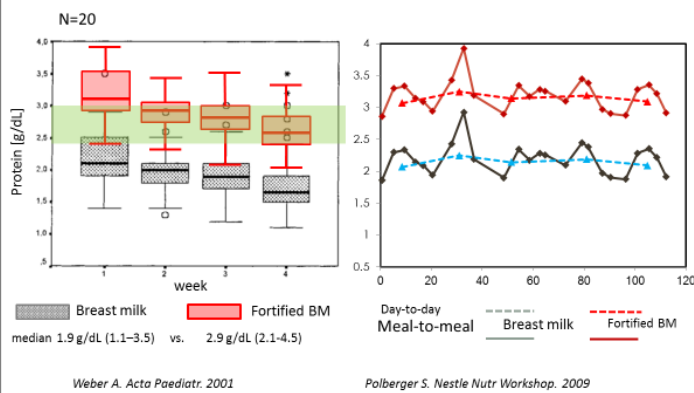
Henriksen C, et al. 2009  
Corvaglia, et al. 2010

Slide 14

What does the standard fortification do? Basically, it shifts up these variations by this 1–1.1 gm/dL, and you see that for the first week, it's fine. Second week, third week is fine, but here [into week 4] already you see a significant amount of milk samples and mothers who have a protein

concentration, despite standard fortification, that is not sufficient.

### Variation of protein content in breast milk and fortified breast milk vs ESPGHAN recommendations



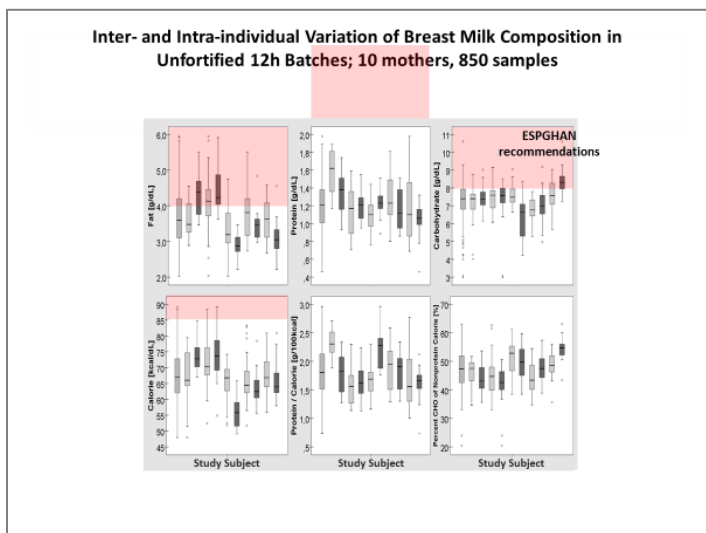
Slide 15

And we are here only on week 3 and 4, and for a 24-weeker, week 4 would be 28 weeks, and that's still a long way to go until term.<sup>4</sup> So, basically the babies here would be depleted with protein intake.

These are data [Slide 16] from our own study where we had 10 mothers from an overall sample of 850 using 12-hour batches, and you see for the different macronutrients here,<sup>6</sup> fat, protein and carbohydrate, the huge variation. The shaded areas are the ESPGHAN recommendations. You see here calories; you see here protein-to-calorie ratio (I will come to that later). That is very important. This is the carbohydrate-to-fat ratio in the non-protein energy, which is also extremely important. It might explain some of the observations that Paul Rozance, MD, did about carbohydrate depletion<sup>7</sup>—what you do if you put insulin into an organism.



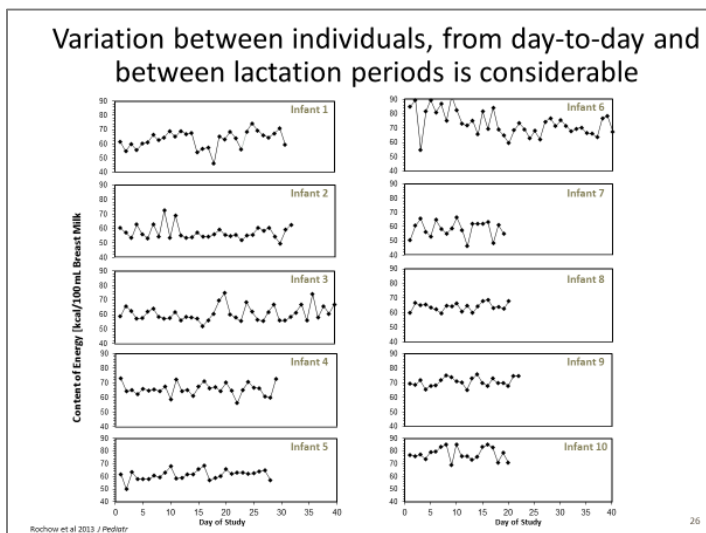
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Slide 16

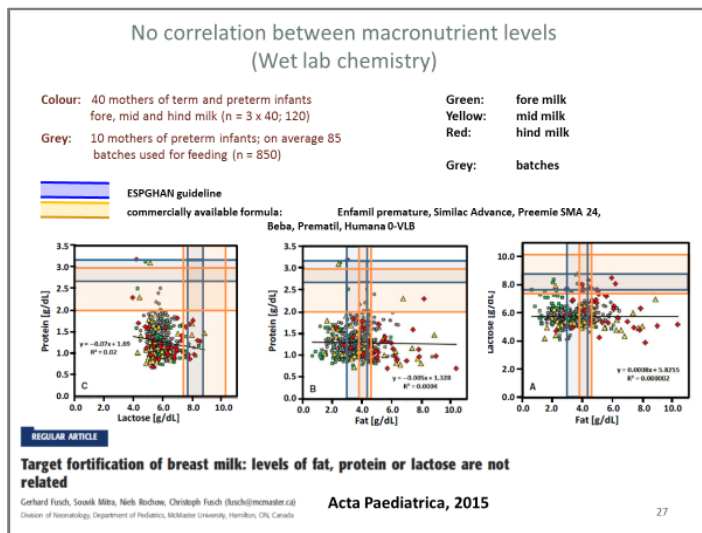
Here are data [Slide 17] on calories. And you see here that this mom produces a breast milk with 65 kcal/100 ml [Infant 8].<sup>8</sup> That is the assumption. This one is close to it, as well [Infant 4]. This mom behaves according to protocol.

This mom, here [Infant 6], does not behave according to protocol. She produces a rich breast milk, 90 kcal, and stays [at day 40] still at 70 kcal/100 ml. Here [With Infant 5] and here [Infant 3] we are seeing that we have only 55 kcal/100 ml, so that is very depleted breast milk which is fed to the baby.



Slide 17

I first thought having all this data together that Mother Nature produces either a rich breast milk or a poor diluted breast milk—it's a matter of dilution. When we did the x-, y-plots of the different macronutrients against each other, we were blown away, and I think that has never been shown before about the huge variability. That is shown in this paper, which we published [in] 2015, *Acta Paediatrica*.<sup>9</sup> What you see here [Slide 18] in these 850 samples plus 120 samples, 3 times from 40 moms, fore, mid, and hind milk, [plot 1] lactose vs protein, which is all over the place. There is not a diluted or undiluted milk, the same for fat and protein [plot 2] and for fat and lactose [plot 3]. There are moms who produce milk that is rich in protein but poor in energy. There are moms who are producing breast milk that is poor in protein and rich in energy. All these babies cannot grow. I will show you, a little later, why. The same is true for fore, hind, and mid milk.

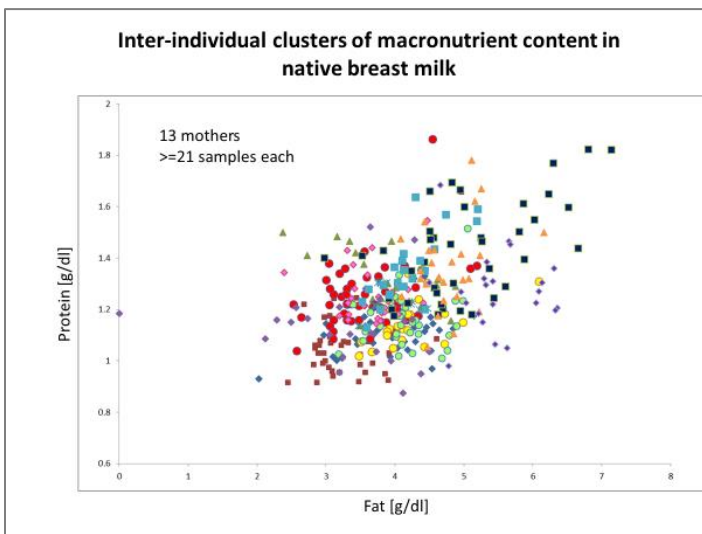


Slide 18

## Individualized Fortification of Breast Milk for Preterm Infants

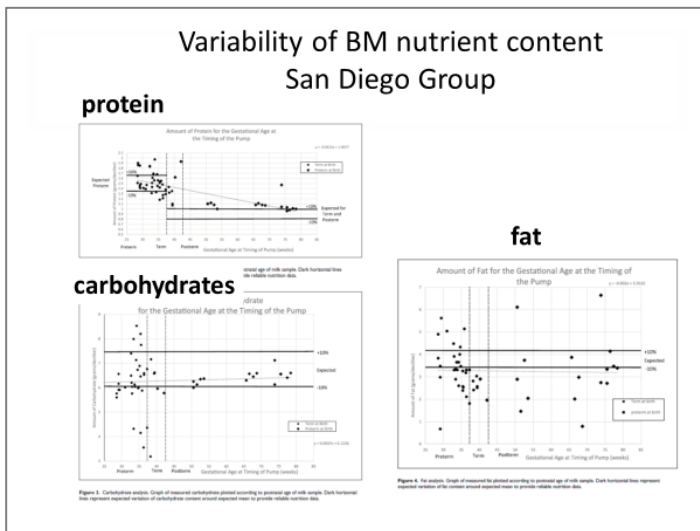
*“There are moms who produce milk that is rich in protein but poor in energy. There are moms who are producing breast milk that is poor in protein and rich in energy. All these babies cannot grow.”*

What you see here [Slide 19] is 13 mothers who had at least 21 samples. [Fat is plotted on the x-axis; protein along the y-axis] These mothers produce milk that is significantly different from [each other]. I’ll come to this graph a little later. Basically, this is energy [x-axis]—fat is a major determinant for energy—and this [y-axis] is protein intake.



Slide 19

We’re not the only ones, fortunately, who have looked into this. Recently, Jae Kim, MD, PhD, from San Diego, published a paper last year<sup>10</sup> that basically confirms what we have published in *Acta Paediatrica*.<sup>11</sup>



Slide 20

Fortifiers now are composed in different ways. Actually, we have 4 products, and there are 2 mainly used in Europe, 2 mainly used in North America. You see that the composition for protein is the same, but to gain the extra fat, the North American fortifiers are heavily fat-based, nearly free from lactose (only a little bit), whereas the European ones are not using fat, but achieve everything with lactose. The question is, where does it lead to?

**Fortifier (cow’s milk), standard**

+ cow’s milk protein  
Increases nutrient intake protein, calories, minerals

- reduces NEC-protective effect of breast milk  
optimum composition of non-protein calories (F : CHO) unknown  
osmolality ^^  
Variability of MN composition still present  
adequate growth for all? assumption of standard composition of all BM

Routine Fortifier	Fat (g/dl)	Protein (g/dl)	Lactose (g/dl)	Energy (kcal/dl)
Nestle - FM 85	0.02	1.0	3.4	17.8
Milupa - FMS	0	1.1	2.7	15.2
Mead Johnson - EnfaCare	1	1.1	0.4	15.0
Abbott - Similac	0.36	1.0	1.8	14.4

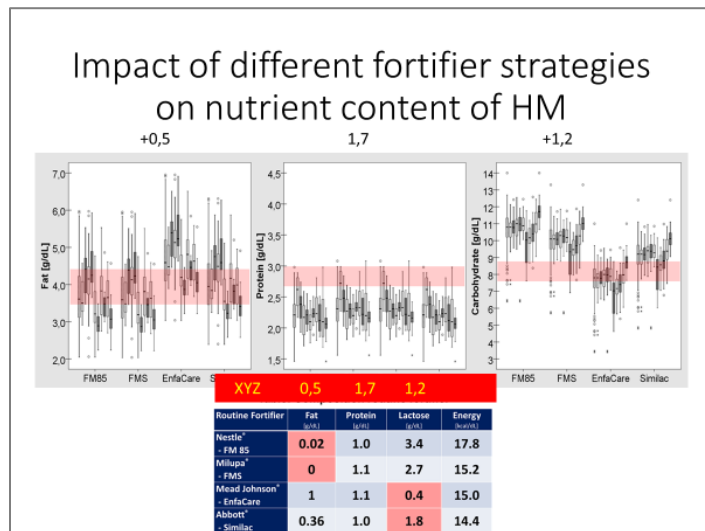
• Target concentrations per 100mL breast milk: fat: 4.4g, protein: 3g, CHO: 8.8g.

Slide 21

We used our data here [Slide 22] and did the calculations.<sup>13</sup> When we fortify milk with these 4

## Individualized Fortification of Breast Milk for Preterm Infants

different fortifiers, where do we come up to with fat, with protein, and with carbohydrates? And you'll see here that for fat, the European fortifiers [are] a little bit under fortified, and the North American fortifiers, over fortified. So, North American babies get a good amount of fat when they are on breast milk. For protein, they all add the same amount, and it is not sufficient. And for carbohydrates, the European ones over fortify with carbohydrates, and the North Americans under fortify, at least this one is a little higher.

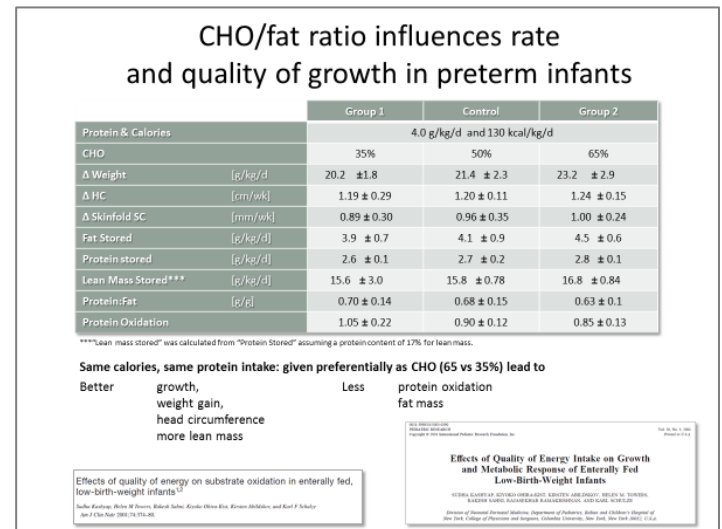


Slide 22

We said, what should the optimum composition be for a fortifier to meet the needs of most [infants]? And then we found that we should have 0.5 g/dL of fat, 1.7 g/dL of protein, and 1.0 g/dL of lactose. That would be the ideal fortifier that at least serves the purpose of most of these individuals. Interestingly, there are 2 fortifiers now in clinical studies that have a composition that is very close to what we thought should be in there. I wonder what results will come in terms of growth.

The carbohydrate-to-fat ratio influences the rate and quality of growth in preterm infants. Sudha Kashyap, MD, from New York, from [Children's Hospital of New York] did a very nice study that was published around 2000 with a few papers.<sup>12,13</sup> She

had 3 groups of stable growing preemie babies. They all got the same amount of protein, 4.0 g/kg/d, and the same amount of calories, 130 kcal/kg/d. She provided the non-protein calories, here [Slide 23, Group 1] 35% as fat, 65% as carbohydrates, here [Group 2] it was 50%:50%, and here [Group 3] it was the other way around.



Slide 23

And what you find interestingly is that the weight gain is significantly different. Three g/kg/d is a significant difference. Head circumference was different, and babies accumulated more lean mass here, but they also accumulated a little bit more fat mass when they were receiving a little bit more carbohydrates. I think that nicely explains what you see when we add insulin, because basically you make the carbohydrates disappear, and babies don't grow so well.

Why did that happen? She measured protein, better said, amino acid oxidation, and she found that the amino acid oxidation here [Group 1, final row] is much higher, when you give the same amount of calories as fat, compared to carbohydrates, than when you do it the other way around [Group 2, final row].

What does amino acid oxidation mean? It means that the proteins, the amino acids, are not being put

## Individualized Fortification of Breast Milk for Preterm Infants

together because that's an energy consuming process that might need quick energy, which means carbohydrates and not fat, but a bigger portion now goes into gluconeogenesis. To do gluconeogenesis with protein means you have to strip off the nitrogen from the skeleton, and you will have the carbon skeleton. Carbon skeleton goes into gluconeogenesis, and the nitrogen is being used to form urea. That is a very costly process. Urea is also a strong osmolyte and binds water.

My hypothesis is that some of the cosmetic edema that we see in healthy growing babies is just an overproduction of urea, and because we give imbalanced nutrition to these kids.

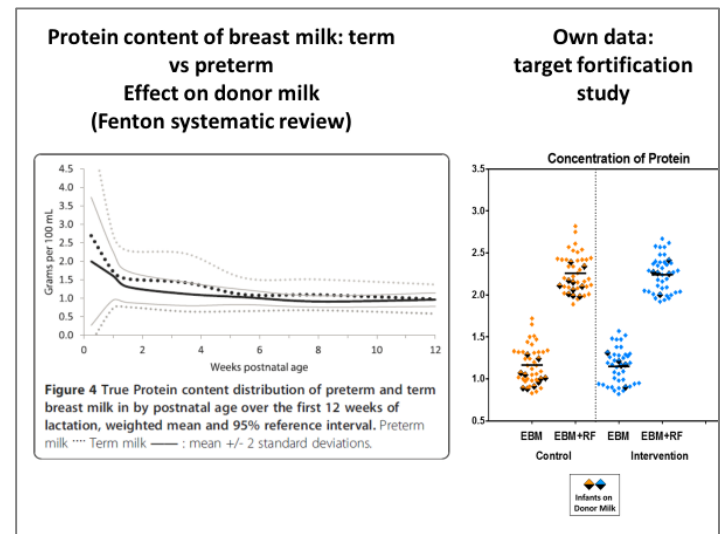
### Donor Milk and Its Components

Now, let's go to donor milk. Donor milk contains natural components, molecules, hormones, and cells. It does the same as mother's milk; however the natural component is less active. Still, the macronutrient content is variable and not balanced. The protein content is even lower because donor milk is usually obtained late in lactation. We do certain procedures with donor milk: we pasteurize [it], we freeze it, which might contribute to—that the components are less active. And donor milk is relatively costly, and you don't get it everywhere.

Donor milk	
+	-
• Natural components molecules, hormones, cells	MN content variable not balanced
• Microbiome	Protein too low for preemies
• Oligosaccharides	Protein content even lower due to late lactation
• Human protein	Pasteurisation
• Natural component less active	Cost
	Availability

Slide 24

These are data [Slide 25, left plot] [showing] how the protein concentration goes down after birth. These are data [right plot] from our own study where you see according to protein content, these diamonds are donor milk babies, so they really receive protein at the lower end.



Slide 25

Donor milk has an inbuilt risk of providing insufficient nutrient intake if you don't take care.<sup>14</sup>

**Donor milk has an inbuilt risk of providing insufficient nutrient intake**

In the systematic review by Quigley and McGuire,<sup>11</sup> infants randomized to receive donor milk had slower growth than infants randomized to receive formula; however, only 2 of 9 trials included in their analyses used donor milk fortified with nutrients. Although no statistically significant differences in growth between groups were observed in the present study, results showed a 0.5- to 1.0-SD decline in weight for age and length for age during the intervention, suggesting that growth and likely nutritional intake were suboptimal in both groups of infants.

11. Quigley M, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2014;4(4): CD002971.

Slide 26

Interestingly, it has been shown in different studies here [Slide 27], that the amount of donor milk



## Individualized Fortification of Breast Milk for Preterm Infants

compared to mother's milk influences growth rates, as in this study.<sup>15</sup>

neurodevelopmental outcome from the group is not better.

### Dose dependent effects of donor milk exposure

Donor milk may differ from mom's milk for preterm infant needs-growth

Median (IQR)	Percentage of maternal milk intake in diet			p value
	<20% mm (n=20)	20-80% mm (n=11)	>80% mm (n=17)	
Weight at study entry, g	1126 (956, 1240)	1180 (1050, 1300)	1240 (1143, 1320)	0.17
Weight at end of study, g	1385 (1235, 1455)	1460 (1360, 1510)	1490 (1430, 1510)	0.012*
Weight gain, g/kg/d	11.4 (9.5, 14.5)	15.0 (13.3, 16.1)	15.6 (12.5, 19.3)	0.016**
Length gain cm/wk	0.9 (0.7, 1.1)	0.9 (0.5, 1)	0.9 (0.7, 1.2)	0.51

\* difference between <20% and >80%  
\*\* difference between <20% and 20-80%

- Compared to >80%, infants fed <20% grew **5.1 g/kg/d slower** than those fed >80%, adjusted for GA at birth, day of first feeding, feeding tolerance, and weight at the first day of full feeding, prenatal steroids, and duration of study. **<20% MM means >80% DM**

Adapted from *Montjoux et al, Acta Paediatrica 2011, vol 100 pg 1548-54*

Slide 27

Also, in this study, the more donor milk you give, the worse the babies grew.

### Growth Outcomes by Type of Human Milk

	Type of Human Milk			P value
	>75% DM n=23	>75% MM n=51	Mixed MM/DM n=14	
Discharge weight, g median (IQR)	2330 (2070, 2720)	2710 (2480, 3050)	2675 (2500, 3090)	0.07
Discharge weight z score median (IQR)	-1.36 (-1.83, -0.48)	-1.04 (-1.43, -0.26)	-0.88 (-1.2, -0.17)	0.24
SGA at discharge	56% (13/23)	35% (18/51)	21% (3/14)	0.08
Human milk fortification, highest level used				
24 kcal/oz	13% (3/23)	20% (10/51)	7% (1/14)	0.82
27 kcal/oz	57% (13/23)	53% (27/51)	57% (8/14)	
30 kcal/oz	30% (7/23)	27% (14/51)	36% (5/14)	
Discharge head circumference, cm n = 56 median (IQR)	32 (31.5, 33.5)	33.5 (32.5, 34.75)	33.25 (32.25, 34.25)	0.23
Discharge head circumference z score median (IQR)	-0.7 (-1.4, -0.2)	-0.4 (-1, 0.4)	-0.9 (-1.15, -0.25)	0.11
Change in weight z score, birth to discharge median (IQR)	-0.84 (-1.00, -0.25)	-0.55 (-0.89, -0.03)	-0.45 (-1.2, -0.15)	0.54

• Low rates of SGA status at discharge for all groups compared to previous studies

• All infants had decrease in wt z score over hospitalization

• Donor milk infants had a non-significantly greater negative change in weight z score

• Growth with donor milk may be compromised?

Slide 28

Interestingly, the DOMINO trial<sup>16</sup> from Deborah O'Connor, PhD, RD, which we also were part of at McMaster University, showed that babies on donor milk have a reduced NEC rate and should have a better outcome, but the neurodevelopmental outcome was not better. So, this is a little bit surprising that you have less NEC rate, but your

### DOMINO trial

Lower NEC rates, but no improvement in neurodevelopmental outcomes

The JAMA Network

From: Effect of Supplemental Donor Human Milk Compared With Preterm Formula on Neurodevelopment of Very Low-Birth-Weight Infants at 18 Months: A Randomized Clinical Trial  
JAMA. 2016;316(18):1897-1905. doi:10.1001/jama.2016.16144

Characteristics	Adjusted Mean (SD) IQ*		P Value	Adjusted Mean (SD) IQ**	P Value
	Donor Milk (n = 151)	Preterm Formula (n = 148)			
Composite score†	92.9 (9.8 to 95.3)	94.5 (9.4 to 97.3)	-1.6 (-3.3 to 0.2)	46	-2.0 (-3.6 to -0.3)
Cognitive primary outcome	82.2 (8.8 to 94.0)	85.2 (8.7 to 93.0)	-3.0 (-4.7 to -1.3)	39	-2.1 (-3.7 to -0.5)
Language	91.8 (8.8 to 94.8)	94.0 (9.8 to 97.0)	-2.2 (-3.8 to -0.6)	27	-3.7 (-5.3 to -2.1)
Neurodevelopment score †§					
Cognitive	41 (21) (27.2)	24 (48) (24.2)	19.4 (3.7 to 29.6)	30	
Language	20 (28) (24.2)	14 (43) (27.2)	3.2 (-1.4 to 20.3)	28	
Motor	38 (49) (25.5)	38 (47) (28.4)	3.1 (-1.2 to 11.8)	41	
Disability score †¶					
Cognitive	14 (11) (9.3)	12 (34) (8.1)	-1.2 (-4.4 to 2.1)	75	
Language	20 (18) (19.8)	22 (44) (19.3)	1.4 (-1.0 to 3.8)	72	
Motor	18 (18) (12.3)	13 (47) (8.8)	2.2 (-1.8 to 6.3)	47	

\*Standardized mean IQ (SD, %). Continuous variables were analyzed by analysis of covariance, with adjustment as indicated. All models were tested for treatment interaction, and model effect indicated were found to be statistically significant. Analyses were run without statistically significant interactions in the models. Categorical variables were analyzed by logistic regression analysis with adjustment as indicated.  
†Adjusted using covariates from model 1.  
‡Adjusted for recruitment center and birth weight group (1000g, 1000-1499g).  
§High school or less, college or vocational diploma, less than high school, and percentage of total annual hours for each infant consumed as mother's milk. For the motor composite score a statistically significant interaction was found with maternal education (P = .03), and this interaction was retained in the model.  
¶Logistic regression analysis of the proportion of participants with scores indicative of neurodevelopment or disability were not performed using model 2 adjustments because of insufficient sample size.

No difference in BSID III scores

Increased risk of Cog < 85

No difference in Risk of Cog < 70

Slide 29

So, I thought about it and said, no, these are the number of infants on donor milk and on the standard preterm formula [n=151 DM, 148 PTF]. This was the reduction in NEC rate [-7]. And if we assume that one baby who has a NEC and a short gut, has an IQ that might be 25 points lower, then the donor milk group would gain 175 IQ points.

If the babies on donor milk grow a little less, and let's say they lose only 2 IQ points, then we are already in a negative balance, and babies on donor milk, all over, have a worse performance for neurodevelopmental outcome because each baby is affected by only a few IQ points. Whereas, here [Slide 30, row 3], few babies are affected with a big number of IQ points.



## Individualized Fortification of Breast Milk for Preterm Infants

### DOMINO trial: balance of outcome parameters

- 151 DM    3 NEC     $\Delta$ IQ NEC vs non-NEC: 25 points
- 148 PTF   10 NEC    $\Delta$ IQ DM vs PTF: 2 points
  
- -7 NEC \* 25 IQP = +175 for DM group
- 148 DM \* -2 IQP = -296 for DM group
- -----
- Grand Total:                    -141 for DM group

Slide 30

Now what does 2 IQ points mean? If we take Dr. Stephen's data that I've shown you in the beginning with 8.2 MDI/g protein/kg/d<sup>17</sup> and *delta* MDI of 2 would mean there is a difference of 0.24 g protein/kg/d. The difference between donor milk and mother's milk is between 0.3 and 0.5 g protein/kg/d. So, the *delta* of 0.24 g/kg/d here would add a growth rate difference of 1.5 g/kg/d.

### DOMINO trial: balance of outcome parameters

- Taking Stephens data (8.2 MDI per 1 g Protein/kg/d)
- ====>  $\Delta$ MDI of 2  $\approx$  0.24 g P/kg/d !!! !
  - ====>  $\Delta$ P of DM vs MM: 0.3 – 0.5 g/kg/d
  - ====>  $\Delta$ P of 0.24 g/kg/d  $\approx$   $\Delta$ growth rate of 1.5 g/kg/d
- Study possibly not powered (2-tailed) to detect differences in growth rates of 1-2 g/kg/d

Slide 31

The study was not powered to see this difference in growth rate. So, it is still an explanation why babies on mother's milk do not do better if we don't take

care of that problem. Same thing is here [Slide 32] in the data from Tufts.<sup>18</sup>

### Tufts BSID III scores at 2 years

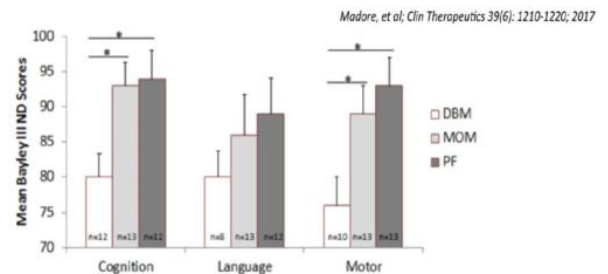


Figure 1: Comparison of mean Bayley III neurodevelopmental scores at two-years corrected age between feeding groups  $\pm$  standard error. Statistical analysis through ANOVA with multiple comparisons. \* Indicates p value <0.05.

Highlights the importance of ongoing randomized trials being completed

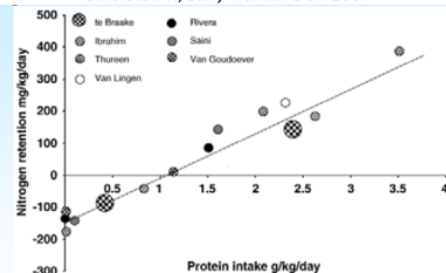
- Infants fed DM grew more poorly in the first month of life than those fed MM or PF

Slide 32

Okay, now how do we grow? We have protein intake [that] determines our nitrogen retention, which is the buildup of lean mass, and you need about 1g not to grow. And then, it's a very linear correlation.<sup>19</sup>

### Amino acids and N2 retention in the first days of life

Embleton N, *Early Human Dev.* 2007



200 mg of N2 retention equal 10g of growth

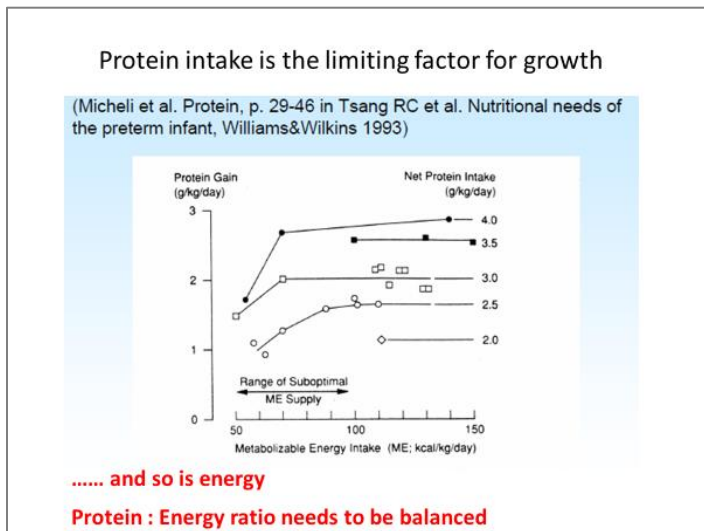
**Protein intake determines growth rates; strong relationship; Ventilator settings and BGA, only slower kinetics**

Slide 33

But you also need energy. So, here, basically [Slide 34], is the intake of protein.<sup>20</sup> This is the net gain of protein. Lean mass and here's metabolizable energy. So, if you don't give enough metabolizable

## Individualized Fortification of Breast Milk for Preterm Infants

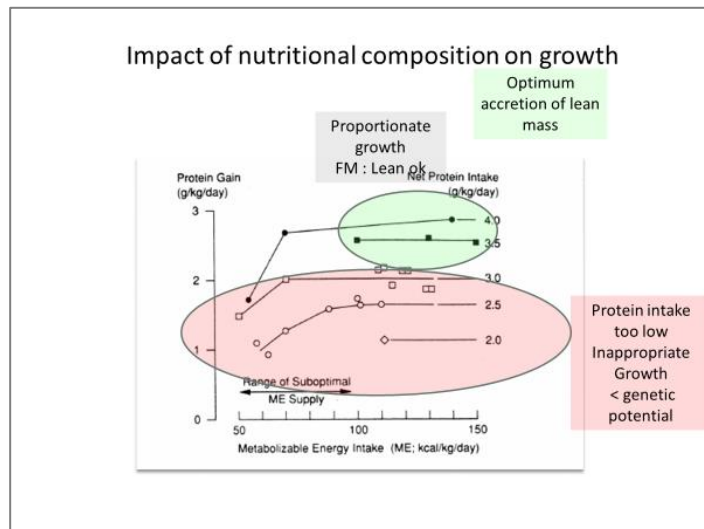
energy, then you won't grow though you get an appropriate amount of protein. You need both.



Slide 34

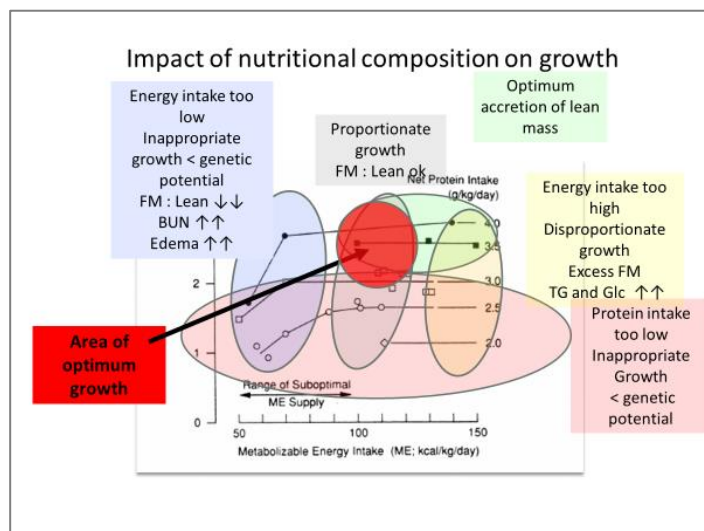
Why does it happen? To build up lean mass is an energy consuming process. To build up 1g costs about 10 kcal. If you don't provide these kcals, you won't grow. Plus, your protein will go down the urea wave because you do protein oxidation. We have seen that in Sudha Kashyap's data.<sup>13,14</sup>

You need both protein and energy, in the right way. If we now look into this graph and see in this area [Slide 35, shaded green], babies might grow appropriately. We get 4 g/kg/d. Three now, 4 g/kg/d. That is fine, and enough energy, so we gain a good amount of protein. Here [shaded pink], we don't grow enough because we don't get enough protein.



Slide 35

You can do it for all other circumstances. You achieve different kinds of growth. The only way where you achieve the best growth is here [Slide 36, shaded red]. This is the area of optimum growth. Where you have so much energy and so much protein. If you give more energy, you become fat [shaded yellow], you will weigh more, but that is maybe not desirable.

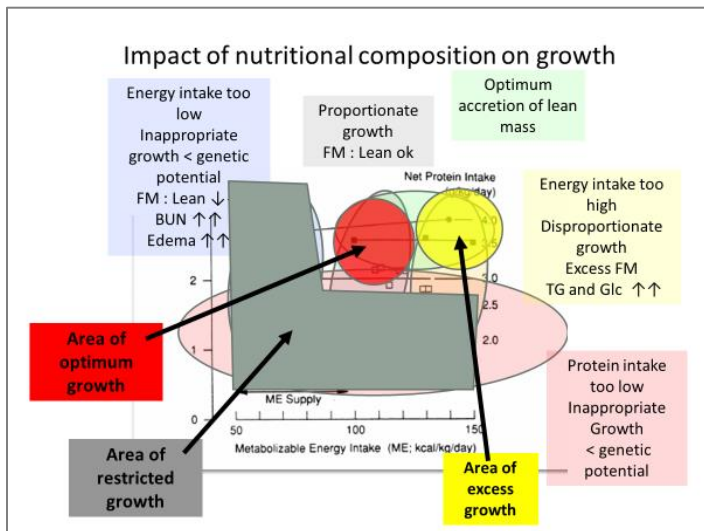


Slide 36

These are areas here of overgrowth [Slide 37, shaded yellow] and these are areas of undergrowth

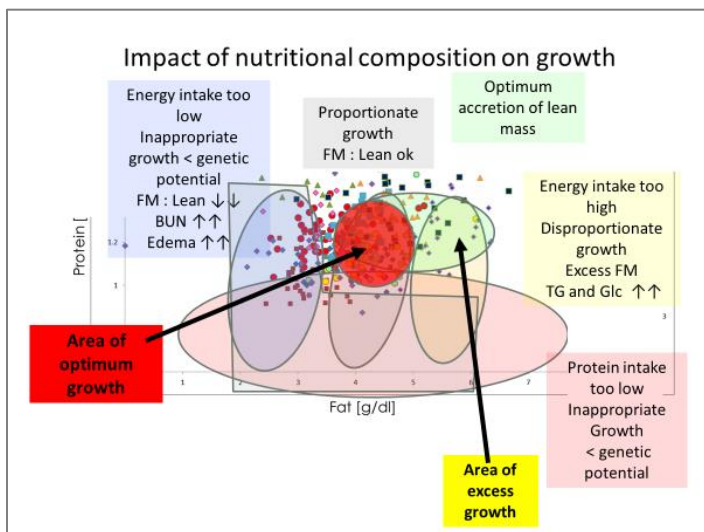
## Individualized Fortification of Breast Milk for Preterm Infants

when you are here [Slide 37, shaded grey]. So, why am I showing that to you?



Slide 37

Going back to this slide here [referencing Slide 19], where we have energy and protein, and I do the overlay of what I've shown to you [Slide 38], then you see that there are, indeed, combinations of breast milk where babies cannot grow. And unfortunately, there are more combinations where babies cannot grow than that they grow too much.



Slide 38

To overcome this, what I would like to say is that breast milk is a highly unstandardized diet that

sometimes can make babies fail to grow. Even if we give more of everything, we cannot fix certain deficits. So, in this study here [Slide 39],<sup>21</sup> adjustable fortification was done. It was a stepwise procedure having 6 strengths of protein, and that was added according to the BUN [blood urea nitrogen levels]. As long as BUN was low, [HMF and protein] was increased. As long as the BUN was high, [HMF and protein] was decreased.

### Adjustable fortification of breast milk improves growth, but not for all subjects

Arslanoglu S (2006) J Perinatol  
Inclusion criteria  
BW 600 – 1750 g  
GA 24 – 34 weeks  
Healthy infants (no NEC, sepsis, IVH)  
no ventilator support on day 21

#### Results

Table 5 Weight, length and head circumference gains during the study period

Outcome variable	STD	Adj	P-value
Weight gain (g/day)	24.8±4.8	30.1±5.8	<.01
(g/kg/day)	14.4±2.7	17.5±3.2	<.01
Length gain (mm/day)	1.1±0.4	1.3±0.5	>.05
Head circumference gain (mm/day)	1.0±0.3	1.4±0.3	<.05

Values are mean±s.d.

Randomisation stratified according to BW  
< 1250 g  
< 1500 g

Table 2 Amount of HMF and protein at the various fortification levels

Fortification level	Amount added (g/100 ml milk)
3	HMF 6.25+prot 0.8
2	HMF 6.25+prot 0.4
1	HMF 6.25
0	HMF 5
-1	HMF 3.75
-2	HMF 2.5

Slide 39

Here's the difference in weight gain [right table]. So, the adjusted fortification gets a better weight gain. You'll see here, 14.4 g/kg/d vs 17.5 g/kg/d. Still, with this variation, you see that there are babies that will grow with 14 g/kg/d and also with 11 g/kg/d, which is not appropriate.

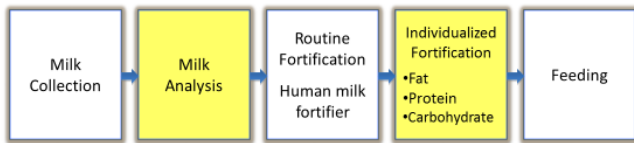
That's why we thought we need to do individualized fortification, which basically means we do milk analysis here, then add, additionally, what is missing.

## Individualized Fortification of Breast Milk for Preterm Infants

### HOW TO FORTIFY BREAST MILK?

New approach: "Individualized fortification"

Analyzing breast milk and individually fortifying it to reach recommended macronutrient amount



Our goal: "standardized" intake for preemies

Slide 40

There's one study from Karen Simmer, MD, PhD, from [University of Western] Australia that did it already in 2009,<sup>22</sup> and didn't find differences doing it. Why?

**Does target fortification work?**  
Study of 2009, publ 2016

Comparing different methods of human breast milk fortification using measured v. assumed macronutrient composition to target reference growth: a randomised controlled trial

Germana McLeod<sup>1</sup>, Jill Maynard<sup>1</sup>, Peter E. Hartmann<sup>2</sup>, Elizabeth Nathan<sup>3</sup>, Franca Cockburn<sup>4</sup> and Karen Simmer<sup>5</sup>

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	Igp (n 20)		RPgp (n 20)		P
	n	%	n	%	
Gestational age (weeks)					
Mean	27.0		27.1		0.781
SD	1.9		2.0		
Birth weight (g)					
Mean	1014.8		1009.2		0.953
SD	269.3		313.1		
Full enteral feeds achieved (d)					
Median	17		17		0.654
Range	8-27		9-29		
Days from birth when feeds were fortified					
Median	20		20		0.903
Range	10-39		10-36		
Weight at start of fortification (g)					
Median	1032		1155		0.925
Range	700-1998		505-1885		

Slide 41

If you look into the data, then you'll see here [Slide 42] protein-to-energy ratio, which she achieved in both groups, the same intake, so there was no difference, and 2.6 g is too low for proper growth. So, that's why she didn't find an intake, a difference in growth.

### No difference in growth . . .

Table 4. Growth data of infants at discharge (Mean values and standard deviations)

	Igp (n 20)		RPgp control (n 20)		P
	Mean	SD	Mean	SD	
Growth at discharge					
Age (weeks)	37.7	2.5	37.8	2.2	0.762
Fat mass (g)	318	111	348	149	0.469
Body fat (%) (without correction for length)	13.7	3.6	13.6	3.5	0.984
Discharge weight (kg)	2294	356	2464	528	0.243
Discharge length (cm)	43.8	2.6	44.6	2.8	0.343
Discharge head circumference (cm)	32.4	1.6	33.1	1.8	0.184
Weight gain velocity from birth (g/kg per d)	11.4	1.4	12.1	1.6	0.135
Weight gain velocity after birth weight regained (g/kg per d)	13.4	1.9	14.3	1.6	0.138

Igp, intervention group; RPgp, routine practice group.

Calculated nutritional intakes after milk was fortified on measured composition

	Igp (n 20)		RPgp (n 20)		P
	Mean	SD	Mean	SD	
Fluid (ml)	158	14	153	9	0.256
Energy (kJ)	524	44	538	47	0.336
Protein (g)	3.3	0.4	3.4	0.5	0.673
PER	2.6	0.3	2.7	0.3	0.751
Lipid (g)	6.8	0.9	6.8	1.0	0.702
CHO (g)	12.9	1.1	13.5	0.9	0.640

But also no difference in intake . . .

Slide 42

There is a trial from Leipzig, where they did a similar thing, but there must be some methodological issues because you cannot, in a blinded trial, get a dichotomous distribution in the same group. That would not work. They didn't find a difference either.

**The LEIPZIG trial (e-Poster EAPS8-1096)**

UNIVERSITÄT LEIPZIG  
Universitätsklinikum Leipzig  
Wissenschaftszentrum für Ernährung

**STANDARD FORTIFICATION (STD) VERSUS TARGET FORTIFICATION (TFO) IN VERY LOW BIRTHWEIGHT INFANTS: EFFECT ON GROWTH AND AMINO ACID PROFILE**

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	STD (n=54)	TFO (n=55)	P
protein content, g/100 ml	3.2 ± 0.3	3.0 ± 0.2	<0.001
< 34 SSW	3.2 ± 0.3	3.2 ± 0.1	0.006
≥ 34 SSW	3.1 ± 0.3	2.8 ± 0.1	<0.001
fat content, g/100 ml	4.3 ± 0.9	4.6 ± 0.5	<0.001
carbohydrate content, g/100 ml	9.8 ± 0.4	9.7 ± 0.4	0.480
fluid intake (ml/kg/d)	148.5 ± 22.0	152.1 ± 19.1	<0.001
energy content (kcal/kg/d)	140.3 ± 19.4	146.3 ± 16.4	<0.001
protein consumption (g/kg/d)	4.7 ± 0.8	4.5 ± 0.6	<0.001
fat consumption (g/kg/d)	6.5 ± 1.5	7.1 ± 1.0	<0.001

P:E (g/100kcal) 3,35 3,08

Slide 43

There are some critiques on these previous trials. I don't want to go into too much detail. They are methodological and also the way how they measure.



## Individualized Fortification of Breast Milk for Preterm Infants

### Summary of critiques of previous trials

#### Perth trial:

Target values for intake too low: P:E 1: 2.6/2.7, prot 3.3 g/kg/d  
No intervention occurring in intervention group, therefore no effect observed

#### Leipzig trial

Protein Intake unreasonably high in control group: 3.2/3.1 g/dL after STF which corresponds to a native protein content of 2.1/2.2 g/dl, not supported by data obtained from other clinical trials, reported values of native BM are 0.9 – 1.4 g/dl (Fenton, Kim, Lonnerdal, Hartmann, Rochow)

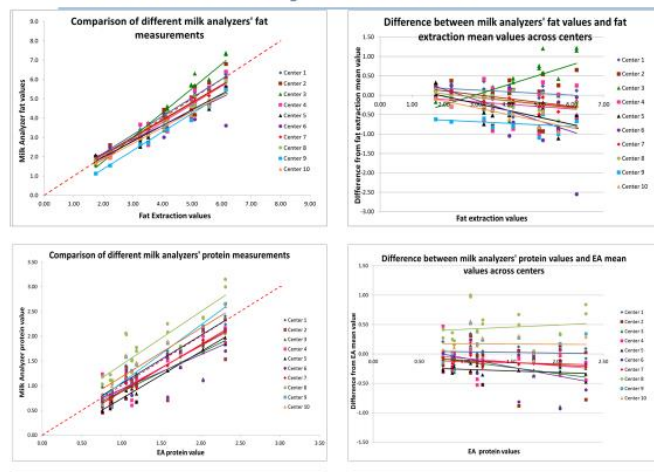
Dichotomous distribution for < and > 34 weeks of gestation, but only in the intervention group, not in control group.

Slide 44

We spend a good amount of time in finding out how can we measure macronutrient content at the bedside? We use these dairy industry milk analyzers, which is a big business, and use them to measure human milk. Unfortunately, you need to do a lot of work to basically tame the shrew. Because human milk and cow's milk are different.

We did that, I can't show you all the data because it would take too much time, but these are recent data from a multicenter study where we sent out the same samples to 13 labs in North America and in Europe, and you see that the data here [Slide 45, lower left plot] were all over the place. So, you can't do target fortification if you have this bad precision.

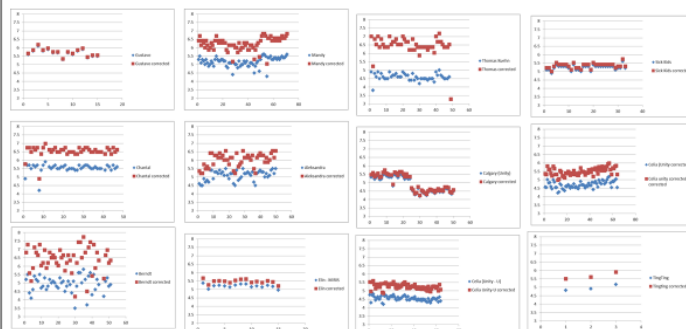
### MAMAS Study Protocol



Slide 45

So, we were training and modifying these devices, and here you see how the different units measured differently at quality controls. Obviously, this unit [Slide 46, 1st column, last plot] will get different results from target fortification compared to this one [3rd column, last plot].

### Comparison of all centers for high QC fat



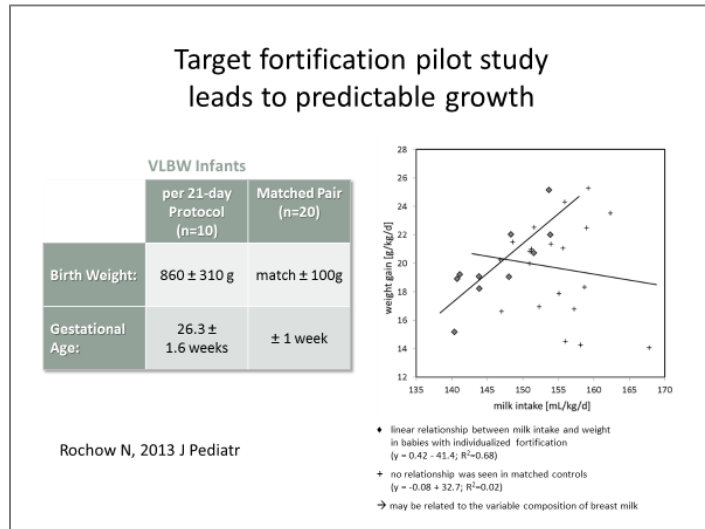
Slide 46

The good thing is we could train them with corrected values. We measure the right thing with a lot of training. That can be overcome, but it's not just buying a milk analyzer and then you have the right values.



## Individualized Fortification of Breast Milk for Preterm Infants

This is a study [Slide 47] that we did as a pilot study about target fortification.<sup>23</sup> And we saw a predictable weight here [plot] in babies that got target fortification and was dependent upon milk intake, which was not the case for babies that were on standard fortification. These babies did not grow, though they got a huge amount of fluid here.



Slide 47

Then we did a randomized controlled trial, and I will show you the data in this last part of my talk.

### Objective

To evaluate the effect of individualized target fortification of breast milk for fat, carbohydrates and protein on the weight gain of preterm infants compared to routine fortification in a randomized controlled double blinded trial (RCT)

Slide 48

The randomized trial in 100 babies. It was double-blinded single-center randomized control, but with

3 weeks of intervention. Randomization was done with sealed envelopes, and the primary outcome was weight at 36 weeks. And we had lots of secondary outcomes.

### Study design

- Double-blinded, Single Centre Randomized Control Trial
- Three weeks intervention period
- Randomisation using sealed envelopes
- Primary outcomes:
  - weight at 36 weeks
- Secondary outcomes:
  - Growth Velocity (GV)
  - Nutritive Efficiency (NE)
  - Anthropometrics
  - Metabolic parameters (Urea, Glucose, Triglycerides)

Slide 49

These are the baseline demographics [Slide 50]: no difference, 27 weeks, 970 g. We started with staggered fortification around 3 weeks of life.

### Baseline demographics

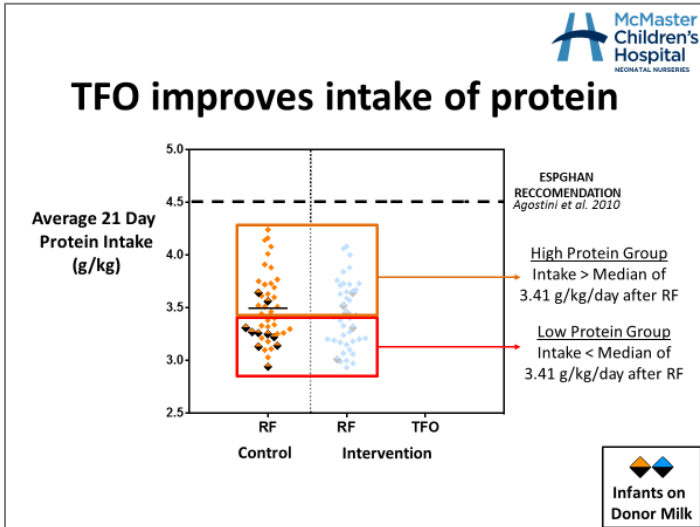
	Control (n=43)	Intervention (n=42)	p-value
Birth Weight (g)	970 ± 260	960 ± 210	0.97
GA at Birth (weeks)	27.0 ± 1.8	27.2 ± 1.2	0.44
GA at start (weeks)	30.4 ± 1.6	30.4 ± 1.1	0.85
DOL at start	22 ± 7	20 ± 6	0.31
Male (n)	24	22	0.75

Slide 50

You see that the TFO (target fortification) improves intake of protein. So, this is the protein intake after standard fortification in the control group. This

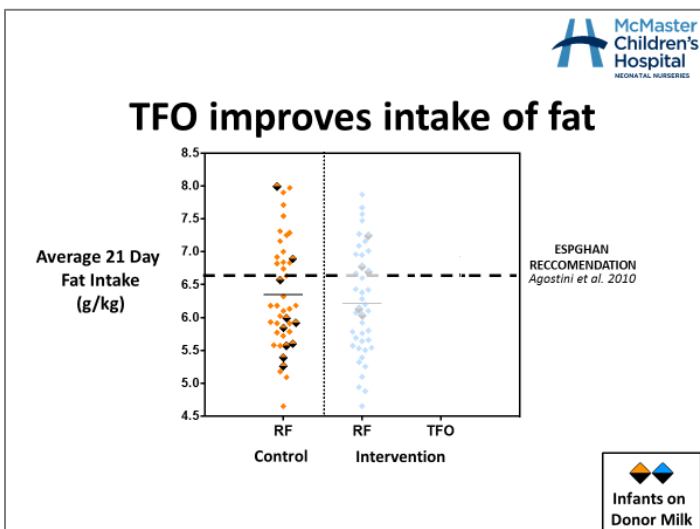
## Individualized Fortification of Breast Milk for Preterm Infants

would have been in the TFO group, but with the extra fortification, we indeed achieved 4.5 g/kg/d.



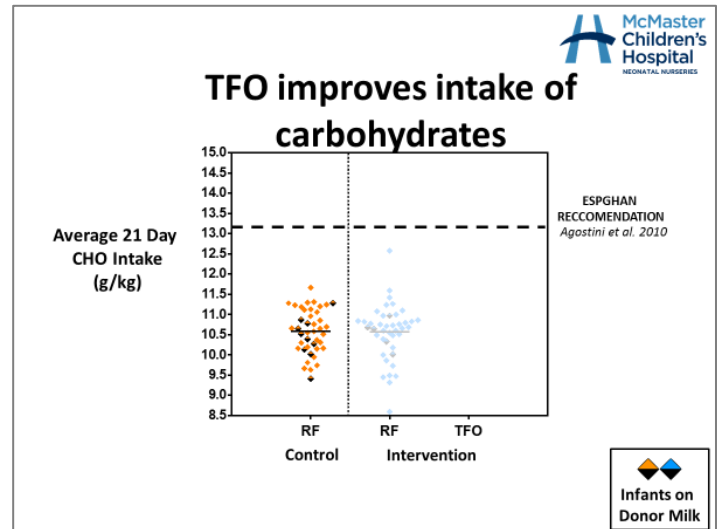
Slide 51

The same happened with fat, but there was not a big effect, because I told you that North American fortifiers already contain so much fat that we came close to the recommendations (was a little bit more).



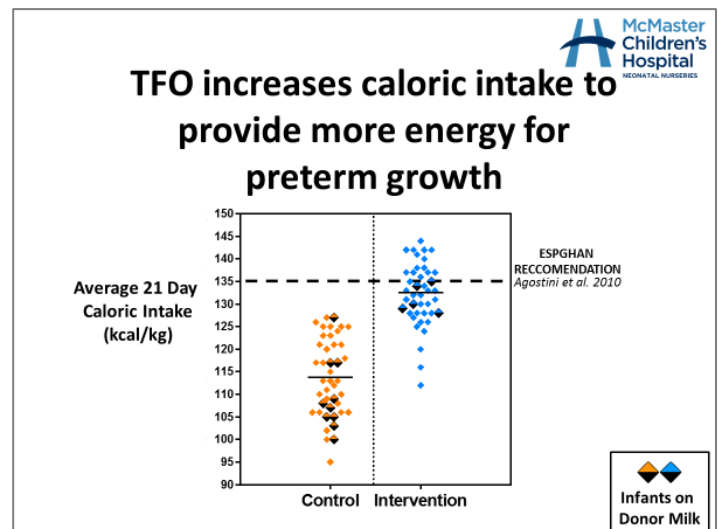
Slide 52

For carbohydrates, it was a big difference.



Slide 53

Overall, the caloric intake also improved.



Slide 54

These are the data: control = 2280 g, intervention = 2510 g, a difference of 230 g. Growth velocity was also different.

## Individualized Fortification of Breast Milk for Preterm Infants



### TFO improves growth outcomes

	Control (n=43)	Intervention (n=42)	p-value
Weight (g)	2280 ± 340	2510 ± 290	0.001
Growth Velocity (g/kg/d)	19.4 ± 2.3	21.2 ± 2.3	<0.001
Nutritive Efficiency (g/dL)	12.6 ± 1.6	13.9 ± 1.7	<0.001
TFI (mL/kg/d)	155 ± 4	153 ± 4	0.008

$$\text{Nutritive Efficiency} = \frac{\text{Growth Velocity}}{\text{TFI}}$$

Babies who received breast milk with low-protein content will definitely benefit from this approach. It is relatively logical, because it is physiology, but we could show it in this trial.

Interestingly, there were also a trend to better outcomes for all NICU outcomes in the TFO group. We had less NEC, less death, less PDA [patent ductus arteriosus]. Feeding intolerance was also lower (maybe because we had a more constant intake for the babies, no variation).

### Perinatal characteristics and NICU Outcomes

	Control Group		TFO Group	
	Randomized	Completed	Randomized	Completed
N -	89	51	90	52
Maternal Diabetes	6 (7)	3 (6)	7 (8)	6 (12)
Hypertension/Preeclampsia	16 (18)	12 (24)	25 (28)	17 (33)
Suspected Chorioamnionitis	32 (36)	15 (29)	25 (28)	13 (25)
Antenatal Corticosteroids	84 (94)	48 (96)	78 (87)	47 (90)
Died	5 (6)	2 (4)	0 (0)	0 (0)
NEC all cases	5 (6)	2 (4)	2 (2)	0 (0)
NEC Bell stage 3	3 (3)	1 (2)	0 (0)	0 (0)
Sepsis clinical	41 (46)	19 (37)	30 (33)	14 (27)
Sepsis culture positive	17 (19)	7 (14)	22 (24)	10 (19)
PDA	57 (64)	30 (59)	51 (57)	29 (56)
PDA treated	35 (39)	21 (41)	31 (34)	14 (27)
BPD mild	22 (25)	17 (33)	20 (22)	15 (29)
BPD moderate/severe	32 (36)	18 (35)	29 (32)	16 (31)
feeding intolerance		14 (27)		8 (15)**

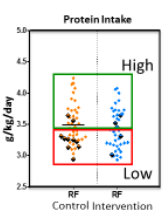
No difference in perinatal characteristics and demographics

Trend towards better NICU outcomes

Better tolerance of enteral nutrition (more balanced intake??)

Slide 55

Then we did a subgroup analysis in the high and low protein group. High and low protein group means the protein content in native, unfortified breast milk. You'll see that in those individuals that receive breast milk from mothers with high-protein content, there's no effect of the intervention. They grow equally well. Why? Because mother has already enough protein in there. In the low-protein group, there is a huge difference, of about 370 g. Also, the growth rates are significantly different.



### Improved growth outcomes in Low Protein group

	High Protein			Low Protein		
	Control (n=22)	Intervention (n=21)	p-value	Control (n=21)	Intervention (n=21)	p-value
Weight (g)	2400 ± 331	2480 ± 265	0.35	2170 ± 316	2540 ± 312	<0.001
Growth Velocity (g/kg/d)	19.7 ± 2.0	21.3 ± 2.0	0.011	19.2 ± 2.7	21.0 ± 2.6	0.030
Nutritive Efficiency (g/dL)	12.7 ± 1.4	14.0 ± 1.6	0.009	12.4 ± 1.9	13.8 ± 1.8	0.019
TFI (mL/kg/d)	155 ± 4	153 ± 3	0.087	155 ± 3	153 ± 4	0.044

Intake after RF > Median of 3.41 g/kg/day      Intake after RF < Median of 3.41 g/kg/day

Slide 56

Slide 57

The clinical chemistry is here [Slide 58], 2.5 BUN vs 4.2 in both groups, a slight increase but not of clinical significance. Interestingly, the triglycerides went down, maybe because we gave more carbohydrates, so we could burn the fat a little better.

## Individualized Fortification of Breast Milk for Preterm Infants

### Clinical chemistry

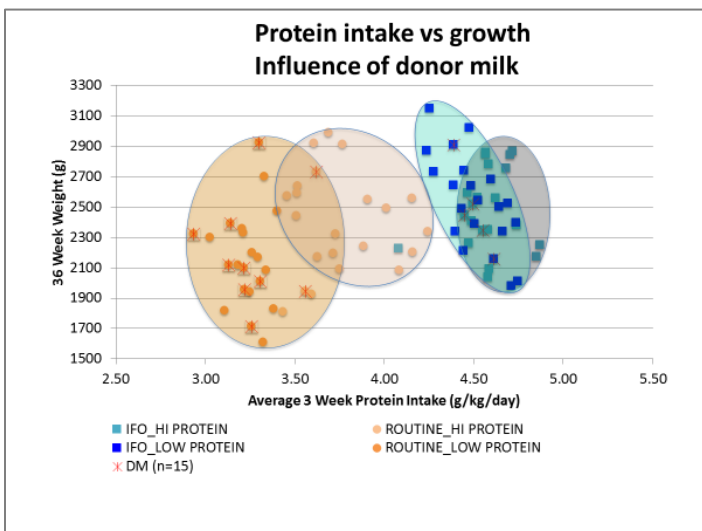
Outcome	All		High protein group		Low protein group	
	Control Group	TFO Group	Control Group	TFO Group	Control Group	TFO Group
Glucose Day 14	4.1 ± 0.8	4.6 ± 0.9*	4.3 ± 0.9	4.6 ± 1.1	4.0 ± 0.8	4.5 ± 0.7*
Glucose Day 21	4.6 ± 2.3	4.3 ± 1.1	4.8 ± 3	4.3 ± 1.2	4.3 ± 0.9	4.2 ± 1
BUN Day 14	2.5 ± 1.1	4.2 ± 1.5***	2.8 ± 0.9	4.1 ± 1.6**	2.3 ± 1.2	4.2 ± 1.4***
BUN Day 21	2.5 ± 1.1	4.7 ± 1.5***	2.8 ± 1.2	4.8 ± 1.1***	2.3 ± 0.8	4.6 ± 1.8***
TG Day 14	0.9 ± 0.4	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.2	0.9 ± 0.6	0.7 ± 0.2
TG Day 21	0.8 ± 0.5	0.7 ± 0.3	0.8 ± 0.3	0.7 ± 0.3	0.9 ± 0.7	0.7 ± 0.2

**Mild increase in glucose and BUN levels without clinical significance**  
**Drop in triglyceride levels**

Slide 58

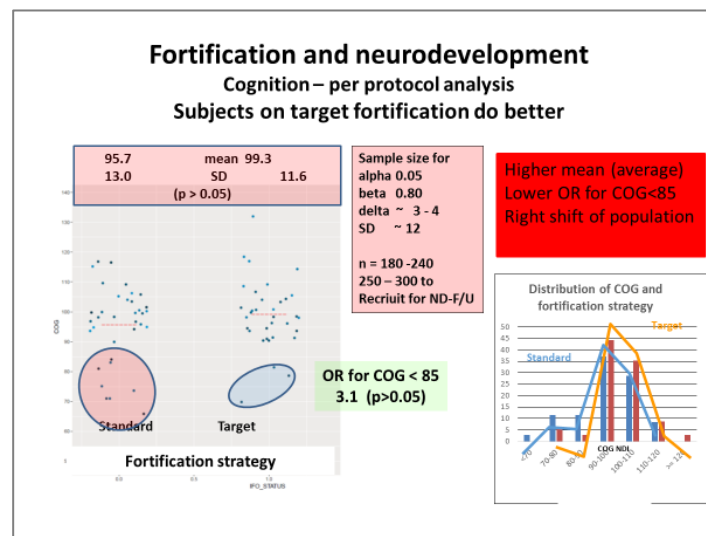
Here [Slide 59] you see all protein intakes vs weight; and these are the 2 groups with low-protein intake. This is without fortification. This is with fortification. You see that these children who do not grow well without target fortification, basically, are moved up here [higher weight].

You see here these babies also on donor milk intake, and they are now also up here. This again visualizes that the effect is mostly pronounced in this group of babies with low-protein content in native breast milk.



Slide 59

These are very recent data [Slide 60]. We got the neurodevelopmental follow-up. It's not shown, yet, at a conference like PAS [Pediatric Academic Societies]. And you'll see here that the intellectual outcome, the difference is about 4 points. The odds ratio for cognitive below 85 is 3.1.



Slide 60

Also, if you look here at the distribution of the IQs, you see a shift to higher IQs, and the effect size again is around 4. Unfortunately, we are not powered to detect this difference, so we didn't find statistical difference. If we wanted to have been powered on these levels, we would have needed to include 250 to 300 babies. So, that's something for the next step for a multicenter randomized control trial.

In summary, preterm formula makes babies grow with predictable and adequate growth rates, including neurodevelopment. Trade-off is microbiome and NEC.

Mother's own milk and donor milk reduce risk for NEC. The trade-off is growth, and neurodevelopmental outcome might not be as good as it could be. Fortification improves growth and neurodevelopmental outcome. Trade-off is NEC protective effects due to the exposition of cow's milk decreasing. The elimination of cow's milk

## Individualized Fortification of Breast Milk for Preterm Infants

protein from fortifiers seems to reduce the NEC rate. Trade-off is growth. (I didn't show you the data on that.) Modern fortifiers should contain more protein, about 0.5–0.7 g/kg/d, and a more balanced mixture of fat and carbohydrates.

For donor milk, additional supplementation using 0.3–0.5 g protein/100 ml seems to be reasonable. I think that may be the most important message.

Adjusted fortification may help to improve growth but is not efficient in all preterm infants. Data about NDI are not available.

The 2 randomized controlled trials on TFO are charged with significant methodological limitations and cannot be generalized to a standard setting. Data from our double-blind randomized controlled trial showed that target fortification improves growth compared to standard fortification,<sup>24</sup> most likely including neurodevelopmental outcome. It's a kind of precision medicine.

For both high-end fortification strategies, modern modular components need to be developed to conserve the NEC protective effect of breast milk, ideally to make it cow's milk protein free and minimize the pro-inflammatory potential by using better lipids. More research and clinical studies are needed, and they need to apply rules of good laboratory practice.

### Summary (1 out of 4)

- Preterm formula makes babies grow with predictable and adequate growth rates (including neurodevelopment)  
Trade-off: microbiome and NEC
- MoM and donor milk reduce the risk for NEC  
Trade-off: growth and NDI ↘
- Fortification improves growth and NDI  
Trade-off: NEC protective effect (due to exposition to cow's milk)
- Elimination of cow's milk protein from fortifiers seems to reduce the NEC-Rate  
Trade-off: growth?

### Summary (2 out of 4)

- Modern fortifiers should contain more protein (ca. 0.5 – 0.7 g/kg/d) and a more balanced mixture of fat and CHO (Rochow, Fusch, 2016)
- For donor milk, additional supplementation using 0.3-0.5 g protein/100ml seems to be reasonable (Simmer 2015)
- Adjusted Fortification may help to improve growth, but is not efficient in all preterm infants. Data about NDI are not available.



## Individualized Fortification of Breast Milk for Preterm Infants

### Summary (3 out of 4)

- The 2 RCTs on TFO are charged with significant methodological limitations and cannot be generalized to a standard setting
- Data from our double-blind RCT show that target fortification improves growth compared to standard fortification (most likely including NDI).  
Precision medicine....

### Summary (4 out of 4)

- For both “high-end” fortification strategies modern modular components need to be developed to conserve the NEC protective effect of breast milk (cow’s milk protein free) and minimize the pro-inflammatory potential (omega 3 : 6, limited MCT).
- More research and clinical studies are needed and they need to apply rules of GCLP.

### Abbreviations

<b>BGA</b>	blood gas analysis	<b>LISA</b>	less invasive surfactant administration
<b>BM</b>	breast milk	<b>MAMAS</b>	maternal adiposity, metabolism, and stress study
<b>BSID</b>	Bayley Scale of Infant Development	<b>MDI</b>	mental development index
<b>BUN</b>	blood urea nitrogen	<b>ME</b>	metabolize energy
<b>BW</b>	birth weight	<b>MM</b>	mother’s milk
<b>CHO</b>	carbohydrate	<b>MN</b>	macronutrients
<b>Cog</b>	cognition	<b>NDI</b>	national death index
<b>CPAP</b>	continuous positive airway pressure	<b>NE</b>	nutritive efficiency
<b>CV</b>	conventional ventilation	<b>NEC</b>	necrotizing enterocolitis
<b>DM</b>	donor milk	<b>NIV</b>	non-invasive ventilation
<b>DOHaD</b>	developmental origins of health and disease	<b>NHFOV</b>	non-invasive high-frequency ventilation

## Individualized Fortification of Breast Milk for Preterm Infants

<b>ELBW</b>	extremely low birth weight	<b>PDA</b>	patent ductus arteriosus
<b>ESPGHAN</b>	European Society for Pediatric Gastroenterology Hepatology and Nutrition	<b>PDI</b>	physical development index
<b>F</b>	fat	<b>PNGR</b>	postnatal growth restriction
<b>FM</b>	fat mass	<b>PTF (or PF)</b>	preterm formula
<b>GCLP</b>	Guidelines for Good Clinical Laboratory Practices	<b>SGA</b>	small for gestational age
<b>GV</b>	growth velocity	<b>TG</b>	triglyceride
<b>HFOV</b>	high-frequency oscillation ventilation	<b>TFI</b>	total fluid intake
<b>HM</b>	human milk	<b>TFO</b>	target fortification
<b>HMF</b>	human milk fortifier	<b>VLBW</b>	very low birth weight
<b>HMO</b>	human milk oligosaccharide		

- Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics*. 1999;104(2 Pt 1):280-289.
- Henriksen C, Westerberg AC, Ronnestad A, et al. Growth and nutrient intake among very low birth weight infants fed fortified human milk during hospitalisation. *Br J Nutr*. 2009; 102, 1179–1186.
- Weber A, Loui A, Jochum F, Bühner C, Obladen M. Breast milk from mothers of very low birthweight infants: variability in fat and protein content. *Acta Paediatr*. 2001;90(7):772-775.
- Henriksen C, Westerberg AC, Rønnestad A, et al. Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation. *Br J Nutr*. 2009;102(8):1179-1186. doi:10.1017/S0007114509371755
- Corvaglia L, Aceti A, Paoletti V, et al. Standard fortification of preterm human milk fails to meet recommended protein intake: Bedside evaluation by Near-Infrared-Reflectance-Analysis. *Early Hum Dev*. 2010;86(4):237-240. doi:10.1016/j.earlhumdev.2010.04.001
- Rochow N, Fusch G, Choi A, et al. Target fortification of breast milk with fat, protein, and carbohydrates for preterm infants. *J Pediatr*. 2013;163(4):1001-7. doi:10.1016/j.jpeds.2013.04.052
- Rozance PJ, Hay WW. Describing hypoglycemia - definition or operational threshold? *Early Hum Dev*. 2010; 86(5): 275–280. doi:10.1016/j.earlhumdev.2010.05.002
- Rochow N, Fusch G, Choi A, et al. Target fortification of breast milk with fat, protein, and carbohydrates for preterm infants. *J Pediatr*. 2013;163(4):1001-7. doi:10.1016/j.jpeds.2013.04.052
- Fusch G, Mitra S, Rochow N, Fusch C. Target fortification of breast milk: levels of fat, protein or lactose are not related. *Acta Paediatr*. 2015;104(1):38-42. doi:10.1111/apa.12804
- Sauer CW, Boutin MA, Kim JH. Wide Variability in Caloric Density of Expressed Human Milk Can Lead to Major Underestimation or Overestimation of Nutrient Content. *J Hum Lact*. 2017;33(2):341-350. doi:10.1177/0890334416672200

## Individualized Fortification of Breast Milk for Preterm Infants

11. Fusch G, Mitra S, Rochow N, Fusch C. Target fortification of breast milk: levels of fat, protein or lactose are not related. *Acta Paediatr.* 2015;104(1):38-42. doi:10.1111/apa.12804
12. Kashyap S, Towers HM, Sahni R, Ohira-Kist K, Abildskov K, Schulze KF. Effects of quality of energy on substrate oxidation in enterally fed, low-birth-weight infants. *Am J Clin Nutr.* 2001;74(3):374-380.
13. Kashyap S, Ohira-Kist K, Abildskov K, et al. Effects of quality of energy intake on growth and metabolic response of enterally fed low-birth-weight infants. *Pediatr Res.* 2001;50(3):390-397.
14. Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2018;6:CD002971. doi:10.1002/14651858.CD002971.pub4
15. Montjoux-Régis N, Cristini C, Arnaud C, Glorieux I, Vanpee M, Casper C. Improved growth of preterm infants receiving mother's own raw milk compared with pasteurized donor milk. *Acta Paediatr.* 2011;100(12):1548-54. doi:10.1111/j.1651-2227.2011.02389.x
16. O'Connor DL, Gibbins S, Kiss A, et al. Effect of supplemental donor human milk compared with preterm formula on neurodevelopment of very low-birth-weight infants at 18 months: A randomized clinical trial. *JAMA.* 2016 Nov 8;316(18):1897-1905. doi:10.1001/jama.2016.16144
17. Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. *Pediatrics.* 2009;123(5):1337-1343. doi:10.1542/peds.2008-0211
18. Madore LS, Bora S, Erdei C, Jumani T, Dengos AR, Sen S. Effects of donor breastmilk feeding on growth and early neurodevelopmental outcomes in preterm infants: An observational study. *Clin Ther.* 2017 Jun;39(6):1210-1220. doi:10.1016/j.clinthera.2017.05.341
19. Embleton ND. Optimal protein and energy intakes in preterm infants. *Early Hum Dev.* 2007 Dec;83(12):831-837.
20. Micheli JL, Schutz T. Protein. In: Tsang RC, Lucas A, Uauy R, Zlotkin S. eds. *Nutritional needs of the Preterm Infant: Scientific Basis and Practical Guidelines.* Baltimore, MD: Williams & Wilkins, 1993: 29-46. 44.
21. Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? *J Perinatol.* 2006 Oct;26(10):614-621.
22. McLeod G, Sherriff J, Hartmann PE, Nathan E, Geddes D, Simmer K. Comparing different methods of human breast milk fortification using measured v. assumed macronutrient composition to target reference growth: a randomised controlled trial. *Br J Nutr.* 2016;115(3):431-439. doi:10.1017/S0007114515004614
23. Rochow N, Fusch G, Choi A, et al. Target fortification of breast milk with fat, protein, and carbohydrates for preterm infants. *J Pediatr.* 2013;163(4):1001-1007. doi:10.1016/j.jpeds.2013.04.052
24. Fusch G, Mitra S, Rochow N, Fusch C. Target fortification of breast milk: levels of fat, protein or lactose are not related. *Acta Paediatr.* 2015;104(1):38-42. doi:10.1111/apa.12804