Diet and Microbiome Influences on Breast Cancer Risk

Katherine L. Cook, Ph.D.
Associate Professor
Department(s) of Surgery, Cancer Biology, Comprehensive Cancer Center
Wake Forest University School of Medicine

@KLCookLab
Microbiome

1. Your body is mostly microbes

2. Microbes are influenced by many factors

3. Obesity reduces microbiome diversity

- Firmicutes/Bacteroidetes

Our body is only 43% human!

39 trillion bacteria cells: 30 trillion human cells
Good to know microbiome terms and explanations

**Alpha-diversity:** richness measure/total number of different microbes in a sample

- **Sample 1:** 5 different types of bacteria
- **Sample 2:** 6 different types of bacteria

**Sample 2** has a higher α-diversity

**Beta-diversity:** difference in communities of microbes

- **Sample 1**
- **Sample 2**
- **Sample 3**

Samples 2+3 are the most different from each other and have a higher β-diversity
Functions of a healthy gut bacterial microbiome

- Internal microbiome control
- Protection from pathogens & infections
- Prevention of allergies
- Improved gut motility

Modulation of central nervous system

Immune system development

Synthesis of vitamins

Insulin/glucose homeostasis

Ruan et al., 2020
What shifts your gut bacterial microbiome?

Consumption of a high saturated fat diet increases Firmicutes phyla.

Mediterranean diet consumption associated with increased Bacteroidetes phyla.

Urbanization associated with decreases in gut diversity.

Commonly used drugs such as proton pump inhibitors, metformin, selective serotonin reuptake inhibitors and laxatives influence gut microbiome composition.

Moderate exercise increases gut diversity.

Dietary pattern

Geography

Early-life factors

Pharmaceuticals

Lifecycle stage

Firmicutes and Proteobacteria phyla increases in elderly populations.
How the gut microbiome composition may affect breast cancer risk

Increased Breast Cancer Risk

- Obesity
  - Bacteriodes
  - Firmicutes

- Intestinal permeability
- Circulating LPS
- Energy harvest
- Deconjugated estrogen metabolite availability
- Modified insulin responsiveness

- Chronic Low-Grade Inflammation
- Metabolism
- Acetaldehyde
- ROS
Exploring the gut microbiome for breast cancer prevention

*Fabian et. al., Prevent Cancer Research 2021*

- **n=46** randomized peri/postmenopausal overweight/obese women
- **n=23** Placebo + Weight loss intervention
- **n=20** Omega-3 (2,200 mg EPA+1,050 mg DHA) + Weight loss intervention

<table>
<thead>
<tr>
<th></th>
<th>Placebo n=16</th>
<th>Omega-3 PUFA n=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>52.7 ± 5.7 (45-63)</td>
<td>51.8 ± 7.0 (43-64)</td>
</tr>
<tr>
<td>BMI baseline, kg/m²</td>
<td>31.7 ± 3.3 (28-40)</td>
<td>32.2 ± 3.6 (28-42)</td>
</tr>
<tr>
<td>% Body fat composition</td>
<td>46.5 ± 4.5 (37.4-54.9)</td>
<td>48.0 ± 3.2 (42.2-51.7)</td>
</tr>
<tr>
<td>10-year Tyrer-Cuzick model, % risk</td>
<td>12.0 ± 8.5 (6.2-37)</td>
<td>9.1 ± 4.8 (1.9-19)</td>
</tr>
</tbody>
</table>

No significant differences between groups at baseline in patients with collected fecal samples
Weight loss and omega-3 supplementation interactions on the phyla level microbiota abundances

**Cook et. al., unpublished data**

- Placebo <10% BW loss
- Omega-3 <10% BW loss
- Placebo >10% BW loss
- Omega-3 >10% BW loss

**Phylum Proportional Abundance (%)**
- Bacteroidetes
- Firmicutes
- Proteobacteria
- Verrucomicrobia

**Firmicutes/Bacteroidetes Ratio**

**Phylum Proportional Abundance (%)**

- Placebo <10% Weight loss
- Omega-3 <10% Weight loss
- Placebo >10% Weight loss
- Omega-3 >10% Weight loss

n=7-10; *p<0.05
Cook et. al., unpublished data
Weight loss and omega-3 supplementation interactions on select microbiota species

<table>
<thead>
<tr>
<th>Microbiota Species</th>
<th>Placebo &lt;10% Weight loss</th>
<th>Omega-3 &lt;10% Weight loss</th>
<th>Placebo &gt;10% Weight loss</th>
<th>Omega-3 &gt;10% Weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phocaeicola dorei</td>
<td>0.0086</td>
<td>0.0589 $^a$</td>
<td>0.0342</td>
<td>0.0184 $^b$</td>
</tr>
<tr>
<td>Bacteroides intestinalis</td>
<td>0.0467</td>
<td>0.0065</td>
<td>0.0014 $^a$</td>
<td>0.0023 $^a$</td>
</tr>
<tr>
<td>Phocaeicola vulgatus</td>
<td>0.0669</td>
<td>0.0389</td>
<td>0.0717</td>
<td>0.0939 $^b$</td>
</tr>
<tr>
<td>Alistipes finegoldii</td>
<td>0.0175</td>
<td>0.0097</td>
<td>0.0214</td>
<td>0.0097</td>
</tr>
<tr>
<td>Alistipes onderdonkii</td>
<td>0.0142</td>
<td>0.0274</td>
<td>0.0098</td>
<td>0.0051 $^b$</td>
</tr>
<tr>
<td>Alistipes putredinis</td>
<td>0.0107</td>
<td>0.0227</td>
<td>0.0364</td>
<td>0.0276</td>
</tr>
<tr>
<td>Alistipes shahii</td>
<td>0.0076</td>
<td>0.0116</td>
<td>0.0099</td>
<td>0.0200</td>
</tr>
<tr>
<td>Phocaeicola massiliensis</td>
<td>0.0017</td>
<td>0.0147</td>
<td>2.0000e-005</td>
<td>0.0145</td>
</tr>
<tr>
<td>Bacteroides stercoris</td>
<td>0.0014</td>
<td>0.0054</td>
<td>0.0099</td>
<td>0.0231</td>
</tr>
<tr>
<td>Bacteroides uniformis</td>
<td>0.0415</td>
<td>0.0637</td>
<td>0.0594</td>
<td>0.0588</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
<td>0.0155</td>
<td>0.0077</td>
<td>0.0139</td>
<td>0.0072 $^a, c$</td>
</tr>
<tr>
<td>Phascolarctobacterium faecium</td>
<td>0.0095</td>
<td>0.0068</td>
<td>0.0073</td>
<td>0.0166 $^a$</td>
</tr>
</tbody>
</table>

n=34

Cook et. al., unpublished data
Omega-3 PUFA supplements modulates inflammation associated microbes

Previous European cohort (n=53) showed a positive correlation between gut *D. formicigenerans* and circulating C-reactive protein in obese postmenopausal women (Brahe et. al., *Nutrition and Diabetes* 2015)

n=16-18; *p < 0.05  
Cook et. al., unpublished data
Snap-shot summary

• Omega-3 PUFA regardless of weight loss reduces inflammation-associated *D. formicigenerans*

• Omega-3 PUFA and >10% weight loss interact to significantly decrease Firmicute phyla proportional abundance compared with all other groups

• Omega-3 PUFA and >10% weight loss interact to increase metabolic health associated microbe *P. faecium*
The breast microbiome and cancer

• Microbiota of human breast tissue. Urbaniak et al, 2014

<table>
<thead>
<tr>
<th>Microbiota Genus</th>
<th>Canadian breast tissue (% of microbiota population)</th>
<th>Irish breast tissue (% of microbiota population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>11.4%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>10%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>8.3%</td>
<td>30.8%</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>6.5%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>6.5%</td>
<td>12.7%</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>5.8%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Prevotella</td>
<td>5%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Listeria</td>
<td>&lt;2%</td>
<td>12.1%</td>
</tr>
</tbody>
</table>

  • Increased Staphylococcus in breast tissue from women with BC when compared with healthy controls

• The microbiome of aseptically collected human breast tissue in benign and malignant disease. Hieken et al, 2016
  • Decreased Lactobacillus in breast tissue from women with malignant BC
Since diet is main determinant of the gut microbiome, can what you eat modify you breast microbiome?

10-fold increase in MG Lactobacillus (genus) proportional abundance
Detangling obesity and diet using NHP cohort

Table 1. Metabolic Parameters by Body Weight Group. Values represent mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Mediterranean-Lean (n=7)</th>
<th>Mediterranean-Heavy (n=7)</th>
<th>Western-Lean (n=7)</th>
<th>Western-Heavy (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>2.5±0.2</td>
<td>4.1±1.0</td>
<td>2.7±0.2</td>
<td>4.9±1.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.7±4.2</td>
<td>48.2±6.9</td>
<td>39.7±4.5</td>
<td>60.2±10.4</td>
</tr>
<tr>
<td>Body Fat Composition (%)</td>
<td>8.4±2.0</td>
<td>23.4±9.0</td>
<td>11.7±4.3</td>
<td>39.1±10.5</td>
</tr>
<tr>
<td>Insulin AUC</td>
<td>1721±335</td>
<td>6456±5703</td>
<td>3431±4043</td>
<td>10526±8427</td>
</tr>
<tr>
<td>TPC (mg/dL)</td>
<td>133.4±29.4</td>
<td>163.7±48.0</td>
<td>159.5±33.1</td>
<td>149.9±28.5</td>
</tr>
<tr>
<td>HDL-C</td>
<td>48.6±8.6</td>
<td>66.7±29.8</td>
<td>78.4±40.6</td>
<td>72.1±17.2</td>
</tr>
<tr>
<td>TPC/HDL-C ratio</td>
<td>2.78±0.50</td>
<td>2.62±0.46</td>
<td>2.38±0.95</td>
<td>2.13±0.16</td>
</tr>
<tr>
<td>Cortisol</td>
<td>36.5±4.2</td>
<td>29.3±5.6</td>
<td>33.7±8.8</td>
<td>41.1±5.7</td>
</tr>
</tbody>
</table>

Cook et. al., unpublished data
Diet and adiposity interact to shift NHP breast microbiome

Cook et. al., unpublished data

n=5-7; *p<0.05; **p<0.01

PERMANOVA Analysis

Western + low adiposity vs Western + high adiposity p=0.006

Western + low adiposity vs Mediterranean + low adiposity p=0.013
Elevating mammary gland *Lactobacillus* modifies tissue metabolism

**Glucose Metabolism**

- Hexokinase 2
- Glucose
- Glucose 6-phosphate
- Glucose 1-phosphate
- Fructose 1,6-bisphosphate
- Glyceraldehyde-3-phosphate
- Dihydroxyacetone
- 1,3-diphosphoglycerate
- 3-phosphoglycerate
- 2-phosphoglycerate
- PEP
- Pyruvate
- Acetyl-CoA
- TCA Cycle

**Pathways**

- Gluconeogenesis (n=10)
- Glycolysis / Gluconeogenesis (n=10)
- Glucagon signaling pathway (n=16)
- Oxytocin signaling pathway (n=21)
- Hypertrophic cardiomyopathy (HCM) (n=13)
- Amphetamine addiction (n=11)
- Staphylococcus aureus infection (n=9)
- C-type lectin receptor signaling pathway (n=14)
- Diabetic cardiomyopathy (DCM) (n=12)
- Tuberculosis (n=17)
- Complement and coagulation cascades (n=11)
- Calcium signaling pathway (n=18)
- CAMP signaling pathway (n=19)
- Aldosterone synthesis and secretion (n=12)
- Tight junction (n=17)
- Adrenergic signaling in cardiomyocytes (n=16)
- Insulin signaling pathway (n=15)
- Central carbon metabolism in cancer (n=9)
- HIF-1 signaling pathway (n=12)
- Cocaine addiction (n=7)
- Arrhythmogenic right ventricular ... (n=9)

**Data**

- n=4-6
- Arnone et. al., unpublished data
Mammary gland *Lactobacillus* reduces tumorigenesis

**MMTV-PyMT Mammary Carcinogenesis Model**

Saline  Lactobacillus

**Ki67 Tumor Proliferation**

Saline  Lactobacillus

**MMTV-PyMT Study**

Tumor-free Survival (%)  Tumor Multiplicity (Tumors per mouse)  Proliferative Index (Relative Ki-67 positive cells)

**n=6-7**

Arnone et. al., unpublished data
Conclusions

• We have identified several pro-health associated species (such as \textit{P. faecium}) that may be a potential novel probiotic to reduce metabolic breast cancer risk factors.

• Diet and adiposity interact to influence the breast tissue microbiome.

• Lower body fat composition was associated with increased breast \textit{Lactobacillus} populations in NHP cohort regardless of dietary pattern.

• Mammary gland \textit{Lactobacillus} enhances normal tissue glucose metabolism.

• Mammary gland \textit{Lactobacillus} prevents breast tumorigenesis and decreased tumor proliferation in a MMTV-PyMT murine model.

• Both the gut and breast microbiome may be a modifiable target for breast cancer prevention.
Thank you to all the patients that participate in clinical trials and donate their samples!
Questions?