Selective enrichment of key bacterial groups within the human colon in response to changes in diet

Alan Walker

Wellcome Trust Sanger Institute
Diet and the human gut microbiota

- A significant proportion of dietary compounds escape digestion in the small intestine.
- Non-digestible carbohydrates are the predominant growth substrates for gut bacteria.
Principal substrates available for utilization by intestinal microbes

Of dietary & intestinal origin:

- Resistant starch
- Non-starch polysaccharides
- Unabsorbed sugars
- Oligosaccharides
- Dietary protein
- Enzymes / secretions / mucus

[from Cummings & Macfarlane (1991)]

Digestibilities for plant cell wall polysaccharides – 7 subjects (Slavin et al J. Nut 1981)

- Pure cellulose (Solka Flok) minimal
- Cellulose (in normal diets) 69.7% (+/-10.7)
- Hemicellulose 71.7% (+/- 5.4)
Microbial metabolism of dietary compounds

complex polysaccharides

polysaccharide degraders

oligo-, mono-saccharides

saccharolytic bacteria

formate

H₂ + CO₂

acetogens

acetate

lactate

succinate

ethanol

methanogens

CH₄

H₂S

sulfate reducers

SO₄²⁻
Regional differences along the length of the GI tract drive the development of distinct microbial communities with differing fermentative activities.

<table>
<thead>
<tr>
<th>Proximal Colon</th>
<th>Transverse Colon</th>
<th>Distal Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active fermentation</td>
<td>Depletion of substrates</td>
<td>Reduced carbohydrate fermentation</td>
</tr>
<tr>
<td>High bacterial growth rates</td>
<td>Reduction in bacterial activity</td>
<td>Increase in protein fermentation</td>
</tr>
<tr>
<td>Total SCFA around 127mM</td>
<td>Total SCFA around 117mM</td>
<td>Total SCFA around 90mM</td>
</tr>
<tr>
<td>pH 5.5-5.9</td>
<td>pH around 6.2</td>
<td>pH 6.5-6.9</td>
</tr>
</tbody>
</table>

Figure adapted from Leser & Mølbak (2009) and Cummings & Macfarlane (1991)
Diet and the human gut microbiota

- A significant proportion of dietary compounds escape digestion in the small intestine.

- Non-digestible carbohydrates are the predominant growth substrates for gut bacteria.

- The ND carbohydrate content of the diet may have a considerable influence on human health.

- “Prebiotic” dietary supplements (e.g. inulin, FOS) have been extensively studied.

- Relatively little understood about the effect of the major dietary ND carbohydrates on microbial growth in vivo.
## Impact of dietary non-digestible carbohydrates

<table>
<thead>
<tr>
<th>High NSP, low starch</th>
<th>High resistant starch, low NSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High NSP</td>
<td>Phenolics Fermentation Bacteria</td>
</tr>
<tr>
<td>Distal colon</td>
<td>Low Proximal colon Amylolytic</td>
</tr>
<tr>
<td>Fibrolytic</td>
<td></td>
</tr>
</tbody>
</table>

### Human volunteer trial – 14 overweight/obese males

<table>
<thead>
<tr>
<th>M</th>
<th>NSP</th>
<th>Starch</th>
<th>Weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(n = 7)</td>
</tr>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week</td>
<td></td>
</tr>
<tr>
<td>NSP</td>
<td></td>
<td>3 weeks</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td></td>
<td>3 weeks</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
<td>3 weeks</td>
<td></td>
</tr>
</tbody>
</table>

- **M** = weight maintenance, mixed diet (55% energy from carbohydrates)
- **NSP** = high non-starch polysaccharides (added bran), minimal starch
- **Starch** = Added resistant starch (Type III), reduced NSP
- **Weight loss** = reduced calorie intake. Increased % protein.

[Walker AW et al ISME Journal (2011)]
Easier to boost resistant starch (Type III) than NSP (bran)
Principal Component Analysis - effect of non-starch (NSP) and resistant starch (RS) diets on fecal metabolites
Microbiota response – experimental design

1. 16S rRNA gene DGGE analysis – time series (14 subjects, all time points)

2. 16S rRNA gene sequencing analysis on final sample of each dietary period (n=6)

3. qPCR analysis on selected bacterial groups, plus methanogens (14 subjects – all time points)
6 overweight male volunteers

Four diets:
M = maintenance
RS = resistant starch
NSP = non-starch polysaccharide
WL = weight loss
16S rRNA gene sequencing - Compositional analysis

• Analysis of individual phylotypes reveals significant differences:

  - Proportional abundance of *Ruminococcus bromii* (*Ruminococcaceae*) + *Eubacterium rectale* (*Lachnospiraceae*) increased on RS diet
  - *Collinsella aerofaciens* proportion reduced on WL diet
  - Used qPCR to monitor selected bacterial groups across all donors and all samples
qPCR results

Mean results across all 14 volunteers and each dietary regime

R-ruminococci

- R-ruminococci increased on RS diet

E. rectale/Roseburia

- E. rectale/Roseburia spp. increased on RS and decreased on WL diet
qPCR results

• Rapid responses to RS diet in most individuals
• ‘Bloom’ of ruminococci (related to *R. bromii*) on resistant starch diet
• Marked inter-individual variation in responses
qPCR results

R-ruminococci spp.

% of universal 16S rRNA gene copies

M | NSP | RS | WL

Volunteer

11 | 12 | 17 | 18 | 19 | 23 | 24

Bifidobacterium spp.

% of universal 16S rRNA gene copies

M | NSP | RS | WL

Oscillibacter spp.

% of universal 16S rRNA gene copies

M | NSP | RS | WL

E. rectale/Roseburia spp.

% of universal 16S rRNA gene copies

M | NSP | RS | WL

[Petra Louis - Rowett Institute of Nutrition and Health]
R-ruminococci

Distribution of glycoside hydrolase families in the genomes of five polysaccharide-utilizing bacteria

[Flint HJ et al Nat Rev Microbiol (2008)]
Phylotypes related to *Ruminococcus bromii* are abundant in the large bowel of humans and increase in response to a diet high in resistant starch

Guy C.J. Abell, Caroline M. Cooke, Corinna N. Bennett, Michael A. Conlon & Alexandra L. McOrist
Partitioning of bacterial 16S rRNA sequences between liquid and particulate fractions of human fecal samples

- R-ruminococci are preferentially associated with fiber particles in stool samples.
- Bacteroidetes partition more into the liquid phase

[Walker AW et al Env Microbiol (2008)]
FISH analysis of liquid and particulate fractions

Liquid fraction

Bacteroides/Prevotella
R-ruminococci
Roseburia/E. rectale

Particulate fraction
Multi-probe FISH analysis of particulate fraction

Donor A
Red = R-ruminococci

Donor B
Green = Lachnospiraceae

Donor C
Blue = DAPI

[Walker AW et al Env Microbiol (2008)]
RS diet responses in 14 volunteers

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>R-ruminococci</th>
<th>E. rec/Roseburia</th>
<th>Bifidobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>++</td>
<td>-</td>
<td>-</td>
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<tr>
<td>17</td>
<td>+</td>
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<td>18</td>
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<td>-</td>
</tr>
<tr>
<td>26</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

acetate, ethanol fibre degrader? | butyrate | lactate, acetate immune modulation?

- Likely to have different consequences for host health

<40% resistant starch fermented

<2-fold
>2-fold
>4-fold
>8-fold

[Walker AW et al ISME Journal (2011)]
Summary

- Specific bacterial groups/species respond strongly to dietary change, but there is inter-individual variation in the groups that respond.
- Ruminococci may be important for resistant starch degradation.
- Other dietary substrates will likely drive different microbiota responses:

**Resistant Starches Types 2 and 4 Have Differential Effects on the Composition of the Fecal Microbiota in Human Subjects**

Inés Martínez, Jaehyoung Kim, Patrick R. Duffy, Vicki L. Schlegel, Jens Walter*

Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska, United States of America

- Implications for human health?
  - Does this affect energy harvest from the diet?
  - Does this impact delivery of SCFA (e.g. butyrate) to the distal colon?

- Implications for therapeutic dietary intervention:
  - Even if rational prebiotics/functional foods are designed the microbiota response may depend on the individual.
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