



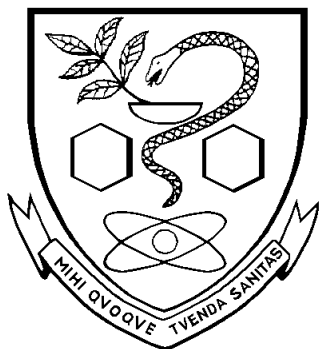
FBA417 - Alimentos e Nutrição II.

DCNT/Cardiovascular : Interação do microbioma e dieta em doenças cardiovasculares

Christian Hoffmann

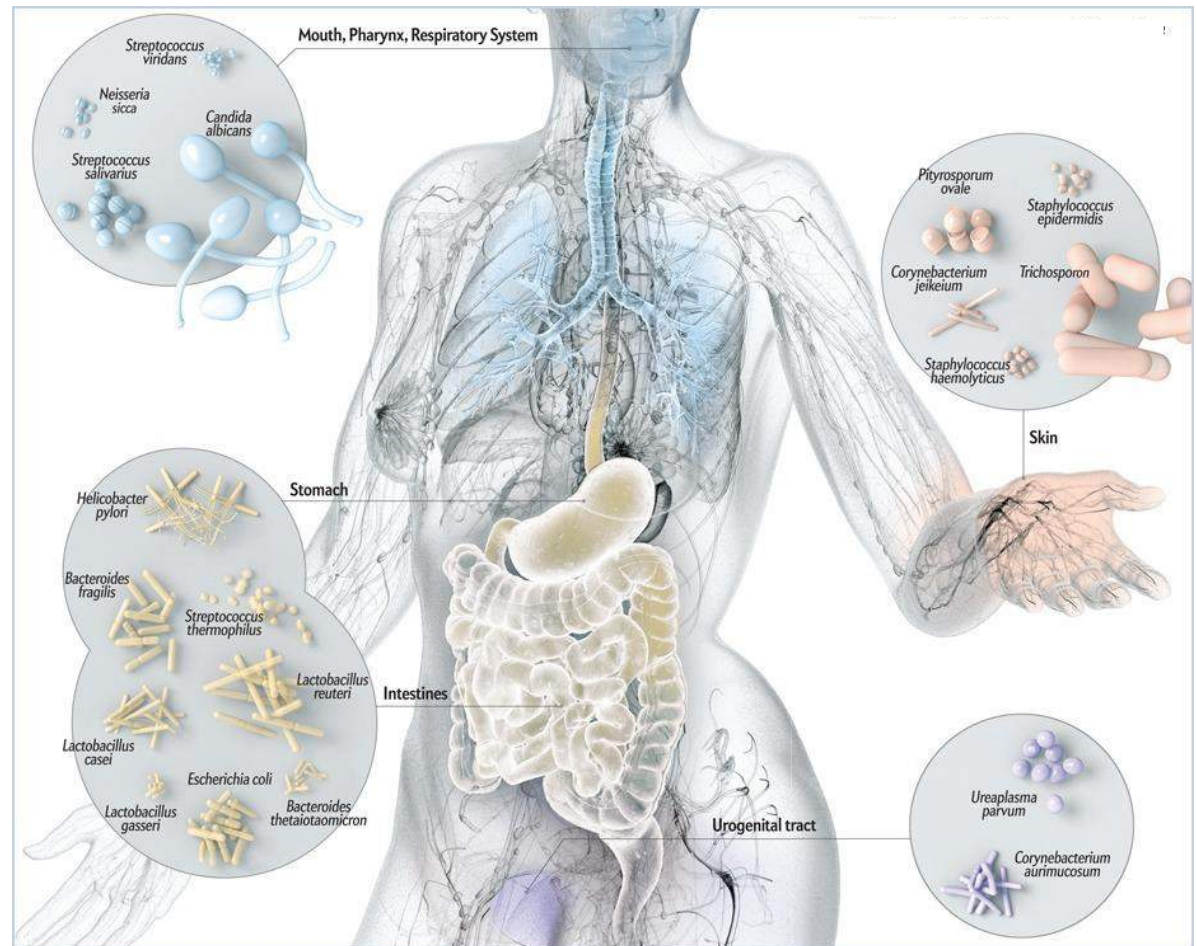
USP/FCF

Março 2015.



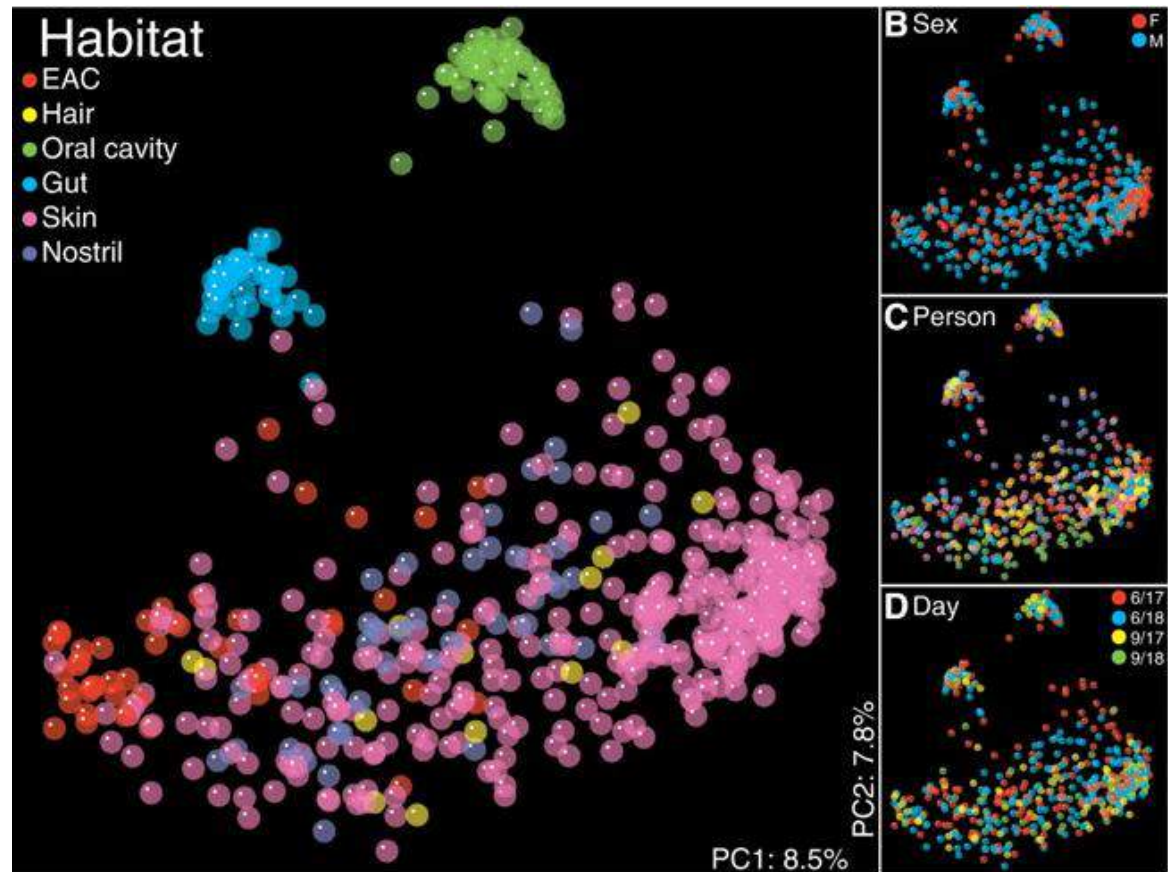
O Microbioma Humano

- Composto de: Bactérias, Arquêas, Eucariotos e Vírus
- Densidade: 10^{11} a 10^{12} células/ml (cólon)
- Número alto de espécies presente
- Um Microbioma para cada parte do corpo (naris, boca, intestino, pele etc.)



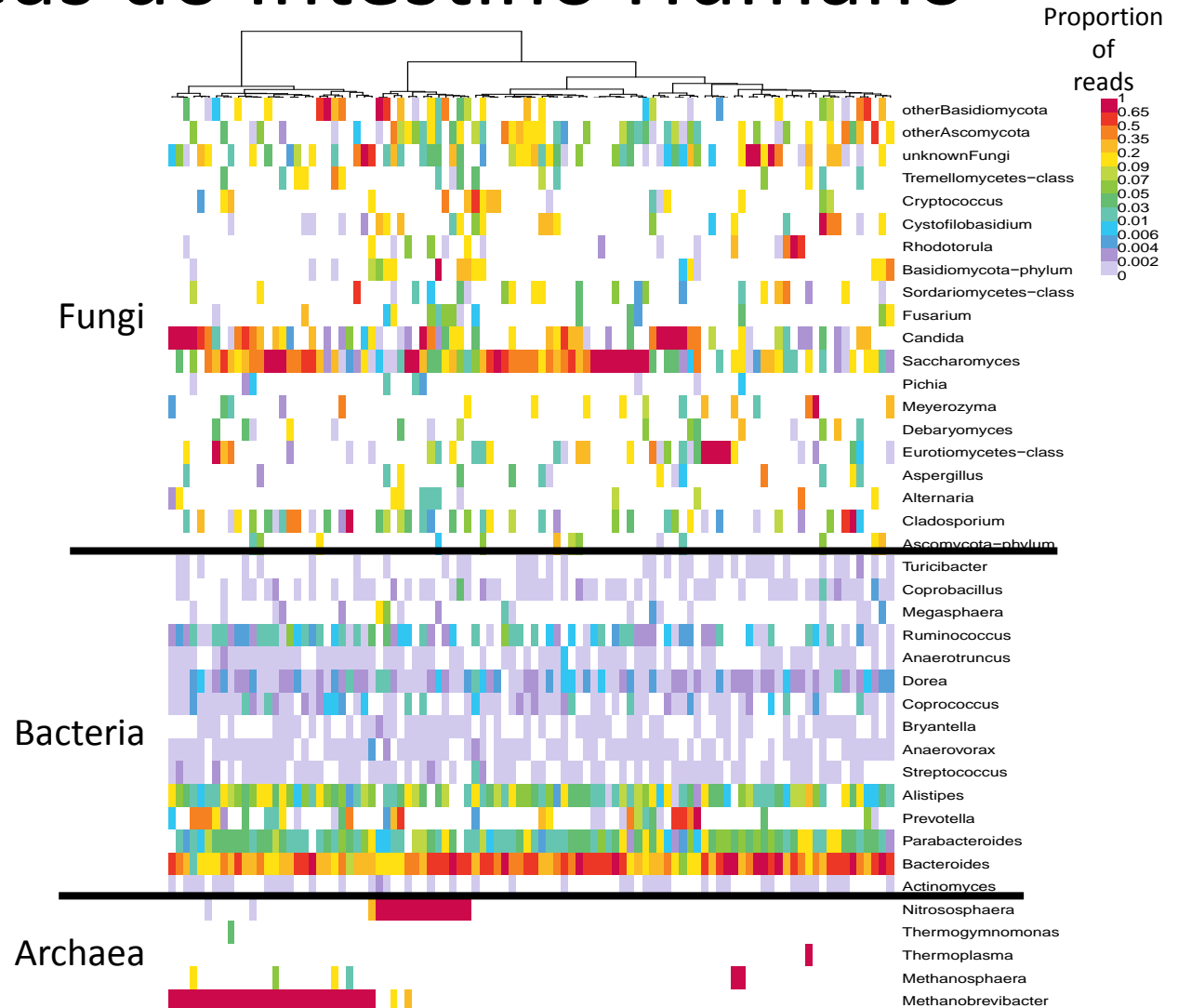
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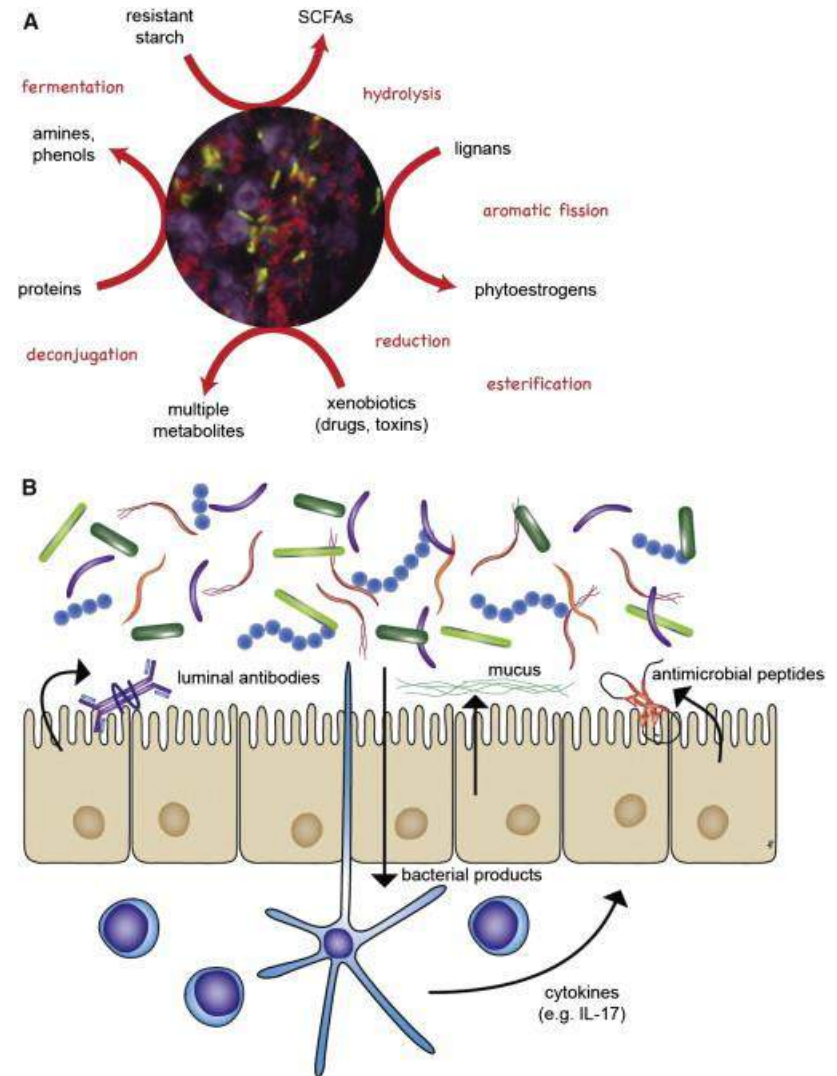
Diversidade: Bactérias, Fungos e Arqueas do Intestino Humano

- 66 gêneros fúngicos
- 5 gêneros Arqueanos



O Microbioma Intestinal Humano

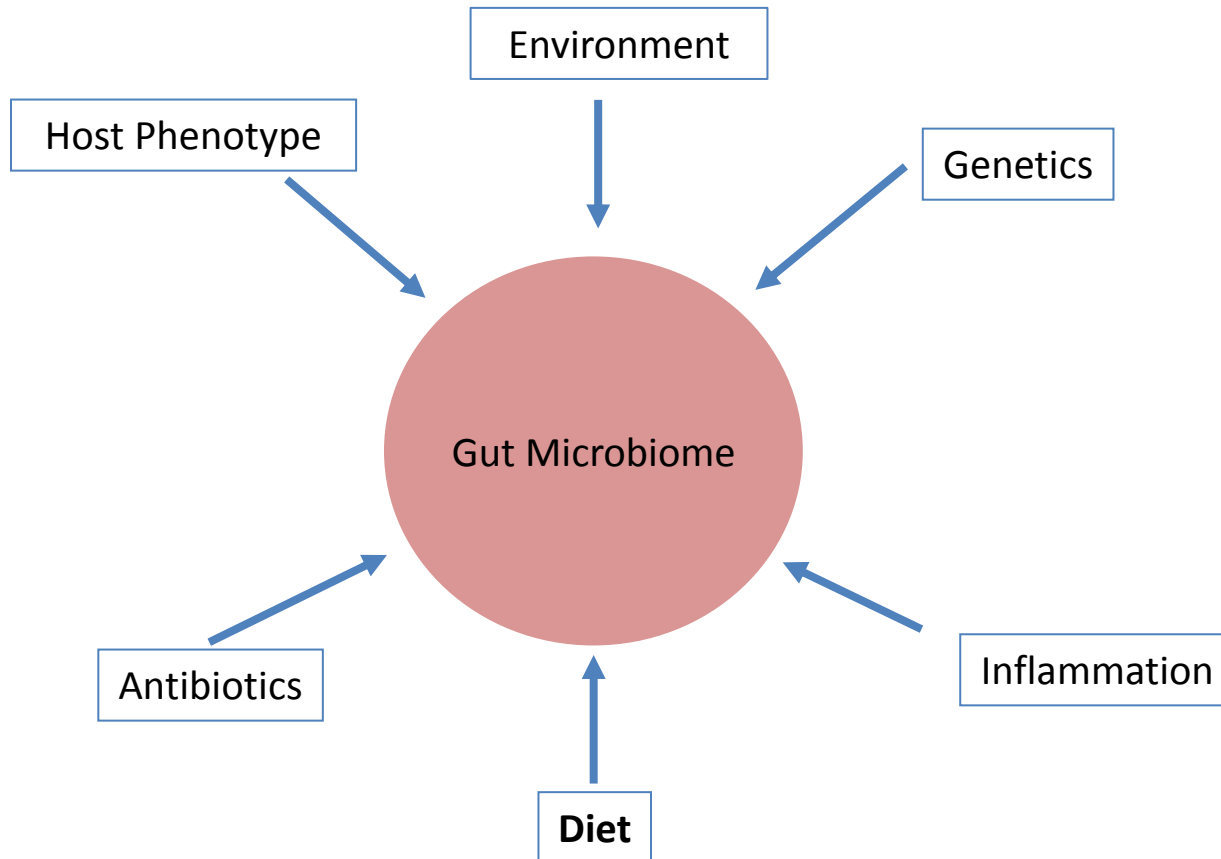
- Proteção ao tecido epitelial
- Regulação do armazenamento de gorduras do hospedeiro
- Estimulação da angiogenese intestinal
- Desenvolvimento do sistema imune
- Digestão
- Etc etc etc



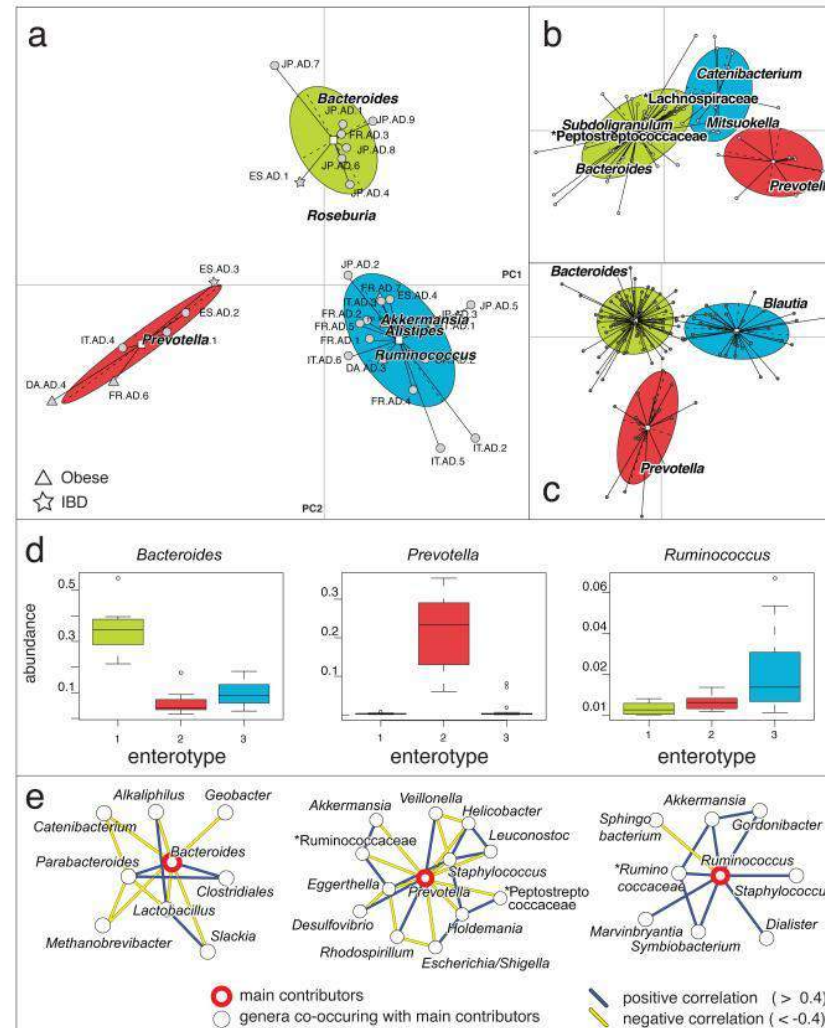
Influências *no* Microbioma

?

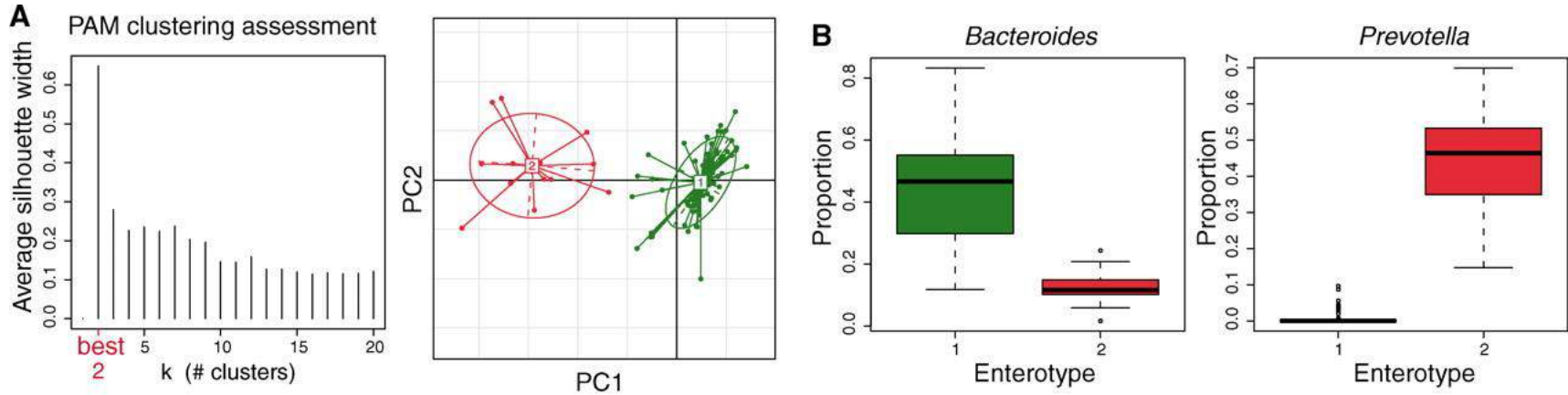
Influências *no* Microbioma



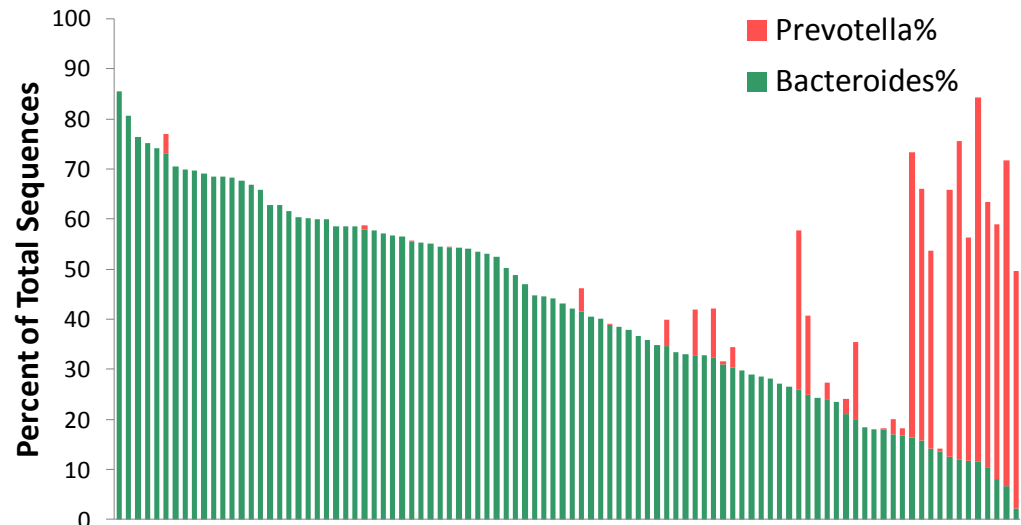
Variação do Microbioma: Enterótipos



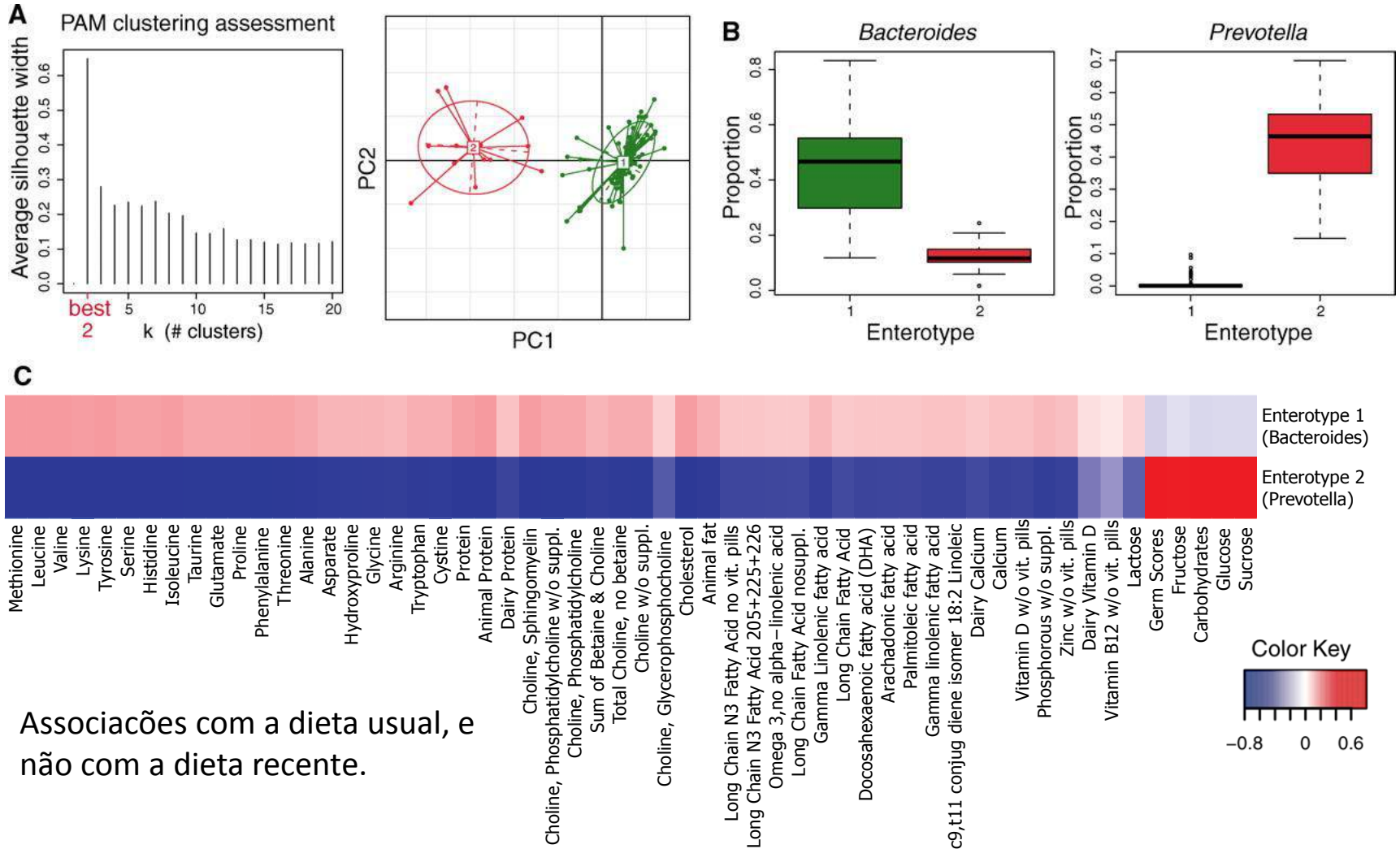
Variação do Microbioma: Enterótipos



- Um balanço entre *Bacteroides* e *Prevotella*



Enterótipos e a Dieta Humana

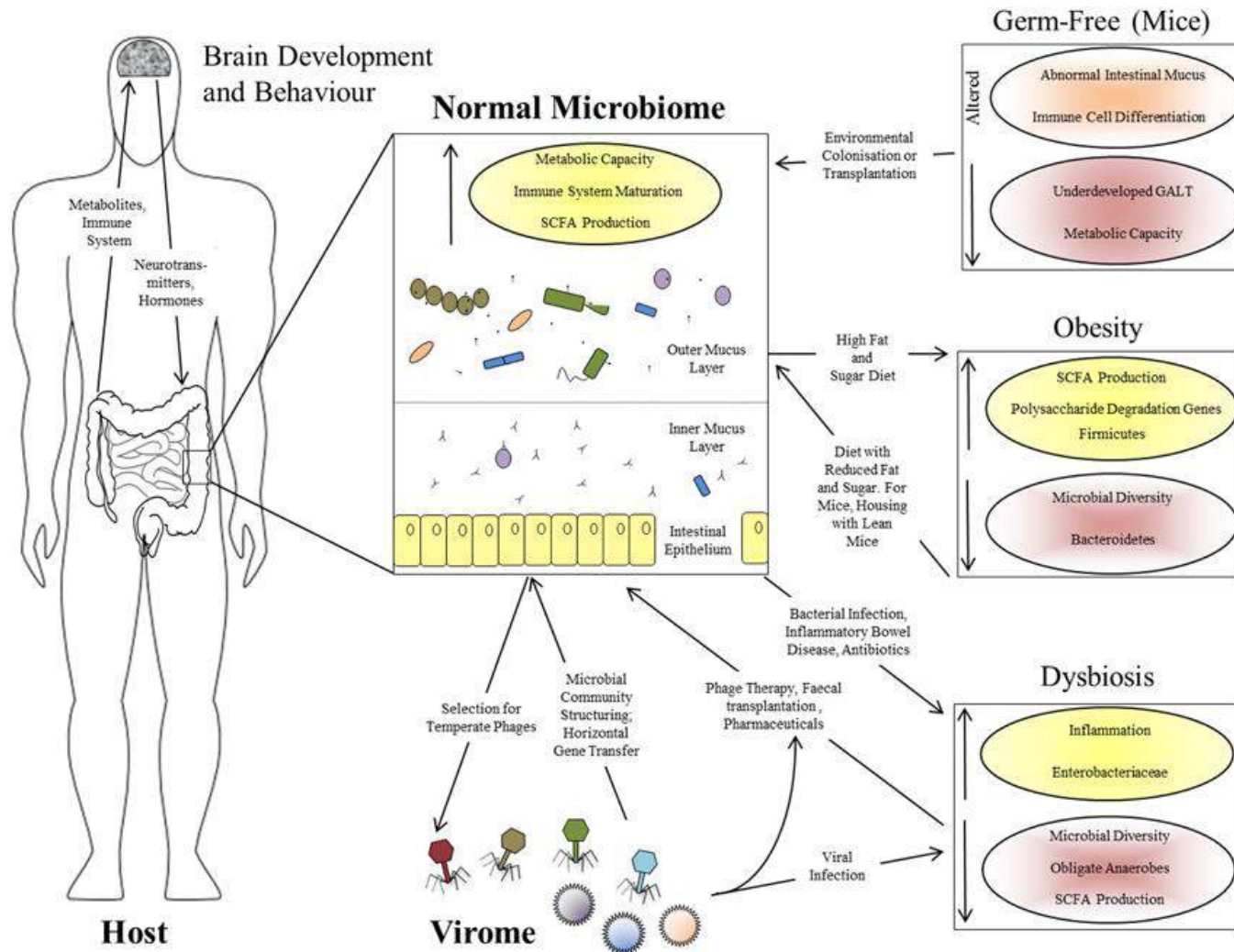


Associações com a dieta usual, e não com a dieta recente.

Influências *do* Microbioma

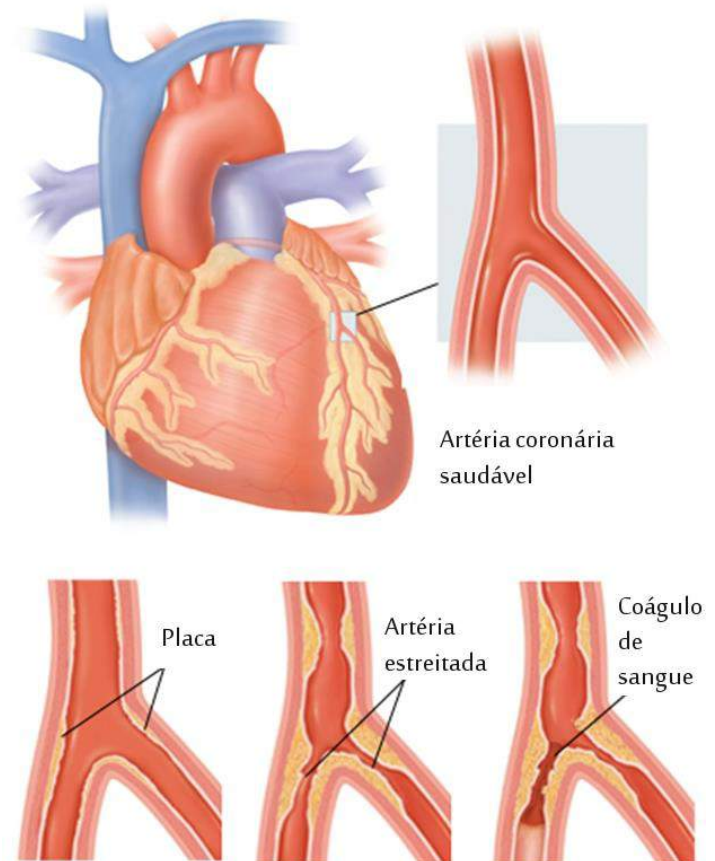
?

O Microbioma e o Organismo Humano: “Simbiose e disbiose”



Doença Cardiovascular – Aterosclerose

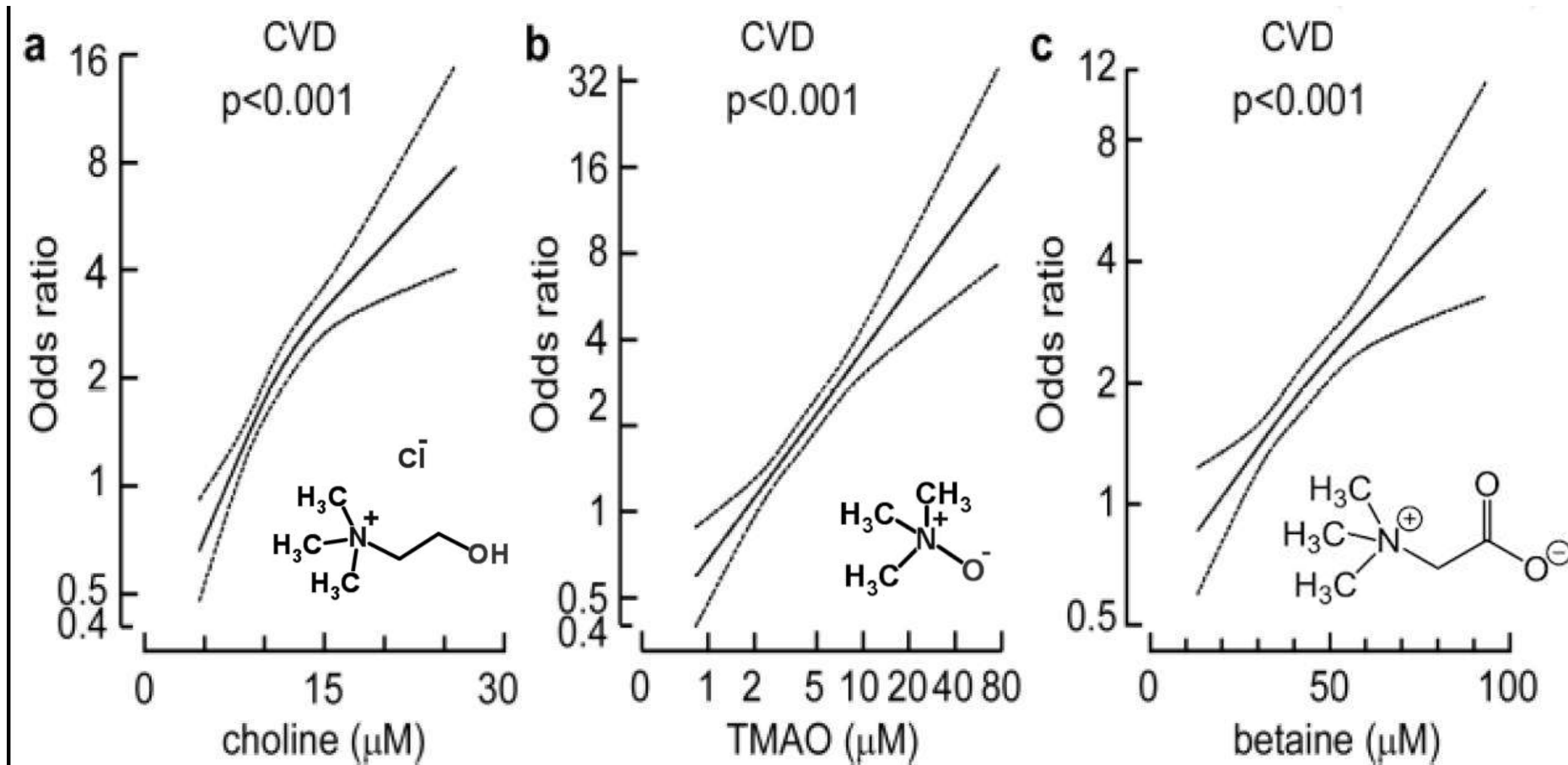
- O que é a aterosclerose?
- Fatores de risco:
 - Colesterol alto
 - Obesidade
 - Diabetes
 - etc



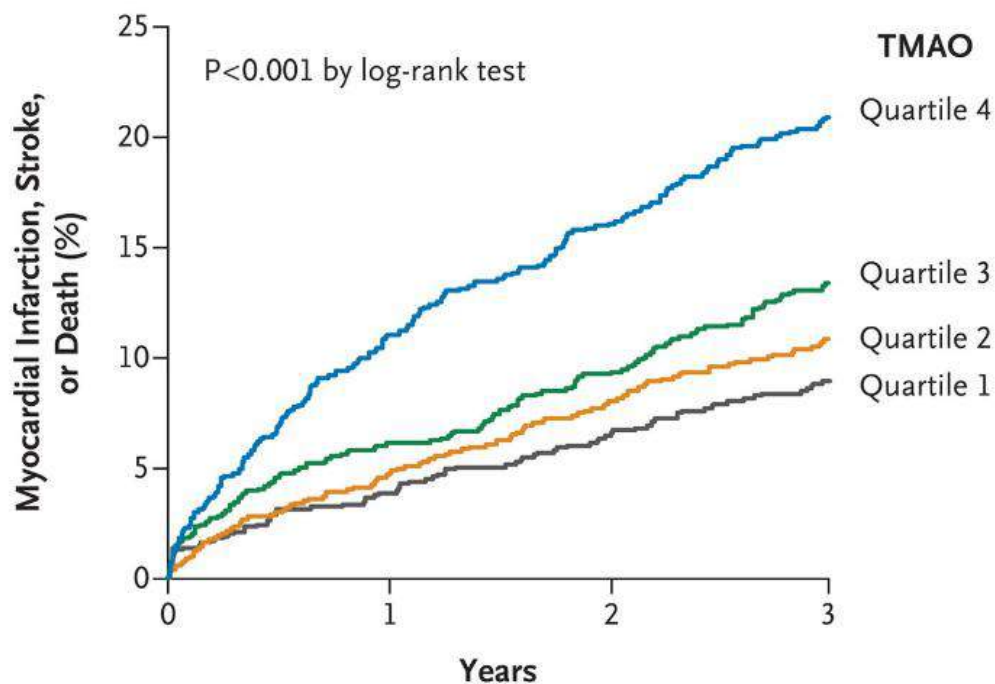
Risco doença cardiovascular

- N = 1865

- Níveis séricos humanos



- TMAO
- N=4007
- Seguidos por 3 anos.

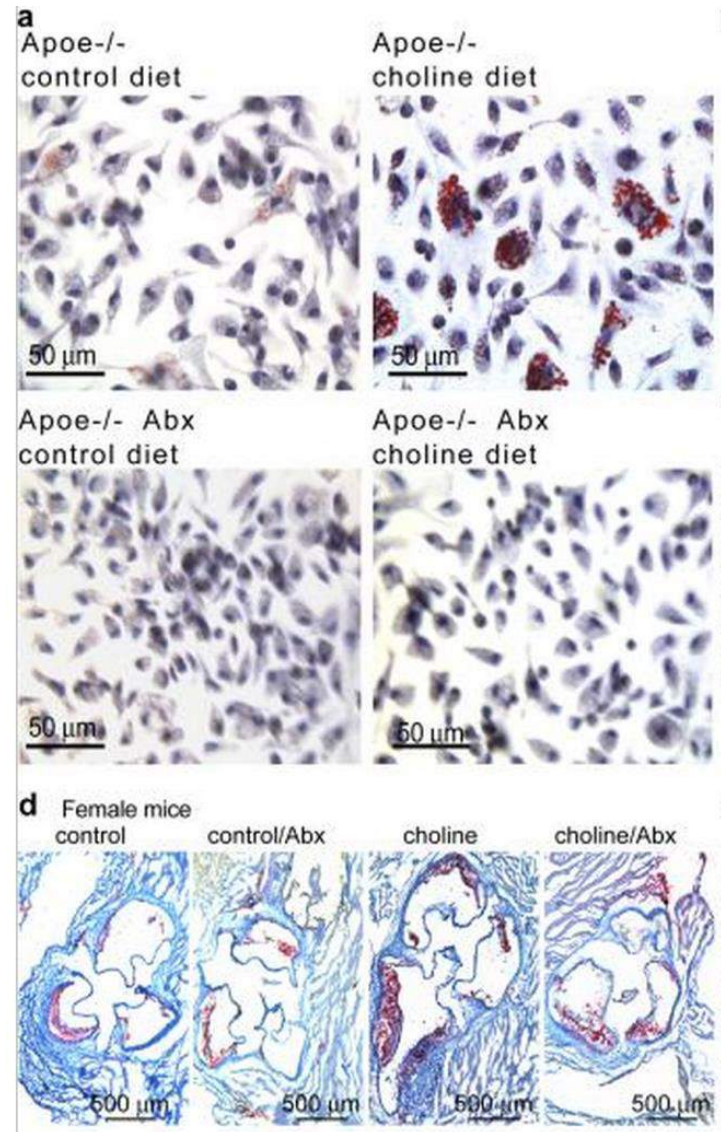


No. at Risk

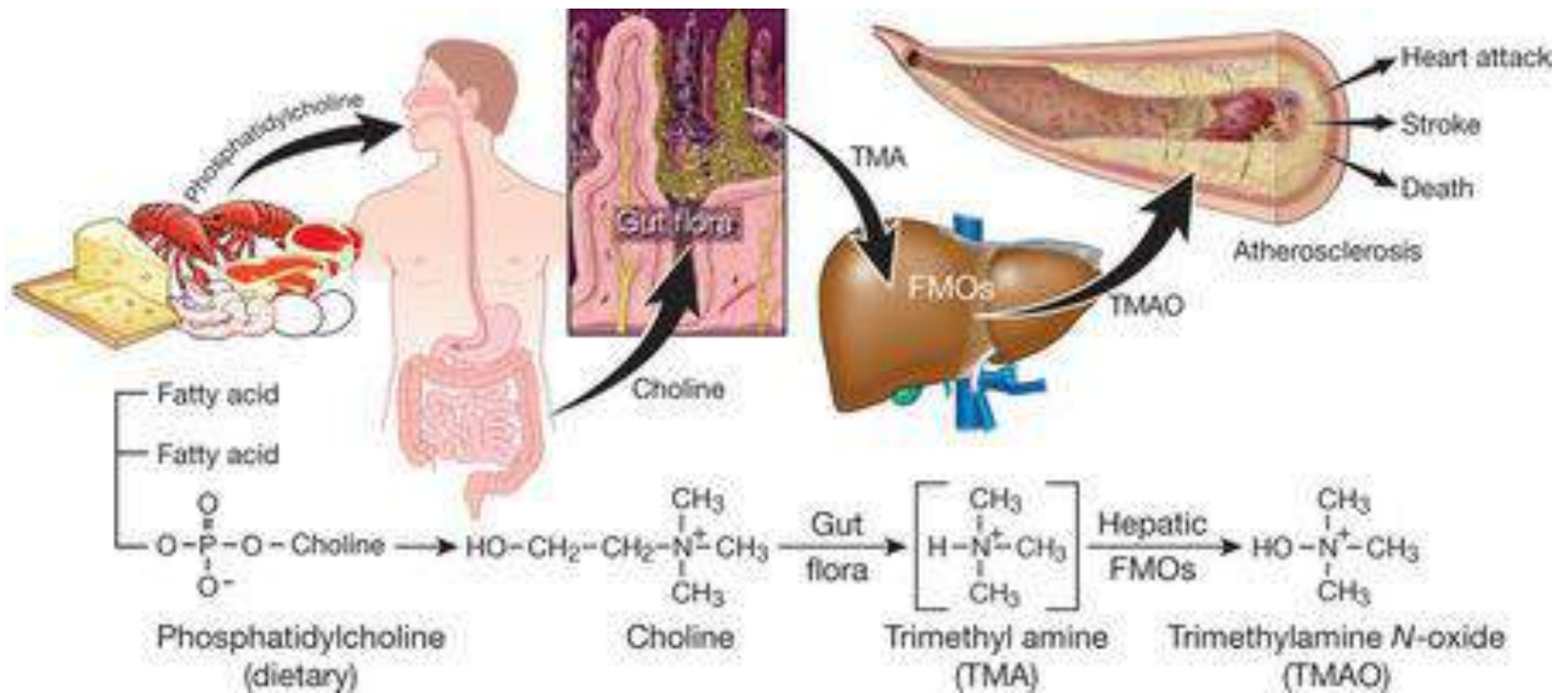
Quartile 1	1001	933	869	827
Quartile 2	998	940	884	843
Quartile 3	1003	938	888	835
Quartile 4	1005	913	849	791

Aterosclerose

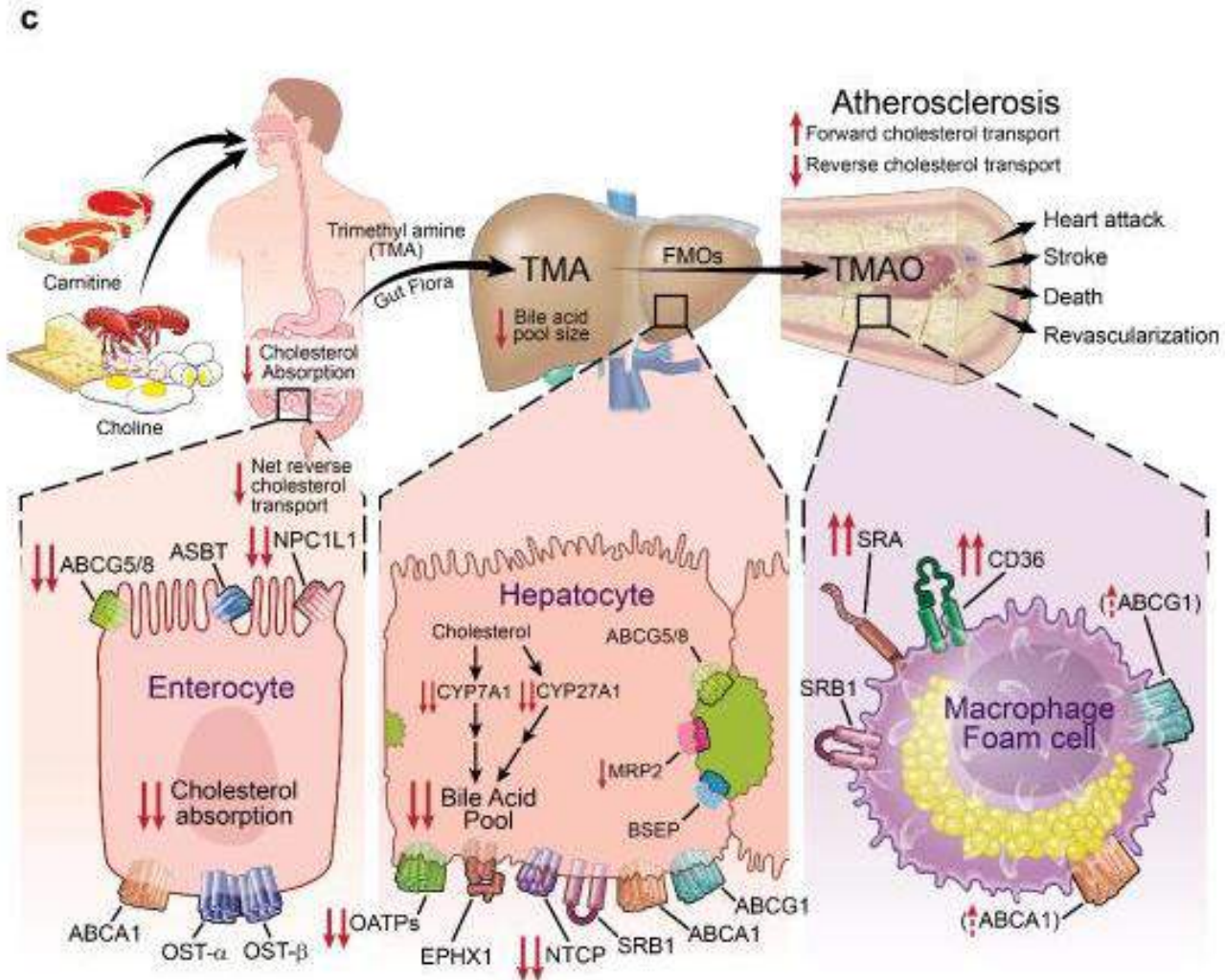
- Modelo animal:
 - Colina
 - células espumosas (macrófago), com e sem antibiótico



Atherosclerosis



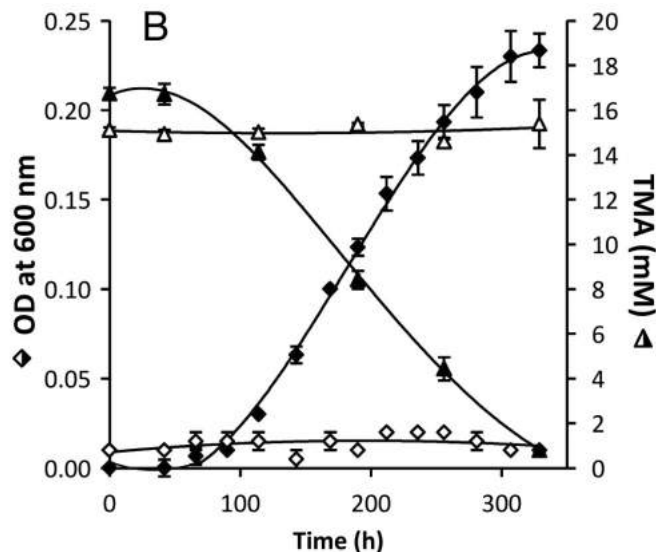
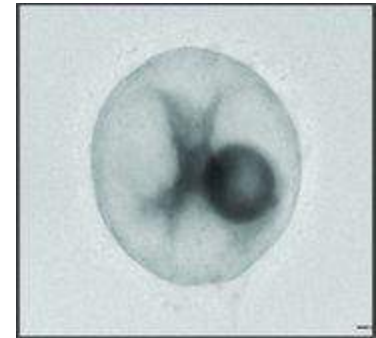
Atherosclerosis



Koeth et al 2013. Nat Med. 2013 May; 19(5): 576–585. Published online 2013 Apr 7

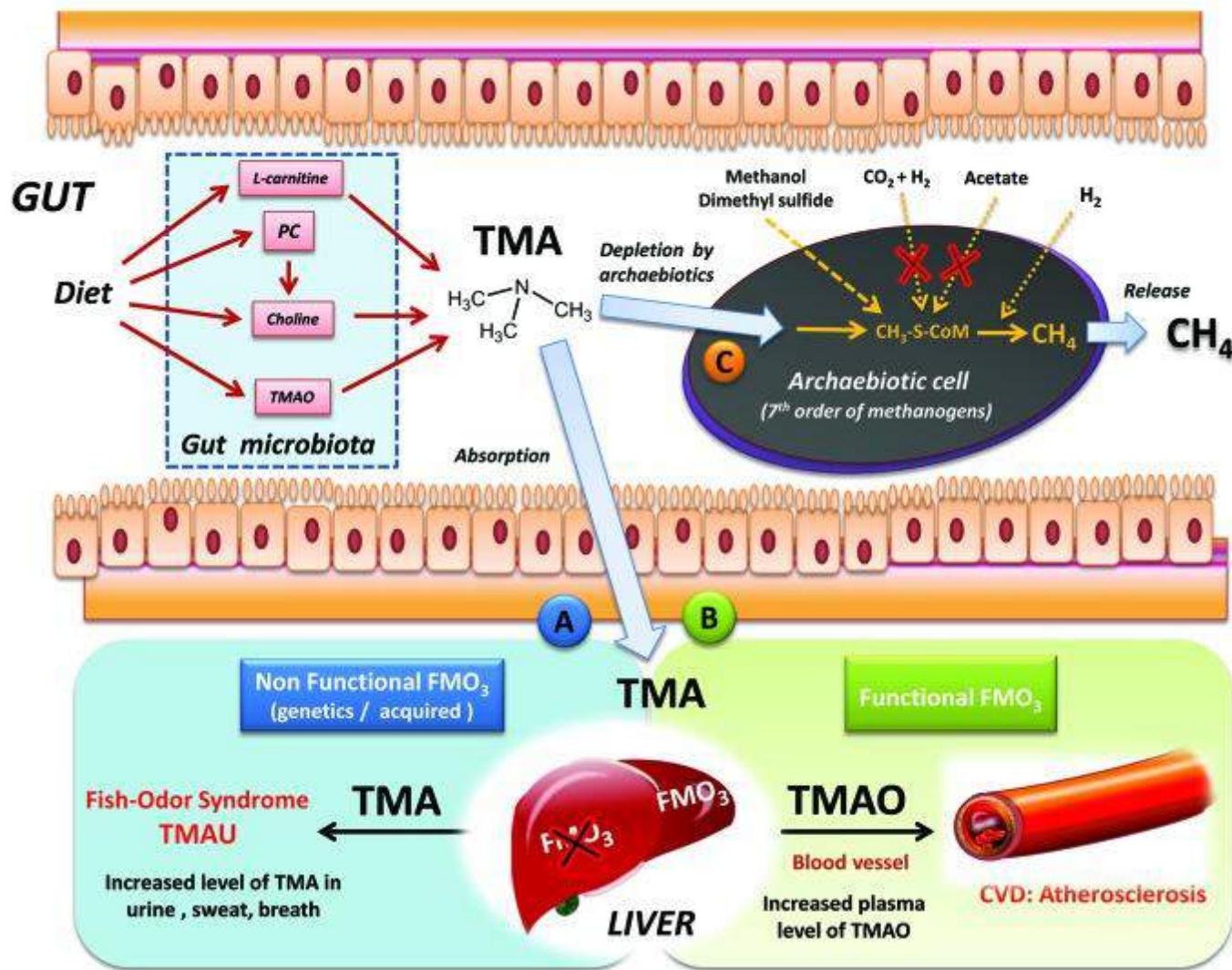
Arqueobiótico X Aterosclerose

- *Methanomassiliicoccus luminyensis*
- Metanogênica
- Isolada do microbioma intestinal de uma pessoa na França
- Cresce usando TMA e produz Metano



- ▲ [TMA] com TMA e H₂
- △ [TMA] sem TMA e H₂
- ◆ Crescimento da *M. lum.* com TMA e H₂
- ◇ Crescimento da *M. lum.* sem TMA e H₂

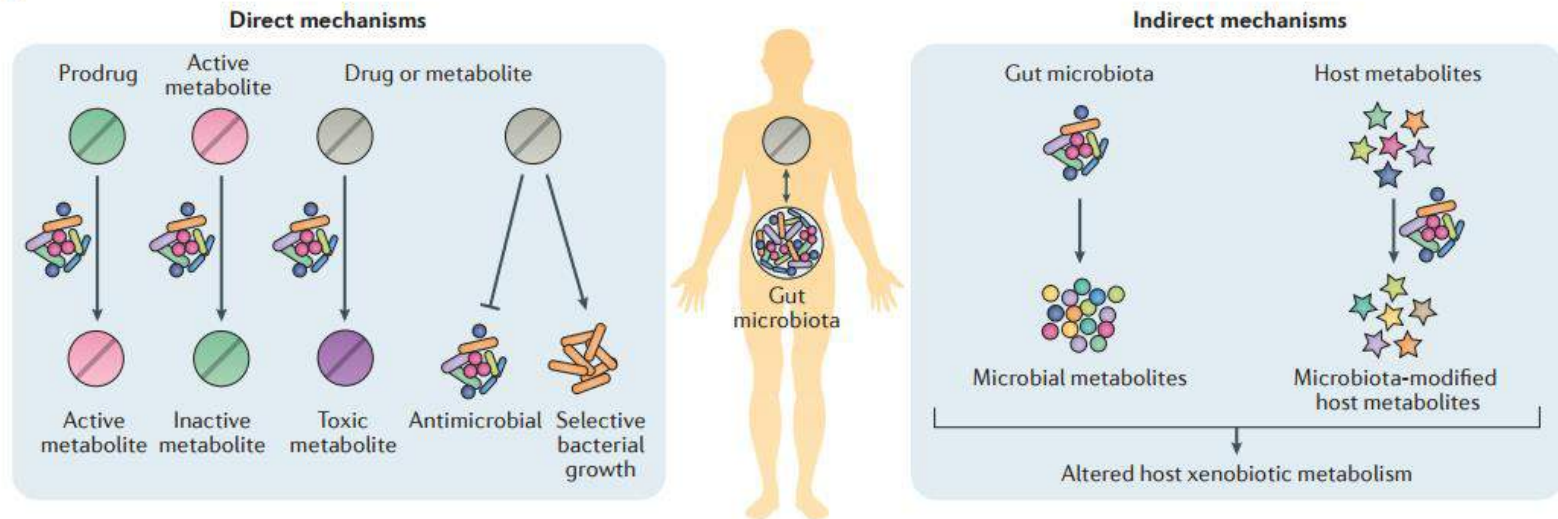
Arqueobiótico X Aterosclerose



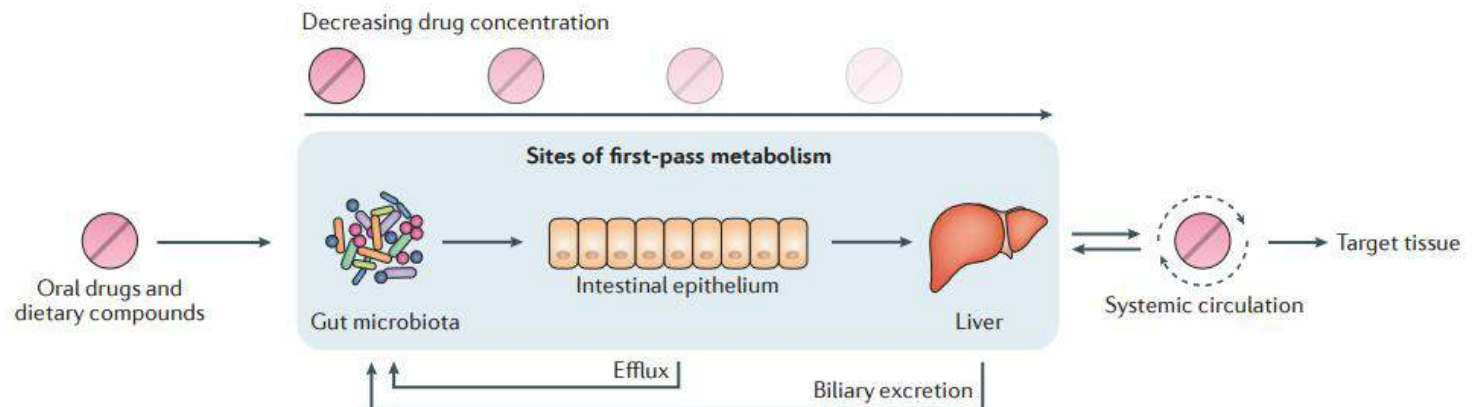
Café!

Interação fármaco-microbioma-dieta

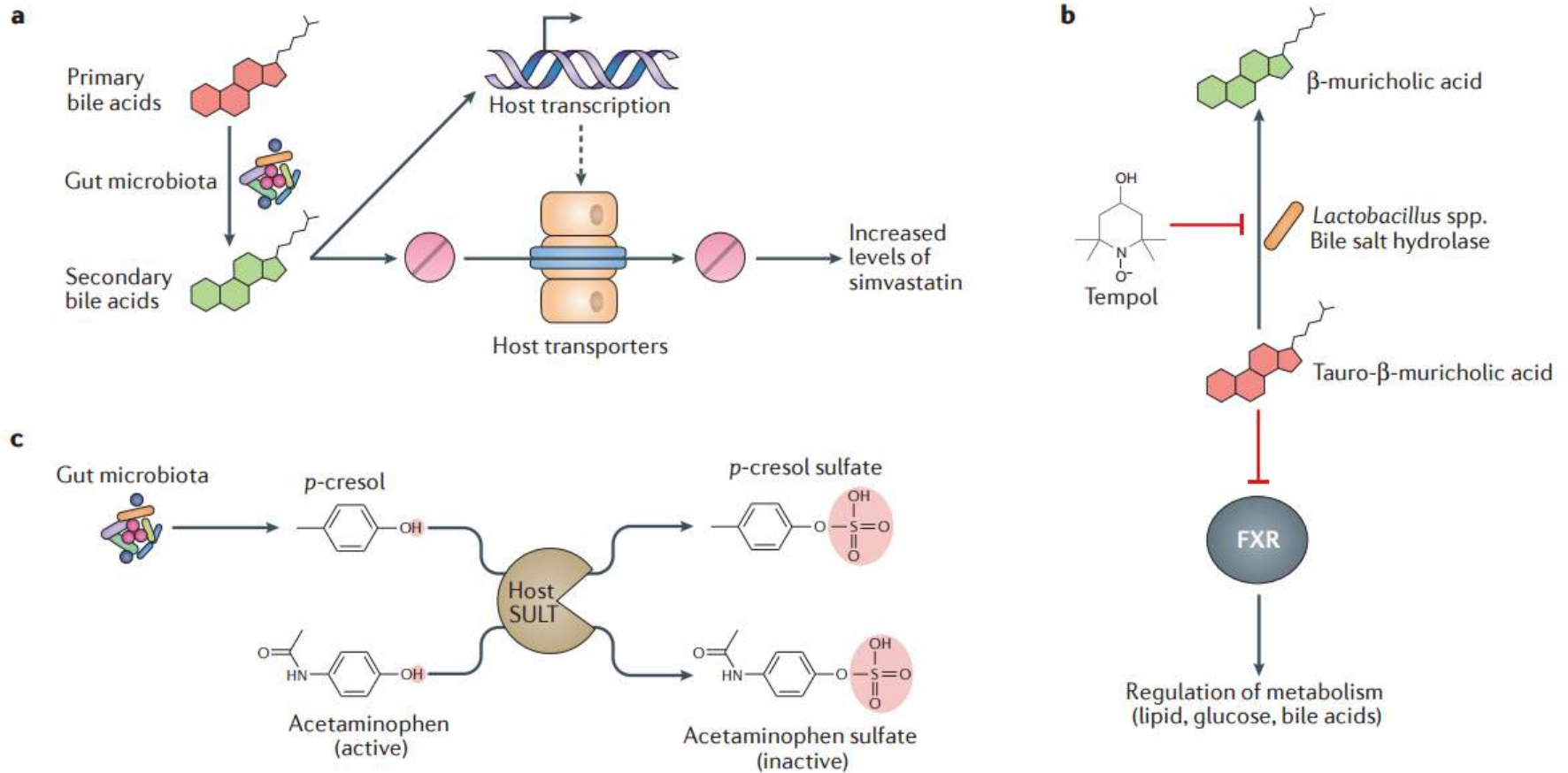
a



b



Interação fármaco-microbioma-dieta



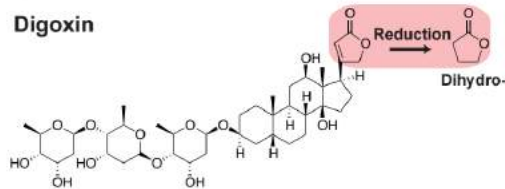
Digoxina

- Digoxin
- Margem terapeutica estreita: (0.5 a 2 ng/mL)
- Tratamento com antibióticos de amplo espectro auxilia na melhor absorção da droga ativa.
- Bacteria redutora conhecida mas sem ser possível correlacionar com a redução *in vivo*



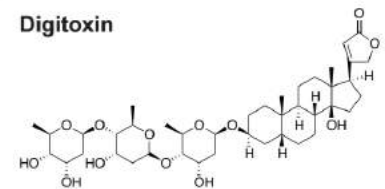
A)

Digoxin



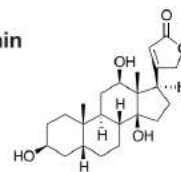
B)

Digitoxin



C)

Digoxigenin



D)

Ouabain



Fig. 2. A microbial biomarker predicts the inactivation of digoxin.

(A) Liquid chromatography–mass spectrometry (LC-MS) was used to quantify digoxin reduction in the fecal microbiomes of 20 unrelated individuals. **(B)** The *cgr* ratio was significantly different between low and high reducers. Data represent QPCR with the *cgr2* gene, and *E. lenta*–specific 16S rDNA primers (table S4). **(C)** Five low-reducing fecal microbial communities were incubated for 5 days in the presence or absence of *E. lenta* DSM2243 or FAA 1-3-56. LC-MS was used to quantify the completion of digoxin reduction. Supplementation with the nonreducing strain of *E. lenta* did not significantly affect digoxin reduction efficiency. **(D)** The *cgr* ratio was obtained for each of the low-reducing microbial communities after incubation. Outliers were identified using Grubbs’ test ($P < 0.01$) and removed. Values are means \pm SEM. Points in (A) and (B) represent biological replicates. Asterisks indicate statistical significance by Student’s *t* test (* $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$).

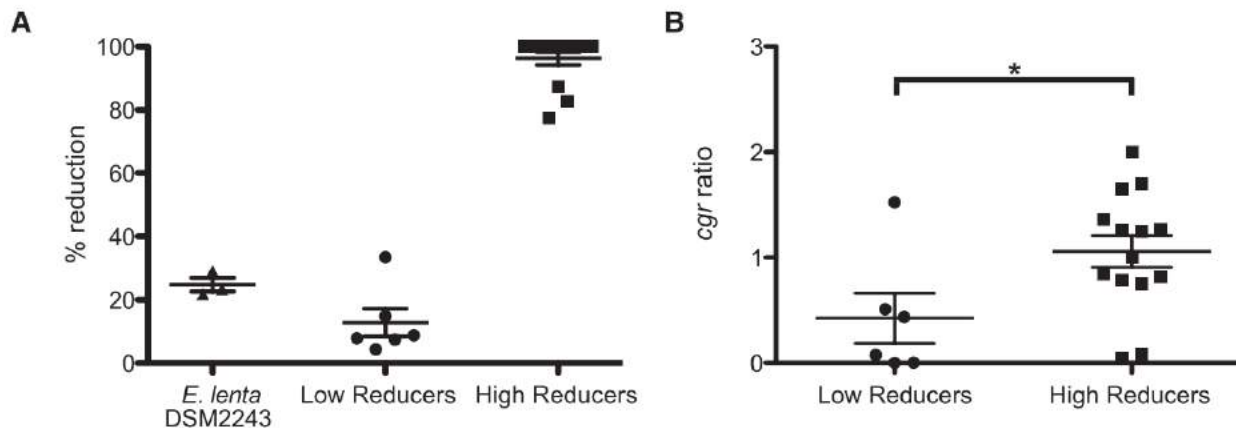


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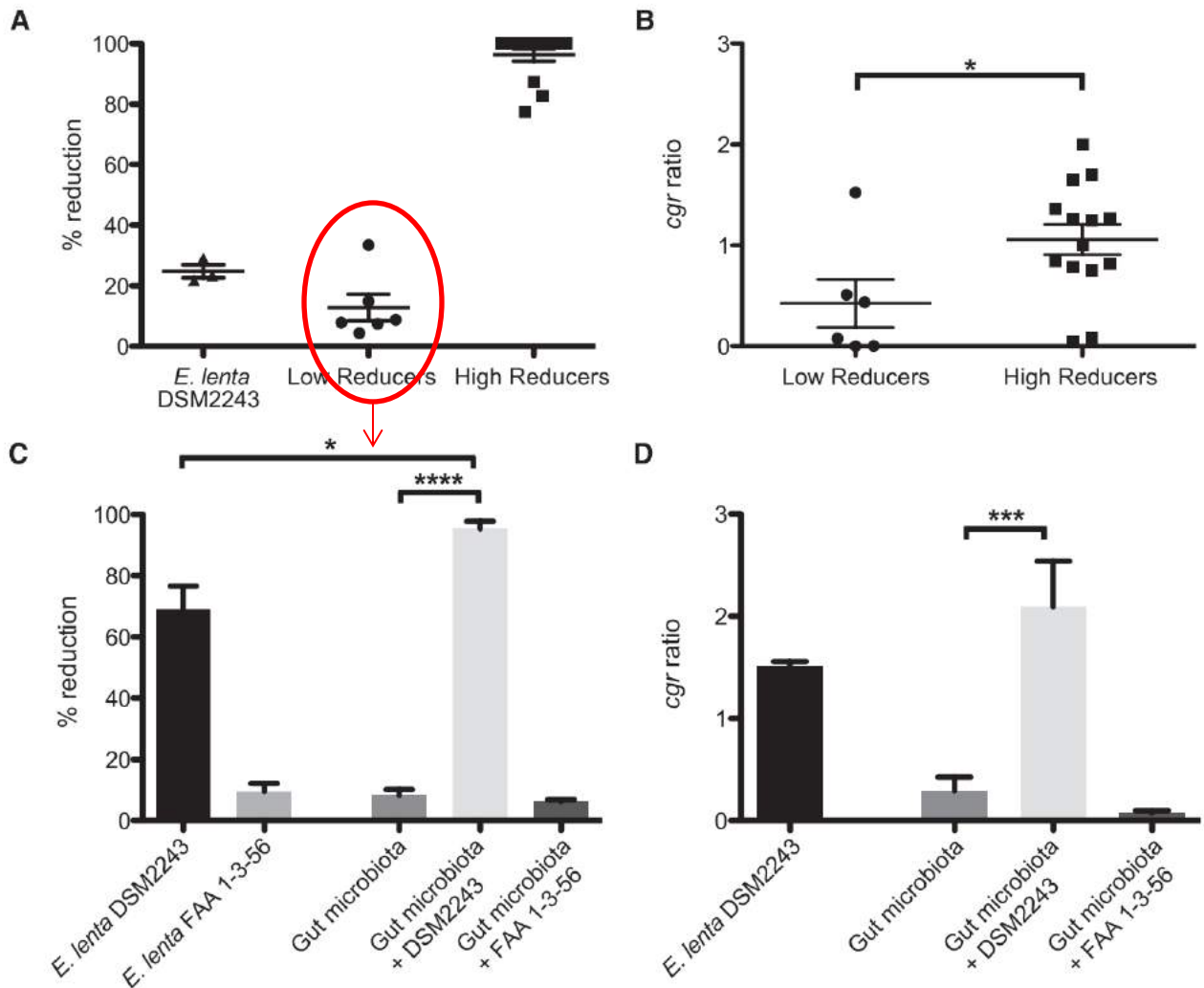
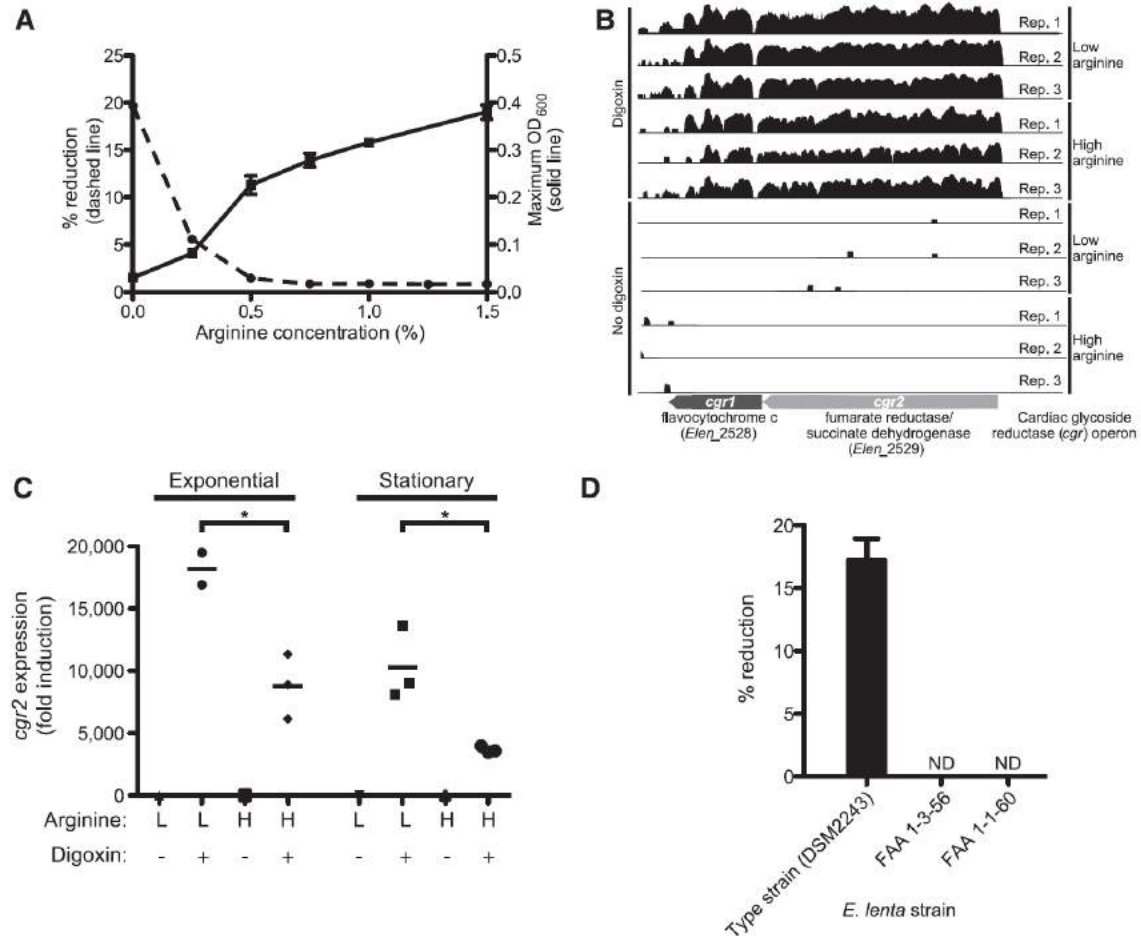


Fig. 1. Discovery of a bacterial operon induced by digoxin. (A) Arginine stimulates the growth of *E. lenta* DSM2243 in vitro while blocking the reduction of digoxin. Maximum optical density (absorbance) at 600 nm (OD₆₀₀) (solid line; values are means ± SEM; *n* = 3) and digoxin percentage reduction efficiency (dashed line; values are means; *n* = 2) after 48 hours of growth. (B) RNA-Seq profiles of the *cgr* operon are shown with and without digoxin during exponential growth in medium containing low or high arginine. The height is proportional to the natural log of the number of unambiguous sequencing reads mapped to each base. (C) *cgr2* transcription as determined by QRT-PCR. Asterisks indicate statistical significance by Student's *t* test (*P* < 0.05). Horizontal lines are means; *n* = 2 to 3. (D) Identification of two strains of *E. lenta* incapable of reducing digoxin. Values are means ± SEM; *n* = 3. ND, no reduction detected.



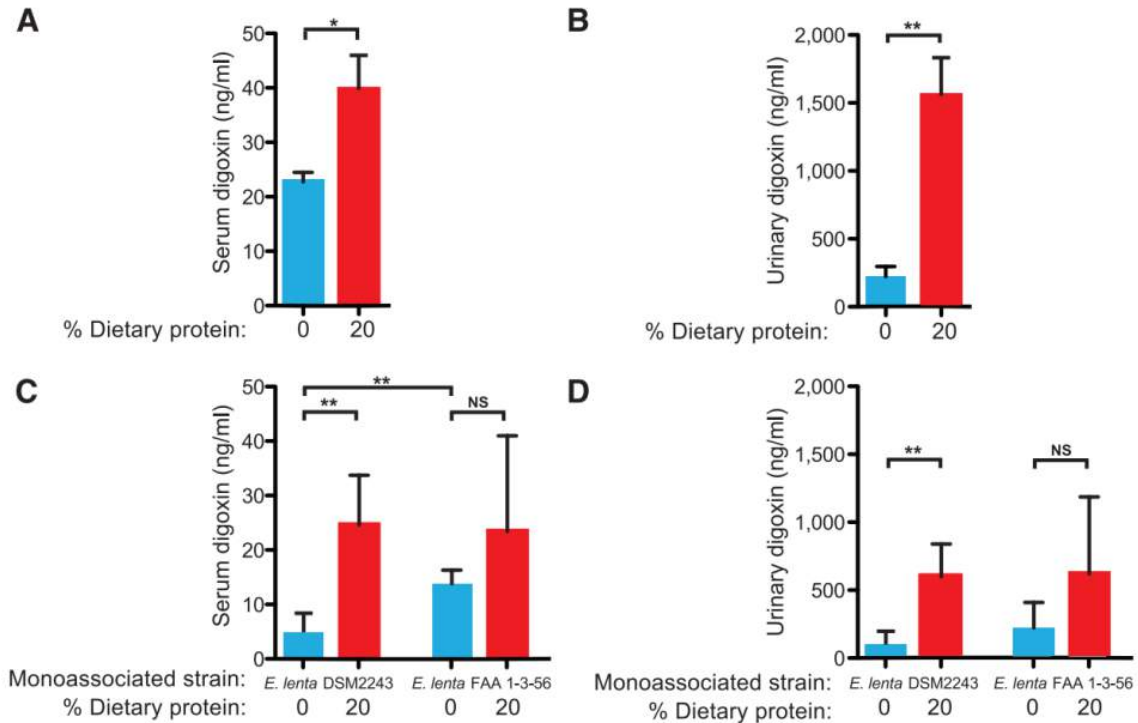
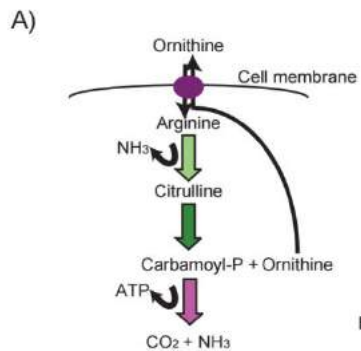
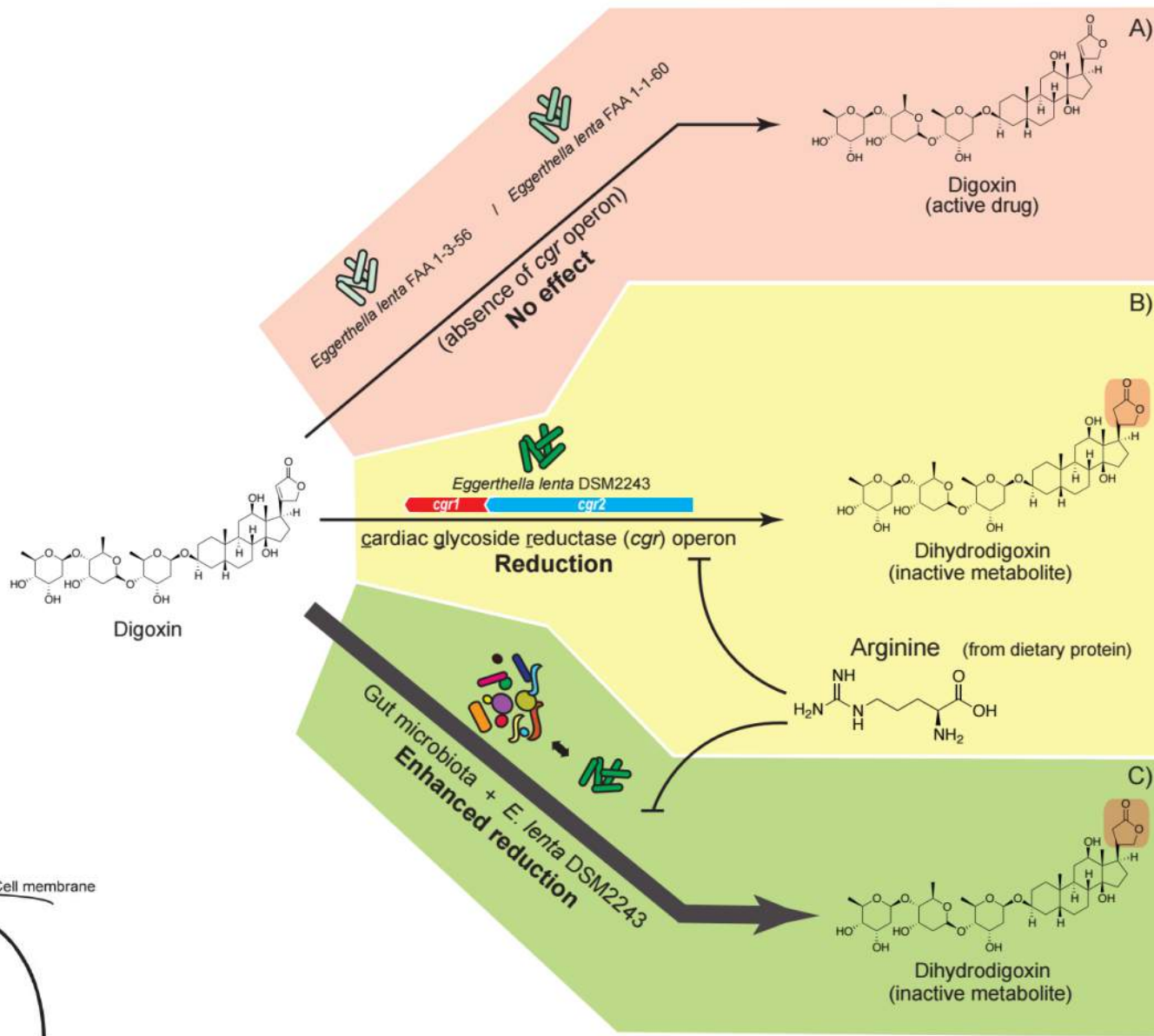


Fig. 3. Dietary protein blocks the inactivation of digoxin. Serum (A) and urinary (B) digoxin levels from the type strain experiment. Fecal digoxin levels showed a consistent trend: the mean area under the curve was 6.226 ng digoxin per hour per ml in germ-free mice, 3.576 for mice fed the 0% protein diet, and 6.364 for mice fed the 20% protein diet. Serum (C) and urinary (D) digoxin levels from each group. Digoxin levels were quantified by enzyme-linked immunosorbent assay (ELISA) (7). Values are means \pm SEM. Asterisks indicate statistical significance by Student's *t* test (**P* < 0.05; ***P* < 0.01). *n* = 4 to 5 mice per group. NS, not significant.



Atividades em sala