Bacteriophage-mediated manipulation of the gut microbiome – promises and presents limitations

Dennis S. Nielsen
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Gut microbiota, health and disease

- "All disease begins in the gut" (Hippocrates, 460-370 BC)
- "A lot of diseases begins in the gut" (Dennis S. Nielsen, 1977-?)
- In his 1910 book "The prolongation of life. Metchnikoff suggested
  d to the influence of "bad"

  of lactic acid bacteria
  effect
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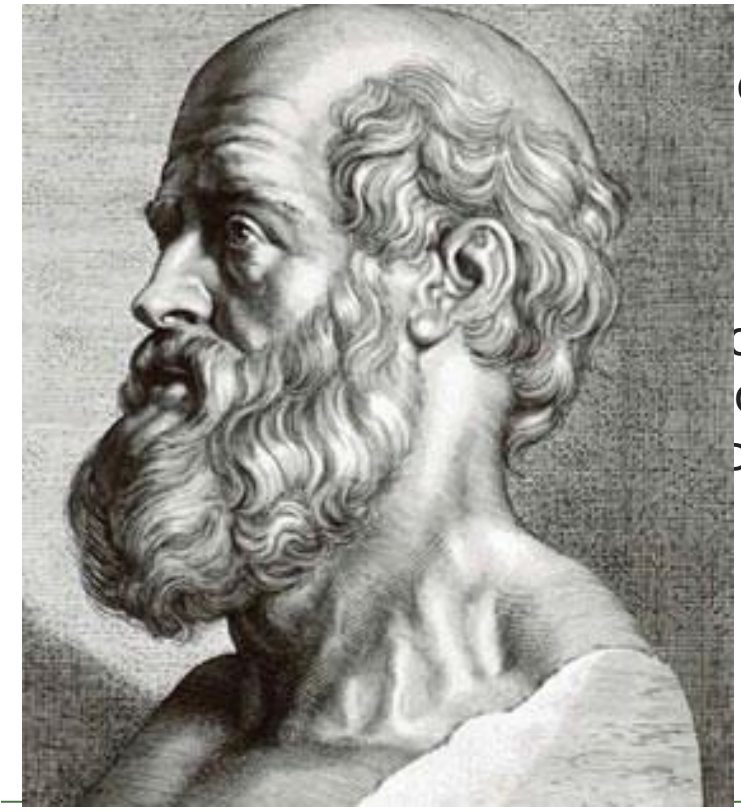
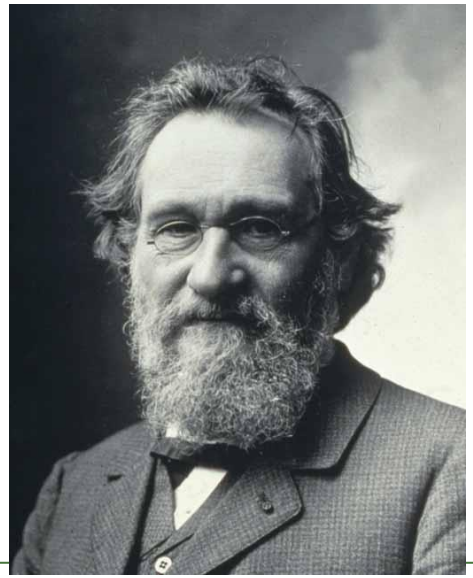
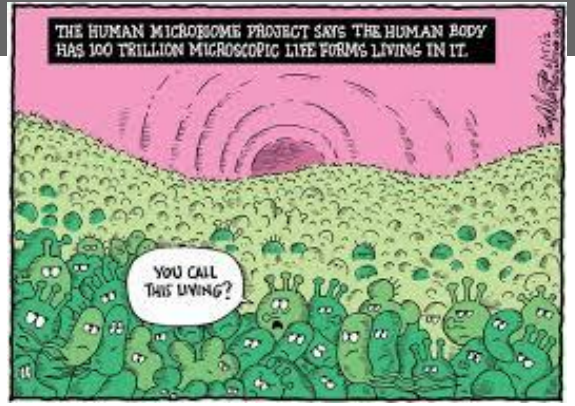
  cteria



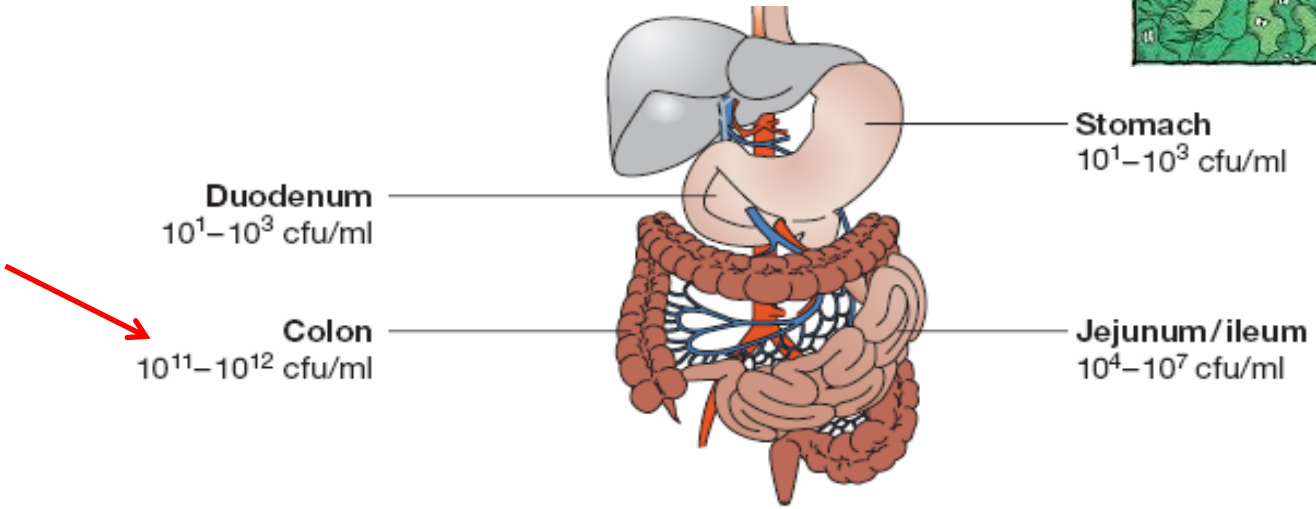




What do we know today

- We know that the gut is densely populated!

1.000.000.000.000 microbes pr. gram of faeces!!



- We also know that a (very) long list of diseases are connected (correlation, sometimes also causal link) to gut microbiome (GM) dysbiosis/imbalance
    - Obesity, type 2 diabetes, asthma, eczema, type 1 diabetes, metabolic syndrome, inflammatory bowel disease, colon cancer, cardiovascular disease, autism, liver disease, behaviour/depression etc. etc.

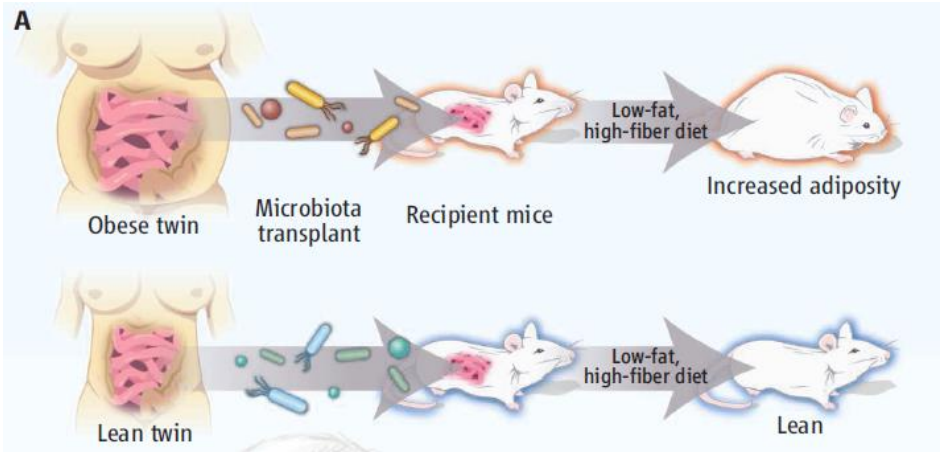
Gut microbiota and obesity

- The fuzz about GM started with some obese mice 16 years ago

Obesity alters gut microbial ecology PNAS | August 2, 2005 | vol. 102

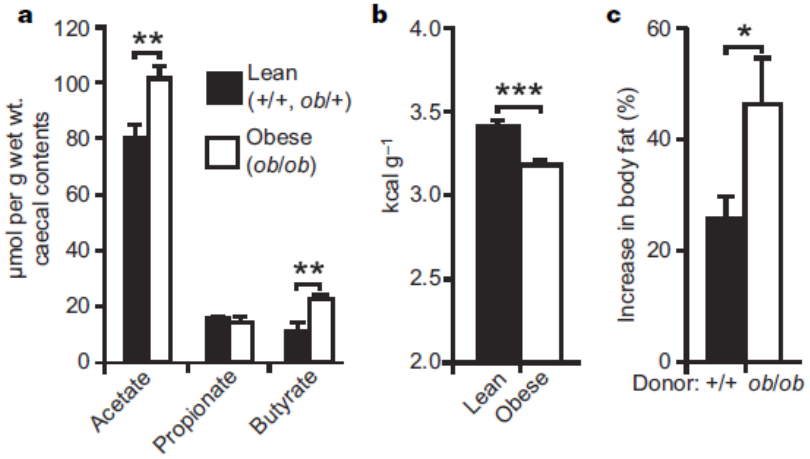
Ruth E. Ley[†], Fredrik Bäckhed[†], Peter Turnbaugh[†], Catherine A. Lozupone[‡], Robin D. Knight[§], and Jeffrey I. Gordon[†¶]

- Then it was shown that the obese GM has an increased capacity for energy harvest
- And that the obese phenotype is transferable with the GM



Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice

Vanessa K. Ridaura,[1] Jeremiah J. Faith,[1] Federico E. Rey,[1] Jiye Cheng,[1] Alexis E. Duncan,[2,3] Andrew L. Kau,[1] Nicholas W. Griffin,[1] Vincent Lombard,[4] Bernard Henrissat,[4,5] James R. Bain,[6,7,8] Michael J. Muehlbauer,[6] Olga Ilkayeva,[6] Clay F. Semenkovich,[9] Katsuhiko Funai,[9] David K. Hayashi,[10] Barbara J. Lyle,[11] Margaret C. Martini,[11] Luke K. Ursell,[12] Jose C. Clemente,[12] William Van Treuren,[12] William A. Walters,[13] Rob Knight,[12,14,15] Christopher B. Newgard,[6,7,8] Andrew C. Heath,[2] Jeffrey I. Gordon[1]*



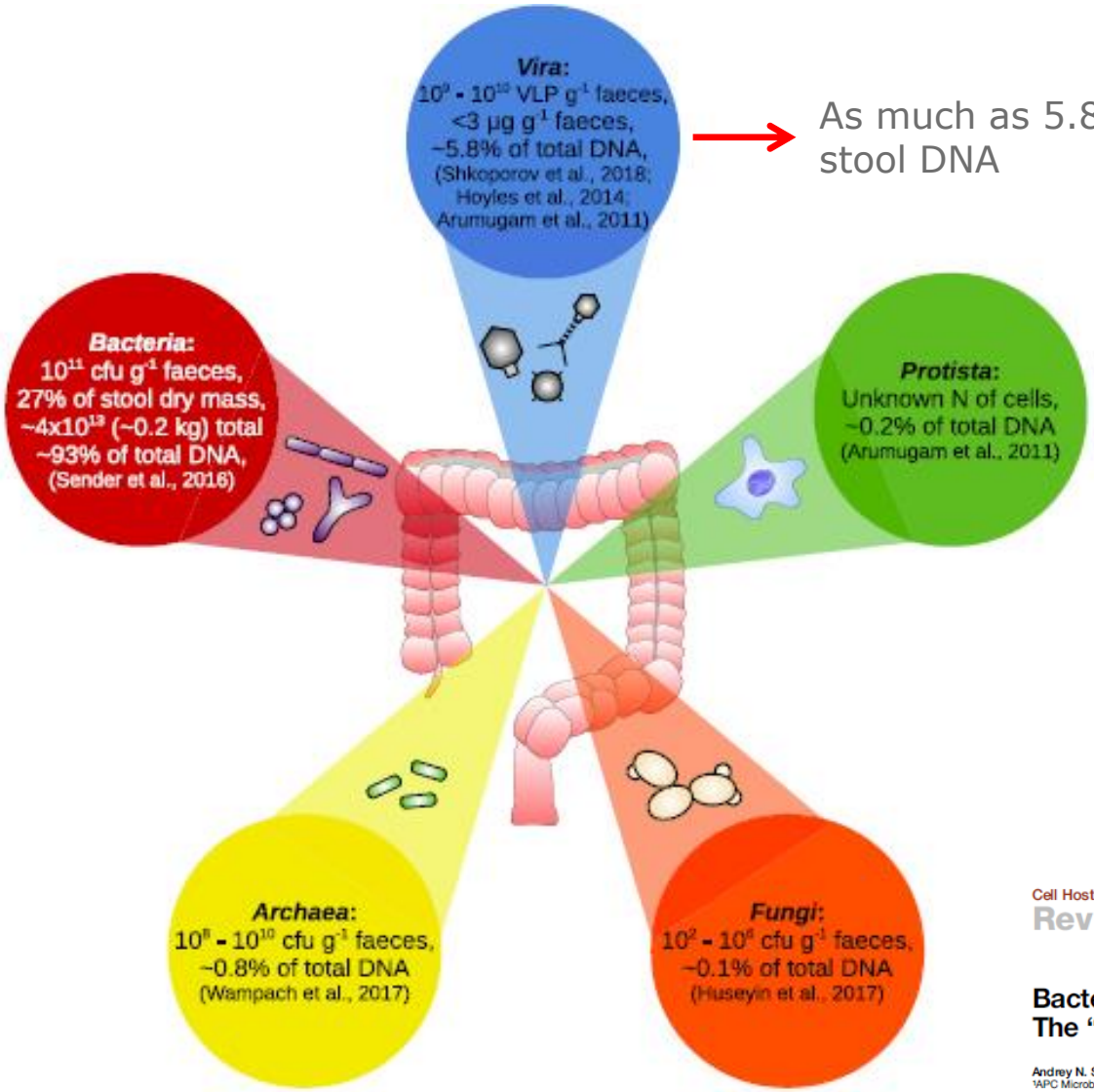
An obesity-associated gut microbiome with increased capacity for energy harvest

Peter J. Turnbaugh[1], Ruth E. Ley[1], Michael A. Mahowald[1], Vincent Magrini[2], Elaine R. Mardis[1,2] & Jeffrey I. Gordon[1]

My gut microbiome got virus-infected…

- There is more to the gut microbiota than ”just” bacteria
    - Parasites, fungi, archea…
- And viruses!

My gut microbiome got virus-infected…



As much as 5.8% of stool DNA

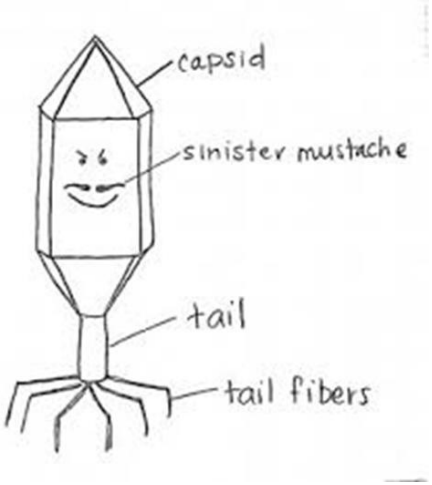
Cell Host & Microbe
Review

Bacteriophages of the Human Gut: The "Known Unknown" of the Microbiome

Andrey N. Shkoporov[1,*] and Colin Hill[1]
[1]APC Microbiome Ireland & School of Microbiology, University College Cork, Co. Cork, Ireland
*Correspondence: andrey.shkoporov@ucc.ie
https://doi.org/10.1016/j.chom.2019.01.017

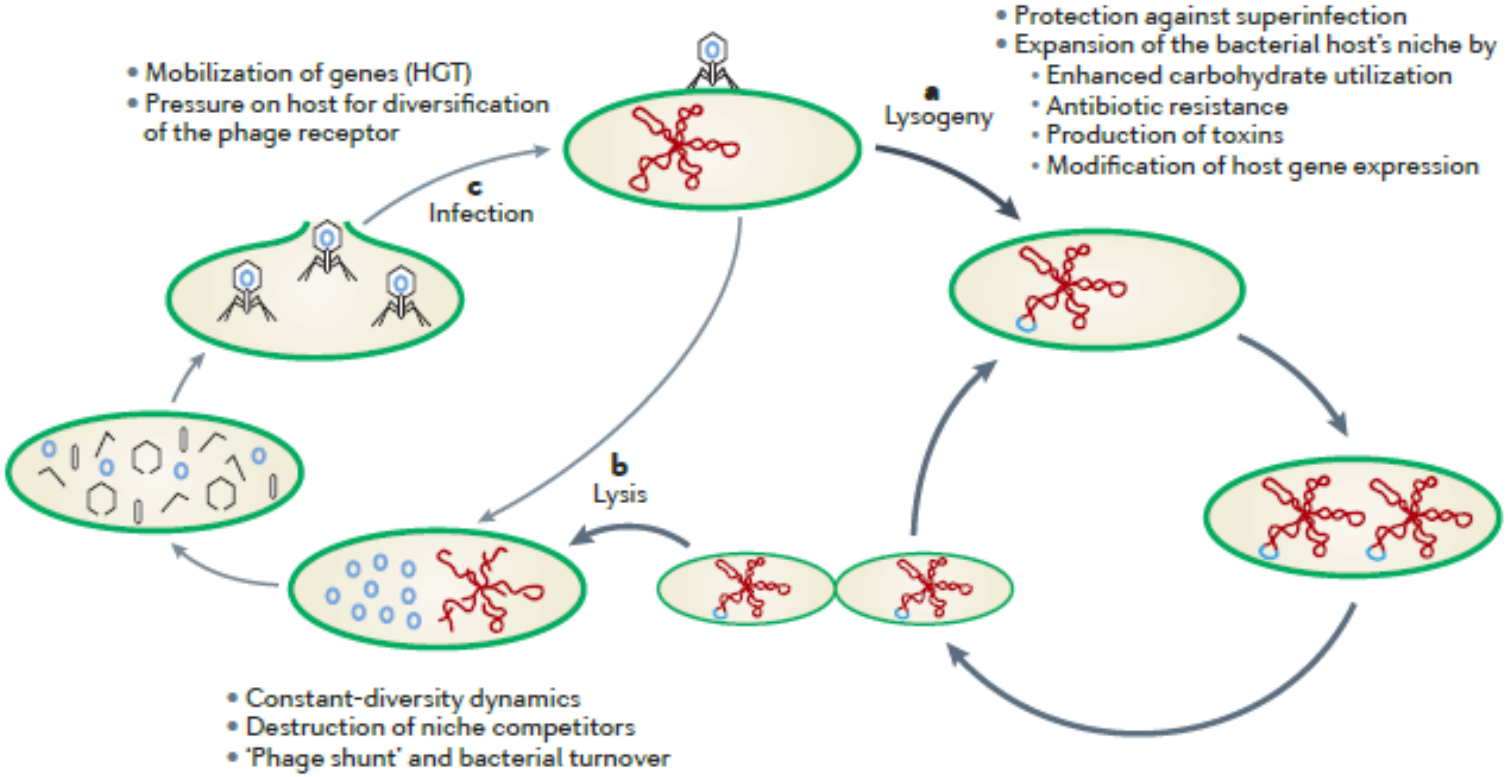
My gut microbiome got virus-infected…

- There is more to the gut microbiota than ”just” bacteria
    - Parasites, fungi, archea…
- And viruses!
- In short (bacterio)phages are viruses attacking bacteria
- Phages are probably the most ”numerous” biological entity on Earth
    - Estimate: 10^{31} phages on the planet
- Rather under-studied in the gut
    - Ratio prokaryotes:phages ≈ 1:1 in the adult gut
- (Most likely) they play important roles in:
    - Shaping/maintaining the gut microbiota
    - Gut microbiota dysbiosis leading to certain disases



Reyes et al. (2012). Nat. Rev. Microbiol.

Phages and phage life cycles



GM dysbiosis ⇔ disease

Phages attack specific bacteria

Can we use phages for GM modulation?

Phages for hitting specific GM members

- Can we use phages for knocking out/reducing specific GM members?
- Yes

GUT MICROBES
2018, VOL. 9, NO. 5, 391–399
https://doi.org/10.1080/19490976.2018.1447291

BRIEF REPORT

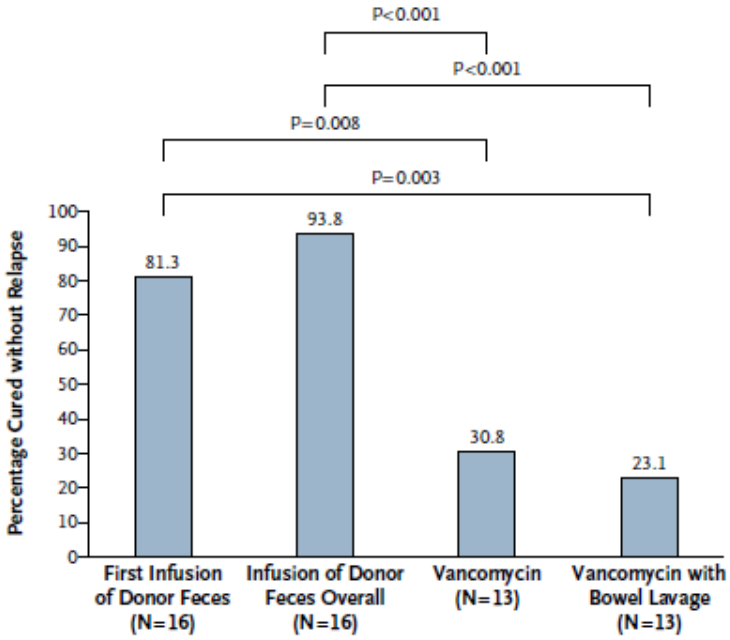
A bacteriophage cocktail targeting *Escherichia coli* reduces *E. coli* in simulated gut conditions, while preserving a non-targeted representative commensal normal microbiota

Tomasz Cieplak [a], Nitzan Soffer [b], Alexander Sulakvelidze [b], and Dennis Sandris Nielsen [a]

- And no…
- To "correct" dysbiosis one would have to hit many obligate anaerobes
  - Hard to cultivate the hosts (low throughput)
  - And even harder to plaque/isolate relevant phages

Faecal transplant has become pretty hot shit…

- Now standard treatment for recurrent *Clostridium difficile* infections in Denmark and numerous other countries
  - If antibiotics does not work FMT has a success rate of ≈90-95%



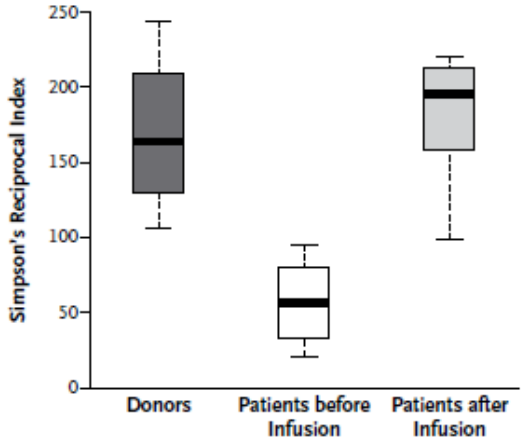


Figure 3. Microbiota Diversity in Patients before and after Infusion of Donor Feces, as Compared with Diversity in Healthy Donors.

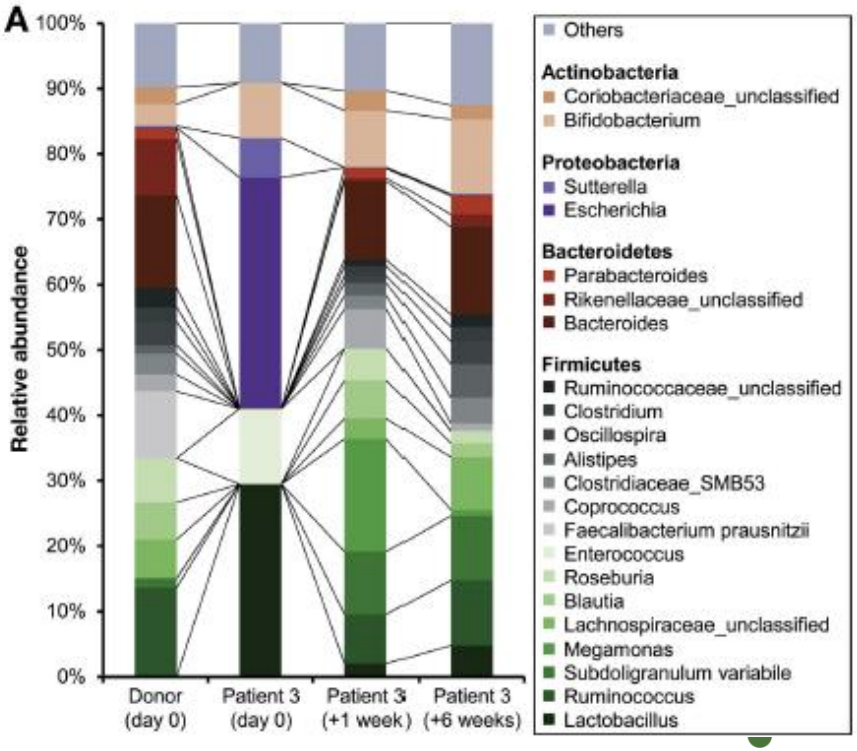
Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.

Faecal transplant – without bacteria

The positive of faecal transplant on *C. difficile* can also be achieved without bacteria

- Fecal filtrate (0.45 µm filter)
    - Contains bacteriophages + metabolites etc.
- Proof of principle study. 5/5 cured.



Gastroenterology 2017;152:799–811

Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With *Clostridium difficile* Infection

Stephan J. Ott,[1,*] **Georg H. Waetzig**,[2,*] **Ateequr Rehman**,[3,*] Jacqueline Moltzau-Anderson,[3,4] Richa Bharti,[3] Juris A. Grasis,[5] Liam Cassidy,[6] Andreas Tholey,[6] Helmut Fickenscher,[7] Dirk Seegert,[2] Philip Rosenstiel,[3,§] and Stefan Schreiber[1,3,§]

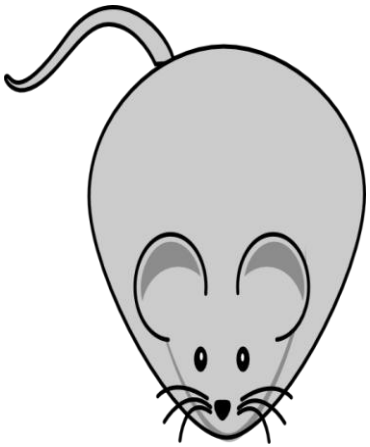
Saving mice from obesity and type 2 diabetes (T2D) by fecal virome transfer (FVT)

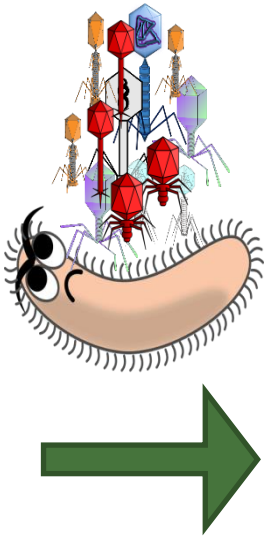
Obesity is causally linked with GM dysbiosis

- Can we alleviate the dysbiosis and reverse the phenotype?

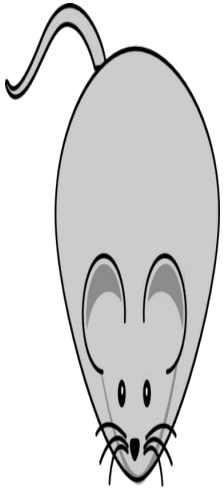
Alter GM with FVT from **lean** donor

Obese mouse





Lean mouse



Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model

Torben Sølbeck Rasmussen, [1] Caroline Märta Junker Mentzel, [2] Witold Kot, [3] Josué Leonardo Castro-Mejía, [1] Simone Zuffa, [4] Jonathan Richard Swann, [4] Lars Hestbjerg Hansen, [3] Finn Kvist Vogensen, [1] Axel Kornerup Hansen, [2] Dennis Sandris Nielsen [1]

Rasmussen TS, *et al. Gut* 2020;**69**:2122–2130. doi:10.1136/gutjnl-2019-320005

viruses

MDPI

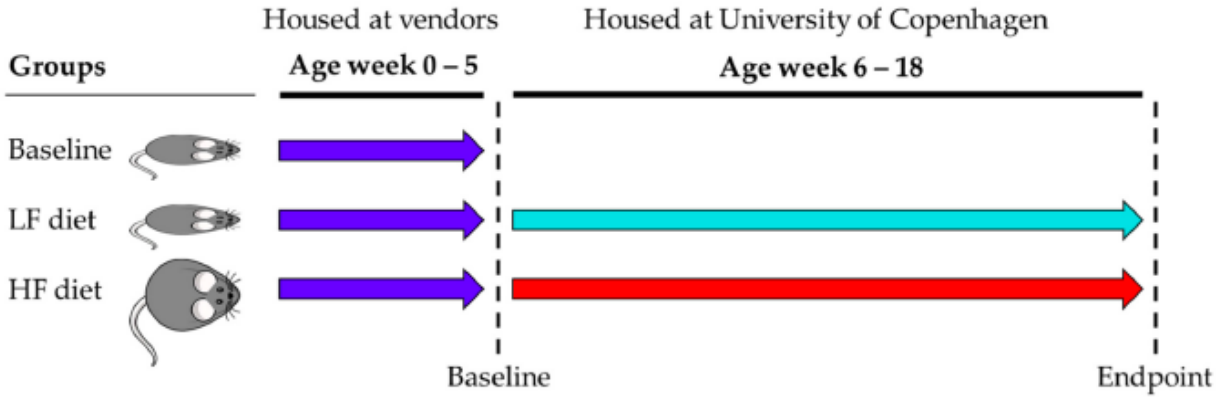
Article

Mouse Vendor Influence on the Bacterial and Viral Gut Composition Exceeds the Effect of Diet

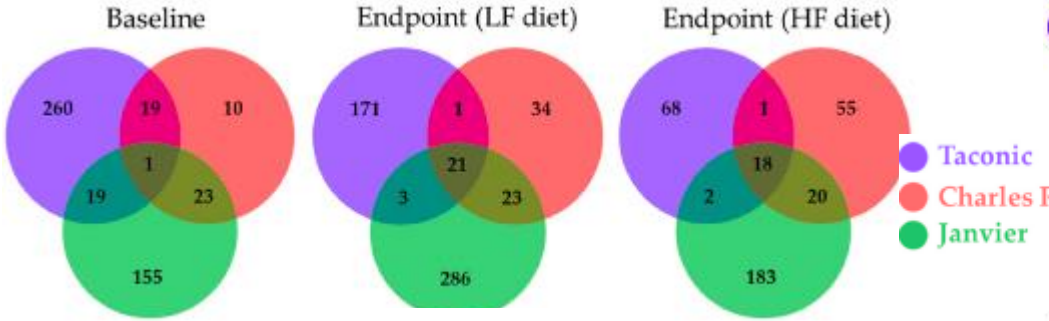
Torben Sølbeck Rasmussen [1,*], Liv de Vries [1], Witold Kot [3], Lars Hestbjerg Hansen [3], Josué L. Castro-Mejía [1], Finn Kvist Vogensen [1], Axel Kornerup Hansen [2] and Dennis Sandris Nielsen [1,*]

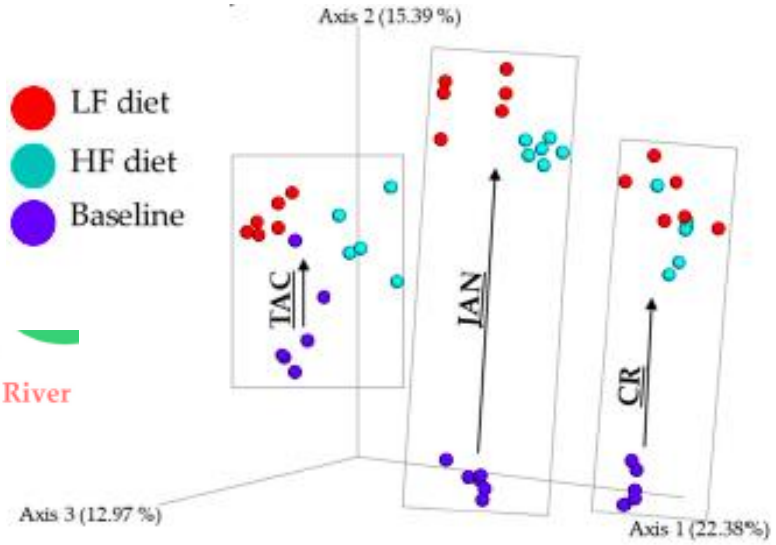
Choice of donor?

- Mice from 3 different vendors
- 13 weeks of high fat or low fat (normal chow) diet

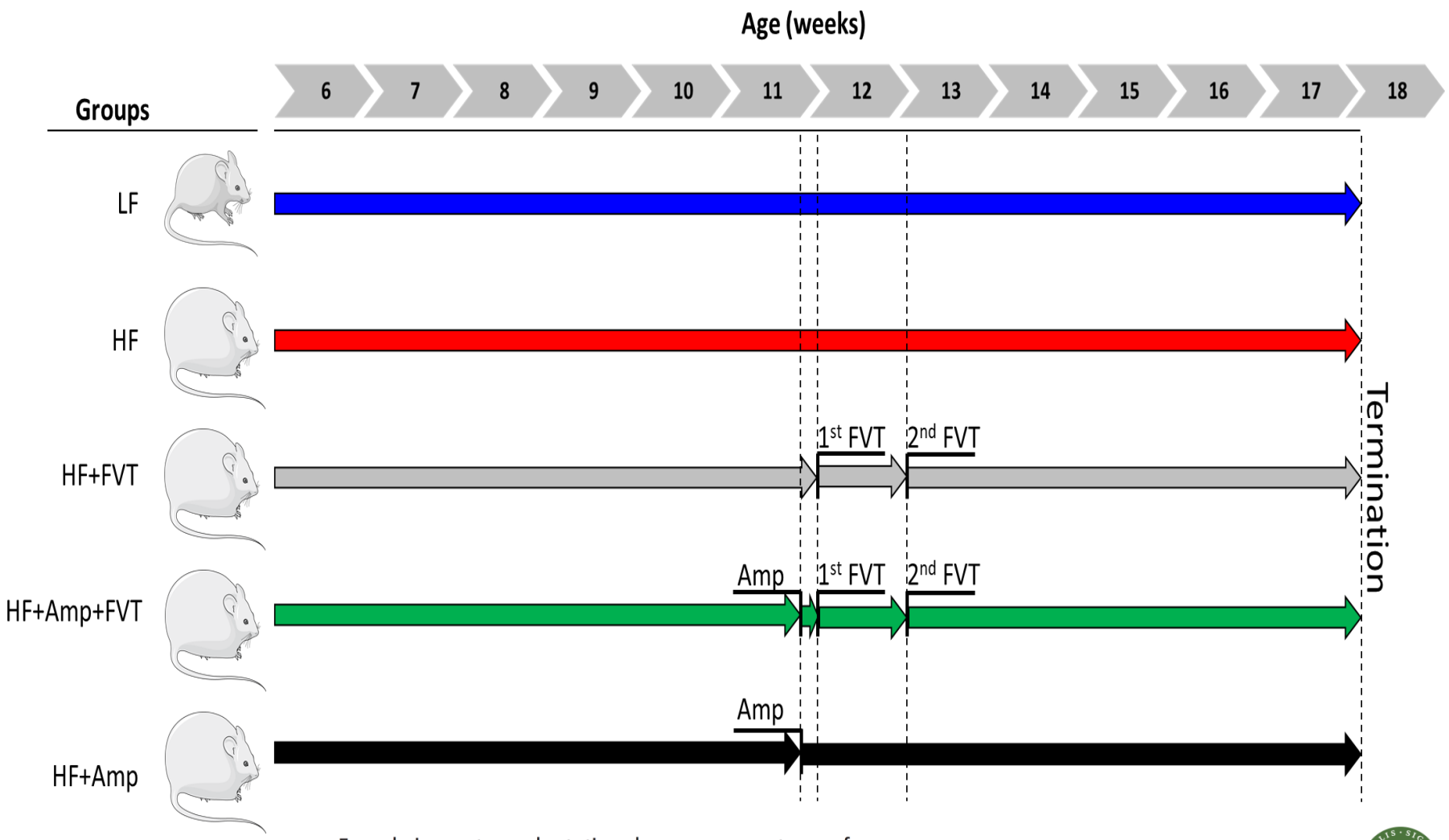


- Large vendor differences – also after 13 weeks on HF/LF diet





Shared vOTUs



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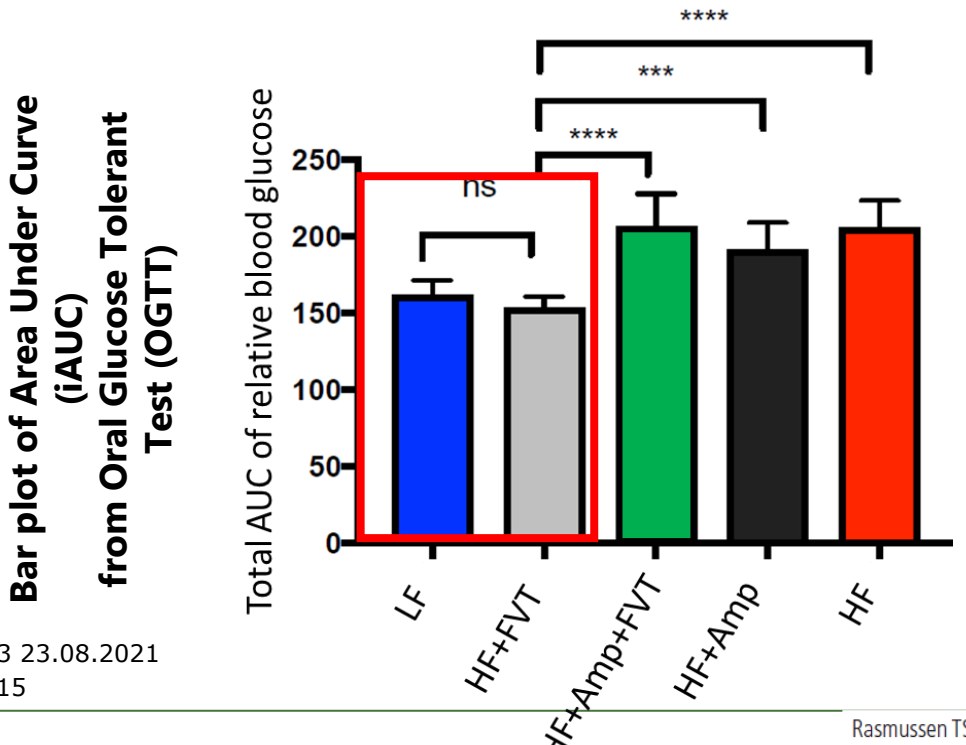
Rasmussen TS, *et al*. *Gut* 2020;**69**:2122–2130. doi:10.1136/gutjnl-2019-320005

Effect of FVT on DIO mice 6 weeks post treatment

- FVT treated high fat fed mice gained ≈15% less weight than sham-treated high fat fed mice
- And their blood sugar metabolism is normalised
- Gut bacterial and gut virome composition, blood metabolome, liver gene expression etc. changed with HF+FVT mice becoming ≈ LF (control) mice

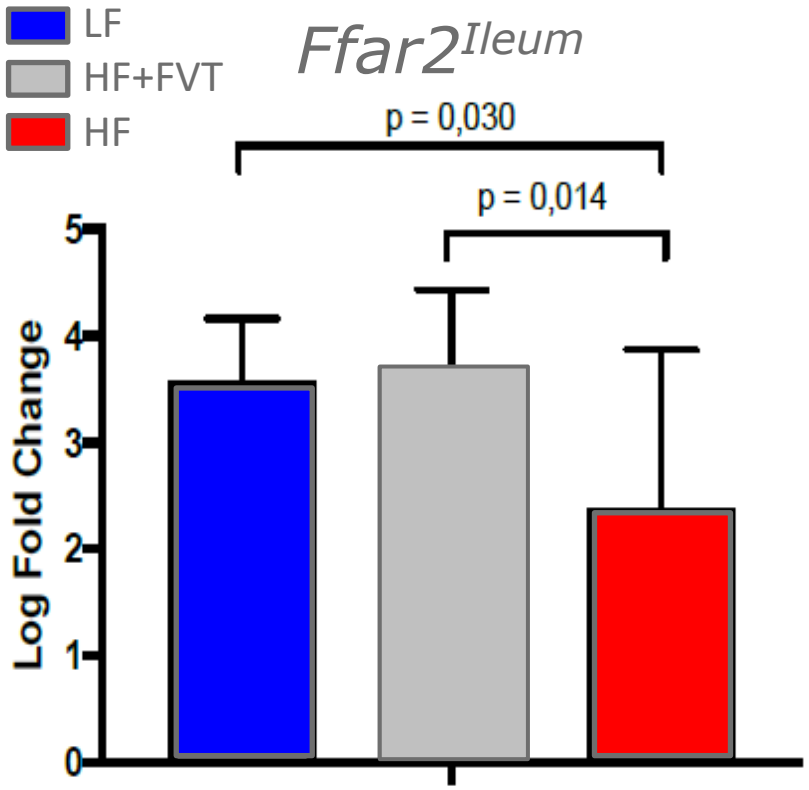


At 6 weeks post-treatment:
HF-FVT vs. LF:
adj. p-value = 0.9648

Rasmussen TS, *et al*. *Gut* 2020;**69**:2122–2130. doi:10.1136/gutjnl-2019-320005

One example: Altered gene expression after FVT

- qPCR-based in house gene expression array focussing on obesity, metabolic syndrome and T2D
  - Liver and ileum



Free fatty acid receptor (Ffar2):
Ffar2 → leptin production

Ffar2 → GLP-1 → glucose homeostasis → GLP-1 enhance insulin production

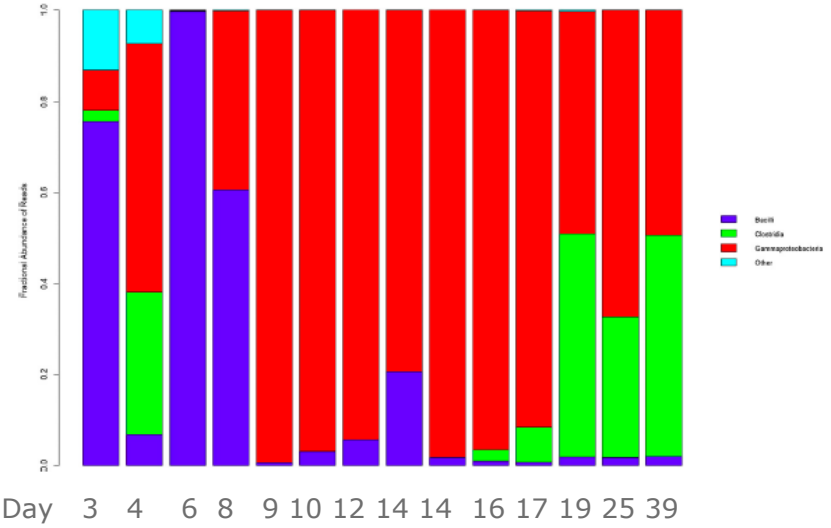
Ffar2 → regulate whole-body homeostasis → adipogenesis + lipid storage

Ffar2 → reacts on SCFA levels (metabolome+ levels of SCFA producers)

Gut 2020;**0**:1–9. doi:10.1136/gutjnl-2019-320005

Preterm birth (week 26-30ish) and gastrointestinal tract challenges

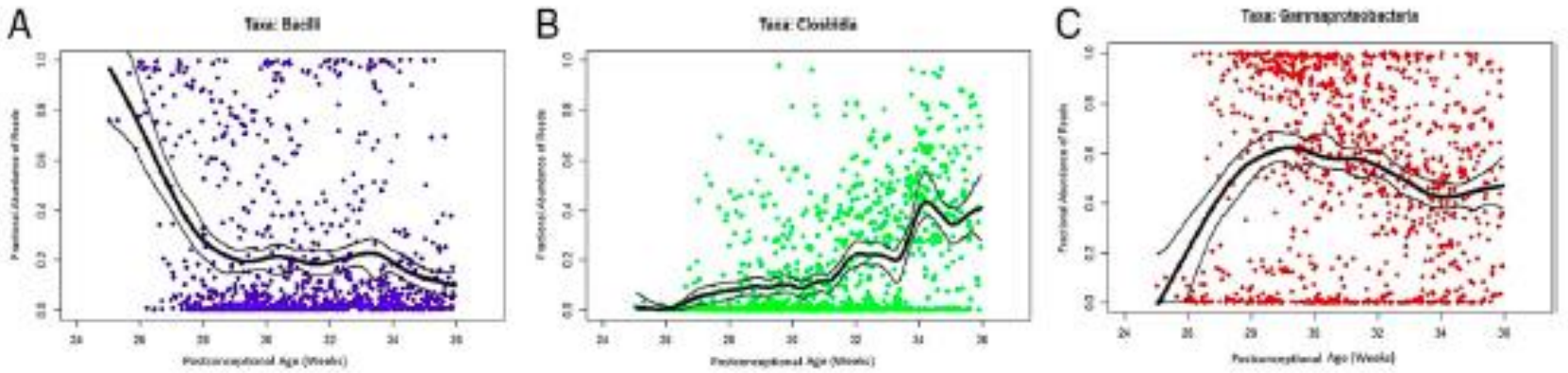
- Immature GI tract, immature organs
- Often limited access to mothers milk
- Extensive use of antibiotics etc.
- ≈5% of VLBW infants develop NEC



Patterned progression of bacterial populations in the premature infant gut

12522–12527 | PNAS | August 26, 2014 | vol. 111 | no. 34

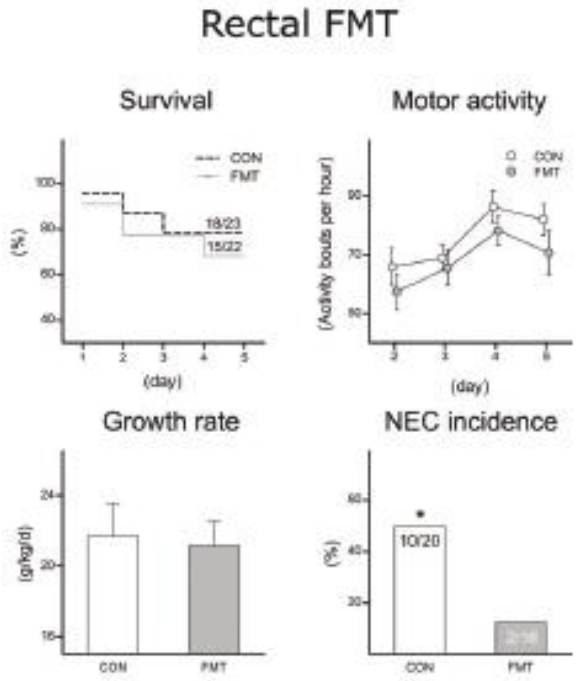
, Erica Sodergren[c,4], Carla M. Hall-Moore[a], Harold J. Stevens[a], William E. Bennett, Jr.[a], Nurmohammad Shaikh[b], Laura A. Linneman[b], Julie A. Hoffmann[b], Aaron Hamvas[b,7], Elena Deych[a], Berkley A. Shands[a], William D. Shannon[a,8], and Phillip I. Tarr[b,8]

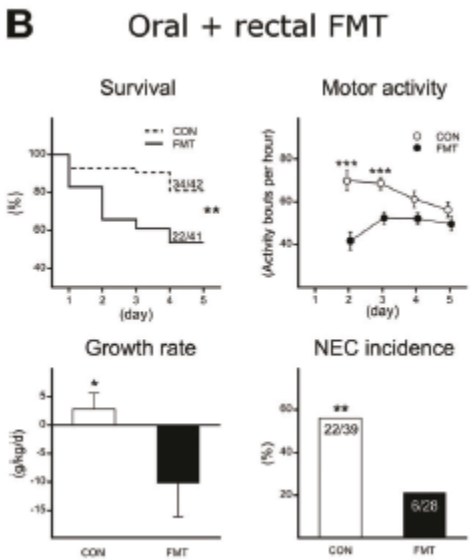


n=58 infants, 922 samples

Fecal microbiota transplant to preterms

- Faecal microbiota transplant (FMT) has proven very efficient for treating *Clostridium difficile* associated diarrhoea in adults
- Here we tested the efficacy of FMT to prevent NEC in preterm piglets





The ISME Journal
https://doi.org/10.1038/s41396-018-0301-z

ISME

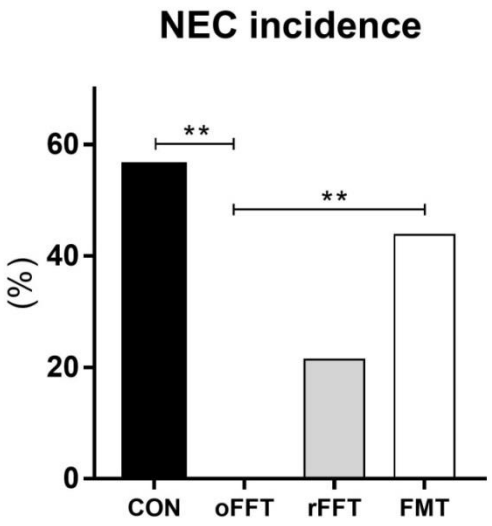
ARTICLE

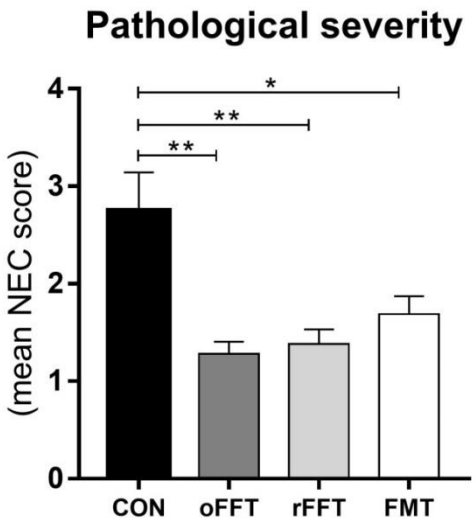
Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs

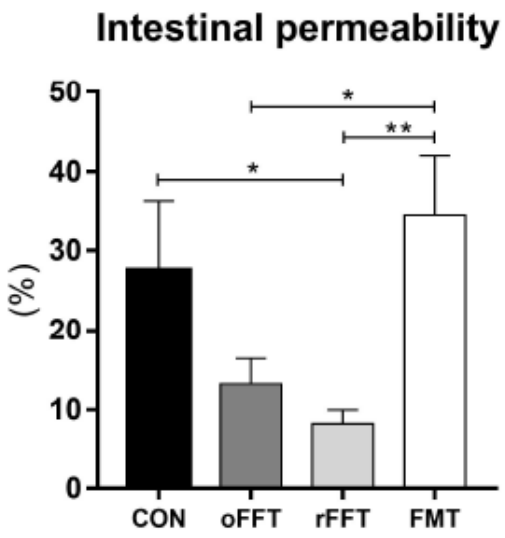
Anders Brunse[1] · Lena Martin[1,2] · Torben Sølbeck Rasmussen[3] · Lars Christensen[1] · Malene Skovsted Cilieborg[1] · Maria Wiese[3] · Bekzod Khakimov[3] · Robert Pieper[2] · Dennis Sandris Nielsen[3] · Per Torp Sangild[1] · Thomas Thymann[1]

But what about FVT?

- Fecal filtrate (Deng et al., 2019) of **colon content from healthy, term piglet** – compared to FMT (same donor) and Control (no treatment)
  - Lowered NEC incidence
  - Lowered pathological severity
  - Improved intestinal permeability







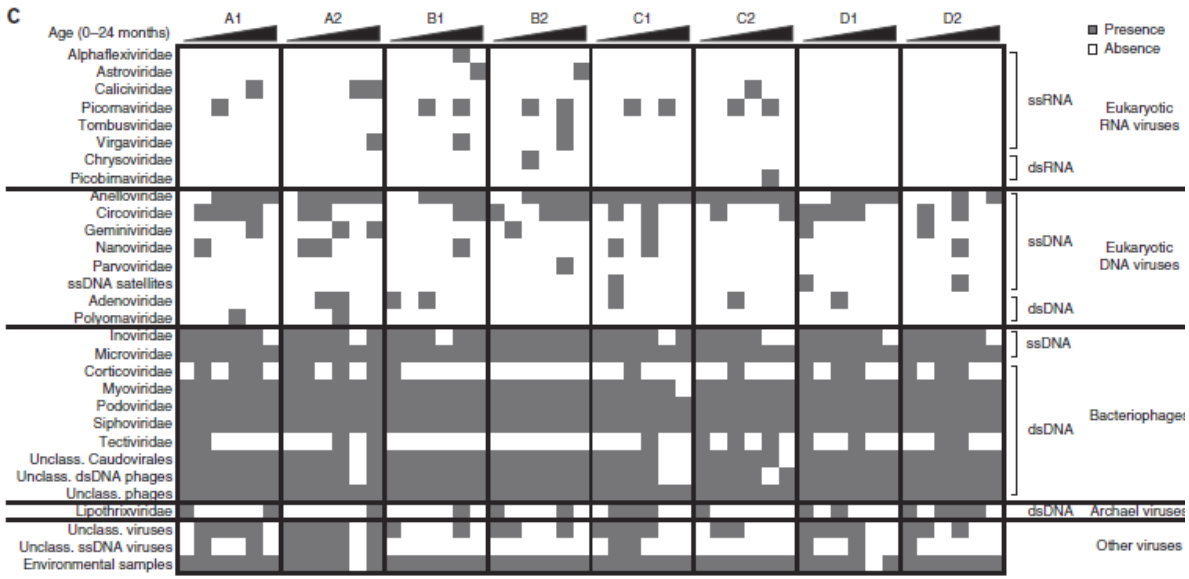
Brunse et al, ISME, accepted for publ.
doi.org/10.1101/2020.05.25.114751

And what about the challenges?

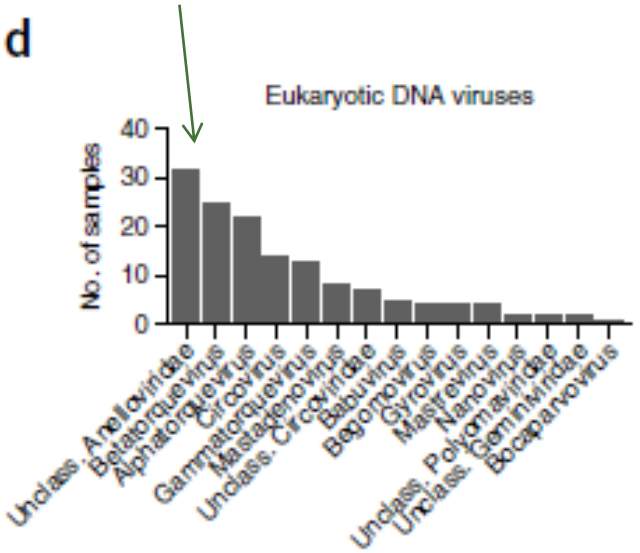
- FVT virome transplant is indeed a promising tool
  - And (likely) safer than FMT as bacterial pathogens are removed
  - Much reduced risk of sepsis in very vulnerable host (preterm infants, cancer etc.)
- But there are also challenges

Eukaryotic viruses

- Not only dsDNA but also ssDNA and RNA viruses



Detected in 32 out of 48 samples



Fecal samples obtained 0, 3, 6, 12, 18 & 24 months of life from 4 twin pairs

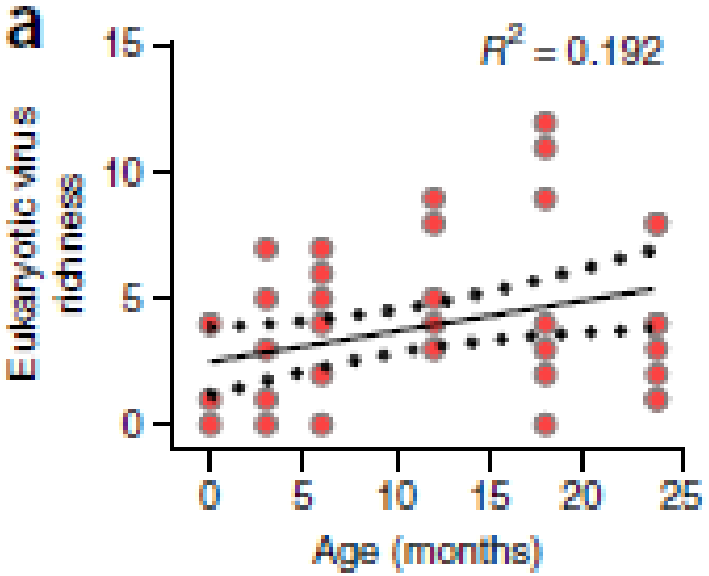
Early life dynamics of the human gut virome and bacterial microbiome in infants

Efrem S Lim[1,2], Yanjiao Zhou[3,4], Guoyan Zhao[1], Irma K Bauer[3], Lindsay Droit[1,2], I Malick Ndao[3], Barbara B Warner[3], Phillip I Tarr[1,3], David Wang[1,2] & Lori R Holtz[3]

nature medicine

Early life gut microbiota/virome establishment

- The eukaryotic virome is simple at birth and increases in complexity from there
  - Environmental exposure



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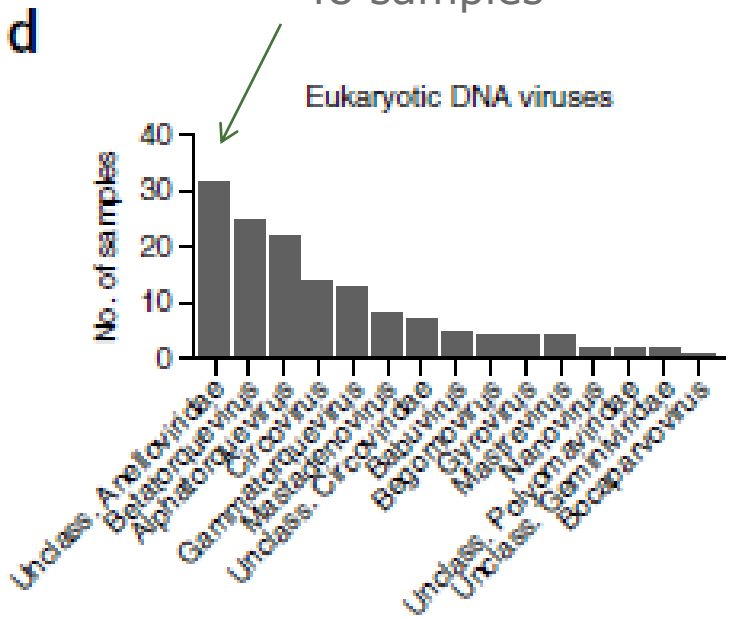


Many viruses of unknown function. Implications for health and disease?

Early life dynamics of the human gut virome and bacterial microbiome in infants

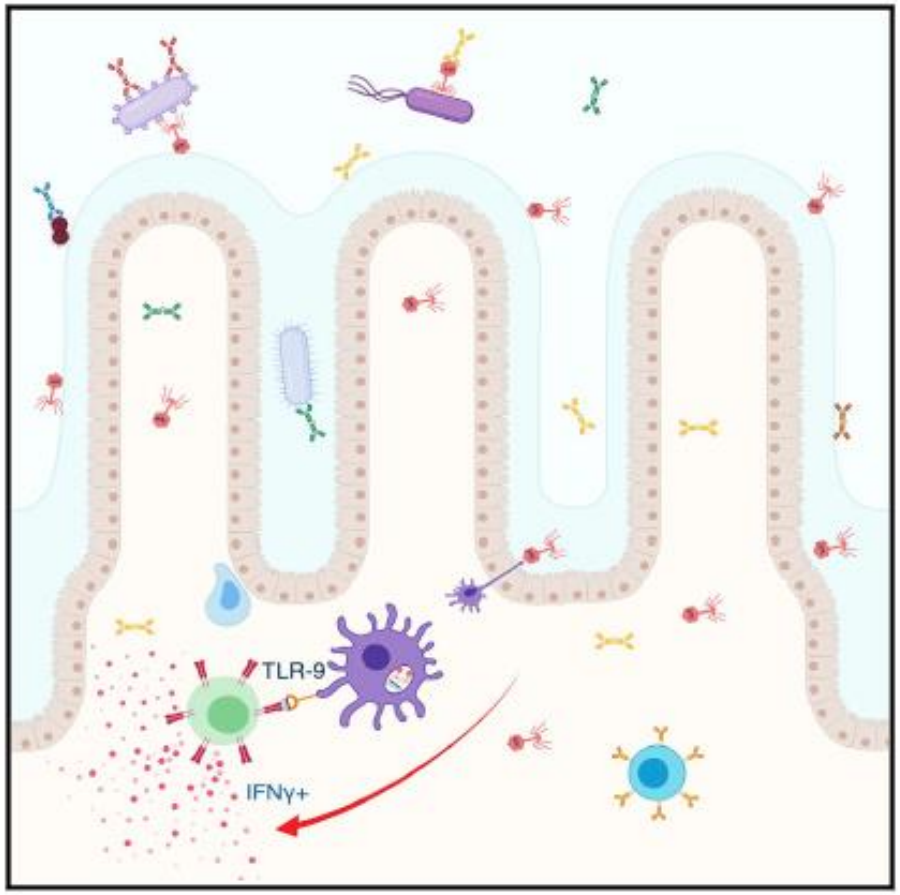
Efrem S Lim[1,2], Yanjiao Zhou[3,4], Guoyan Zhao[1], Irma K Bauer[3], Lindsay Droit[1,2], I Malick Ndao[3], Barbara B Warner[3], Phillip I Tarr[1,3], David Wang[1,2] & Lori R Holtz[3]

nature medicine

Phages and the immune system

Not only specific bacteria are recognised by phages

- Toll like receptor (TLR) 3 and 9 recognise phages/phage DNA
- => IFNγ production
- Prophage induction?
- => (Unwanted) GM community shulfing/ changes?



Cell Host & Microbe
Article

Expansion of Bacteriophages Is Linked to Aggravated Intestinal Inflammation and Colitis

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To sum up

- Gut microbiome dysbiosis is associated with numerous diseases
- Fecal virome transfer is (at least in the 3 cases investigated here) able to reverse a "negative" phenotype
    - E.g. by reducing weight gain and normalising blood glucose metabolism
    - Lowering NEC
    - And curing *C. difficile* infections
- But some (many) questions remain:
    - By doing FVT, we do not run the risk of transferring pathogenic bacteria
    - But what about the eukaryotic viruses (we carry a lot – and for most we do not know what they do…)
    - (Unintended) immune system stimulation

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