MICROBIOTA AND DEVELOPMENT OF GI FUNCTION AND INFLAMMATION

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The Versatile Intestine: The intestine is not only a digestive-absorptive organ

- Largest immune organ of the body.
- Harbors a huge microbial ecosystem.
- Harbors the enteric nervous system
Intestinal Surface Area

The intestinal surface area is the largest of the body and approximates that of a tennis court (250 m²).
INTESTINAL FUNCTIONS

- Digestion and absorption of nutrients.
- Maintenance of hydroelectric homeostasis
- Regulation of peristalsis
- Modulation of immune response
- Production of neuroendocirinal signals
- Microbiota
Growth in Length

• Human fetal intestine elongates 1000 fold from 5-40 weeks.
• Length doubles in last 15 weeks of gestation.
• Mean length at birth (term) is 275 cm. (small intestine is about 200cm).
Capability for Digestion, Absorption and Assimilation

- **Digestion**-occurs in lumen.
- **Absorption**-occurs at the enterocyte interface (microvillus membrane).
- **Assimilation**-occurs in the enterocyte and beyond.
Digestion, absorption and motility in relation to fetal and neonatal development

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<tr>
<th>Gestational week</th>
<th>Very preterm newborns</th>
<th>Preterm newborns</th>
<th>Full-term newborns</th>
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**Digestion**
- Progressive detecting of sucrose, lactase and GI peptides
- Sucrase 70% of adult
- Lactase peak

**Absorption**
- Glucose transporter presence
- Gastric lipase increase

**Motility**
- Swallowing present
- Disorganized motility
- Fetal mot
- MMC
- Mature motility

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<tr>
<th>% survival</th>
<th>17</th>
<th>39</th>
<th>50</th>
<th>80</th>
<th>90</th>
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The Intestinal Microbiome

Neish, A. Gastroenterology vol. 136, No. 1, 2009
Gut-Associated Lymphoid Tissue structures are strategically situated in relation to the greatest concentration of microbiota

- **Peyer’s patches**: distal ileum (nos. 100-250)
- **Isolated lymphoid follicles (ILFs)**: large bowel (nos. ~30,000)

*Brandtzaeg, Immunological Investigations 2010*
Alterations in the Vaginal Microbiome by Maternal Stress Are Associated With Metabolic Reprogramming of the Offspring Gut and Brain
Human Milk

- Increased Bifidobacteria
- Increased Folate
- Increased DNA Methylation
- Decreased Gene Transcription

Formula

- Increased Firmicutes
- Increased Histone Deacetylation
- Increased Gene Transcription

Epigenetically Induced Metabolic Changes
Th1 cells
TNFα
INFγ
IL-2

ICC

Enterocytes
Smooth muscle cells

Enteric neuron

IL-6
Enteric glia
IL-1β

Capillaries
Key steps in ENS development

- **Migration** of neural crest precursor cells (NCC) into gut
- **Proliferation** of NCC within the gut to ultimately form millions of ENS cells
- **Assembly** of NCC into groups (myenteric ganglia, submucosal ganglia)
- **Differentiation** of NCC into a range of enteric neuronal types and glial cells
- **Formation of the functional circuitry** necessary for controlled gut activity
- **Post-natal modifications** to adapt to a changing gut environment
Development of smooth muscle and ENS in human gut

GREEN - $\alpha$SMA
RED – p75 (NCC)
Nerves / Muscles / ICCs present

Swallow

Intestinal motor activity

Organised activity

Sucking

10 16 22 28 34 40

Weeks Gestation

Courtesy of Prof. Peter Milla
POSTNATAL MODIFICATION OF ENS

- Dietary modifications - effects on proportions of neurons and gut motility

- Stress/inflammation – changes in ENS in e.g. maternal separation model

- ENS damage/injury – chemical ablation of myenteric plexus
SCFAs are generated in the large intestine as a result of bacterial fermentation of dietary fiber and resistant starch.

Butyrate increased the proportion of ChAT-immunoreactive (IR) myenteric neurons in vivo.

Butyrate also increased colonic transit and neuronally mediated contractile response in the colon.

From: Soret et al., 2010. Gastroenterology 138:1772–1782
NEUROIMMUNE INTERACTION
LONG-TERM ALTERATIONS OF COLONIC NERVE–MAST CELL INTERACTIONS INDUCED BY NEONATAL MATERNAL DEPRIVATION IN RATS

Close apposition between mast cells and PGP9.5 fibres – similar to that seen in IBS

Barreau F et al. Gut 2008;57:582-590
Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice

Nobuyuki Sudo\textsuperscript{1,2}, Yoichi Chida\textsuperscript{1}, Yuji Aiba\textsuperscript{3,4}, Junko Sonoda\textsuperscript{1}, Naomi Oyama\textsuperscript{1}, Xiao-Nian Yu\textsuperscript{1}, Chiharu Kubo\textsuperscript{1} and Yasuhiro Koga\textsuperscript{3}

Commensal microbes affect the neural network responsible for controlling stress responsiveness

![Graph showing ACTH and Corticosterone levels](image)

**Figure 3. Effects of restraint stress on plasma ACTH and corticosterone levels in gnotobiotic mice**

Plasma ACTH and corticosterone levels were measured before or immediately after 1 h restraint in GF (n = 20), SPF (n = 18) and monoassociated mice (n = 18–24 per group) at 9 weeks of age. *P < 0.05, ***P < 0.001 by Dunnett's test.
Extrinsic Neural Regulation of Antroduodenal Motor Activity in Preterm Infants
Youhanna Al-Tawil, George Klee and Carol Lynn Berseth
A Centenary of Gastrointestinal Endocrinology

J. F. Rehfeld

Horm Metab Res 2004; 36(11/12): 735-741
THE INTESTINE THE NEGLECTED ORGAN....
ILEAL EXPRESSION OF TYROSINE HYDROXILASE AND VASOACTIVE INTESTINAL PEPTIDE (neuronal density) AFFECTED BY DIET (prebiotic composition)
GUT BRAIN COMMUNICATION
Structural and functional features of central nervous system lymphatic vessels

Antoine Louveau¹,², Igor Smirnov¹,², Timothy J. Keyes¹,², Jacob D. Eccles³,⁴,⁵, Sherin J. Rouhani³,⁴,⁶, J. David Peske³,⁴,⁶, Noel C. Derecki¹,², David Castle², James W. Mandell⁸, Kevin S. Lee¹,²,⁹, Tajie H. Harris¹,² & Jonathan Kipnis¹,²,³
INTESTINAL PERMEABILITY

- Altered microbiota (low diversity caused by antibiotics)
- Intact intercellular junction
- Mucus
- Immature intestinal barrier
  - Decreased mucus
  - Decreased IgA
  - Low intercellular junction integrity and increased permeability
- Exaggerated inflammation and tissue injury
Assessment of Intestinal Permeability in (Premature) Neonates by Sugar Absorption Tests

Willemijn E. Corpeleijn, Ruurd M. van Elburg, Ido P. Kema, and Johannes B. van Goudoever

Figure 2 Lactulose/mannitol (L/M) ratio in 102 preterm infants measured within two days of birth (test 1) and three to six days later (test 2). It was higher in test 1 (0.427) than in test 2 (0.182) (p < 0.001).

Figure 3 Lactulose/mannitol (L/M) ratio in 116 preterm infants and 16 healthy term infants measured within two days of birth (test 1). It was significantly higher in the preterm than the term infants (0.404 and 0.170 respectively, p < 0.001).
DNA methylation in genetically identical C57 wild-type mice under either conventional (CNV) or germ-free (GF) conditions. Different methylation happen in the whole colon

Yu et al 2015
Transcriptional Modulation of Intestinal Innate Defense/Inflammation Genes by Preterm Infant Microbiota in a Humanized Gnotobiotic Mouse Model

A

Relative mRNA expression levels in \( M_p \)-L and \( M_p \)-H ailum

<table>
<thead>
<tr>
<th>Gene</th>
<th>( M_p )-L</th>
<th>( M_p )-H</th>
<th>GF (1)</th>
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<tbody>
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<td>IL1β</td>
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<td>PPARγ</td>
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* \( p < 0.05 \)

B

VCAM-1

Magnification 400x 50 μm

C

MCP-1

Magnification 400x 50 μm

Magnification 1000x 20 μm
Different DNA methylation and Protein expression in Fetal and Pediatric intestinal epithelium

Kraiczy et al 2015
Relation between intestinal function and microbiota
60 preterm (GA 28-32wks) were enrolled in the study and double-blind assigned to receive either *L. reuteri* at dose of 1x10^8 CFU a day or placebo for 30 days.

Was recorded:

- Days on parenteral nutrition,
- Days of hospital stay
- Number of days of antibiotics
- Gastric emptying rate at 1 month of life
- Fecal cytokines and fecal calprotectin at 1 month of life
## DEMOGRAPHICAL AND CLINICAL CHARACTERISTICS

<table>
<thead>
<tr>
<th></th>
<th>Tot n = 60</th>
<th>L. <em>Reuterii</em> = 30</th>
<th>Placebo = 30</th>
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<tbody>
<tr>
<td>Gestational age</td>
<td>30,2 (±1,2)</td>
<td>30,1 (±1,2)</td>
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<tr>
<td>Gender (M/F)</td>
<td>13/17</td>
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<td>16/14</td>
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<td>Delivery (VB/CS)</td>
<td>4/26</td>
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<td>5/25</td>
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<tr>
<td>Birth weight (g)</td>
<td>1471,5 (±455,1)</td>
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<td>1406,6 (±536,4)</td>
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RESULTS ON MOTILITY

Gastric emptying rate was significantly increased and fasting antral area reduced in the newborns receiving supplementation with *L. reuteri*.
Results on fecal cytokine

[L. Reuteri bar graph with Placebo comparison for IL-1B, IL-10, IL-17, Calprotectin, TNFalpha, IL-8, IL-6]
CLINICAL FEATURES

![Bar chart showing the comparison between L. Reuteri and Placebo for time in days for Parenteral nutrition, Hospitalization, and Antibiotic treatment.](chart.png)
Take Home Messages

• The Developing GI tract is complex and is much more than an organ of digestion and absorption.

• The premature intestine is capable of significant but limited digestion and absorption. Motility is be limiting but all of these functions are stimulated with enteral feeding.

• The role of microbiota is crucial and driving the changes in the intestinal colonization could improve intestinal maturation.
SAVE the DATE

4th INTERNATIONAL CONGRESS OF
Probiotics, Prebiotics in Pediatrics

MAY 3 - 5, 2018
BARI, Italy