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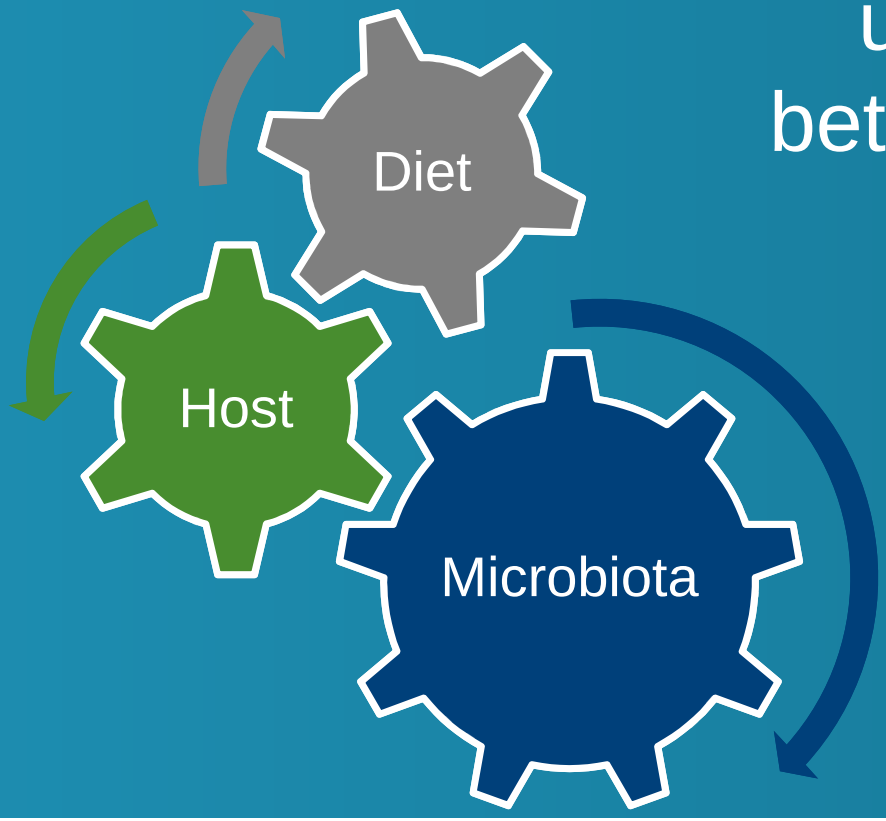


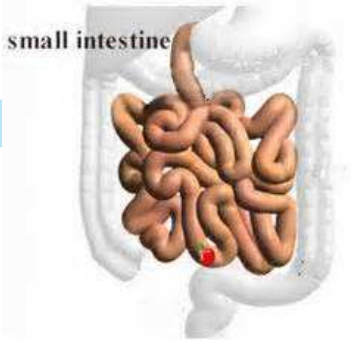
How metabolomics can increase our understanding of the interaction between the diet, the microbiota and the host

Kristin Verbeke

Translational Research in Gastrointestinal Disorders (TARGID) and
Leuven Food Science and Nutrition Centre (LFoRCe)

KU Leuven, Belgium

The human metabolome

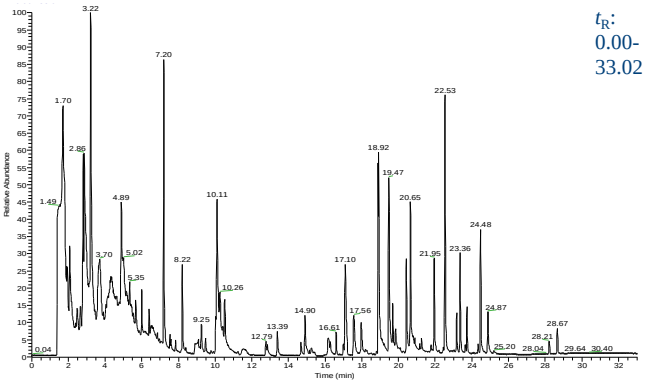


Endogenous metabolome

⇨ Highly complex and highly variable

Targeted versus non-targeted analysis

Hypothesis-driven



Data-driven

	1H-NMR	GC-MS	LC-MS
Sensitivity	+	+++	+++
Quantitative	+	+	+
robustness	+++	+	+
Sample preparation	no	yes	yes

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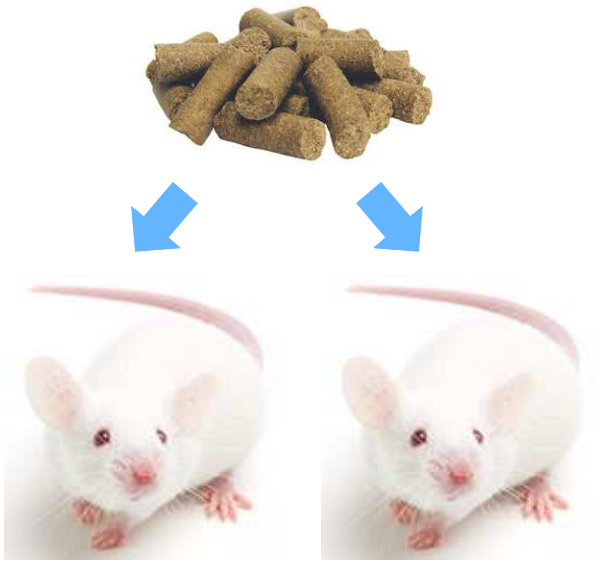
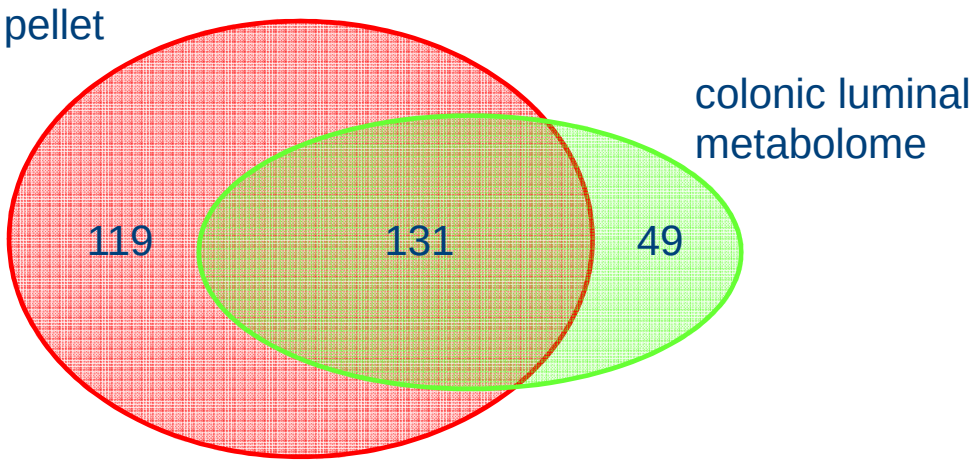
Impact of the diet and the microbiota on the fecal metabolome



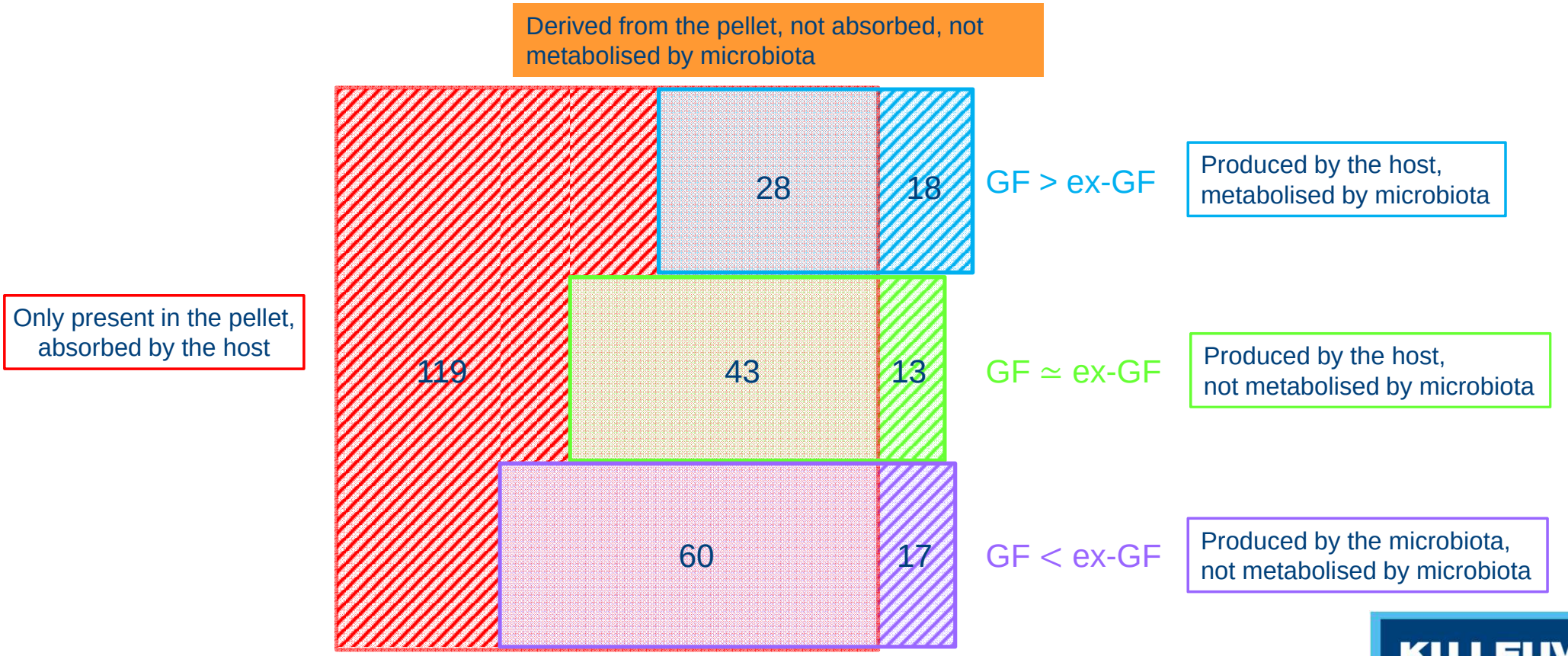
germfree mice

conventionalised mice



Matsumoto, Science reports 2012, 2:233.doi:10,1038

Impact of the diet and the microbiota on the fecal metabolome



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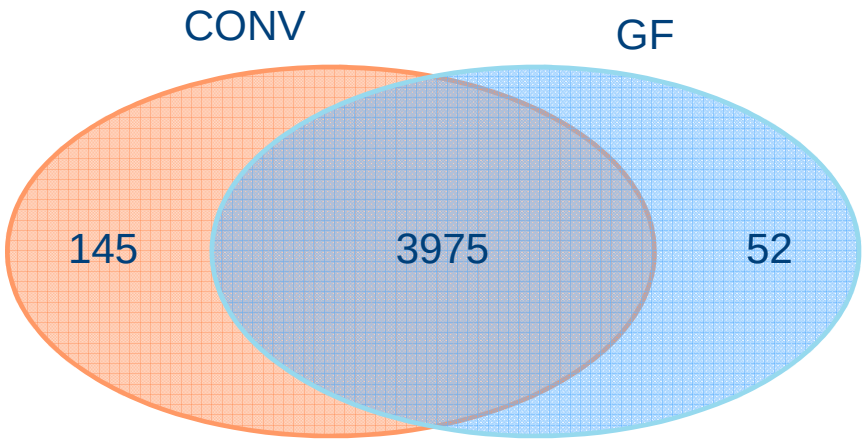
Matsumoto, Science reports 2012, 2:233.doi:10,1038

Impact of gut microbiota on blood metabolites



Metabolite	Fold change	P value
Indole derivatives		
Tryptophan	1.7, GF	8.42×10^{-12}
N-acetyltryptophan	2.4, GF	3.56×10^{-4}
Indoxyl sulfate	conv	1.34×10^{-7}
Serotonin	2.8, conv	1.27×10^{-10}
IPA	conv	7.69×10^{-7}
Phenyl derivatives		
Phenylalanine	1.05, GF	0.3
Tyrosine	1.44, GF	1.14×10^{-4}
Hippuric acid	17.4, conv	1.98×10^{-9}
Phenylacetylglycine	3.8, conv	4.70×10^{-8}
Phenyl sulfate	conv	9.85×10^{-7}
p-Cresol sulfate	conv	0.002
Phenylpropionylglycine	conv	3.07×10^{-7}
Cinnamoylglycine	conv	2.93×10^{-7}
Flavones		
Equol sulfate	conv	1.44×10^{-5}
Methyl equol sulfate	conv	2.18×10^{-6}
Others		
Urate	1.99, conv	1.51×10^{-6}
Creatinine	1.08, conv	0.071
Dihydroxyquinoline glucuronide	conv	7.64×10^{-6}
12-Hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid	4.0, conv	8.20×10^{-5}
3-Carboxy-4-methyl-5-pentyl-2-furanpropionic acid glucuronide	3.4, conv	1.37×10^{-6}

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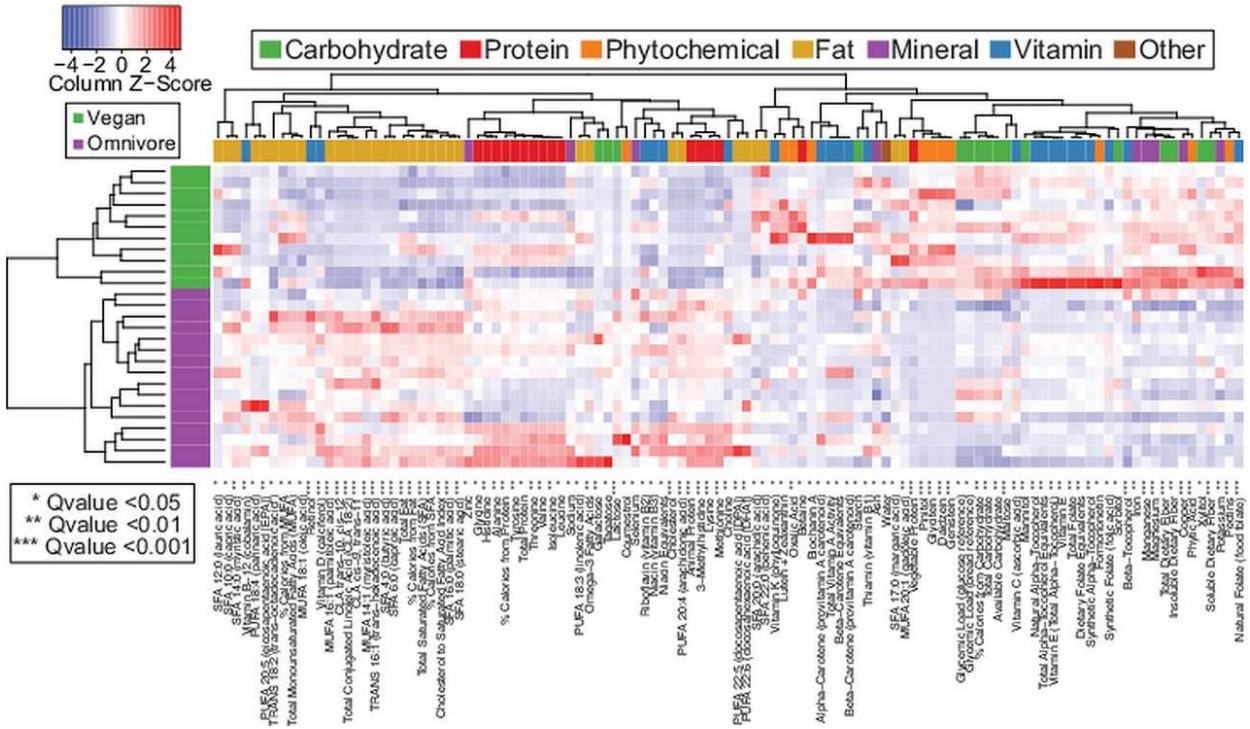
Witkoff WR, PNAS 2009, 106:3698-3703

Impact of diet on the human metabolome

- 15 vegans (> 6 months)
- 16 omnivores

- analysis of dietary intake
- metabolome analysis
  - feces
  - plasma
  - urine
- analysis of microbiota composition



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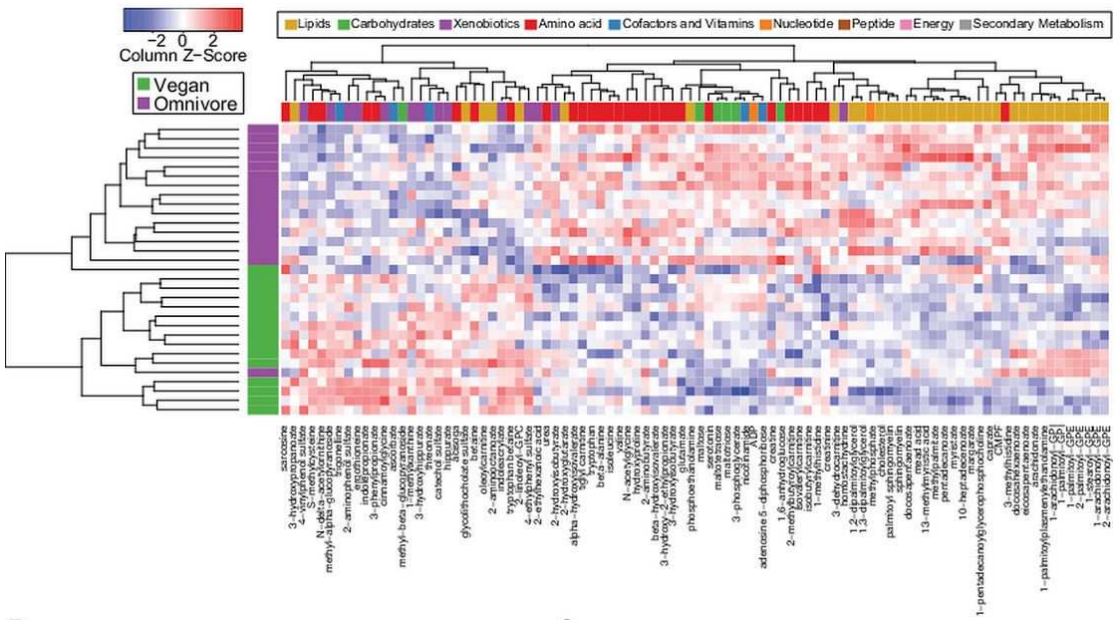
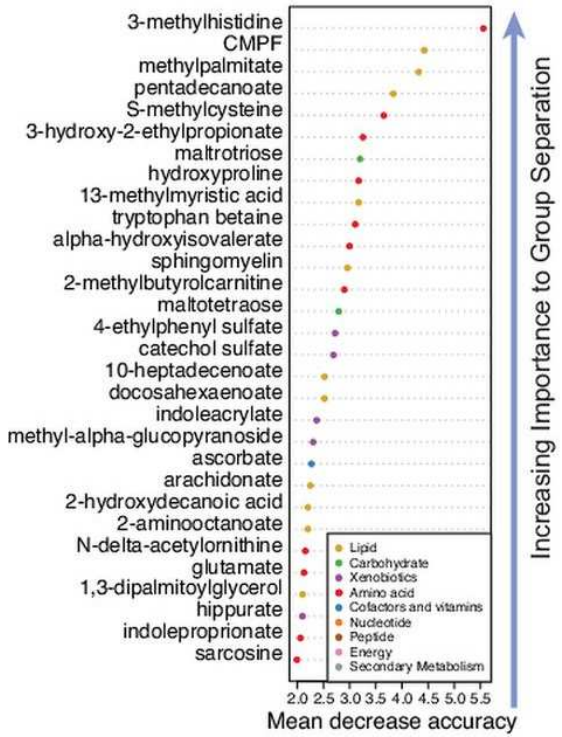
Wu G, Gut 2014, Published Online First:Nov 26, doi:10.1136/gutjnl-2014-308209

Impact of diet on the plasma metabolome





- metabolome of omnivores particularly reflects the higher intake of meat and fat
- a few metabolites are microbial-human cometabolites

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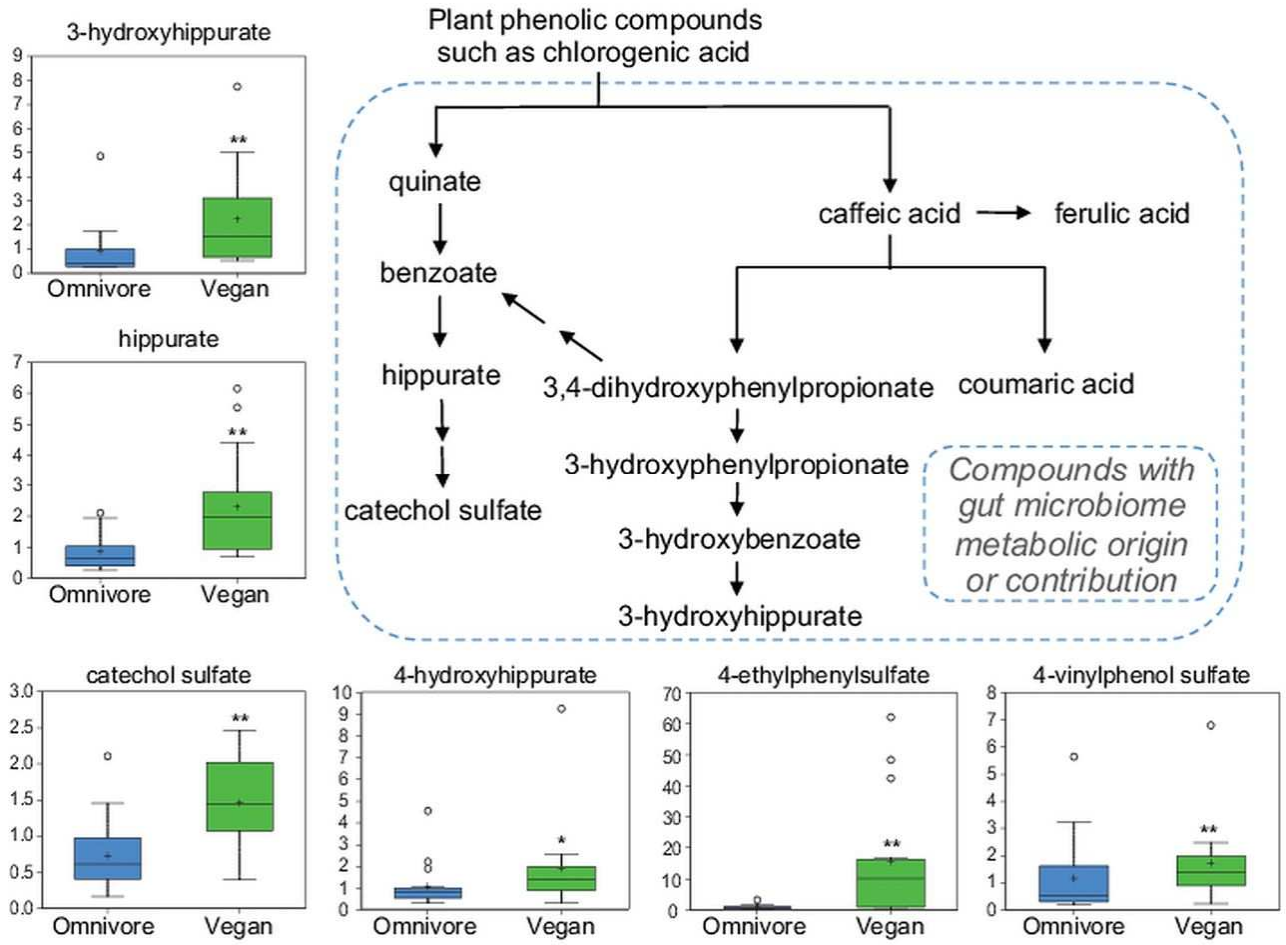
Diet-dependent metabolites produced by the gut microbiota of vegans



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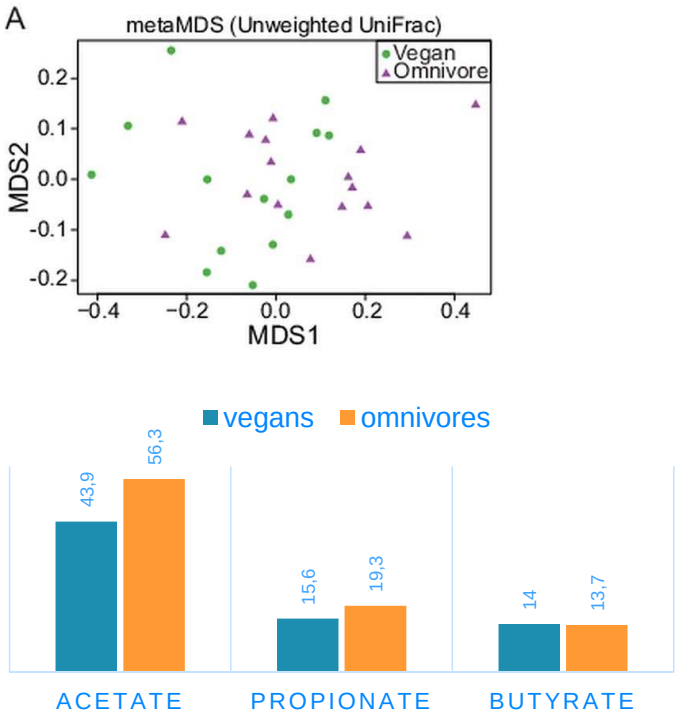
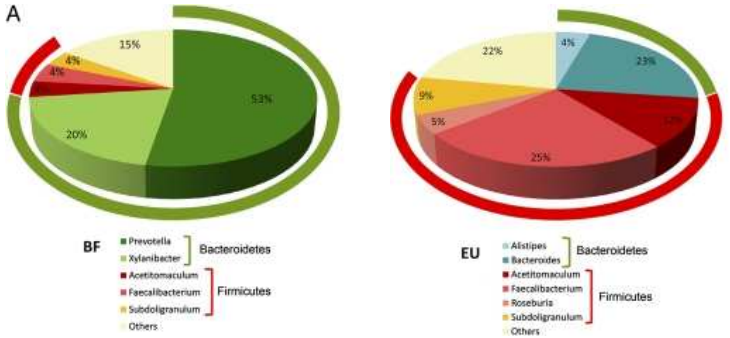
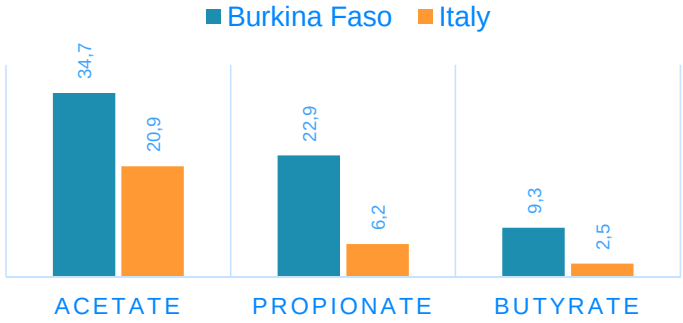
Fecal short chain fatty acids

vegians and omnivores, both in western society



agrarian society vs western society





⇨ The production of gut bacteria-derived metabolites from dietary substrates is constrained by the composition of the gut microbiota

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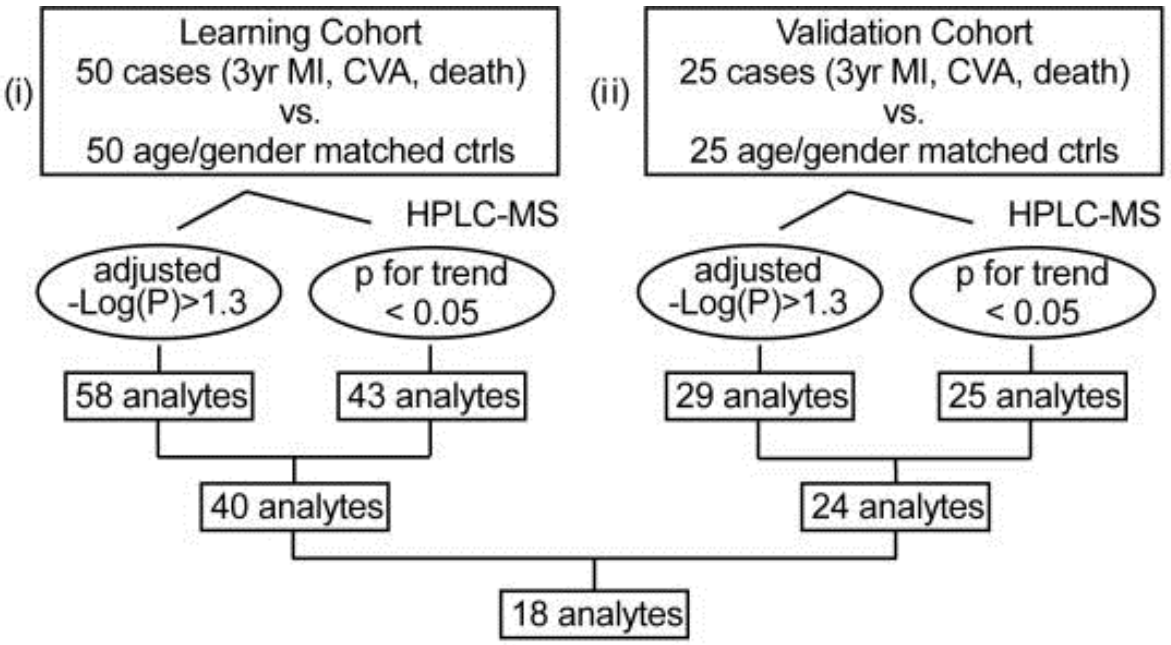
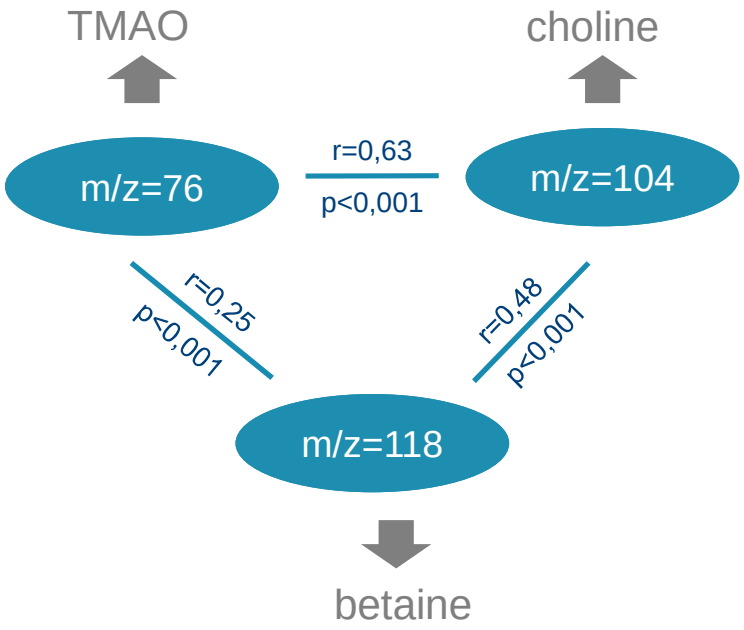
De Filippo, et al. PNAS 2010; 107:14691-14696

Identification of TMAO as a risk factor for cardiovascular disease





⇨ correlation suggests common biochemical pathway

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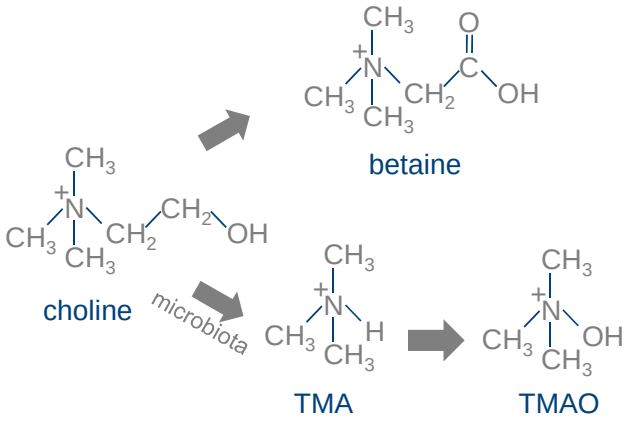
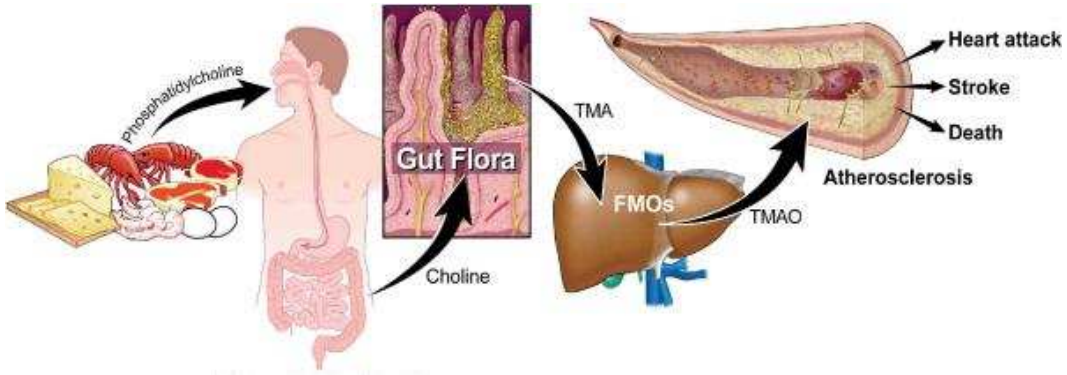
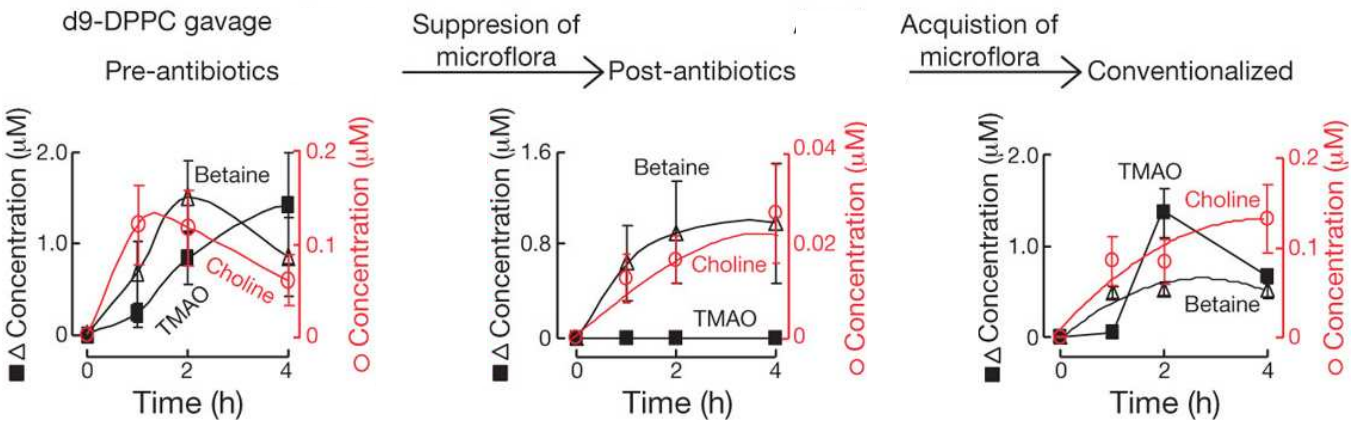
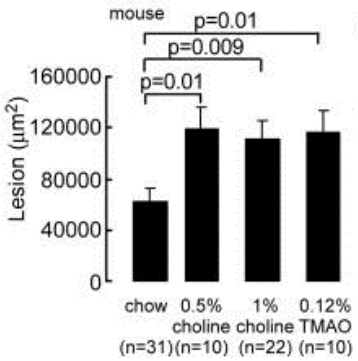
Wang Z, Nature 2011; 472;57-65

TMAO and cardiovascular disease risk





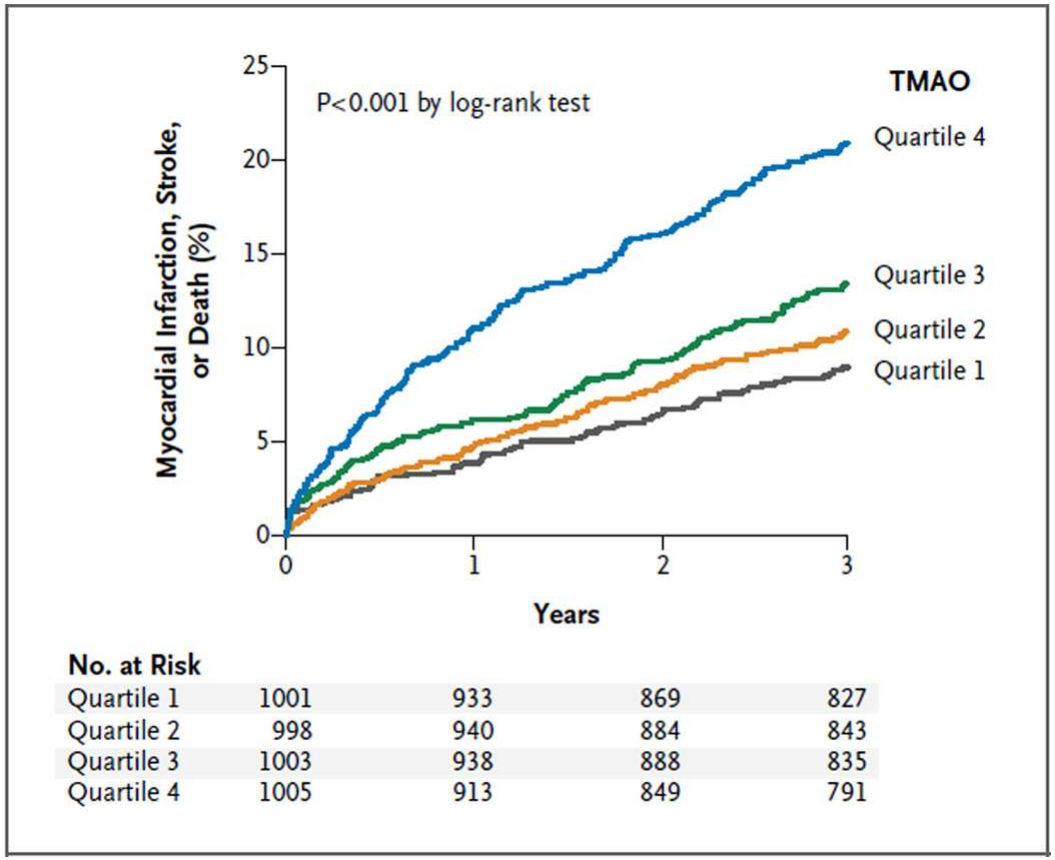




Wang Z, Nature 2011; 472;57-65; Koeth RA, Nat Med 2013; 19:576-585

Confirmation in humans

- 4007 patients with no evidence of coronary syndrome
- Undergoing diagnostic cardiac catherisation
- Follow-up for 3 years
- Quantification of plasma TMAO



No. at Risk				
Quartile 1	1001	933	869	827
Quartile 2	998	940	884	843
Quartile 3	1003	938	888	835
Quartile 4	1005	913	849	791

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Tang et al., N Eng J Med 2013; 368:1575-1584

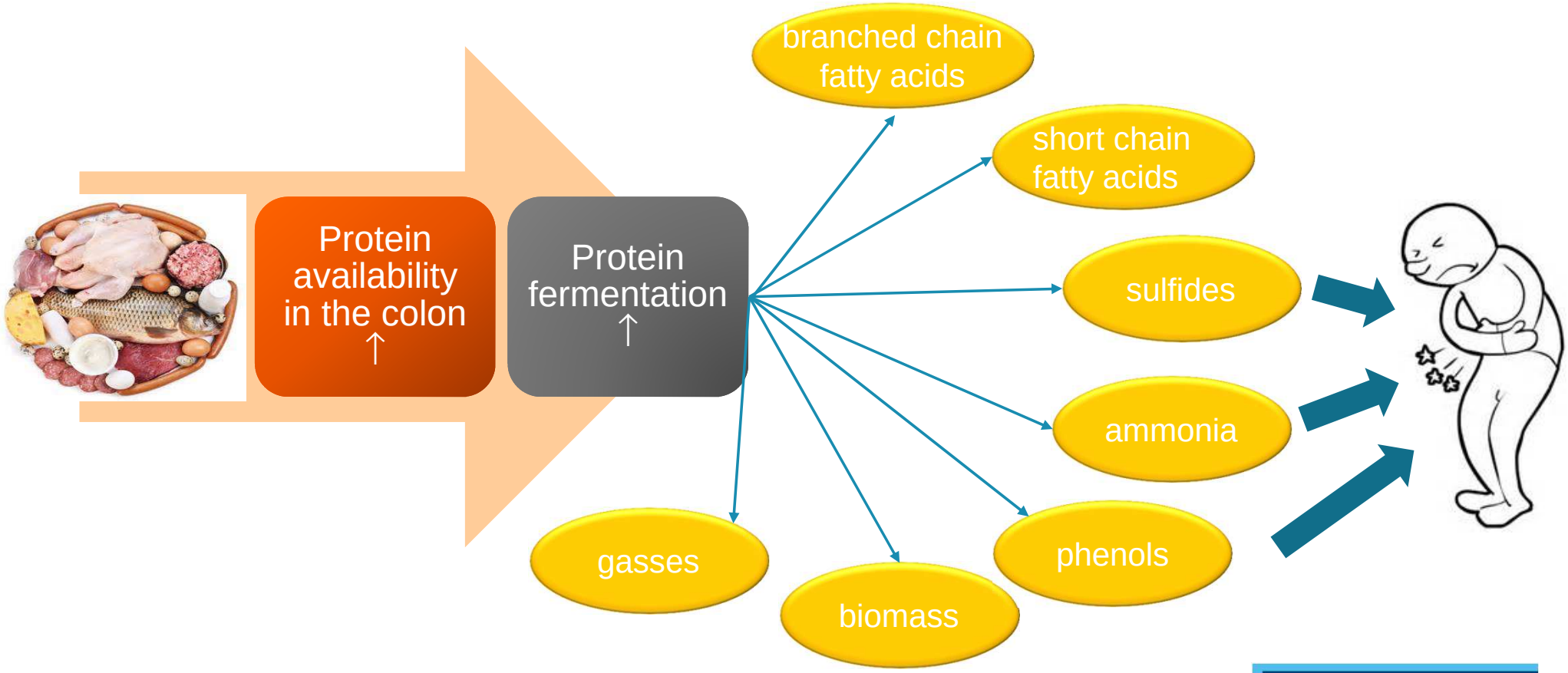
High protein diets

- highly popular because of increased weight loss (at least on short term)

- concerns about safety
  - cardiovascular risk?
  - kidney function?
  - bone health?

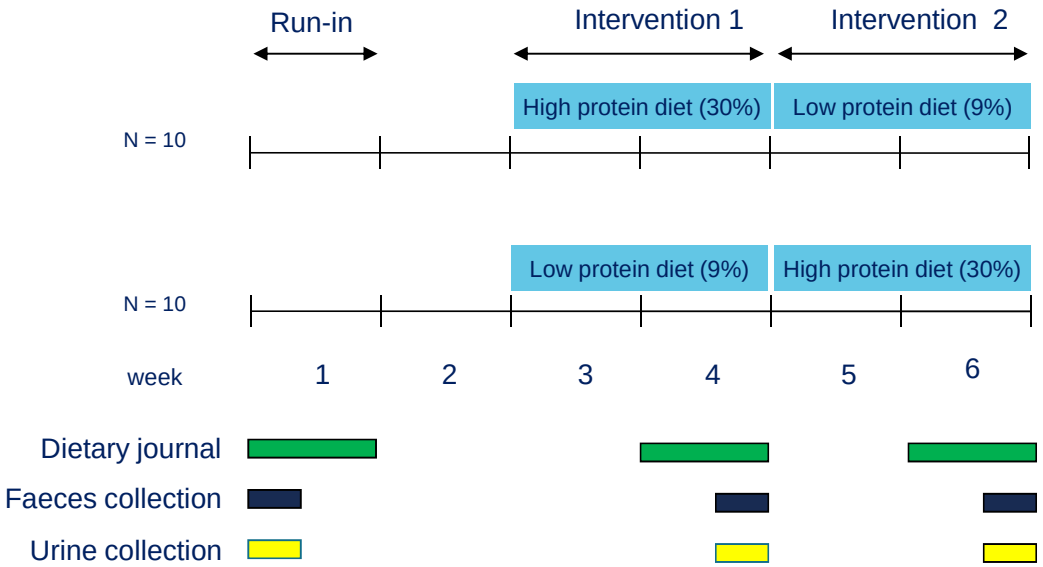
- what about gut health?

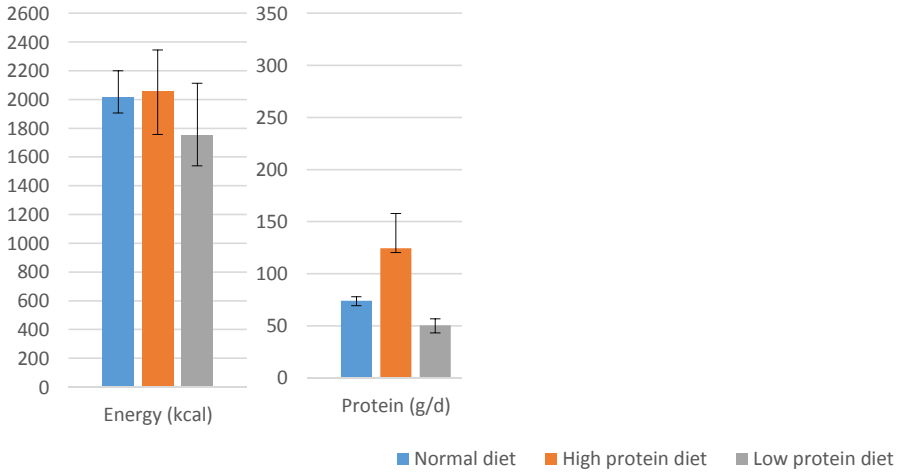
The impact of high protein diets on gut health



The impact of high protein diets on fecal metabolite patterns

Isocaloric high protein diet

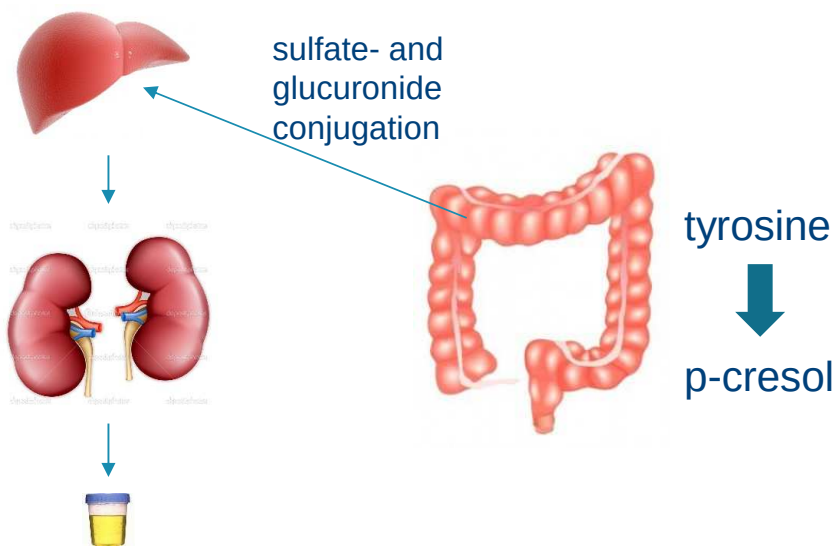


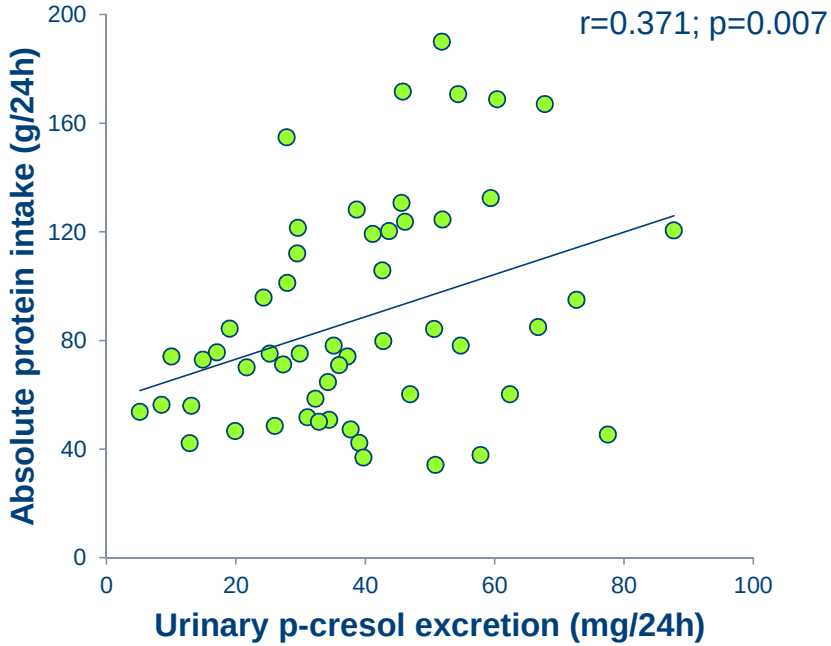


High protein diets increase colonic protein fermentation

Urinary p-cresol excretion:

- Unique bacterial metabolite of tyrosine
- biomarker for protein fermentation





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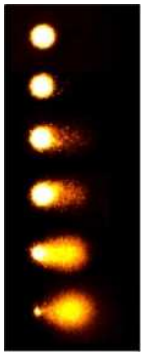
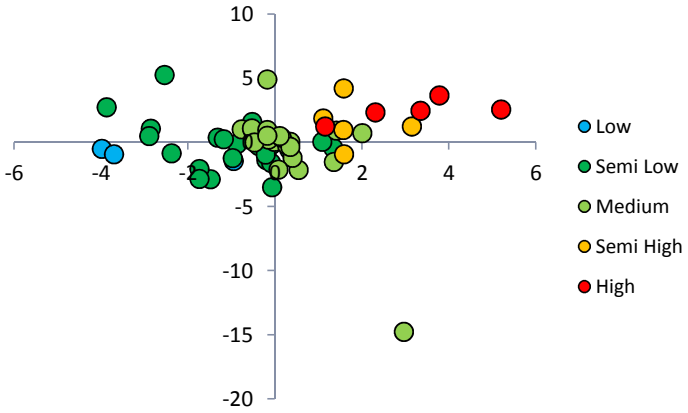
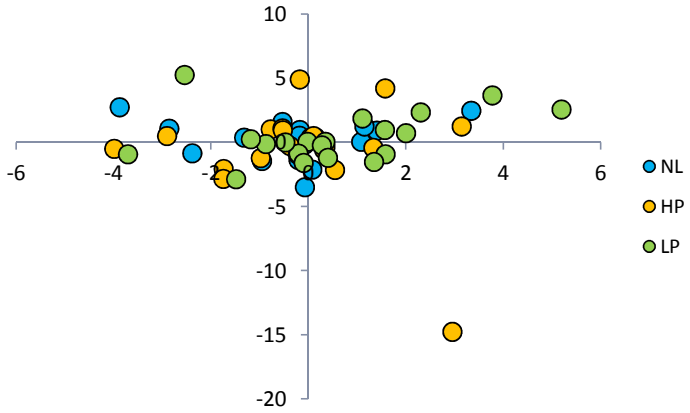
Impact of protein intake on fecal water toxicity

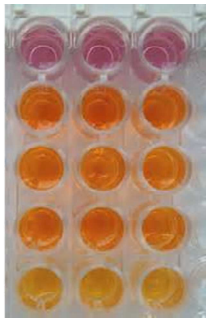
genotoxicity

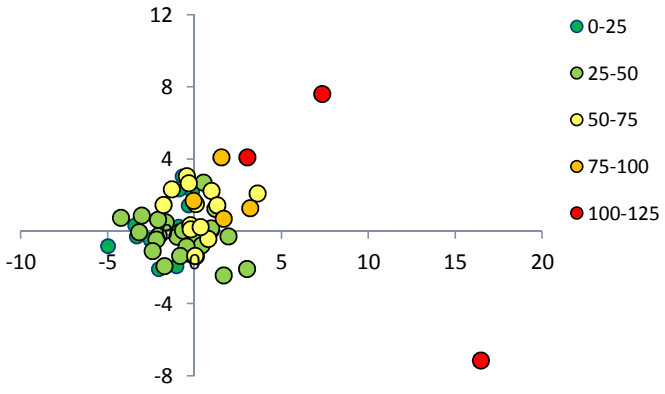


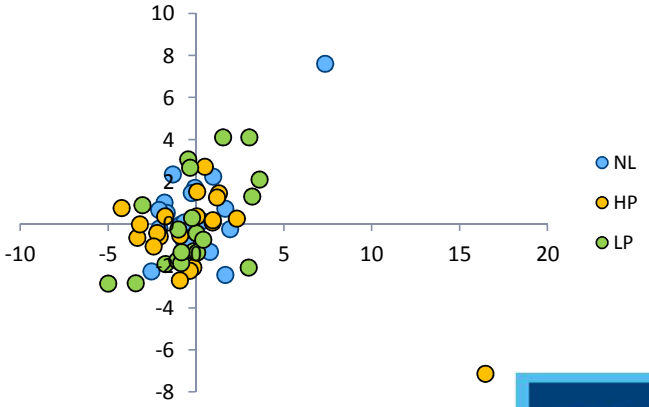




cytotoxicity







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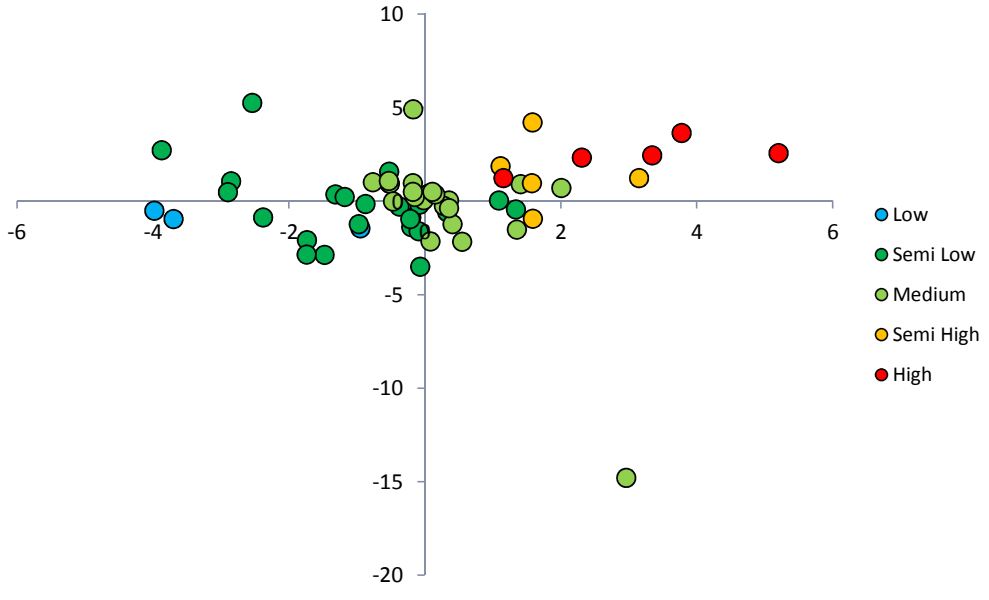
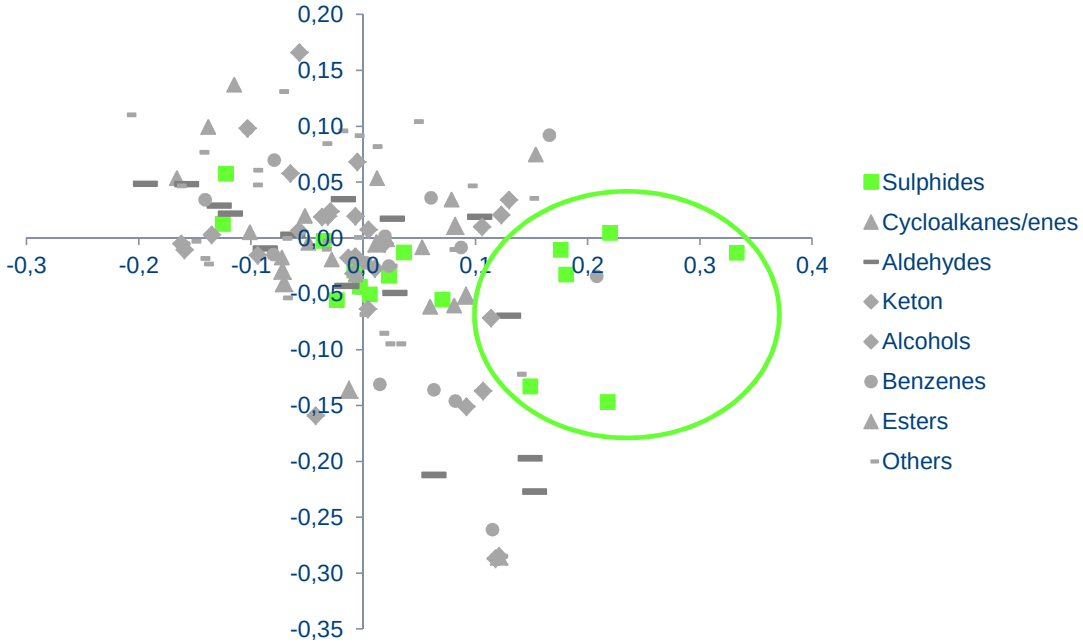
Windey K, PlosOne 2012, 12: e52387 DOI: 10.1371/journal.pone.0052387

High genotoxic samples are associated with sulfides





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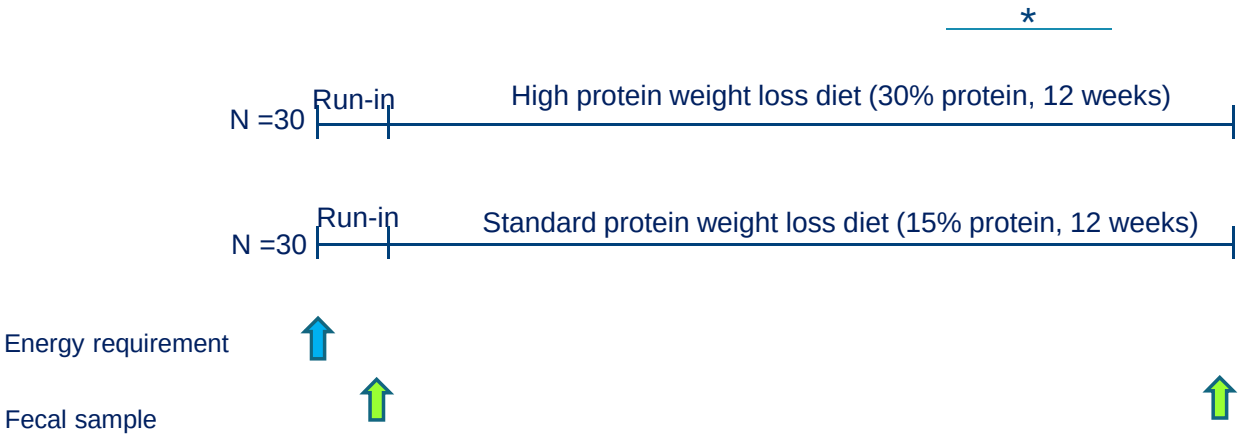
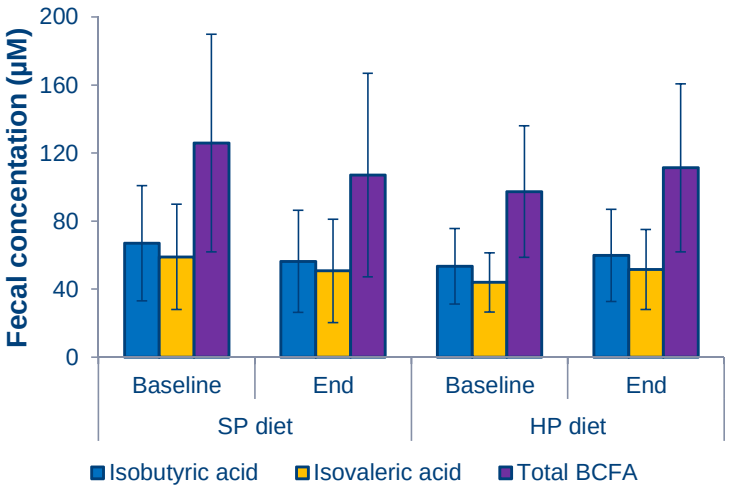
Impact of a high protein diet with caloric restriction on fecal metabolites



Dietary intake

	Run-in	HP weight loss diet	SP weight loss diet
Energy intake	Isocaloric		
Protein (%)	15	30	15
Carbohydrates (%)	55	40	55
Fat (%)	30	30	30



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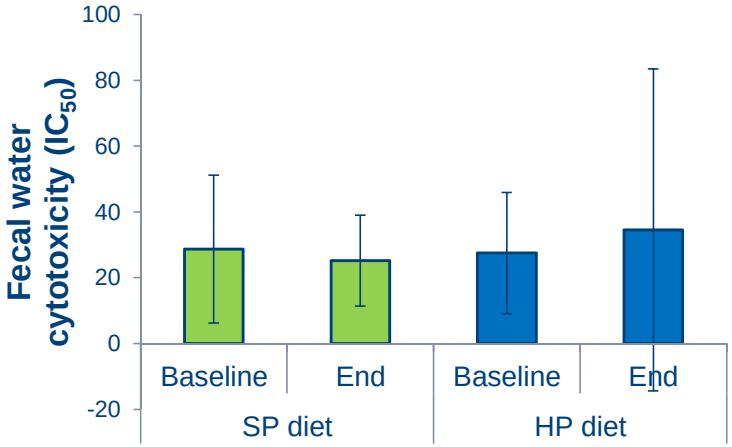
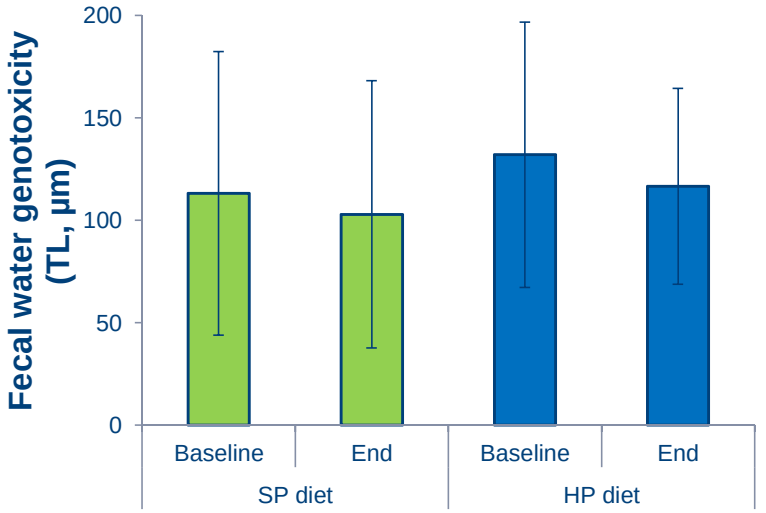
Fecal water toxicity was not affected by the diets





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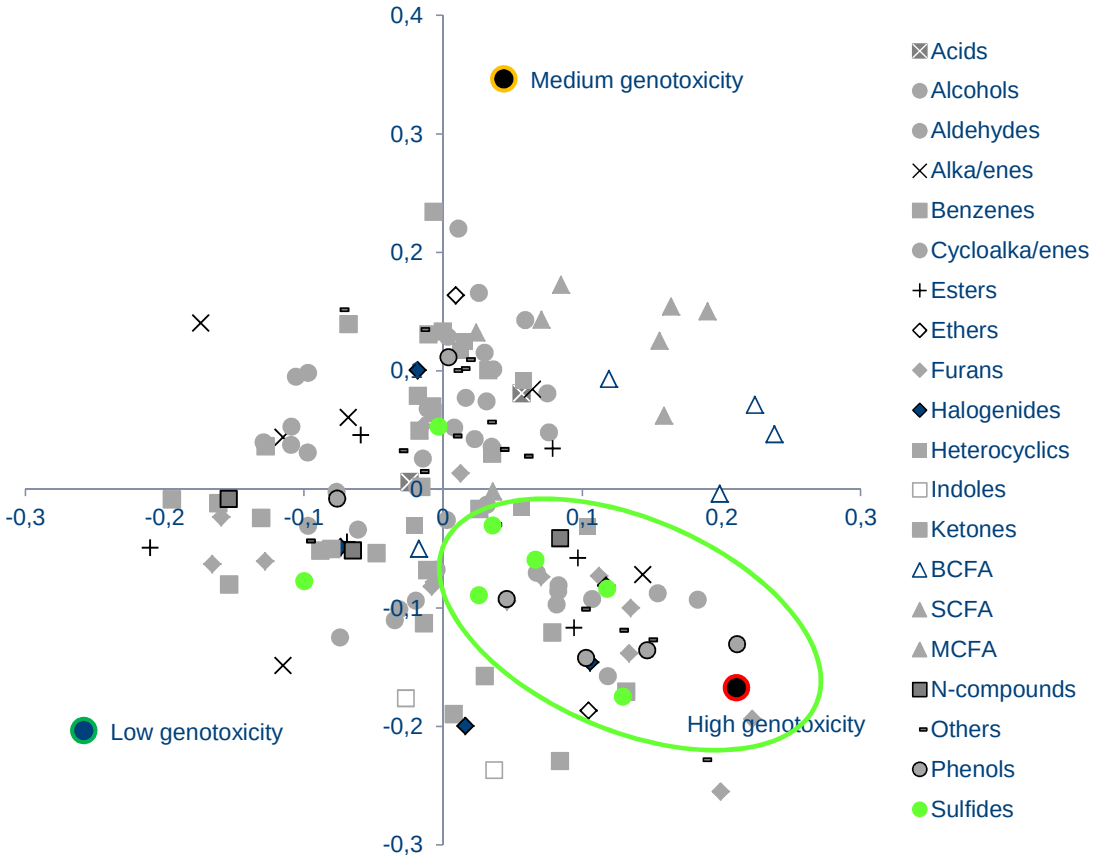
Sulfides are associated with high genotoxic samples



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Role of sulfate-reducing bacteria (SRB) in fecal water genotoxicity

S-containing AZ
(cysteine, methionine)

Inorganic sulfur

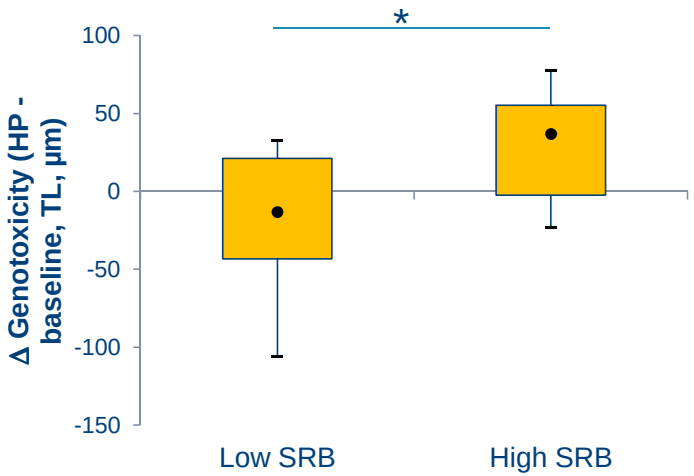
↓

Sulfate

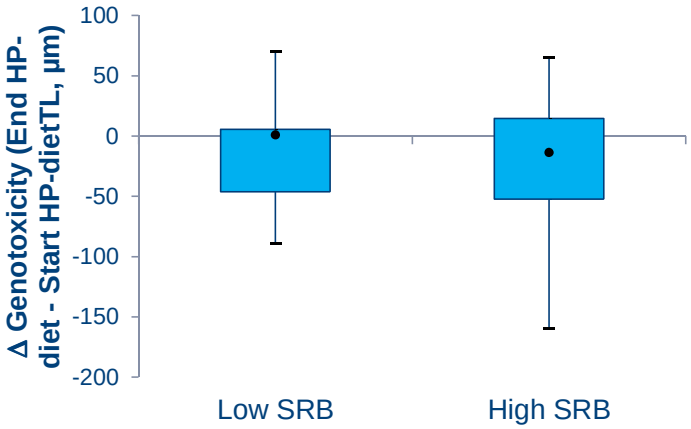
↓ SRB

H_2S

Isocaloric HP diet

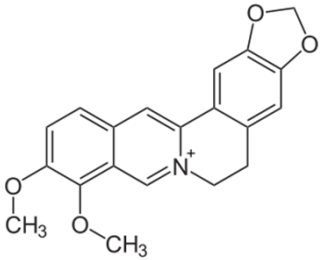


HP weight loss diet



- ⇨ baseline number of SRB predisposes to increased fecal water toxicity only after an isocaloric high protein diet
- ⇨ minimal absolute protein intake seems required

The lipid-lowering effect of orally administered berberine



Coptis chinensis

- Antimicrobial activity against bacteria, viruses, fungi, protozoans and helminths
- Lipid-lowering effect

BUT

- Poorly absorbed from the gut ⇨ levels in blood and tissues far below effective concentrations used in cell culture studies

- Accumulation of berberine in the gut may affect the microbiota

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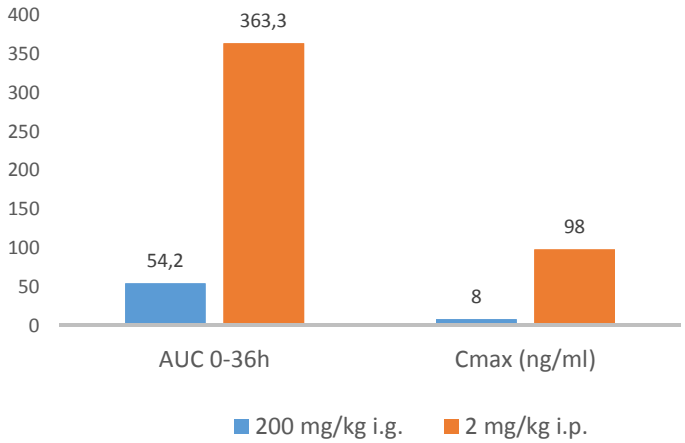
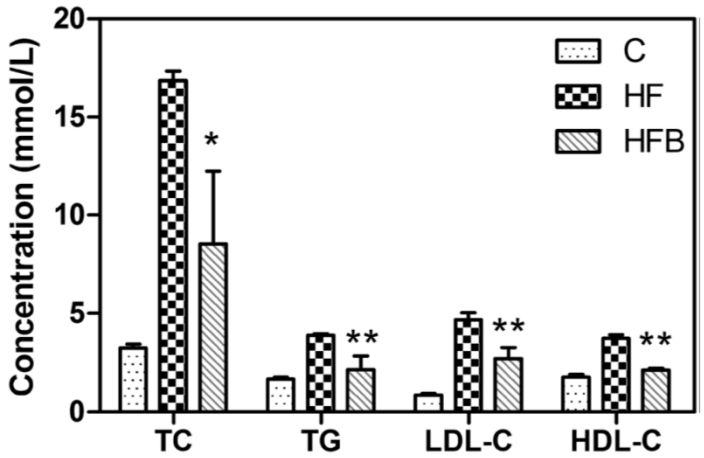
Gu, Mol Biosyst 2014, epub nov 20

The lipid-lowering effect of orally administered berberine



Control diet (C)
High fat diet (HF)
High fat diet + berberine (HFB)

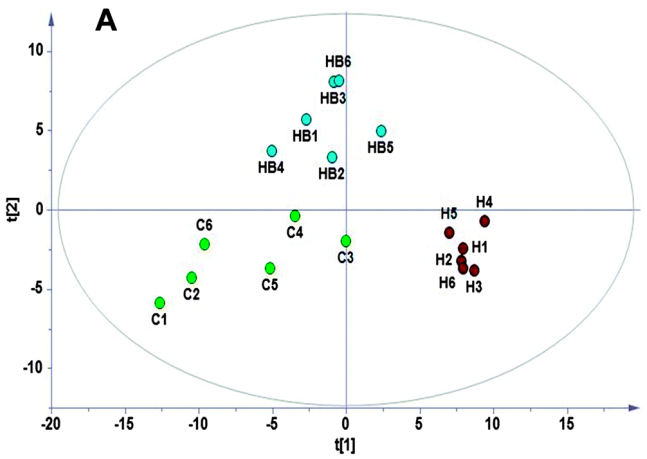


⇨ Metabolomic analysis of serum and cecal samples

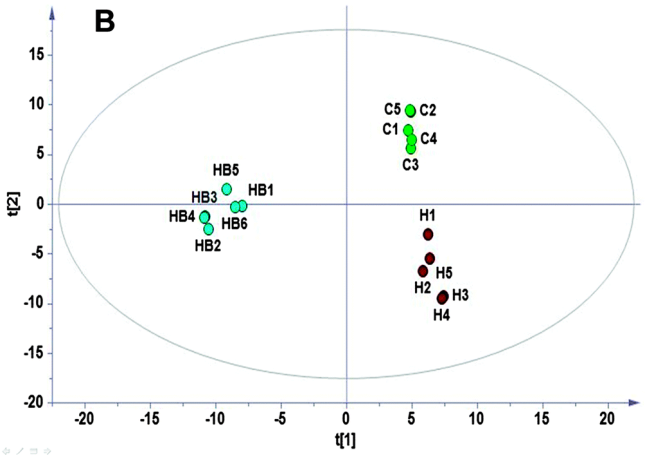
Gu, Mol Biosyst 2014, epub nov 20

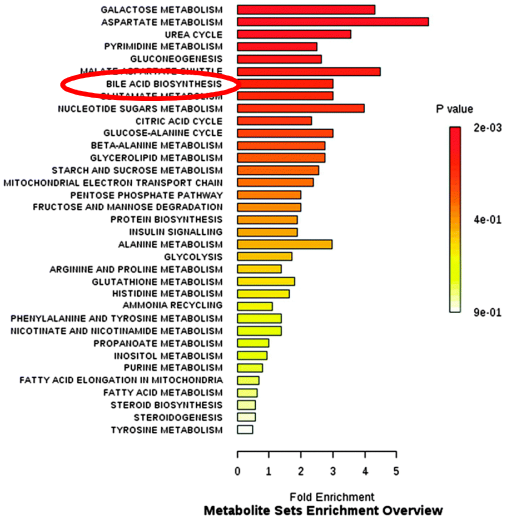
Effect of berberine on the metabolites in serum and gut content

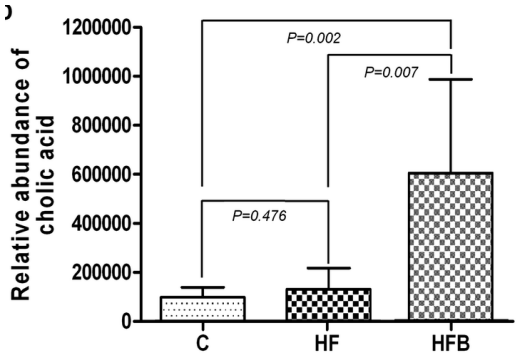
serum



gut content







⇨ Metabolic impact analysis: significant impact on metabolism of cholesterol and/or biosynthesis of bile acids

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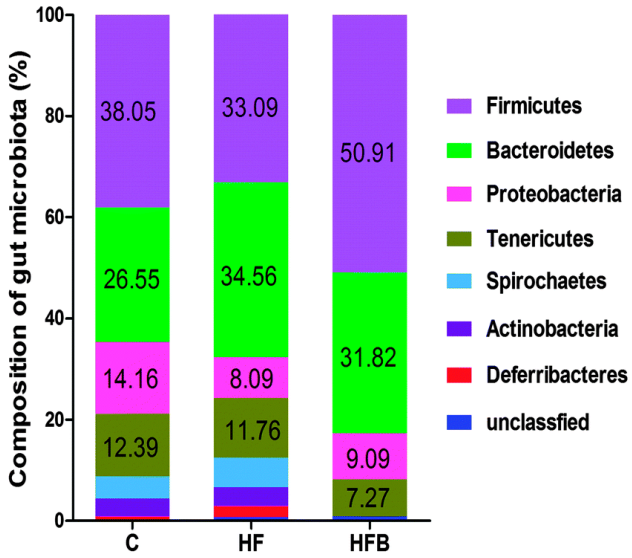
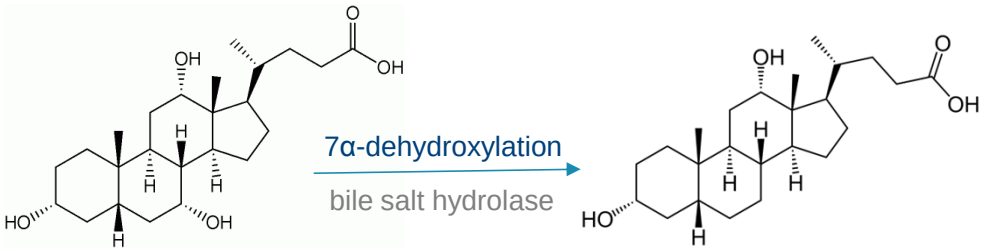
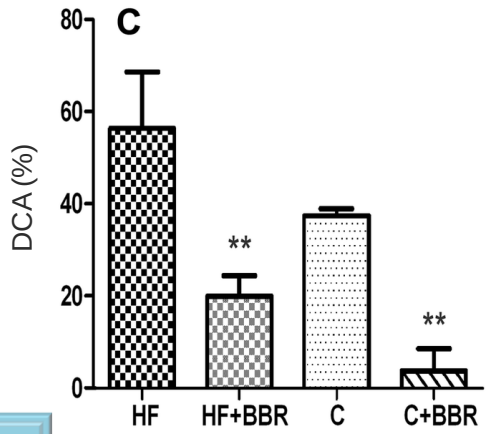
Effect of berberine on microbiota composition and activity





cholic acid

deoxycholic acid (DCA)



Elimination of cholic acid is significantly inhibited

lipid lowering effect of berberine was attributed to increased bile acid synthesis and inhibition of its degradation by the microbiota

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Gu, Mol Biosyst 2014, epub nov 20

Conclusions

- metabolomics holds much promise to increase our understanding of physiological processes
- Impact of the diet on the metabolome is more pronounced in animals than in humans
- main goal is to generate hypotheses about metabolic mechanisms
  - ⇨ need further validation in targeted studies