A metabolomic analysis of the mammalian gut microbiota

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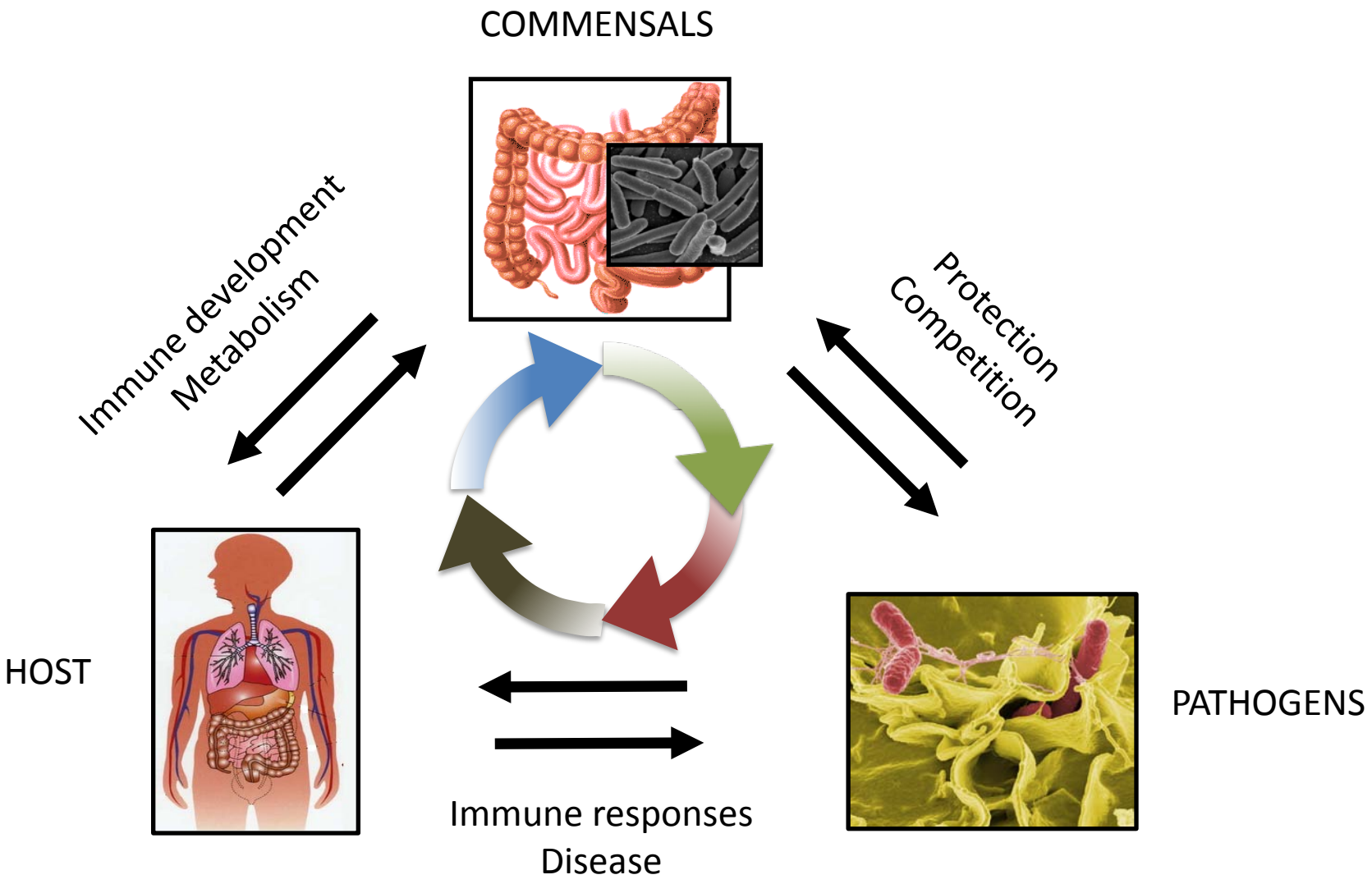
Humans are constantly engaging in complex interactions with microbes



The mammalian gut microbiota

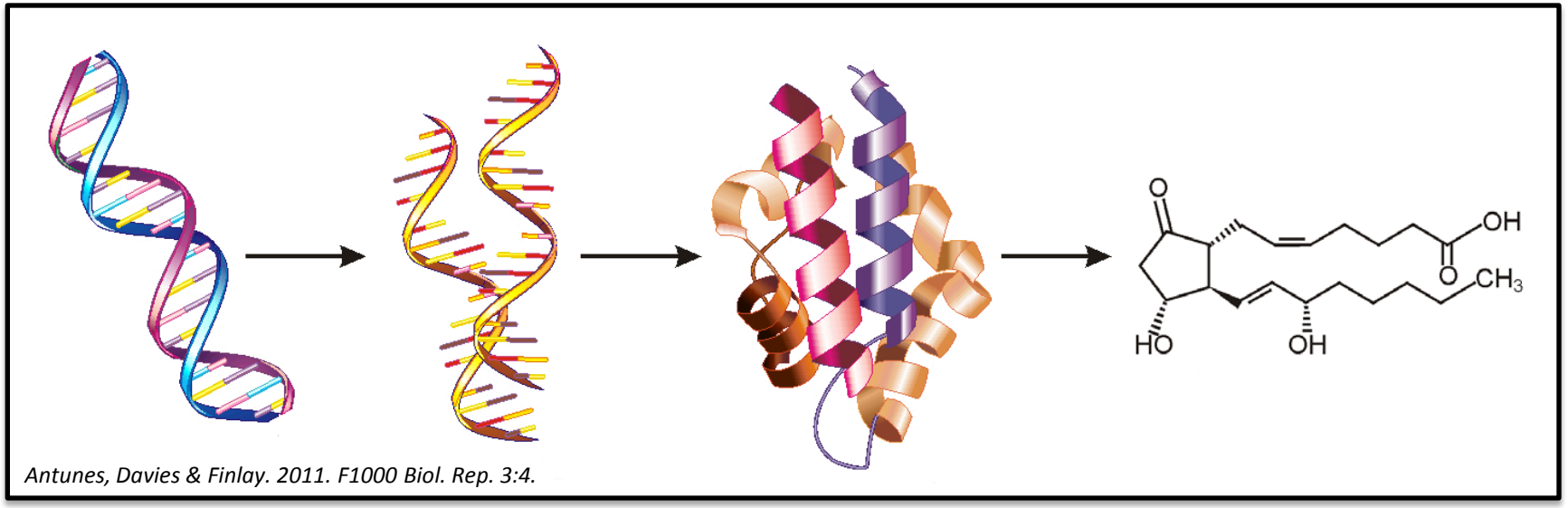
- Consortium of commensal microorganisms
  - 100 trillion cells
  - 10x the number of human cells, 100x the number of human genes
  - >200 genera, >1000 species, >7000 strains
  - Collectively >35,000 species

- Development of the gut-associated immune system

- Energy balance
  - Degradation of complex carbohydrates
  - Activation of nutrient assimilation
  - Synthesis of vitamins

- Protection against pathogens – “Colonization resistance”
  - Competition for nutrients and colonization sites
  - Production of antibacterial molecules

'Omics' technologies provide a powerful way of probing host-microbe interactions



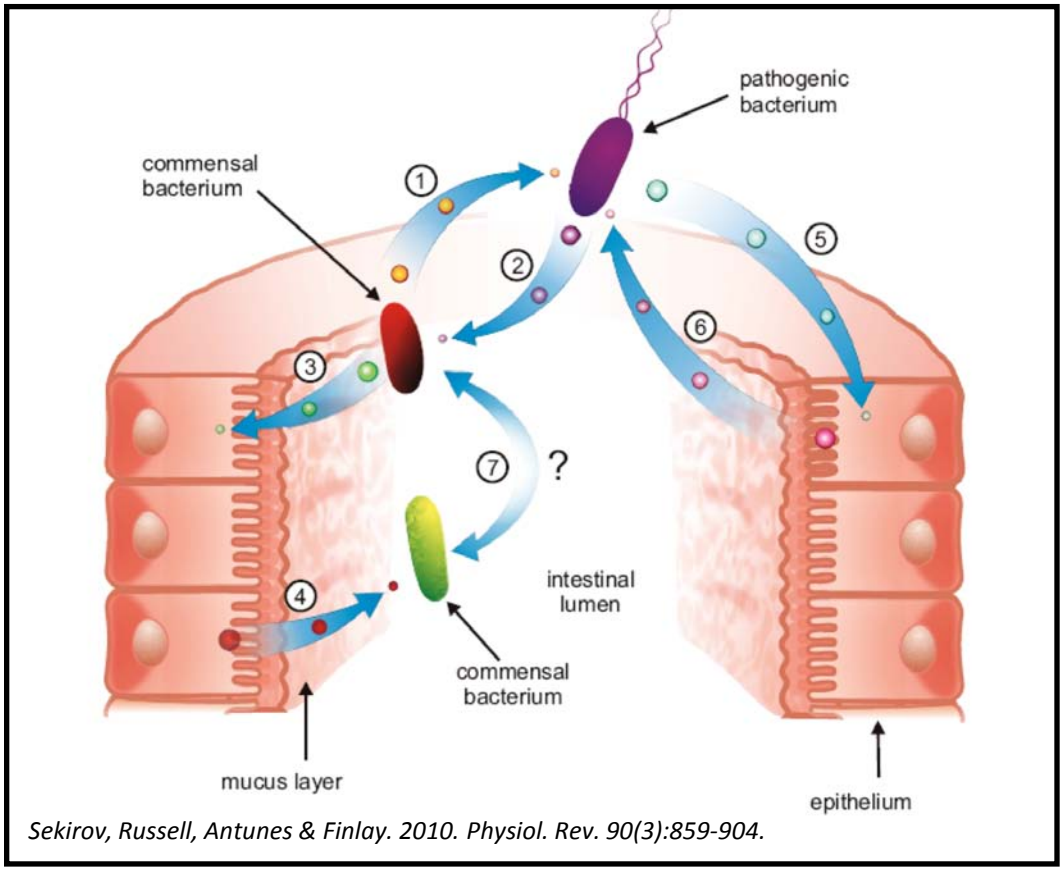
Antunes, Davies & Finlay. 2011. F1000 Biol. Rep. 3:4.

Genomics Transcriptomics Proteomics Metabolomics

SYSTEMS BIOLOGY

Small molecules play important roles in the lifestyle of all organisms

- Endocrine signaling in mammals
  - Homeostasis
  - Response to insult
- Microbial communication
  - Quorum sensing
  - Competition
  - Cooperation
- Metabolic interrelationships
  - Microbial consortia
  - Secondary metabolites



Sekirov, Russell, Antunes & Finlay. 2010. Physiol. Rev. 90(3):859-904.

What are the roles played by small molecules in commensal host-microbe interactions?

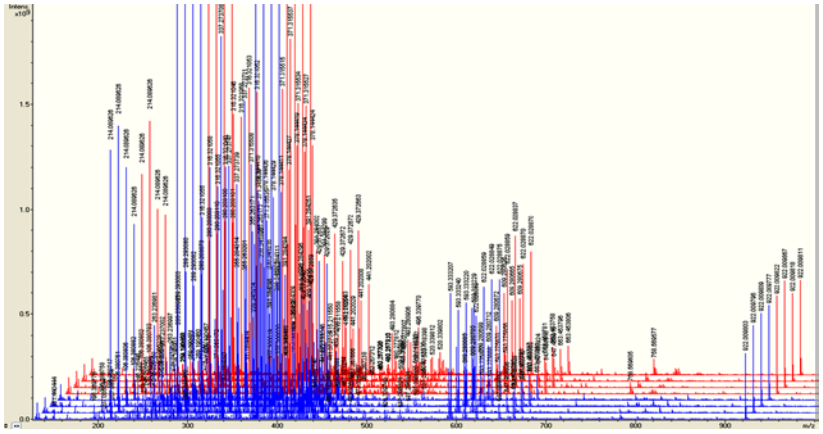
What is the impact of the microbiota on the chemical composition of the gastrointestinal tract?

Collect feces
Acetonitrile extract

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

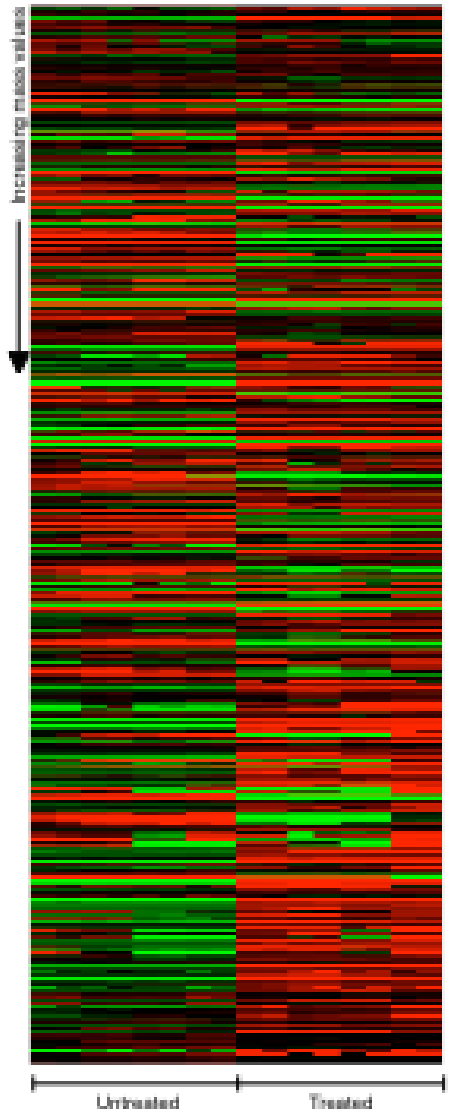
Streptomycin

Metabolic profiling



DATABASE SEARCHES

The levels of several hundred fecal metabolites are affected by antibiotic treatment

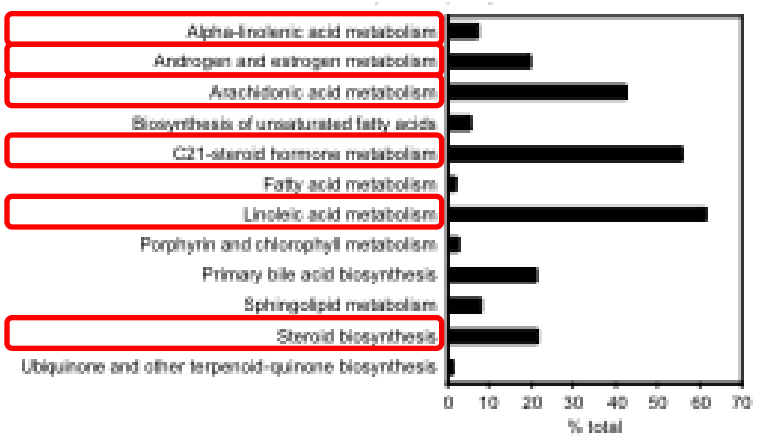


Metabolites detected	
Negative ionization	1043
Positive ionization	1386
Overlap	199
Total	2230
Metabolites changed	
Untreated > Treated	793
Treated > Untreated	1165
Total changed	1958
% total	87.8

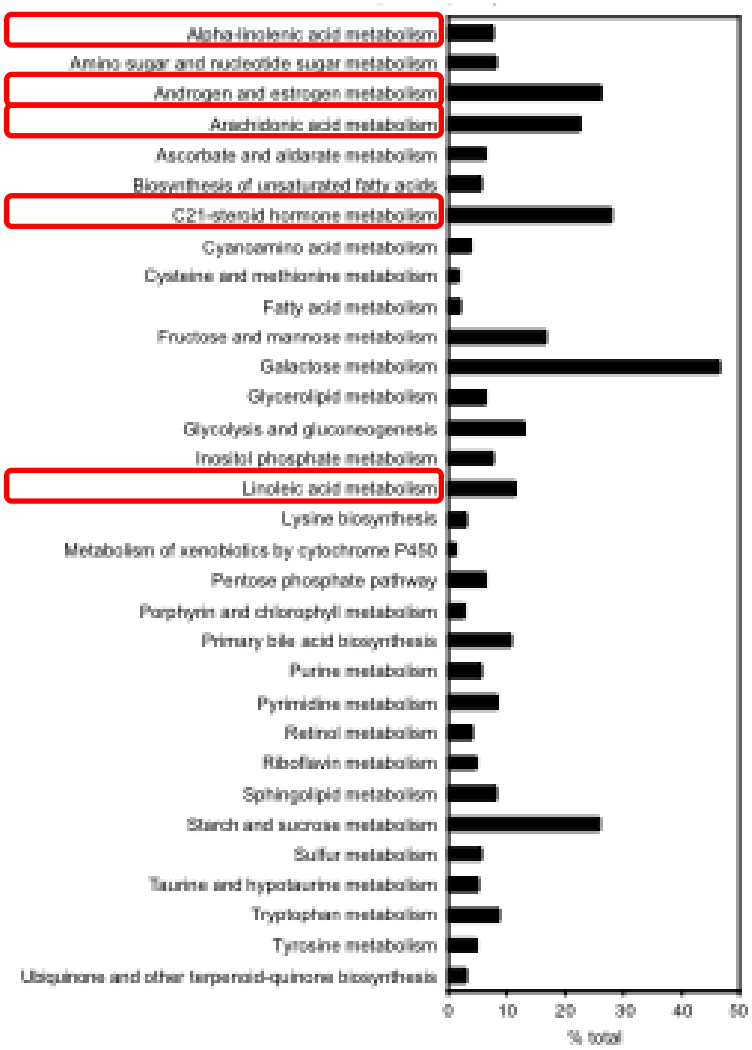
Antunes et al. 2011. Antimicrob. Agents Chemother. Accepted.

Multiple host metabolic pathways are affected by antibiotic treatment

Decreased after antibiotic treatment



Increased after antibiotic treatment



Steroids

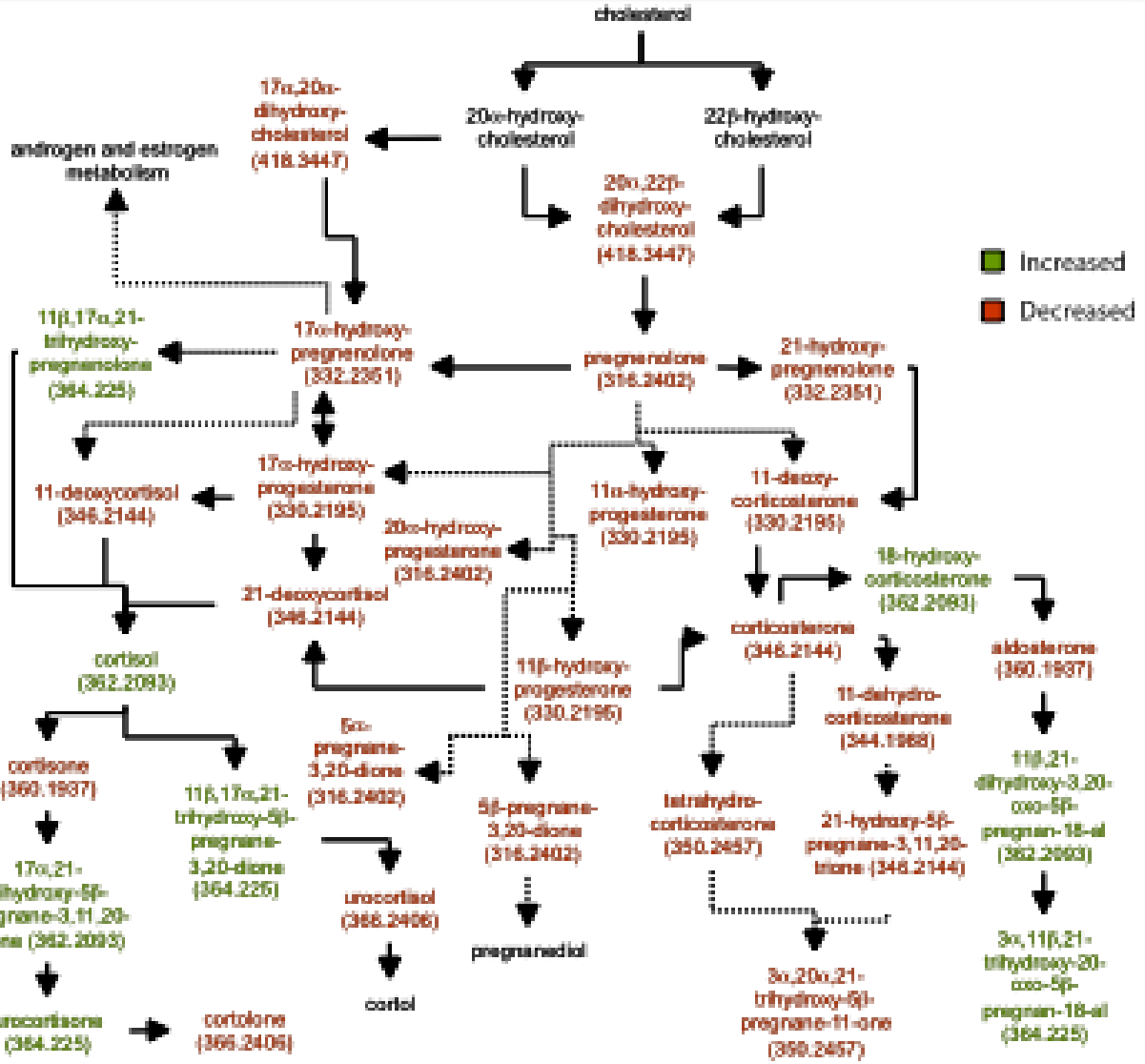
- Androgen and estrogen metabolism
- C21-steroid hormone metabolism
- Steroid biosynthesis

Eicosanoids

- Alpha-linoleic acid metabolism
- Linoleic acid metabolism
- Arachidonic acid metabolism

Antunes et al. 2011. Antimicrob. Agents Chemother. Accepted.

Steroid hormone metabolism is affected by antibiotic treatment



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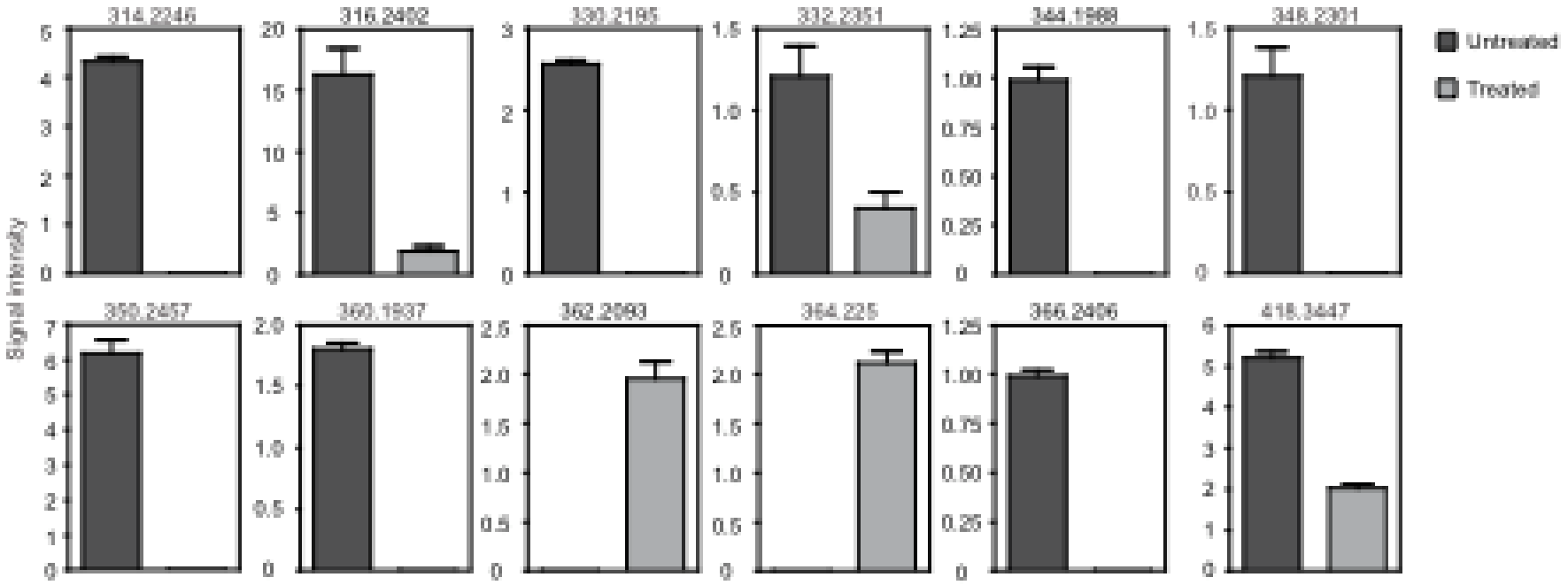
Steroid hormone metabolism is affected by antibiotic treatment



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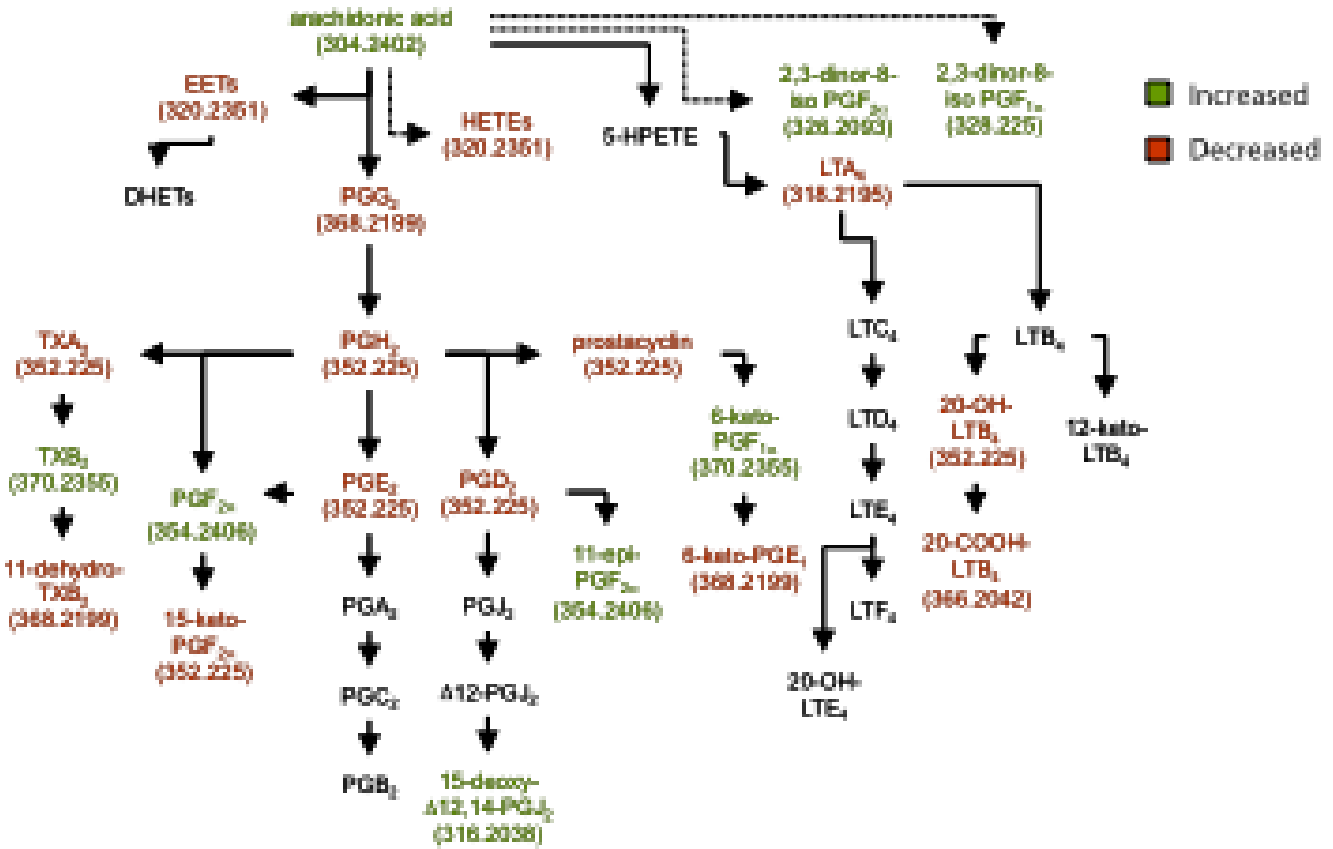
Eicosanoid hormone metabolism is affected by antibiotic treatment



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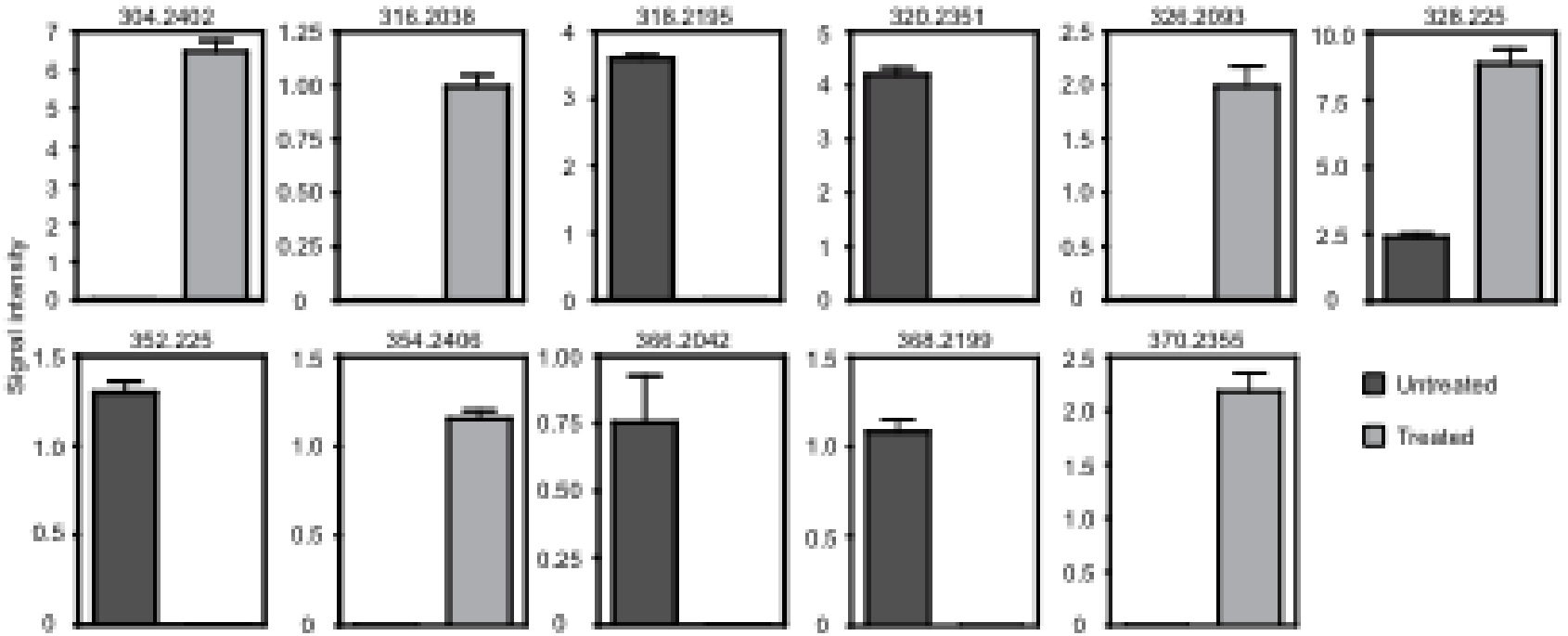

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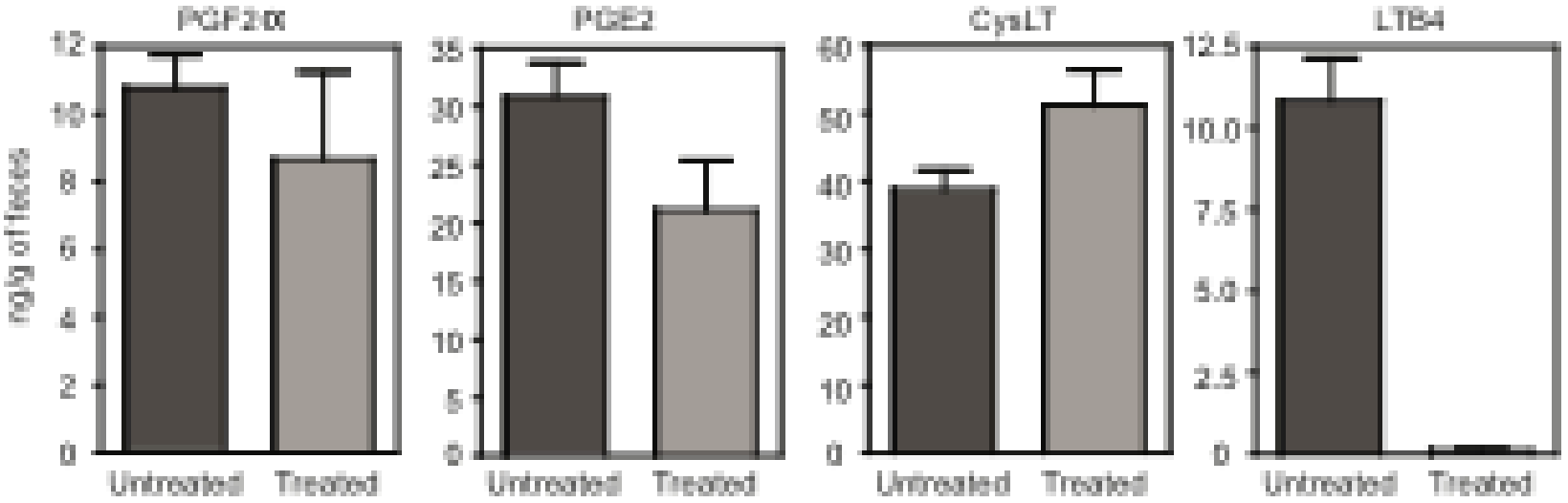
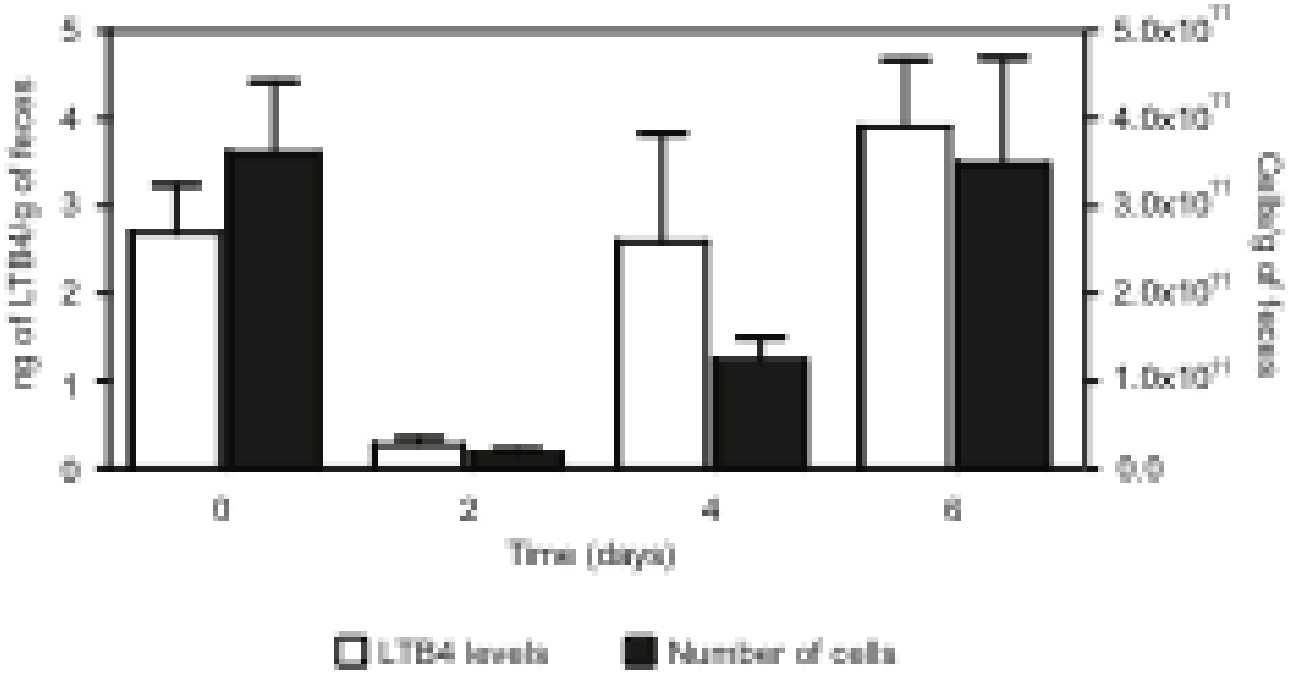
Leukotriene B4 levels are highly impacted by antibiotic treatment



Antunes et al. 2011. Antimicrob. Agents Chemother. Accepted.

Leukotriene B4 levels correlate with the number of bacteria colonizing the gastrointestinal tract



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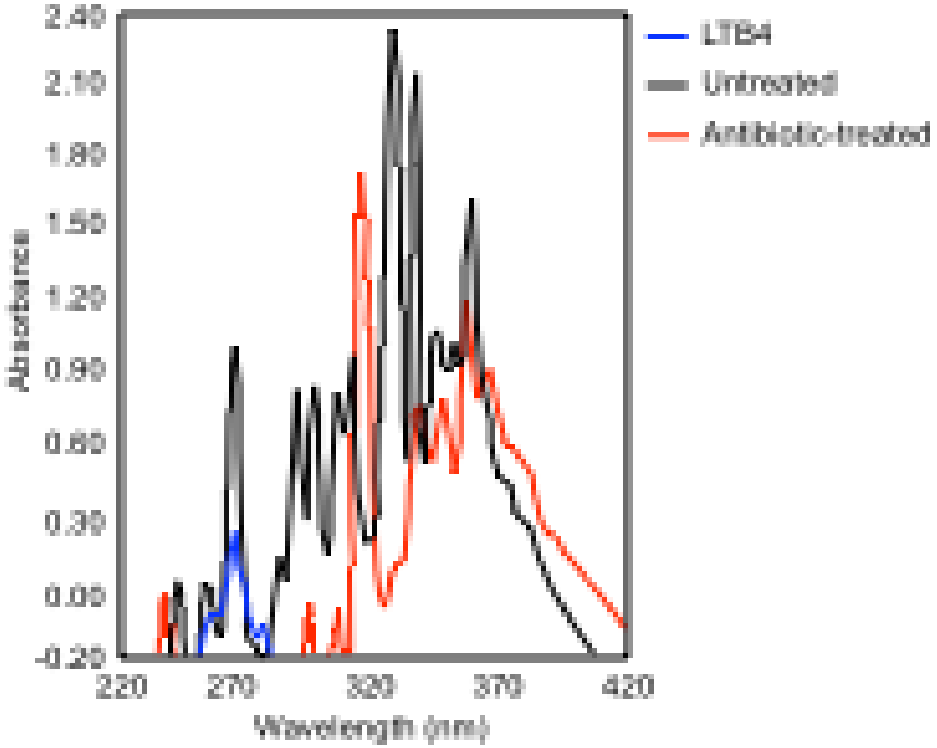
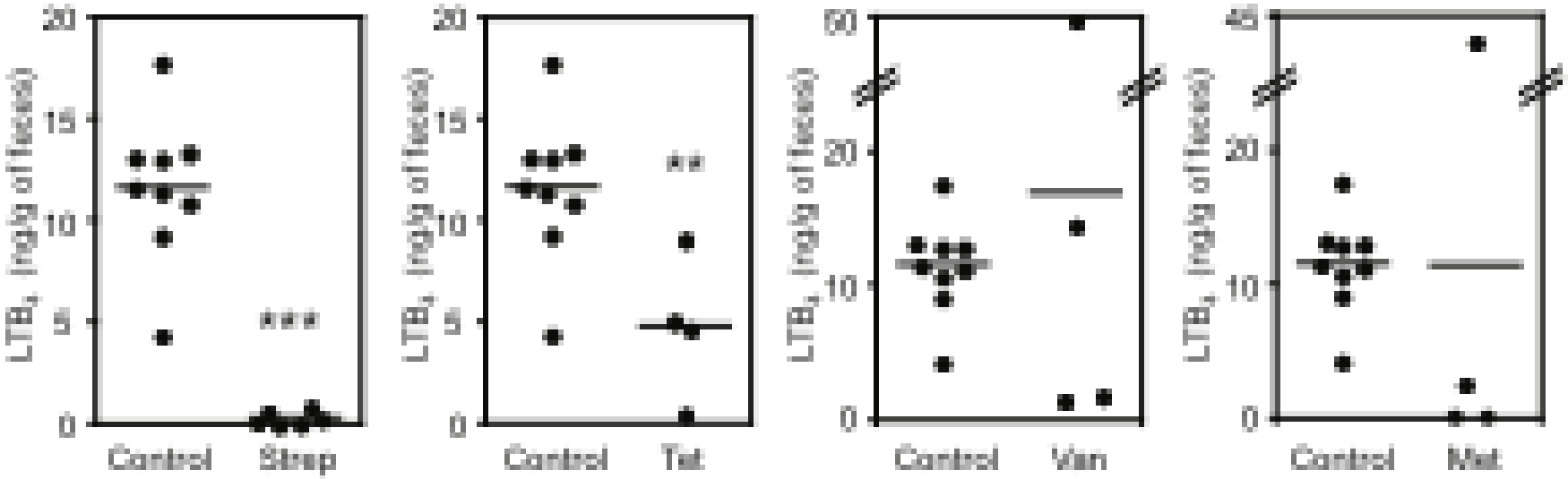


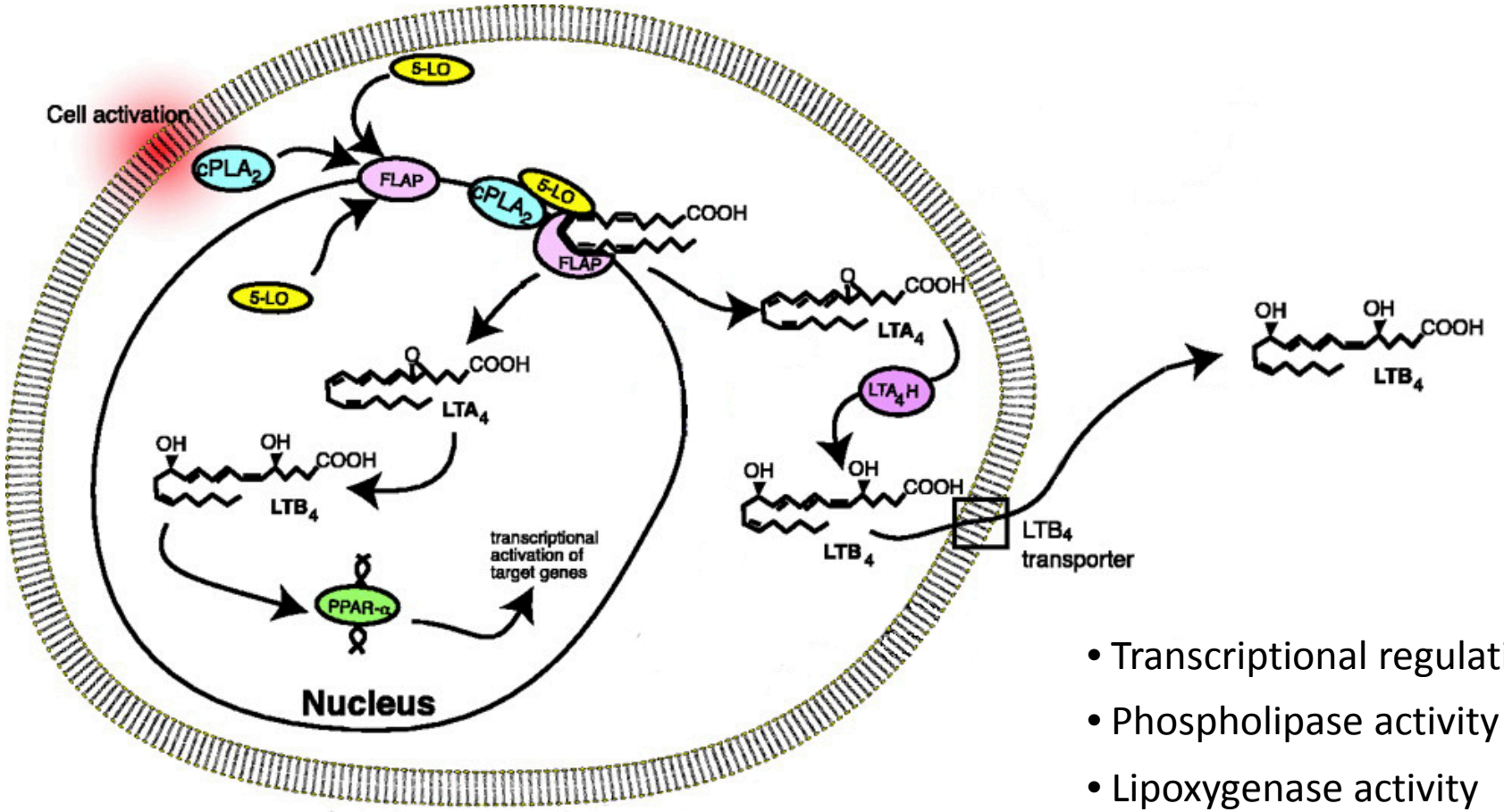
Clinically-relevant doses of antibiotics can impact fecal levels of leukotriene B4



Streptomycin: 450 mg/L
Tetracycline: 50 mg/L
Metronidazole: 750 mg/L

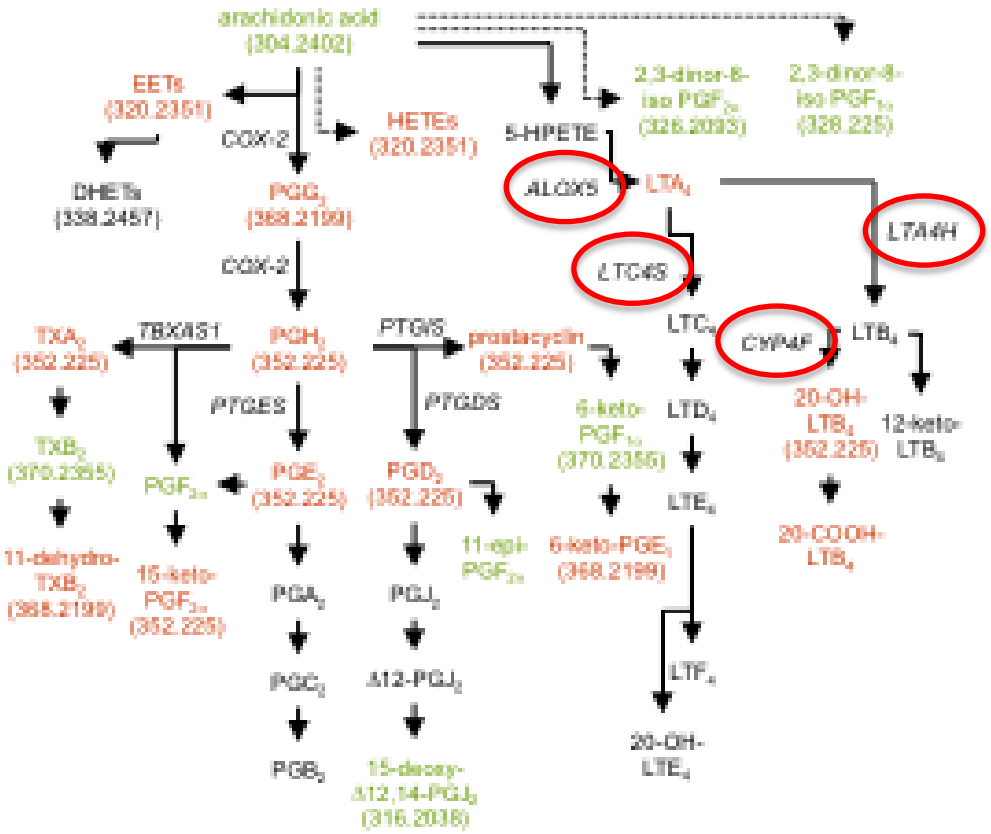
Antunes et al. 2011. Antimicrob. Agents Chemother. Accepted.

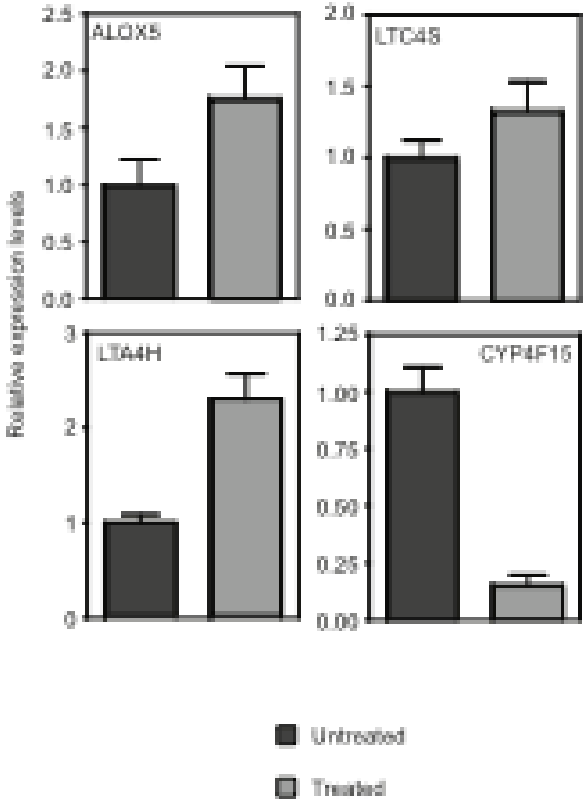
How does the gut microbiota affect leukotriene B4 production?



- Transcriptional regulation
- Phospholipase activity
- Lipoxygenase activity
- Transport

Transcriptional regulation alone does not explain the effect of antibiotic treatment on leukotriene B4 metabolism





Eicosanoids are involved in host responses to infection

Phagocytosis and bactericidal action of mouse peritoneal macrophages treated with leukotriene B4.

Demitsu *et al*. 1989. Int. J. Immunopharmacol. 11(7):801-8.

Signal transduction and invasion of epithelial cells by *S. typhimurium*.

Pace *et al*. 1993. Cell. 72(4):505-14

***Salmonella* infection induces a hypersecretory phenotype in human intestinal xenografts by inducing cyclooxygenase 2.**

Bertelsen *et al*. 2003. Infect. Immun. 71(4):2102-9.

***Salmonella enterica* serovar Typhimurium infection induces cyclooxygenase 2 expression in macrophages: involvement of *Salmonella* pathogenicity island 2.**

Uchiya *et al*. 2004. Infect. Immun. 72(12):6860-9.

The gut microbiota confers resistance to colonization by pathogens

Antibiotic treatment increases mouse susceptibility to *Salmonella* infection

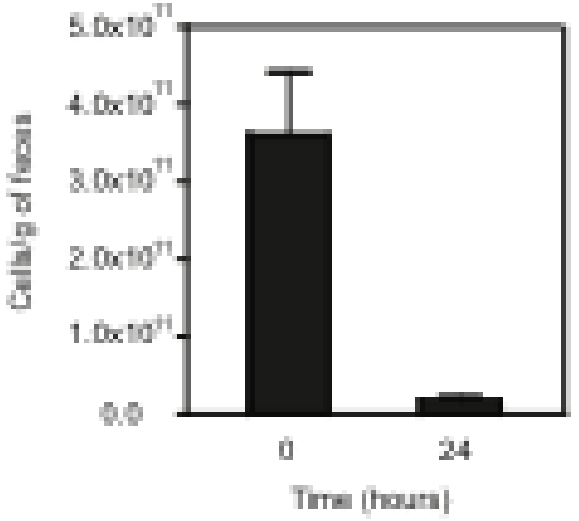
20 mg streptomycin +

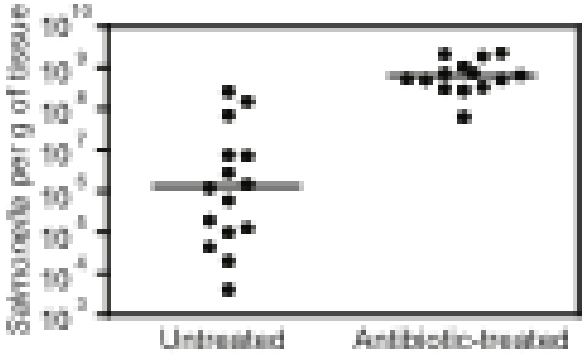
24 hours ↓

Salmonella Typhimurium

1-5 days ↓

Salmonella pathogenesis
- Bacterial loads
- Inflammation
- Transmission





Leukotriene B4 can partially rescue resistance to Salmonella infection in antibiotic-treated mice

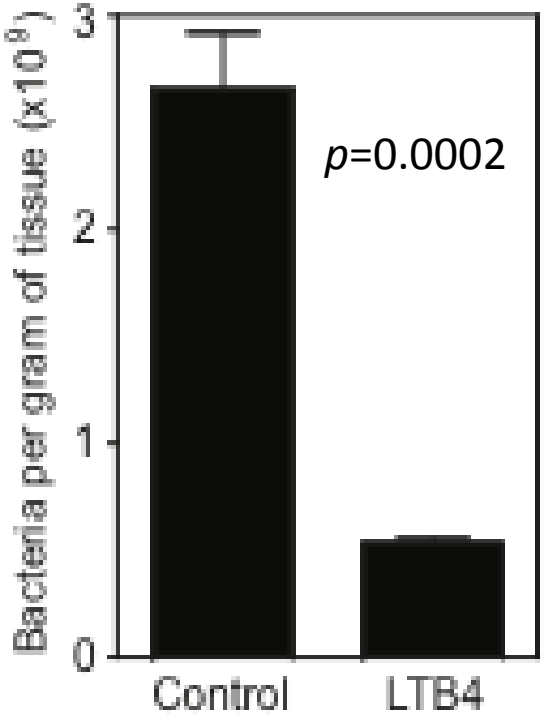
20 mg streptomycin +

24 hours ↓

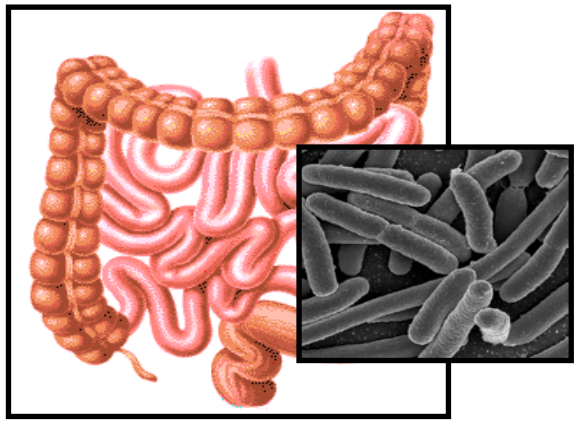
Salmonella Typhimurium

1-5 days ↓ **± LTB4 orally**

Intestinal colonization by *Salmonella*

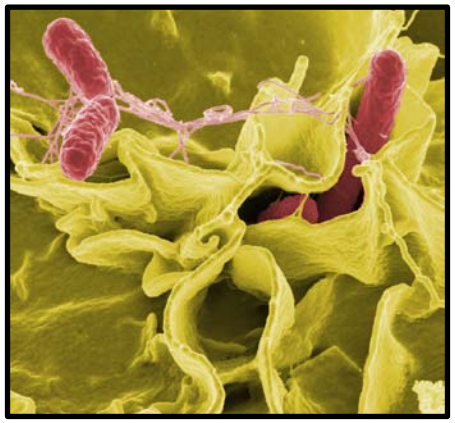


What are the roles of the other 1000's of small molecules present in the mammalian gut?



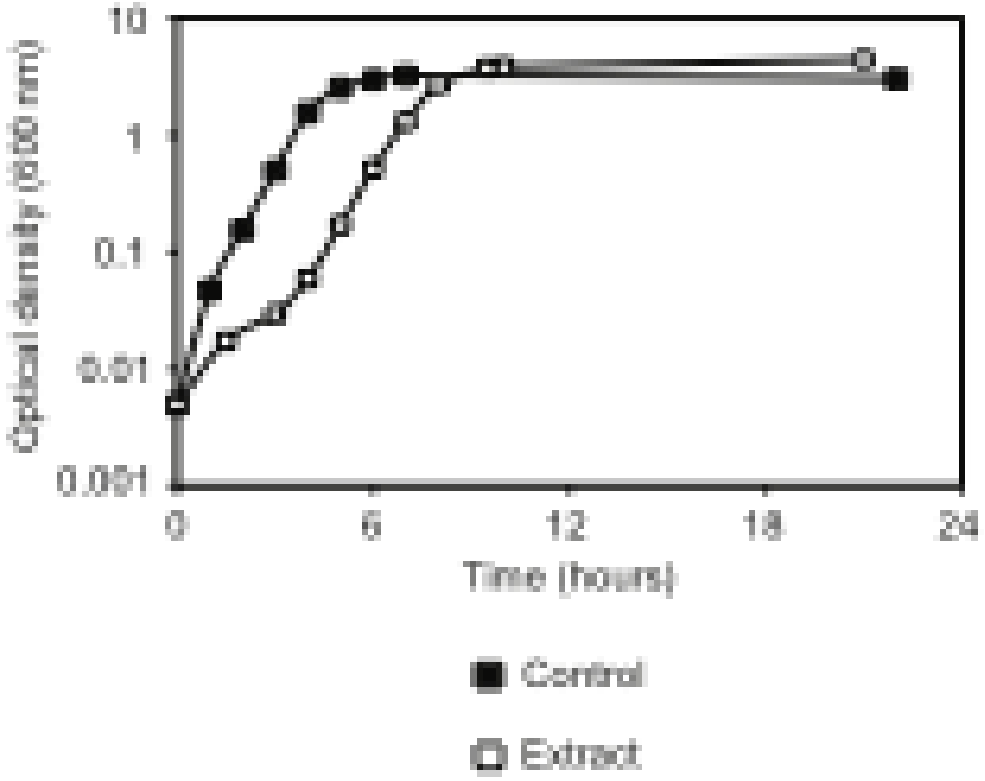
Human feces

Ethyl acetate
extraction
≈16 hours
↓
Centrifuge
↓
Supernatant
-20 °C



Growth
Gene expression

The gut metabolome is a potential source of small molecules with antibiotic activity

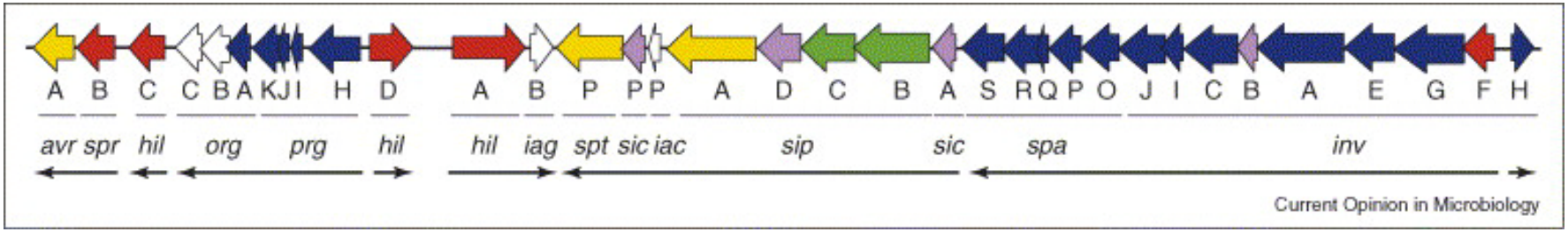


The gut metabolome contains small molecules that control Salmonella gene expression

Gene	Common Name of Primary Target	Fold-change
fliC	flagellin	7.1
flgL	flagellar hook-associated protein FlgL	3.8
spoT	bifunctional (p)ppGpp synthetase/hydrolase	3.8
flgD	flagellar basal body rod modification protein	3.6
flgN	putative FlgK/FlgL export chaperone	3.4
flgM	anti-sigma28 factor FlgM	3.3
lon	DNA-binding ATP-dependent protease La	2.8
cheY	chemotaxis regulatory protein CheY	2.7
flgB	flagellar basal body rod protein FlgB	2.3
iagB	invasion protein precursor	2.3
hilD	invasion protein regulatory protein	-9.2
fljB	flagellin	-7.1
invB	secretion chaperone	-6.7
sopA	secreted effector protein	-5.1
prgK	needle complex inner membrane lipoprotein	-4.0
orgB	needle complex export protein	-3.7
invH	needle complex outer membrane lipoprotein precursor	-2.2
fis	DNA-binding protein Fis	-2.1

The gut metabolome is a potential source of small molecules with anti-virulence activity

The Salmonella Pathogenicity Island 1 is repressed by small molecules from human feces

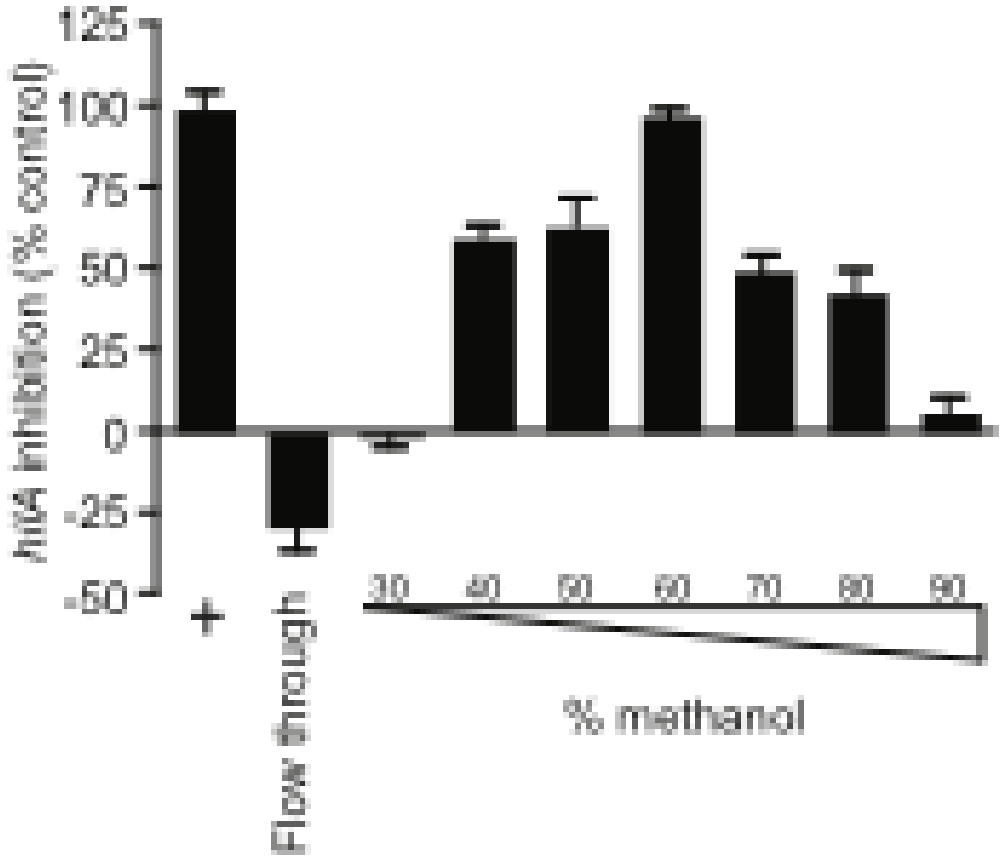


The bioactive molecule from human feces binds C_{18} resin and elutes at 60% methanol

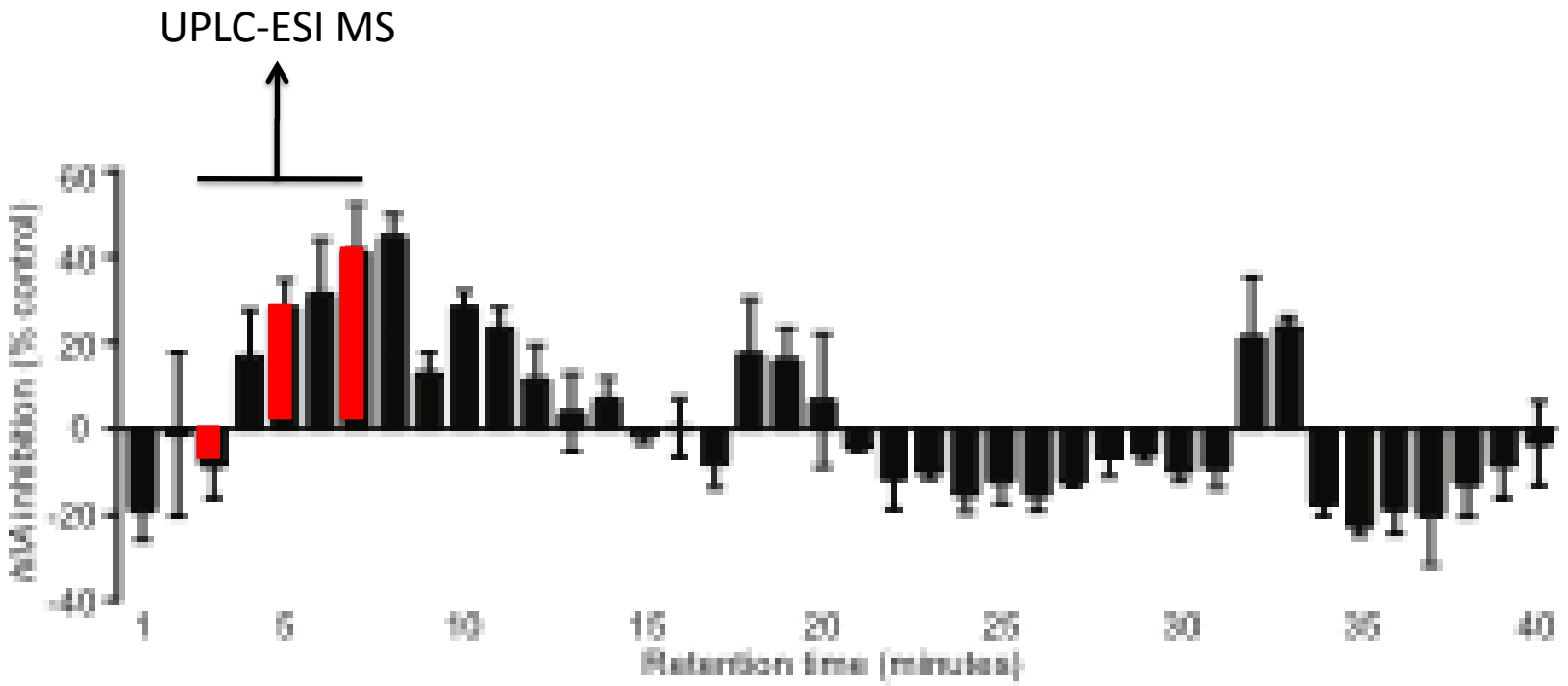
Fecal extract

C18 cartridge

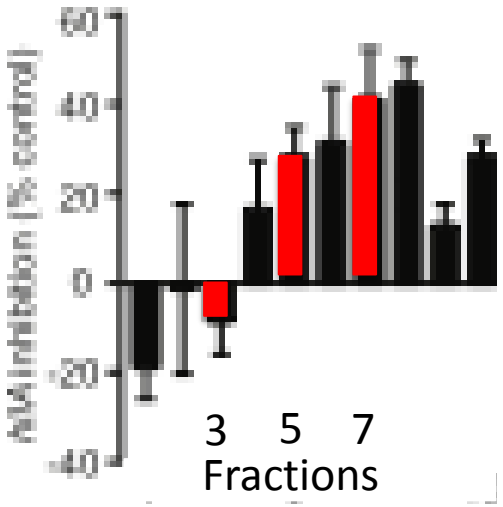
Gradual elution
in methanol

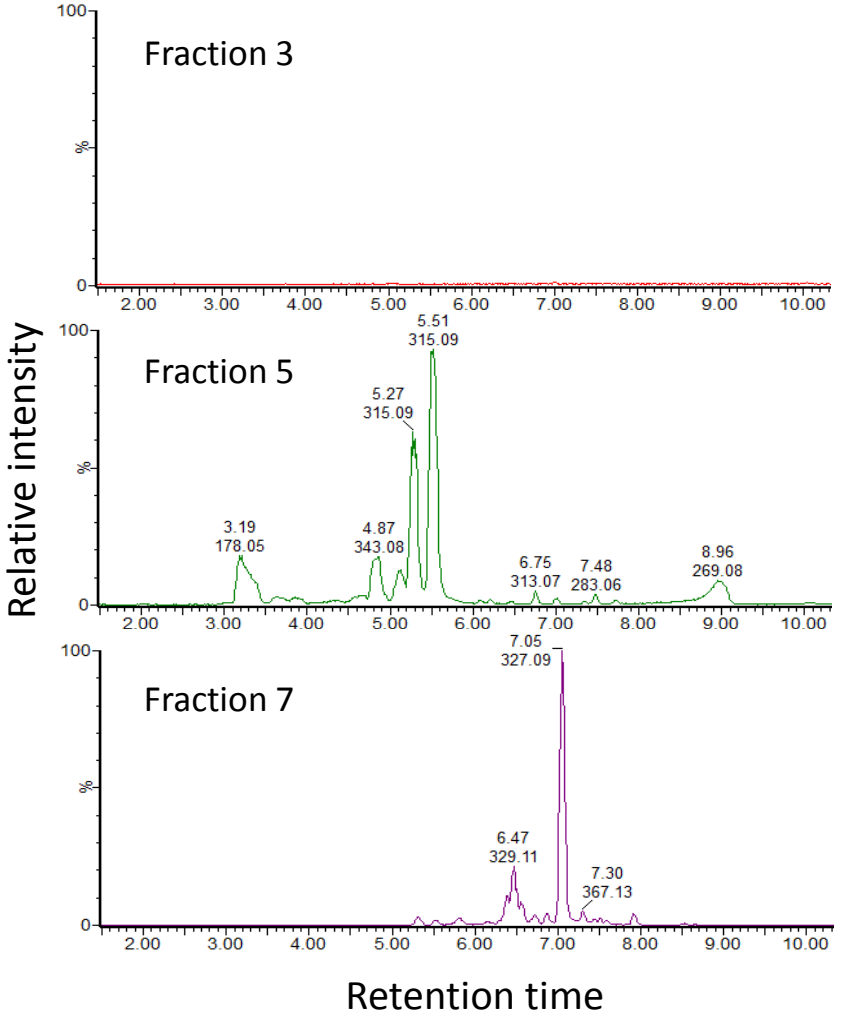


The bioactive molecule from human feces can be purified through C_{18} reverse-phase HPLC



UPLC-ESI MS profiles can be used to identify potential candidates with bioactivity





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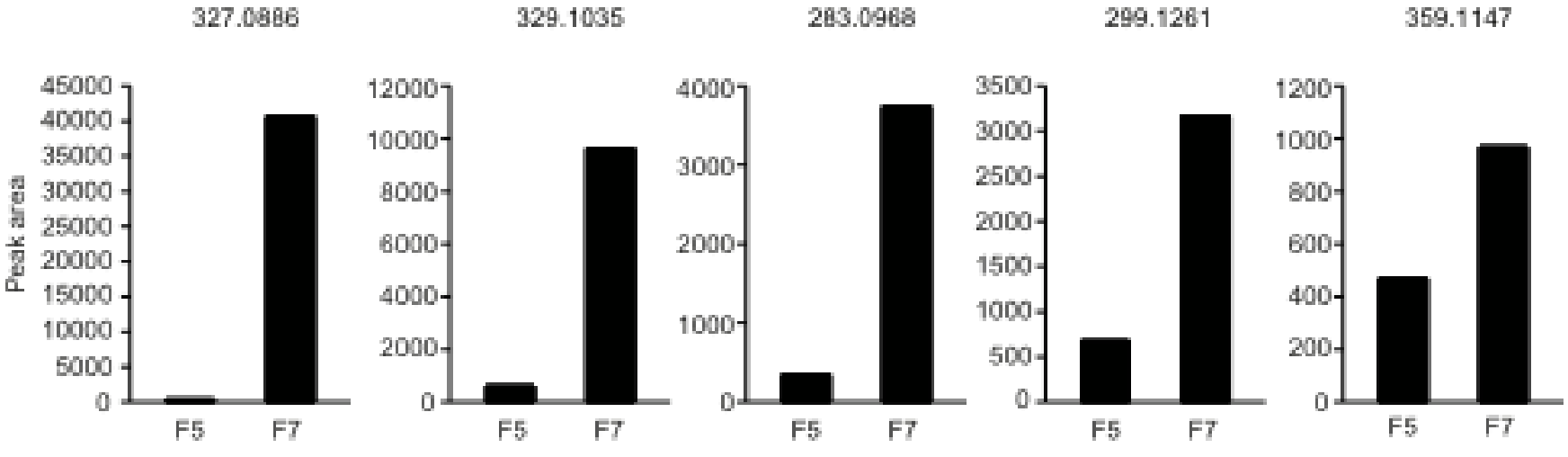

Accurate mass determination
Database searches

327.086843	**329.103062**	**283.097583**	**299.128867**	**359.113457**
2-Hydroxycinnamic acid *Enol-phenylpyruvate* *Phenylpyruvic acid* *m-Coumaric acid* *4-Hydroxycinnamic acid*	*Hydroquinone* *Pyrocatechol*	*Heme A*	*Hydrocinnamic acid* *2-Phenylpropionate* *4-Coumaryl alcohol*	*Galactinol dihydrate*

The gut microbiota is required for the production of the bioactive molecule from human feces

Collect feces
Acetonitrile extract

hilA expression

Streptomycin

What's next?

- What are the molecular mechanisms involved in microbe interactions with the mammalian endocrine system?
- What are the molecular details of signaling between the intestinal microbiota and incoming pathogens?
- Can we use this systems biology approach to identify the molecular determinants of interactions between humans and other microbiomes?
- What are the roles of the other 1000's of small molecules present in the mammalian gut?
- Can the intestinal metabolome be explored as a source of bioactive molecules?
  - Antibiotic
  - Anti-virulence
  - Anti-inflammatory
  - Prebiotic

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