Diet, intestinal microbial communities and host health

Alan Walker
Rowett Institute of Nutrition and Health, University of Aberdeen

8/9/15
The human intestinal microbiota

- Human large intestine hosts an enormous number of microbes ("microbiota")
  - 100,000,000,000,000 (10^{14}) bacterial cells
  - Greater than the number of human cells

- Thousands of different species colonise
  - Most are strict anaerobes
  - Each host have a unique and largely stable microbiota

- The cumulative “microbiome” of these cells contains 400x more unique genes than the human genome
  - est. 8 million vs ~20-25,000

- Plays a number of key roles in maintaining host health
  - Enhances resistance against infection
  - Immune system development/maintenance
  - Beneficial compound production
  - Breakdown dietary fibre
Principal substrates available for utilisation by intestinal microbes

Of dietary & intestinal origin:

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

Adapted 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)
Metabolise dietary components that escape digestion by human enzymes
- Endows host with degradative capabilities they have not needed to evolve themselves

Vitamin production
- K, riboflavin (B₂), biotin (B₇), folic acid (B₉), cobalamin (B₁₂)

Release of phytochemicals
- Phenolic compounds etc

Primary end products are short chain fatty acids
- Acetate (C₂), propionate (C₃) and butyrate (C₄)

SCFAs are symbiotic compounds
- Gut epithelial cells grow on products of bacterial metabolism
- Derive up to 70% of energy needs from bacterially-produced butyrate
- Increases energy yield from diet (5 to 10% of caloric intake per day)
Impact of gut bacterial short chain fatty acids on the host

- Inhibition of histone deacetylase (*butyrate, propionate*)
- Altered mucosal gene expression, cell differentiation
- Protection against colorectal cancer, colitis (*butyrate*)
- Energy source for the colonic epithelium (*butyrate*)
- Anti-inflammatory effects (including stimulation of Tregs)
- Suppression of colitogenic pathogens (*acetate*)
- Stimulation of host receptors (FFAR2, FFAR3, GPR109)
- Influence on gut hormones (e.g. GLP-1, PYY) and satiety
- Influences upon gut transit, gut barrier function
- Peripheral energy supply, lipogenesis (*acetate*)
- Promote intestinal gluconeogenesis (*butyrate, propionate*)

Lactate accumulation shown to be due mainly to reduced lactate utilization by other bacteria at pH 5.2 ($^{13}$C lactate)

Many diseases are caused by microbes that normally live asymptptomatically on the host

- *Staphylococcus aureus* (MRSA), Strep throat, gingivitis, acne, meningitis, pneumonia, *C. difficile* diarrrhoea, thrush, UTIs, gastric cancer (*Helicobacter pylori*).

A general imbalance (“dysbiosis”) in microbiota composition has been implicated in many disorders

- Inflammatory bowel diseases, bowel cancer, irritable bowel syndrome, diabetes, liver disease, allergies, atherosclerosis
Impact of microbially derived metabolites on the host

**Damaging**
- Heterocyclic Amines
- N-Nitrosamines
- Polyamines

**Protective**
- Indoles
- Anti-Inflammatory Molecules
- Phytoestrogens
- Short Chain Fatty Acids
- Anti-oxidant Molecules
The “Western” diet, microbiota and host health

High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health

Wendy R Russell, Silvia W Gratz, Syliva H Duncan, Grietje Holtrop, Jennifer Ince, Lorraine Scobie, Garry Duncan, Alexandra M Johnstone, Gerald E Lobley, R John Wallace, Garry G Duthie, and Harry J Flint

Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in II10−/− mice

Suzanne Devkota1, Yunwei Wang2, Mark W. Mushch3, Vanessa Leone4, Hannah Fehlner-Peacht5, Anuradha Nadimpalli6, Dionysios A. Antonopoulos7, Bizu Jiber8 & Eugene B. Chang9

Dietary cholesterol directly induces acute inflammasome-dependent intestinal inflammation

Fraenze Progatzky1, Navjot J. Sangha1, Nagisa Yoshida1, Marie McBrien1, Jackie Cheung1, Alice Shia1,2, James Scott2, Julian R. Marchesi3,4,5,6, Jonathan R. Lamb1, Laurence Bugeon1,* & Margaret J. Dallman1,*

Artificial sweeteners induce glucose intolerance by altering the gut microbiota

Joshua Sager1, Tal Korem2, David Zeevi2, Gill Zilberman Schapira2, Christoph A. Thaiss2, Ori Mazar2, David Brand3, Niv Zmora4,5, Shlomit Gibril4, Adina Weinberger4, Yad Kuneroman5, Alon Harmelin5, Ilana Kobolkin Gal2, Haitt Shamir2, Zanir Hakim2,3, Eran Segal2 & Eran Elinav4

Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome

Benoit Chassaing1, Ornry Koren1, Julia K. Goodrich1, Angela C. Poole2, Shanthi Srinivasan4, Ruth E. Ley3 & Andrew T. Gewirtz2

Tang W.H. et al. (2013) NEJM 368; 1575-1584

Altering the intestinal microbiota

- The aim of all of these approaches is to shift the composition of the microbiota to a more beneficial state
  - Is targeted manipulation possible via alterations in host diet?
Short- v long-term impacts of diet on the intestinal microbiota

LETTER

Diet rapidly and reproducibly alters the human gut microbiome

Lawrence A. David1,14, Corinne F. Maurice1, Rachel N. Carmody1, David B. Gootenberg1, Julie E. Button1, Benjamin E. Wolfe1, Alisha V. Ling3, A. Sloan Devlin4, Yug Varna5, Michael A. Fischbach6, Sudha B. Biddinger7, Rachel J. Dutton8 & Peter J. Turnbaugh9

Figure 1: Short-term diet alters the gut microbiota.

- Short term dietary regimes can result in reproducible but limited microbiota response

ARTICLE

Gut microbiota composition correlates with diet and health in the elderly

Marcus J. Glasson1,14, Ian B. Jeffery1,14, Susana Conde1, Susan F. Power1, Elizabeth M. O'Connor2,3, Siobhan Casack1, Hugh M. E. Harris1, Maurod Coakley1, Bhavaneshwar Lakshmanan1, Greg O’Sullivan1, Gerald P. Fitzgerald1, Jennifer Deane1, Michael O’Connor2,3, Norman Herriott1,2, Megan O’Connor2,3, Denk O’Mahony1,3, Douve van Sinderen1,5, Martina Wallace1, Lorraine Brennan1, Catherine Stanton1,4, Julian R. Marchesi1, Anthony P. Fitzgerald1,5, Fenggu Shanahan1,5, Colin Hill1,4, R. Paul Rees1,3 & Paul W. O’Toole1

Figure 2: Dietary patterns in community location correlate with separations based on microbiota composition.

- Changes in long-term dietary patterns illicit extensive changes in microbiota composition
Disappearing human microbiota?

- Have host behavioural changes in the urbanisation era introduced changes in microbiota composition?

ESSAY

What are the consequences of the disappearing human microbiota?


Martin J. Blaser and Stanley Falkow

The ‘disappearing microbiota’ hypothesis [...] suggests [...] developments over the past century [...] contributed to a shift in the [...] species and types of microorganism in the gut.
Link between diet, oral microbiota and health

- Calcified plaque – one of the only preserved records of bacteria
- Densely colonised by oral microbiota

- Increased caries rate in skeletal records is linked to increased consumption of carbohydrates
- Unknown whether or not these changes were accompanied by shifts in microbiota composition


Dobney et al., 1994

Image: Dr Jo Buckburry (re-plotted from Moore and Corbett 1978)
http://www.leeds.ac.uk/yawya/bioarchaeology/Dental%20disease.html
Oral microbiota changes through history

- Non-pathogenic *Ruminococcaceae* associated with hunter-gatherers
- Decay-associated *Veillonellaceae* increase post-farming

Changes in predominant oral pathogens as a result of diet and culture

- These pathogens do not appear to be prominent in hunter-gatherers
- *Streptococcus mutans* became dominant relatively recently
- Shifts correlate with human behavioural changes (e.g. diet)
Regional variations in gut microbiota composition

- We may need to reconsider what we think of as a normal “healthy” intestinal microbiota

[Cooper, P. et al., (2013) PLOS ONE 8,e76573]

![Pie chart and bar graphs showing regional variations in gut microbiota composition.](image)

Many novel bacteria

Children in:
- Kenya
- Mali
- The Gambia
- Bangladesh

Pop, M. et al., Genome Biology (2014), 15:R76

De Filippo et al. PNAS (2010)
Links between host diet and microbiota structure

- Microbiota structures are associated with long term dietary consumption patterns
O’Keefe et al performed 2-week food exchanges

- African Americans were fed a high-fibre, low-fat African-style diet and rural Africans a high-fat, low-fibre western-style diet
- Resulted in measurable changes in health biomarkers
  - \( \uparrow \) butyrogenesis, \( \downarrow \) secondary bile acid synthesis in the African Americans
Impact of low carbohydrate weight loss diets

** P < 0.001

- Low CHO diet leads to reduction in butyrate-producing bacteria
- Reduced faecal butyrate levels

** P < 0.001
Major fibre derived phenolics in faecal samples

- Low carbohydrate, high protein intake resulted in reduced concentrations of potentially cancer-protective plant phenolic derivatives

- Ferulic acid derivatives
  - Ferulic acid
  - 3OMe4OHPPA
  - 34OHPPA
  - 3OHPPA

- Concentration (μg cm⁻³)

- P < 0.05
- P < 0.001

- 8 volunteers
  - Maintenance Diet
  - Low Carb. Diet 20+/−1 days
  - Low Carb. Diet 27+/−1 days

- Hydrogenation
- Demethylation
- Dehydroxylation

- Russell WR et al., AJCN (2011) 93, 1062-72
Resistant starch vs non-starch polysaccharide diet

14 obese male volunteers with metabolic syndrome (mean age 54 years, mean BMI 39.4 kg/m²)

<table>
<thead>
<tr>
<th>Collection of faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
</tr>
<tr>
<td>1 wk</td>
</tr>
</tbody>
</table>

Mean dietary intake [g/d]:

<table>
<thead>
<tr>
<th>Diet</th>
<th>CHO</th>
<th>Starch</th>
<th>RS</th>
<th>NSP</th>
<th>Protein</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>427</td>
<td>230</td>
<td>5</td>
<td>28</td>
<td>103</td>
<td>126</td>
</tr>
<tr>
<td>NSP</td>
<td>427</td>
<td>138</td>
<td>2</td>
<td>42</td>
<td>102</td>
<td>136</td>
</tr>
<tr>
<td>RS</td>
<td>434</td>
<td>275</td>
<td>26</td>
<td>13</td>
<td>109</td>
<td>127</td>
</tr>
<tr>
<td>HPMC</td>
<td>201</td>
<td>110</td>
<td>3</td>
<td>22</td>
<td>144</td>
<td>63</td>
</tr>
</tbody>
</table>

CHO: carbohydrate

M: Weight maintenance, mixed diet (55% energy from carbohydrates)

NSP: High non-starch polysaccharides (added bran), low RS

RS: High resistant starch (Type III), reduced NSP

HPMC: Reduced calorie intake. Increased % protein, moderate carbohydrate
Resistant starch vs non-starch polysaccharide diet

- Samples cluster by donor, not by diet
- Sub-group of bacteria are highly responsive to both the NSP and RS-enriched diets
  - More *Ruminococcaceae* species increase with RS
  - More *Lachnospiraceae* species increase with NSP

Keystone species within the microbiota

**Ruminococcus spp. - qPCR**

% of universal 16S rRNA gene copies

<table>
<thead>
<tr>
<th>Time [days]</th>
<th>M</th>
<th>NSP</th>
<th>RS</th>
<th>WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td></td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td></td>
<td></td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>40-50</td>
<td></td>
<td></td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>50-60</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>60-70</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Volunteer M, NSP, RS, WL with different colors representing each volunteer.

**Residual faecal starch in volunteers**

% residual faecal starch

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>M</th>
<th>NSP</th>
<th>RS</th>
<th>WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>10</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td></td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td></td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>80</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td></td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>50</td>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>70</td>
<td></td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>90</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Volunteer 25 in vitro faecal starch utilisation**

% of residual starch

<table>
<thead>
<tr>
<th>Time [h]</th>
<th>M</th>
<th>NSP</th>
<th>RS</th>
<th>WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td></td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td></td>
<td></td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>40-50</td>
<td></td>
<td></td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>50-60</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>60-70</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

V25 faecal sample with + B. adolescentis, + E. rectale, + B. thetaiotaomicron, + R. bromii to indicate keystone species for starch utilisation.

Walker et al. ISME J (2011); Ze et al. ISME J (2012)
Links between diet, microbiota and health

Wheat, oats
Resistant starch
Pectin
Protein

NSP-degraders
- Butyrivibrio fibrisolvens (Bu)
- Bacteroides ovatus (Pr, Ac, Su)
- Ruminococcus spp. (Ac)
- Bacteroides xylanisolvens (Pr, Ac, Su)
- Bacteroides cellulosilyticus (Pr, Ac, Su)

Starch degraders
- Ruminococcus bromii (Cu)
- Eubacterium rectale (Cu)
- Bifidobacterium adolescentis (Cu, Ac)
- Bacteroides thetaiotaomicron (Pr, Ac, Su)

Pectin degraders
- Bacteroides thetaiotaomicron (Cu, Ac, Su)
- Faecalibacterium prausnitzii (Pr, Ac, Su)

Peptide/amino acid utilisers
- Bacteroides spp. (Ac, Su, Pr)
- Clostridium bififormans (Fo, Ac)

Cross-feeding of breakdown products and metabolites. Main products detected in the colon are (Pr, Ac, Bu)

LUMEN

EPITHELIAL CELLS

LAMINA PROPRIA

Receptors GPR 41, 43 and 109A
Effector T-cells

IL-10

Inflammatory response is blocked

NFκB

PPARγ

Walker AW & Duncan SH. MNFR (In Prep.)
Conclusions

• Long-term dietary patterns have significant impacts on both intestinal microbiota composition and activity
  — Fibre consumption appears to be a major driver

• Microbiota composition and activity are strongly correlated with markers of host health/disease
  — Mechanistic studies now starting to emerge that link diet/microbiota/health

• Specific bacterial groups/species respond strongly to dietary change, but there is inter-individual variation in the groups that respond

• Many gut bacteria appear to be nutritionally specialised; these species are likely to show the greatest responses to dietary manipulation
  — ‘Keystone’ species may determine the ability to ferment insoluble substrates

• Implications for therapeutic dietary intervention:
  — The response may depend on the underlying microbiota composition of a given individual
  — Need continued and improved characterisation of the microbiota in order to predict responses to dietary manipulation

Contact: alan.walker@abdn.ac.uk
Protection against colorectal cancer and colitis

Interplay between diet and microbiota

Fermentable carbohydrates:

Nutrient supply to mucosa
Barrier against infection
Release of phytochemicals

Exposure to metabolites and bacteria that promote disease
phenols, amines, indoles, N-nitroso compounds, H$_2$S, amines, bile acids, faecapentaenes, heme

Supply of short chain fatty acids
Phytochemicals in colon
Colonic pH

Interplay between diet and microbiota on gut health
Acknowledgements

Rowett Institute of Nutrition and Health/University of Aberdeen

Microbiology Group
Harry Flint
Sylvia Duncan
Gillian Donachie
Petra Louis
Jennifer Ince
Lucy Webster
Xiaolei Ze
Aurore Bergerat
Freda McIntosh
Karen Scott
Jenny Martin

David Brown (Analytical Chem)
Wendy Russell (Mol Nutr)
Alex Johnstone (OMH)
Gerald Lobley (OMH)
Sylvia Stephen (HNU)
Grietje Holtrop (BioSS)

University of Aberdeen
Keith Dobney

Pathogen Genomics Group
Julian Parkhill
Paul Scott

Bacterial Pathogenesis Group
Trevor Lawley
Mark Stares

Core Sequencing Teams
Carol Churcher
David Harris
Richard Rance

All other members of the Sanger Institute’s sequencing teams

University of Modena and Reggio Emilia
Maddalena Rossi
Andrea Quartieri

University of Adelaide
Alan Cooper
Christina Adler
Laura Weyrich
John Kaidonis
Wolfgang Haak
Corey Bradshaw
Grant Townsend

University of Warsaw
Arkadiusz Sołtysiak

Johannes Gutenberg University of Mainz
Kurt Alt

St. George’s, University of London
Phil Cooper

U. Maryland
Colin Stine
Mihai Pop

Funding Sources:

wellcome trust
MRC Medical Research Council
The Scottish Government
Riaghaltas na h-Alba