Synbiotics and the human intestinal microbiota

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The gut microbiota

What are synbiotics, prebiotics, probiotics

Can we use dietary supplementation to modify the composition of the gut microbiota and improve health?

Comparison of the effects of different supplements:

How consistent are the responses of different individuals

Are Synbiotics effective??
Introduction: Human Gut Microbiota

- $10^{10} - 10^{12}$/ml gut contents (large intestine)
- Outnumber human cells in the body by 10 : 1

- Complex and diverse bacterial community
- >500 hundred different bacterial species
- Colonise each individual

- Most are highly oxygen sensitive, but can be cultured

- Molecular approaches (mainly based on 16S ribosomal genes) allow analysis of bacterial communities without cultivation

- Until recently >70 % of ‘phytotypes’ belonged to no known cultured species
Function of the gut microbiota

- Metabolism of dietary components
- Modification of host secretions (mucin, bile, gut receptors..)
- Immune function, inflammation
- Pathogenesis - dysbiosis
- Barrier function
Function of the large intestinal microbiota

**metabolism of dietary components**

**Potential health benefits** –
- Prevention of colorectal cancer
- Barrier against pathogen infection;
- Stimulate beneficial gut bacteria = prebiotic effects
- Metabolic health (insulin response)
- Vascular health (metabolism of lipids, cholesterol)

Dietary polysaccharides + FOS, RS, NSP

Absorption

Right Side (Proximal)

Left Side (Distal)

Short Chain Fatty Acids + $\mathrm{H}_2 + \mathrm{CO}_2 + \mathrm{CH}_4 +$ phytochemicals

Fermentation
~99 % of the colonic bacteria belong to 4 phyla
• Bacteroidetes
• Firmicutes
• Actinobacteria
• Proteobacteria

[Duncan and Flint, 2009]
Carbohydrate utilization in the gut – functional bacterial groups

Diet

- Insoluble complex carbohydrates
  - Primary degraders
    - H-utilisers (acetogens, methanogens, SRB)
    - lactate utilisers

- Soluble polysaccharides
  - Polysaccharide utilisers

- Oligosaccharides, sugars
  - Oligosaccharide/sugar utilisers

Metabolic products

(Flint et al. Env Micro 2007)
Definitions

• **Probiotics**
  live microorganisms which, when administered in adequate amounts confer a **health benefit** on the host (FAO/WHO 2001)

• **Prebiotics**
  A selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring **benefit(s) upon host health**. (Gibson et al. 2010)

**Synbiotics**
Combination of a probiotic and a (selected) prebiotic to facilitate growth of the probiotic – applied to **improve host health**
The gut microbiota

What are synbiotics, prebiotics, probiotics

Can we use dietary supplementation to modify the composition of the gut microbiota and improve health?

Comparison of the effects of different supplements:
- starch and non-starch polysaccharides (NSP)
- Inulin/FOS
- probiotics

How consistent are the responses of different individuals

Are Synbiotics effective??
Effect of reduced carbohydrate intake on gut microbiota

Human dietary study – effects of Atkin’s type diets in obese subjects

[Rowett Human Nutrition Unit]

<table>
<thead>
<tr>
<th>Maintenance (M)</th>
<th>Carbohydrate g</th>
<th>NSP g</th>
<th>Starch g</th>
<th>Protein g</th>
<th>Fat g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance (M)</td>
<td>400</td>
<td>28</td>
<td>187</td>
<td>94</td>
<td>123</td>
</tr>
<tr>
<td>Moderate carbohydrate (HPMC)</td>
<td>170</td>
<td>12</td>
<td>95</td>
<td>127</td>
<td>74</td>
</tr>
<tr>
<td>Low carbohydrate (HPLC)</td>
<td>23</td>
<td>6</td>
<td>3</td>
<td>120</td>
<td>126</td>
</tr>
</tbody>
</table>

M (3 days) → HPMC (4 weeks) → HPLC (4 weeks)
M (3 days) → HPLC (4 weeks) → HPMC (4 weeks)

[Duncan et al (2007) AEM 73; 1073-1078]
Effect of dietary carbohydrate on faecal short-chain fatty acid production

Production of SCFA as dietary carbohydrate

[Duncan *et al* (2007) AEM 73; 1073-1078]
Response of specific bacterial groups to changes in carbohydrate intake

Detection by fluorescent in situ hybridisation (FISH) using group-specific probes

% total eubacterial (Eub338) count in faeces

Bacterial group

Diet:
- Maintenance
- Moderate carbohydrate
- Low carbohydrate

A, B, C: $P < 0.05$

[Duncan et al (2007) AEM 73; 1073-1078]
Response of specific bacterial groups to changes in carbohydrate intake

Detection by fluorescent in situ hybridisation (FISH) using group-specific probes

% bacterial (Eub338) count in faeces

Diet:
- Maintenance
- Moderate carbohydrate
- Low carbohydrate

A, B, C: $P < 0.05$

Numbers of *E. rectale/Roseburia* group as dietary carbohydrate

[Duncan *et al* (2007) *AEM* 73; 1073-1078]
Direct positive correlation between faecal butyrate concentration and numbers of *E. rectale/Roseburia* group

Diet M – maintenance; medium carbohydrate (HPMC); low carbohydrate (HPLC)

Butyrate helps protect against colon cancer and levels increase as carbohydrate content of diet increases

[Duncan *et al* (2007) *AEM* 73; 1073-1078]
Does the type of carbohydrate matter?

Comparison of the effect of resistant starch (RS) and non-starch polysaccharide (NSP) on gut microbiota

Study design:
14 overweight volunteers, fed fixed diet; cross-over design

Faecal samples

<table>
<thead>
<tr>
<th>1 week</th>
<th>3 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
</table>

- **M**
  - NSP
  - RS

- **M**
  - RS
  - NSP

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total CHO</th>
<th>Non-starch polysaccharide (g/day)</th>
<th>Resistant starch (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSP</td>
<td>427</td>
<td>41.7</td>
<td>2.5</td>
</tr>
<tr>
<td>RS</td>
<td>434</td>
<td>16</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Faecal samples analysed for:
- total microbial composition (DGGE, clone library sequencing)
- SCFA production
- Enumeration of specific bacterial groups (Q-PCR)

Diet does induce a shift in the microbiota – within a few days

Profiles individual-specific

But

Changes in certain bacterial groups occur rapidly following diet shift; some increase, some decrease

### Bacterial populations estimated by qPCR expressed as % relative of total bacteria

<table>
<thead>
<tr>
<th>Bacterial group (%)</th>
<th>Diet</th>
<th>P diet (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>NSP</td>
</tr>
<tr>
<td>Bacteroides/Prevotella</td>
<td>27.8</td>
<td>25.7</td>
</tr>
<tr>
<td>E. rectale/Roseburia</td>
<td>7.3</td>
<td>6.5</td>
</tr>
<tr>
<td>F. prausnitzii</td>
<td>11.2</td>
<td>14.4</td>
</tr>
<tr>
<td>Ruminococci</td>
<td>6.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Oscillibacter</td>
<td>0.74</td>
<td>0.77</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Methanogens</td>
<td>0.080</td>
<td>0.034</td>
</tr>
</tbody>
</table>

One mean value is shown per diet for the 14 volunteers.

No significant bacterial changes in response to the NSP diet

The E. rectale/Roseburia and R. bromii groups both increase significantly in response to increased RS starch intake
Individual-specific response to RS supplementation
Numbers of *R. bromii* increase on RS diet in 10/14 volunteers

Influence of diet upon dominant human colonic bacteria (16S rRNA gene sequencing)

Numbers of *E. rectale* and *R. bromii* increase transiently in response to starch supplementation (RS III)

Effect of prebiotics (inulin) on the faecal microbiota

Q-PCR quantification of dominant groups of the human gut microbiota

Stimulation of bifidobacteria **and** *Faecalibacterium prausnitzii*

Fuller et al, BJN (2007); Ramirez et al, BJN (2009)
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Are Synbiotics effective??
Children admitted to hospital, treated antibiotics, on discharge receive READY-TO-USE THERAPEUTIC FOOD = RUTF (WHO approved standard diet for severely malnourished children) -composition: peanut butter; milk powder; oil; sugar; micronutrients

-In this study mixed with SYNBIOTIC 2000 Forte (Medipharm, Sweden) Mixture of 4 PROBIOTICS (>10^{10} cfu bacteria / day) (Lactobacillus casei sp paracasei; Lactobacillus plantarum; Leuconostoc sp.; Pedicoccus sp.) 4 PREBIOTICS: (betaglucans; inulin; pectin; resistant starch)
Upon discharge, ~400 per group
(396 RUTF (control), 399 RUTF plus synbiotic)

Supplementation with RUTF has positive health outcomes
(alleviates malnutrition and reduces infant mortality)

BUT - No significant differences between groups
(no added effect of synbiotic)

WHY?

Poor combination of prebiotic/probiotic strains?
Poor survival of probiotic strains?

Appropriate choice of prebiotic enhances probiotic survival

Clinical study – Crohns disease patients, a randomized double-blind study;
Synbiotic consumption (*B. longum* plus Synergy1) improved clinical symptoms

H. Steed, Macfarlane et al 2010 Aliment Pharmacol Ther 32: 872-883
Potential probiotic/prebiotic combinations = novel synbiotics

Essential to have evidence based combinations

RS III plus *Ruminococcus bromii*

RS III plus *E. rectale*

Inulin plus *R. inulinivorans or F. prausnitzii*

FOS plus most (not all) *Bifidobacterium spp.* - but non-specific

But – bacterial cross-feeding – can confuse results

Test in pure culture, then in mixed ecosystem
Why do we need new probiotics/synbiotics?

25 cultured species accounted for approximately 50% of 16S rRNA sequences (6 obese males)

NONE of them are Bifidobacteria or Lactobacilli

Current probiotics are the less abundant gut microbial species

Walker AW et al
ISME J (2011)
Conclusions

❖ SCFA production (including butyrate) and numbers of the *E.rectale/Roseburia* group decline as carbohydrate intake is reduced

❖ Numbers of *R. bromii* and the *E.rectale/Roseburia* group increase significantly when resistant (RS III) is included in the diet

❖ Supplementation with wheat bran NSP did not significantly affect numbers of any bacterial group

❖ combinations of probiotics and prebiotics must be carefully considered in the creation of new synbiotics

❖ Dietary modulation can change the composition of the gut microbiota to benefit health
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