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

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DTU Food
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Introduction

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

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

- An increased system retention time has *in vitro* been associated with more extensive protein fermentation (Macfarlane et al., 1989; Tottey et al., 2016)
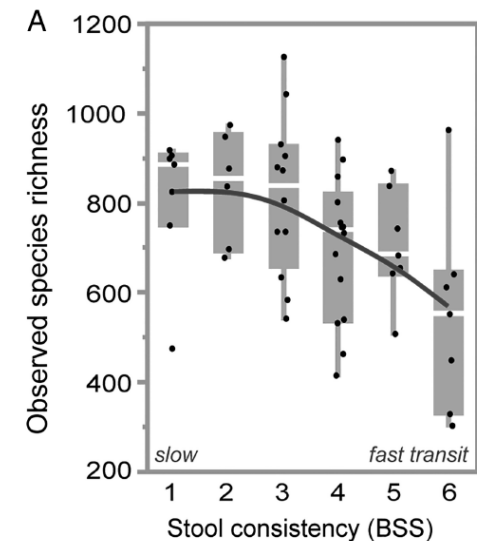
(Vandeputte et al., 2015; Gut)

Introduction
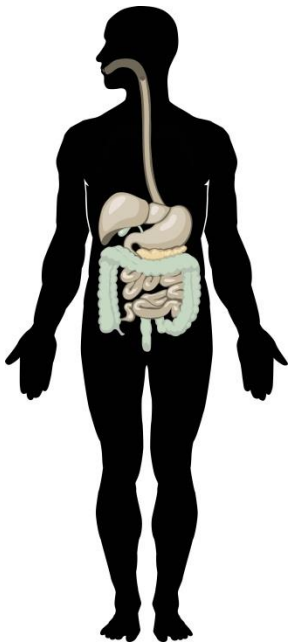
Bristol stool scale important measure that reflects gut microbiota composition
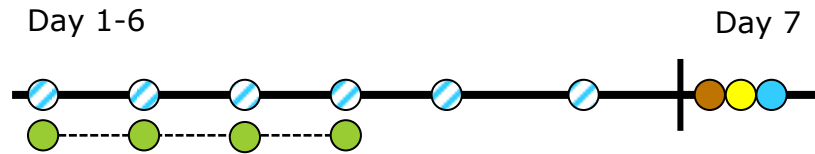↳ check Gut publication, 2015 Jeroen Raes

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

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

Professor
Tine Rask Licht

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

Sample collection

Day 1-6

Day 7

- 🟢 4-days habitual dietary intake recorded
- 🔵 Ingestion of radio-opaque transit markers
- 🔵 Abdominal X-rays obtained
- 🟤 Fecal sample collected
- 🟡 Urine sample collected

🔵 Abdominal X-rays

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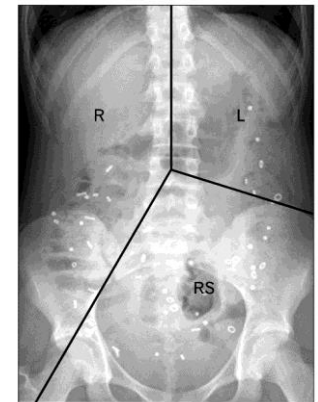
🟤 Fecal sample

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

🟡 Urine sample

Urine metabolite profiling
Ultra performance liquid chromatography mass spectrometry

How does colonic transit time relate to gut microbial diversity and composition?

● Fecal sample

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

Colonic transit time correlates to gut microbial alpha-diversity
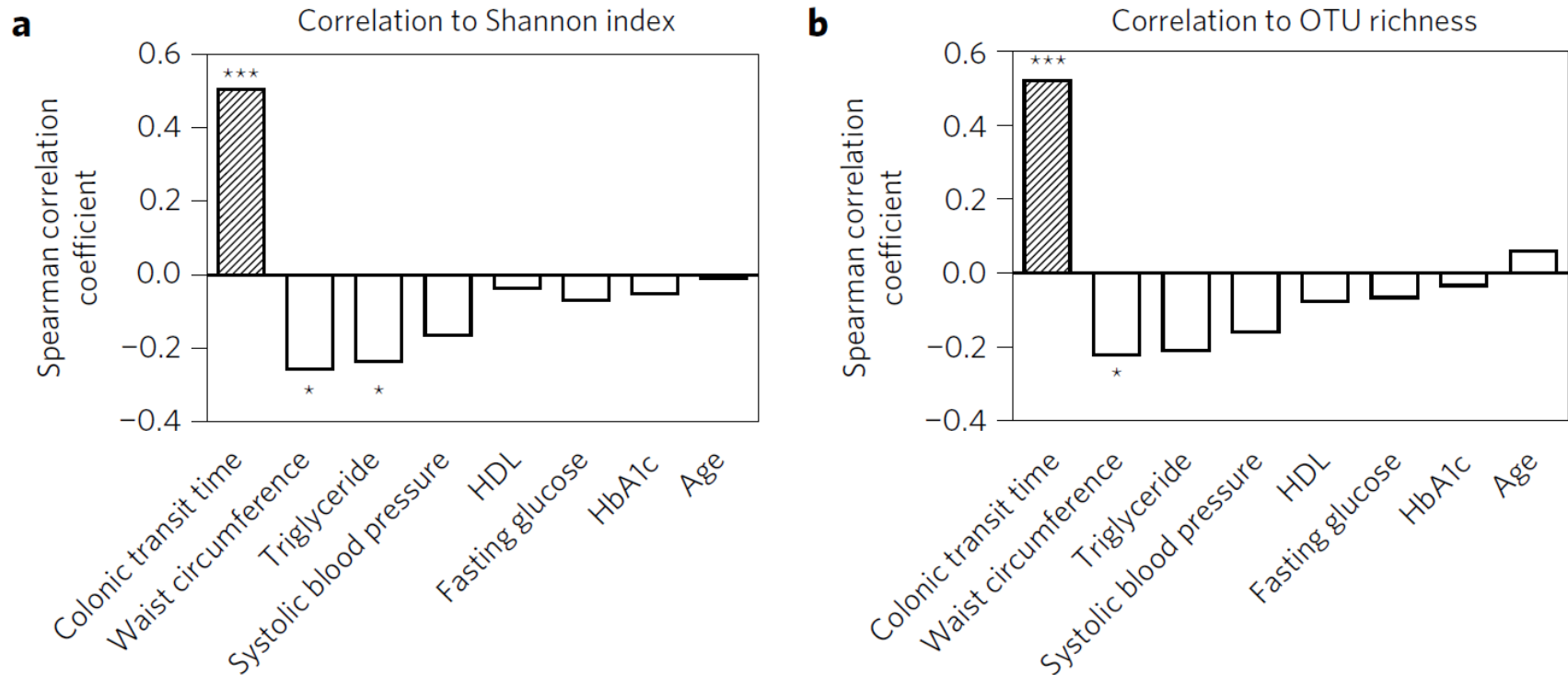
Correlations were calculated based on Spearman's Rank correlation (n=85).
Significant associations are represented by asterisks (* $p<0.05$, *** $p<0.000001$).

A long colonic transit time reduces gut microbial beta-diversity
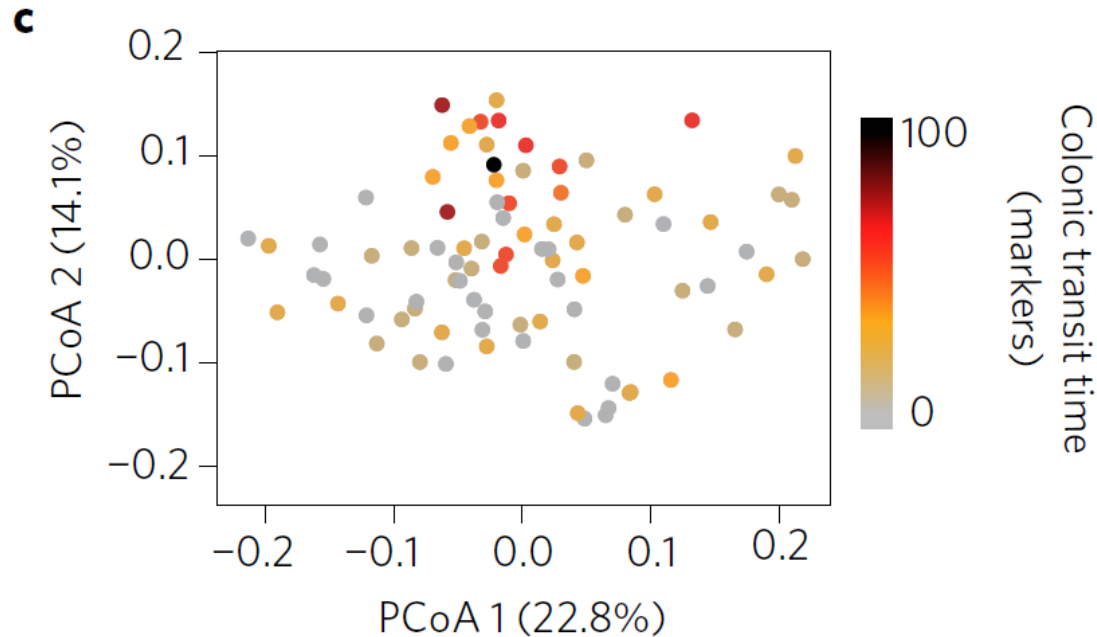
Beta-diversity assessed by weighted UniFrac distances
(Adonis, $P < 0.001$, $R^2 = 0.07$)

Bacterial groups significantly associated with colonic transit time

Positive associated
Negative associated

Phylum	Class	Order	Family	Genus
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaeceae	cc115 (q=0.05)
		SHA-98 (q=1.3x10^{-6})		
	Clostridia	Clostridiales	Christensenellaceae (q=1.0x10^{-5})	
			Dehalobacteriaceae (q=4.0x10^{-4})	Dehalobacterium (q=8.0x10^{-4})
			Eubacteriaceae (q=0.04)	Anaerofustis (q=0.02)
			Ruminococcaeceae (q=0.01)	Oscillospira (q=1.3x10^{-5})
				Ruminoccocus (q=3.9x10^{-3})
				Faecalibacterium (q=2.0x10^{-4})
			Tissierellaceae (q=0.007)	WAL_1855D (q=0.009)
			Mogibacteriaceae (q=0.01)	
			Lachnospiraceae (q=2.1x10^{-3})	
			Clostridiaceae (q=0.03)	
Bacteroidetes	Bacteriodia	Bacteroidales	Porphyromonadaceae	Porphyromonas (q=6.4x10^{-4})
			Odoribacteraceae	Butyricimonas (q=0.005)
			S24-7 (q=0.04)	
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae (q=0.04)	
		Pseudomonadales	Pseudomonadaceae (q=0.04)	
	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio (q=0.005)
Tenericutes (q=0.02)				
Euryarchaeota (q=0.0001)	Methanobacteria (q=0.0002)	Methanobacteriales (q=0.0002)	Methanobacteriaceae (q=2.9x10^{-4})	Methanobrevibacter (q=3.7x10^{-4})

How does colonic transit time relate to colonic metabolism?

🟡 Urine sample

Urine metabolite profiling
Ultra performance liquid chromatography mass spectrometry
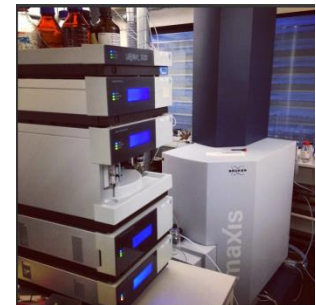
Global metabolic profiling of urine using UPLC-QTOF-MS

- **Column**
  - C18 column

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

- **Run sequence**
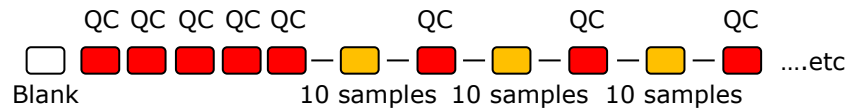
Based on Want *et al*., 2010; Nature Protocols

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

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

Colonic transit time associates with the urinary metabolic profile

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

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

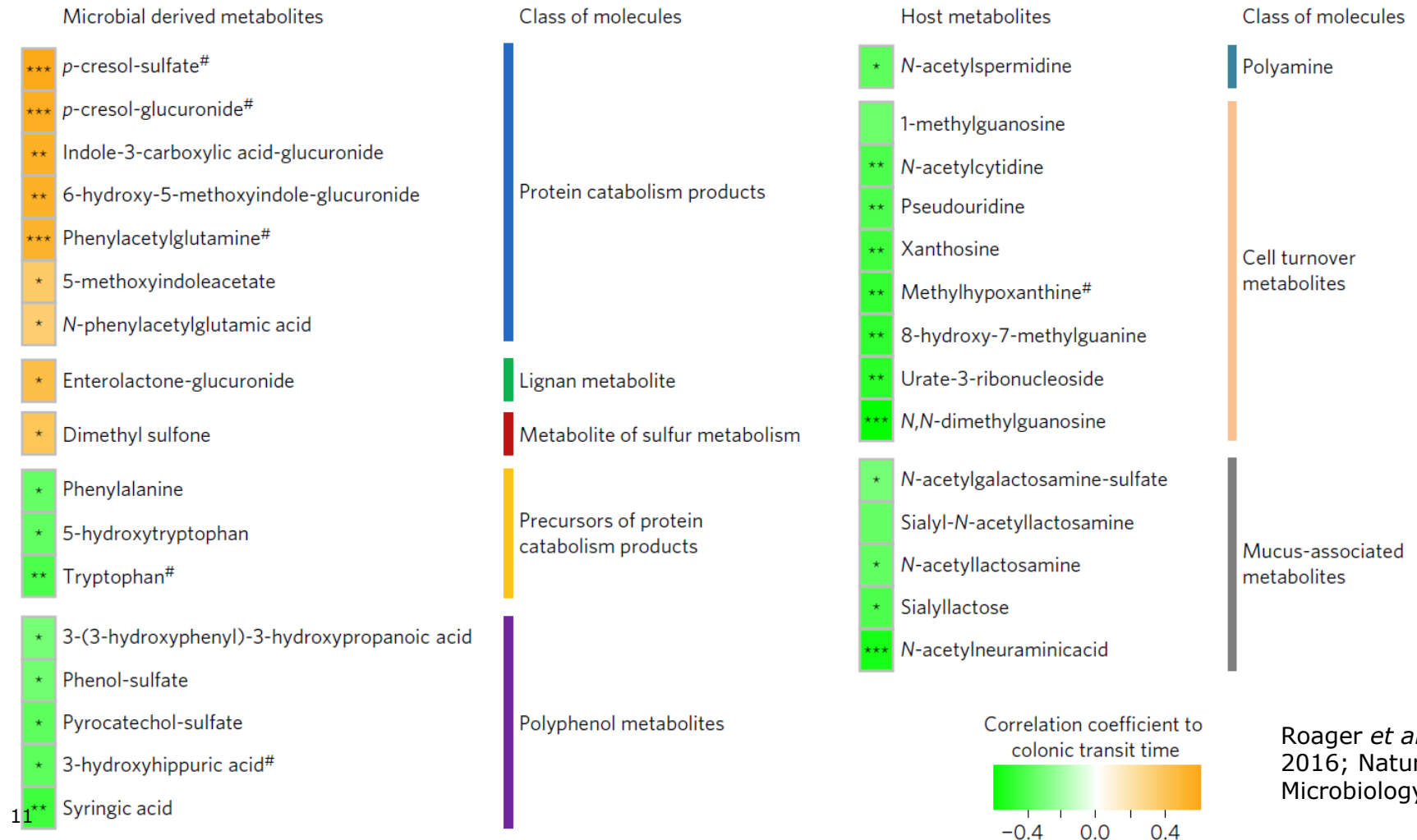| Microbial derived metabolites	Class of molecules
*** p-cresol-sulfate#	
*** p-cresol-glucuronide#	
** Indole-3-carboxylic acid-glucuronide	Protein catabolism products
** 6-hydroxy-5-methoxyindole-glucuronide	
*** Phenylacetylglutamine#	
* 5-methoxyindoleacetate	
* N-phenylacetylglutamic acid	
* Enterolactone-glucuronide	Lignan metabolite
* Dimethyl sulfone	Metabolite of sulfur metabolism
* Phenylalanine	Precursors of protein catabolism products
* 5-hydroxytryptophan	
** Tryptophan#	
* 3-(3-hydroxyphenyl)-3-hydroxypropanoic acid	Polyphenol metabolites
* Phenol-sulfate	
* Pyrocatechol-sulfate	
* 3-hydroxyhippuric acid#	
** Syringic acid	

Host metabolites	Class of molecules
* N-acetylspermidine	Polyamine
1-methylguanosine	Cell turnover metabolites
** N-acetylcytidine	
** Pseudouridine	
** Xanthosine	
** Methylhypoxanthine#	
** 8-hydroxy-7-methylguanine	
** Urate-3-ribonucleoside	
*** N,N-dimethylguanosine	
* N-acetylgalactosamine-sulfate	Mucus-associated metabolites
Sialyl-N-acetyllactosamine	
* N-acetyllactosamine	
* Sialyllactose	
*** N-acetylneuraminicacid	

Correlation coefficient to colonic transit time

−0.4   0.0   0.4

Roager *et al.*, 2016; Nature Microbiology

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Transit time relates to bacterial metabolism and mucosal turnover

Portal circulation

Shift in colonic metabolism

Decreased polyphenol conversion

Polyphenols → Phenols / Pyrocatechol

Increased lignan conversion

Lignans → Enterolactone

Increased protein catabolism

Tyrosine → P-cresol

Phenylalanine → Phenylacetate

Tryptophan → Indole / Indoleacetate / Indole-3-carboxylic acid

Methionine → Methanethiol

Reduced mucus secretion / increased bacterial catabolism

lumen Mucus

epithelium

Methionine → Putrescine

Reduced epithelial cell turnover

Liver/mucosal enzymes

urine

3-(3-hydroxyphenyl)-3-hydroxypropanoic acid ↓
Phenol-sulphate
Pyrocatechol-sulphate
3-hydroxyhippuric acid
Syringic acid
Enterolactone-glucuronide ↑

P-cresol-sulphate ↑
P-cresol-glucuronide

Phenylacetylglutamine ↑
Phenylacetylglutamic acid

6-Hydroxy-5-methoxyindole-glucuronide
5-Methoxyindoleacetate ↑
Indole-3-carboxylic acid -glucuronide

Dimethyl sulfone ↑

N-acetylneuraminic acid ↓
N-acetyllactosamine
N-acetylgalactosamine
Sialyllactose
N-acetylspermidine ↓
Dimethylguanosine ↓
Urate-3-ribonucleoside
8-hydroxy-7-methylguanine
Methylhypoxanthine
Xanthosine
Pseudouridine
N-acetylcytidine

Vegetables

Digestion enzymes

Dietary protein

GI tract

Roager *et al.*, 2016; Nature Microbiology

Transit time relates to the gut microbial composition and diversity

Diet

Increased colonic transit time

Changed gut microbiota composition

- alpha-diversity
- OTU richness ↑

- Ruminococcaceae
- Christensenellaceae
- Methanobrevibacter ↑
- Lachnospiraceae
- Faecalibacterium prausnitizii ↓

Slower passage of food

Shift in colonic metabolism

Conclusions

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

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

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

Roager *et al*., 2016; Nature Microbiology
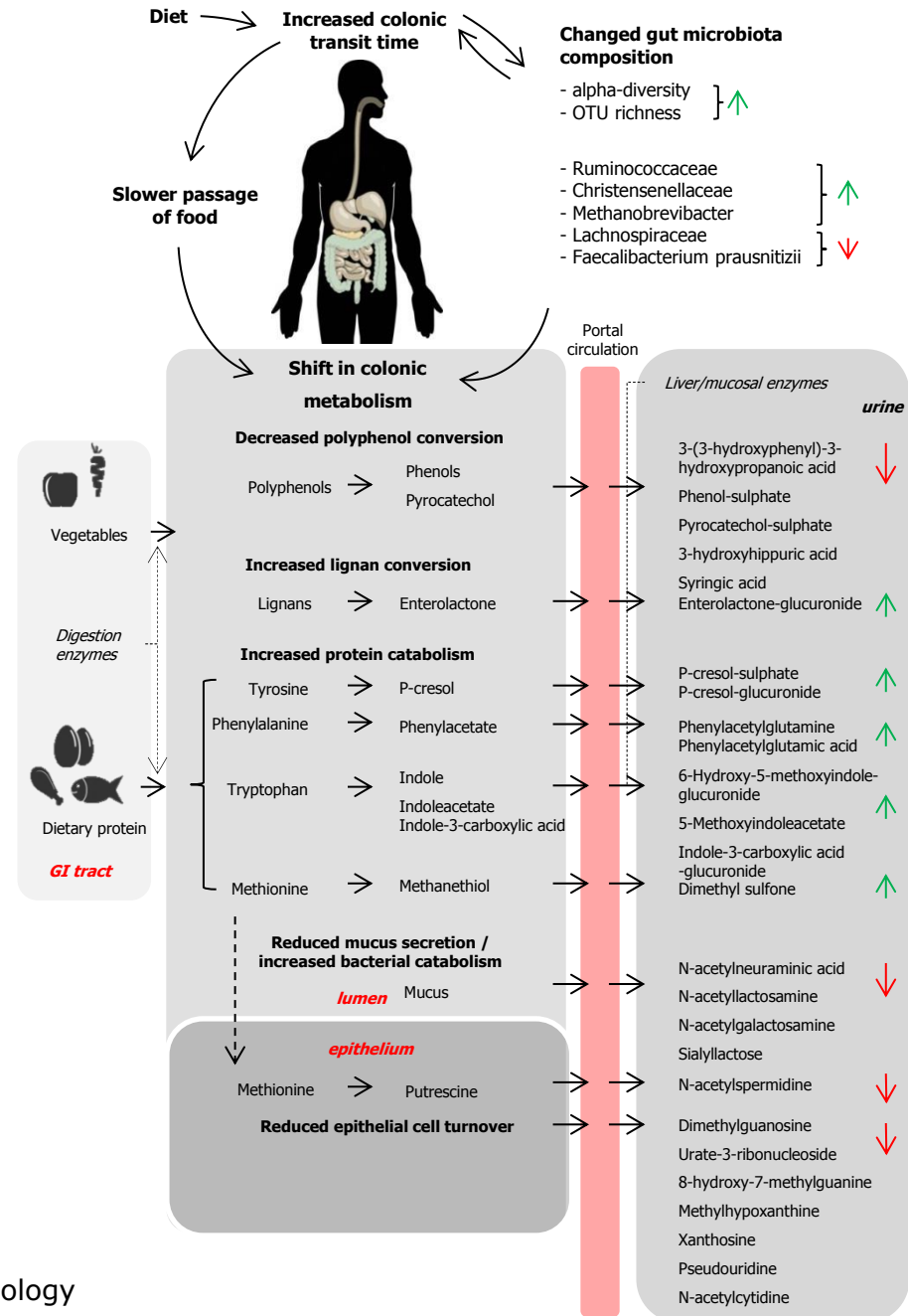
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