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# MOTHER'S MILK AND THE MICROBIOME

Tiffany Weir, PhD  
Associate Professor  
Food Science and Human Nutrition  
Colorado State University

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# The Gut Microbiome

Stomach:  $10^{10}$  Lactobacilli, Clostridia, Streptococcus, Helicobacter pylori, Proteobacteria

Duodenum:  $10^8$  Streptococcus, Lactobacilli

Small Intestine:  $10^{11}$  Streptococcus, Bacteroides, Actinobacteria, Clostridia

Large Intestine:  $10^{14}$  Bacteroides, Clostridium groups IV and XV, Bifidobacterium, Enterobacteriaceae

Proximal Ileum:  $10^9$  Streptococcus, Lactobacilli

Distal Ileum:  $10^{11}$  Streptococcus, Bacteroides, Actinobacteria, Clostridia

Am J Gastroenterol Suppl (2012) 1:15-21

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# Gut Microbiota: Fun Facts

Getting to know your gut microbiota

A large quantity (trillions) of bacteria and other microorganisms inhabit your intestines, fulfilling key functions for your health and well-being.

- Gut microbiota's weight can reach 1 kg
- 1 to 2 million bacterial cells per gram of stool
- 95% of gut bacteria live in the gastrointestinal tract
- Bacteria are 10 to 50 times greater than human cells
- In our body, bacteria outnumber human cells 10:1
- The total surface area of the gut is 400 m<sup>2</sup> (as if it were laid out)
- Last year, more acid-lipolytic bacteria were found in the same 2.5 grams

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# Protection

I. Direct inhibition

II. Nutrient/receptor competition

III. Stimulation of immune defenses

Legend:   
 - red: peptidoglycan   
 - blue: lipoteichoic acid   
 - green: lipopolysaccharide   
 - purple: flagellin   
 - yellow: PAMP receptor   
 - orange: toll-like receptor   
 - pink: NOD1   
 - light blue: NOD2

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# Digestion

- Indigestible carbohydrates
  - Bacteria encode enzymes we lack
  - SCFA
- Fat
  - Can modify bile acids
  - Certain bacteria are thought to aid in fat digestion
- Protein
  - Proteolytic processes degrade proteins into amino acids
- Synthesis of essential amino acids
  - Microbially synthesized can contribute 19-22% of daily requirements.
- Synthesis of vitamins
  - vitamin K and B
  - B12: site of synthesis vs absorption
- Absorption of ions (Calcium, Magnesium, iron)

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# Obesity

- Germ-free mice required 30% more calories to maintain same weight as normal littermates
- Germ-free mice transplanted with normal microflora gained weight.
- Increased energy harvest associated with higher levels of the phylum Firmicutes

\* Turnbaugh and Backed studies from Gordon lab (2006)

### Obesity

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The diagram shows two human figures, one lean and one obese, with their respective gut microbiomes. The bar chart compares 'Number of OTUs' for 'Obese' (blue) and 'Lean' (red) groups across 'Genus' and 'Species' levels. The obese group shows significantly higher diversity in both categories.

Ley et al. 2006. Nature

### Obesity

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#### Gut dysbiosis = loss of community balance

The diagram illustrates the gut wall with 'Eubiosis' (balanced) and 'Dysbiosis' (imbalanced) states. Dysbiosis leads to an 'Inflammatory Immune Response' involving 'Immune cells'.

Sheflin et al. (2014) Curr. Oncol. Reports 16: 406.

### Obesity

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The diagram shows 'Obese mice' with 'Microbiota transfer' to 'Recipient mice', resulting in 'Increased adiposity'. Conversely, 'Lean mice' result in 'Lean' recipient mice.

When co-housed the "lean" mice transferred their microbes and their phenotype to the "obese" animals!

Ridaura, V. K. et al. Science 341, 1241214 (2013).

### Developing Tolerance

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The diagram shows the gut barrier with 'permeability' and 'proinflammatory' responses. It details cytokines like IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, IL-18, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, IL-37, IL-38, IL-39, IL-40, IL-41, IL-42, IL-43, IL-44, IL-45, IL-46, IL-47, IL-48, IL-49, IL-50, IL-51, IL-52, IL-53, IL-54, IL-55, IL-56, IL-57, IL-58, IL-59, IL-60, IL-61, IL-62, IL-63, IL-64, IL-65, IL-66, IL-67, IL-68, IL-69, IL-70, IL-71, IL-72, IL-73, IL-74, IL-75, IL-76, IL-77, IL-78, IL-79, IL-80, IL-81, IL-82, IL-83, IL-84, IL-85, IL-86, IL-87, IL-88, IL-89, IL-90, IL-91, IL-92, IL-93, IL-94, IL-95, IL-96, IL-97, IL-98, IL-99, IL-100, IL-101, IL-102, IL-103, IL-104, IL-105, IL-106, IL-107, IL-108, IL-109, IL-110, IL-111, IL-112, IL-113, IL-114, IL-115, IL-116, IL-117, IL-118, IL-119, IL-120, IL-121, IL-122, IL-123, IL-124, IL-125, IL-126, IL-127, IL-128, IL-129, IL-130, IL-131, IL-132, IL-133, IL-134, IL-135, IL-136, IL-137, IL-138, IL-139, IL-140, IL-141, IL-142, 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[https://www.youtube.com/watch?v=gnZEge78\\_78](https://www.youtube.com/watch?v=gnZEge78_78)

### Microbiota and Health

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The diagram shows a human silhouette with four key areas of impact: 1. Dietary intake (Protein, Fat, Carbs, Fiber, etc.), 2. Altered gut bacteria (Dysbiosis, etc.), 3. Biologic effects (Acid, Heat, etc.), and 4. Host disease (Cancer, etc.).

Singh et al. 2017. J. Transl. Med.

### Microbiota and Health

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The tree diagram shows 'Gut Microbiota' at the base, branching into 'Metabolic Syndrome', 'Cardiovascular disease', 'Immune-mediated diseases', 'Neurodegenerative diseases', 'Psychiatric diseases', 'Autoimmune diseases', 'Cancer', 'Diabetes', 'Obesity', 'Allergies', 'Hypertension', 'Insulin resistance', 'Colorectal cancer', 'Dementia/Alzheimer's/ Parkinson's', 'IBD/ IBS/ IBD', and 'Crohn's disease'.

Nagpal et al. 2014 Front Med. dx.doi.org/10.3389/fmed.2014.00015

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## HOW DO WE GET THESE MICROBES?

Birth influences and early feeding patterns are CRITICAL for a healthy adult microbiome.



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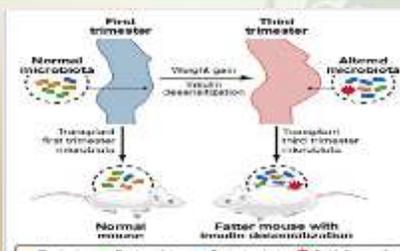
## Colonization Influences



Putignani et al (2014) Ped Res 76, 2-10.

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## Gestational Diabetes

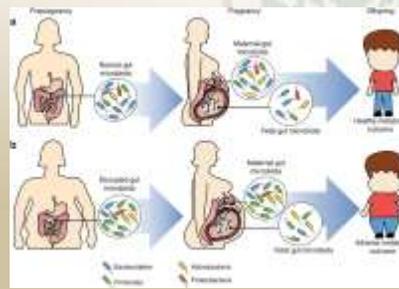


Koren et al (2012) Cell 150:470

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## Fetal Programming

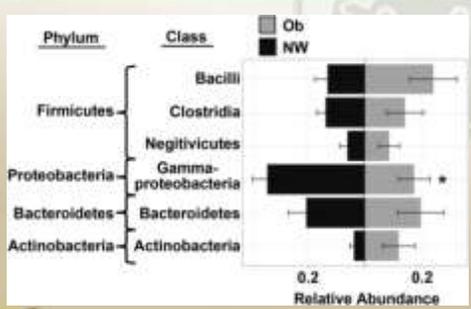
Maternal obesity is a strong predictive factor of childhood obesity and may be due to microbiota effects.



Gohir et al (2015) Ped Res 77:196.

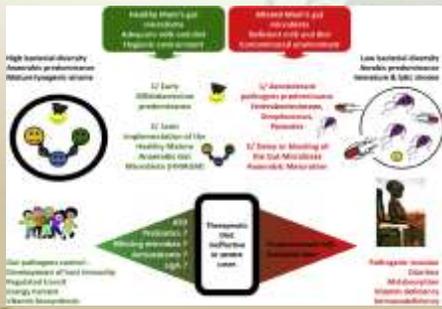
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## Maternal Weight and Microbiota



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## Malnutrition and Microbiota



## Placental Microbiome Colorado State University

Small populations of microbes have been detected in the placenta.

Composition most closely mimics the mother's oral microbiota.

May lead to pre-natal exposures to microbes that start priming the immune system.

Link peridontal health and pre-term infancy?

**Placental**

Maternal pre-probiotic supplements

Microbial microbiota

Placenta

## Mode of Delivery Colorado State University

Disease	OR	95% CI
<b>Allergic Rhinitis</b>		
All Countries	1.37	(1.14-1.63)
Hygiene Countries Only	1.78	(1.36-2.31)
<b>Asthma</b>		
All Countries	1.24	(1.05-1.53)
Finland	1.53	(1.10-2.12)
Finland & Hygiene Countries <sup>3</sup>	1.83	(1.12-2.97)
<b>Celiac Disease</b>	1.89	(1.12-3.16)
<b>Diabetes Mellitus (Type 2)</b>	1.18	(1.04-1.34)
<b>Gastroenteritis<sup>4</sup></b>	1.48	(1.24-1.78)
<b>Gastroenteritis AND Asthma</b>	1.14	(1.08-1.21)

1. Hakkarinen et al. 2011, PLoS One; 2. Besser et al. 2012, JAMA Pediatrics; 3. Hakkarinen et al. 2011, PLoS One; 4. Besser et al. 2012, JAMA Pediatrics

Nature Rev Microbiol (2010) 9:27-38 | Clin Perinatol. 2011; 38:321-331

## Restoring Microbiota Colorado State University

1 Birth

2 Sterile container

3 Mouth Face Rest of body

Image: M.J. Schwan

Dominguez-Bello et al. (2016) Nature Med 22:250

## Microbial Dynamics Colorado State University

<https://www.youtube.com/watch?v=Pb272zsixSQ>

Birth 1 month 6 months 12 months 2-3 years

Microbial diversity

Arrieta et al. Front. Immunol. | <http://dx.doi.org/10.3389/fimmu.2014.00427>

## Breast vs. Formula Colorado State University

**Breast-fed (BF)**

**Formula-fed (FF)**

BF had more than Proteobacteria and Bacteroidetes

FF had more Firmicutes and/or Bacteroidetes

Donovan et al. (2012) Advances in Nutrition 3(3):450S-455S.

## Milk Microbiome Colorado State University

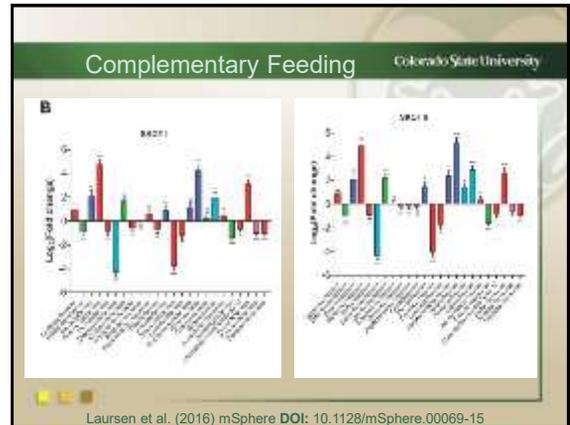
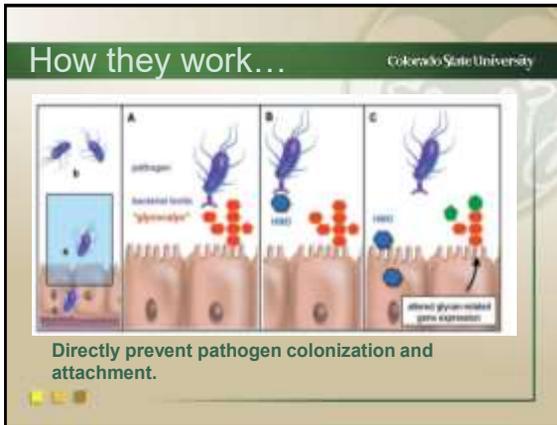
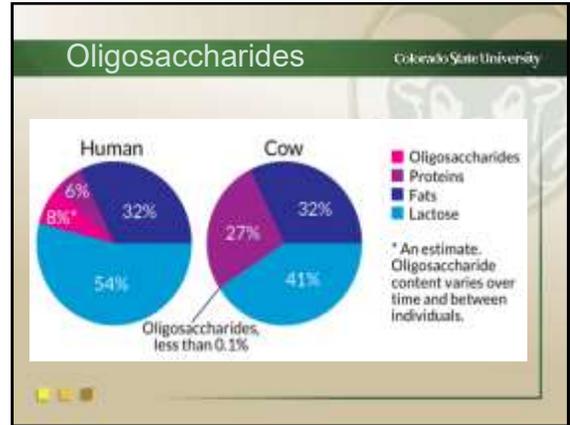
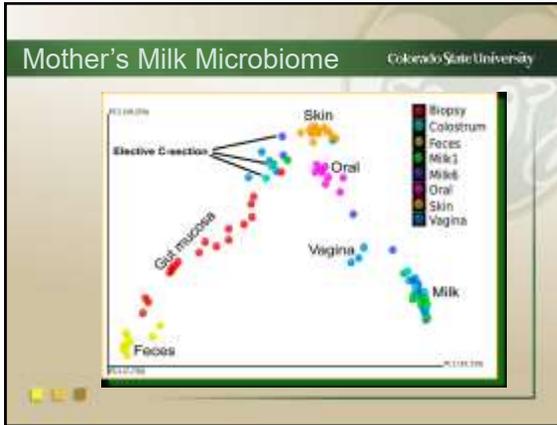
Maternal gut

Extracellular vesicles

Mammary gland

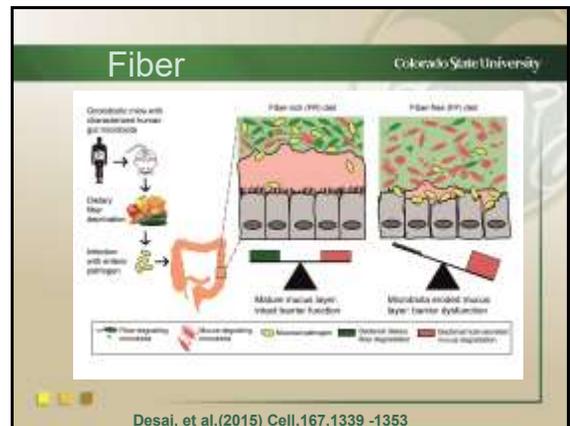
Infant gut

Constrains milk microbiota



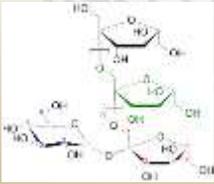
### What should I eat?

Feeding the Microbiome of Mom and Baby.



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**Prebiotics** are non-living indigestible polysaccharides (food components) that stimulate the growth of beneficial bacteria (*Bifidobacterium*).



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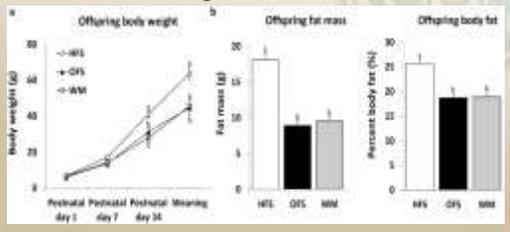
### Prebiotic Sources

- Diet
  - Major dietary sources are consumed in limited amounts in a typical American diet
- Supplements
- Fortification in foods
  - Yogurt
  - Infant formula
  - Artificial sweeteners

Food with Prebiotics	Prebiotic fiber by weight	Amount needed for 1g serving
Oatmeal	64.5%	8.3 g
Immature Artichoke	35.5%	19 g
Dandelion greens	24.3%	24.7 g
Raw garlic	17.3%	34.3 g
Raw leek	15.7%	31.3 g
Raw onion	9.6%	69.8 g
Cooked asparagus	5%	118 g
Raw asparagus	3%	178 g
Raw wheat bran	3%	118 g
Whole wheat flour, unbleached	4.2%	115 g
Raw banana	1%	409 g

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### Maternal Prebiotics Reduce Offspring Weight Gain in Rats



Paul et al. 2016. *Scientific Reports*. doi:10.1038/srep20683

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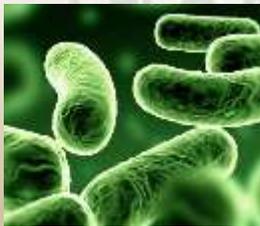
### Omega 6:3 in BM

Increased ratios of Omega 6:3 in breast milk associated with higher INFLAMMATION, INSULIN RESISTANCE, OBESITY, and reduced NEUROPROTECTIVE EFFECTS.

<b>Animal Sources:</b> Sardines Wild Salmon Sablefish/Black Cod Flaxseed/Flaxseed oil Grass-fed Meats Grass-fed Dairy Pasture-raised Eggs	<b>Plant Sources:</b> Walnuts Chia Seeds Hemp Seeds Tofu+ Tempeh Cauliflower Broccoli Dark leafy greens
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**Probiotics** are live bacteria or yeast that when eaten in sufficient amounts can be beneficial for intestinal health.



Slide from Katie McGirr, CSU Extension

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### Probiotic sources

- Food sources:
  - Fermented dairy foods like yogurt, kefir products, and aged cheeses
  - Some fermented non-dairy foods including kimchi, sauerkraut, and kombucha
  - Supplemented non-fermented foods: Good Belly



Slide from Katie McGirr, CSU Extension

## Summary:Pre/Probiotics

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- Prebiotic supplementation (2 months prenatal and during lactation) prevented excess adiposity and improved insulin sensitivity in offspring.
- Probiotics 2 weeks pre-natally activates immune responses and alters meconium microbiota.
- Probiotics given during the 3<sup>rd</sup> trimester and during lactation to mothers with skin allergies reduced eczema incidence in infants.

## Summary (cont)

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- Probiotics administered to C-section babies in the first 6 months of life reduced allergies in children at 5 years.
- Systematic review of probiotic use in pre-term infants suggest little risk and possible benefits of probiotic supplementation on NEC.

## Cautions

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- Antibiotics should not be overused, but have a lifesaving, important place in modern medicine.
- In the wrong place, even good bacteria go bad.
- We still have a long way to go
  - Many studies in animals rather than humans
  - Human studies on limited population size, ethnic/geographic/socioeconomic backgrounds

## Key Points

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- Disturbances in the maternal microbiota are heritable and can influence child health outcomes.
- Breastfeeding provides the infant with pre- and probiotics necessary for early immune development.
- Dietary management of the microbiota is critical for mother and child in the pre and early postnatal period.

## Useful info

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- Microbial changes during pregnancy, birth, and infancy. Nuriel-Ohayon et al (2016) Frontiers in Microbiology. Doi:10.3389/fmicb.2016.01031
- Consumerlabs: <https://www.consumerlab.com/>
- Natural Standards: <https://naturalmedicines.therapeuticresearch.com/>
- International Scientific Asscn of Probiotics and Prebiotics: <https://isappscience.org/>



Mothers' Milk Bank

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a program of Rocky Mountain Children's Health Foundation

<https://rmchildren.org/?gclid=CNJhtyc4MCFdO3wAodK78GJg>

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