Dietary modulation of the human gut microbiota – taming the beast within!

Kieran Tuohy
Fondazione Edmund Mach, Italy
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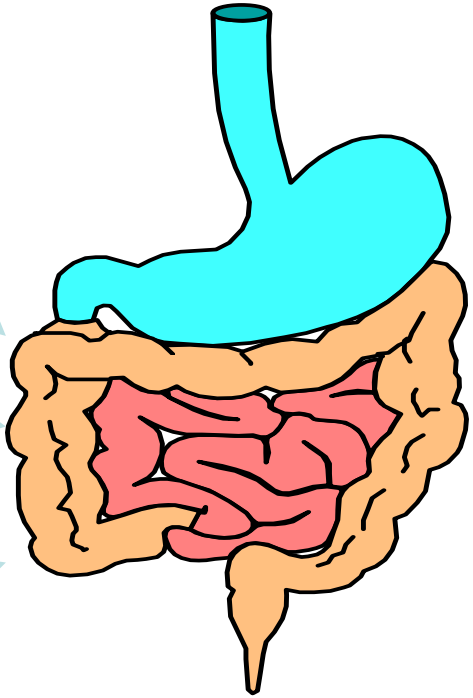
Provincia di Trento

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

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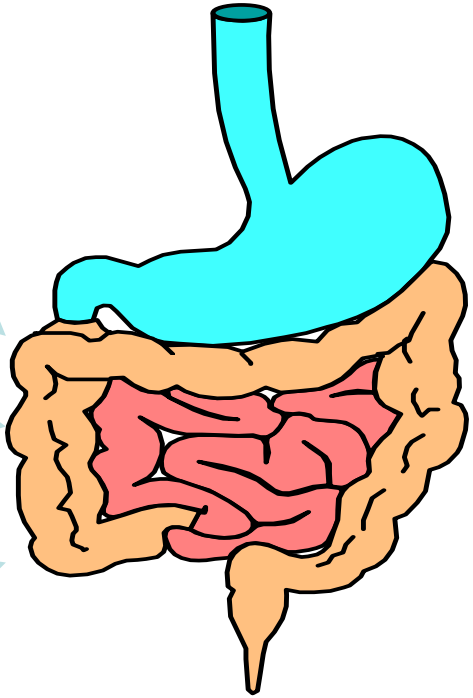

Interactions with;

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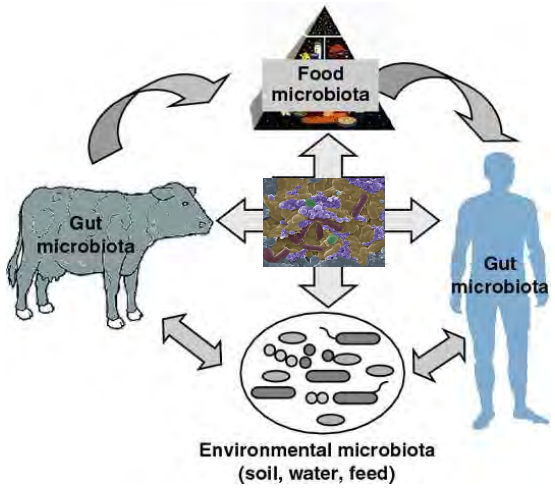
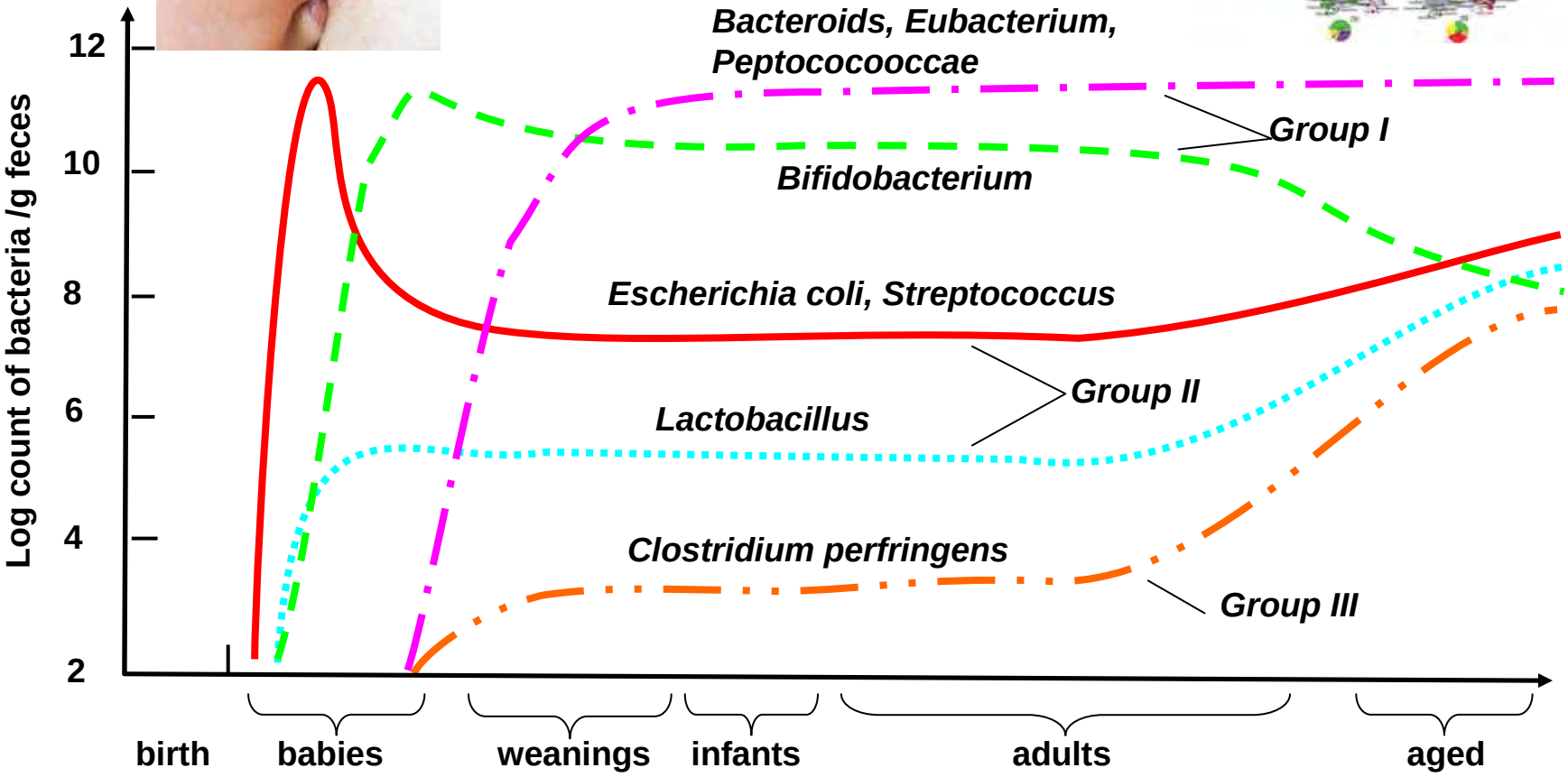
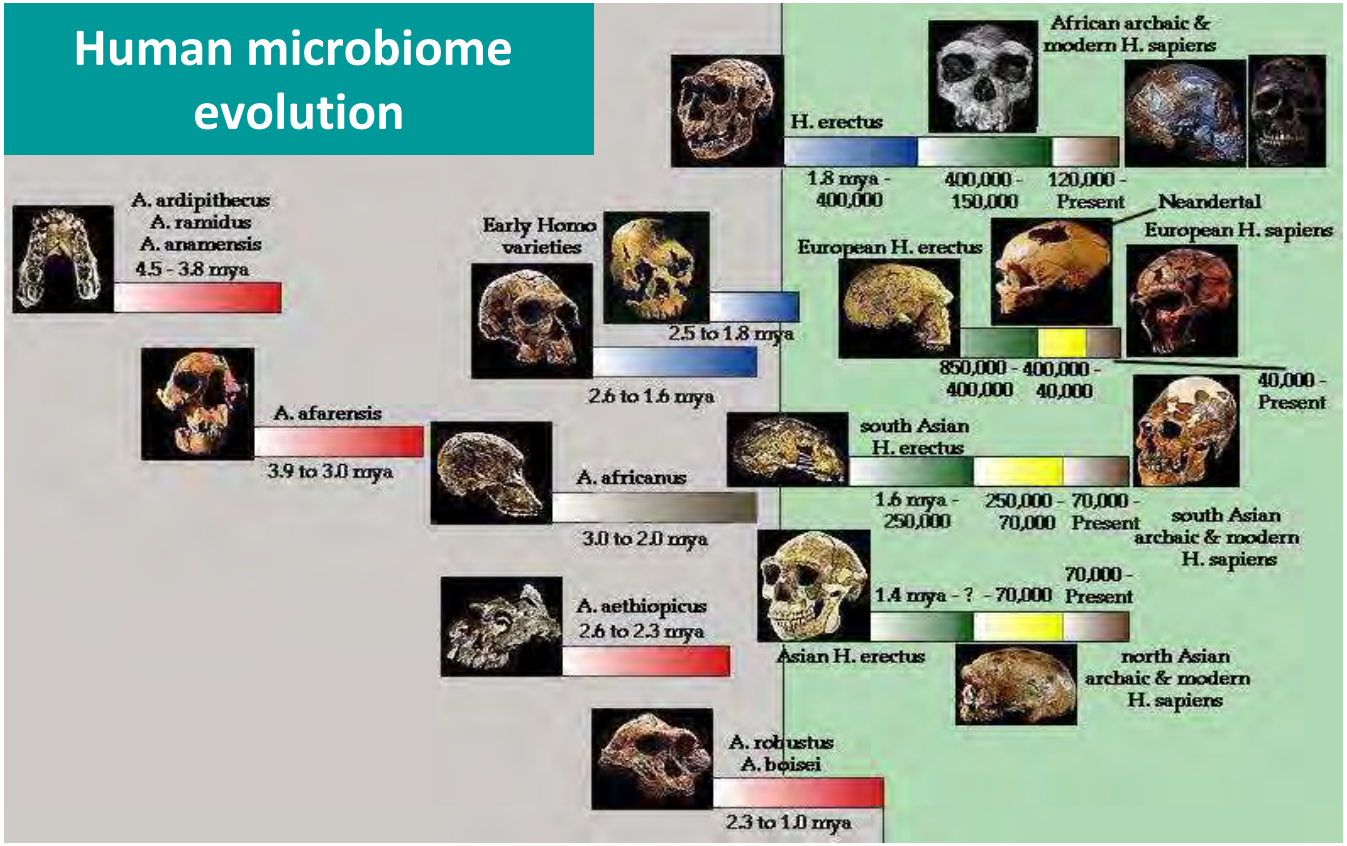
Gut microbiota and essential organ within the human system – we have become an ecosystem


Food microbiota
Gut microbiota
Gut microbiota
Environmental microbiota (soil, water, feed)


Log count of bacteria /g feces
12
10
8
6
4
2
Bacteroids, Eubacterium, Peptococooccae
Group I
Bifidobacterium
Escherichia coli, Streptococcus
Group II
Lactobacillus
Clostridium perfringens
Group III
birth
babies
weanings
infants
adults
aged

Human diet shaped our closely co-evolved human:microbe ecosystem

Human microbiome evolution



Dietary evolution

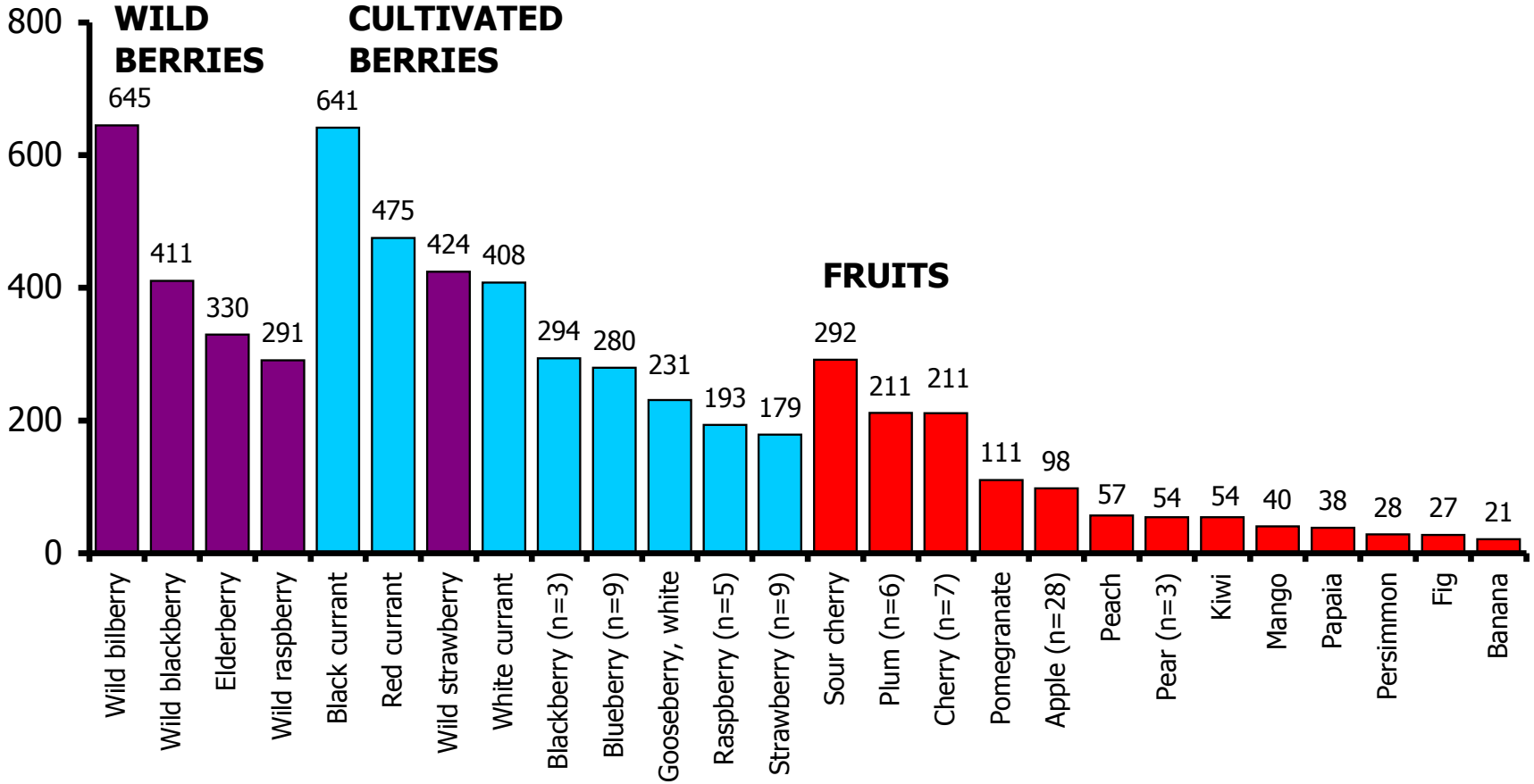
- **Neolithic times: ~10,000 yrs BP (birth of agriculture)**
- **Agricultural/Industrial revolutions: Late 18th and early 19th century**
- **Recent changes: Over the last 50 yrs (Western-style diet)**

Estimated daily fiber intake in Palaeolithic /Traditional diets and Modern diet

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

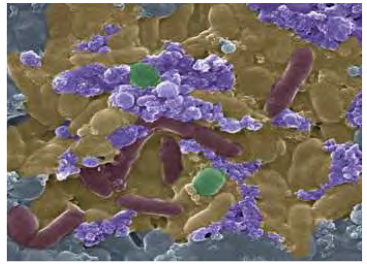
(Tuohy et al. Current Pharmaceutical Design, 2009)

Total polyphenols (catechin equivalents, mg/100 g)



Redrawn from: Mattivi F., Dietas Mediterráneas: La evidencia científica, 2004, 99-111

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



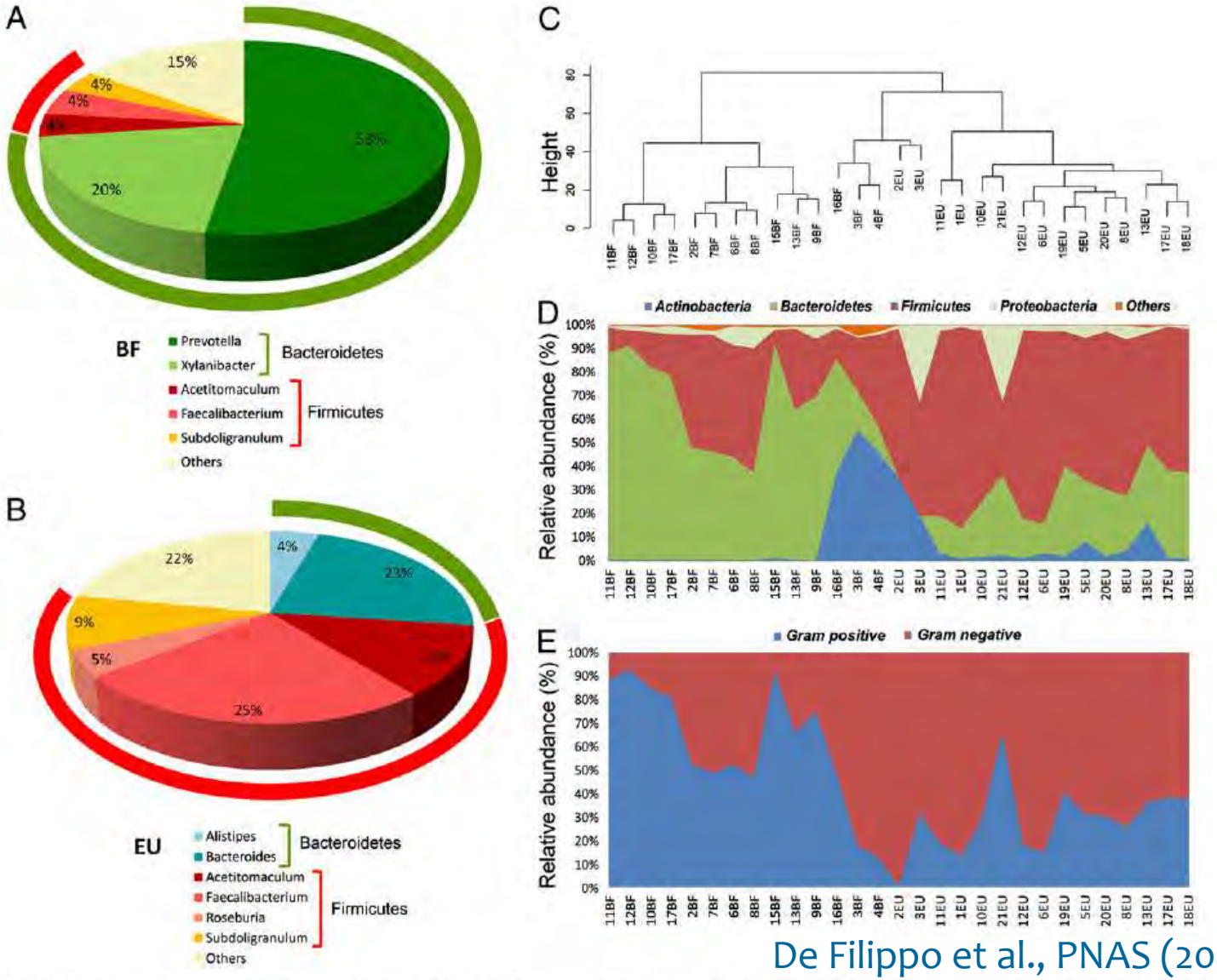
PNAS

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

Carlotta De Filippo[a], Duccio Cavalieri[a], Monica Di Paola[b], Matteo Ramazzotti[c], Jean Baptiste Poullet[d], Sebastien Massart[d], Silvia Collini[b], Giuseppe Pieraccini[e], and Paolo Lionetti[b,1]

[a]Department of Preclinical and Clinical Pharmacology, University of Florence, 50139 Firenze, Italy; [b]Department of Pediatrics, Meyer Children Hospital, University of Florence, 50139 Firenze, Italy; [c]Department of Biochemical Sciences, University of Florence, 50134 Firenze, Italy; [d]DNA Vision Agrifood S.A., B-4000 Liège, Belgium; and [e]Centro Interdipartimentale di Spettrometria di Massa, University of Florence, 50139 Firenze, Italy

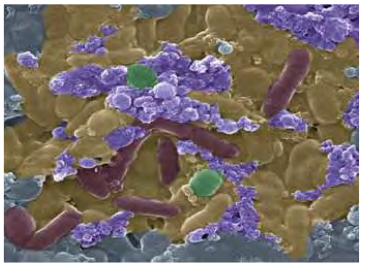
De Filippo et al., PNAS (2010)



De Filippo et al., PNAS (2010)

Fig. 2. 16S rRNA gene surveys reveal a clear separation of two children populations investigated. (*A* and *B*) Pie charts of median values of bacterial genera present in fecal samples of BF and EU children (>3%) found by RDP classifier v. 2.1. Rings represent corresponding phylum (Bacteroidetes in green and Firmicutes in red) for each of the most frequently represented genera. (*C*) Dendrogram obtained with complete linkage hierarchical clustering of the samples from BF and EU populations based on their genera. The subcluster located in the middle of the tree contains samples taken from the three youngest (1–2 y old) children of the BF group (16BF, 3BF, and 4BF) and two 1-y-old children of the EU group (2EU and 3EU). (*D*) Relative abundances (percentage of sequences) of the four most abundant bacterial phyla in each individual among the BF and EU children. Blue area in middle shows abundance of Actinobacteria, mainly represented by *Bifidobacterium* genus, in the five youngest EU and BF children. (*E*) Relative abundance (percentage of sequences) of Gram-negative and Gram-positive bacteria in each individual. Different distributions of Gram-negative and Gram-positive in the BF and EU populations reflect differences in the two most represented phyla, Bacteroidetes and Firmicutes.

Aberrant gut microbiota associated with Western-style diet



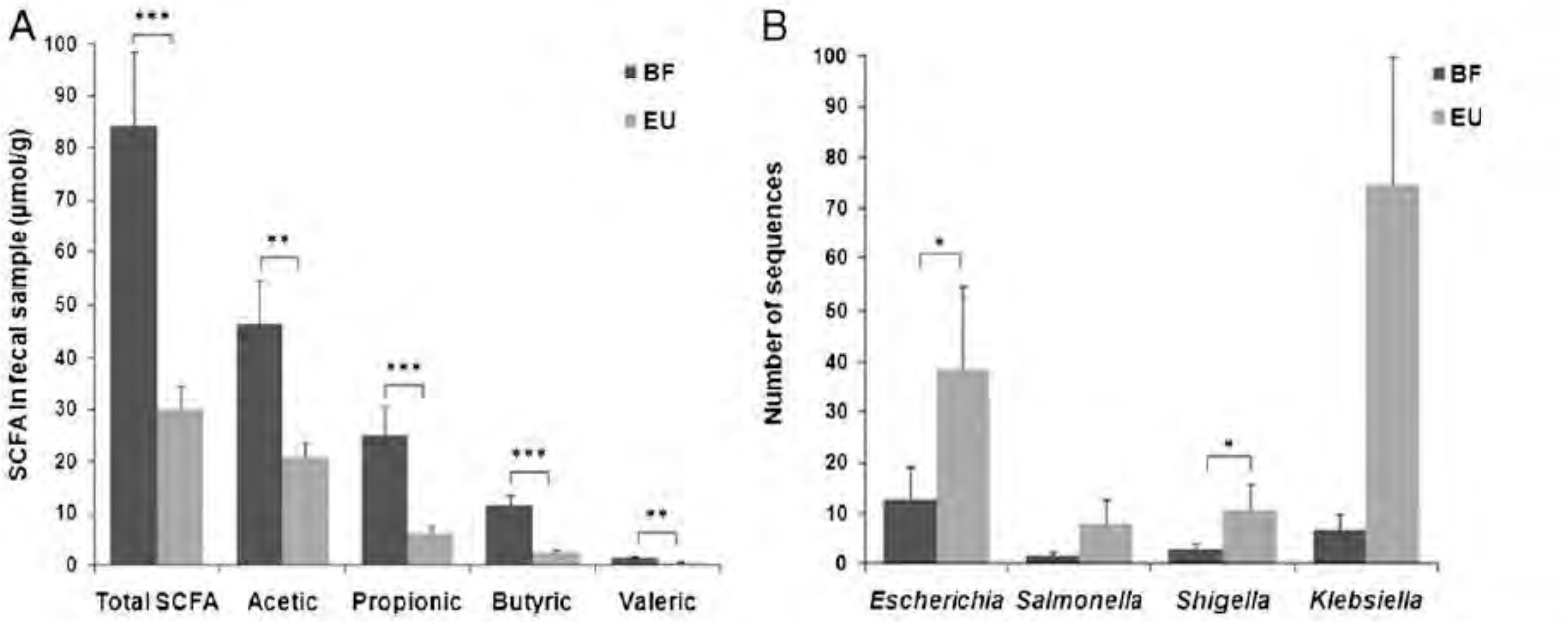


Fig. 3. SCFA-producing bacteria could help to prevent establishment of some potentially pathogenic intestinal bacteria. (*A*) Quantification of SCFAs in fecal samples from BF and EU populations by SPME-GC-MS. (*B*) Number of sequences relative to principal *Enterobacteriaceae* genera, in BF and EU children microbiota. Mean values (±SEM) are plotted. Asterisks indicate significant differences (one-tailed Student *t* test of all data points: $*P < 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).

- 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)

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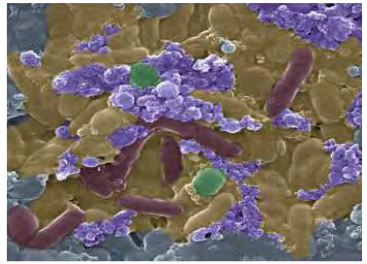

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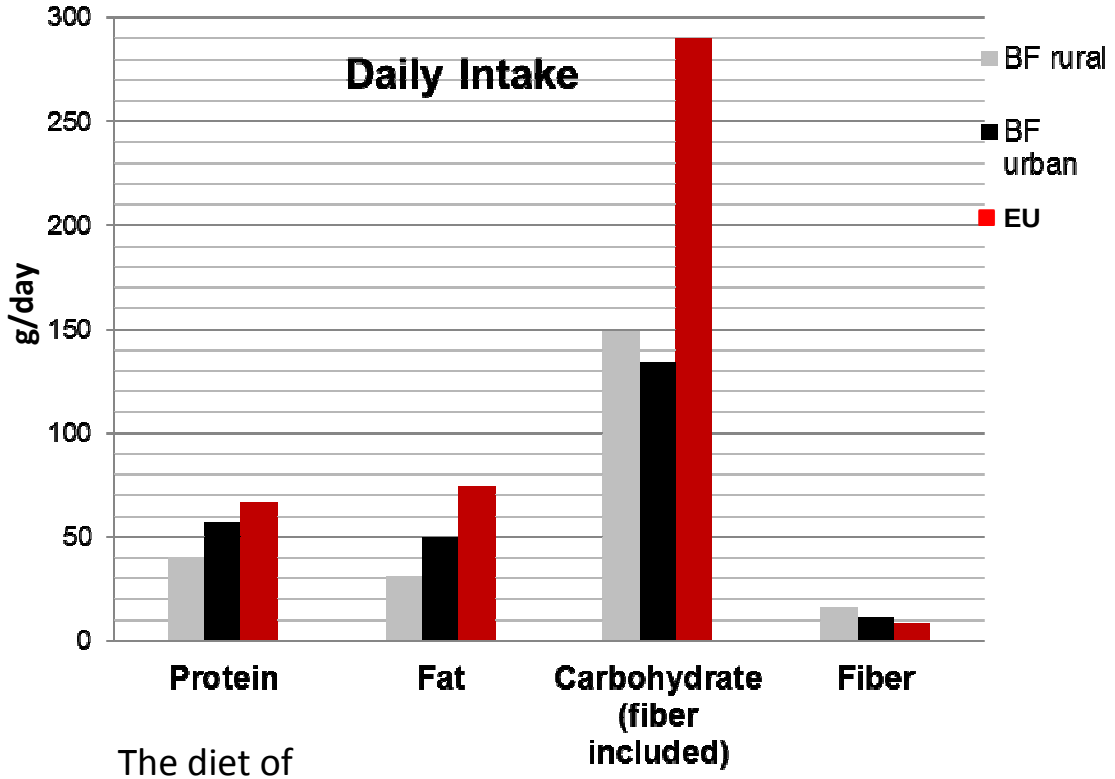
Revisited

De Filippo et al., PNAS (2010)

Total daily food intake in relation to the average of maximum quantity ingested per day



The diet of

-**BF rural** children is low in fat and rich in fibers and plant-polysaccharides and predominantly vegetarian

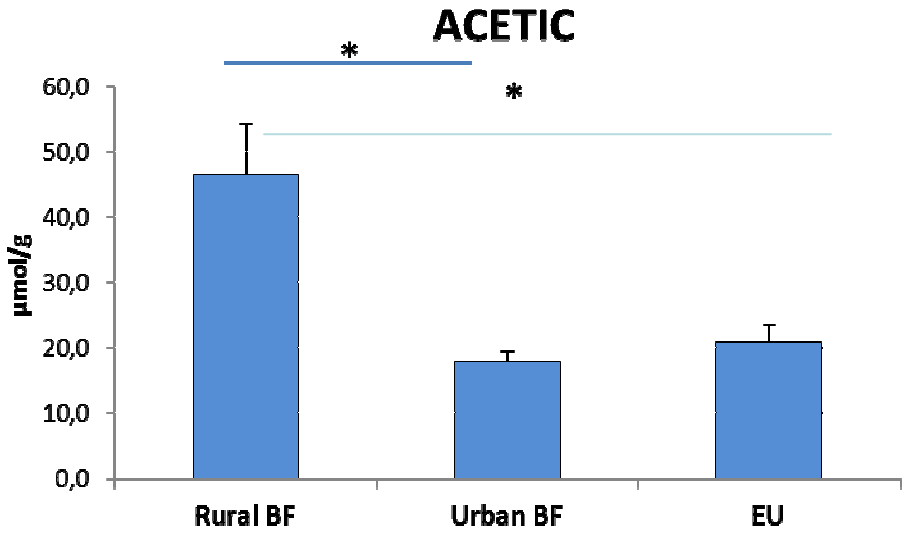
-**BF urban** children maintain the consumption of cereals and legumes but introduces milk, meat, fish, egg and peanuts.

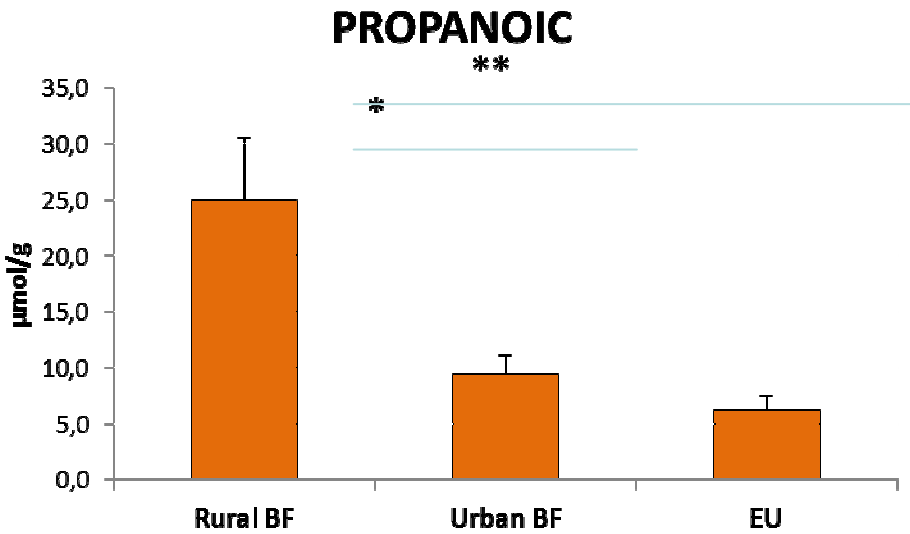
- **EU** is a a typical western diet high in animal protein, sugar, starch, and fat and low in fiber.

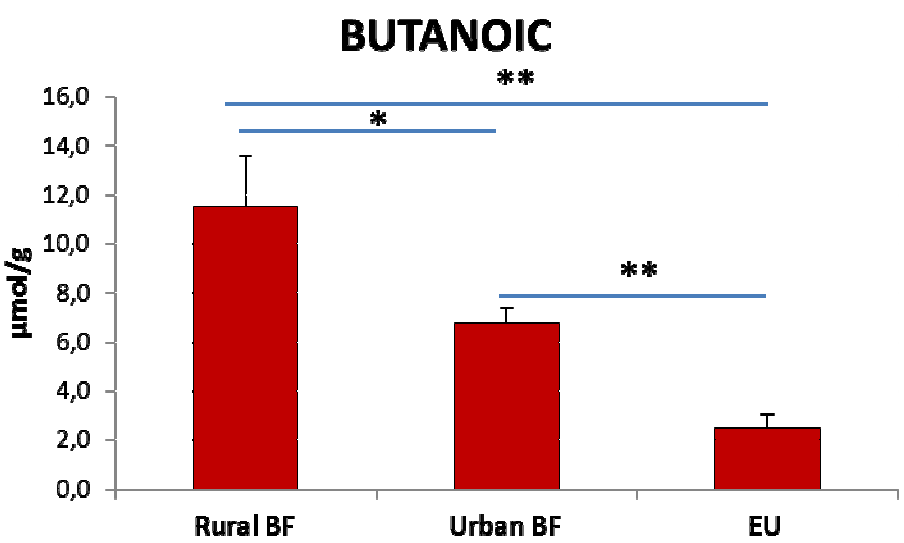
a) Millet; b) Millet flour; c-d) black-eyed peas, Niebè, e) *Parkia biglobosa* tree (Néré); f) Soumbalà , Nerè fruits fermented.

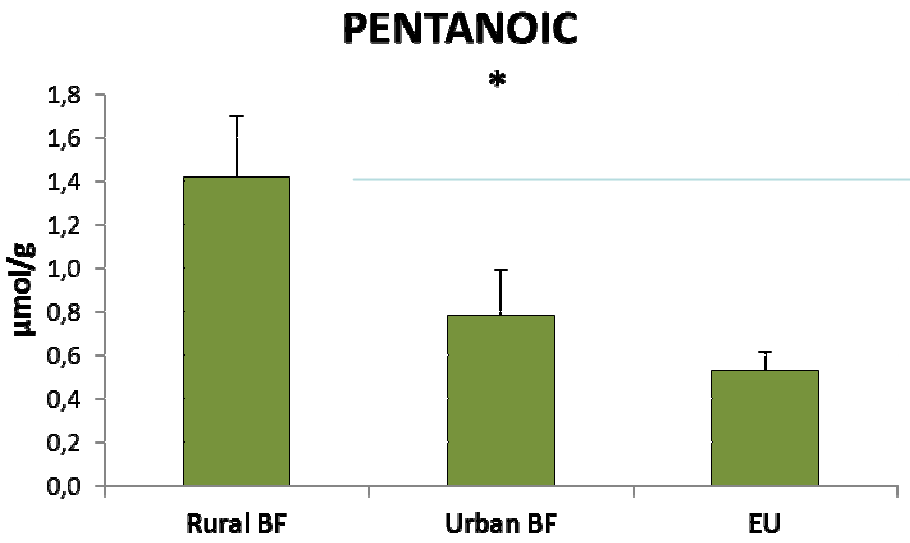
Nutritional composition of foods is available from http://www.inran.it for EU and http://www.fao.org for BF

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

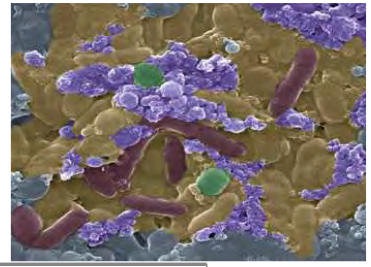


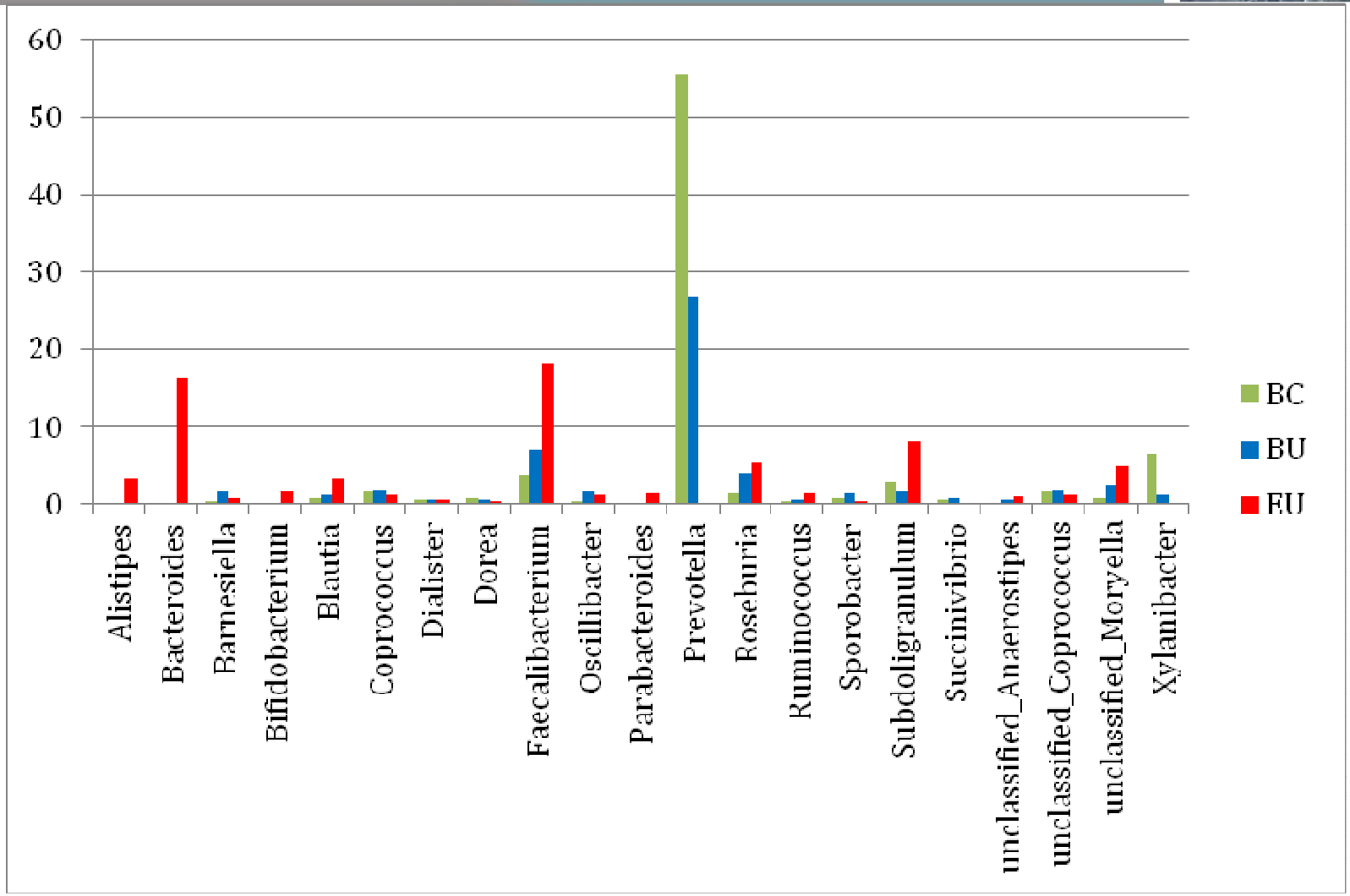






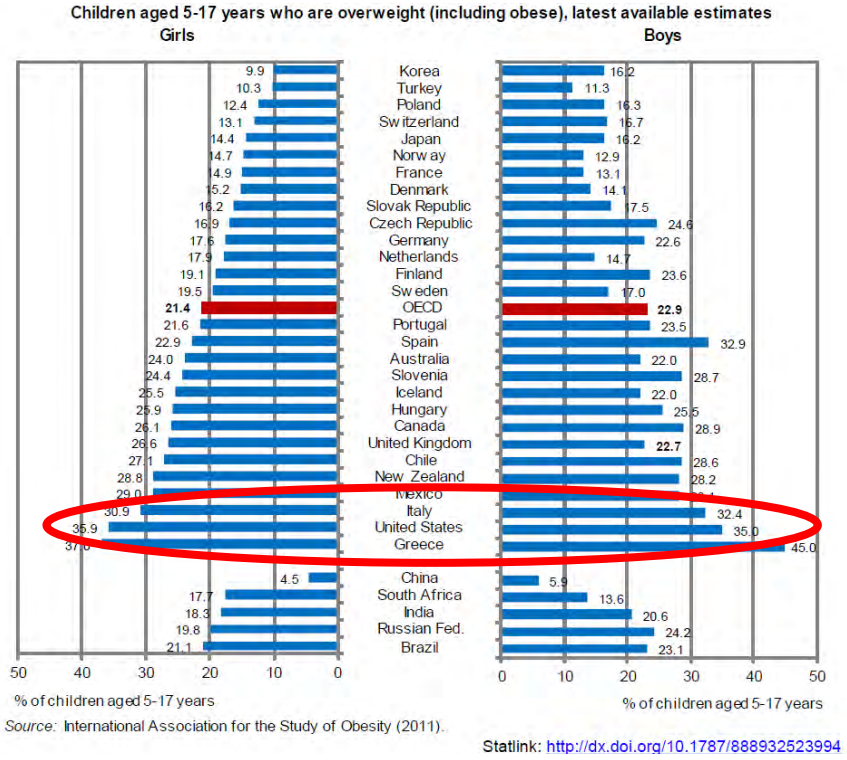
Gut microbiota…… but not as we know it!





OBESITY EPIDEMIC

- Currently 300 million people obese world-wide
- 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 dieing from cancer



Nature. 2011 April 7; 472(7341): 57–63. doi:10.1038/nature09922.

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Zeneng Wang[1,2], **Elizabeth Klipfell**[1,2], **Brian J. Bennett**[3], **Robert Koeth**[1], **Bruce S. Levison**[1,2], **Brandon DuGar**[1], **Ariel E. Feldstein**[1,2], **Earl B. Britt**[1,2], **Xiaoming Fu**[1,2], **Yoon-Mi Chung**[1,2], **Yuping Wu**[4], **Phil Schauer**[5], **Jonathan D. Smith**[1,6], **Hooman Allayee**[7], **W. H. Wilson Tang**[1,2,6], **Joseph A. DiDonato**[1,2], **Aldons J. Lusis**[3], and **Stanley L. Hazen**[1,2,6,8]

[1]Department of Cell Biology, Cleveland Clinic, Cleveland, OH 44195

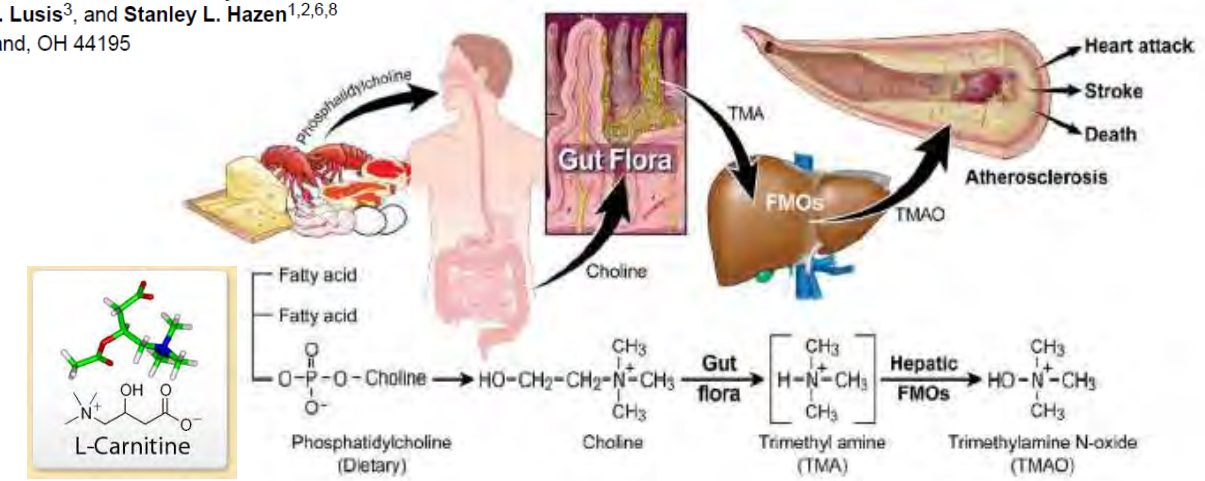


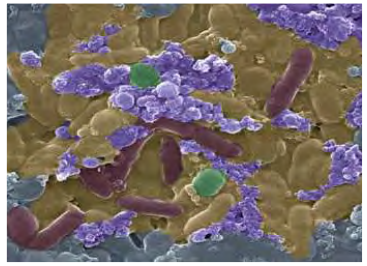
Figure 6. Gut flora dependent metabolism of dietary PC and atherosclerosis

Schematic summary illustrating newly discovered pathway for gut flora mediated generation of pro-atherosclerotic metabolite from dietary PC.

Koeth et al 2013 Nature Medicine

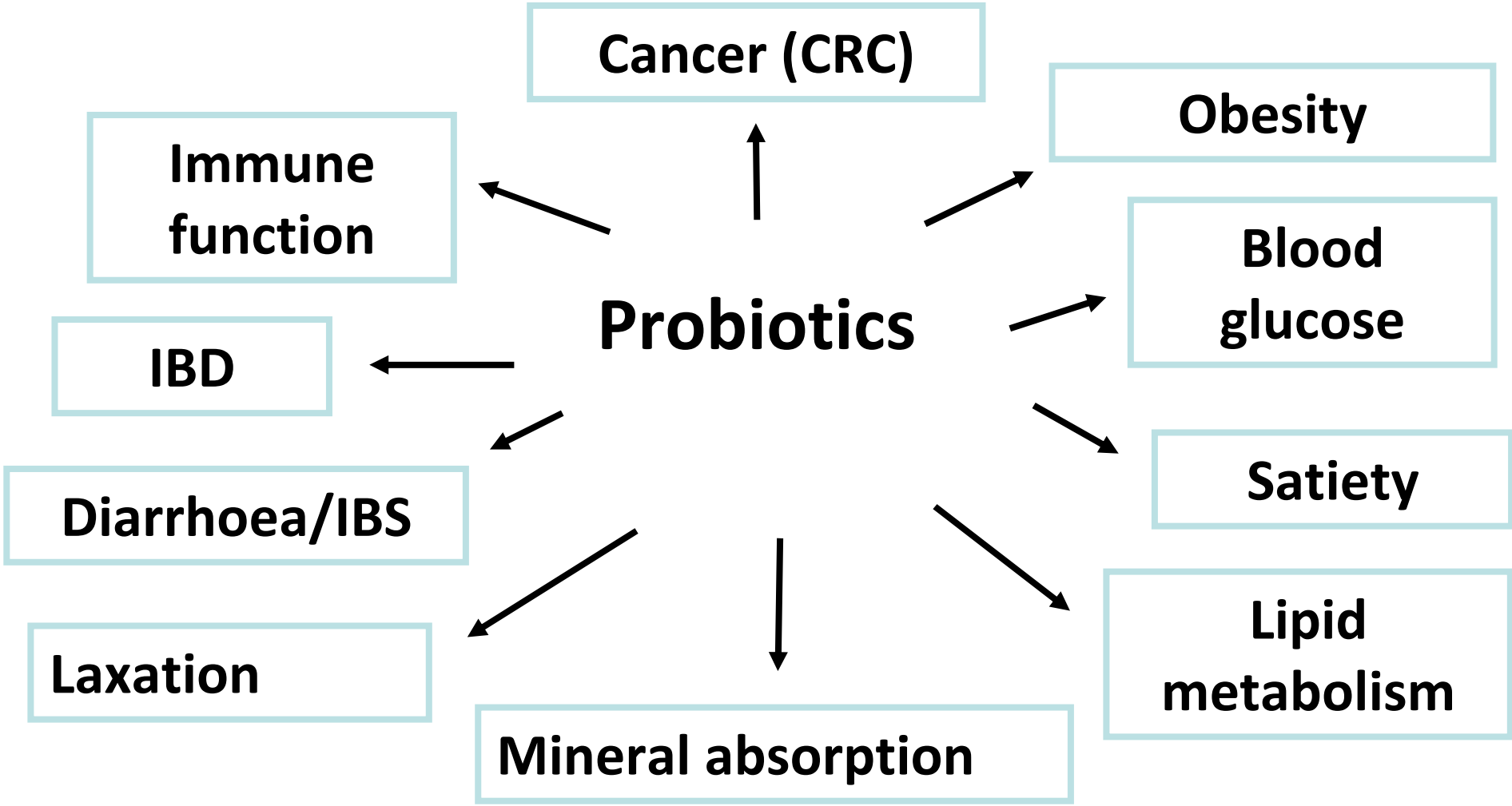
- TMA/TMAO confirmed strong link with CVD in patients
- confirmed microbiota metabolism of L-carnitine/choline → TMA→TMAO
- TMA not produced in vegans
- confirmed inflammatory activity & linked to macrophages reverse cholesterol transport
- TMAO reduced bile acid pool

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*, *Enteorcoccus faecium*, *Clostridium butyricum*, *Saccharomyces ceriviseae*
- **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



European Journal of Clinical Nutrition (2012) **66**, 1234–1241
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www.nature.com/ejcn

ORIGINAL ARTICLE

Cholesterol lowering and inhibition of sterol absorption by *Lactobacillus reuteri* NCIMB 30242: a randomized controlled trial

ML Jones[1,2], CJ Martoni[2] and S Prakash[1,2]

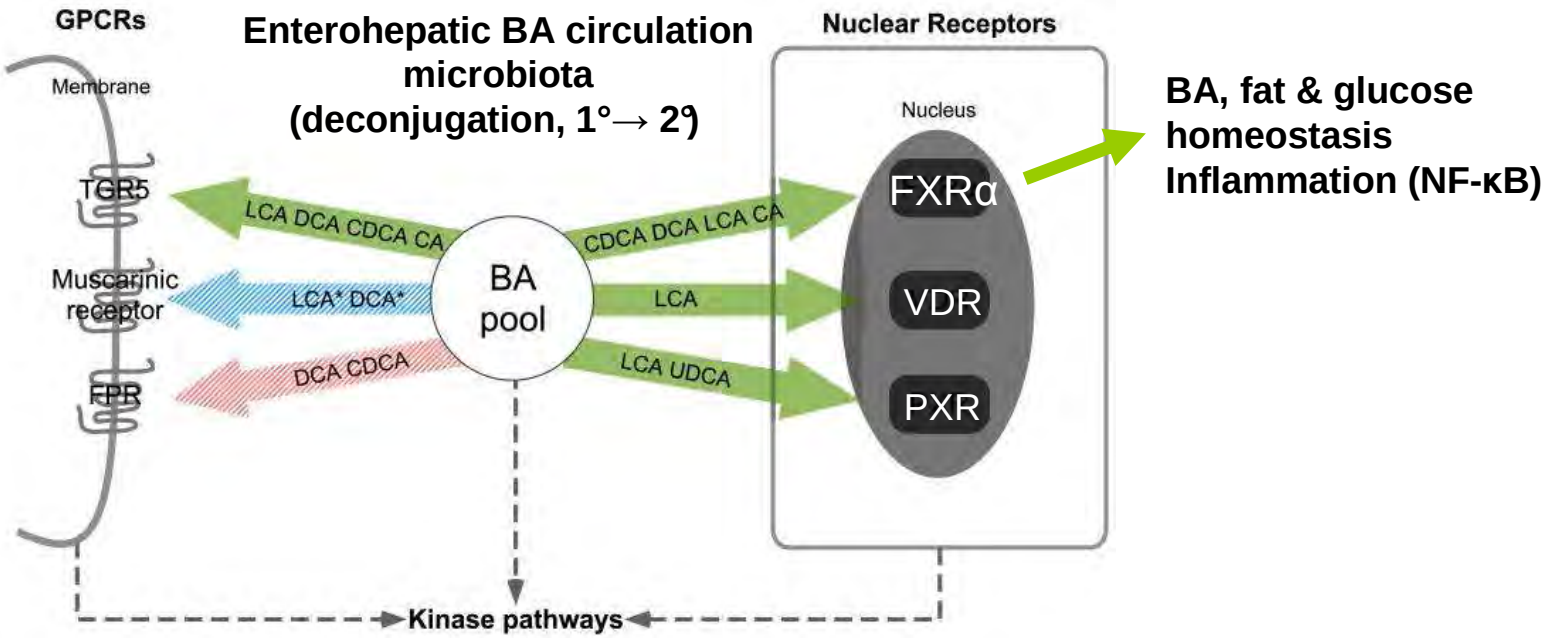
- *Lb. reuteri* selected for Bile Salt Hydrolase activity (2 capsules/day at 2 x 10^9 CFU/capsule) for 9 weeks
- Randomized, double-blind, placebo-controlled, parallel-arm, multicenter study
- N=127 hypercholesterolemic patients
- Probiotic reduced plasma
  - TC by 9.14%
  - LDL-C by 11.64%
  - LDL-C/HDL-C 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

The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation

Thijs W.H. Pols, Lilia G. Noriega, Mitsunori Nomura, Johan Auwerx, Kristina Schoonjans*

Laboratory of Integrative and Systems Physiology (LISP), Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Journal of Hepatology (2011)



Cell type	Species	Cellular action	References
Macrophages*	Human / Rabbit / Rat	Inhibition of cytokine production	[62, 68]
Enteroendocrine cells	Human / Mouse	Secretion of GLP-1	[71, 79]
Brown adipocytes	Mouse	Increase in energy expenditure	[69]
Sketelal muscle cells	Human	Increase in energy expenditure	[69]
Sinusoidal endothelial cells	Rat	Regulation of endothelial NO synthase	[67]
Biliary epithelial cells	Mouse	Promotion of chloride secretion	[65]
Astrocytes	Rat	Generation of ROS	[101]
Enteric neurons	Mouse	Release of NO and suppression of intestinal motility	[70]
Gallbladder smooth muscle cells	Mouse	Decrease of gallbladder smooth muscle cell function	[73]

Table 1. Cellular actions described for TGR5 in different cell types. ∗Macrophages include alveolar macrophages, Kupffer cells and THP-1 cells.

The 3Ps: Probiotics, Prebiotics & Polyphenols

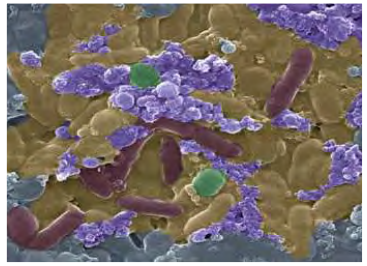


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Table 2. Human dietary interventions using prebiotic functional foods*

Prebiotic	Microbiological methods	Dose	Design	Results	References
Inulin	Culture	15 g/day for 15 days	Placebo-controlled, crossover study	Bifidobacteria ↑ and Gram-positive cocci ↓	Gibson *et al.* 1995
Inulin	Culture	20–40 g/day for 19 days	Placebo-controlled, crossover study	Bifidobacteria ↑, enterococci ↓ and enterobacteria ↓	Kleesen *et al.* 1997
Inulin	Culture	9 g/day for 28 days	Placebo-controlled, crossover study	Bifidobacteria ↑ and total facultative anaerobes ↓	Brighenti *et al.* 1999
Inulin	FISH	Up to 34 g/day for 64 days	Placebo-controlled, parallel study	Bifidobacteria ↑	Kruse *et al.* 1999
Inulin (long chain)	FISH	8 g/day for 14 days	Placebo-controlled, crossover study	Bifidobacteria ↑	Tuohy *et al.* 2001a, 2001b
Inulin	FISH	5 g/day and 8 g/day for 14 days	Placebo-controlled, crossover study	Bifidobacteria ↑	Kolida *et al.* 2007
Jerusalem artichoke inulin or chicory inulin	FISH	7.7 g/day for 7 days	Placebo-controlled, parallel study	Bifidobacteria ↑, bacteroides ↓ and clostridia ↓	Kleessen *et al.* 2007
Inulin	Culture	0.75, 1.00 or 1.25 g/day for 35 days	Placebo-controlled, parallel study	Bifidobacteria ↑, clostridia ↓ and Gram-positive cocci ↓	Yap *et al.* 2008
Inulin	qPCR	10 g/day for 16 days to 12 healthy adults	Treatment compared with no treatment period	Bifidobacteria ↑ and *Faecalibacterium prausnitzii* ↑	Ramirez-Farias *et al.* 2009
FOS	Culture	15 g/day for 15 days	Placebo-controlled, crossover study	Bifidobacteria ↑, bacteroides ↓, clostridia ↓ and fusobacteria ↓	Gibson *et al.* 1995
FOS	Culture	0–20 g/day for 7 days	Placebo-controlled, parallel study	Bifidobacteria ↑	Bouhnik *et al.* 1999
scFOS (Actilight™)	Culture	Daily dose of 2.5, 5.0, 7.5 and 10 g/day for 7 days	Placebo-controlled, crossover study	Bifidobacteria ↑	Bouhnik *et al.* 2006
FOS + PHGG	FISH	6.6 g/day FOS and 3.4 g/day PHGG for 21 days	Placebo-controlled, crossover study	Bifidobacteria ↑	Tuohy *et al.* 2001a, 2001b
FOS + GOS	Culture	4 g/L FOS and 8 g/L GOS for 28 days	Placebo-controlled, parallel study	Lactobacilli ↑ and bifidobacteria ↑	Moro *et al.* 2002
FOS + GOS	Culture	10 g/L for 28 days	Placebo-controlled, parallel study	Bifidobacteria ↑	Boehm *et al.* 2002
FOS + GOS	Culture	8 g/L for 42 days	Placebo-controlled, parallel study	Percentage of bifidobacteria ↑	Knol *et al.* 2005
GOS (TOS)	Culture	0–10 g/day for 56 days	Placebo-controlled, parallel study	Bifidobacteria ↑ and lactobacilli ↑	Ito *et al.* 1990
GOS (TOS)	Culture	2.5 g/day for 21 days	Feeding study	Bifidobacteria ↑	Ito *et al.* 1993
GOS (TOS)	Culture	10 g/day for 21 days	Feeding study	Bifidobacteria ↑	Bouhnik *et al.* 1997
GOS (TOS)	Culture	8.5 g/day and 14.4 g/day for 21 days	Placebo-controlled, parallel study	Bifidobacteria ↔	Alles *et al.* 1999
B-GOS	FISH	7 g/day for 7 days	Placebo-controlled, crossover study	Bifidobacteria ↑	Depeint *et al.* 2008
B-GOS	FISH	5.5 g/day for 10 weeks	Placebo-controlled, crossover study	Bifidobacteria ↑	Vulevic *et al.* 2008
GOS (TOS)	FISH	3.5 g/day and 7 g/day for 12 weeks	Placebo-controlled, parallel study	Bifidobacteria ↑	Silk *et al.* 2009

(*Continued*)

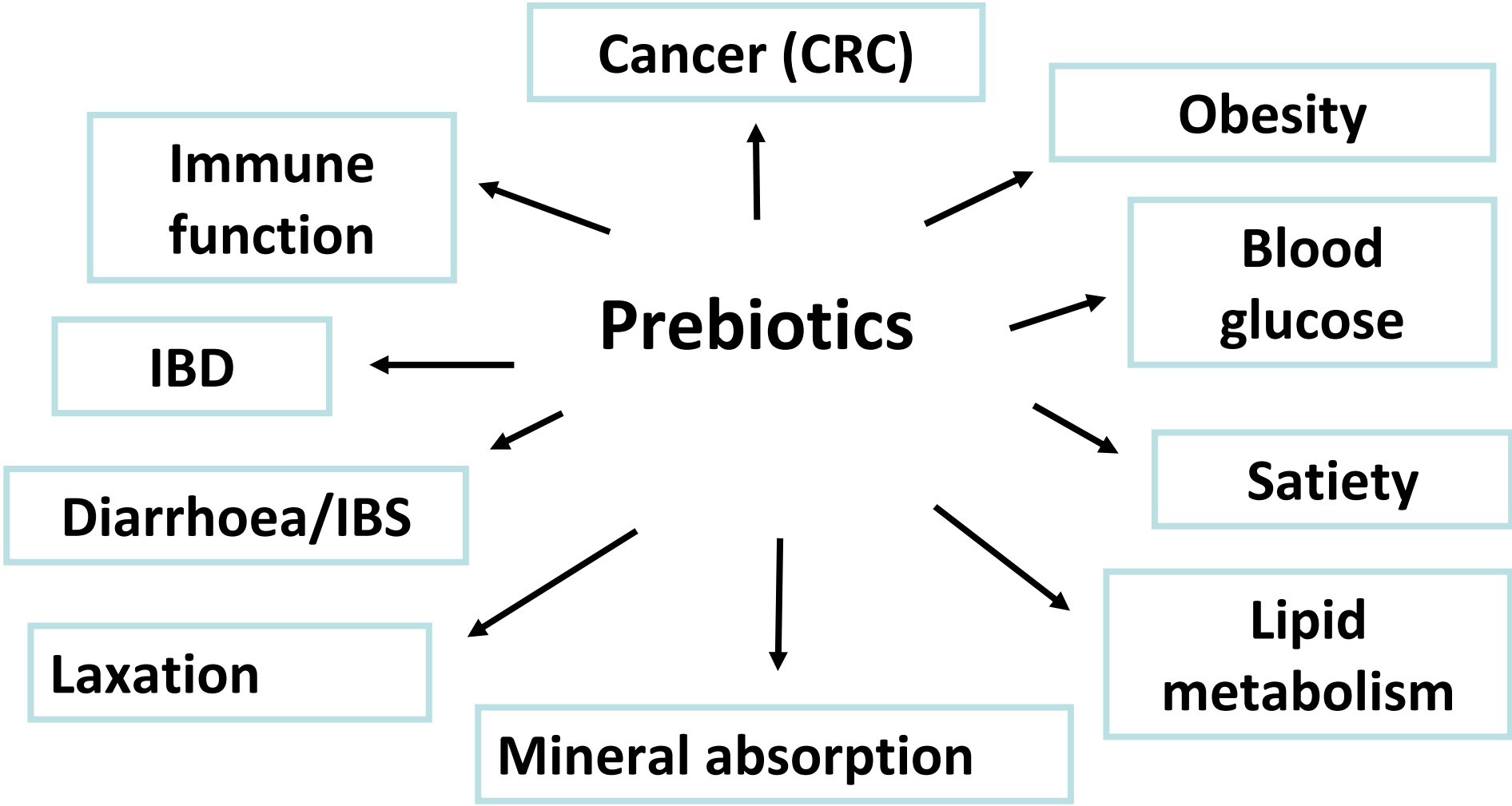
Table 2. (*Continued*)

Prebiotic	Microbiological methods	Dose	Design	Results	References
Lactulose	Culture	3 g/day for 14 days	Feeding study	Bifidobacteria ↑ and lecithinase-positive clostridia ↓	Terada *et al.* 1992
Lactulose	Culture	5 g/L and 10 g/L for 21 days	Feeding study	Bifidobacteria ↑ and clostridia ↓	Nagendra *et al.* 1995
Lactulose	Culture	20 g/day for 4 weeks	Placebo	Bifidobacteria ↑ and lactobacilli ↑	Ballongue *et al.* 1997
Lactulose	FISH + Culture	10 g/day for 26 days	Placebo-controlled, parallel study	Bifidobacteria ↑	Tuohy *et al.* 2002
Lactulose	Culture	10 g/day for 6 weeks	Placebo-controlled, parallel study	Bifidobacteria ↑	Bouhnik *et al.* 2004a, 2004b
IMO	Culture	13.5 g/day for 14 days	Feeding study	Bifidobacteria ↑	Kohmoto *et al.* 1988
IMO	Culture	5–20 g/day (variable dose) for 12 days	Feeding study	Bifidobacteria ↑	Kaneko *et al.* 1994
IMO	Culture	15 g/day for 7 days	Feeding study	Bifidobacteria ↑, lactobacilli ↑ and *Clostridium perfringens* ↓	Gu *et al.* 2003
SOS	Culture	3–5 g/day and 15 g/day	Placebo-controlled, crossover study	Bifidobacteria ↑, clostridia ↓ and bacteroides ↓	Benno *et al.* 1987
SOS	Culture	10 g/day for 21 days	Placebo-controlled, crossover study	Bifidobacteria ↑ and clostridia ↓	Hayakawa *et al.* 1990
Raffinose	FISH	2 g/day for 4 weeks	Placebo-controlled, crossover study	Bifidobacteria ↑	Dinoto *et al.* 2006
Resistant starch	Culture	10 g/day for 7 days	Placebo-controlled, parallel study	Bifidobacteria ↑	Bouhnik *et al.* 2004a, 2004b
Acacia gum	Culture	10 g/day and 15 g/day for 10 days	Placebo-controlled, parallel study	Bifidobacteria ↑	Cherbut *et al.* 2003
Whole grain wheat	FISH	48 g/day for 21 days	Placebo-controlled, crossover study	Bifidobacteria ↑ and lactobacilli ↑	Costabile *et al.* 2008
Gum Arabic	qPCR	Dose response (5–40 g/day) for 4 weeks	Parallel, different doses, negative control (water), positive control 10 g/day inulin	Bifidobacteria ↑, lactobacilli ↑ and bacteroides ↑ at 10 g/day	Calame *et al.* 2008
Arabinoxylan-oligosaccharides	qPCR	10 g/day for 21 days	20 healthy adults, placebo-controlled, crossover study	Bifidobacteria ↑ (bifidobacteria ↑ with placebo)	Cloetens *et al.* 2010

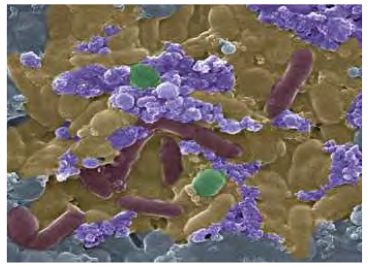
*The microbiological methods are listed together with the dose of prebiotic, the study design and the main microbiological results in terms of faecal microbiota modulation.

FISH, fluorescent *in situ* hybridisation; qPCR, quantitative PCR; FOS, fructo-oligosaccharides; scFOS, short-chain fructo-oligosaccharides; PHGG, partially hydrolysed guar gum; GOS, galacto-oligosaccharides; TOS, trans-galacto-oligosaccharides; B-GOS, Bimuno-GOS; IMO, isomalto-oligosaccharides; SOS, soybean oligosaccharides.

Gut microbiota and systemic health



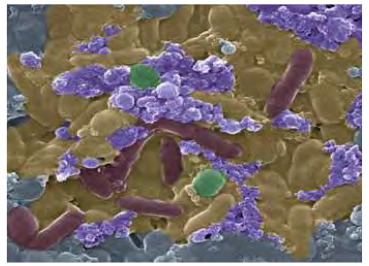
Delaying the progression of obesity with fermentable carbohydrates and prebiotics

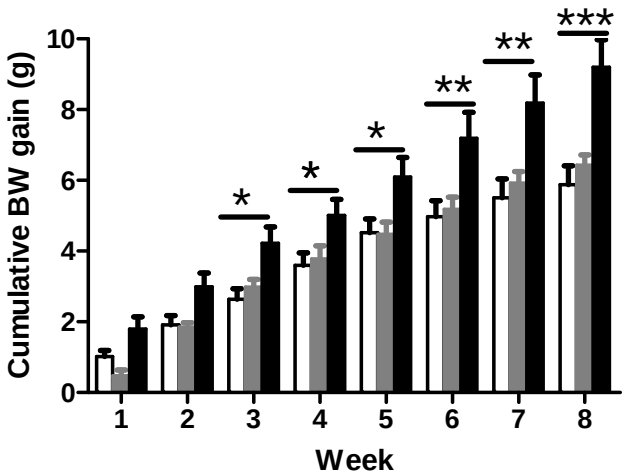


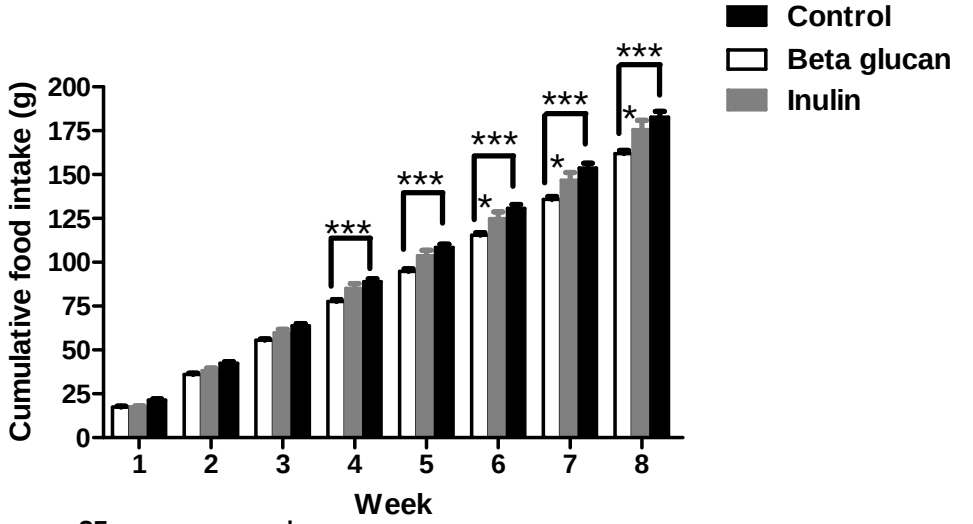
- Does dietary supplementation with prebiotics or fermentable CHO/fiber reduce body weight through enhanced satiety
- High fat fed animals (control)
- High fat supplemented with Inulin (Synergy 1 (10% w/w)
- High fat supplemented with β-glucan (10% w/w)
  - Diets were isoenergetic with cellulose used 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

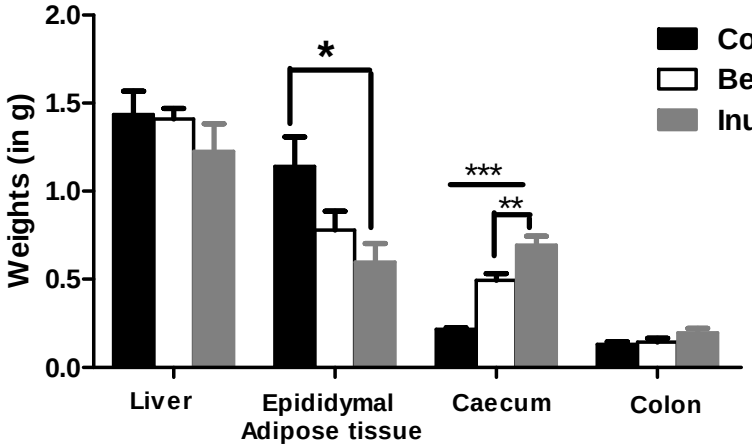
Tulika Arora, Gary Frost et al. PLoS One 2012

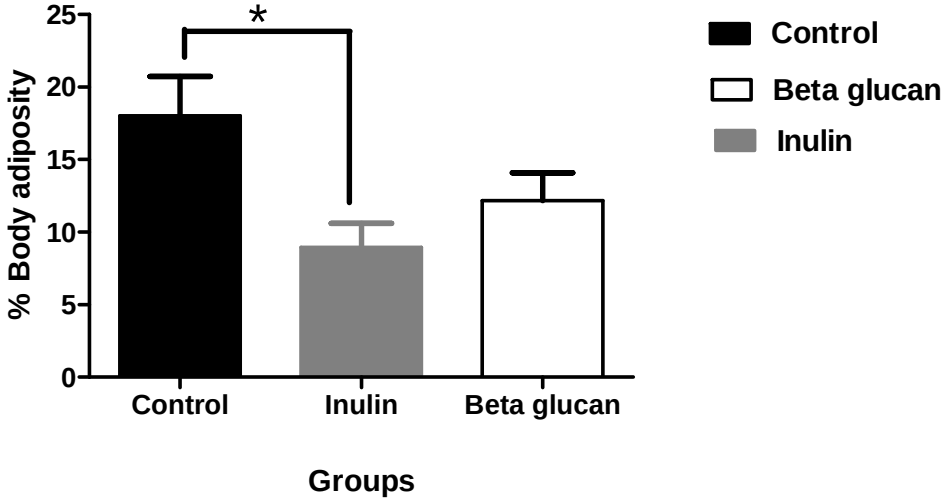
Inulin and β-glucan reduce body weight gain



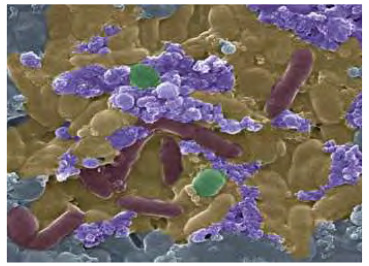








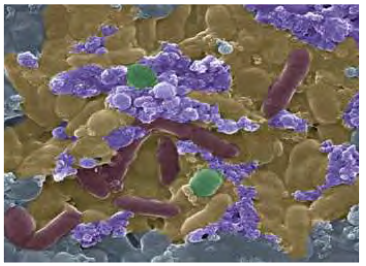
Effect of inulin and β-glucan supplementation on adiposity parameters and PYY level in high fat fed mice.

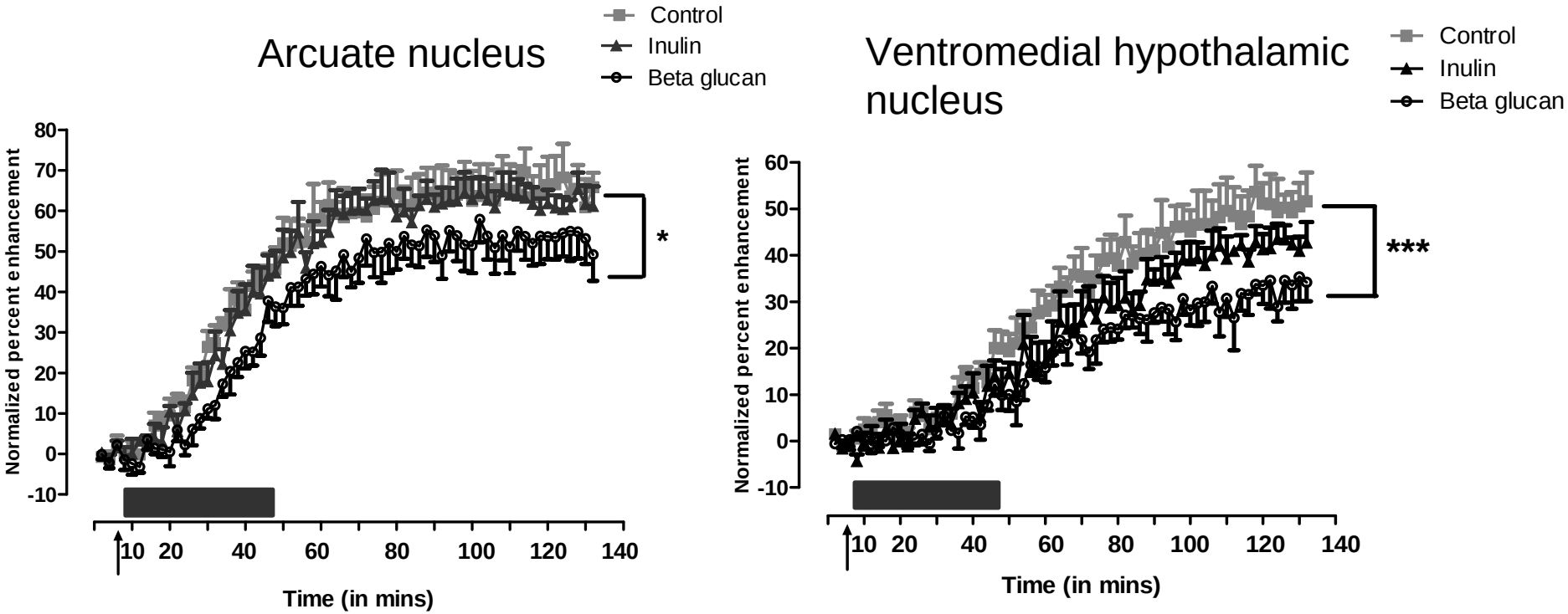


	HFD-C	HFD-I	HFD-BG
Epididymal adipose tissue (g)	1.14±0.16[a]	0.59±0.10[b]	0.77±0.10[a]
Whole body adiposity (%)	18.03±2.72[a]	8.95±1.66[b]	12.17±1.92[a]
Liver lipid content (%)	6.30±1.62[a]	6.02±1.97[a]	6.02±1.36[a]
Muscle lipid content (%)	0.96±0.149[a]	0.72±0.05[a]	1.29±0.57[a]
Visceral fat (g)	2.17±0.46[a]	1.23±0.17[a]	1.49±0.27[a]
Subcutaneous fat (g)	3.40±0.53[a]	2.08±0.13[a]	2.44±0.28[a]
Adipocyte size (μm)	122.25±10.2[a]	72.95±8.72[b]	111.19±4.03[ac]
Adipocyte number (x10^7)	1.43E+08[a]	1.31E+08[a]	1.86E+08[a]
Liver size (g)	1.43±0.13[a]	1.23±0.15[a]	1.40±0.06[a]
Caecum (g)	0.21±0.01[a]	0.69±0.05[b]	0.49±0.03[c]
Colon (g)	0.13±0.01[a]	0.19±0.02[a]	0.14±0.02[a]
PYY (pmol/ml)	0.10±0.012[a]	0.10±0.008[a]	0.13±0.016[a]
Colonic PYY	27.3 3.7	22.8 5.3	19.9 1.6

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

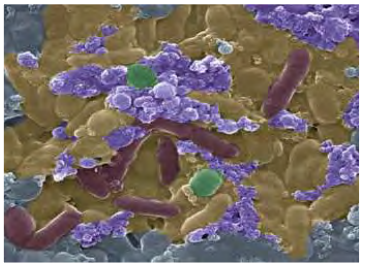
Effect of inulin and β-glucan supplementation on changes in signal intensity in the appetite centres of the brain measured by MRI

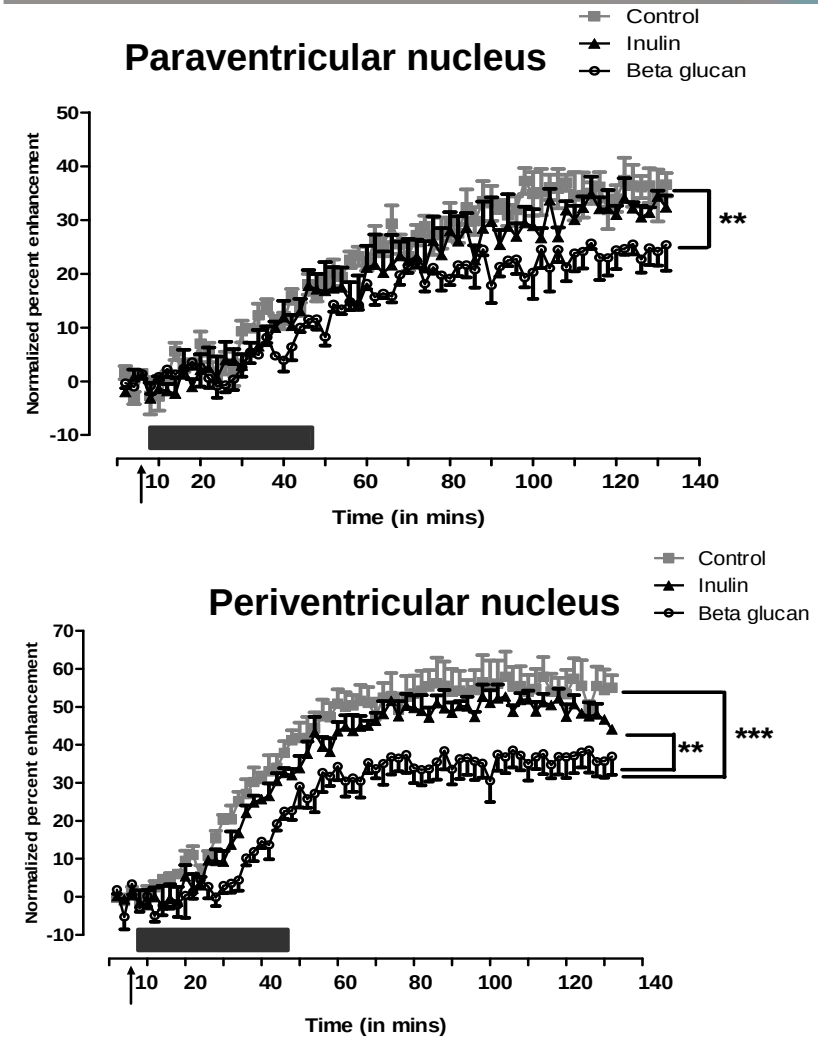


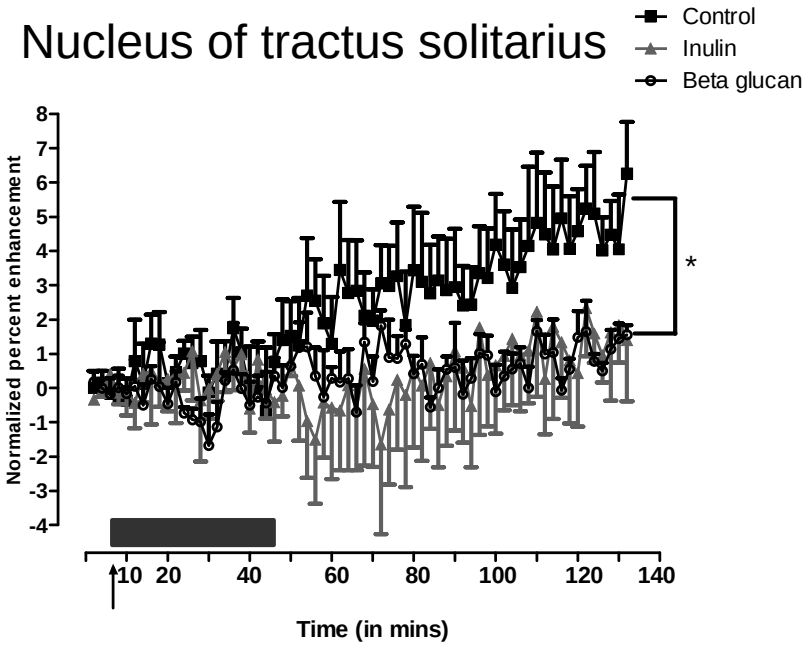


The arrow shows the start of Mn2+ infusion and grey bar represents the duration of Mn2+ infusion.

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

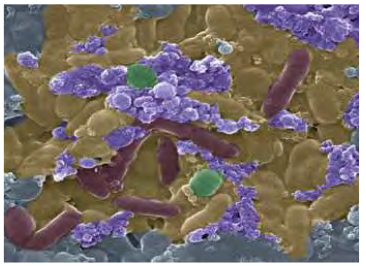


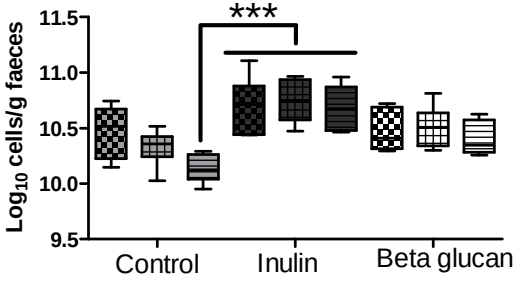




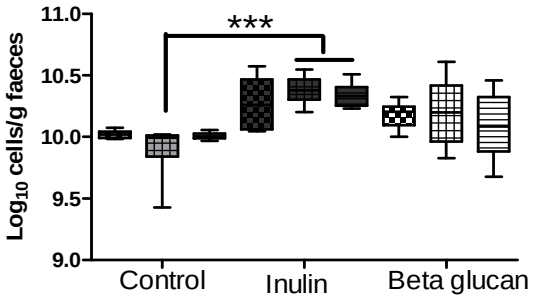
The arrow shows the start of Mn2+ infusion and grey bar represents the duration of Mn2+ infusion.

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

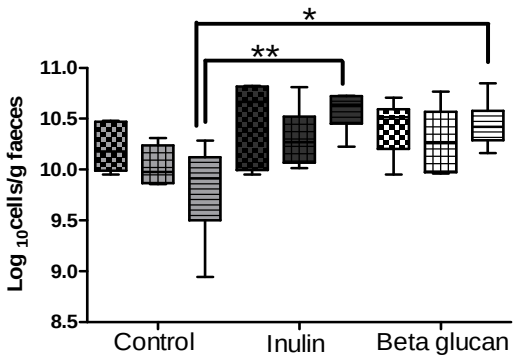




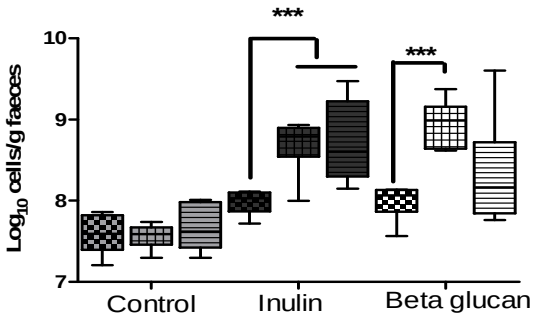
A: Total bacteria



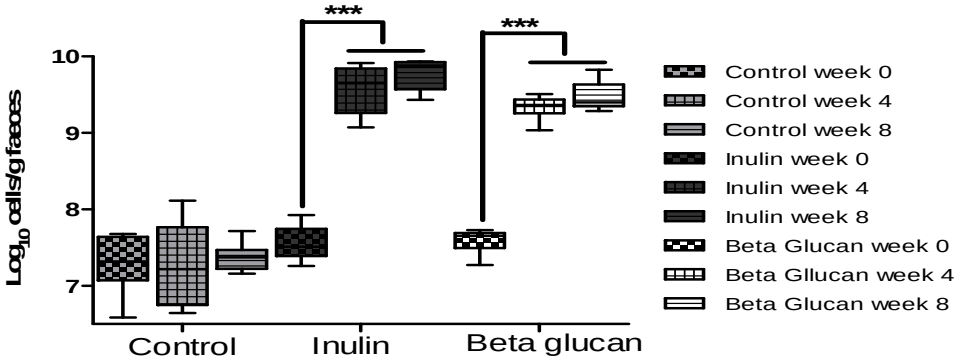
B: Mouse Intestinal Bacteria



C: *Eubacterium rectale-Clostridium coccoides*



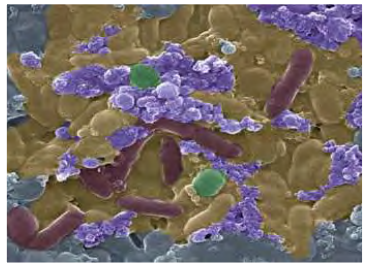
D: *Lactobacillus*

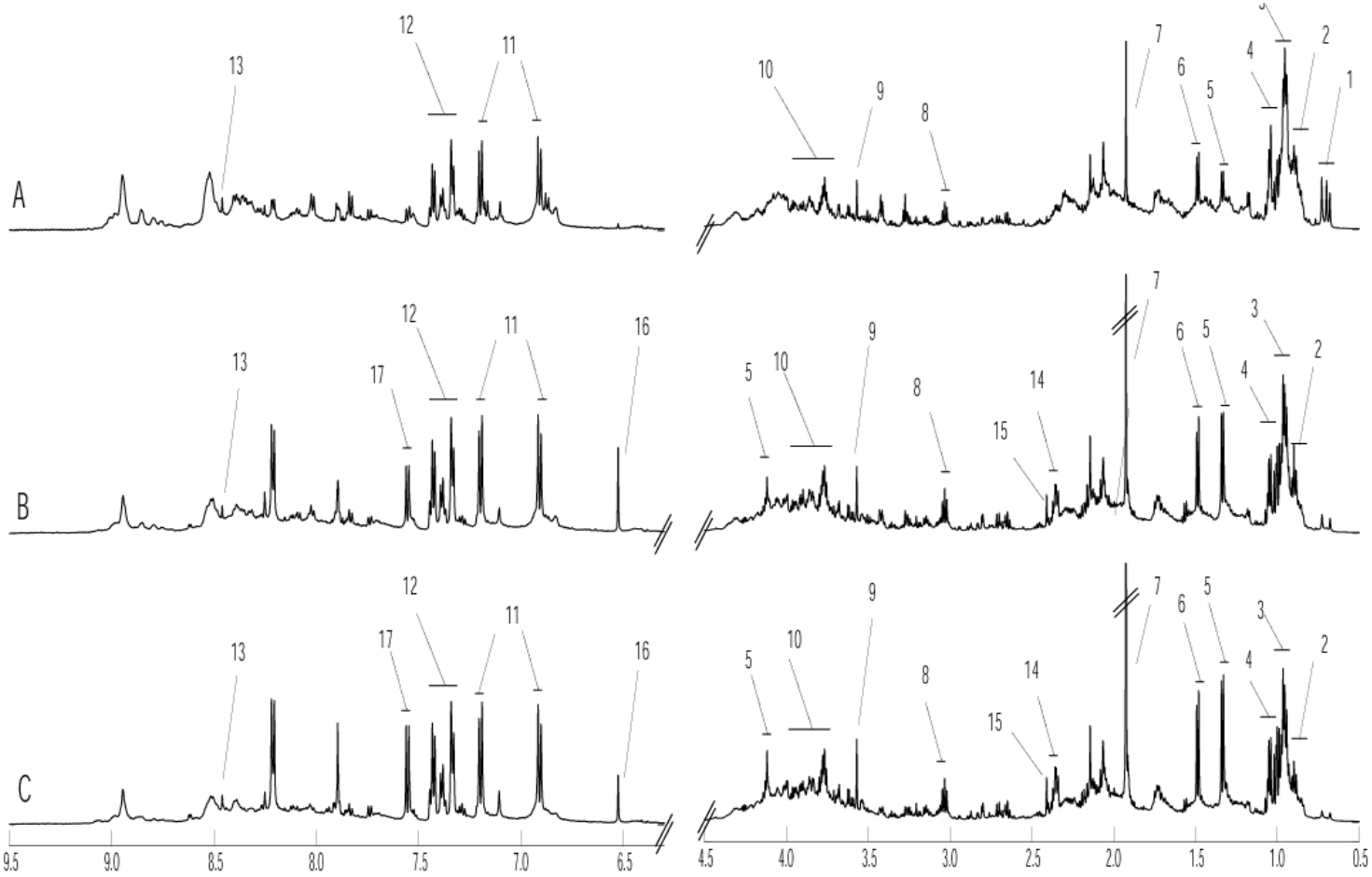


E: *Bifidobacterium*

Similar findings observed for caecal contents at week 8.

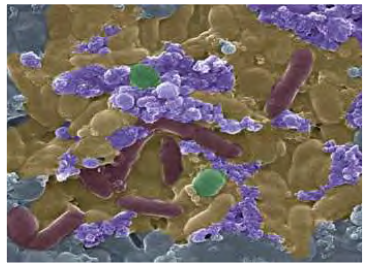
Effect of inulin and β-glucan supplementation on murine faecal metabolite profiles (NMR) compared to high fat diet supplemented with cellulose



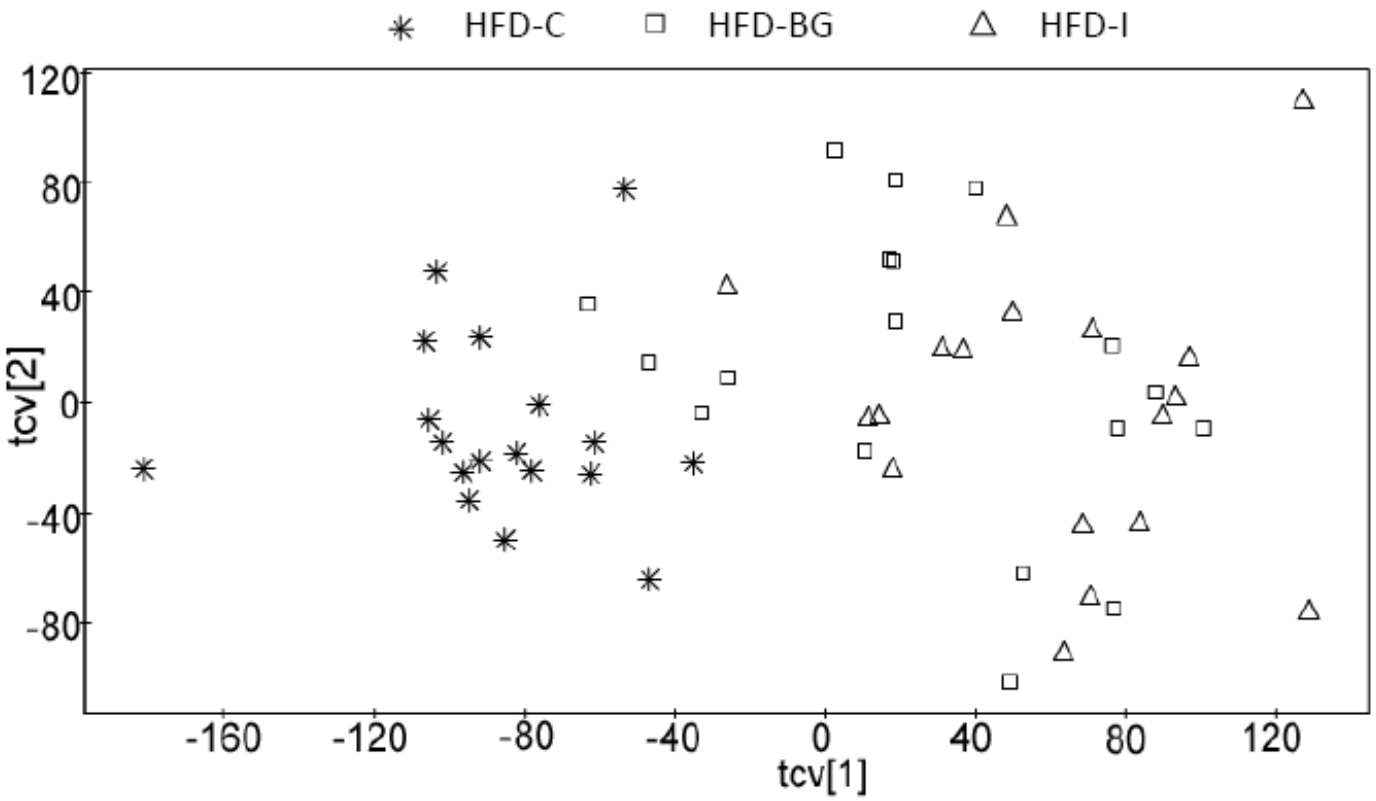


Key: 1, Bile acids; 2, Butyrate; 3,Isoleucine, leucine and valine; 4, Propionate; 5, Lactate; 6, Alanine; 7, Acetate; 8, Lysine; 9, Glycine; 10, Glucose and amino acids; 11, Tyrosine; 12, Phenylalanine, 13, Formate; 14, Glutamate; 15, Succinate; 16, Fumarate; 17, Uracil.

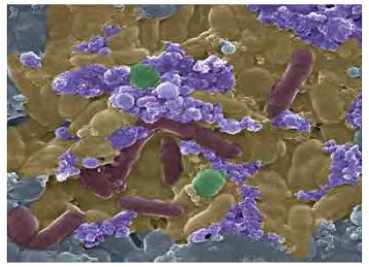
NMR based metabolomics separates cellulose from inulin or β-glucan supplemented animals on high fat diets



PCA scores plot of fecel metabolite profiles showing clear clustering patterns for mice fed with HFD-C, HFD-BG and HFD-I groups.



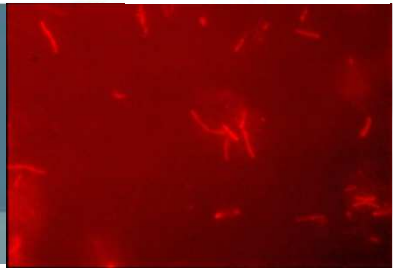
Fermentable fibers/prebiotics reduce body weight but by different mechanisms



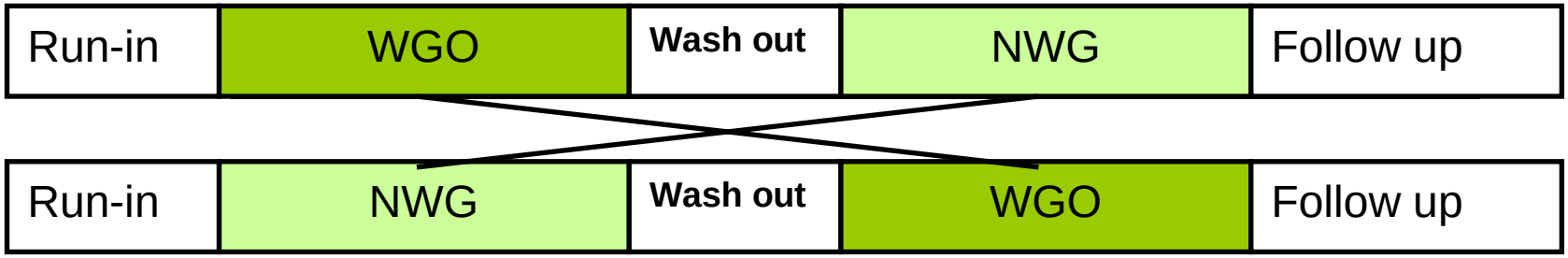
- β-glucan reduced cumulative body weight apparantly through reduced stimulation of hypothalamic appetite centres, increased satiety and reduced food intake.
- Inulin appeared to reduce cumulative body weight gain through reduced adipocyte size and whole body adiposity
- SCFA concentrations in the caecum 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

Arora et al. PLoS One 2012

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

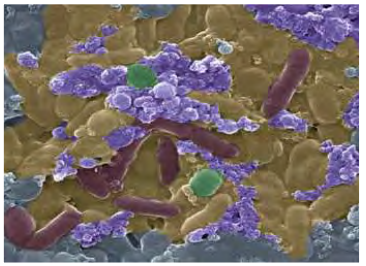


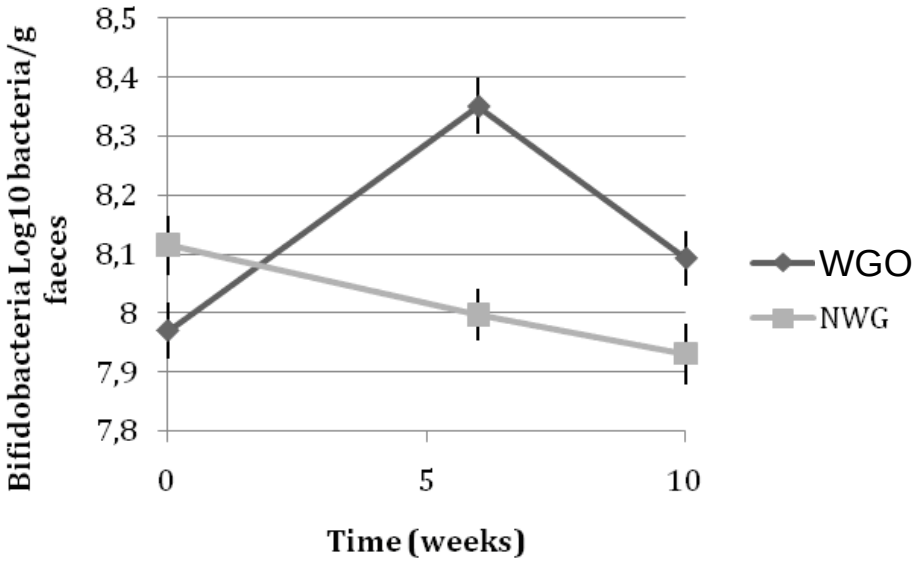
- Two 6 week treatment periods separated by 4 week washout periods.
- Whole oat grain (WGO) vs non-whole grain cereal (NWG)
- Samples collected before and after cereal consumption and then 4 weeks following end of consumption.
- Blood (fasted), 24 hour urine, saliva and fecal samples

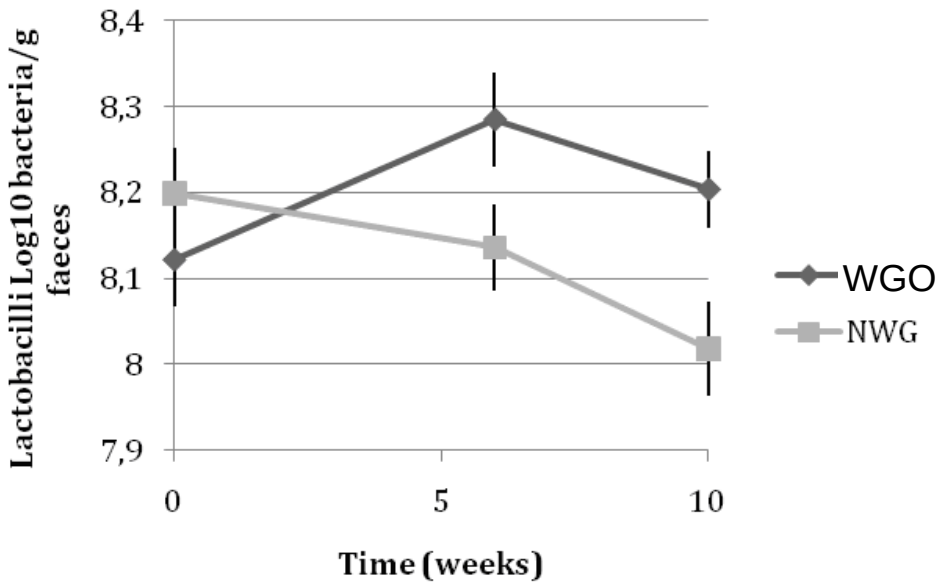
Connolly et al. In preparation

Supported by **Jordans Cereals**

Whole grain oats modified gut microbiota in beneficial manner compared to non-whole grain cereal

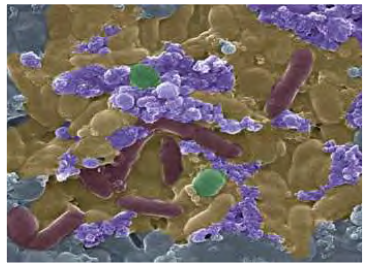


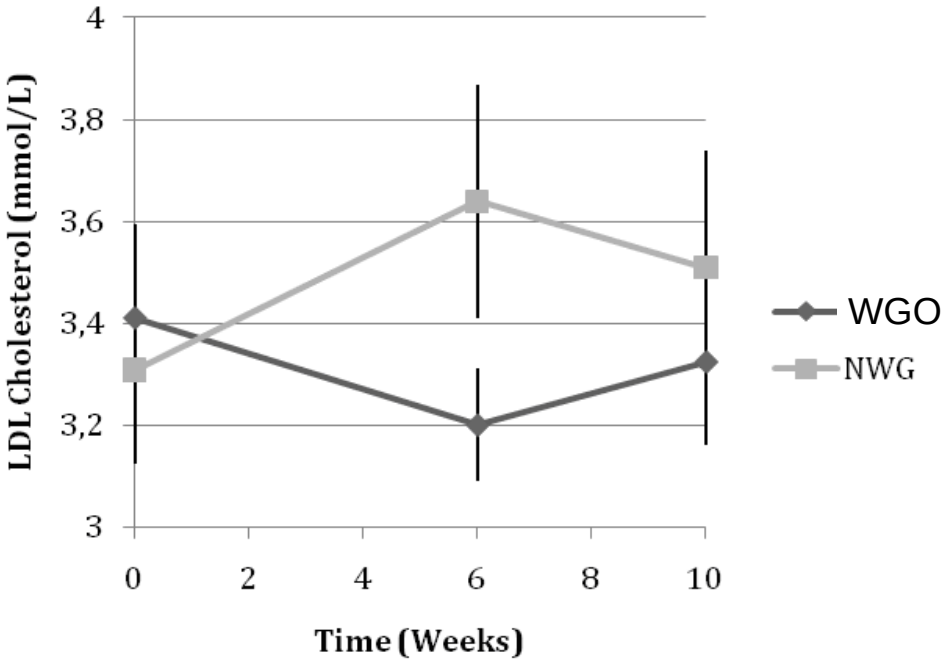


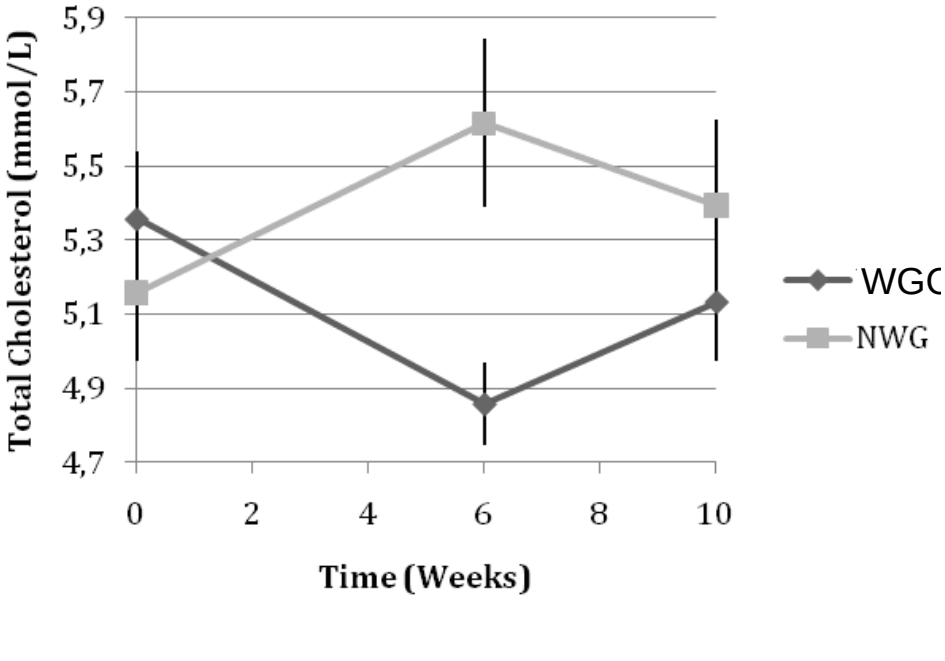


Whole grain oats significantly increased faecal bifidobacteria and lactobacilli but no other bacterial groups measured.

Whole grain oats improved blood cholesterol profiles

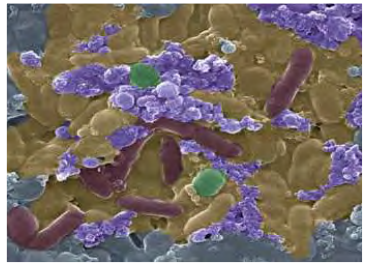






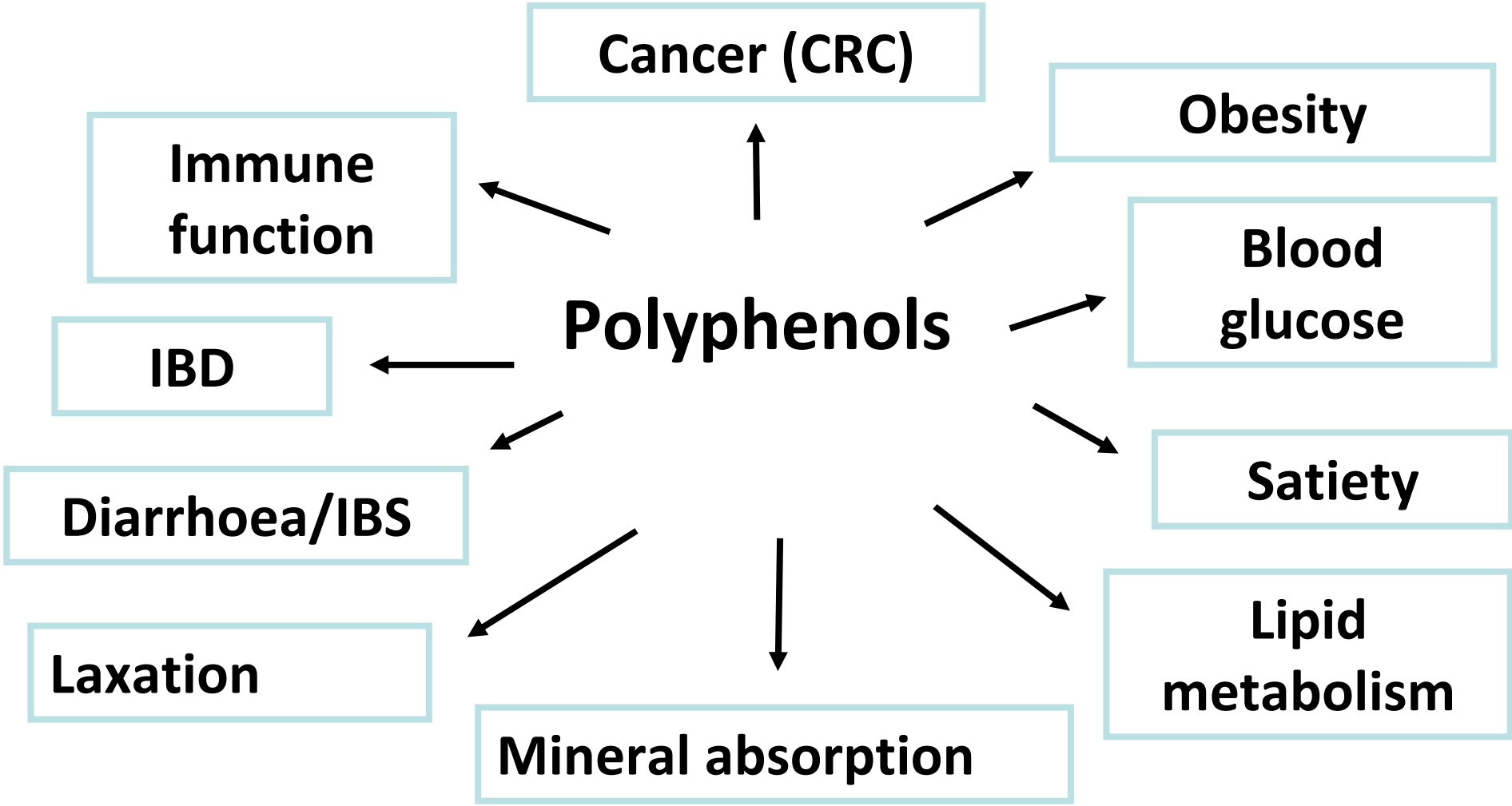
- Whole grain oats significantly reduced LDL and total cholesterol, reversing a trend towards elevated LDL and TC in the non-whole grain breakfast cereal treatment.

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*, *Enteorcoccus faecium*, *Clostridium butyricum*, *Saccharomyces ceriviseae*
- **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

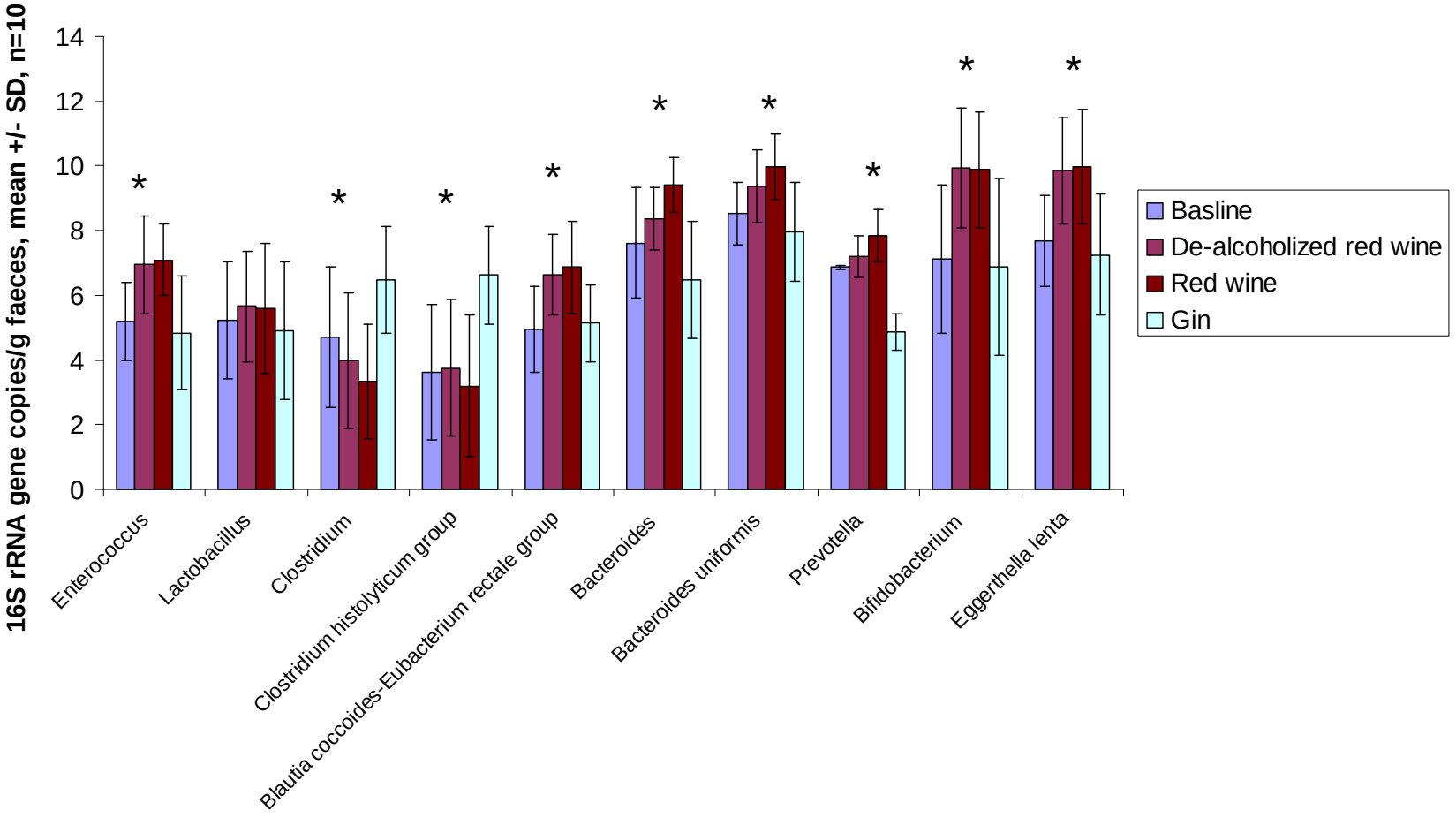
Gut microbiota and systemic health



Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers[1–4]

María Isabel Queipo-Ortuño, María Boto-Ordóñez, Mora Murri, Juan Miguel Gomez-Zumaquero, Mercedes Clemente-Postigo, Ramon Estruch, Fernando Cardona Diaz, Cristina Andrés-Lacueva, and Francisco J Tinahones

American Journal of Clinical Nutrition, 2012



Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers[1–4]

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The American Journal of Clinical Nutrition

TABLE 4
Anthropometric and biochemical variables during the study[1]

	Baseline (washout period)	De-alcoholized red wine period	Red wine period	Gin period	P^2
Weight (kg)	97.8 ± 21.3	97.8 ± 19.4	96.4 ± 20.6	97.2 ± 19.6	0.306
Waist (cm)	106.7 ± 14.3	106.5 ± 14.4	105.1 ± 14.5	105.7 ± 13.5	0.392
Hip (cm)	111.0 ± 10.4	109.0 ± 12.8	110.2 ± 11.1	110.8 ± 10.3	0.908
DBP (mm Hg)	97.4 ± 15.2^a	91.0 ± 12.9^a	86.5 ± 11.6^b	98.4 ± 14.3^a	0.026
SBP (mm Hg)	145.4 ± 23.9^a	135.1 ± 24.6^b	129.5 ± 17.6^b	142.7 ± 22.3^a	0.026
BMI (kg/m^2)	27.6 ± 3.2	27.6 ± 3.1	27.5 ± 2.9	27.6 ± 2.8	0.241
Glucose (mg/dL)	111.3 ± 23.1	104.5 ± 24.2	108.5 ± 16.4	108.8 ± 17.2	0.772
Uric acid (mg/dL)	5.7 ± 1.1^a	5.3 ± 1.0^a	5.0 ± 0.8^b	5.4 ± 1.5^a	0.018
GOT (mg/dL)	22.0 ± 7.3^a	14.3 ± 4.0^b	17.6 ± 13.4^b	19.1 ± 8.0^a	0.021
GPT (mg/dL)	46.4 ± 12.6	41.2 ± 7.7	42.0 ± 9.3	43.1 ± 6.9	0.888
GGT (mg/dL)	36.9 ± 25.6^a	30.1 ± 13.5^b	36.1 ± 16.3^b	38.0 ± 27.7^a	0.012
Triglycerides (mg/dL)	245.4 ± 231.7^a	171.7 ± 206.7^b	179.4 ± 177.1^b	190.1 ± 222.5^b	0.001
Cholesterol (mg/dL)	257.5 ± 88.6^a	241.2 ± 94.9^a	188.6 ± 61.6^b	235.3 ± 91.4^a	0.008
LDL cholesterol (mg/dL)	129.6 ± 41.9	123.5 ± 28.1	125.7 ± 30.3	130.6 ± 22.0	0.266
HDL cholesterol (mg/dL)	58.5 ± 16.7^a	48.8 ± 17.1^b	49.7 ± 14.3^b	52.3 ± 16.5^a	0.001
CRP (mg/L)	6.9 ± 2.6^a	4.3 ± 2.3^b	4.6 ± 2.5^b	6.8 ± 3.7^a	0.001

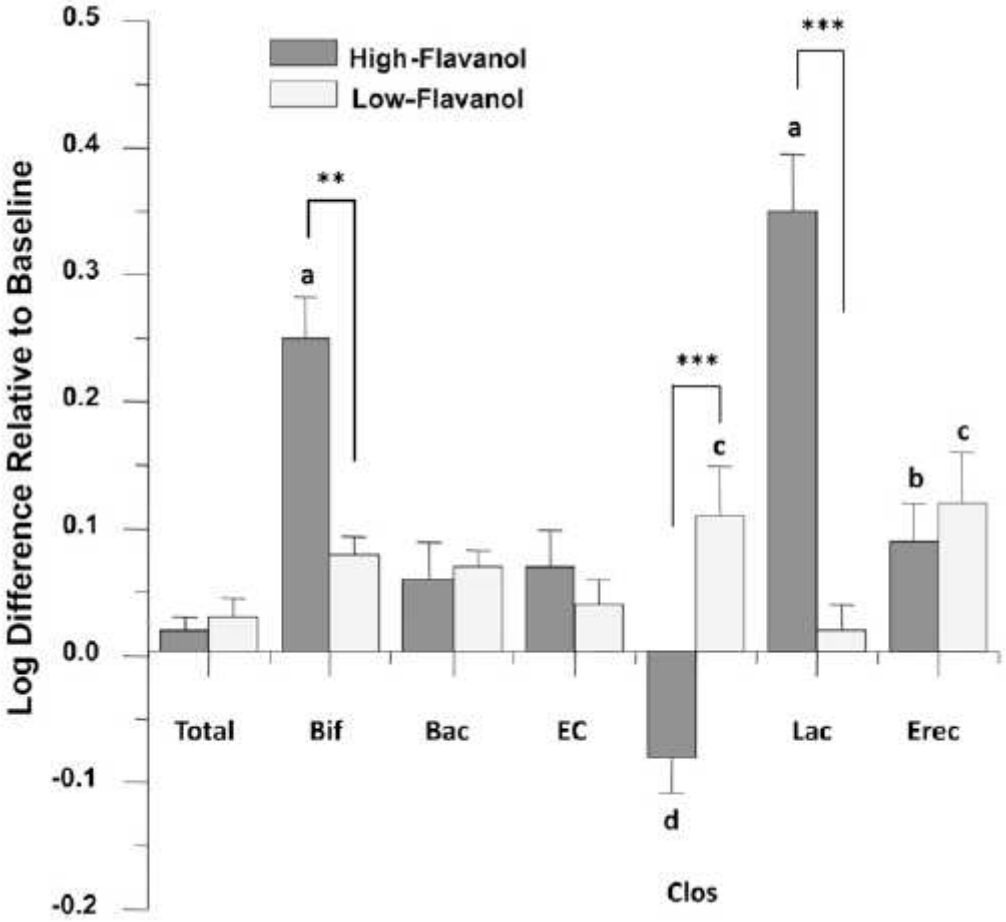
[1] All values are means ± SDs; $n = 10$ subjects. Means in a row with different superscript letters are significantly different, $P < 0.05$ (Wilcoxon's signed-rank test with post hoc Bonferroni test). CRP, C-reactive protein; DBP, diastolic blood pressure, GGT, γ-glutamyl transferase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; SBP, systolic blood pressure.

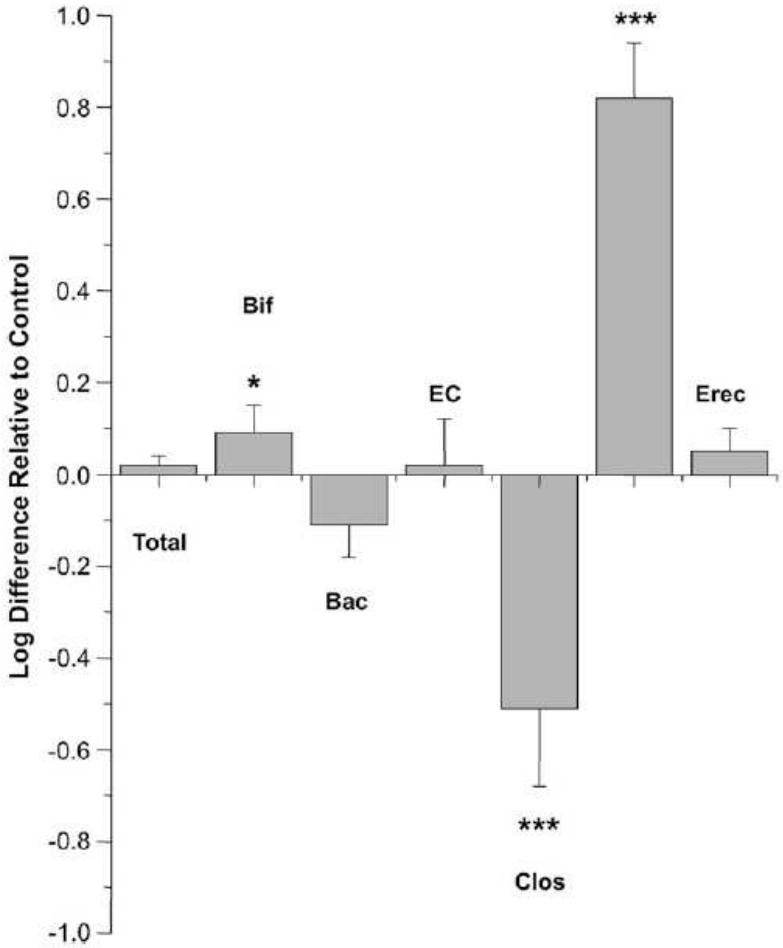
[2] Derived by using the Friedman test.

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

Xenofon Tzounis, Ana Rodriguez-Mateos, Jelena Vulevic, Glenn R Gibson, Catherine Kwik-Uribe, and Jeremy PE Spencer

The American Journal of Clinical Nutrition **(2011) 93:62-72.**





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

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The American Journal of Clinical Nutrition

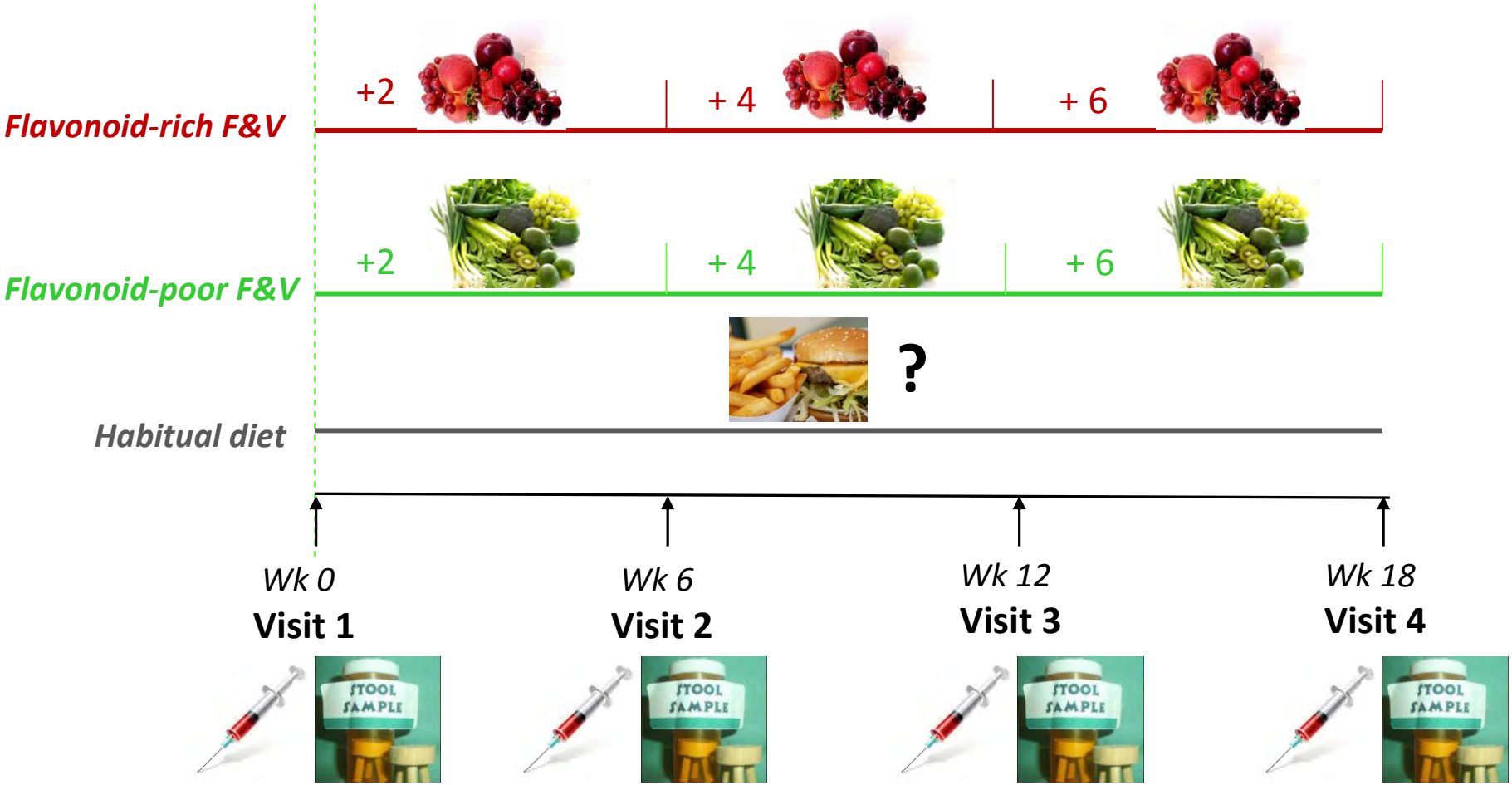
TABLE 3
Anthropometric and biochemical variables before (Pre) and after (Post) the 4-wk intervention with either the low–cocoa flavanol or high–cocoa flavanol drink ($n = 20$)[1]

	Low-flavanol cocoa		High-flavanol cocoa	
	Pre	Post	Pre	Post
BMI (kg/m^2)	23.1 ± 2.02	23.2 ± 2.06	23.2 ± 2.1	23.2 ± 2.1
Diastolic BP (mm Hg)	71.4 ± 12.95	72.0 ± 11.75	69.8 ± 6.9	68.8 ± 7.2
Systolic BP (mm Hg)	107.3 ± 7.81	105.8 ± 11.32	110.0 ± 11.6	105.7 ± 12.8
Total cholesterol (mmol/L)	4.70 ± 0.19	4.31 ± 0.1[2]	4.77 ± 0.2	4.34 ± 0.2[2]
HDL cholesterol (mmol/L)	1.36 ± 0.08	1.29 ± 0.08	1.39 ± 0.09	1.33 ± 0.11
LDL cholesterol (mmol/L)	2.63 ± 0.16	2.50 ± 0.14	2.57 ± 0.17	2.48 ± 0.16
Triacylglycerol (mmol/L)	1.06 ± 0.08	1.05 ± 0.07	1.07 ± 0.08	0.87 ± 0.09[2,3]
Glucose (mmol/L)	5.13 ± 0.10	5.08 ± 0.10	5.13 ± 0.13	5.18 ± 0.12
C-reactive protein (mg/mL)	0.26 ± 0.11	0.31 ± 0.14	0.27 ± 0.12	0.19 ± 0.09[2,3]
Fecal water TAC (mmol/L Trolox[4])	479.2 ± 48.3	459.7 ± 41.3	487.2 ± 46.6	473.2 ± 55.6

[1] All values are means ± SDs. BP, blood pressure; TAC, total antioxidant capacity. Significance was calculated by the Tukey-Kramer test after 2-factor repeated-measures ANOVA with time and treatment as the 2 factors.
[2] Significantly different from baseline, $P < 0.05$
[3] Significantly different from low–cocoa flavanol interventions, $P < 0.01$.
[4] Trolox (Sigma Chemical Co, Poole, United Kingdom).

Increasing fruit and vegetable intake *in vivo* – FLAVURS project

FLAVURS
Flavonoids University of Reading Study



High Flavonoid group

FLAVURS
Flavonoids University of Reading Study

Apple crumble
Dried cranberries/ blueberries

Fruit smoothies
(Strawberry and raspberry/ Blackberry and blueberry)

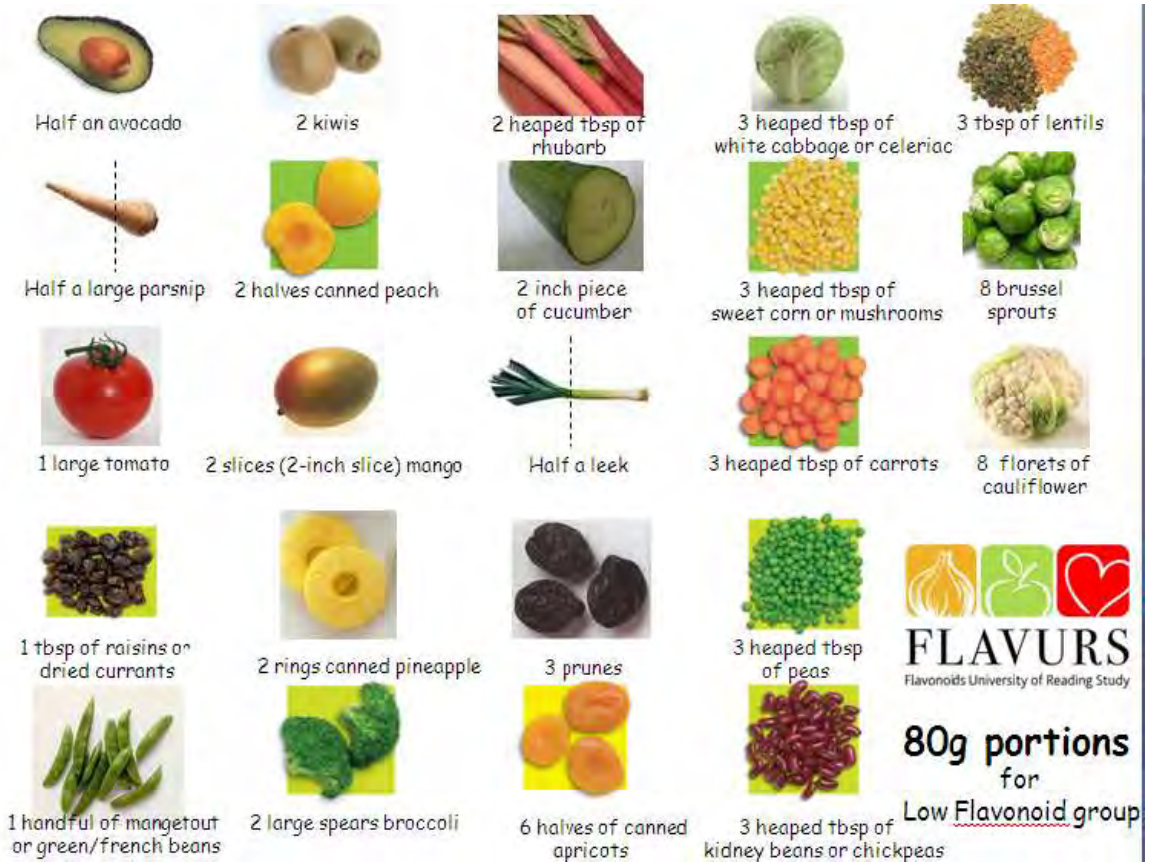
Fruit juices
(Blackcurrant /apple/cranberry /orange)

Roasted peppers
Pepperdew cherry peppers

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

Low Flavonoid group

FLAVURS
Flavonoids University of Reading Study



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

Rhubarb crumble
Dried fruits (raisins, currants, mango)

Fruit smoothies (tropical mix)

Fruit juices (mango/ pineapple)

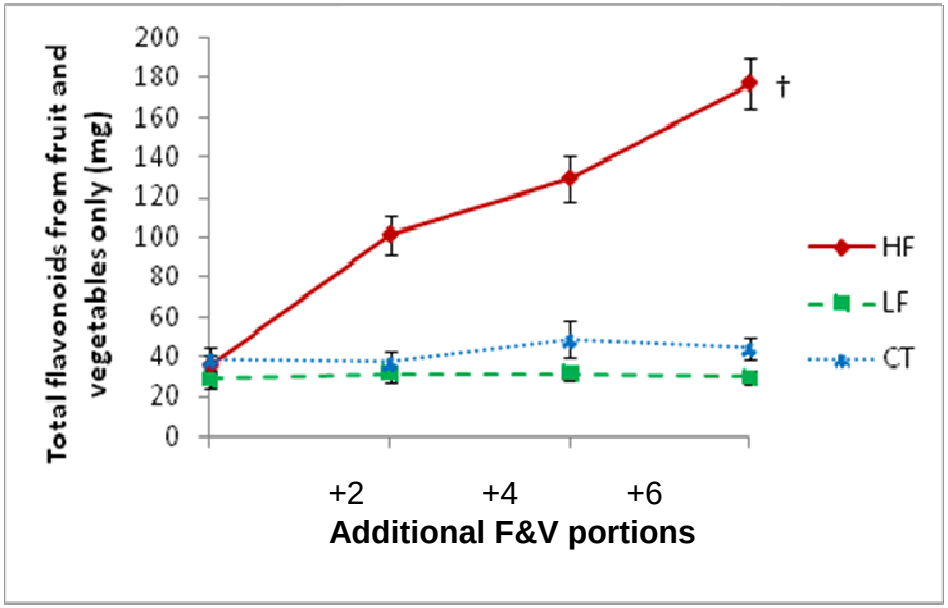
Guacamole
Houmous

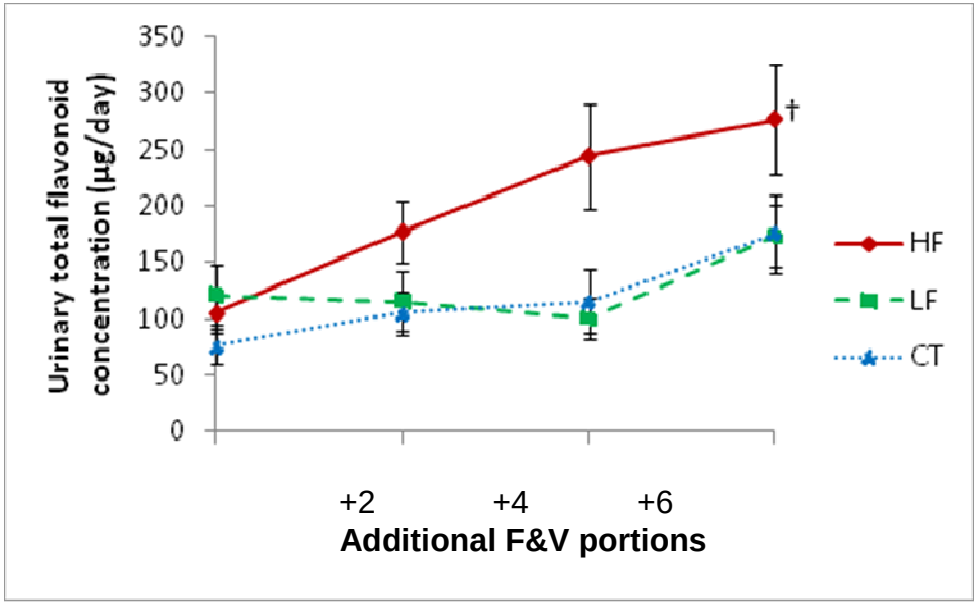
Soups
(Carrot & coriander/broccoli & stilton)

Canned chopped tomatoes

FLAVONOIDS

FLAVURS
Flavonoids University of Reading Study



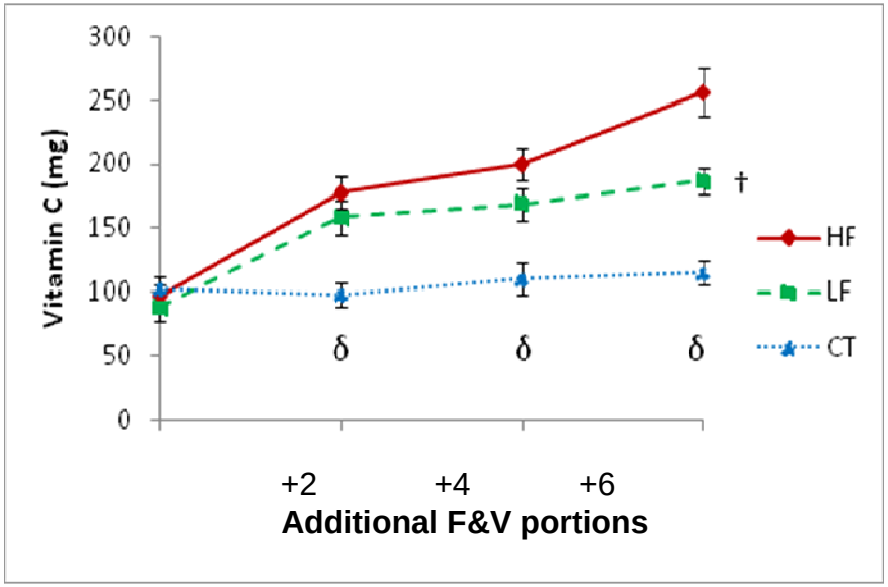


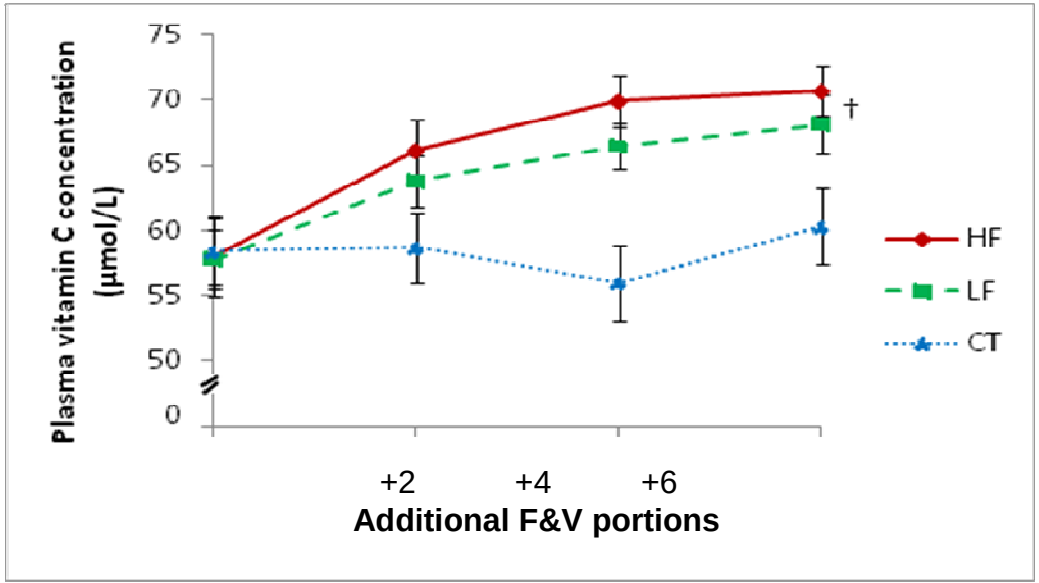
Dietary intake: HF dose dependent increase
HF higher vs LF & CT +2,+4,+6
Time x treatment (P=0.006)

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

FLAVURS
Flavonoids University of Reading Study



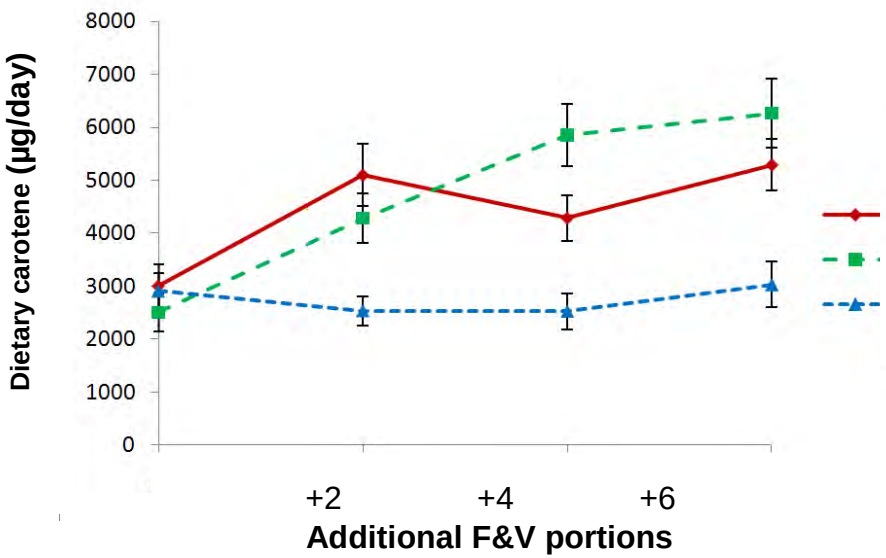


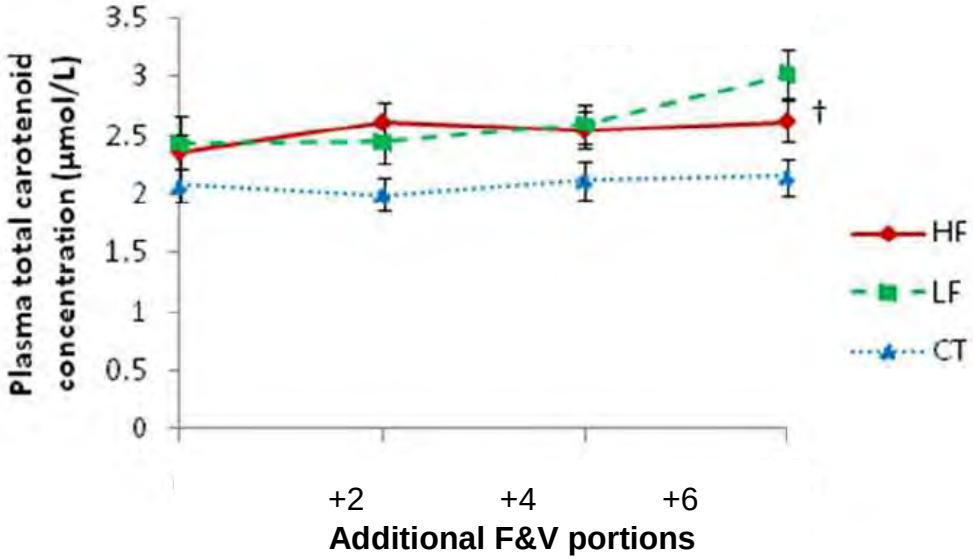
Dietary intake: HF & LF dose increase
HF & LF vs CT higher +2, +4, +6
Time x treatment (P=0.0001)

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

CAROTENOIDS

FLAVURS
Flavonoids University of Reading Study



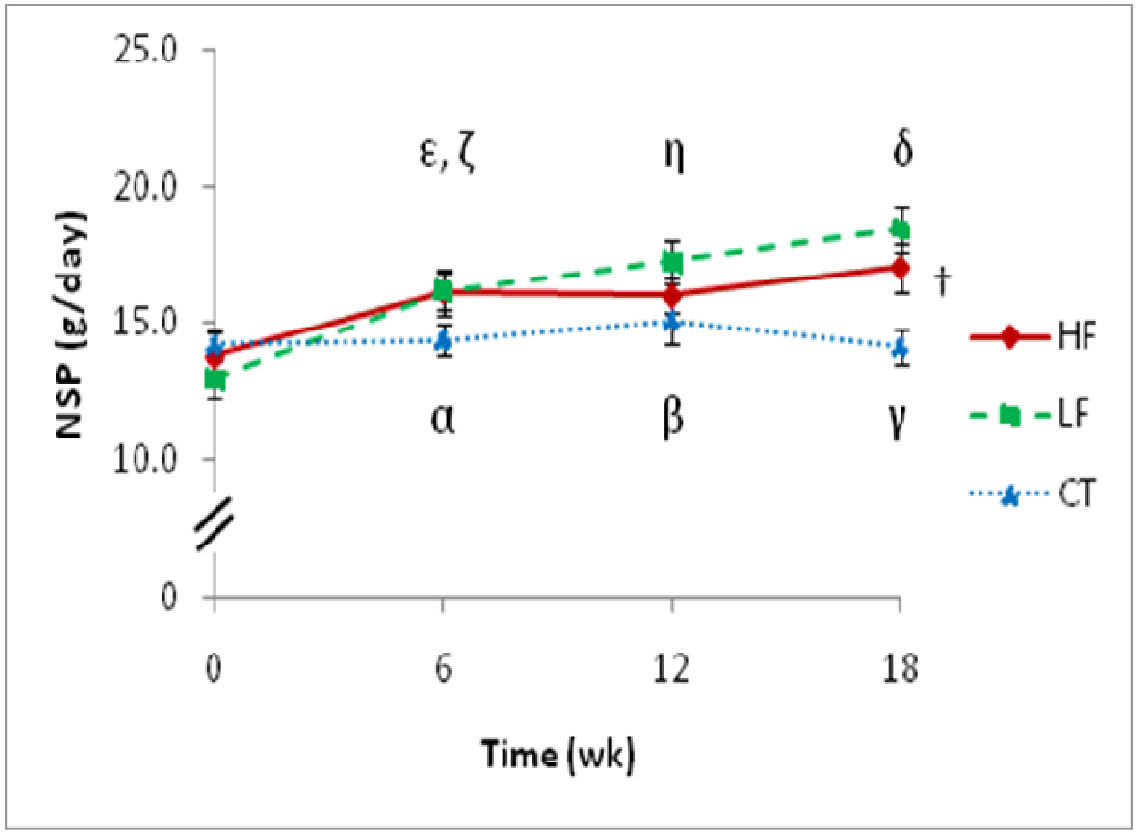


Dietary intake : LF dose dependent increase
HF & LF higher CT all points
Time x treatment (P=0.001)

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

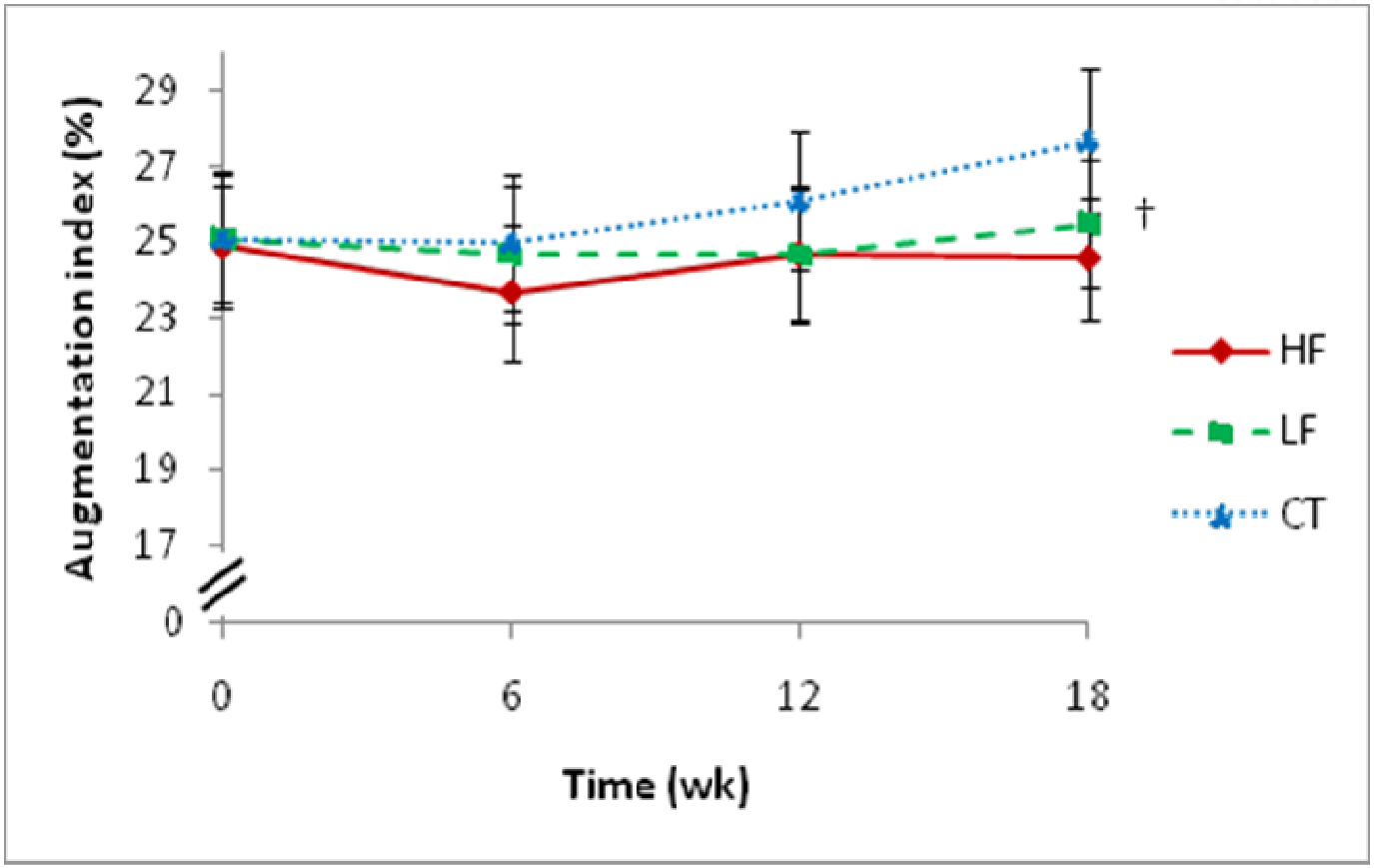
FLAVURS
Flavonoids University of Reading Study



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

FLAVURS
Flavonoids University of Reading Study



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

FLAVURS
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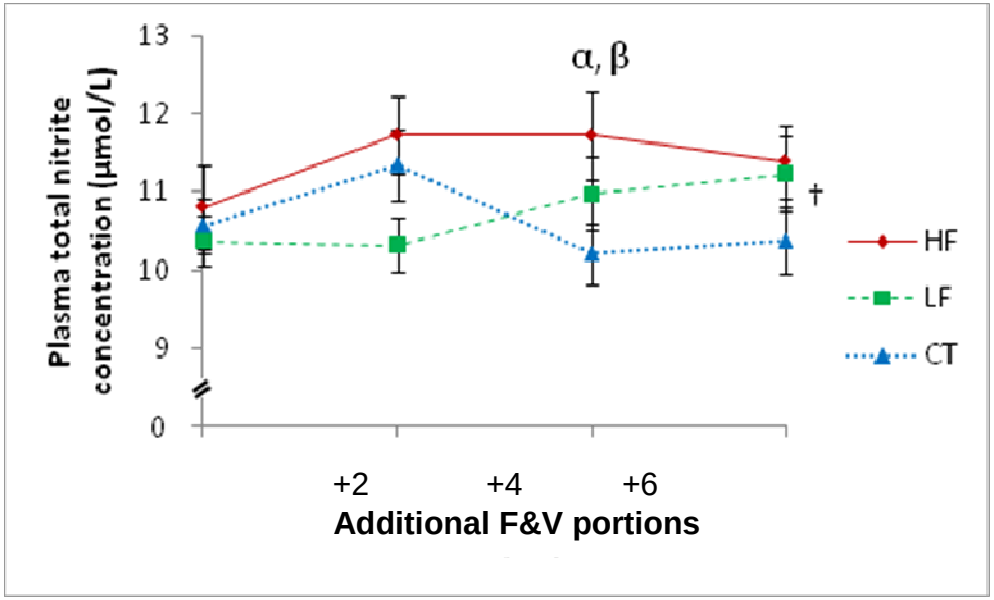
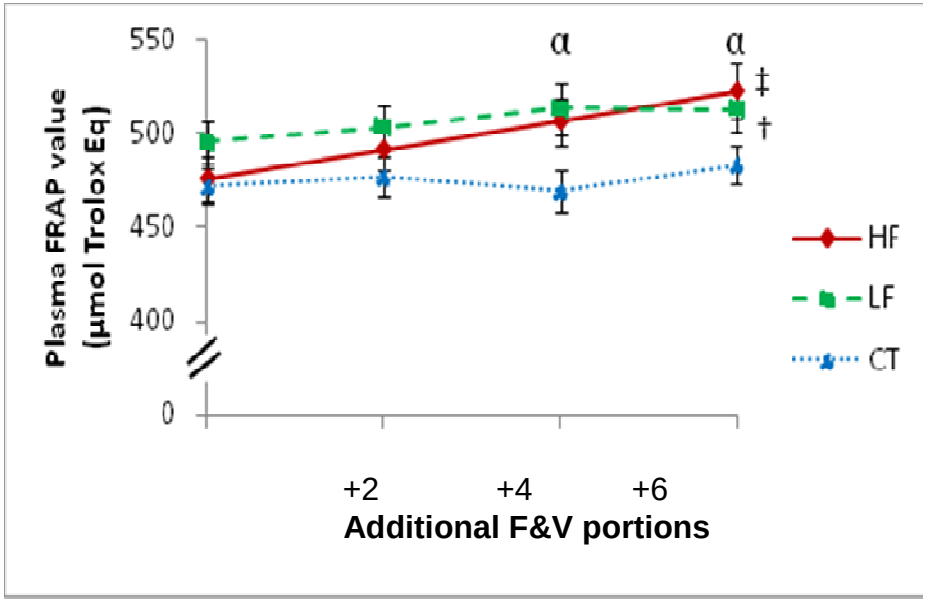


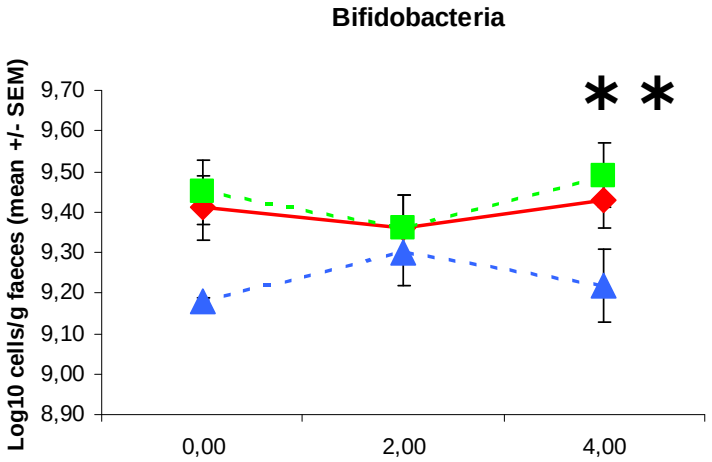


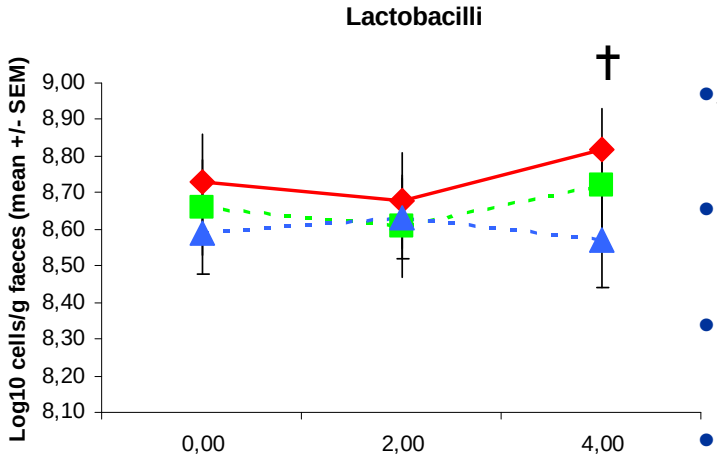
Total plasma nitrate/nitrite
HF higher than LF & CT +6
Time x treatment (p=0.03)

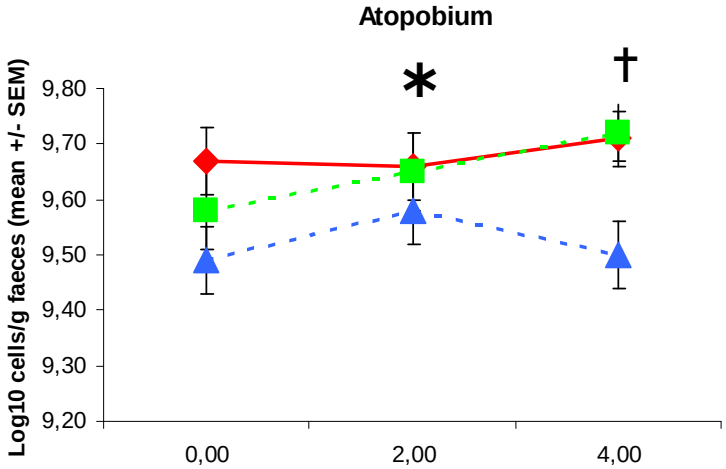
Plasma FRAP
HF dose dependent increase
LF higher +4 & +6 vs baseline
Time x treatment (P=0.009)

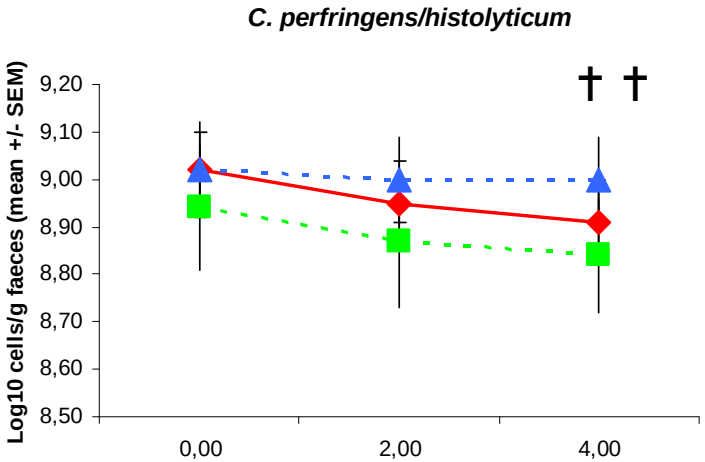
High fruit and veg diet appears to modulate gut microbiota in "benificial"manner

FLAVURS
Flavonoids University of Reading Study









- Small changes
- In "right"direction
- HF ↑ *Eu. rectale*
- LF ↑ *F. prausnitzii*
- LF ↑ *Bacteroides*

◆ HF
■ LF
▲ CT

† P<0.05 Sig diff to wk 0 Wilcoxon Test; ∗ P<0.05 sig diff to CT Kruskal-Wallis Test

UNTARGETED METABOLOMIC ANALYSIS OF URINE

- Urine dilution 1:5

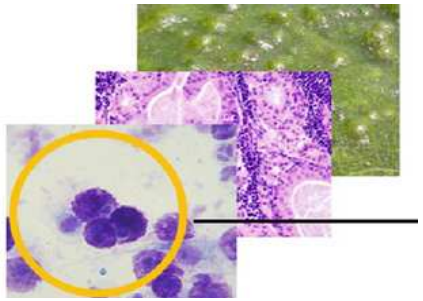
- HPLC Analysis on RP column in positive and negative ionization mode

- XL Orbitrap in Full Scan MS and MS/MS within high resolution and mass accuracy Approaches

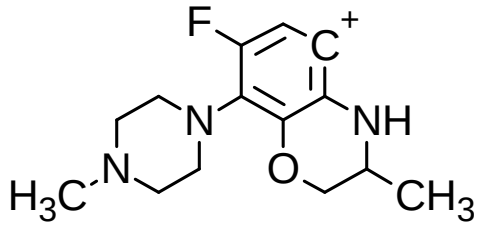
- Substances considered as biomarkers when *p<0.005 (t-test)*
- *Annotation of metabolites:*
  - *Mass accuracy of precursor ion [M+H]+ (< 3 ppm error)*
  - *Isotopic pattern distribution*

- *Databases used for annotation: In-house data base, Human Metabolome Database, Metlin, MAssBank, LipidMaps*

Metabolomics workflow



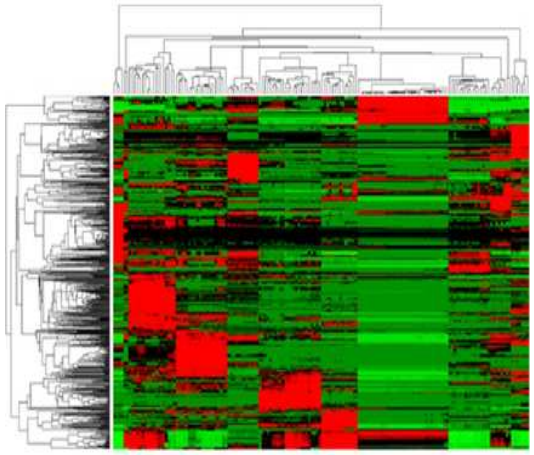
Samples: urine, plasma, fecal water



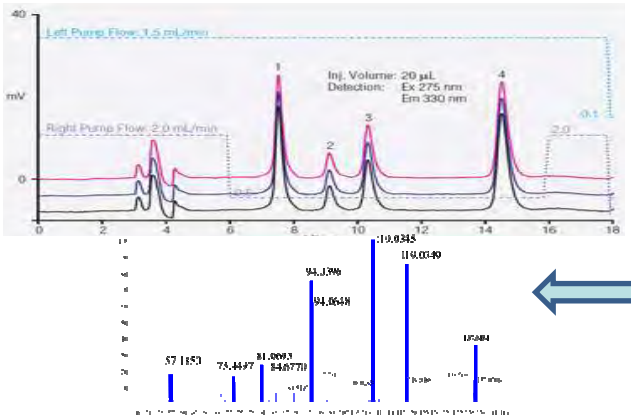
Sample preparation: extraction of all analytes

Separation on LC column

Untargeted analysis wit use of HR mass



Statistic analysis

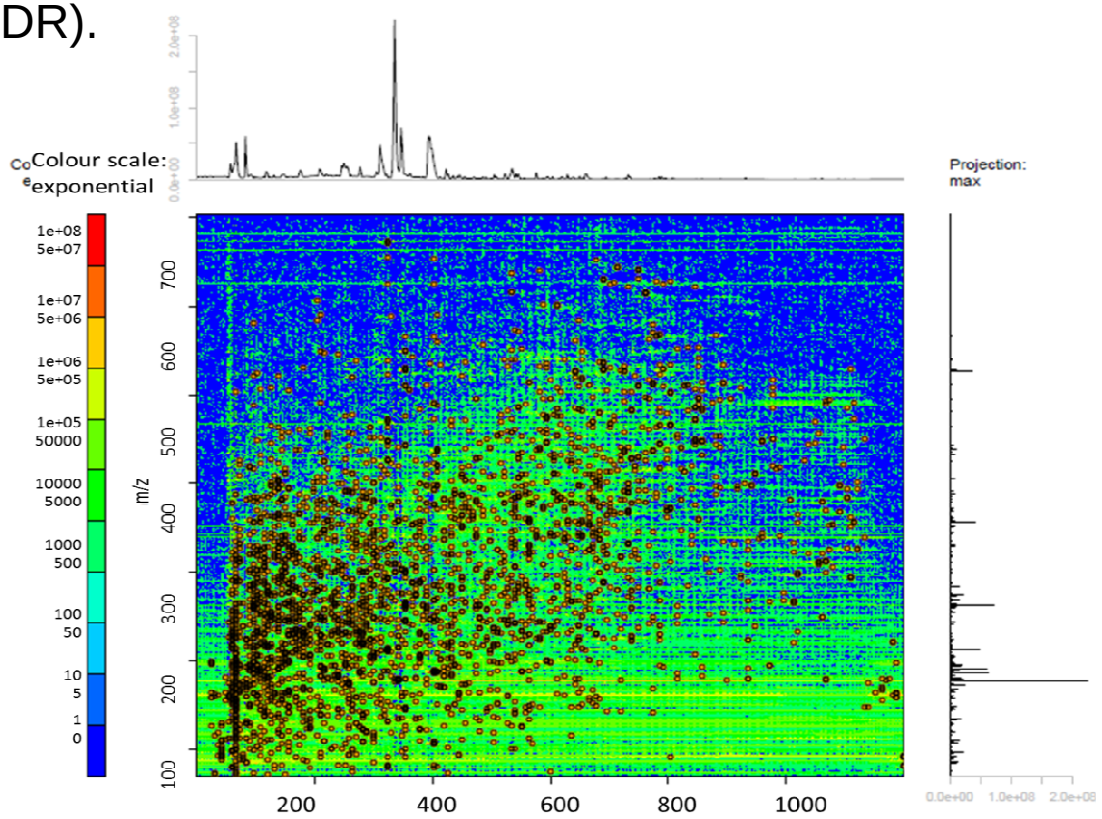


Biomarker identification

ALLIGNMENT 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).



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

BMJ open

Adherence to a Mediterranean diet is associated with a better health-related quality of life: a possible role of high dietary antioxidant content

Marialaura Bonaccio,[1,2] Augusto Di Castelnuovo,[1] Americo Bonanni,[1,3] Simona Costanzo,[1] Francesca De Lucia,[1] George Pounis,[1] Francesco Zito,[1] Maria Benedetta Donati,[2] Giovanni de Gaetano,[2] Licia Iacoviello,[2,4] on behalf of the Moli-sani project Investigators*

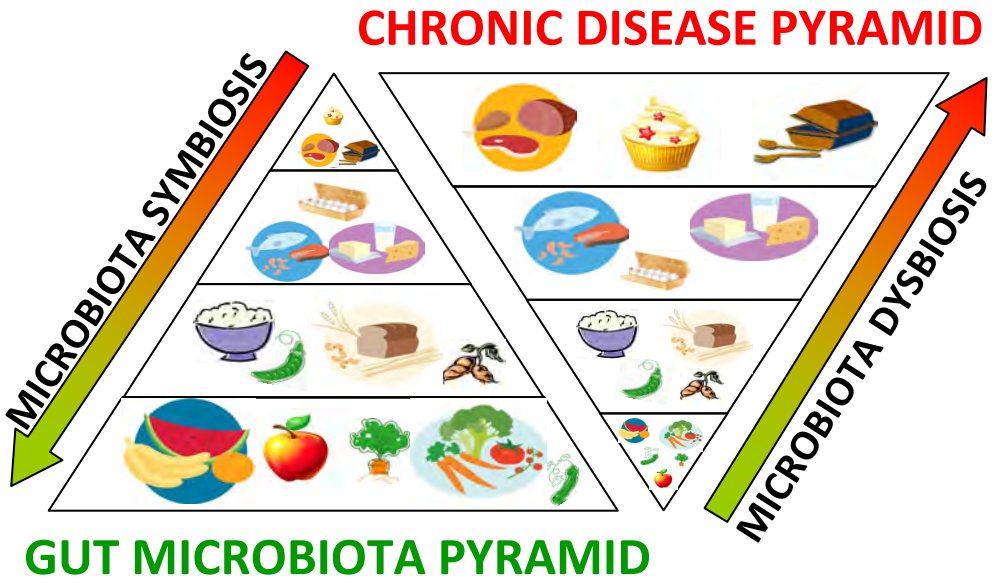
Table 2 Multivariate regression coefficients (95% CI) for the association of Mediterranean diet scores or other dietary patterns with mental and physical component scores and further adjustment for food antioxidant content (FAC) or dietary fibre

	β*	95% CI	p Value**	β* Further adjusted for FAC	95% CI	p Value**	B* Further adjusted for dietary fibre	95% CI	p Value**
Mental component score									
Mediterranean diet	0.33	0.18 to 0.49	<0.0001	0.08	−0.09 to 0.25	0.35	0.13	−0.04 to 0.29	0.13
Italian Mediterranean index	0.36	0.20 to 0.51	<0.0001	0.03	−0.14 to 0.22	0.67	0.15	−0.01 to 0.32	0.07
Olive oil and vegetables pattern	0.50	0.34 to 0.65	<0.0001	0.19	−0.003 to 0.38	0.05	0.32	0.15 to 0.50	0.0004
Meat and pasta pattern	0.07	−0.10 to 0.24	0.44	0.05	−0.12 to 0.21	0.59	0.14	−0.03 to 0.31	0.11
Eggs and sweets pattern	−0.33	−0.52 to −0.14	0.001	−0.18	−0.39 to 0.01	0.06	−0.16	−0.36 to 0.04	0.11
Physical component score									
Mediterranean diet	0.15	0.06 to 0.24	0.001	0.13	0.03 to 0.21	0.01	0.16	0.07 to 0.26	0.001
Italian Mediterranean index	0.08	−0.003 to 0.16	0.06	0.06	−0.04 to 0.15	0.26	0.08	−0.01 to 0.17	0.09
Olive oil and vegetables pattern	0.15	0.06 to 0.24	0.001	0.15	0.04 to 0.26	0.01	0.17	0.06 to 0.27	0.0010
Meat and pasta pattern	−0.11	−0.20 to −0.02	0.02	−0.12	−0.22 to −0.03	0.01	−0.11	−0.20 to −0.01	0.03
Eggs and sweets pattern	−0.02	−0.13 to 0.08	0.71	0.004	−0.11 to 0.12	0.94	−0.01	−0.12 to 0.10	0.90

*Regression coefficients represent the variation in mental or physical component scores for a one standard deviation change in MDS, IMI or dietary patterns.
**p for trend values obtained from fully adjusted model for age, sex, BMI, total energy intake, total physical activity, education, income, total socioeconomic status, smoking, diabetes, hypertension, hypercholesterolemia.

"**Conslusions:** 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".

Dietary patterns – Mediterranean diet & Gut Microbiome "ecosystem support"



INRAN, FAO Double Pyramid

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

http://www.barillacfn.com/en/position-paper/pp-doppia-piramide-alimentazione/

Fondazione Edmund Mach

- **Thank you**: Dr Paolo Formentini, Ordine dei Medici Reggio Emilia

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- Gary Frost, Imperial College London, Chris Gill, University of Ulster