Exploring the role of the major gut microbiota clusters on nutritional and functional benefits of nutrients and non-nutrients

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Conflict of Interest Disclosure

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ILSI Europe group: ‘Role of microbiota on nutritional & functional benefits of nutrients & non-nutrients’

Aims of the expert group

• Evaluate role of microbiota in metabolism of dietary compounds
• Review mechanisms and pathways involved
• Identify main types of microorganisms involved
• Consider the methodologies for investigating gut microbiota metabolism

Outputs
Shortt et al ‘Systematic review of the effects of the intestinal microbiota on selected nutrients and non-nutrients’ Eur J Nutr. 2017

Human gut microbiota – phyla & genera

- **Firmicutes**
  - Clostridium
  - Roseburia
  - Faecalibacterium
  - Blautia
  - Dorea
  - Lactobacillus
  - Peptostreptococcus
  - Eubacterium
  - Streptococcus
  - Staphylococcus
  - Butyrvibrio

- **Bacteroidetes**
  - Bacteroides
  - Prevotella

- **Verrucomicrobia**
  - Akkermansia

- **Proteobacteria**
  - Escherichia
  - Klebsiella
  - Desulfovibrio

- **Actinobacteria**
  - Bifidobacterium
  - Collinsella

90% of bacteria are in Bacteroidetes/Firmicutes phyla
Gut microbiota - metabolism

- Large metabolic potential - equivalent to, but different from that of liver

- Microbiota metabolic range
  - Reduction
  - Hydrolysis
  - Polysaccharide fermentation
  - Dehydroxylation
  - Methylation
  - Demethylation
  - Deamination
  - Nitrosation
  - Ring fission
  - Aromatization
  - Oligomer breakdown
Dietary components reviewed

- Carbohydrates
- Energy homeostasis
- Proteins
- Lipids
- Bile acids
- Vitamins
- Phytochemicals/polyphenols
**Carbohydrate fermentation**

- **Polysaccharides**
  - NSP/Resistant starch

- **Oligosaccharides**

- **Mucins**

**Microbial fermentation**

- \( \text{H}_2 \), \( \text{CO}_2 \)
- \( \text{CH}_4 \)
- Acetate
- Propionate
- Butyrate
- Lactate

**Short chain fatty acids (SCFA)**

- Methanobrevibacter smithii
- Most gut organisms
- Eubacterium hallii
- Roseburia
- Coprococcus catus
- Megasphaera elsdenii
- Veillonella spp
- Ruminococcus obeum
- Bacteroidetes
- Eubacterium rectale
- Eubacterium hallii
- Roseburia spp
- Coprococcus spp
- Faecalibacterium prausnitzii
- Bifidobacterium spp
- Lactobacillus spp

**ILSI Europe**
Energy homeostasis

Studies in humans and animals suggest microbiota is involved in assimilation, storage & expenditure of energy obtained from dietary substrates.

- Some studies report higher Firmicutes/Bacteroides ratio in obese subjects
- Higher BMI associated with increased abundance of *Eubact. ventrosium* and *Roseburia intestinalis, E. rectale*
- Weight-loss regimes (diet or gastrectomy) associated with ↑ Bacteroidetes ↓ Firmicutes inc *Clostridium, Eubacterium, Coprococcus*).

Potential mechanisms

- Gut microbes break down non-digestible carbohydrates to SCFA allowing the host to salvage energy from indigestible dietary substrates.
- SCFA ↓ energy intake: Pr & Bu increase the levels of satiety hormones PYY and GLP-1, Ac & Pr increase expression of leptin

Role of specific gut microbes and SCFA in energy balance remains to be clarified
Protein metabolism

Branched chain fatty acids (BCFA)
- Isobutyrate (from val)
- 2-methylbutyrate (i-leu)
- Isovalerate (leu)

Davila et al. Pharmacological Res. 69:114-126 2013
Lipid metabolism

- Linoleic acid reduced to stearic acid (via CLA and vaccininc acid) in vitro by range of gut bacteria

- Phosphatidylcholine to TMA
Microbiota metabolism of choline to TMA and TMAO

TMA produced by solely by gut microbiota
High-fat diets lead to increased production of TMA and TMAO, - risk factors for cardiovascular disease

Choline metabolizers:
**Proteobacteria**: *Desulfovibrio desulfuricans*, *Klebsiella spp*, *Escherichia spp*, *Edwardsiella tarda*, *Proteus penneri*
**Firmicutes**: *Clos sporogenes*, *hathewayi*, *asparagiforme*

Martinez-del-Campo mBio 6(2) e00042 (2015)
Romano et al mBio 6(2) e02481 (2015)
Bile acids

1. Deconjugation by bacterial bile salt hydrolases (BSH)

**Bile salt hydrolase**
- BSH genes identified in the main bacterial genera including *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, and *Listeria*
- Most hydrolyze both glyco and tauro-conjugates.
- Reduces toxicity of bile acids, releases N, S and C atoms
- Deconjugation reduces efficiency of BA for emulsifying lipids and micelle formation
Bile acids – further metabolism

7α-dehydroxylation
- Main bacterial genera include Clostridium, Eubacterium

Epimerization
- Main genera: Bacteroides, Clostridium, Egghertella, Peptostreptococcus, Ruminococcus, Eubacterium

DCA & LA are more cytotoxic and genotoxic, to colon mucosa than CA & CDA
Vitamin synthesis

• Vitamin synthesis genes common esp. vitamin K and B group vitamins - biotin, cobalamin, folate, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine

• For riboflavin & biotin, all tested microbes in Bacteroidetes Fusobacteria and Proteobacteria phyla had required pathways, fewer Firmicutes and Actinobacteria had the pathways

• Bacteroidetes is most important phyla for vitamin synthesis

• Many of these vitamins are utilized by other bacteria
## Vitamin synthesis

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Intracellular concentration [mmol/gDW]</th>
<th>Dietary reference intake [mg/day]</th>
<th>%DRI from gut microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>$9.0 \times 10^{-7}$</td>
<td>0.03</td>
<td>4.5</td>
</tr>
<tr>
<td>Cobalamin</td>
<td>$8.5 \times 10^{-8}$</td>
<td>0.0024</td>
<td>31</td>
</tr>
<tr>
<td>Folate</td>
<td>$5.0 \times 10^{-5}$</td>
<td>0.4</td>
<td>37</td>
</tr>
<tr>
<td>Niacin</td>
<td>$3.3 \times 10^{-3}$</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>Pantothenate</td>
<td>$2.3 \times 10^{-6}$</td>
<td>5</td>
<td>0.078</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>$5.8 \times 10^{-4}$</td>
<td>1.3</td>
<td>86</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>$9.0 \times 10^{-6}$</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Thiamin</td>
<td>$8.7 \times 10^{-6}$</td>
<td>1.15</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Phytochemicals

**Flavonoids**
(Fruit, veg.)

**Carotenoids**
(Carrot, pepper, tomato)

**Stilbenes**
(Wine, nuts)

**Polyphenols**
Lignans, ferulic acid, isoflavones
(seeds, cereals, legumes)

**Glucosinolates/Isothiocyanates**
(cruciferous vegetables)

**Organosulphur Compounds**
(Garlic, onions)
Polyphenols

- Often poorly absorbed in small intestine ➔ colon
- Parent polyphenols are extensively metabolized by the microbiota, (deglycosylation, ring fission, dehydroxylation) - can impact bioactivity
- Metabolism often requires consortia or 2 or more microbes
- Large interindividual variations in absorption and excretion ascribed to differences in gut microbiota
Pathways of colonic degradation of the flavonoid rutin

Rutin → Deglycosylation → Quercetin →
- Ring fission, water elimination, dehydroxylation
- Further degradation

Rutin → Dehydroxylation →
- 3,4-dihydroxyphenylacetic acid →
- 3-hydroxyphenylacetic acid →
- 3,4-dihydroxyphenylacetic acid

Absorption from the colon

Further degradation → Protocatechuic acid

β-Oxidation + glycination → 3-hydroxyhippuric acid
Gut microbiota & inter-individual variation in polyphenol metabolism

• Differences in composition of microbiota between individuals can have significant effects on extent of metabolism and metabolite profile

• Examples:
  • Isoflavonoids (daidzein to equol)
  • Naringin
  • Anthocyanins
  • Lignans
  • Tea catechins
  • Rutin
Isoflavone metabolism by gut bacteria

Clostridium spp.
Eubacterium ramulus

Lactobacillus mucosae +
Enterococcus faecium +
Finegoldia magna +
Veillonella sp

Slakia isoflavoniconvertens,
Slakia equolifaciens,
Adlercreutzia equolifaciens

Blaut et al 2003; Decroos et al 2005;
Matthies et al 2009, 2012)
Equol excretion in subjects consuming soy isoflavonoids

Subjects consumed soy burgers (56mg IF) - 1/day for 17 days (Rowland et al 2003)
Methodologies

- Isolated cultures
- Gut microbial enzyme activity
- Omics approaches
  - Metagenomics
  - Metatranscriptomics
  - Metaproteomics
  - Metabolomics
- Mathematical modeling
Omics approaches for studying gut microbial metabolism/function

- **Metagenomics** – study functional genes associated with specific microbial types,
- **Meta-transcriptomics** – monitor active bacteria, reveals functional roles (e.g., CHO metabolism) info on functional dysbiosis,
- **Meta-proteomics** – confirming microbial function (faecal meta proteome is subject-specific and stable)
- **Metabonomics** – pathway analysis, metabolic biomarkers of disease risk
Conclusions

• Gut microbiota metabolism enlarges the capacity of host to metabolize range of dietary components and extends the range of metabolites formed.
• CHO metabolism is major function of microbiota – pathways and microbes well studied.
• Microbial metabolites of nutrients and non-nutrients can be important cell signaling molecules (SCFA, bile acids) and have impacts on health (SCFA, TMA, phenolics).
• Large interindividual variation in microbiota – consequences for metabolism of dietary compounds and health.
• ‘Omics’ provide insight into microbiota function at high resolution.
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