Gut microbiota, metabolic syndrome, obesity and the nutrient sensor pathways

Department of Gastroenterology, Endocrinology & Metabolism
Medical University Innsbruck

Herbert Tilg

Nothing to disclose
Fig. 1 The gut microbiota in development and disease.
Overview

• Microbiota regulates hepatic steatosis

• Innate immunity critically involved: TLRs, inflammasome

• Hepatic steatosis/metabolic dysfunction: transmissible via the microbiota

• Nutrient sensor pathways

• Conclusions
Ob/ob mouse has intestinal stasis
Intestinal bacterial overgrowth
Endogenous ethanol production
Damaged GI epithelial barrier
Permit EtOH/LPS escape?
Induce inflammatory cytokines?

Cope et al., Gastro, 1999

Link microbiota and NAFLD-related immune dysfunction
Role of small intestinal bacterial overgrowth in NASH (Gut 2001)

- 22 patients with NASH studied (\(^{14}\)C-D-Xylose and lactulose breath test)
- Higher prevalence of bacterial overgrowth in patients with NASH compared to controls
- In parallel, NASH patients demonstrated higher circulating TNF-\(\alpha\) levels (no increase in circulating endotoxin)
Probiotics and anti-TNF improve NASH

Li et al. Hepatology 2003
Gut microbiota regulates fat storage

Bäckhed F et al. PNAS 2004

Three groups of mice:
- germ-free state
- Those allowed to acquire a microbiota from birth to adulthood (CONV-R)
- Raised GF until adulthood and then colonized for 2 weeks with an unfractionated cecal microbiota from CONV-R donors (CONV-D)
A 14-d conventionalization of WT GF mice increases circulating leptin levels and decreases sensitivity to insulin

(A) Leptin, insulin, glucose levels; (B) Glucose tolerance, (C) Insulin tolerance
Gut microbiota regulates fat storage

Bäckhed F et al. PNAS 2004

Conventionalization induces hepatic lipogenesis and nuclear import of the basic helix-loop-helix transcription factor (ChREBP)

A) Hepatic oil-red staining
B) Liver TG levels
C) qRT-PCR assays of liver mRNAs
D) IH studies
Gut microbiota regulates fat storage
Bäckhed F et al. PNAS 2004

Fasting-induced adipocyte factor (Fiaf)

Conventionalization promotes adipocyte hypertrophy by suppressing *Fiaf* expression in the intestine: A) epididymal fat pads, B) qRT-PCR assays of epididymal mRNAs, C) LPL activity, D) *Fiaf* expression, E) generation of a fiaf −/− mouse, F) absence of Fiaf markedly attenuates the increase in total body fat content after a 14-d conventionalization.
Gut microbiota regulates fat storage

Bäckhed F et al. PNAS 2004

**Microbial colonization of the gut**

- Processing of dietary polysaccharides
  - Increased hepatic lipogenesis (ChREBP/SREBP-1)
- Suppression of Fiaf in the gut epithelium
  - LPL activity
  - Triglyceride storage in adipocytes and liver

**LINK:** Gut microbiota – energy harvest – energy storage – HEPATIC STEATOSIS
Mechanisms underlying the resistance to diet-induced obesity in germ-free mice

Bäckhed F et al. PNAS 2007

GF mice are protected against diet-induced obesity

Gut microbiota suppresses AMPK activity (‘fuel gauge’ that monitors cellular energy status)
Potential interactions of the microbiota with metabolic (liver) pathways

Tilg H. Gastroenterology 2009
Evolution of Steatosis/Metabolic Syndrome: A role for other factors?

Gut Microbiota?
Cytokines?

Innate immunity?
Inflammasome?
Fig. 1 T5KO mice develop obesity.
Fig. 2 T5KO exhibit hyperglycemia/insulin resistance.
Fig. 4 T5KO gut microbiota is necessary and sufficient to transfer metabolic syndrome phenotype to germ-free mice.

A-C: Antibiotic therapy

D-H: 4 week old GF WT mice received intragastrically cecal content from WT or T5KO mice

M Vijay-Kumar et al. Science 2010;328:228-231
• 2 different colonies with TLR5-deficiency studied

• Intestinal inflammatory disease and metabolic dysfunction not evident

• Only observed an impaired CD4 T cell response to flagellated pathogens

• … differences in the gut microbiota between institutional animal facilities or differences obtained during rederivation of these animals might explain divergent phenotypes
Liver toxicity is mediated via TLR9 and Nalp3 inflammasome

J Clin Invest 2009

ASC (apoptosis associated speck-like protein containing CARD)
Inflammasome consists of an NLR (nucleotide-binding domain, leucine-rich repeat-containing), ASC and caspase 1
Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity

Jorge Henao-Mejía, Eran Elina, Chengcheng Jin, Liming Hao, Wajahat Z. Mehat, Till Strowig, Christoph A. Thaiss, Andrew L. Kau, Stephanie C. Eisenbarth, Michael J. Jurczak, João-Paulo Camporez, Gerald I. Shulman, Jeffrey I. Gordon, Hal M. Hoffman, & Richard A. Flavell

Increased severity of NASH in inflammasome-deficient mice

Increased severity of NASH in \textit{Asc-} and \textit{Il18}-deficient mice is transmissible to co-housed wild-type animals

\textbf{DISEASE TRANSFER!}

Increased severity of NASH in Asc-deficient and co-housed wild-type animals is mediated by TLR4, TLR9 and TNF-α

d) Glucose

e) Insulin levels

f) i.p. glucose challenge after 11 weeks of HFD

3 weeks of antibiotic therapy before 12 weeks of HFD
Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity

- Inflammasome-deficiency-associated changes in the microbiota lead to hepatic steatosis and inflammation through input of TLR4 and TLR9 agonists leading to enhanced TNF liver expression
- Co-housing leads to disease in WT mice
- Relationship between microbiota, metabolic and auto-inflammatory (liver) disease
Antibiotic therapy (Cipro + Metro) significantly reduced the severity of NASH in Asc⁻/⁻ mice, and abolished transmission of the phenotype to WT animals.

Significant increase in the family of *Porphyromonadaceae*

*Porphyromonas* has been associated with several components of MS both in mice and humans, including atherosclerosis and T2DM.

Expansion of this taxa is strongly associated with complications of chronic liver disease.
A metagenome–wide association study of gut microbiota in type 2 diabetes

Junjie Qin*, Yingrui Li*, Zhiming Cai*, Shenghui Li*, Jianfeng Zhu*, Fan Zhang*, Suisha Liang†, Wenwei Zhang‡, Yuanlin Guan*, Dongqian Shen†, Yangqin Peng‡, Dongya Zhang‡, Zhuye Jie‡, Wencian Wu‡, Youwen Qin‡, Wenbin Xue‡, Junhua Li‡, Lingshuan Hani†, Donghui Lu†, Peixian Wu†, Yali Dai†, Xiaojuan Sun‡, Zesong Li‡, Aifa Tang‡, Shilong Zhong‡, Xiaoping Li, Weineng Chen†, Ran Xu†, Mingbang Wang‡, Qiang Feng‡, Meihua Gong‡, Jing Yu‡, Yanyan Zhang‡, Ming Zhang‡, Torben Hansen‡, Gaston Sanchez‡, Jeroen Raes‡, Gwen Falony‡, Shujiro Okuda‡, Mathieu Almeida‡, Emmanuelle LeChatelier†, Pierre Renault†, Nicolas Pons†, Jean-Michel Batto⁰, Zhaoxi Zhang‡, Hua Chen†, Ruifu Yang‡, Weimu Zheng†, Songgang Li†, Huanming Yang‡, Jian Wang‡, S. Dusko Ehrlich⁰, Rasmus Nielsen‡, Oluf Pedersen²,³,¹,¹,¹, Karsten Kristiansen¹,²,³ & Jun Wang²,³,¹,¹

Metagenome-wide association study (MGWAS)
345 chinese subjects
60.000 type-2 diabetes-associated markers identified
Identification of T2D-associated markers from gut metagenome.

No significant relationship between enterotype and T2D

JJ Qin et al. Nature 2012. doi:10.1038/nature11450
Taxonomic and functional characterization of gut microbiota in T2D.

MLGs, Metagenomic linkage group

Red text = Enriched functions

Blue text = Decreased functions
Gut microbiota of T2D patients show a moderate degree of dysbiosis

3.2 +/- 0.2% of gut microbial genes are associated with T2D in an individual

JJ Qin et al. Nature 2012 doi:10.1038/nature11450
Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse

**Fig. 1** Cumulative diabetes incidence in NOD female mice. Mice received pure water (*n*=15, solid line) or vancomycin treatment as adults VA from 8 weeks of age (*n*=15, dashed line) or vancomycin treatment as pups VP from birth and until 4 weeks of age (*n*=21, dotted line). Mice were diagnosed as diabetic and killed when blood glucose levels exceeded 12 mmol/l on two consecutive days. Comparisons of survival curves were tested by logrank test; *p*=0.026, VP vs untreated; *p*=0.169, VA vs untreated

Hansen et al. Diabetologia 2012
Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse

- Insulitis score and blood glucose levels significantly lower in the adult group
- Vancomycin treatment decreased many major genera of Gram-positive and – negative microbes while *Akkermansia muciniphila* became dominant
- Protective role for *Akkermansia*?

Hansen et al. Diabetologia 2012
• Many immune and metabolic changes occur during normal pregnancy
• 91 pregnant women of varying pregnancy BMIs and gestational diabetes status and infants studied
• Gut microbiota changed dramatically between first (T1) and third (T3) trimesters
• Vast expansion of diversity between mothers and an overall increase in *Proteobacteria* and *Actinobacteria*
• T3 stool showed strongest signs of inflammation and energy loss
• When transferred to GF mice, T3 microbiota induced greater adiposity and insulin sensitivity compared to T1
Figure 1. 16S rRNA Gene Surveys Reveal Changes to Microbial Diversity during Pregnancy. Microbial communities clustered using PCoA of the weighted UniFrac matrix.
Figure 4  High Between-Individual Microbial Diversity in T3 Persists in the Women Postpartum and Is Observed in Their Neonates  (A) Mean weighted (SEM) UniFrac distances between bacterial communities of women (sampled at T1, T3, and 1 month postpartum).
Koren et al. Host Remodeling of the Gut Microbiome and Metabolic Changes during Pregnancy

Cell 2012, 150: 470 - 480
Nutrient sensor pathways – link to metabolic inflammation

Genetic Predisposition

Gut Microbiota

Dietary Factors

Pro-inflammatory?
Anti-inflammatory

Metabolic syndrome, diabetes
Phosphatidylcholine - Atherosclerosis

Pro-inflammatory diet

Phosphatidylcholine (enriched in eggs, liver, meat etc) Drives inflammation

... concept of pro-inflammatory diets!!

Wang et al, Nature 2011
Products of gut microbiota such as SCFA, peptidoglycan or LPS activate different receptors of innate immune cells.
Multiple parallel „HITs“ Hypothesis

1st hits:
Nutritional aspects
Overweight
Inflammation?
Microbiota?

Normal

1st hit

Steatosis/MS

2nd hit

Diabetes/Steatohepatitis

2nd hits:
Diet?
Mediators?
Innate immunity?
Microbiota?
Extrahepatic cytokine sources?

Inflammation
Microbiota

3rd hit

Cirrhosis
Conclusions: Gut microbiota and obesity/diabetes

• Gut microbiota considered key factor regulating hepatic steatosis, MS and diabetes

• Diet: key „environmental factor“ modulating gut´s microbiota

• Involved mechanisms: TLRs, Fiaf, AMPK, ChREBP, cytokines, inflammasomes, ER stress
Conclusions

• Obesity, Metabolic Syndrome and NAFLD: is the cause in the GI Tract?

• May at least play an important role

• Microbiota, cytokines and inflammasome as major players including as therapeutical targets