Colonic transit time relates to bacterial metabolism and mucosal turnover in the human gut

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Introduction

• Bristol stool scale correlates with gut microbial richness (Vandeputte et al., 2015; Gut)

• High bacterial richness and diversity is generally thought to characterize a healthy gut microbial ecosystem (Le Chaterlier et al., 2013; Lozupone et al., 2012)

• An increased system retention time has in vitro been associated with more extensive protein fermentation (Macfarlane et al., 1989; Tottey et al., 2016)
Introduction

• Two human intervention studies within the Gut, Grain and Greens (3G) Center

• Baseline samples from 98 subjects
  – 61 female and 37 male
  – Aged 22-66 years

(Ibrügger et al., 2014; J. Clin. Trials)
Sample collection

Day 1-6

- Abdominal X-rays
- Ingestion of radio-opaque transit markers
- Fecal sample collected
- Urine sample collected

Day 7

- 4-days habitual dietary intake recorded
- Abdominal X-rays obtained

Colonic transit time

A dose of 24 radio-opaque markers is given to the subject on each of six consecutive days with breakfast. On day 7 abdominal X-rays are obtained. Number of markers are counted.

Fecal microbiota composition

DNA extraction followed by 16S rRNA gene sequencing

Urine metabolite profiling

Ultra performance liquid chromatography mass spectrometry

Roager et al., 2016; Nature Microbiology
How does colonic transit time relate to gut microbial diversity and composition?

- Fecal sample

**Fecal microbiota composition**
DNA extraction followed by 16S rRNA gene sequencing

Roager *et al.*, 2016; Nature Microbiology
Colonic transit time correlates to gut microbial alpha-diversity

Correlations were calculated based on Spearman’s Rank correlation (n=85). Significant associations are represented by asterisks (* p<0.05, *** p< 0.000001).

Roager et al., 2016; Nature Microbiology
A long colonic transit time reduces gut microbial beta-diversity

Beta-diversity assessed by weighted UniFrac distances
(Adonis, P<0.001, $R^2=0.07$)

Roager et al., 2016; Nature Microbiology
Bacterial groups significantly associated with colonic transit time

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>Erysipelotrichi</td>
<td>Erysipelotrichales SHA-98</td>
<td>Erysipelotrichaeae</td>
<td>cc115 (q=0.05)</td>
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<tr>
<td></td>
<td>Clostridia</td>
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<tr>
<td></td>
<td></td>
<td>Clostridiales</td>
<td>Christensenellaceae (q=1.0x10^-5)</td>
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<td></td>
<td></td>
<td></td>
<td>Dehalobacteriaceae (q=4.0x10^-4)</td>
<td>Dehalobacterium (q=8.0x10^-4)</td>
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<td></td>
<td></td>
<td>Eubacteriaceae (q=0.04)</td>
<td>Anaerostis (q=0.02)</td>
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<td>Ruminococcaeae (q=0.01)</td>
<td>Oscillospira (q=1.3x10^-5)</td>
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<td></td>
<td>Tissierellaceae (q=0.007)</td>
<td>Ruminococcus (q=3.9x10^-3)</td>
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<td>Mogibacteriaceae (q=0.01)</td>
<td>Faecalibacterium (q=2.0x10^-4)</td>
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<td>Lachnospiraceae (q=2.1x10^-3)</td>
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<td>Clostridiaceae (q=0.03)</td>
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<tr>
<td>Bacteroidetes</td>
<td>Bacteriodia</td>
<td>Bacteroidales</td>
<td>Porphyromonadaceae</td>
<td>Porphyromonas (q=6.4x10^-4)</td>
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<td>Odoribacteriaceae</td>
<td>Butyricimonas (q=0.005)</td>
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<td>S24-7 (q=0.04)</td>
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<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Pasteurellales</td>
<td>Pasturellaceae (q=0.04)</td>
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<td>Pseudomonadales</td>
<td>Pseudomonadaceae (q=0.04)</td>
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<td>Desulfovibrio (q=0.005)</td>
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<td>Deltaproteobacteria</td>
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<tr>
<td>Tenericutes</td>
<td>(q=0.02)</td>
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<tr>
<td>Euryarchaeota</td>
<td>Methanobacteria</td>
<td>Methanobacteriales</td>
<td>Methanobacteriaceae (q=2.9x10^-4)</td>
<td>Methanobrevibacter (q=3.7x10^-4)</td>
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<tr>
<td>(q=0.0001)</td>
<td>(q=0.0002)</td>
<td>(q=0.0002)</td>
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</tbody>
</table>

Roager et al., 2016; Nature Microbiology
How does colonic transit time relate to colonic metabolism?

- Urine sample

**Urine metabolite profiling**
Ultra performance liquid chromatography mass spectrometry

Roager et al., 2016; Nature Microbiology
Global metabolic profiling of urine using UPLC-QTOF-MS

- **Column**
  - C18 column

- **Settings**
  - m/z-range: 50-1000
  - Positive and negative ionization

- **Run sequence**

  - QC QC QC QC QC QC QC QC QC
  - Blank 10 samples 10 samples 10 samples

- **Data processing included**
  - The data were normalized to total ion intensity
  - Features should be detected in at least 80% of samples
  - Features with a CV% above 30% in QC-samples were excluded

- **Metabolite identification levels according to Sumner et al., 2007**
  - Human Metabolome Database
  - Tandem mass spectrometry
  - Authentic standards

Based on Want et al., 2010; Nature Protocols
Colonic transit time associates with the urinary metabolic profile

- 1553 molecular features measured by UPLC-MS
  - 82 were associated with a long colonic transit time
  - 121 were associated with a short colonic transit time

Spearman’s Rank correlation + correction for multiple testing using Benjamini-Hochberg approach ($q<0.05$)

Roager et al., 2016; Nature Microbiology
Transit time relates to bacterial metabolism and mucosal turnover

Roager et al., 2016; Nature Microbiology
Transit time relates to the gut microbial composition and diversity

Diet

Increased colonic transit time

Slower passage of food

Shift in colonic metabolism

Changed gut microbiota composition

- alpha-diversity
- OTU richness

- Ruminococcaceae
- Christensenellaceae
- Methanobrevibacter
- Lachnospiraceae
- Faecalibacterium prausnitizii

Roager et al., 2016; Nature Microbiology
Conclusions

- Colonic transit time is a confounder in gut microbiota and metabolomics studies.

- High microbial richness at the OTU level may not per se be indicative of a healthy ecosystem.

- Our findings may contribute to understand diseases where constipation is a risk factor, e.g. colorectal cancer.

Roager et al., 2016; Nature Microbiology
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