Impact of the gut microbiota on metabolism of nutrients and non-nutrients

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

Aims of the 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
Human gut microbiota – phyla & genera

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

- **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
- Microbe-host co-metabolism

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

• Carbohydrates
• Proteins
• Fats
• Bile acids
• Vitamins
• Phytochemicals/polyphenols
Carbohydrate fermentation

**Polysaccharides**
- NSP/Resistant starch

**Oligosaccharides**
- Mucins
  - NSP/Resistant starch
  - Microbial fermentation

**Microbial fermentation**

- Acetate
  - H₂, CO₂
  - Methanobrevibacter smithii
  - Most gut organisms

- Propionate
  - CH₄
  - Desulfovibrio spp

- Butyrate
  - H₂S

- Lactate
  - Eubacterium rectale
  - Eubacterium hallii
  - Roseburia spp
  - Coprococcus spp
  - Faecalibacterium prausnitzii
  - Eubacterium hallii
  - Roseburia spp
  - Coprococcus spp
  - Faecalibacterium prausnitzii

- Bacteroidetes
  - Bifidobacterium spp
  - Lactobacillus spp
Bacterial proteolysis

- Branched chain fatty acids (BCFA)
  - Isobutyrate (from valine)
  - 2-methylbutyrate (isoleucine)
  - Isovalerate (leucine)

Davila et al. Pharmacological Res. 69:114-126 2013
Fat metabolism
- Linoleic acid
- Bile salts
Pathways of LA metabolism by bacterial species isolated from the human gut.

Eubacterium siraeum
Roseburia faecis
Roseburia intestinalis
F. prausnitzii
Bifidobacterium breve
Propionibacterium freudenreichii

Lactobacillus spp

Bifidobacterium breve
Propionibacterium freudenreichii

Bile acids

Deconjugation by bacterial bile salt hydrolases (BSH)

<table>
<thead>
<tr>
<th>Bile salt hydrolase (BSH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• BSH genes identified in the main bacterial genera including <em>Bacteroides, Bifidobacterium, Clostridium, Lactobacillus</em>, and <em>Listeria</em></td>
</tr>
<tr>
<td>• Most hydrolyze both glyco and tauro-conjugates.</td>
</tr>
<tr>
<td>• Reduces toxicity of bile acids and releases nitrogen, sulphur and carbon atoms</td>
</tr>
<tr>
<td>• Deconjugation reduces efficiency of BA for emulsifying lipids and micelle formation</td>
</tr>
</tbody>
</table>
Bile acids

7α-dehydroxylation

7-a-dehydroxylase
- main bacterial genera include Clostridium, Eubacterium
DCA & LA are more toxic to colon mucosa than CA & CDA

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

Ursodeoxycholic acid
Vitamin synthesis

- Vitamin synthesis genes common esp. vitamin K and B group vitamins - biotin, cobalamin, folate, nicotinic acid, panthothenic 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 utilised 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>$1.3 \times 10^{-4}$</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>

Magnusdottir et al. Front Genet. 2015
Phytochemicals

Flavonoids
(Fruit, veg.)

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

Carotenoids
(Carrot, pepper,
tomato)

Glucosinolates/
Isothiocyanates
(cruciferous
vegetables)

Stilbenes
(Wine, nuts)

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

Deglycosylation

Rutinose

Rutin

Deglycosylation

O-Rutinose

Deglycosylation

Quercetin

Ring fission, water elimination, dehydroxylation

Protocatechuic acid

3,4-dihydroxyphenyl-acetic acid

3-hydroxyphenyl-acetic acid

Dehydroxylation

3-(3-hydroxyphenyl)-propionic acid

β-Oxidation + glycination

3-hydroxyhippuric acid

Absorption from the colon
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  
Blaut et al 2003

Lactobacillus mucosae + Enterococcus faecium + Finegoldia magna + Veillonella sp

Slakia isoflavoniconvertens, Slakia equolifaciens, Adlercreutzia equolifaciens

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 extends metabolic flexibility of host to process wide range of dietary nutrients and non-nutrients.
• CHO metabolism major function of microbiota – pathways well studied.
• Large amount of functional redundancy so composition variation may not result in functional variation.
• Interindividual variation in microbiota – consequences for metabolism of dietary compounds and health.
• ‘Omics’ provide insight into microbiota function at high resolution.
• In future important to integrate omics datasets to fully exploit potential and develop mathematical models.
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