Intestinal microbiota, diet and health.

Kieran Tuohy
Fondazione Edmund Mach, TN

The human gut microbiota

Up to 1000 species

70% unculturable

Closely co-evolved microbial partners

Interactions with:
- Diet
- Drugs
- Immune system
- Gut physiology
- Bile acids/liver
- Systemic metabolism
- Adipose tissue
- Brain development & function
Impact of Western style diet on colonic fermentation

**Proximal colon** ~ saccharolytic

- SCFA
  - Acetate
  - Propionate
  - Butyrate

Energy source

- Apoptosis
- Differentiation
- Epigenetics
- Gene expression
- Gut hormones
- Gut permeability

**Distal colon** ~ proteolytic

- Amines
- Indoles
- Ammonia
- Sulphides
- N-nitroso

- DNA damage
- Tumours
- Cytotoxicity
- Leaky gut
- Liver disease

Altered mucosal permeability and systemic inflammation?

Modified from Gero Macfarlane
Impact of traditional diets rich in fiber, polyphenols on colonic fermentation

Proximal colon
- ~ saccharolytic
- SCFA
  - Acetate
  - Propionate
  - Butyrate
- Energy source
- Apoptosis
- Differentiation
- Epigenetics
- Gene expression
- Gut hormones
- Gut permeability

Distal colon
- ~ proteolytic
- Amines
- Indoles
- Ammonia
- Sulphides
- N-nitroso
- DNA damage
- Tumours
- Cytotoxicity
- Leaky gut
- Liver disease

High fiber diets, Paleolitic, Mediterranean, rural African and Asian
Enhanced mucosal barrier function and immune homeostasis

Modified from Gero Macfarlane

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Koeth et al 2013 Nature Medicine
- TMA/TMAO confirmed strong link with CVD in patients
- Confirmed microbiota metabolism of L-carnitine/choline → TMA → TMAO
- TMA not produced in vegans
- Confirmed inflammatory activity & linked to macrophages reverse cholesterol transport
- TMAO reduced bile acid pool
Human diet shaped our closely co-evolved human:microbe ecosystem

**Human microbiome evolution**

- **Bacteroids, Eubacterium, Peptococcaceae**
- **Bifidobacterium**
- **Escherichia coli, Streptococcus**
- **Lactobacillus**
- **Clostridium perfringens**

**Dietary evolution**

- Neolithic times: ~10,000 yrs BP (birth of agriculture)
- Agricultural/Industrial revolutions: Late 18th and early 19th century
- Recent changes: Over the last 50 yrs (Western-style diet)
Estimated daily fiber intake in Palaeolithic / Traditional diets and Modern diet

<table>
<thead>
<tr>
<th>Dietary pattern</th>
<th>Fiber content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palaeolithic diet first reported in 1985 (Eaton SB)</td>
<td>45.7g</td>
</tr>
<tr>
<td>Palaeolithic diet modified in 1990 (Eaton SB)</td>
<td>&gt;100g</td>
</tr>
<tr>
<td>Palaeolithic diet reported in 1996/1997 (Eaton SB)</td>
<td>104g</td>
</tr>
<tr>
<td>Rural Chinese diet</td>
<td>77g</td>
</tr>
<tr>
<td>Rural African diet</td>
<td>120g</td>
</tr>
<tr>
<td>Current US diet</td>
<td>10-20g</td>
</tr>
<tr>
<td>Recommended fiber content in US</td>
<td>25-38g</td>
</tr>
<tr>
<td>Current UK diet</td>
<td>12g</td>
</tr>
<tr>
<td>Recommended fiber content in UK</td>
<td>18g</td>
</tr>
</tbody>
</table>


Total polyphenols (catechin equivalents, mg/100 g)

Gut microbiota differs between children following Western-style diet in Italy and children in rural Africa following traditional diet.

Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlootta De Filippo*, Duccio Cavaliere*, Monica Di Paola*, Matteo Ramazzotti*, Jean Baptiste Pouletet†, Sebastien Massart†, Silvia Caffini‡, Giuseppe Peracino‡ and Paolo Lionetti*§∥

*Department of Preventive and Clinical Pharmacology, University of Florence, 50134 Florence, Italy; †Department of Pediatrics, Meyer Children Hospital, University of Florence, 50134 Florence, Italy; ‡Department of Biochemical Sciences, University of Florence, 50134 Florence, Italy; §TNO Vision Agrifood B.V., 61490 Lage, Belgium; and ‖Centro Interdipartimentale di Sottoscrizione di Massa, University of Florence, 50022 Massa, Italy

De Filippo et al., PNAS (2010)
Aberrant gut microbiota associated with Western-style diet

SCFA about 3-4 fold higher in African children than Italian children
Abundance of Enterobacterial groups commonly associated with gastrointestinal disease higher in EU/Italian children

De Filippo et al., PNAS (2010)

Gut microbiota differs between children following Western-style diet in Italy and children in rural Africa following traditional diet.

Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

De Filippo et al., PNAS (2010)
The diet of
- BF rural children is low in fat and rich in fibers and plant-poly saccharides and predominantly vegetarian
- BF urban children maintain the consumption of cereals and legumes but introduces milk, meat, fish, egg and peanuts
- EU is a typical western diet high in animal protein, sugar, starch, and fat and low in fiber

Nutritional composition of foods is available from [http://www.inran.it](http://www.inran.it) for EU and [http://www.fao.org](http://www.fao.org) for BF

Quantification of SCFAs in fecal samples from BF and EU populations by SPME-GC-MS.

**ACETIC**

**PROPANOIC**

**BUTANOIC**

**PENTANOIC**
Gut microbiota….. but not as we know it!

OBESITY EPIDEMIC

- Currently 300 million people obese worldwide
- Obese adults are up to 80 times more likely to develop type 2 diabetes than non-obese adults
- Obese adults are 2-3 times more likely to develop heart disease
- Obese adults have a 40% increased risk of dying from cancer
The 3Ps: Probiotics, Prebiotics & Polyphenols

- **PROBIOTICS**...“live microorganisms which when administered in adequate amount confer a health benefit on the host” (FAO, 2001).
  - *Lactobacillus*
  - *Bifidobacterium*
  - *Escherichia coli Nissle 1917, Bacillus sporogenes, Entercoccus faecium, Clostridium butyricum, Saccharomyces cerevisae*

- **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)
  - Inulin, oligofructose, fructooligosaccharides, galactooligosaccharides, lactulose, arabinogalactan, arabinofuranosyl, pectic-oligosaccharides, glucooligosaccharides
  - Resistant starch and certain whole plant foods including whole grain wheat, whole grain oats

- **POLYPHENOLS**... 90% resistant to digestion and reach the colon, plant secondary metabolites, usually antioxidant, antimicrobial activities, enzyme/nutrient binding properties and possibly prebiotic type properties, e.g. red-wine polyphenols, apple tannins

---

Gut microbiota and systemic health

```
Cancer (CRC)  Obesity
  ↙          ↗
  Probiotics  Blood glucose
  ↓          ↘
  Diarrhoea/IBS  Satiety
  ↘          ↙
  Laxation  Mineral absorption
```

- Probiotics
- Immune function
- IBD
- Diarrhoea/IBS
- Laxation
- Cancer (CRC)
- Obesity
- Blood glucose
- Satiety
- Mineral absorption
- Lipid metabolism
**Lb. reuteri** selected for Bile Salt Hydrolase activity (2 capsules/day at 2 x 10⁹ CFU/capsule) for 9 weeks

- Randomized, double-blind, placebo-controlled, parallel-arm, multicenter study
- N=127 hypercholesterolemic patients
- Probiotic reduced plasma
  - TC by 9.14%
  - LDL-C by 11.64%
  - LDL-C/HDL-C ratio by 13.39%
  - Non-cholesterol plant sterols
  - Increased circulating deconjugated bile acids
- Proposed new cholesterol lowering activity of probiotics via modified absorption of lipids from the gut

### Table 1. Cellular actions described for TGR5 in different cell types.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Species</th>
<th>Cellular action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages*</td>
<td>Human / Rabbit / Rat</td>
<td>Inhibition of lipoprotein production</td>
<td>[60, 61]</td>
</tr>
<tr>
<td>Enterococcal cells</td>
<td>Human / Mouse</td>
<td>Secretion of CL-P1</td>
<td>[71, 72]</td>
</tr>
<tr>
<td>Brown adipocytes</td>
<td>Mouse</td>
<td>Increase in energy expenditure</td>
<td>[66]</td>
</tr>
<tr>
<td>Glutametabolizing cells</td>
<td>Rat</td>
<td>Increase in energy expenditure</td>
<td>[65]</td>
</tr>
<tr>
<td>Enterohepatic cells</td>
<td>Mouse</td>
<td>Increase of enterohepatic NO synthesis</td>
<td>[67]</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>Mouse</td>
<td>Promote of bile acid secretion</td>
<td>[70]</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Rat</td>
<td>Generation of ROS</td>
<td>[103]</td>
</tr>
<tr>
<td>Enteric neurons</td>
<td>Mouse</td>
<td>Release of NO and suppression of intestinal motility</td>
<td>[71]</td>
</tr>
<tr>
<td>Colonic smooth muscle cells</td>
<td>Mouse</td>
<td>Decrease of gutlapheric smooth muscle cell function</td>
<td>[71]</td>
</tr>
</tbody>
</table>

* Macrophages include alveolar macrophages, Kupffer cells and THP-1 cells.
Influencing the Gut:brain axis

99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Psycho-neuroimmunology and the intestinal microbiota: clinical observations and basic mechanisms

Clinical and Experimental Immunology

Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo1, 2, Paul Fonythe3, 4, Marianne V. Chew, 5, Emily Escaravage, 5, Hélène M. Savignac, 6, Timothy G. Dinan3, 7, John Bienenstock, 8, 11, and John F. Cryan1, 8, 9
Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo, Paul Fonythe, Marianne V. Chew, Emily Escaravage, Hélène M. Savignac, Timothy G. Dinan, John Bienenstock, and John F. Cryan

Oral intake of γ-aminobutyric acid affects mood and activities of central nervous system during stressed condition induced by mental tasks

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo, Paul Fonythe, Marianne V. Chew, Emily Escaravage, Hélène M. Savignac, Timothy G. Dinan, John Bienenstock, and John F. Cryan

γ-Aminobutyric acid production by culturable bacteria from the human intestine

E. Barrett, R.P. Ross, P.W. O'Toole, G.F. Fitzgerald, and C. Stanton
GABA..... An effective immunomodulatory molecule

GABA is an effective immunomodulatory molecule
Zhu Zin - Sarah Kumar Moshio - Ryoichi Hira

- GABA receptors also on human PBMC, monocytes and neutrophils

Oral Treatment with γ-Aminobutyric Acid Improves Glucose Tolerance and Insulin Sensitivity by Inhibiting Inflammation in High Fat Diet-Fed Mice
Jide Tian†, Hoa N. Dang, Jing Yong, Wing-Sheung Chui, Matthew P. G. Dizon, Catherine K. Y. Yaw, Daniel L. Kaufman
Department of Molecular and Medical Pharmacology, University of California Los Angeles, Los Angeles, California, United States of America

GABA treatment improves IN sensitivity
Lactobacillus plantarum/brevis FEM 1874: GABA producer and BSH positive

GABA PRODUCTION (mg/L)

Not all cheeses are equal.
Smelly cheeses – live cheeses!

Anti-pathogen activity of Trentino cheese lactic acid bacteria

<table>
<thead>
<tr>
<th>LAB Strain</th>
<th>Escherichia coli DSM 2183</th>
<th>Listeria monocytogenes 309</th>
<th>Listeria innocua DSM 306</th>
<th>Staphylococcus aureus ATCC 25923</th>
<th>Bacillus subtilis DSM 435</th>
<th>Candida albicans ATCC 10233</th>
<th>Salmoella enterica DSM 14221</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus paracasei Vi44 (6M)</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
</tr>
<tr>
<td>Lactobacillus paracasei 1580</td>
<td>+++</td>
<td>Ni</td>
<td>+++</td>
<td>+++</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
</tr>
<tr>
<td>Lactobacillus paracasei 7210 (1M)</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides Vi 47 (3M)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Acid (pH 2) and bile tolerance of Trentino cheese lactic acid bacteria

Survival of selected Trentino cheese lactic acid bacteria under simulated gastrointestinal conditions of pH and bile – a first screen for potential as probiotics

In vitro Digestion

Mouth
Food ground in buffer

Stomach
↓ pH to 2.5
Add pepsin gastric and gastric lipase → 37 °C/2h.

Liver & Pancreas
↑pH (6N NaOH) to 6.5
Add bile salts
Amylase, trypsin, chymotrypsin, colipase → 37 °C/1h

Large Intestine
Selection of putative probiotics from lactic acid bacteria isolated from Trentino cheeses

Aim: to select putative probiotics suitable for cheese applications from Trentino dairy Microbiome

TrentinoGut - Lorenza Conterno

University of Camerino, Stefania Silvi and Alice de Angelis

Strains kindly isolated and provided by Elena Franciosi

Putative Trentino probiotic survival under gastrointestinal conditions in presence and absence of cheese matrix

<table>
<thead>
<tr>
<th>Strain</th>
<th>Survival %</th>
<th>Culture Broth</th>
<th>Culture in Model Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>2689</td>
<td>60</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>512</td>
<td>70</td>
<td>65</td>
<td>70</td>
</tr>
<tr>
<td>2360</td>
<td>50</td>
<td>45</td>
<td>50</td>
</tr>
</tbody>
</table>

2689 = Lactobacillus paracasei
512 = Lactobacillus casei paracasei rhamnosus
2360 = Lactobacillus rhamnosus

TrentinoGut Lorenza Conterno and Alice de Angelis
The 3Ps: Probiotics, Prebiotics & Polyphenols

- **PROBIOTICS**...“live microorganisms which when administered in adequate amount confer a health benefit on the host” (FAO, 2001).
  - *Lactobacillus*
  - *Bifidobacterium*
  - *Escherichia coli Nissle 1917, Bacillus sporeogens, Entecococcus faecium, Clostridium butyricum, Saccharomyces cerevisiae*

- **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)
  - Inulin, oligofructose, fructooligosaccharides, galactoooligosaccharides, lactulose, arabinogalactan, arabinofuranosyl, pectic-oligosaccharides, glucooligosaccharides
  - Resistant starch and certain whole plant foods including whole grain wheat, whole grain oats

- **POLYPHENOLS**... 90% resistant to digestion and reach the colon, plant secondary metabolites, usually antioxidant, antimicrobial activities, enzyme/nutrient binding properties and possibly prebiotic type properties, e.g. red-wine polyphenols, apple tannins

---

**Table 2. Human dietary interventions using probiotic functional foods**

<table>
<thead>
<tr>
<th>Prebiotic</th>
<th>Microbiological methods</th>
<th>Design</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin</td>
<td>Cultures 25 g/day for 15 days</td>
<td>Placebo-controlled, cross-over study</td>
<td>Bilbithenium 1 and Glu-road probe</td>
<td>Gibson et al. 1995</td>
</tr>
<tr>
<td>Inulin</td>
<td>Cultures 20-40 g/day for 14 days</td>
<td>Placebo-controlled, cross-over study</td>
<td>Bilbithenium 2 and Glu-road probe</td>
<td>Kimmel et al. 1997</td>
</tr>
<tr>
<td>Inulin</td>
<td>Cultures 5 g/day for 24 days</td>
<td>Placebo-controlled, cross-over study</td>
<td>Bilbithenium 1 and Glu-road probe</td>
<td>Bright et al. 1998</td>
</tr>
<tr>
<td>Inulin (long chain)</td>
<td>FER 5 g/day for 5 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Kettel et al. 1999</td>
</tr>
<tr>
<td>Inulin</td>
<td>FER 5 g/day for 8 days</td>
<td>Placebo-controlled, cross-over study</td>
<td>Bilbithenium 1</td>
<td>Wady et al. 2004, 2010</td>
</tr>
<tr>
<td>Inulin</td>
<td>FER 5 g/day for 5 days</td>
<td>Placebo-controlled, cross-over study</td>
<td>Bilbithenium 1</td>
<td>Kolb et al. 2007</td>
</tr>
<tr>
<td>Inulin</td>
<td>FER 5 g/day for 5 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1, Lactobacillus acidophilus and chitosan</td>
<td>Klemm et al. 2007</td>
</tr>
<tr>
<td>Inulin</td>
<td>FER 5 g/day for 5 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1 and Galacto-oligosaccharides</td>
<td>Topp et al. 2008</td>
</tr>
<tr>
<td>Inulin</td>
<td>FER 5 g/day for 5 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1, Lactobacillus caseinii and chitosan</td>
<td>Rosell-Mora et al. 2009</td>
</tr>
<tr>
<td>Inulin</td>
<td>FER 5 g/day for 5 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1, Lactobacillus caseinii and chitosan</td>
<td>Gibson et al. 1995</td>
</tr>
<tr>
<td>FOS</td>
<td>Cultures 0-20 g/day for 7 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Falzner et al. 1998</td>
</tr>
<tr>
<td>FOS (Ambin™)</td>
<td>Cultures 0-20 g/day for 7 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Bodin et al. 2006</td>
</tr>
<tr>
<td>FOS + FOSG</td>
<td>FEI 0.4 g/day for 205 days</td>
<td>Placebo-controlled, cross-over study</td>
<td>Bilbithenium 1</td>
<td>Bodin et al. 2006</td>
</tr>
<tr>
<td>FOS + GOS</td>
<td>Culture 35 g/day for 26 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Boeckel et al. 2002</td>
</tr>
<tr>
<td>FOS + GOS</td>
<td>Cultures 5 g/day for 14 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Kind et al. 2010</td>
</tr>
<tr>
<td>GOS (70%w/w)</td>
<td>Cultures 2.5 g/day for 21 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Ito et al. 1990</td>
</tr>
<tr>
<td>GOS (70%)</td>
<td>Cultures 2.5 g/day for 21 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Ito et al. 1993</td>
</tr>
<tr>
<td>S-OS (70%)</td>
<td>Cultures 2.5 g/day for 21 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Bodin et al. 1993</td>
</tr>
<tr>
<td>S-OS (70%)</td>
<td>Cultures 2.5 g/day for 21 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Alme et al. 1999</td>
</tr>
<tr>
<td>S-OS (70%)</td>
<td>Cultures 2.5 g/day for 21 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Depeuter et al. 2008</td>
</tr>
<tr>
<td>GOS (70%)</td>
<td>Cultures 2.5 g/day for 21 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bilbithenium 1</td>
<td>Volk et al. 2008</td>
</tr>
</tbody>
</table>
Gut microbiota and systemic health

- Prebiotics:
  - Immune function
  - IBD
  - Diarrhoea/IBS
  - Laxation
  - Cancer (CRC)
  - Obesity
  - Blood glucose
  - Satiety
  - Lipid metabolism

Mineral absorption

Table 2. (Continued)

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Microbiological method</th>
<th>Dose</th>
<th>Design</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactulose</td>
<td>Culture</td>
<td>5 g/kg for 14 days</td>
<td>Feeding study</td>
<td>Bifidobacteria</td>
<td>Tentori et al. 1992</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Culture</td>
<td>5 g/kg and 10 g/kg for 21 days</td>
<td>Feeding study</td>
<td>Bifidobacteria, C. leptolyticum</td>
<td>Negus et al. 1993</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Culture</td>
<td>20 g/kg for 4 weeks</td>
<td>Placebo-controlled, parallel study</td>
<td>Bifidobacteria, C. leptolyticum</td>
<td>Ballingale et al. 1997</td>
</tr>
<tr>
<td>Lactulose</td>
<td>FED + Culture</td>
<td>10 g/kg for 26 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bifidobacteria</td>
<td>Trudel et al. 2002</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Culture</td>
<td>10 g/kg for 4 weeks</td>
<td>Placebo-controlled, parallel study</td>
<td>Bifidobacteria</td>
<td>Bodusk et al. 2004a, 2004b</td>
</tr>
<tr>
<td>IMO</td>
<td>Culture</td>
<td>15.5 g/kg for 14 days</td>
<td>Feeding study</td>
<td>Bifidobacteria</td>
<td>Kalliomaki et al. 1998</td>
</tr>
<tr>
<td>IMO</td>
<td>Culture</td>
<td>5-20 g/kg (continuous dose) for 12 days</td>
<td>Feeding study</td>
<td>Bifidobacteria</td>
<td>Kassee et al. 1994</td>
</tr>
<tr>
<td>IMO</td>
<td>Culture</td>
<td>15 g/kg for 7 days</td>
<td>Feeding study</td>
<td>Bifidobacteria, Lactobacilus</td>
<td>Or et al. 2003</td>
</tr>
<tr>
<td>DDS</td>
<td>Culture</td>
<td>5-5 g/kg and 15 g/kg</td>
<td>Placebo-controlled, crossover study</td>
<td>Bifidobacteria, Lactobacillus</td>
<td>Bomke et al. 1997</td>
</tr>
<tr>
<td>SCOS</td>
<td>Culture</td>
<td>10 g/kg for 21 days</td>
<td>Placebo-controlled, crossover study</td>
<td>Bifidobacteria</td>
<td>Hydonen et al. 1990</td>
</tr>
<tr>
<td>Bifidusone</td>
<td>FED</td>
<td>2 g/kg for 4 weeks</td>
<td>Placebo-controlled, crossover study</td>
<td>Bifidobacteria</td>
<td>Dufresne et al. 2000</td>
</tr>
<tr>
<td>Resistin starch</td>
<td>Culture</td>
<td>10 g/kg for 7 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bifidobacteria</td>
<td>Bodusk et al. 2004a, 2004b</td>
</tr>
<tr>
<td>Acacia gum</td>
<td>Culture</td>
<td>10 g/kg and 15 g/kg for 10 days</td>
<td>Placebo-controlled, parallel study</td>
<td>Bifidobacteria</td>
<td>Chetrit et al. 2003</td>
</tr>
<tr>
<td>Whole grain wheat</td>
<td>FED</td>
<td>40 g/kg for 21 days</td>
<td>Placebo-controlled, crossover study</td>
<td>Bifidobacteria, Lactobacillus</td>
<td>Comstock et al. 2000</td>
</tr>
<tr>
<td>Gas Anise</td>
<td>qPCR</td>
<td>Does response (5-8 g/kg) for 6 weeks</td>
<td>Parallel, different doses, negative control (water) positive control (10 g/kg milk)</td>
<td>Bifidobacteria, Lactobacillus, Bacteroides</td>
<td>Calame et al. 2006</td>
</tr>
<tr>
<td>Acidophilus-</td>
<td>qPCR</td>
<td>10 g/kg for 21 days</td>
<td>30 healthy adults, placebo-controlled, crossover study</td>
<td>Bifidobacteria, Lactobacillus, Bacteroides</td>
<td>Chuntos et al. 2010</td>
</tr>
</tbody>
</table>

*The microbiological methods are listed together with the dose of probiotic, the study design and the main microbiological results in terms of functional microbiota modulation.

FED, freeze-dried; qPCR, quantitative PCR; FOS, fructooligosacharides; aFOS, short-chain fructooligosacharides; PEGG, partially hydrolysed guar gum; GOS, galactooligosaccharides; TOS, trans-galacto-oligosaccharides; R-GOS, Bifidogalactooligosaccharides; IMO, inulin-type oligofructans; SCOS, syrup of oligofructans.
Delaying the progression of obesity with fermentable carbohydrates and prebiotics

- Does dietary supplementation with prebiotics or fermentable CHO/fiber reduce body weight through enhanced satiety
- High fat fed animals (control)
- High fat supplemented with Inulin (Synergy 1 (10% w/w))
- High fat supplemented with β-glucan (10% w/w)
  - Diets were isoenergetic with cellulose used to reduce calorie load of control, high fat diet.
  - Measures: magnetic resonance imaging (whole body fat deposition and stimulation of hypothalamus appetite centres), PYY, gut microbiota and caecal/faecal metabolites


Inulin and β-glucan reduce body weight gain
Effect of inulin and β-glucan supplementation on adiposity parameters and PYY level in high fat fed mice.

<table>
<thead>
<tr>
<th></th>
<th>HFD-C</th>
<th>HFD-I</th>
<th>HFD-BG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal adipose tissue (g)</td>
<td>1.14±0.16a</td>
<td>0.59±0.10b</td>
<td>0.77±0.10a</td>
</tr>
<tr>
<td>Whole body adiposity (%)</td>
<td>18.03±2.72a</td>
<td>8.95±1.66b</td>
<td>12.17±1.92a</td>
</tr>
<tr>
<td>Liver lipid content (%)</td>
<td>6.30±1.62a</td>
<td>6.02±1.97a</td>
<td>6.02±1.36a</td>
</tr>
<tr>
<td>Muscle lipid content (%)</td>
<td>0.96±0.14a</td>
<td>0.72±0.05b</td>
<td>1.29±0.57a</td>
</tr>
<tr>
<td>Visceral fat (g)</td>
<td>2.17±0.46a</td>
<td>1.23±0.17a</td>
<td>1.49±0.27a</td>
</tr>
<tr>
<td>Subcutaneous fat (g)</td>
<td>3.40±0.53a</td>
<td>2.08±0.13a</td>
<td>2.44±0.28a</td>
</tr>
<tr>
<td>Adipocyte size (μm)</td>
<td>122.25±10.2a</td>
<td>72.95±8.72c</td>
<td>111.19±4.03c</td>
</tr>
<tr>
<td>Adipocyte number (x10⁸)</td>
<td>1.43±0.08a</td>
<td>1.31±0.09b</td>
<td>1.86±0.08a</td>
</tr>
<tr>
<td>Liver size (g)</td>
<td>1.43±0.13a</td>
<td>1.23±0.15a</td>
<td>1.40±0.08a</td>
</tr>
<tr>
<td>Caecum (g)</td>
<td>0.21±0.01a</td>
<td>0.25±0.05a</td>
<td>0.49±0.03c</td>
</tr>
<tr>
<td>Colon (g)</td>
<td>0.13±0.01a</td>
<td>0.19±0.02b</td>
<td>0.14±0.02a</td>
</tr>
<tr>
<td>PYY (pmol/ml)</td>
<td>0.10±0.012a</td>
<td>0.10±0.008a</td>
<td>0.13±0.016a</td>
</tr>
<tr>
<td>Colonic PYY</td>
<td>27.3 3.7</td>
<td>22.8 5.3</td>
<td>19.9 1.6</td>
</tr>
</tbody>
</table>

The values with different superscripts letters are significantly different from each other.

Effect of inulin and β-glucan supplementation on changes in signal intensity in the appetite centres of the brain measured by MRI.

The arrow shows the start of Mn2+ infusion and grey bar represents the duration of Mn2+ infusion.
Effect of inulin and β-glucan supplementation on changes in signal intensity in the appetite centres of the brain measured by MRI

The arrow shows the start of Mn2+ infusion and grey bar represents the duration of Mn2+ infusion.

Effect of inulin and β-glucan supplementation on murine gut microbiota compared to high fat diet supplemented with cellulose

Similar findings observed for caecal contents at week 8.
Effect of inulin and β-glucan supplementation on murine faecal metabolite profiles (NMR) compared to high fat diet supplemented with cellulose.

NMR based metabolomics separates cellulose from inulin or β-glucan supplemented animals on high fat diets.

PCA scores plot of fecal metabolite profiles showing clear clustering patterns for mice fed with HFD-C, HFD-BG and HFD-I groups.
Fermentable fibers/prebiotics reduce body weight but by different mechanisms

- β-glucan reduced cumulative body weight apparently through reduced stimulation of hypothalamic appetite centres, increased satiety and reduced food intake.

- Inulin appeared to reduce cumulative body weight gain through reduced adipocyte size and whole body adiposity

- SCFA concentrations in the caecum β-glucan > inulin > high fat control

- Inulin gave increased caecum weight

- β-glucan had higher excretion of glucose in faeces while high-fat control had higher excretion of butyrate and propionate


Whole grain oats vs non-whole grain breakfast cereal dietary intervention in subjects “at risk” of developing the metabolic syndrome

- Randomized, crossover study, 30 volunteers, male and female with slightly elevated levels of either total cholesterol or fasting glucose at risk of developing metabolic disorders

- Two 6 week treatment periods separated by 4 week washout periods.

- Whole oat grain (WGO) vs non-whole grain cereal (NWG)

- Samples collected before and after cereal consumption and then 4 weeks following end of consumption.

- Blood (fasted), 24 hour urine, saliva and fecal samples

Connolly et al. In preparation

Supported by Jordans Cereals
Whole grain oats modified gut microbiota in beneficial manner compared to non-whole grain cereal

Whole grain oats significantly increased faecal bifidobacteria and lactobacilli but no other bacterial groups measured.

Whole grain oats improved blood cholesterol profiles

- Whole grain oats significantly reduced LDL and total cholesterol, reversing a trend towards elevated LDL and TC in the non-whole grain breakfast cereal treatment.
Impact of wheat bran fibre (WBF) on gut microbiota & markers of CVD in overweight adults

<table>
<thead>
<tr>
<th>Run-in</th>
<th>WBF (bread, biscuits, breakfast cereals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run-in</td>
<td>Cellulose (bread, biscuits, breakfast cereals)</td>
</tr>
<tr>
<td>2 weeks</td>
<td>8 weeks</td>
</tr>
</tbody>
</table>

- Subjects: n=80, BMI > 27
- FEM & Santa Chiara Hospital (Dr Carlo Pedrolli), APSS, Trento
- Biomarkers of CVD risk
- Gut microbiota (454-pyrosequencing, FISH, qPCR)
- MS based metabolomics (targeted and untargeted)

The 3Ps: Probiotics, Prebiotics & Polyphenols

- **PROBIOTICS**...“live microorganisms which when administered in adequate amount confer a health benefit on the host” (FAO, 2001).
  - *Lactobacillus*
  - *Bifidobacterium*
  - *Escherichia coli* Nissle 1917, *Bacillus sporogenes*, *Enterococcus faecium*, *Clostridium butyricum*, *Saccharomyces cerevisae*
- **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)
  – Inulin, oligofructose, fructooligosaccharides, galactooligosaccharides, lactulose, arabinogalactan, arabinoxylan, pectic-oligosaccharides, glucooligosaccharides
  – Resistant starch and certain whole plant foods including whole grain wheat, whole grain oats
- **POLYPHENOLS**... 90% resistant to digestion and reach the colon, plant secondary metabolites, usually antioxidant, antimicrobial activities, enzyme/nutrient binding properties and possibly prebiotic type properties, e.g. red-wine polyphenols, apple tannins
Gut microbiota and systemic health

Cancer (CRC)  
Obesity  
Blood glucose  
Satiety  
Lipid metabolism

Polyphenols  

Immune function  
IBD  
Diarrhoea/IBS  
Laxation  
Mineral absorption

Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers

Maria Isabel Quiroz-Ortiz, Maria Boto-Ordóñez, Mora Murri, Juan Miguel Gomez-Zamaquero, Mercedes Clemente-Ponsio, Ramon Estrach, Fernando Cunlina Diaz, Cristina Andreis-Lacueva, and Francisco J Timón

Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers\textsuperscript{1–4}

Maria Isabel Queipo-Ortuno, María Bote-Ordóñez, Mora Marri, Juan Miguel Gomez-Zumaquero, Mercedes Clemente-Postigo, Ramon Estruch, Fernando Cardona Diaz, Cristina Andrés-Lacueva, and Francisco J Tilgner

Table 4: Anthropometric and biochemical variables during the study\textsuperscript{1}

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (washout period)</th>
<th>De-alkalized red wine period</th>
<th>Red wine period</th>
<th>Gin period</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>97.8 ± 21.3</td>
<td>97.8 ± 19.4</td>
<td>96.4 ± 20.6</td>
<td>97.2 ± 19.6</td>
<td>0.306</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>106.7 ± 14.3</td>
<td>106.5 ± 14.4</td>
<td>105.1 ± 14.5</td>
<td>105.7 ± 13.5</td>
<td>0.392</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>111.0 ± 10.4</td>
<td>109.0 ± 12.8</td>
<td>110.2 ± 11.1</td>
<td>110.8 ± 10.3</td>
<td>0.908</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>97.4 ± 15.2</td>
<td>91.0 ± 12.9</td>
<td>86.5 ± 11.6\textsuperscript{a}</td>
<td>98.4 ± 14.3\textsuperscript{b}</td>
<td>0.026</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>145.4 ± 23.9\textsuperscript{a}</td>
<td>135.1 ± 24.6\textsuperscript{b}</td>
<td>129.5 ± 17.6\textsuperscript{a}</td>
<td>142.7 ± 22.3\textsuperscript{b}</td>
<td>0.026</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>27.6 ± 3.2</td>
<td>27.6 ± 3.1</td>
<td>27.5 ± 2.9</td>
<td>27.6 ± 2.8</td>
<td>0.241</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>111.3 ± 23.1</td>
<td>104.5 ± 24.2</td>
<td>108.5 ± 16.4</td>
<td>108.8 ± 17.2</td>
<td>0.772</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.7 ± 1.4</td>
<td>5.3 ± 1.0\textsuperscript{a}</td>
<td>5.0 ± 0.8\textsuperscript{b}</td>
<td>5.4 ± 1.5\textsuperscript{a}</td>
<td>0.018</td>
</tr>
<tr>
<td>GOT (mg/dL)</td>
<td>22.0 ± 7.3\textsuperscript{a}</td>
<td>14.3 ± 4.0\textsuperscript{b}</td>
<td>17.6 ± 13.4\textsuperscript{a}</td>
<td>19.1 ± 8.0\textsuperscript{b}</td>
<td>0.021</td>
</tr>
<tr>
<td>GPT (mg/dL)</td>
<td>46.4 ± 12.6</td>
<td>41.2 ± 7.7</td>
<td>42.0 ± 9.3</td>
<td>43.1 ± 6.9</td>
<td>0.888</td>
</tr>
<tr>
<td>GGT (mg/dL)</td>
<td>36.9 ± 25.6\textsuperscript{a}</td>
<td>30.1 ± 13.5\textsuperscript{b}</td>
<td>36.1 ± 16.3\textsuperscript{a}</td>
<td>38.0 ± 27.7\textsuperscript{b}</td>
<td>0.012</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>245.4 ± 231.7\textsuperscript{a}</td>
<td>171.7 ± 206.7\textsuperscript{b}</td>
<td>179.4 ± 177.9\textsuperscript{a}</td>
<td>190.1 ± 222.8\textsuperscript{b}</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>257.5 ± 88.6\textsuperscript{a}</td>
<td>241.2 ± 94.9\textsuperscript{b}</td>
<td>188.6 ± 61.6\textsuperscript{a}</td>
<td>239.3 ± 91.4\textsuperscript{b}</td>
<td>0.008</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>129.6 ± 41.9</td>
<td>123.5 ± 28.1</td>
<td>125.7 ± 30.3</td>
<td>130.6 ± 22.0</td>
<td>0.266</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>58.5 ± 16.7\textsuperscript{a}</td>
<td>48.8 ± 17.1\textsuperscript{b}</td>
<td>49.7 ± 14.3\textsuperscript{a}</td>
<td>52.3 ± 16.5\textsuperscript{b}</td>
<td>0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.9 ± 2.6\textsuperscript{a}</td>
<td>4.3 ± 2.3\textsuperscript{b}</td>
<td>4.6 ± 2.5\textsuperscript{a}</td>
<td>6.8 ± 3.7\textsuperscript{b}</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\textsuperscript{1} All values are means ± SDs; n = 10 subjects. Means in a row with different superscript letters are significantly different, P < 0.05. (Wilcoxon’s signed-rank test with post hoc Bonferroni test). CRP: C-reactive protein; DBP: diastolic blood pressure; GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; SBP: systolic blood pressure.

\textsuperscript{2} Derived by using the Friedman test.

Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study\textsuperscript{1–3}

Kenyon Tyssens, Ana Rodriguez-Mateos, Jelena Valerie, Glenn R Gibson, Catherine Kuek-Urba, and Jeremy PE Spencer

Measuring the impact of raspberries of different polyphenol content on gut microbiota

- Raspberries digested and fermented
  - Alpen Gold (yellow), Tulameen (red), Anne (yellow), Sugana Yellow (yellow), Sugana Red (red)
- Controls (inulin and cellulose)

qPCR (total, Enterobacteriaceae, LAB, *Bifidobacterium* spp.)
FISH (totals, *Bifidobacterium* spp., Bacteroidaceae e Prevotellaceae (Bac303), *Clostridium hystolyticum* (Chis), *Clostridium coccoides-Eubacterium rectale* (Erec), Faecalibacterium prausnitzii (Fpra)
ARISA

Targeted Metabolomic:
Anthocyanins and Ellagitannins (UPLC-MS)
SCFA (GC-MS)
Significant increase in bifidobacterial abundance as measured by FISH

Total anthocyanins and anthocyanins profile during fermentation for Tulameen or Sugana Red

a: average of five significantly higher

pg: pelargonidin
cy: cyanidin
glu: glucoside
gal: galactoside
samb: sambubioside
rut: rutinoside
soph: sophoroside
Ellagitannins measured during raspberry fermentation

Casuaricin: monomer
Sanguin: dimer
Lambertianin: trimer

Increasing fruit and vegetable intake in vivo – FLAVURS project

Flavonoid-rich F&V
Flavonoid-poor F&V
Habitual diet

Wk 0
Visit 1

Wk 6
Visit 2

Wk 12
Visit 3

Wk 18
Visit 4

03/11/2014
High Flavonoid group

Apple crumble
Dried cranberries/ blueberries

Fruit smoothies
(Strawberry and raspberry/ Blackberry and blueberry)

Fruit juices
(Blackcurrant /apple/cranberry /orange)

Roasted peppers
Pepperdew cherry peppers

All fruits and vegetables contain ≥ 15mg/100g of flavonoids

Low Flavonoid group

Rhubarb crumble
Dried fruits (raisins, currants, mango)

Fruit smoothies (tropical mix)

Fruit juices (mango/ pineapple)

Guacamole
Houmous

Soups
(Carrot & coriander/broccoli & stilton)

Canned chopped tomatoes

All fruits and vegetables contain < 5mg/100g of flavonoids
FLAVONOIDS

Dietary intake: HF dose dependent increase
HF higher vs LF & CT +2, +4, +6
Time x treatment (P=0.006)

Biomarker: 24h urinary flavonoid & metabolites
HF dose dependent increase
HF higher vs LF & CT +2, +4, +6
Time x treatment (P=0.0001)

VITAMIN C

Dietary intake: HF & LF dose increase
HF & LF vs CT higher +2, +4, +6
Time x treatment (P=0.0001)

Biomarker: Plasma vitamin C
HF & LF dose increase
HF & LF vs CT higher +2, +4, +6
Time x treatment (P=0.0001)
CAROTENOIDS

Dietary intake: LF dose dependent increase
HF & LF higher CT all points
Time x treatment (P=0.001)

Biomarker: Total plasma carotenoids
LF dose dependent increase
HF & LF higher CT all points
Time x treatment (P=0.0001)

Non-starch polysaccharide (NSP) changes

HF & LF higher than the CT all time points
LF dose dependent increase
Time x treatment interaction (P=0.0001).
F&V impact on arterial stiffness measured by PWA

HF and LF attenuated increase shown in CT group
Time x treatment $P=0.009$ when standardised for HR75 $P=0.03$

Other blood parameters

**Total plasma nitrate/nitrite**
HF higher than LF & CT +6
Time x treatment ($p=0.03$)

**Plasma FRAP**
HF dose dependent increase
LF higher +4 & +6 vs baseline
Time x treatment ($P=0.009$)
High fruit and veg diet appears to modulate gut microbiota in “beneficial” manner

**Bifidobacteria**

- Small changes
- In “right” direction
- HF ↑ *Eu. rectale*

**Lactobacilli**

- HF → *F. praunitzii*
- LF ↑ *Bacteroides*

**Atopobium**

**C. perfringens/histolyticum**

Untargeted Metabolomic Analysis of Urine

- Urine dilution 1:5
- HPLC Analysis on RP column in positive and negative ionization mode
- XL Orbitrap in Full Scan MS and MS/MS within high resolution and mass accuracy Approaches
- Substances considered as biomarkers when \( p < 0.005 \) (t-test)
- Annotation of metabolites:
  - Mass accuracy of precursor ion [M+H]+ (< 3 ppm error)
  - Isotopic pattern distribution
- Databases used for annotation: In-house database, Human Metabolome Database, Metlin, MAssBank, LipidMaps
**Metabolomics workflow**

Samples: urine, plasma, fecal water

Sample preparation: extraction of all analytes

Separation on LC column

Biomarker identification

Statistic analysis

Untargeted analysis with HR mass spectrometer

ALIGNMENT OF CHROMATOGRAMS, BATCH CORRECTIONS, PEAK PICKING

UNIVARIATE ANALYSIS with XCMS

Data processing - XCMS using the “matchedFilter” peak picking method with Spectra Filter Window Mower function.

For each mass feature two linear mixed models were fitted, diet-time interaction and time alone.

Both models were adjusted for baseline. p values for all features were corrected for multiple testing according to the two-stage Benjamini and Hochberg step-up false discovery rate (FDR).
**Nutrition and Nutrigenomics**

**Apples Gut Microbiota Modulation**

Adding value to the food chain

<table>
<thead>
<tr>
<th>.Annotation</th>
<th>Elemental Composition, MW, adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.10 ProlineBetaine, MMW: 143.0946, p 0.002 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>2.20 N-acetyl-S-(2-hydroxypropyl) cysteine, MMW: C8H15NO4S; p</td>
<td></td>
</tr>
<tr>
<td>3.80 Hydroxy Hippuric Acid (isomer), MMW: 195.0531, p 0.02 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>4.40 Hydroxy Hippuric Acid (isomer), MMW: 195.0531, p 0.002 ↑Diet A.</td>
<td></td>
</tr>
<tr>
<td>4.82 Vanilloylglycine, MMW: 225.0637; p 0.03 ↑ Diet A; B;</td>
<td></td>
</tr>
<tr>
<td>5.70 Hippuric Acid, MMW: 179.0582; p 0.002 ↑Diet A</td>
<td></td>
</tr>
<tr>
<td>5.89 Phenylacetylglutamine, MMW: 264.1110, p 0.04 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>6.15 FerulicAcid Sulfate, MMW: 274.0731, p 0.04 ↑Diet A; B</td>
<td></td>
</tr>
<tr>
<td>6.26 Dihydroxyphenyl-γ-valerolactone-O-sulphate MMW:288.0306 p 0.0003 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>6.56 Dihydroxyphenyl-γ-valerolactone-O-methyl-O-GLC, p 0.01 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>7.14 Cresol-Glucuronide, MMW: 284.0896; p 0.001 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>7.35 Hydroxy Hippuric Acid (isomer), MMW: 195.0531, p 0.01 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>7.78 Hydroxy-tridecenoic acid GLC, MMW: 404.2046, p 0.001 ↑Diet A;</td>
<td></td>
</tr>
<tr>
<td>12.38 Iberin N-acetyl-cysteine MMW: p 0.0001 ↑Diet A &amp; p 0.001 ↑Diet B</td>
<td></td>
</tr>
</tbody>
</table>

**Biological samples:** Blood, Urine and Faecal samples

**Measurements**
- % body fat composition, DEXA
- Blood pressure,
- Vascular stiffness (pulse wave analysis, PWA)
- and vascular reactivity (laser Doppler imaging, LDI)
- Gut microbiota (454-pyrosequencing)
- Untargeted metabolomics.

**Food Quality and Nutrition Department**
Conclusions: Adherence to an MD pattern is associated with better HRQL. The association is stronger with mental health than with physical health. Dietary total antioxidant and fibre content independently explain this relationship.

INRAN, FAO Double Pyramid

Barilla Centre for Food Nutrition: Double Pyramid: healthy food for people, sustainable food for the planet

Thank you: SINU, Professor Brighenti

Fulvio Mattivi, Duccio Cavalieri and Roberto Viola, FEM-IASMA

NN Group: Lorenza Conterno, Francesca Fava, Elena Franciosi, Carlotta de Filippo, Athanasios Koutsos, Ilaria Caraffa, Florencia Ceppa, Andrea Manchini

University of Reading, Glenn Gibson, Bob Rastall, Julie Lovegrove, Parveen Yaqoob, Christine Williams, Ian Rowland, Michael Connolly

Gary Frost, Imperial College London, Daniele Del Rio, University of Parma