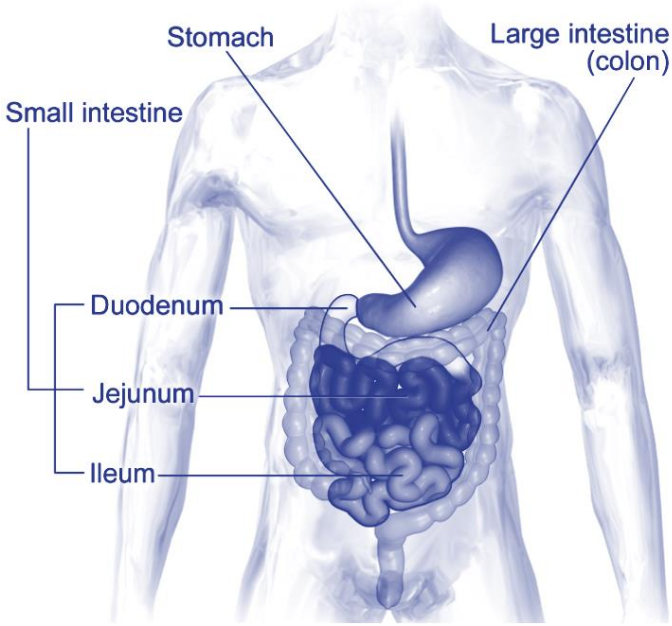
Amsterdam UMC
Universitair Medische Centra

Microbiota Center Amsterdam

Gutday 2019



December 5th Amsterdam

WINCLOVE PROBIOTICS

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Welcome

On behalf of the organizers of the 21th Annual Gut Day, Max Nieuwdorp, Joost Wiersinga, Wouter de Jonge, Hauke Smidt, Hermie Harmsen, Erwin Zoetendal, Hilde Herrema,Floor Huigenholtz, Jorn Hartman and Mark Davids as well as the final meeting of JPI HDHL DINAMIC consortium meeting we welcome you to Amsterdam.

The annual Gut Day is a major event that yearly takes place in the Netherlands, and during this meeting researchers in all fields of microbiota research (from basic to clinical science) come together to discuss new data and future directions. The Gut Day was able to attain contributions from key opinion leaders such Stanislav Dusko Ehrlich, Cyriel Ponsioen and Fredrik Bäckhed.

In this years' event, the Gut Day event is combined to JPI HDHL "DINAMIC" consortium end meeting. The JPI consortium's is lead by prof Dirk Haller and prof Tom Clavel, and will present the results regarding the role of healthy (mediterrranean) diet on human microbiota during the Gut Day.

The generous contributions of the sponsors of the Gut Day's event have made it possible to organize the Gut Day as a free event for participants. In addition, there are Award prizes for best seminar

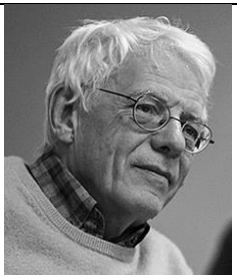
We think this year's edition will be an unforgettable event and hope you will all enjoy this 21th annual gut day 2019.

Program

Collegeroom 4

08:30-09:00	Registration			
09:00-09:10	Welcome by Max Nieuwdorp and Joost Wiersinga			
09:10-09:55	**Keynote lecture Stanislav Dusko Ehrlich "Microbiota analyses techniques: from past to present"**			
09:55-10:20	Coffee & tea			
10:20-11:20	Platform presentations, chair Joost Wiersinga			
	Gabriela Bravo-Ruiseco	Silicon inclusion in *Faecalibacterium prausnitzii* : a survival mechanism?		
	Prokopis Konstanti	Treatment of eosinophilic esophagitis with an elemental diet is associated with changes in the microbial communities of the upper gastrointestinal tract		
	Maries Lissens	Inhibiting *Salmonell*a EPS production is evolutionarily robust		
	Marjolein Klaassen	Anti-inflammatory gut microbial pathways are decreased during Crohn's disease exacerbations		
11:20-12:05	**Keynote Lectrue Cyriel Ponsioen "FMT for IBD, is there light at the end of the tunnel?"**			
12:05-12:45	Lunch			
12:45-13:30	Poster walk			
13:30-15:00	Platform presentations, chair Hermie Harmsen			
	Bastiaan Haak	Transkingdom analysis of the intestinal ecosystem of critically ill patients on the ICU		
	Benoit Marsaux	Application of the SHIME platform to assess the interplay between fungi and bacteria in the human gut		
	Quinten Ducarmon	The bacterial gut microbiota during controlled human infection with *Necator americanus* larvae		
	Matthew Davies	Does our microbiome travel well? Microbiome resilience and acquisition of multidrug resistant bacteria in travellers		
	Gerben Hermes	Why cross-sectional microbiota analysis do not provide consensus observations: a case for the need of temporal data		
	Poster Pitches	**Carlijn Bruggeling**	**Ellen De Paepe**	**Gianluca Galazzo**
		Annelies Kers	**Jannie Henderickx**	**Lieven Van Meulebroek**
15:00-15:20	Coffee & tea			
15:30-16:30	**Keynote lecture Fredrik Bäckhed "Microbiota in human metabolism: from bench to clinic and back"**			
16:30	Awards and closing by Wouter de Jonge, Max Nieuwdorp and Joost Wiersinga			
16:30	Drinks			

Keynote speakers



Stanislav Dusko Ehrlich

Professor

King's College London

"Microbiota analyses techniques: from past to present"

Cyriel Ponsioen

Professor

Amsterdam UMC
Universitair Medische Centra

"FMT for IBD, is there light at the end of the tunnel?"

Fredrik Bäckhed

Professor

Göteborgs Universitet

"Microbiota in human metabolism: from bench to clinic and back"

Microbiota analyses techniques: from past to present

Stanislav Dusko Ehrlich

A microbiologist whose primary research interest is the human microbiome's role in human biology and its impact on human health and well-being.

He is Research Director Emeritus at INRA, where he coordinated the EU-funded project MetaHIT and is the PI of the Metagenopolis project. Professor at King's College London, where he is Director of the Centre for Host Microbiome Interactions.

Ehrlich's team has developed an approach known as 'quantitative metagenomics,' which is based on high throughput sequencing of total DNA extracted from stools and powerful bioinformatics. This approach describes the composition of the neglected gut microbiome in high detail. It has shown that this organ comes in three main types (known as enterotypes); that one person in four has lost a significant proportion of its richness (40 per cent of its species), and therefore has an increased risk of developing chronic metabolic syndromes (diabetes, liver and heart complications); and that the richness can be restored, at least in part, by nutritional interventions.

FMT for IBD, is there light at the end of the tunnel?

Cyriel Ponsioen

Fecal microbiota transfer (FMT) is a very old therapeutic modality. The first formal account of FMT for inflammatory bowel disease was reported as a case report in the Lancet in 1989. In the past two decades several favorable observations have been reported, mainly in ulcerative colitis (UC), which prompted us to embark on the very first, formal, randomized, placebo-controlled trial. While this trial was negative on its primary endpoint, it yielded several exciting observations, which encouraged us to further pursue and refine this treatment modality. Notwithstanding, there is still a possibility that the positive associations between restoration of a dysbiotic microbial signature to a more healthy state are merely correlations and bear no causal relationship. Whether FMT for IBD is merely a hype or indeed a new and promising treatment modality for IBD will be discussed in this key-note lecture.

Microbiota in human metabolism: from bench to clinic and back

Fredrik Bäckhed

The microbial ecosystem, microbiota, of the human gut consists of trillions of bacteria and recent data have demonstrated that an altered gut microbiota can be associated with a number of diseases, ranging from obesity and inflammatory diseases to behavioural abnormalities. There is extensive microbiota-host cross talk that generates signals to extraintestinal organs and it is becoming more evident that a miss-configured microbiota may produce signals that contribute to metabolic diseases. Identification and understanding of these signals is a daunting task especially as the microbiota is modulated by environmental cues and interact with dietary macronutrients to produce bioactive compounds. These microbiota-derived metabolites can signal directly to distant organs in the body where they activate cellular receptors. However, they may also signal by modulating enterendocrine cell function or nervous signalling from the gut. Thus diet-microbiota interactions may regulate host metabolism at several levels.

Abstacts platform presentations

Gabriela Bravo-Ruiseco	Silicon inclusion in *Faecalibacterium prausnitzii* : a survival mechanism?
Prokopis Konstanti	Treatment of eosinophilic esophagitis with an elemental diet is associated with changes in the microbial communities of the upper gastrointestinal tract
Maries Lissens	Inhibiting *Salmonell*a EPS production is evolutionarily robust
Marjolein Klaassen	Anti-inflammatory gut microbial pathways are decreased during Crohn's disease exacerbations
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Matthew Davies	Does our microbiome travel well? Microbiome resilience and acquisition of multidrug resistant bacteria in travelers
Gerben Hermes	Why cross-sectional microbiota analysis do not provide consensus observations: a case for the need of temporal data

Silicon inclusion in Faecalibacterium prausnitzii: a survival mechanism?

Gabriela Bravo-Ruiseco[1], Lu Wang[1], Eleni Sibbald-Tsompanidou[1], Daan J. Touw[2], Jan IJmker[2], Gabriela Ruiseco-Gutierrez[3], Jeroen Kuipers[4], Ben N. G. Giepmans[4], Jan Maarten van Dijl[1], Hermie J.M. Harmsen[1]

Faecalibacterium prausnitzii is a strict anaerobic, rod shape, gram-positive bacterium and it is a part of the human gut microbiome. As one of the many beneficial bacteria in our gut, it represents between 5 to 15% of the bacterial content in the human colon. So far is considered a marker of health, since low counts of *F. prausnitzii* have being associated with auto-immune and inflammatory diseases, such as Crohn's disease and diabetes.
As a strict anaerobe, *F. prausnitzii* is extremely sensitive to oxygen, making the study of this bacterium challenging. This especially applies for studying the survival mechanisms that it uses, to maintain the high numbers in the colon or to survive outside the host.
In this study we describe an intracellular inclusion produced by Faecalibacterium prausnitzii in response to aging and how may confer protective effects to the bacteria, allowing it to survive longer.
Even though there is no evidence of this new survival mechanism happening in human hosts, it could have also big environmental effects by the depletion of silicon of the environment.

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TREATMENT OF EOSINOPHILIC ESOPHAGITIS WITH AN ELEMENTAL DIET IS ASSOCIATED WITH CHANGES IN THE MICROBIAL COMMUNITIES OF THE UPPER GASTROINTESTINAL TRACT

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Introduction & objectives: Eosinophilic esophagitis (EoE) is an allergic disease of the esophagus characterized by eosinophil predominant inflammation. The course of disease can be ameliorated by elimination diets or an elemental diet. Given the big effect of diet on microbiota and its implication with allergic diseases, we examine the gastrointestinal (GI) microbiota composition from EoE subjects with active disease and study their response to an elemental diet.
Material and methods: We profiled the GI microbiota composition of healthy non-EoE adults (n=8) and EoE subjects with active disease (n=17) before and after the elemental diet, using biopsies from esophagus and duodenum along with saliva and fecal samples. Clinical symptoms and dietary intake were used to assess correlations with the microbiota data.
Results and discussion: GI microbiota from the EoE subjects at baseline was different from the healthy controls. After the elemental diet, the microbial communities from the upper GI became more similar to that of healthy controls, and a significant reduction of the genera *Streptococcus* in saliva, *Veillonella* in esophagus and the mucolytic bacteria i.e. *R. torques* in duodenum was observed. On top of this iron and calcium intake negatively correlated with the abundance of *Streptococcus* while protein intake was positively correlated with *Veillonella*. Interestingly, the esophageal microbiota composition between subjects classified as responders and partial responders was different. These findings suggest that (1) an elemental diet changes the microbial communities of the upper GI tract and (2) only esophageal microbiota is associated with treatment response. These results warrant further investigation into the mechanisms behind these findings and their clinical consequences.

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Inhibiting *Salmonella* EPS production is evolutionarily robust

Lise Dieltjens[1], Kenny Appermans[1], Maries Lissens[1], Bram Lories[1], Wook Kim[3], Erik Van der Eycken[2], Kevin R. Foster[3], Hans P. Steenackers[1]

Salmonella is an important food-borne pathogen that is able to form biofilms both in the gut and outside the host. Bacteria in biofilms are typically enclosed in a matrix of self- produced exopolymeric substances (EPS), which mediate attachment and offer high protection against antibiotics and disinfectants. This urges the need for strategies that inhibit biofilm formation and render microbes susceptible to treatment. As for antibiotics, the problem with any long-term treatment strategies, however, is the potential for resistance evolution. We need anti-biofilm strategies, therefore, that also limit the evolution of resistance.
We hypothesize that the reliance of biofilms on shared EPS may also be their weakness as social evolution theory predicts that inhibiting shared, cooperative traits can select against drug resistance. We here test this hypothesis based on inhibitors of EPS production in *Salmonella* biofilms.
We show that EPS of *Salmonella* biofilms is a cooperative trait whose benefit is shared among cells, and that EPS inhibition reduces both cell attachment and antimicrobial tolerance in a biofilm assay. We then compare an EPS inhibitor to conventional antibiotics in an evolutionary experiment. While resistance against conventional antimicrobials rapidly evolves, we see no evolution of resistance to EPS inhibition. We further show that a resistant strain is outcompeted by a susceptible strain under EPS inhibitor treatment, explaining why resistance does not evolve. Finally, we validate our findings in a well-established *Salmonella* gut infection model: the nematode *Caenorhabditis elegans*. We show that EPS inhibition strongly reduces colonization of the worm intestinal lumen, reduces *Salmonella* virulence and prolongs worm survival, supporting the potential of EPS inhibitors as therapeutic agents.
Our work indicates that targeting cooperative traits is a viable solution to the problem of antimicrobial resistance.

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Anti-inflammatory gut microbial pathways are decreased during Crohn's disease exacerbations

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BACKGROUND AND AIMS
Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract characterized by alternating periods of exacerbation and remission. We hypothesized that changes in the gut microbiome are associated with CD exacerbations, and therefore aimed to correlate multiple gut microbiome features to CD disease activity.
METHODS
Fecal microbiome data generated using whole-genome metagenomic shotgun sequencing of 196 CD patients were of obtained from the 1000IBD cohort (one sample per patient). Patient disease activity status at time of sampling was determined by re-assessing clinical records three years after fecal sample production. Fecal samples were designated as taken 'in an exacerbation' or 'in remission'. Samples taken 'in remission' were further categorized as 'before the next exacerbation' or 'after the last exacerbation', based on the exacerbation closest in time to the fecal production date. CD activity was correlated with gut microbial composition and predicted functional pathways via logistic regressions using MaAsLin software.
RESULTS
In total, 105 bacterial pathways were decreased during CD exacerbation (FDR<0.1) in comparison to the gut microbiome of patients both before and after an exacerbation. Most of these decreased pathways exert anti-inflammatory properties facilitating the biosynthesis and fermentation of various amino acids (tryptophan, methionine and arginine), vitamins (riboflavin and thiamine) and short-chain fatty acids (SCFAs).
CONCLUSION
CD exacerbations are associated with a decrease in microbial genes involved in the biosynthesis of the anti-inflammatory mediators riboflavin, thiamine and folate and SCFAs, suggesting that increasing intestinal abundances of these mediators might provide new treatment opportunities. These results were generated using bioinformatic analyses of cross-sectional data and need to be replicated using time-series and wet lab experiments.

KEYWORDS
Crohn's disease activity; fecal microbiome; whole genome metagenomic shotgun sequencing

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*Equal contribution

Transkingdom analysis of the intestinal ecosystem of critically ill patients on the ICU

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Background The bacterial microbiota plays a critical role in enhancing local and systemic immunity, and disturbances of these micro-organisms have been linked to altered outcomes in critical illness. However, emerging data indicate that other intestinal organisms, such as eukaryotic viruses, bacteriophages and fungi, are closely integrated with the bacterial microbiota and its host through processes coined transkingdom interactions. Despite the recent establishment of the importance of these other kingdoms to the intestinal ecosystem, their role in critically ill patients remains to be elucidated.
Methods: In this observational cohort study we collected fecal samples from 34 patients admitted to the intensive care unit (ICU). Fourteen healthy subjects that provided fecal samples pre- and post-broad-spectrum antibiotic exposure served as controls. The bacterial microbiome, fungiome, eukaryotic - and prokaryotic virome was subsequently characterized using 16S rRNA, Internal Transcribed Spacer (ITS) and VIDISCA-NGS sequencing, as well as a targeted parasite, 16s and 18s qPCRs. In addition, fecal short-chain fatty acid levels were determined using nuclear magnetic resonance (1H-NMR) spectroscopy.
Results: Critical illness in ICU patients was characterized by a marked shift in fecal bacterial, fungal, and prokaryotic virome composition when compared to healthy controls, with extreme inter-individual differences. Sharp increases in fungal overgrowth as well as a depletion of short-chain fatty acid levels were seen in both critically ill patients and healthy volunteers post-antibiotics, whereas communities remained in balance when absolute bacterial abundance and diversity was maintained.
Conclusions: In this first transkingdom analysis of the intestinal ecosystem on the ICU, we observed highly heterogeneously altered patterns of the bacterial microbiome, fungiome, and prokaryotic virome in both critically ill patients and healthy volunteers treated with antibiotics. The short- and long-term consequences of these disturbances remain to be determined.

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Application of the SHIME platform to assess the interplay between fungi and bacteria in the human gut.

Benoît Marsaux[1,2,*], Pieter Van den Abbeele[2]

Fungal infections have a major impact on human health, infecting about 2 billion people and killing more than malaria or breast cancer each year. In particular, *Candida* species impose a high clinical and economic burden upon the European population. They frequently cause fatal hospital-acquired bloodstream infections, but also oral thrush and vaginitis. Most women have suffered an episode of vulvovaginal candidiasis, with ~ 8 % enduring recurrent infections. The initiation and severity of a *Candida* infection depends on an intricate interplay between the infecting fungal strain, the individual's immune status and its microbiota, all of which can display significant variability.

Therefore, the European FunHoMic project aims at defining and exploiting the Fungal-Host-Microbiota interplay to identify novel biomarkers (fungal or host genetic polymorphisms, microbiota profiles, metabolites or immune markers) for the stratification of a patient's risk of serious fungal infection.

In this frame, there is a need for a representative *in vitro* model to study the interplay between fungi and bacteria in a human gut-like environment. Having a stable *C. albicans*-infection model will allow further understanding the role of dysbiosis in the pathogenicity of this commensal species of the gut; as to testing specific treatments for preventing or curing *Candida* infections. For this purpose, the SHIME® (Simulator of the Human Intestinal Microbial Ecosystem), represents a valid dynamic model of the complete gastrointestinal tract. It simulates the bacterial part of the microbiota with differences both longitudinally (ileum, ascending, transverse and descending colon) and laterally (mucus versus lumen). Yet, prior to develop a *C. albicans*-long term infection model, there is a need to characterize the mycobiome part of the SHIME® which might imply adjustments of certain parameters to better mimic the *in vivo* situation.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 812969.
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The bacterial gut microbiota during controlled human infection with *Necator americanus* larvae

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Hookworms are soil-transmitted helminths which use immune-evasive strategies to persist in the human duodenum where they are responsible for anemia and protein loss. Despite mass drug administration, hookworm is still responsible for a high burden of disease globally, underscoring the need for an effective vaccine. A controlled human hookworm infection (CHHI) model was developed to aid vaccine development. Apart from their pathogenic role, hookworms are increasingly investigated for attenuating auto-immune disease, as they induce immunoregulation. Currently, little is known about the bacterial-helminth relationship during helminth infection. The current study explores temporal changes in the gut microbiota in response to human infection with *Necator americanus* in a CHHI model with healthy volunteers.

Twenty-four healthy volunteers were included in a randomized CHHI, of which 20 volunteers completed the study. Volunteers were exposed to cumulative doses of 50, 100 and 150 larvae. The 150 larvae group received first infection at trial week 0, the 100 group at trial week 2 and the 50 larvae group at trial week 4. Fecal samples were collected for microbiota profiling through 16S rRNA gene amplicon sequencing at weeks 0 (baseline), 4, 8, 14 and 20 of the trial.

All volunteers developed patent infection. Heavy gastrointestinal (GI) complaints were observed in 11 volunteers and were not associated with the larval dose. No differences in alpha diversities (Chao1-richness and Shannon-diversity) or stability measures (Bray-Curtis and Jaccard) were found between the three study groups, nor were significant differences in abundance of individual bacterial taxa found. Overall, bacterial richness increased from week 8 to week 20 ($p=0.017$) of the trial. Volunteers with heavy GI had transient instability of the microbiota during the first eight weeks ($p=0.047$) and a rapid recovery at week 20 ($p=0.004$). Additionally, eosinophil count significantly correlated with microbiota stability (Jaccard, $r=-0.26$, $p=0.02$). *Barnesiella*, amongst other taxa, was found to be more abundant in the heavy complaints group throughout the study ($p<0.05$).

In conclusion, this study investigated longitudinal changes in the gut microbiota during *N. americanus* infection in healthy individuals. We found a remarkable stability of the gut microbiota in response to this infection over the twenty-week study period, although transient instability was observed in individuals with heavy GI complaints. This instability correlated with eosinophil counts, suggesting a relationship between microbiota and eosinophilic enteritis. These data open new avenues for exploring helminth-bacterial interaction in the human intestine.

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Does our microbiome travel well? Microbiome resilience and acquisition of multidrug resistant bacteria in travellers

Matthew Davies[1,2]*, Willem van Schaik[1], Alan McNally[1], Petra Wolffs[2], the COMBAT-consortium and John Penders[2,3]

The international spread of antimicrobial resistance poses a serious health risk, compounded by approximately 1.4 billion travellers in 2018, many of which to countries that are hotspots of resistance. A previous study focussing on the carriage of multidrug resistant bacteria after travel showed that there is extensive acquisition and persistence of extended spectrum beta lactamase producing Enterobacteriaceae (ESBL-E) in the gut of travellers visiting Asia and Africa. Using shotgun sequencing data from 190 of these travellers, the metagenomics profile of the gut microbiome has been analysed to understand its role in this context.

A metagenomics species concept approach was used to determine the taxonomic composition, population diversity and metabolism of the microbiome at baseline (before travel) and how these are altered longitudinally. Predicted genes are clustered by their abundance profile across multiple samples, providing a more powerful signal for analysing metagenome data. Here we show that these aspects at baseline do not significantly differ between travellers that were or were not subsequently colonised by ESBL-E, so are not predictive of the risk of acquiring ESBLs. Alternatively, there were longitudinal changes detected in the taxonomy and functional profile which were specific to the travel destination.

The lack in predictive power of the baseline microbiome suggests that a traveller's risk of ESBL acquisition is difficult to determine before travel. Alternatively, the longitudinal results highlight the taxa and metabolic processes that may have a role in the protection against, or clearance of, ESBL producing Enterobacteriaceae. These are therefore potential targets as a prophylactic treatment or as adjuvants in the decolonisation of ESBL-E. However, the destination of travel is be a key factor to focus on, as this is a significant contributor to how the gut microbiome is altered.

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Why cross-sectional microbiota analyses do not provide consensus observations: a case for the need of temporal data.

Gerben DA Hermes[1], Hauke Smidt[1], Ben JM Witteman[2,3], Ellen Kampman[2], Willem M de Vos[1,4], Erwin G Zoetendal[1]

Introduction: Functional Gastrointestinal Disorders like irritable bowel syndrome (IBS) constitute one of the most common reasons for seeking healthcare. The pathophysiological mechanisms underlying these disorders are complex, because they typically display a complex multifactorial aetiology and biological heterogeneity. In the last years, the gastrointestinal (GI) microbiota has been increasingly implicated in the direct involvement in the pathogenesis of IBS. Most microbiota research focusses on compositional differences between the fecal microbiota of healthy controls and IBS subjects and several microbial signatures have been reported. However, observations from different studies have been highly inconsistent. These inconsistencies may relate to methodological differences, lack of rigorous statistics and outliers due to small sample sizes or uncontrolled environmental exposures which are known to influence the microbiota. There are also intrinsic biological factors related to the microbiota, such as functional redundancy and intra-individual temporal variability. Despite this, many studies describe comparisons between different groups of subjects on the basis of a single fecal sample per subject. Aim: Our aim was to study the temporal dynamics in microbiota composition in subjects diagnosed with IBS and asymptomatic control (AC) subjects. Methods: For this, the COMIC study was set up. It contains clinically relevant host characteristics and microbial profiles generated by HITChip for 575 samples. 155 participants were sampled at baseline of which 100 were repeatedly sampled at 2 month intervals over the course of 1 year of which 84 (41 IBS and 43 AC) completed the study period with all fecal samples collected. Results: Intra-individual microbiota variation was lower than that between random samples (P<10E-5), but not different between IBS and AC. A cross-sectional analysis of all 575 samples revealed a pattern of IBS associated taxa. However, a temporal analysis of the same individuals over time revealed that the microbiota of IBS and AC was different at only half of the intervals. More surprisingly, at each interval the representative taxa were different. This was not only due to large microbiota changes within IBS, but also within the AC group. Conclusion: Our results show that the microbiota was individual specific, but not rigid and showed fluctuations over time in both IBS and AC subjects. These fluctuations were so large that even when comparing the same individuals over time, cross-sectional snapshot analyses resulted in different pictures of the IBS microbiota compared to AC. We argue that temporal microbiota datasets are vital in the quest for a validated diagnostic microbial signal for IBS and possibly other microbiota associated disorders.

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Abstracts posters presentations

Poster Pitches

Carlijn Bruggeling

Ellen De Paepe

Gianluca Galazzo

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Lieven Van Meulebroek

Right-sided colonic biofilms are associated with adenoma formation in patients with Lynch syndrome

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Patients with Lynch syndrome carry a pathogenic variant in one of their DNA mismatch repair genes; MLH1, MSH2, MSH6 or PMS2. These variants increase the risk to develop adenomas and cancer of the colon and rectum (CRC). Adenoma frequency is highly variable between Lynch patients, which cannot only be explained by genetic variants. Hence, other factors may influence the risk for adenoma formation. Interestingly, right-sided sporadic CRCs often have bacterial biofilms (89%), while these are rarely found on left-sided CRC (12%). Here we investigate whether right-sided colonic biofilms pose a risk-factor for adenoma formation in Lynch patients.
One-hundred Lynch patients undergoing regular screening colonoscopies at the Radboudumc Nijmegen were included. During colonoscopy, forceps biopsies were taken from colon ascendens (right colon) and descendens (left colon). Biopsies were screened for bacterial biofilms using fluorescent *in situ* hybridization by targeting bacterial 16s rRNA. The frequency of colorectal adenomas (tubular adenomas and (tubule)villous adenomas) before and during the colonoscopy was registered.
A total of 172 adenomas were recorded from the first screening colonoscopy until inclusion in the study. Right-sided biofilms were more common (45%) than left-sided biofilms (35%) and were associated with a higher risk for right-sided adenoma formation (odds ratio: 3.15 (CI: 1.28; 7.79). Interestingly, left-sided bacterial biofilms were not associated with left-sided adenoma formation (odds ratio: 0.85 (CI: 0.36; 2.02). Our data show that biofilms are frequent in Lynch patients and suggest that we can identify high-risk Lynch patients through examining microbial biofilm formation in the right-sided colon. It remains to be investigated whether biofilms are also associated to adenoma formation prospectively.

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Multi-biofluid Metabolomics as a Tool to Discover Metabolite Biomarkers for Cow's Milk Allergy

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In industrialized nations, food allergies are a growing epidemic and are considered a major thread to our wellbeing. Cow's milk allergy (CMA) is one of the first allergies to occur in early childhood and early life sensitization has been associated with an increased risk to develop the atopic march, including eczema, asthma and other food allergies later in life. As such, more research is urgently needed to gain more insights into this disease and to improve current diagnostics. Therefore, this pilot study evaluated a unique multi-biofluid platform, applying UHPLC-Q-Orbitrap-HRMS for polar metabolic fingerprinting as well as the innovative LA-REIMS for rapid metabolomics screening. The use of urine and feces was preferred in this research, as these biofluids are readily available, while the collection of plasma is problematic, especially in young children. Additionally, the fecal content is influenced by genes, diet, environment and microbiome, of which the latter is implicated in oral tolerance development and is known to differ between allergic children and their healthy controls. Metabolic fingerprinting was applied on simultaneously collected fecal and urine samples from children, below the age of 5 years, with IgE mediated CMA (n = 5), non-IgE mediated CMA (n = 3) and their healthy brothers and sisters (n = 5). The established OPLS-DA models for feces, urine and the combination thereof were able to discriminate according to allergy state. Most biomarker candidates could be linked to other microbiome-related diseases, such as asthma, inflammatory bowel disease, and autism, while other metabolites could be associated to the immune system. Finally, a rapid metabolomics screening approach was evaluated on simultaneously collected fecal and urine samples of children, below the age of 5 years, with IgE mediated CMA (n = 6), non-IgE mediated CMA (n = 4), IgE mediated food allergy other than cow's milk (n = 5) and their healthy brothers and sisters (n = 11). The associated LDA models displayed clear clustering according to allergy state, the latter was confirmed following validation, which suggested a correct classification for respectively 94.96% and 79.12% of the fecal and urine samples.
As such, the multi-biofluid strategy entails the unprecedented potential to reveal more significant results in food allergy research, including the discovery of biomarkers and unraveling of mechanistic information. Additionally, the innovative LA-REIMS technique can be considered an excellent rapid metabolomics screening methodology.

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Infant gut microbial community assembly and maturation is linked to subsequent atopy development during childhood

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Gut microbiota maturation in early life is a dynamic process that is still incompletely understood. It has been hypothesized that inadequate microbiota maturation can cause immune deregulations linked to manifestation of non-communicable diseases, such as atopic dermatitis, later in life.

We investigated the gut microbiota maturation of children from birth up to school-age in association to the subsequent development of atopic dermatitis (AD) in a deeply phenotyped cohort.

Fecal samples from 311 children were collected at ages 5, 13, 21, 31 weeks and 6-12 years and profiled by amplicon sequencing of the 16S rRNA V4 gene region
Our findings show that the complexity of the microbiota gradually increases over time. Changes in complexity and composition were mostly driven by the duration of breast feeding (p=0.001) and the age of introduction of solid food (p=0.003), whereas the impact of environmental and genetic factors were less pronounced.
Next, we introduced the application of a joint model that enables the use of repeatedly collected infant fecal samples (longitudinal analysis) and link its variations with the probability to develop AD at a given age (survival analysis). The results show that a decrease in microbial richness (Shannon index p=0.0001) is associated with an increased risk to develop AD. Furthermore, several bacterial genera were associated with a reduced risk of
AD, including Lachnospira and Faecalibacterium.

Altogether our findings suggest that adequate gut microbiota maturation during the first year of life is crucial to reduce the risk of atopic dermatitis later in life

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Inoculation of newly hatched broiler chickens with adult microbes accelerated the development of the intestinal microbiota and increased the activation of natural killer cells

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Immediately after hatch, intestinal colonization by microorganisms is initiated. It is generally accepted that the development of the hosts' immune system depends on interactions with intestinal microorganisms. Since commercial chickens hatch under strict hygiene practices in hatcheries, they are exposed to a diverse range of microorganisms from environmental rather than from parental sources. This study investigated the effects of inoculation with adult chicken microbes (AM) or PBS in commercial Ross 308 broilers directly post-hatch (day 0), on maturation of the intestinal microbiota and activation of natural killer (NK) cells. The microbiota composition of cecal content samples was assessed by 16S ribosomal RNA gene amplicon sequencing at ages 1, 3, 7, 14, 21 and 35 days. In parallel, activation of intestinal NK cells was determined by flow cytometry. Dirichlet multinomial mixtures modelling was applied to sequencing data and revealed three distinct clusters between broilers. The first cluster (A) included both 1 day old AM and PBS inoculated broilers, and 3 days old PBS inoculated broilers. Cluster B consisted of AM chicks of 3 days, and most broilers of 7 days old. In broilers in cluster B higher frequencies of gut NK cells were found and the activation of intestinal NK cells was increased compared to cluster A. All broilers of 14, 21 and 35 days old were in cluster C, independent of inoculation type. This suggests that inoculation of newly hatched broilers with AM accelerated the maturation of the microbiota in cecal content and affects NK cell activation within the first week of life. Knowledge of the microbe-immune crosstalk is essential to develop new strategies to improve the resilience of the immune system in the defense against pathogens to improve broiler health and reduce the need for antibiotics.

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Characterising the gastric and faecal proteome to unravel gastrointestinal function and maturation in preterm infants

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The nutritional requirements of preterm infants are unique and challenging to meet in neonatal care, yet crucial for their growth, development and health. As such, it is relevant to increase understanding of how dietary inputs are being processed by the immature and developing gastrointestinal tract of preterm infants. In this study, we therefore investigated gastrointestinal function and maturation during early life of preterm infants, including functioning of the gut microbiota.
Gastric aspirates (n = 40 infants) and faecal samples (n = 10 infants) were collected during the first two and six postnatal weeks of life respectively, and analysed with metaproteomics through LC-MS/MS. Differences in the gastric proteome were mainly driven by the percentage of human milk in enteral feeding (22.7%, p = 0.002) and sample pH (11.8%, p = 0.002). Proteins involved in digestive and immune functioning were significantly more abundant at times of human milk-predominated feeding. Composition of the faecal proteome was associated with gestational and postnatal age (10.4% and 6.3% respectively, p = 0.002). Specific human digestive enzymes in faeces were more abundant with increasing postnatal age. Bacterial digestive enzymes in faeces were analysed across gestational and postnatal age.
In conclusion, our data provides insights in the gastric and faecal proteome of preterm infants including digestive functioning of the gut microbiota. Deeper understanding of gastrointestinal maturation and functioning in preterm infants might contribute to the improvement of current nutrition support strategies.

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Rapid LA-REIMS and comprehensive UHPLC-HRMS for fecal metabolomics to assess metabolic disorders

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Metabolomics offers unique possibilities to characterize the pathophysiological state of an individual and is therefore assigned valuable potential for point-of-care applications and personalized healthcare provision. However, the conventional analytical approaches of liquid-chromatography mass spectrometry (LC-MS) are not ideally suited to translate metabolomics into a decentralized clinical setting, as being often associated with extensive workflows that are not compliant with the conditions for non-laboratory testing. In this regard, our study explored the concept of laser-assisted rapid evaporative ionization mass spectrometry (LA-REIMS) for rapid metabolic fingerprinting of biofluids. With this type of ambient ionization, the process of laser ablation initiates matrix-assisted desorption and ionization of intact biomolecules, whereby only minimal sample pre-treatment is needed and real-time data acquisition is possible. To date, a wide range of LA-REIMS applications have already been actualized, mainly relating to surgical intervention (*i.e.* tissue analysis), but not yet for biofluids.

The LA-REIMS platform comprised a Quadrupole Time-of-Flight mass spectrometer and Opolette™ HE2940 pump that was equipped with a Nd:YAG infra-red laser (2940 nm). Methods were optimized for various biofluids, namely saliva, urine, plasma, and feces, whereby the time for data acquisition was only 0.5 min per sample. These methods were successfully applied to assess cow's milk allergy in infants, obesity in adolescents, and type 2 diabetes in adults.

With respect to the latter, fecal samples were collected from treated type 2 diabetes patients (n = 38, HbA1c > 60 mmol/mol) and healthy controls (n = 36), and subjected to LA-REIMS analysis[1]. Fecal fingerprints enclosed 4,923 compounds ions and allowed discrimination according to pathophysiological state, as indicated by the valid supervised multivariate models ($Q^2(Y)$ of 0.734 and *p*-value of 1.93 e^{-17}) and classification accuracy (90.5%). It was hereby noted that the majority (54%) of the marker molecules were in the 450 - 650 Da mass range, typically populated by fatty acids, pphosphoglycerolipids, sphingolipids, and glycerolipids. Cross-platform validation was achieved by ultra-high performance LC high-resolution Q-Exactive™ MS, applying polar metabolomics[2] and lipidomics[3]. Also here, significant metabolic alterations were revealed ($Q^2(Y) \geq 0.665$ and *p*-value $\leq 4.11\ e^{-5}$), which were mechanistically assessed based on the in-depth metabolite characterization that is possible with this kind of LC-MS approach. Intriguing metabolization paths were defined for metformin (the first-line drug in type 2 diabetes treatment), for which a microbial involvement was proposed.[1] In conclusion, this work presents LA-REIMS for rapid discriminative fingerprinting and comprehensive UHPLC-HRMS for in-depth biological interpretation, whereby the complementarity of the analytical platforms offers unique opportunities to establish metabolomics in a clinical environment.

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Starch source altered ecological network, functional profile, and short-chain fatty acid production in a porcine gut microbiota

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Keywords: *in vitro* colon model, pig, gut microbiota, starch, resistant starch

Abstract
Several validated dynamic *in vitro* models of the colon have been developed for humans, but there is no dynamic *in vitro* fermentation model for pigs. This study was conducted to modify the human, dynamic, computer-controlled TNO *in vitro* model of the colon (TIM-2) for pigs and investigate effects of different starches and polysaccharides on swine microbiota structure, ecological network, predictive functional profile, and short-chain fatty acids production. Our study showed that three different types of starch (weighted and unweighted UniFrac: P = 0.001), or two polysaccharides (weighted UniFrac: P = 0.034, unweighted UniFrac: P = 0.003) greatly impact microbiota composition. Co-occurrence network analysis indicated that microbiota fed with different sources of starch changed the network topological properties. Functional profiles were predicted to vary significantly among the three starch treatments, and the original pig fecal inoculum was more similar to maize starch treatment. On the other hand, compared with maize starch and arabinoxylans (AX), the microbial composition of the original inoculum was more similar to AX-XG (arabinoxylans and xyloglucan), and the functional profile of the original inoculum also clustered with AX-XG. The cumulative productions of acetic (P = 0.006), propionic (P = 0.008), and butyric acid (P = 0.034) on maize starch were significantly higher than those on potato starch and wheat starch. In conclusion, supplementation maize starch as the starch source together with AX and XG, leads to the bacteria being more stable and closer to the original microbial composition and function compared to potato starch, wheat starch and AX.

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Linking blood metabolite and colonic metabolite and microbiota profiles to sanitary conditions in starter pigs

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Low environmental sanitary conditions can have negative effects on health and performance in pigs. To further study the effects of sanitary conditions on nutrient metabolism and health, we evaluated effects of a contrast in sanitary status of pigs on their blood and colon metabolites and microbiota composition in colon. Here, pigs were kept under high (HSC) or low sanitary conditions (LSC) from weaning onwards. HSC was induced via applying a strict hygiene protocol, pigs receiving a preventative antibiotic injection and vaccinations against a number of pathogens. LSC were obtained by omitting cleaning of pens prior to stocking, not applying a hygiene protocol, and spreading of foreign manure. In addition, LSC pigs did not receive an antibiotic treatment and vaccinations. At week 13 of age, 18 animals from both the HSC and LSC group were sacrificed to obtain blood and colon digesta samples. In these samples metabolite profiles were acquired by employing both Nuclear Magnetic Resonance (NMR) and triple quadrupole mass spectrometry (TQMS). In addition, in colon digesta samples the microbiota composition was determined by sequencing the 16S hypervariable regions V3-V4. The NMR analysis showed eight metabolites in blood and 32 metabolites in colon digesta significantly different ($P<0.05$) between SC treatments. The TQMS showed differences for five different metabolites in both blood and colon digesta. These blood metabolites could be linked to protein accretion, nutrient metabolism, immunity, and responses to stress. The microbiota diversity in colon digesta (Shannon index) was significantly higher in LSC compared to HSC ($p<0.05$), values being 2.99 and 2.85, respectively. Abundance of 36 significant bacterial genera (FDR < 0.05 and ARC > 0.01%) was mostly lower in colon digesta from HSC compared to LSC pigs. In conclusion, metabolites in blood and colon digesta and microbial groups in colon show a link with the sanitary conditions in which pigs are housed.

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Pelvic radiation induces multimodal responses in the mouse gut microbiome and intestine

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Background: Dysbiosis of the gut microbiome is well known to be evoked by pelvic radiotherapy. Yet, modulating the resident communities by probiotic consumption has become an appealing means to promote host health by either restoring the host-microbe balance or preventing dysbiosis. Unfortunately, human trials testing adjuvant microbial therapies have been yielding contradictory results.
Objective: The aim of this study was to develop a mouse model of pelvic irradiation-induced intestinal toxicity and microbial dysbiosis for future microbial therapy development to prevent the adverse effects of pelvic irradiation.
Methods: Eight weeks old, male C75Bl/6 mice were exposed to pelvic irradiation with an acute X-ray dose of 12 Gy whilst closely monitored for food intake and body weight. Fecal samples were longitudinally collected before, and one day and seven days after exposure. Microbiota profiles were characterized based on 16S rRNA sequencing using the Illumina MiSeq platform. In parallel, intestinal toxicity was evaluated one, three and seven days post-irradiation on mid jejunum, distal ileum and proximal colon.
Results: Dysbiosis was observed in irradiated mice, which was characterized by both an increase in α- and β-diversity. Linear discriminative analysis effect size (LEfSe) analysis showed that members of *Bacteroides* and *Desulfovibrionaceae*_were differentially abundant (LDA score>4) one and seven days following pelvic irradiation, respectively. Additionally, irradiated mice showed maximum 16.8% decreased body weight seven days after 12 Gy of X-rays exposure, which was at least partly due to a compromised food intake. Furthermore, the crypt apoptosis index in jejunum, ileum and colon was increased one day following irradiation, which does not affect jejunum and ileum villi length three days post-irradiation. However, an increase in ileum and colon crypt depth was observed indicative of repair after injury. Seven days following pelvic irradiation, ileum villi length and total mucosal thickness had increased, suggesting that our procedure did not affect the ability of stem cells to regenerate damaged crypts in the long term. This observation was confirmed by a significant increase in proliferative ($Ki67^+$) crypt cells.
Conclusion and perspective: This study provides a model for future microbial therapy development to prevent the adverse effects of pelvic irradiation. To make this model conclusive, near future research will focus on metagenomic biomarker discovery, detection of inflammation as well as functional assays evaluating intestinal permeability and bacterial translocation. These results will lead to a better understanding of pelvic irradiation-induced effects to the healthy intestine.

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A cross-sectional observational study of the fecal microbiome composition in adult laying hens with and without access to an outdoor range

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Abstract
Laying hens with access to outdoor ranges are exposed to additional environmental micro-organisms, including the potential pathogens of wild birds. Alterations in fecal microbiome community parameters or the relative abundance of individual genera of outdoor-housed layers may serve as an indicator for risks of exposure to potential pathogens. We therefore performed a cross-sectional field study to evaluate differences in the fecal microbiomes of outdoor-housed vs indoor-housed layers across different farms. Eight layer flocks (four indoor and four outdoor) were sampled once. Indoor and outdoor flocks were matched based on the rearing flock of origin and age. Sampled flocks were kept on five poultry farms. For each flock, cloacal swabs were taken from ten layers and were analysed with 16S rRNA amplicon sequencing.
Bacterial diversity was not different between indoor- and outdoor-housed layers; however, housing type (indoor vs outdoor), rearing farm, farm and house significantly contributed to the observed variation in microbiome composition. Distance based redundancy analysis and variation partitioning showed that house, housing type and rearing farm explained 31.8% of the variation in community composition. The combination of farm and rearing flock explained most variation (10.6%). Housing type explained only 0.5% of the variation. Using a random forest classifier, one amplicon sequence variant (ASV) identified as *Dietzia maris* was only found in hens with an outdoor range and is classified a soil bacteria.
Our study shows that the microbial composition of adult laying hens in field conditions is to a limited extent, affected by the access to an outdoor range, but that the house, farm, and rearing flock play a greater role in determining their microbiomes. Overall, measuring differences in fecal microbiota of layers as an indicator for the level of exposure to potential pathogens and biosecurity seems of little practical use.

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Effect of antibiotic-induced gut microbiota disruption on LPS-induced acute lung inflammation

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Abstract
Introduction: An increasing body of evidence is indicating that the gut microbiota, referring to the trillions of microorganisms in mammalian intestines, modulates pulmonary inflammatory responses. This so-called gut – lung axis might be of importance in a whole spectrum of inflammatory pulmonary diseases such as acute respiratory distress syndrome, chronic obstructive pulmonary disease and pneumonia. Here, we investigate the effect of antibiotic disruption of gut microbiota on immune responses in the lung after a intranasal challenge with lipopolysaccharide (LPS); a well-established immunogen derived from the outer membrane of gram-negative bacteria.
Methods: We treated C57Bl/6 mice for two weeks with broad-spectrum antibiotics (ampicillin, metronidazole, neomycin and vancomycin) supplemented to their drinking water. Subsequently, these mice and untreated control mice were inoculated intranasally with 10 µg ultrapure LPS from Klebsiella pneumoniae. Mice were sacrificed 2 and 6 hours post-challenge, after which bronchoalveolar lavage fluid (BALF) and lung tissue were taken. Pulmonary cell influx and cytokine responses were examined. Gut microbiota was analysed by Miseq sequencing of the V3-V4 region of 16s rRNA gene.
Results: Gut microbiota analysis showed that antibiotic-treated mice had a pronounced reduction in numbers and diversity of bacteria. Trends towards increases in total cell numbers and, specifically, neutrophils at t=6 hours were observed in antibiotic treated mice. Conversely, these mice showed significantly decreased levels of myeloperoxidase (MPO) in lung homogenate, which indicates a decrease in the neutrophil numbers. A modest, but time consistent, significant increase of interleukin (IL)-6 release was seen in BALF of antibiotic treated mice. Release of tumour necrosis factor alpha (TNFα), however, was not statistically different between groups.
Conclusion: Antibiotic induced microbiota disruption is associated with alterations in host responses during LPS-induced lung inflammation. Further studies are required to determine the clinical relevance of the gut-lung axis in pulmonary infection and inflammation.

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Carbohydrate boosted control of intestinal immunity in chickens

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Antimicrobial resistance (AMR) can cause an increased incidence of antibiotic treatment failure. It is estimated that the number of human deaths because of AMR will increase more than ten-fold by 2050. To reduce these risks, restrictions on antimicrobial additives in feed and also the use of therapeutic antimicrobials in the poultry sector are in place. However, protozoal infections with *Eimeria* (resulting in the gut disease coccidiosis) and in some cases complicated by secondary overgrowth with *Clostridium perfringens* (causing necrotic enteritis) are highly prevalent in broilers. To combat these infections, coccidiostatic antimicrobials are standardly applied in the broiler feed and some flocks are treated with antibiotics when they develop necrotic enteritis. Therefore, alternative approaches to combat these gut infections are of great importance.
Prebiotic carbohydrates have shown beneficial effects in broilers regarding gut health and can reduce the impact of these intestinal infection. Knowledge about the mode-of-action of these carbohydrates is limited. We want to elucidate the mode-of-action by making use of *in-vitro* screening tools, such a Chicken ALIMEntary tRact mOdel (CALIMERO), which is based on the TNO gastro-intestinal Model (TIM) and co-cultures of broiler intestinal organoids in combination with innate immune cells exposed to microbiota. By testing the effects of specific carbohydrates on the gut microbiota, the intestinal barrier, and immune functions, we will provide an efficient approach to evaluate their potential anti-infective and gut health-promoting effects, this will help identify successful carbohydrates that can be applied to improve broiler gut health and will reduce the use of antimicrobials.

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Vendor associated differences in murine gut microbiota and its effect on lipopolysaccharide induced lung inflammation and Gram-negative pneumonia

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Background: Limited reproducibility of experimental animal models of pulmonary inflammation and pneumonia across different laboratories is a concern. We hypothesized that differences in microbiota composition across vendors can explain a part of this observed variation in phenotypes.
Methods: In order to address this question and to study the effect of different bacterial gut microbiota, a multi-vendor approach was used with genetically similar mice derived from three different vendors (Janvier (Jan), Envigo (Env), and Charles River (CR)). This model was employed to study the effect on the host response to a pulmonary lipopolysaccharide (LPS) challenge (1 µg K. pneumoniae LPS intranasal), as well as experimental Klebsiella pneumoniae infection (ATCC43816, 1x10e4 CFU intranasal). Gut microbiota was analysed using 16s sequencing.
Results: Gut microbiota analysis revealed profound intervendor differences in bacterial composition, shown by beta diversity and at various taxonomic levels. In a first set of experiments, tumor necrosis factor (TNF)-α and interleukin (IL)-6 release in lung and bronchoalveolar lavage fluid (BALF) was determined 6 and 24 hours after intranasal treatment with LPS. No differences were found between the groups, with the exception for Envigo, showing a higher level of TNF-α in lung and BALF at 6 hours. In a second set of experiments, mice from different vendors were subjected to a clinically relevant model of Gram-negative pneumonia (K. pneumoniae). At 12 and 36 hours post infection, no intervendor differences were found in bacterial dissemination, or TNF-α and IL-6 levels in the lungs.
Conclusion: Although there is a marked variation in the gut microbiota composition of mice from different vendors, the effect on the pulmonary host response was limited. Except for an influence on the early TNF-α response during LPS induced lung inflammation, we could not demonstrate a vendor effect during experimental K. pneumoniae pneumonia.

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Early-life feeding accelerates gut-microbiome colonization and intestinal maturation in piglets

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Early-life microbiome perturbations have been suggested to have important effects on host development, physiology and behaviour, which can persist throughout life. We hypothesise that early feeding (access to customised fibrous pre-weaning diet) can affect gut microbiome colonisation and corresponding intestinal development in piglets. In the first experiment, rectal swabs were used to investigate gut microbiota colonisation over time by metataxonomic analysis (n=20 selected from 10 litters). In a second experiment, piglets were sacrificed (at weaning; four weeks of age) to collect intestinal tissue samples from both groups (n=28 selected from 12 litters). Early feeding was observed to enhance the rate of microbial diversity pre-weaning ($P<0.05$). Multivariate analyses indicated that early feeding stimulates pre-weaning colonization of post-weaning signature microbes like *Prevotella, Ruminococcus, Faecalibacterium, Megasphaera, Catenibacterium, Subdoligranulum*. Notably, eating behaviour of the piglets quantitatively correlated with acceleration of the microbiome. The intestinal chyme analysis established that the microbiota acceleration effects were restricted to the colon and were not observed in the jejunal or ileal ecosystem. Nevertheless, early feeding did lead to an accelerated development of mucosal morphometry in jejunum, and also increased epithelial proliferation in colon. In a third experiment, the molecular mucosal development was assessed by transcriptome profiling in jejunal and colonic tissue samples. Preliminary analyses show that colonic mucosa displayed the largest transcriptome response to early feeding at the time of weaning, and the data support the conclusion that early feeding accelerates gut-mucosal development also from a molecular point of view.

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Curdlan feeding changes microbiota composition and improves DSS colitis in mice

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Introduction
β-glucan consumption is known for its beneficial effects in reducing inflammation. Humans lack the required enzymes to digest β-glucans, but certain intestinal microbiota species can digest β-glucans and thus trigger gut microbial changes, improving human health. In this study, we determined curdlan (bacterial β-glucan) induced microbial changes, and followed its influence on the course of intestinal inflammation in the Dextran Sodium Sulfate (DSS) colitis.
Methods
C57BL/6 mice were pre-treated with vehicle (5% glucose) or curdlan (10 mg/ml) through oral gavage for 14 days. Subsequently, mice were taken off curdlan and colitis was induced by administering 2% fresh DSS daily to the drinking water for 7 days. Control (non-colitis) groups received normal drinking water. To determine inflammation, colon weight and length was measured and histology scoring and gene expression study were performed. Colon content was collected for 16S amplicon (V3-V4) sequencing of microbiota composition. Differences in amplicon sequence variance (USEARCH) composition were visualized based on the Bray-Curtis-Dissimilarity Index. Fold differences were studied using DESeq2.
Results
In colitis condition, disease activity index, weight loss and inflammation score of the curdlan pre-treated group were improved compared to the vehicle treated group. Concomitant with improved colitis, the bacterial populations exhibited a higher alpha diversity of the curdlan fed animals compared to vehicle. Beta diversity analysis indicated large differences ($R^2 = 0.46$) in the bacterial community structure (Bray-Curtis) between the colitis and non-colitis condition. While curdlan feeding did not induce any global community changes, specific taxa did show significant differences in relative abundance. Interestingly, a specific *Bifidobacterium* was observed to be 10 to 100-fold more prevalent in the curdlan fed group in both colitis and non-colitis conditions respectively.
Conclusion
Curdlan feeding improved DSS-induced colitis and our data suggests that alterations in the gut microbiome may mediate the observed beneficial effects.

Keywords: Curdlan, Microbiota, Colitis

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Assessment of Colorectal Cancer Progression and Gut Microbiome Dynamics in a Mouse Model

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Colorectal cancer (CRC) is the third most common identified malignancy and thus represents an important health and socioeconomical burden. Various studies have systemically showed that CRC patients are confronted with intestinal dysbiosis, a significant shift in the composition of the gut microbiome. In addition, follow-up studies based on experimental models have shown that a dysbiotic gut community provokes enhanced CRC development. To put a halt to this vicious cycle, re-establishment of a healthy gut microbiome could be key. As a first step towards this goal, experimental data on microbial composition needs to be acquired through the use of a reproducible CRC mouse model.
The objective in this study was the implementation of a CRC mouse model through characterisation of the microbial, histological, molecular and clinical status. In parallel, the microbial variation between non-homogenised aliquots of mice faecal pellets was assessed.
Mice received a single intraperitoneal injection of the carcinogen azoxymethane (AOM) followed by three cycles of water-administered dextran sodium sulphate (DSS). Mice were sacrificed and colon tissue was collected at 6 dedicated time points to allow temporal follow up of tumour number, histopathological hallmarks and RNA/protein alterations, while fresh faecal pellets were collected daily for microbial analysis. Animals were clinically monitored throughout the course of the experiment.
In accordance to literature, AOM-injected mice showed consistent body weight loss following each round of DSS treatment. Additionally, clinical signs of inflammation such as diarrhoea and rectal bleeding were present after each round of DSS treatment. The groups exposed to three rounds of DSS had a median of 6 macroscopically identified tumours per mouse. The majority of these tumours were located in the distal colon and rectum. Preliminary analysis of 16S sequencing data showed little variation in microbial community between aliquots of the same faecal pellet, allowing for multiple analyses on a single faecal pellet. Interestingly, a clear shift was observed when comparing baseline samples and samples collected after three cycles of DSS.
Based on the available data, characterisation of the AOM/DSS mouse model for colitis-associated cancer was successful and reproducible. Additional analysis is required for full characterisation of the chronic inflammatory state, temporal dysbiosis and how these are linked to treatment regimes.

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Dietary modulation of the human gastrointestinal tract with a focus on the mycobiome

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The microbial community in the human gut has a big influence on the health of the host, with evidence of bacterial- and fungal-dysbiosis shown for several diseases. Several studies show that fungal-dysbiosis could be restored by dietary strategies. The aim of this research was to optimize the TIM-2 model to be able to study the gut fungal population, fungal:bacterial interactions, and to use interventions and to modulate these. A validated, computer-controlled *in-vitro* model of the colon (TIM-2) was used to study (modulation of) the human gut fungal population, inoculated with pooled microbiota from healthy volunteers. Samples were taken during the adaptation-period (-18 to 0 hr), and test-period (up to 72 hr). Different dietary interventions were carried out: standard medium, high/low carbohydrate, glucose and simple sugars. Additionally, interventions with antibiotics or fungicides were performed. The mycobiome was analyzed by amplicon-sequencing of the internal-transcribed-spacer-region-2 (ITS2).
TIM-2 was successfully used to simulate the gut fungal population. Stability of the fungi in the model was shown to be affected primarily in the first hours of the adaptation period. Different dietary interventions led to a diet-dependent shift in the fungal population, where a high glucose diet showed the clearest pattern. Removal of either the fungal or the bacterial population led to a change in fungal:bacterial interactions.
The use of an *in-vitro* model of the colon in combination with NGS of ITS2 is a successful way to mechanistically study the effect of diet on gut fungi. Fungal:bacterial interactions were demonstrated.
Key words: Mycobiome, fungi, NGS, *in vitro* model

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Dietary emulsifiers affect the gut microbiota: Perspectives from in vitro techniques

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Background and aims
Dietary additives are widely used in processed food products and have become a target for research in terms of their impact on the gut microbiome. Recent studies with dietary emulsifiers in both in vitro and murine models have indicated a strong impact on both the gut microbiome and host health, possibly related to chronic gut inflammation and other western ailments (Chassaing 2015, Chassaing 2017, Swidsinski et al 2009, Suez et al 2015).
This study aimed at characterizing the effects of 5 dietary emulsifiers on the composition and the activity of the gut bacteria. A comparison was made between 2 mainstream chemical ones (carboxymethylcellulose and tween80) and 3 natural or biotechnological examples (soy lecithin, sophorolipids and rhamnolipids). A second aim was to investigate interindividual variability.
Materials and Methods
The short term impact of 5 emulsifiers on the gut microbiota of 10 individuals was tested in a series of 48h batch-incubations. Emulsifiers were combined with fecal slurry in 4 concentrations: 0, 0.005, 0.05, 0.5 m%. The endpoints measured were the bacterial activity (SCFA-production), the intact cell population (SGPI-staining and Flow cytometry) and community composition (MiSeq sequencing). Also, the bacterial metagenome was assessed using PICRUSt.
Results and Discussion
The 5 emulsifiers showed clearly different effects on all 3 parameters. Sophorolipids and Rhamnolipids were indicated as most harmful towards the microbiota. They diminished the live fractions of the bacteria and increased sharply the abundance of 1 OTU, identified as Escherichia/Shigella, which belongs to the category of opportunistic pathogens. Both also decreased the butyrate production, in favour of propionate. Carboxymethylcellulose and Tween80 show limited effects on SCFA-production, cell counts or microbial composition. Soy Lecithin decreased live/dead ratio's and increased the same OTU of Escherichia/Shigella. It also gave rise to increasing levels of propionate production. Its impact was also slower than the immediate impacts of sophorolipids and rhamnolipids. With regards to the motilty of the microbial community, the PICRUSt analysis indicated strongly increased levels of motility genes. All of these results are confirming earlier findings by B. Chassaing. Finally, clear differences were noted in the response of the different donors to the emulsifiers.

keywords: Gut microbiota, Dietary Emulsifiers, Non-communicable diseases, Interindividual variability, Microbiome composition, Butyrate, Flagellin

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Effects of bile acid dysmetabolism on intestinal health in an inflamed in vitro model of Caco-2 and HT29-MTX-E12 cells

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Abstract
Inflammatory bowel disease (IBD) comprises a set of disorders that causes chronic and relapsing inflammation of the gastrointestinal tract. The gut microbiota are thought to play a major role in the onset and progression of IBD. Several studies have shown that microbial dysbiosis alters the production of microbial metabolites, such as bile acids (BAs). It was previously shown that IBD patients had a lower abundance of secondary BAs and an increase in primary BAs and conjugated BAs as compared to healthy subjects. Since these BAs are involved in several inflammatory signalling pathways, BA dysmetabolism could cause perturbations in immune responses and ultimately to aggravation of IBD progression. BA dysmetabolism during IBD is also characterized by a decreased desulfation-activity, leading to an increased abundance of sulfated BAs. Sulfation is an important modification to detoxify and eliminate BAs. However, the exact effects of an accumulation of sulfated BAs on IBD progression are not widely investigated yet. Therefore, the aim of this study is to investigate the effects of sulfated BAs on the progression of IBD. We will use an in vitro model (Caco-2 and HT29-MTX-E12 cells). In order to simulate a situation of active IBD, the models will be exposed to activated THP-1 cells to induce a diseased state. After treatment with sulfated BAs, the effects on intestinal permeability and immune response will be investigated. Besides, we will also focus on mucus production and composition. Additionally, underlying molecular mechanisms will be investigated to unravel pathways that are affected by sulfated BAs. New insights into the role of BA dysmetabolism in IBD could contribute to the discovery of novel therapies that add to the prevention of IBD.

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Untargeted metabolomics reveals elevated levels of L-carnitine and derived metabolites in the HT29 colon cancer cell line, pig and rat colon tissue samples upon red meat intake

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The consumption of red as opposed to white meat has been associated with diabetes mellitus type II, cardiovascular disorders and particularly colorectal cancer (CRC). Thus, it is of paramount importance to investigate the compositional differences between red and white meat as well as the metabolic products that result from their digestion, allowing to gain more insights in the pathogenesis of the abovementioned diseases. Whereas the nutritional composition of red and white meat has already been analyzed in detail, gastro-intestinal end product formation and absorption remain unexplored. Yet, exactly the latter is crucial for understanding the potential contribution of red meat to disease development. Therefore, we set out to elucidate red meat-associated mechanisms, initiated in colon cells upon gastro-intestinal digestion and absorption of meat, by applying a validated mass spectrometry-based polar metabolomics and lipidomics fingerprinting method. Owing to its untargeted and unbiased nature, this workflow allows pinpointing novel molecules associated with red meat intake and linking gastrointestinal metabolites with metabolic profiles of colon cells.
To do so, both *in vitro* and *in vivo* experiments were performed, whereby the cancer cell line HT29 was incubated for 24h with human *in vitro* colon digests (obtained from three human volunteers) of beef or chicken meat, whereas rats (n = 20) and pigs (n = 32) were fed a red or white meat-based diet. More specifically, pig's feed consisted of red/processed or white meat in addition to a western or recommended background diet that were representative for average human dietary intake.
Red meat intake was specifically associated with increased levels of L-carnitine, acylcarnitines and 3-dehydroxycarnitine in gastro-intestinal colon digests, intracellular extracts and colon tissue. Also, trimethylamine-N-oxide (TMAO) was elevated upon red meat intake in rat colon tissue. *In vitro* colon digestions with L-carnitine have demonstrated that 3-dehydroxycarnitine, which is much more abundant in red meat as compared to white meat, can be transformed into 3-dehydroxycarnitine and trimethylamine. In the liver, the latter is metabolized into TMAO that has been associated with atherosclerotic plaque formation, inflammation and oxidative stress.
To conclude, by linking gastro-intestinal with colon cell metabolic profiles using a comprehensive metabolic fingerprinting approach, we have identified new red-meat associated metabolites that may have relevance to chronic diseases and could serve as novel biomarkers and/or nutri-/therapeutic targets.

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Interleukin-28A Induces Epithelial Barrier Dysfunction in IBD Patient-derived Intestinal Organoids

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Background: Intestinal barrier is recently designated as another hallmark of IBD pathogenesis. Interleukin-28A (IL-28A) is a newly identified member of the IL-10/interferon cytokine family, with its most implicated function being antiviral and anti-proliferative properties. However, the role and underlying mechanisms of IL-28A in the regulation of epithelial barrier in IBD remain so far unexplored. Methods: Levels of IL-28A were measured in the plasma of 11 healthy subjects, 15 active CD, 12 active UC, 14 inactive CD and 13 inactive UC patients using ELISA assay. 3D intestinal organoids were generated from proximal colon biopsies of 7 inactive CD patients, characterized by the expression of differentiation gene markers using qPCR or immunofluorescence staining. Intestinal organoids were exposed to TNF-α, IFN-γ and IL-1β (20 ng/mL) or LPS (100 ng/mL), or IL-28A (500 ng/mL) for 24 h with or without GLPG0634. Epithelial permeability was assessed by the flux of FITC-D4 from the basal to luminal compartment. Expression of IL-28A, IL-28AR, IL-10R2 and junctional components were analyzed by qRT-PCR, immunofluorescence staining or western blotting. JAK-STAT pathway activity were analyzed using western blotting. Results: Plasma levels of IL-28A were significantly increased in active CD and UC patients when compared to healthy subjects. IL-28A and its receptor complex IL-28AR/IL-10R2 were detected in CD patient-derived intestinal organoids and showed a selective response to the stimulation of IFN-γ. IL-28A triggered epithelial barrier disruption, accompanied by reduced expression of ZO-1 and E-Cadherin. This effect was mediated by the activation of JAK-STAT1 signaling. Pre-incubation with the JAK1 inhibitor GLPG0634 ameliorated the barrier dysfunction induced by IL-28A. Conclusion: These results identified cytokine IL-28A as a novel contributor to the pathogenesis of IBD through converging epithelial barrier function, and could be a putative target for IBD treatment. We also provide new basic evidence that restoring intestinal barrier is a potential mechanism that contributes to the clinical benefits of JAK1 inhibitor in CD patients.

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Effect Of Ultra-Filtration After *In Vitro* Digestion Of Sustainable Food Proteins On SCFA Production By Microbiota Fermentation*

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Introduction: Proteins from different sources will be degraded differently in the GI-tract. This influences the absorption of the degradation products in the GIT. During passage along the small intestine, amino acids and peptides from highly digested protein will be absorbed by the intestinal cells and will not reach the colon. However, proteins that are not or partly hydrolysed will pass into the colon and serve as a substrate for proteolytic activity or fermentation by its microbiota.
Objective: Analyse the effect of ultra-filtration (UF) on the SCFA production by microbiota compared to fermentation of whole digests.
Methodology: Animal- and plant/fungal-derived proteins were *in vitro* digested according to an adapted INFOGEST static consensus protocol[1]. The digests were ultra-filtrated using a disk membrane with 1 kDa cut off. The retentate was washed 3 times with buffer after which it was used for mini-fermentation experiments with human microbiota. UF retentate and whole digestions were fermented by the same distal colon microbiota, stabilised by the SHIME system. During the fermentation pressure, SCFA and pH were measured.
Results: Differences in degree of hydrolysis comparing the different sources were observed as well as differences in protein amounts that were retained after UF in the retentate. There were no differences observed in SCFA between plant/fungal and animal derived sources using the whole digest. After UF, microbiota produced more butyrate from animal than from plant/fungal sources. The production of total SCFA was higher in whole digests used compared to UF, although this varied among individual SCFA. Comparing whole digest and retentate from the plant dataset the acetic acid, butyric and iso-valeric levels were significant lower in the retentate. In the animal dataset iso-valeric acid is lower in the retentate.
Conclusion: UF has an effect on SCFA production. Whole digests give an overestimation of the total SCFA production.

Key words: *in vitro* digestion, microbiota, sustainable food proteins

[1] Minekus, M., et al. (2014). Food Funct 5(6): 1113-1124.
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Development of a long-term ileum simulation model using the SHIME® technology

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The small intestinal microbiota has a large potential to influence human health. While 70% of immune cells are situated in this area, which is also the first site where ingested compounds encounter a dense microbiome), research regarding the human gut microbiome has mainly been focusing on the large intestine. A key reason is the inaccessibility of this region in vivo together with a lack of decent in vitro simulations. This study therefore aimed to develop and validate a dynamic, semi-continuous, long-term ileum simulation using the SHIME® (Simulator of Human Intestinal Microbial Ecosystem) technology. After determining relevant physiological conditions and the key colonizers of the ileum, a synthetic consortium was subjected to a multitude of nutritional and environmental conditions for a period of 10 days to identify the specific conditions that allowed the colonization of a stable microbiota with representative composition and metabolic activity. In correspondence with literature data, a specific condition was identified that resulted in the colonization of a stable microbiota mainly comprised of Streptococcaceae, Veillonellaceae, Enterococcaceae and Lactobacillaceae (as detected via 16S-targeted Illumina sequencing) at cell densities that were considerably lower versus those obtained in colonic simulations incubations (as detected via flow cytometry). Upon nutrient administration, this community initially produced high levels of lactate that was subsequently crossfed towards acetate and/or propionate, likely driven by Veillonellaceae. With respect to bile salt metabolism, the ileal microbiota was primarily able to contribute to the initial conversions, i.e. bile salt deconjugation. As proof-of-concept studies, an ileal pathogen (adhesive-invasive Escherichia coli (AIEC)), and a golden standard probiotic (Lactobacillus rhamnosus GG (LGG)), were shown to effectively colonize the ileal community. Finally, an intriguing observation was that upon integration of the ileal compartment in the SHIME model, the preceding ileal microbiota beneficially affected the colon microbiota. While the SHIME model is well-validated, it has been shown that, like for other in vitro models, two key groups are typically depleted in vitro (Lachnospiraceae and Ruminococcaceae), while two others are typically enriched in vitro (Bacteroidetes and Veillonellaceae). While inclusion of a simulation of the mucosal microbiota (M-SHIME) has previously proved to balance Lachnospiraceae and Bacteroidetes levels, inclusion of the ileal microbiota during the current study strong enriched Ruminococcaceae over Veilllonellaceae, so that overall, the colon microbiota in the SHIME model approached the in vivo situation much better. Overall, it can thus be concluded that while the novel ileal microbiota simulation has great potential to provide a deeper understanding in the behavior of ingested compounds in the small intestine as such, its integration in models that simulate the entire gastro-intestinal tract might result in more representative overall in vitro simulations.

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A human small intestine organoid model to study the translocation of *Streptococcus suis* in the human small intestine

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Streptococcus suis is a zoonotic pathogen that can cause septic shock and meningitis in pigs and humans. Human infections are mainly caused by *S. suis* serotype 2, while many other serotypes can be found in pigs. In humans, the consumption of undercooked or raw pig products contaminated with *S. suis* can lead to systemic infections, implying that the gastrointestinal tract is a potential entry point for the pathogen. We previously used Caco-2 cells as a model for the human gut epithelium and found that the ability to translocate through the Caco-2 cell monolayer correlated with the *S. suis* genotype, in which *S. suis* clonal complex 1 translocated better than clonal complex 16 or 20. To further advance our research and gain novel insights in the host-pathogen interaction, we developed a two-dimensional human organoid derived small intestinal epithelium model. The epithelial monolayer represents the intestinal epithelium as it is polarized, has tight and adherent junctions and consist out of enterocytes, paneth cells, goblet cells, enteroendocrine cells and stem cells. We show that *S. suis* strains from clonal complex 1, 16 and 20 are able to translocate across the epithelial monolayer, although there are differences between donors. All three tested clonal complexes induce an equal IL-8 response. A trend that *S. suis* clonal complex 1 is better in translocating than strains belonging to clonal complex 16 and 20 is observed, although all three able to translocate. Interestingly, *S. suis* can translocate without increasing monolayer permeability, suggesting a transcellular route of translocation. Taken together, we generated an intestinal epithelium model that appears suitable to unravel host-pathogen interactions leading to *S. suis* translocation in the human small intestine.

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MUC1 is a receptor for the *Salmonella* SiiE adhesin that enables apical invasion into enterocytes

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Abstract
The cellular invasion machinery of the enteric pathogen *Salmonella* consists of a type III secretion system (T3SS) with injectable virulence factors that induce uptake by macropinocytosis. *Salmonella* invasion at the apical surface of intestinal epithelial cells is inefficient, presumably because of a glycosylated barrier formed by transmembrane mucins that prevents T3SS contact with host cells. We observed that *Salmonella* is capable of apical invasion of intestinal epithelial cells that express the transmembrane mucin MUC1. Knockout of MUC1 in HT29-MTX cells or removal of MUC1 sialic acids by neuraminidase treatment reduced *Salmonella* apical invasion but did not affect lateral invasion that is not hampered by a defensive barrier. A *Salmonella* deletion strain lacking the SiiE giant adhesin was unable to invade intestinal epithelial cells through MUC1. SiiE-positive *Salmonella* closely associated with the MUC1 layer at the apical surface, but invaded *Salmonella* were negative for the adhesin. Our findings uncover that the transmembrane mucin MUC1 is required for *Salmonella* SiiE-mediated entry of enterocytes via the apical route.

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Dietary nutrient concentration acts as a driver of microbial load and diversity

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Until today, the majority of gut microbiome research has focused on the microbial community composition, often in relation to disease. A deeper understanding of the latter is lacking, while the existing data is mainly qualitative. As the quantity or 'microbial load' is important for the microbial activity, Quantitative Microbiome Profiling (QMP), combining flow cytometry and Illumina sequencing, is a novel approach to revisit outstanding research questions in the field of gut microbiota research. In this study, the effect of nutrient concentration as a driver of gut microbial load variation and diversity was examined.
The effects of altering nutrient concentration were evaluated in six different microbial backgrounds, derived from six different donors, sustained in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), *in vitro* gut model. The stabilized microbial communities were left for five days with an undiluted feed as a control period. Next, the concentration was gradually decreased in five day intervals to 33%, 20% and finally 10% of the initial feed concentration. The feeding pattern was kept constant throughout the experiment. Gas composition, metabolite production and cell density, as well as the microbial community composition were assessed daily.
Decreasing the nutrient concentration resulted in decreased microbial loads irrespective of the donor and colon region, while maintaining an equal ratio of living and dead cells. The metabolite production declined with a decreased nutrient concentration, with the slopes showing inter-individual variability. Surprisingly, flow cytometry fingerprinting portrayed an increasing alpha diversity with a reduced feed concentration in the living cells, with the gradient strongly dependent on the donor and colon regions. These results suggest that nutrient load acts as an important driver of microbial load, metabolic output and diversity.

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Modulation of intestinal health by food ingredients; Taking up the challenge for health benefit substantiation

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Objective:
Gut microbial colonization in early life runs in parallel with immune system maturation and plays a role in intestinal physiology and regulation. Aging on the other hand leads to alterations in gut microbial diversity, immunity and metabolism resulting in an increased susceptibility to infections and disease. Studies indicate that nutrition, including pre-, pro- and synbiotics, plays an important role in development and maintenance of a balanced gut environment.
Method:
The present study describes an extensive “Gut Health Discovery Platform (GHDP)” studying 3 main factors contributing to gut health: 1) The microbiome, including assays on anaerobic culturing, 16S sequencing, shotgun metagenomic and metabolite analysis, 2) The intestinal epithelial cells, including assays on anti-adhesion, barrier integrity, barrier enforcement and chemokine/ cytokine profiling, 3) The immune cells, including assays on immune responsiveness and cytokine profiling. The effects of a range of new oligosaccharides (OS) were studied in a part of the GHDP followed by integrative analysis.
Results: In the microcolon model, using infant faces as inoculum, specific OS inhibited, stimulated or were ineffective in modulating Bifidobacteria growth. Intestinal cell assays showed a differential capability for each of the tested OS in inhibiting E. coli adhesion and E. coli induced intestinal barrier disruption.
Conclusions:
Extensive in vitro screening will contribute to the knowledge and selection of present and new microbiome modulators. The present study shows that OS can be grouped based on different readouts, allowing to focus on a specific group, or the selection of a representative member from each group. Based on this knowledge the most suitable components can be identified and taken into clinical trials in the relevant target population or in healthy volunteer challenge models.

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Breast milk microbiome in the Lifelines NEXT cohort

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Lifelines NEXT is a birth cohort of the Northern Netherlands aiming to include 1,500 mother-father-infant trios. Biomaterials including faeces and breast milk and questionnaire data are collected during pregnancy and up to 1 year after birth. The Lifelines NEXT cohort aims to investigate the microbiome patterns of early life, how environmental, maternal and infant factors shape the microbiome and how the microbiome affects maternal and infant health. A pilot experiment with 30 mother-infant pairs in which the maternal and infant gut microbiome was analysed with metagenomic sequencing has been completed. Additionally, we are interested in studying the breast milk microbiome with shotgun metagenomics sequencing. However, milk metagenomic sequencing is challenging due to the presence of large numbers of host cells and small numbers of microbial cells. In this study we tested five DNA isolation strategies for their suitability for shotgun metagenomics sequencing of five human breast milk samples. We further used conventional cultivation to validate the results in two of the five samples.
Shotgun sequencing revealed a large variation in bacterial composition between samples and low bacterial diversity. Detected bacteria corresponded with those previously found in human breast milk samples using 16S rRNA sequencing. No significant differences in species richness and Shannon diversity indices were observed between the different DNA isolation methods. In a principal coordinate analysis, samples clustered by donor and not by isolation method, indicating that the results were independent of the DNA extraction method. Although one DNA isolation method had a significantly higher percentage of microbial reads compared to the other methods, only 4–8% of all high-quality reads were of microbial origin. The concordance of results from shotgun sequencing and from cultures was low and did not differ between DNA extraction strategies.
In line with literature, our study finds high inter-individual variability and low microbial content in breast milk. One DNA isolation method performed slightly better than the other tested methods. Nevertheless, further endeavours are needed to improve the quality of sequencing results from milk samples.

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Enterohepatic cooperation in the establishment of the neonatal gut microbiota

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Following birth, the neonatal enteric mucosa becomes exposed to maternal and environmental bacteria that rapidly evolve into a dense and highly dynamic microbiota. Concomitantly, the liver undergoes functional transition from a hematopoietic organ to a central organ of metabolic regulation and immune surveillance. These age-dependent changes are expected to significantly influence the environmental conditions within the intestinal lumen and might thus exert a major influence on early bacterial colonization. Aim of the present study is to analyse the influence of the developing hepatic function on the early enteric microbiota.
We used a combination of transcriptomic and metabolomic as well as microbial profiling combined with metagenomic prediction to characterize the complex site-specific host-microbial interplay and colonization dynamics in the neonate murine intestine.
We observed major age-dependent microbial and metabolic changes. Multivariate analysis identified sugars and bile acids as potent drivers of the early enteric microbiota development. Consistently, increased hepatic expression of bile acid synthesis genes were accompanied by an increase in bacteria capable to deconjugating bile salts. To proof causality, subsequent analysis by oral administration of tauro-muricholic acid-beta, ursodeoxycholic acid or cholic acid to newborn mice confirmed their role in postnatal microbiota maturation.
In conclusion, our results provide insight into the development of the early enteric microbiota and identify bile acids as host factors that drive microbiota maturation.

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Bowel movement: Identifying fibre-functional entities within the intestinal microbiota.

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Functional constipation (FC) in children is a common gastrointestinal (GI) disorder with a worldwide prevalence ranging from 0.7% to 29.6%. Complaints include infrequent bowel movement, painful defecation and abdominal pain, which all have a big impact on the quality of life. Prebiotic fibres have been shown to relive constipation symptoms in young adults and elderly. However, sufficient evidence is lacking linking additional fibre intake to improve symptoms in children with FC. Moreover, the gut microbiota composition, which is highly influenced by dietary intake, has been correlated with various diseases and has become an important therapeutic target. Several studies with prebiotic fibres but also with faecal microbiota transplants in adults imply a role for the microbiota and modulation thereof in constipated patients. Taking both physiological and microbiota changing properties of these prebiotic fibres into account, we hypothesize that representative prebiotic fibres might be able to relief symptoms of constipation in children as well. Therefore a placebo-controlled randomized double-blind trial will be conducted in children with FC of 1-3 years old. Outcomes will include parameters for bowel habit, quality of life, and potentially some gut microbiome related outcomes.

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Intestinal archaea inversely associated with childhood asthma

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Background:
Methanogenic archaea are a key part of the gut microbiota alongside bacteria. However there is comparatively little research on the role of archaea in health.
Objective:
As in-vitro and animal experiments have demonstrated immunological effects of archaea, we hypothesised that intestinal exposure to archaeal species would influence the risk of asthma and other allergic diseases. We present the first human study connecting gut archaea with childhood asthma.
Methods:
We performed a cross-sectional analysis nested within the Dutch KOALA Birth Cohort Study. DNA from two common intestinal archaeal species, *Methanosphaera stadtmanae* and *Methanobrevibacter smithii*, was quantified in faecal samples from 472 children at school age, using qPCR.
Our primary outcome was parent-reported asthma at 6-10 years. Secondary outcomes were questionnaire-reported eczema, total serum IgE levels, sensitisation to aero- and food-allergens and lung function (FEV1/FVC).
Associations between the presence/absence of each archaeal species and outcome were assessed with logistic or linear regression models, adjusted for potential confounders.
Results:
Presence of *M. stadtmanae* was significantly associated with a lower risk of asthma, adjusted OR 0.32 (0.08 – 0.98). In addition, asthma risk decreased monotonically across three categories of increasing *M. stadtmanae* abundance (adjusted p-for-trend = 0.035). We also observed a non-significant tendency for less eczema and IgE sensitisation amongst children with *M. stadtmanae*. *M. smithii* was not associated with any outcome.
Conclusion:
Further longitudinal and experimental research is needed to explore whether archaea could be directly linked to asthma risk, or if archaeal abundance is indicative of other health-relevant variation in microbiota composition.

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Can early gut microbiota composition predict executive functioning in childhood?

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Animal models suggest that the gut microbiota can influence cognitive development and functioning. A first human study found associations between infant gut microbiota composition and cognition at 2 years of age. We investigated whether gut microbiota composition in infancy and at 6 years of life is associated with executive functioning (EF) at 8 and 10 years of age. EF was assessed using the Behavior Rating Inventory for executive functioning (BRIEF) and the Digit Span memory test. We analyzed the data using Partitioning around Mediods, Extremely Randomized Trees and Bayesian Robust Linear Models (LM). There was no clustering of microbial samples. We found minor evidence for an association between microbial composition and EF using Bayesian LMs and tree based methods warranting future investigations. These should include the assessment of the functionality of the gut microbial ecosystem.

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Sweeteners and sweetness enhancers: long-term effects on the gut microbiota and metabolic health

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Energy overconsumption is suggested to be the main dietary factor driving the obesity epidemic. As a key nutritional component, sugar contributes to the overall energy density of diets, and may therefore promote overeating and a positive energy balance. Dietary sugars can be replaced by non-caloric sweeteners and sweetness enhancers (S&SE). However, the safety and efficacy of these components have recently been topic of scientific and public debates and concerns. In this multicentre project, we aim to investigate the effects of combined and prolonged S&SE use –as part of a healthy diet– on body weight control (primary outcome), gut-microbial composition, satiety signalling and metabolic disease (including liver fat) in overweight adults and children. A total of 330 overweight adults (18-65 years of age) and 330 children (6-12 years of age) will be recruited for the study. Families (consisting of at least one adult and one child) are randomized into two different dietary intervention groups and will follow a healthy diet either with or without S&SE containing foods and beverages. This intervention will last for 22 months, and during the first year of the study faeces samples of both adults and children will be collected at baseline and after 2, 6, and 12 months to assess the effect of S&SE use on gut-microbial composition. Furthermore, the effects of S&SE use on anthropometry (body weight, body composition), satiety signalling (gut-brain signalling markers such as GLP-1 and PYY, brain-reward response by fMRI), glucose homeostasis (oral glucose tolerance tests), liver fat (by ^{1}H-MRS) and adipose tissue function (i.e. markers of adipogenesis in adipose tissue biopsies) will be assessed. We hypothesize that S&SE use as part of a healthy diet leads to improved body weight control compared to a healthy diet without consumption of S&SE products. Additionally, we expect that there will be no safety concerns using S&SE on the long-term regarding gut-microbial composition and related metabolic health parameters.

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Development of the gut microbiome in early life: rationale and design of a prospective cohort study within the LucKi Birth Cohort in the Netherlands

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Early life exposures may alter the course of gut microbial colonization leading to differences in metabolic and immune regulation throughout life. The aim of this study is to examine the dynamics of the gut microbiome composition and its determinants in the first 14 months of life, and to link the infant microbiome composition to the subsequent development of (over)weight, asthma and allergies. This study is embedded in the ongoing prospective observational LucKi Birth Cohort in The Netherlands.
A prospective cohort of 300 mother-infant pairs is being established by enrolling pregnant woman from midwifery practices and hospitals in South Limburg, the Netherlands. Eligibility includes low-risk women, planning a vaginal birth and able to communicate in Dutch. Women are excluded if they have a multiple pregnancy or a preterm birth (< 37 weeks). Study questionnaires and fecal samples of the infant are collected at ages 1 to 2, 4, 8 weeks, 4, 5, 6, 9, 11 and 14 months. In addition, one maternal fecal sample is collected. The microbial composition of fecal samples will be analyzed by next-generation sequencing and linked to perinatal determinants, diet, medication use, life style factors and disease outcome.
Results from this study will contribute to our knowledge on the gut microbiome colonization in early life and its influence on the future development of overweight, asthma and allergy. Eventually, this may have implications for best practices to support the establishment of the microbiome at birth.

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Optimization of fecal NMR-based metabolomics to study the developing infant gut

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Background. While substantial efforts have been made to optimize and standardize fecal metabolomics for studies in adults[1], the development of a standard protocol to analyze infant feces is still lagging behind. Research of the infant fecal metabolome is gaining interest since it contributes to our functional understanding of the complex diet-microbiota interactions in the gastrointestinal tract, and its impact on host health[2]. We therefore aimed to develop and implement a robust pipeline for ^{1}H NMR metabolic profiling of infant feces. This pipeline will subsequently be applied to quantify changes in the metabolic profile of the developing infant gut.

Methods. Fecal samples were collected from seven infants born at term as part of the ongoing Lucki Gut Study in the Netherlands. Samples were subjected to different preparation conditions in order to examine the impact of extraction solvent, dilution ratio, homogenization method, filtration and duration of centrifugation on the metabolite profile. The reference sampling protocol was developed based upon previous literature[3,4], and consisted of extracting the fecal sample with two volumes of UPLC-grade water, vortex shaking for 20 minutes at 4°C and final centrifugation at 16000g for 30 minutes at 4°C. Fecal samples from seven infants were collected at 8 weeks, 4 and 9 months postpartum, and analyzed using ^{1}H NMR spectroscopy. Spectral preprocessing and comparisons of the spectra were performed using Mathworks Matlab R2018a (The Mathworks Inc., USA) and Bruker TopSpin 4.0. Multivariate statistical modeling was performed using Umetrics SIMCA 15 (Umetrics, Sweden).

Results. Simplicity in sample preparation and maximum achievable NMR spectral resolution appear optimal in samples that were diluted 1:5 as compared to 1:2 and 1:10 ratios, respectively, irrespective of the extraction solvent used. Visual inspection of the ^{1}H NMR spectra shows that overall metabolite concentrations are higher in samples diluted in UPLC-grade water as compared to samples extracted with phosphate buffer. Sample homogenization by bead beating and final centrifugation for 30 minutes was further preferred in order to obtain the highest relative concentration of short-chain fatty acids and the maximal signal-to-noise ratio. The individual fecal metabolomes of infants aged 8 weeks resembles that of 4 months but is clearly different from the metabolome at 9 months of age. The profound influence of diet on the fecal metabolome likely explains this change, with mostly milk oligosaccharides derivatives (i.e. fucose, galactose, fructose and glucose) present in 8 weeks and 4 months samples, in contrast to the 9 months samples that mainly contained elevated levels of short-chain fatty acids (i.e. butyrate and acetate). A large inter-individual variability exists between the infants' fecal metabolomes at time points 8 weeks and 4 months of age, however, metabolomes at age 9 months are more similar.

Conclusions. We propose an optimized pipeline for ^{1}H NMR spectroscopic analysis of infant feces to ensure robust data generation and to facilitate inter-laboratory comparison of infants metabolic profiles. Our results show that the fecal metabolome of infants undergoes dynamic, rapid and large intra-individual shifts during the first year of life, especially after transition from milk to solid foods. The application of NMR-based metabolomics in microbiome research may pave a way to the development of new monitoring tools for studying the impact of nutrition on the infant gut microbiome and its functional status.

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Gut metabotyping of obese and overweight children towards early prevention and prognosis of metabolic diseases

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During the past decade, obesity has surfaced as one of the biggest global health problems today. The enormous number of obese adults has already reached over 650 million and while obesity rates continue to rise, Europe's adult population is forecasted to be overweight or obese by the next decade. Besides adults, also children are increasingly facing the obesity pandemic. Due to such emerging early onset of excessive weight gain, long-term health care and (socio)economic burden imposed on both patients and the community is forecasted to mount tremendously. In this respect, effective interventions and preventions are of crucial importance, yet have been associated with enormous relapse. Pediatric obesity might anticipate the onset of impaired fasting glucose and/or impaired glucose tolerance to teen age and full-blown DMT2 to early adulthood, whereby waiving traditional paradigms including DMT2 as adult-onset diabetes. Regarding the dysregulated glucose metabolism involved in obesity, insulin resistance (IR) plays a key role since it already rises years before pubertal onset. At current, internationally agreed standards for (ab)normal insulin sensitivity during childhood are lacking, hence the urging quest for diagnostic, prognostic and/or predictive biomarkers for metabolic diseases in pre-pubertal youth as to hinder long-term (cardio)metabolic complications impacting healthy growth and functionality. Metabolomics reveals a comprehensive metabolic signature compared to reluctant blood analyses. In this regard, attention is emerging towards fingerprinting of stool, in that it unravels the symbiotic interplay between the host, diet and microbiota characterizing responsive metabolic discrepancies regarding (patho)physiology. Therefore, this research provisions a holistic top-down approach mapping the faecal metabolome of overweight and obese children as to stratify the different metabotypes in parallel with the correlation to clinical parameters and laboratory findings. Preliminary proof of concept data enabled faecal fingerprinting (UHPLC-HRMS) of obese children (n = 9; healthy, n = 9, children aged 6-16 years) and demonstrated valid sample classification and thus metabolome discrepancies (OPLS-DA model performance parameters: $R^2(Y)$ 0.996, $Q^2(Y)$ 0.902, p-value $6.9e^{-4}$). Recent advances in the era of ambient ionization HRMS introduced Rapid Evaporative Ionization Mass Spectrometry (REIMS), which has been applied *in vivo* during surgical interventions. The REIMS hyphenated with the laser ablation (LA) technique significantly reduces time (<10s) and workload (minimal to no sample preparation required), whereby enabling simple routine analysis of the 'intact' faecal metabolome. In this context, LA-REIMS fingerprinting offers unique opportunities for healthcare practice as on-site pediatric screening could manifest and hence fostering diagnosis of disease and selection of personalised treatment as early as possible. This multi-centric study will primarily focus on elucidating discriminative metabolites in the pediatric cohorts. Secondly, the candidate biomarkers' predictive and prognostic value towards the development and progression of IR and concurrent metabolic disorders will be qualified during a longitudinal follow-up period of 2 years. Within the worldwide epidemic of obesity, this study offers compelling perspectives in providing hallmarks of phenotypes at high risk for the development of metabolic diseases allowing its early prevention and progression by means of patient-centered intervention.

Keywords: metabolomics, lipidomics, faecal fingerprinting, ambient mass spectrometry, childhood obesity, insulin resistance, metabolic diseases, personalised interventions.

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EARLY LIFE EFFECTS OF HUMAN MILK OLIGOSACCHARIDES AND PREBIOTICS ON ANTIBIOTIC ASSOCIATED GUT MICROBIOTA CHANGES AND HEALTH

M.F. Endika, K. Venema, H. Smidt

Abstract
The microbial colonization of infant gastrointestinal tract plays a key role in human health. Consequently, disruptions during this development of the infant gut microbiota have been reported to increase susceptibility to disease later in life. In early infancy, antibiotic use by the infant is one of the important external factors that influences the gut microbiota composition, associated with decreased numbers of bifidobacteria and Bacteroides. Therefore, promoting specific early feeding practices, such as breastfeeding and supplementation with prebiotics, could be seen as an opportunity to steer the disturbed microbial community towards a more beneficial and resilient state. In this study, TNO large-intestinal model (TIM-2) will be used to examine to what extent the intake of specific prebiotics, before and during antibiotic administration, can improve the resilience of infant gut microbiota. The type of prebiotic will be selected based on the combination of human milk oligosaccharides (HMOs) and prebiotics from KOALA study, as well as novel prebiotics. Samples will be analysed for HMO/prebiotic utilization, microbiota activity (lactate, succinate, short chain fatty acid, branch chain fatty acid, and ammonia), microbiota composition (16S rRNA gene), and function (metatranscriptomics).

Keywords: prebiotics, human milk oligosaccharides, antibiotics, infants, gut microbiome

VEZEL study – Impact of a complex and mixed fibre product in a randomized placebo-controlled trial in prediabetic adults

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Background Prediabetes, defined as impaired fasting glucose, affects about one third of the adult population. Observational and clinical studies found that a higher intake of dietary fibres through vegetables, fruits and whole grains decreased incidence of type 2 diabetes mellitus and related metabolic risk factors. Therefore, increasing daily dietary fibre intake by means of a complex, mixed fibre product might be an attractive way to intervene prediabetes advancement.
Objective In this exploratory study we assessed the effect of a three-week dose of 30g/day dried chicory root on classical glucose homeostasis markers and intestinal health in subjects with elevated fasting glucose levels in the prediabetic range.
Methods Randomized parallel single-blinded placebo-controlled trial. Sixty men and women between 40 - 75 years, with fasting glucose between 5.0 - 5.6 mmol/L and diabetes risk score ≥ 9, or between 5.6 - 6.9 mmol/L (according to ADA) were randomly assigned to an intervention (n=30) or a control group (n=30). After a run-in period of two weeks with half of the treatment, subjects consumed three-weeks long on a daily basis dried, ground chicory roots (30g/day) or a placebo (Maltodextrin). Fasting blood glucose, fasting insulin, HOMA-ir, anthropometric measures and faecal short chain fatty acid (SCFA) levels were assessed at baseline (before the run-in) and after the intervention. Stool consistency and frequency were assessed weekly using the Bristol Stool Scale.
Results Fasting blood glucose, fasting insulin and HOMA-ir decreased slightly in the intervention group. However, none of the biomarkers' changes was statistically significant different from baseline and compared to the control group. Weight and waist circumference did not change in both groups. Chicory root fibre increased faecal SCFA levels significantly in the intervention group ($p=0.0406$). Chicory root fibre also improved stool consistency with an increase of 1.07 on the Bristol Stool Scale ($p = 0.0008$) and on stool frequency with an increase from 1.3 to 1.9 time per day ($p = 0.0002$) as compared to the placebo.
Conclusion Increased intake of a complex and mixed fibre product based on whole chicory roots showed a significant improvement of markers related to intestinal health, including increased faecal SCFA production, stool consistency and frequency. While a slight decrease of classical glucose homeostasis markers was observed in the prediabetic adults, these were not significant in this short term intervention. Further studies are ongoing on differentiating responders and non-responders as well as detailed analysis of the glucose response. Furthermore, longer intervention periods than the presently used 3 weeks may be considered.

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Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit

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Abstract
Longer colonic transit time and hard stools are associated with increased gut microbiota diversity. Here, we investigate to what extent quantitative measures of (segmental) colonic transit time were related to gut microbiota composition, microbial metabolites and gut-related parameters in a human cross-sectional study. Using radio-opaque markers, (segmental) colonic transit time (CTT) was measured in 48 lean/overweight participants with long colonic transit yet without constipation. Fecal microbiota composition was determined using 16S rRNA gene amplicon sequencing. Associations between GI transit (segmental CTT, stool frequency and consistency), microbiota diversity and composition, microbial metabolites (short-chain and branched chain fatty acids [SCFA, BCFA], breath hydrogen), habitual diet and gut-related host parameters (lipopolysaccharide-binding protein (LPB), fecal calprotectin) were investigated using univariate and multivariate approaches. Long descending i.e., distal colonic transit was associated with increased microbial alpha-diversity but not with stool consistency. Using unweighted and weighted UniFrac distance, microbiota variation was not related to (segmental) CTT but to demographics, diet, plasma LBP and fecal calprotectin. Bray Curtis dissimilarity related only to stool consistency. Rectosigmoid and descending colonic transit were negatively associated with fecal SCFA and plasma acetate, respectively. This study suggests that the distal colon transit may affect not only microbiota diversity but also microbial metabolism.

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Quantifying the impact of Human Leukocyte Antigen on the human gut microbiome

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Abstract
The gut microbiome is affected by a number of factors, including the innate and adaptive immune system. The major histocompatibility complex (MHC), or the human leukocyte antigen (HLA) in humans, performs an essential role in vertebrate immunity. HLA determines the specificity of T lymphocyte responses, including against the commensal bacteria present in the human gut. Thus, the adaptive immune response has been suggested to shape the composition of our microbiome. To identify measurable parameters that contribute to a predictive model of the human microbiome, we investigated the effect of HLA haplotype on the microbiome composition. We performed HLA typing and microbiota composition analyses on 3002 public human fecal metatranscriptomic datasets. Indeed, we found that (i) individuals with similar HLA types also had similar microbiomes, (ii) individuals with HLA molecules presenting similar peptides are also similar in their microbiota, and (iii) HLA homozygosity correlated with microbiome diversity, suggesting that diverse immune responses limit microbiome diversity. Our findings indicate that host HLA haplotype influences gut microbiome composition. Because the HLA haplotype is a readily measurable parameter of the human immune system, these results open the door to incorporating the immune system into predictive microbiome models.

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Microbiome GWAS meta-analysis: MiBioGen consortium initiative

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The gut microbiome composition is determined by numerous environmental and intrinsic factors related to the host. The initial genome-wide association studies on the microbiome composition published in recent years provided an evidence that host genome is one of these factors.
High inter-individual variability in the gut microbiome and its capacity to respond to environmental exposures requires large, multi-ethnic, population-based study designs to ensure sufficient statistical power for detecting genotype-microbe associations. To this end, we have established a collaborative initiative, MiBioGen, that joins 20 population-based cohorts comprising more than 18,000 samples for which both genetic (genome-wide genotyping platforms) and microbiome (16S rRNA gene taxonomic profiling) are available. We developed a standardized pipeline to harmonize the different platforms and methods used to generate the microbiome data, such as the method of faecal DNA purification and the 16S gene domain sequenced and confirmed that this pipeline successfully reduces the technical bias between the cohorts.
The preliminary consortium results of GWAS on the microbiome traits, including alpha diversity, presence and abundance of bacterial taxa, revealed 30 genomic loci significantly associated with microbiome composition. The majority of these associations are novel and were not covered by previous studies; in addition, we also replicated well-established microbe-SNP associations, such as the functional LCT-Bifidobacterium linkage.
To our knowledge, MiBioGen is the largest collaboration devoted to microbiome GWAS, and we aim to provide a solid background for further studies in a field of microbiome research.

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Disruptions of the intestinal microbiota are coupled with altered systemic cytokine production profiles in patients with community-acquired pneumonia

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Background: Preclinical studies have shown that the gut microbiota contributes to resistance against viral and bacterial pathogens in the lung. However, it remains unexplored whether intestinal microbiota composition and associated changes in microbe-derived metabolites contribute to an increased risk and altered outcome of community-acquired pneumonia (CAP) in humans.
Methods: In this observational cohort study we collected blood and rectal swabs of 116 CAP patients at hospital admission and 28 days following admission, as well as 51 age and sex matched volunteers without infectious symptoms. The gut bacterial microbiome was subsequently characterized by 16S rRNA sequencing. In addition, the inflammatory cytokine production capacity of monocytes was established using ex vivo stimulation assays, and associations with clinical outcome parameters were evaluated.
Results: The impact of CAP on the gut microbiota was profound, with a loss of alpha and beta diversity and drastic shifts in microbiota community composition, independently of prior antibiotic exposure. In addition, patients with CAP harbored significantly reduced obligate anaerobic bacterial taxa with important metabolic functions, such as the production of butyrate. Monocytes of CAP patients with low abundance of butyrate-producing bacteria produced significantly higher TNF-alpha and lower amounts of IL-10, which strongly correlated with elevated plasma CRP and procalcitonin levels. In addition, low abundance of these microbial communities in the gut was associated with an elongated duration of admission.
Conclusions: CAP is associated with a strong dysbiosis of the intestinal microbiota. Patients with a lower representation of butyrate-producing bacteria in the gut displayed an altered inflammatory cytokine production capacity, higher inflammatory plasma parameters, and delayed recovery to clinical stability, of which the underlying mechanisms remain to be established.

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Bacteroides fragilis is more prevalent in Crohn's disease exacerbations while strengthening the intestinal epithelial barrier in a strain-dependent manner

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Background: Crohn's disease (CD) is a chronic relapsing inflammatory gastro-intestinal disease with a high disease burden. Impaired intestinal integrity and microbial dysbiosis seem to play a role in the pathophysiology. Among others, Bacteroides fragilis has frequently been associated with CD. In addition, recombinant B. fragilis toxin (Bft) was found to disrupt the intestinal epithelial barrier in vitro. Furthermore, Ubiquitin was found as a potential virulence factor, acting on host immune response. This study therefore aims to investigate the role of B. fragilis and its virulence factors in the pathophysiology of CD, focusing on prevalence and its interaction with the intestinal epithelial barrier.
Methods: To investigate the presence of B. fragilis, B. fragilis toxin (Bft) and Ubiquitin, we selected 181 CD patients with active or remissive state from our extensive population-based IBD South Limburg cohort. Disease activity was determined by faecal calprotectin levels (<100 µg/g = remission; ≥250 µg/g = exacerbation) and faecal DNA was investigated by qPCR. Data were analysed using Chi-square test and logistic regression analysis.
To examine the impact of B. fragilis on the intestinal epithelial barrier, we subsequently cultured six B. fragilis strains with various genetic profiles of bft and ubiquitin. Differences in genome, proteome and metabolome were examined. Next, bacteria-free culture supernatant as well as outer membrane vesicles (OMVs) were isolated and luminally applied to colonic adenocarcinoma-derived Caco-2 cell monolayers. After 24 h incubation, the difference in transepithelial electrical resistance (TEER) was determined.
Results: B. fragilis prevalence was 15 % higher ($p<0.023$) in active CD patients compared to remission. Bft and ubiquitin prevalence was comparable in both groups. B. fragilis carriage was further associated with a stricturing disease course and less likely present in patients with previous intestinal resections.
Isolated OMVs of bft positive or bft negative strains did not show any alterations in TEER. Interestingly, the culture supernatant of bft positive B. fragilis strains increased the TEER up to 160 % ($p<0.001$) compared to bft negative strains, even in presence of TNF-α and IFN-γ, suggesting an improved epithelial integrity. However, genome, proteome and metabolome analyses could not identify a responsible factor for the observed TEER increase.
Conclusion: This study confirms in a large well-defined patient cohort that B. fragilis, but not bft or ubiquitin positive strains specifically, is more prevalent in active CD, suggesting that B. fragilis might play a role in exacerbations. Surprisingly, B. fragilis components did not impair the epithelial barrier and components of bft positive strains even improved intestinal barrier function, which warrants further investigation. This unexpected finding stresses the relevance of extending current research on the functional role of relevant microorganisms.

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Longitudinal analysis of the gut microbiome reveals dynamic changes in relation to medications and phenotypes

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Interaction between the human genome and gut microbiome is vital to human health. While the human genome is set at birth, the gut microbiome can undergo dynamic changes over the course of an individual's life. However, we still know little about temporal shifts in the human gut microbiome, nor about the causes and consequences of temporal shifts.

We performed a longitudinal analysis on the gut microbiome of 341 participants in the Dutch population-based cohort Lifelines-DEEP, each individual having metagenomics and deep phenotypic data at two time points (~ 5 years apart). Significant temporal changes in the gut microbiome were detected at the levels of microbial composition, function and antibiotic resistance. Abundance difference was observed for 40% of taxonomies, 60% of functional pathways and 40% of antibiotic resistance genes. Furthermore, genetic stability analysis at strain level has revealed several species are under high evolution rate, including gastrointestinal disease associated *Ruminococcus torques*. Notable, temporal changes were associated to individual phenotypic variation and lifestyle factors. For instance, the genetic variation in *Coprococcus sp ART55/1* strains was higher in individuals with larger changes in BMI, while *Streptococcus thermophilus* and *Bifidobacterium longum* showed higher mutation rates in proton pump inhibitor and non-steroidal anti-inflammatory users, as compared to non-users.

Our data show that the gut microbiome sees dynamic changes not only in microbial composition and functional profiles but also in microbial genetic variation and antibiotic resistance. Our findings yield novel insights into the gut microbiome's impact on the development of complex diseases and traits over time.

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Carbohydrate-induced resilience of the gut microbiota after exposure to antibiotics

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For years, antibiotics have been widely used to treat bacterial infections successfully. Antibiotics however also disturb commensal bacteria in the human gut, thereby also affecting host gastro-intestinal and metabolic health. The exact link between the two still remains unclear. Another emerging issue due to the misuse and overuse of antibiotics is the rise in antibiotic resistance among bacteria, rendering some antibiotics ineffective. One way to positively influence microbiome composition is via prebiotics, e.g. indigestible carbohydrates that can only be metabolized by the gut microbiota. The effect of both antibiotics and prebiotics on gut microbiota composition varies considerably between individuals, and the factors involved require further study.
The aim of this project is therefore to elucidate subject-specific impact of antibiotics on gut microbiota and its resilience level, and to study whether administration of non-digestible carbohydrates can improve resilience and metabolic health in subjects with slow microbiota recovery. In order to achieve these aims, microbiome composition and activity will be profiled first, specifically targeting known antibiotic resistance genes and opportunistic pathogens in samples from our previous study, where prediabetic individuals were treated with amoxicillin or vancomycin. Secondly, indigestible carbohydrates will be screened using in vitro fermentation models for their capacity to improve resilience of slowly recovering microbiotas. Lastly, based on the in vitro fermentation findings, a human intervention trial will be conducted. Healthy, overweight adults will receive, after one week of vancomycin treatment, the selected non-digestible supplement or placebo for eight weeks. After antibiotics treatment and eight-week follow-up, microbiome composition and activity, substrate metabolism, metabolic profile, inflammatory profile and whole-body insulin resistance will be determined.

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Gut Microbial Co-abundance Networks Identify Functional Hubs in Inflammatory Bowel Disease and Obesity

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ABSTRACT

The gut microbiome is an ecosystem that involves complex metabolic interactions. While our knowledge on microbial compositional level has grown substantially, less is known about microbial interactions in the context of human diseases. We therefore constructed and compared microbial co-abundance networks using 2,379 metagenomes from four human cohorts: two population-based cohorts, an obese cohort and an inflammatory bowel disease (IBD) cohort. We show that microbial co-abundance relationships are remarkably different depending on the host's physiological status, particularly for the pathway co-abundance network. We report 1,430 IBD-specific and 342 obesity-specific pathway co-abundance relationships that are significantly enriched compared to population-based cohorts (Fisher's test P_{OB} = $1.5x10^{-14}$, P_{IBD} = 0). Some network edges are highly interconnected to specific pathways that formed network hubs, pinpointing potential keystone pathways that may play important roles in functional dysbiosis in disease. Our study provides evidence that microbial dysbiosis in disease can be seen at microbial co-abundance level and identifies several disease-relevant pathway hubs that might represent potential therapeutic targets for disease prevention and treatment.

Keywords: gut microbiome, dysbiosis, networks, obesity, inflammatory bowel disease

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Carbohydrate-induced resilience of the gut microbiota after exposure to antibiotics

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Although antibiotics have drastically reduced infection-associated morbidity and mortality, their use is also associated with detrimental health effects through perturbations of the gut microbiota. The extension and degree of the impact of antibiotics on the microbial community does not only depend on the type of antibiotics, but also on individual microbiota composition and dietary intake, among other factors. The mechanisms underlying inter-individual variations in antibiotic-induced microbial perturbations and whether faster recovery of the microbiota can be induced by stimulation of specific microbial groups via non-digestible carbohydrates are largely unknown. Therefore, the aim of our research is to identify the subject-specific impact of antibiotics on the gut microbiota and its subsequent resilience level, and to study whether administration of non-digestible carbohydrates can improve resilience and metabolic health in subjects with slow microbiota recovery. To realize this, the first part of our study include analyses of the samples obtained from a previous human vancomycin and amoxicillin intervention trial to determine how these antibiotics induced individual-specific effects on microbiota composition and activity, host metabolism, and their resilience. The second part consists of *in vitro* experiments to test whether the supplementation of non-digestible carbohydrates can counteract the negative effects of vancomycin on the gut microbiota of healthy and pre-diabetic individuals. Finally, on the third part, we will conduct a human intervention trial to evaluate *in vivo* the efficacy of the interventions proposed and tested *in vitro*. Observations from parts 1 and 2 of this study will be presented and discussed.

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The Clinical Link between Human Intestinal Microbiota and Systemic Cancer Therapy

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Abstract
Clinical interest in the human intestinal microbiota has increased considerably. However, an overview of clinical studies investigating the link between the human intestinal microbiota and systemic cancer therapy is lacking. This systematic review summarizes all clinical studies describing the association between baseline intestinal microbiota and systemic cancer therapy outcome as well as therapy-related changes in intestinal microbiota composition. A systematic literature search was performed and provided 23 articles. There were strong indications for a close association between the intestinal microbiota and outcome of immunotherapy. Furthermore, the development of chemotherapy-induced infectious complications seemed to be associated with the baseline microbiota profile. Both chemotherapy and immunotherapy induced drastic changes in gut microbiota composition with possible consequences for treatment efficacy. Evidence in the field of hormonal therapy was very limited. Large heterogeneity concerning study design, study population, and methods used for analysis limited comparability and generalization of results. For the future, longitudinal studies investigating the predictive ability of baseline intestinal microbiota concerning treatment outcome and complications as well as the potential use of microbiota-modulating strategies in cancer patients are required. More knowledge in this field is likely to be of clinical benefit since modulation of the microbiota might support cancer therapy in the future.

Keywords
Human intestinal microbiota; systemic cancer therapy; chemotherapy; immunotherapy; hormonal therapy; clinical relevance; baseline microbiota sampling; longitudinal microbiota sampling; 16S rRNA gene sequencing; metagenomic sequencing

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Probiotic-induced changes in gut microbial composition relate to its buffering effect against the negative consequences of stress on cognitive performance.

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Probiotics affect emotional processing (including emotional memory and decision making), as well as reduce sensitivity for sad mood in humans. On top of this, we recently showed in healthy female volunteers that a 28-day multispecies probiotics intervention buffers against stress-induced decline in working memory performance. Moreover, probiotics reduce stress-related inflammatory cytokines and circulating cortisol levels in animal studies. The mechanisms by which probiotics influence behavior and neurobiological measures may include the gut microbiota. However, evidence for the involvement of the gut microbiome in the effect of probiotics on stress-related cognitive performance is lacking in humans. Here, we asked whether the relative abundance of the gut microbiome is altered by probiotics and whether these changes are associated with behavioral and neurobiological variation. We isolated bacterial DNA from feces donated by the volunteers before and after intervention and sequenced the 16S rRNA gene. Bacterial taxa were identified using the NG-tax pipeline. We assessed the effect of probiotics on gut microbial diversity measures using and composition using Mann-Whitney U tests. Subsequently, we selected the genera showing an intervention effect to assess their relation with behavior and neurobiological measures. This analysis resulted in seven genera with increased relative abundance after intervention. In general, these genera are plant degraders contributing to gut health. From the seven, relative abundance of Ruminococcacceae_UCG-003 positively correlated with the buffering effect on working memory performance ($r_s(27)= .565$, $p= .002$). That is, participants who's relative abundance of Ruminococcacceae_UCG-003 was increased after probiotics also had the strongest benefit of probiotics on working memory after stress. This effect was corrected for multiple comparisons (seven genera) and adjusted for relevant covariates. More importantly, this effect was not observed in the placebo group. Our results (in healthy volunteers) suggest that multispecies probiotics could exert a protective effect of cognitive performance (when measured under stress conditions) and that this effect is mediated by the gut microbial composition. Our findings can guide future research on the potential for probiotics in protecting cognitive performance through the gut microbiome in a healthy population.

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From reads to microbial analysis: towards a standardized pipeline for the gut 16S metagenetic analysis

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Introduction: Bacteria plays a vital role in human health, being involved in the immune responses and food digestion and contributing to the pathogenicity of various diseases. Several important aspects of bacteria are poorly understood, making the identification and monitoring microbial communities of utter importance to patient care. Recent developments in new high-throughput sequencing technologies have revolutionized the study of microorganisms without the need for culturing them in the lab, an approach often referred to as metagenomics. Such applications allow the high-throughput analysis of genetic material of most of the microbes present, without the need for culturing the bacteria first. Although it has nowadays been adopted in many projects, it is far from straightforward necessitating various bioinformatics optimization.
Objective: We aimed at optimizing and standardizing the analysis of 16S metagenomics data analysis with a final aim to develop a comprehensive pipeline from the raw reads up to a microbial diversity reporting.
Methods and applications: Therefore, different tools were developed to deal with chimera (Mysara, Saeys, *et al.*, 2015) and sequencing errors (Mysara, Leys, *et al.*, 2015; Mysara *et al.*, 2016), each of them found to outperform the other existing state-of-the-art tools. Additionally, a new method was introduced to bring closer correspondence between the number of microorganisms detected and the actual diversity within the samples (Mysara, Vandamme, *et al.*, 2017). A one stop-shop software, named OCToPUS, assembles these various algorithms, thereby leading to a highly accurate assessment of microbial diversity starting from the raw sequencing reads (Mysara, Njima, *et al.*, 2017). Lastly, we have been working on an interactive reporting system, that allow the user to interact with the output and draw statistically verified observation from the data. These tailored algorithms have already been successfully applied to assess complex microbial communities in a wide range of environments (Byloos *st al* 2018*,* Su *et al* 2019, Mijnendonckx *et al 2019*) including samples from humans, as in ANTICIPATE 1000-patient clinical trial (Berkell *et al,* in preparation), or animal studies (Charlotte *et al,* in preparation).
Conclusion: We believe that this work would help standardize and optimize the 16S metagenetic analysis and would maximize the information gained from this type of analysis.

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Evaluation of 16S rRNA gene microbial profiling for low biomass samples and proposal to determine validity of the obtained profiles

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Objective
While 16S rRNA gene profiling has become relatively mature, its use for low biomass samples, such as from human skin, is hampered due to their low DNA yield and the bias from contamination or technical artifacts. To that end, we evaluated the microbial profiling methodology for correct representation of the microbial community using low DNA mock communities. The PCR assay was furthered to monitor product formation, as a quality assessment step preceding sequencing, to identify samples for which profiles may not reflect the biomass derived microbial composition.
Method
The DNA mock community, including DNA from 8 bacterial strains, was used to prepare 10-fold serial dilutions ranging from 10^{1} to 10^{-5} ng/µl. Each dilution was used as template for triplicate amplification of the V3-V4 region of 16S rRNA gene. PCR reactions were performed using reagents with or without Sybr Green. Subsequently, PCR products were sequenced on the Illumina platform (on two different runs) followed by quality control and classification of the sequence reads.
Results & Conclusions
Pearson correlations between profiles from triplicate amplifications with DNA concentrations up to 10^{-3} ng/µl were higher than 0.99 and corresponded well to the theoretical mock community composition. This indicates a relatively high repeatability of the profiling methodology even for low DNA concentrations that cannot be accurately measured using fluorescence based technologies.
Although similarity between the microbial profiles and theoretical mock community composition seemed to depend on PCR template concentration for PCRs with Sybr Green, its use facilitated the identification of DNA samples for which the amplified DNA, while possible to be sequenced, would prove to incorrectly represent the microbial composition.

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Gluten-free diet influences the virome composition

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Background: Dietary preferences are known to influence the composition of the gut bacterial communities, however, the influence of a diet on the composition of bacteriophages inhabiting the human gut remains underexplored. In this study, we aimed to explore the influence of a gluten-free diet (GFD) on the gut virome and microbiome.
Methods: We studied changes in the gut virome of 11 healthy European volunteers who followed a GFD for four weeks. Three stool samples were collected from each participant: one at baseline, 1 during the GFD, and one after the washout period, making a total of 33 samples. We performed metagenomic sequencing of DNA from virus-like particles (VLP) and total nucleic acid (TNA). Metagenomic reads were assembled by metaSPAdes per sample; obtained scaffolds were assigned as viral if they were VirSorter positive, contained pVOGs, were circular or had hits to viral RefSeq.
Results: In total, 1903 out of 303,085 non-redundant contigs were assigned as viral, ranging from 10kbp to 641kbp. From the 1903 viruses, only 1.2% could be assigned taxonomy using ViralRefSeq. We observed an extremely high individual specificity of the virome: only 1 viral contig was shared amongst all individuals. In contrast to this, the virome composition in the same individuals at different time points was more stable: on average 33% of contigs were retained at 2 time points in the same individual. Timepoints before and after the GFD clustered together, while the virome during the GFD was significantly shifted. We observed that highly diverse viromes restore better than less diverse ones.
Conclusion: Individual gut virome is highly diverse across individuals. GFD influences the virome composition, these changes are reversible after returning to the baseline diet, and the restoration of the virome is dependent on the initial viral diversity.

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DEVELOPMENT OF A 3D-ORGANOID MODEL OF METHOTREXATE-INDUCED MUCOSITIS

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Introduction: Gastrointestinal mucositis is a side-effect of chemotherapy that causes significant gut toxicity. This results in clinical manifestations that affect the course of chemotherapy. Currently, there is a limited number of in vitro systems suitable to study the mucosal damage. Therefore, we aimed to validate a chemotherapeutics-induced model of mucositis using organoids grown in a 3D fashion.
Materials and Methods: Intestinal organoids derived from mouse ileum were grown for 7 days and incubated with different concentrations of methotrexate (MTX), ranging from 0-1000 ng/ml. Metabolic activity, citrulline levels and cytokine/chemokine production were measured to determine the optimal dosage and incubation time. To link the model to clinical practices, folinic acid (0.0005-50 µg/mL) was added in combination with MTX. To evaluate the effects of short-chain fatty acids in the organoid model, different concentrations of butyrate (0.25-2mM) were added for 96 hours.
Results: MTX (100-1000 ng/ml) treatment resulted in reduced cell metabolic activity and citrulline levels ($p<0.001$). However, recovery after 96 hours was only observed with a MTX dose of 100 ng/ml, showing that 100 ng/ml is the optimal concentration in this model. Folinic acid treatment was able to restore organoid function when applied simultaneously or/up to 24 hours after treatment. Simultaneous addition of 0.25-1mM butyrate showed a protective effect on MTX toxicity.
Conclusion: MTX causes significant organoid damage, which can be reverted upon removal of MTX. The protective effects of folinic acid suggest that the model is clinically relevant. Treatment with butyrate might be a valuable strategy for mucositis treatment.

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Development of a human *in vitro* co-culture model for the gut to investigate inflammation and the role of the microbiome during inflammatory bowel diseases

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Inflammatory bowel diseases (IBD) are chronic disorders with relapsing inflammation periods affecting the gastrointestinal tract. The etiology is not fully understood but the hypothesis states that an aberrant immune response against gut microbiota, with the involvement of host genetic background and environment, plays an important role. Nowadays, the incidence of IBD is increasing worldwide and to increase quality of life in patients, there is a need for effective preventive and therapeutic treatments to induce remission in the disease course. Current intestinal *in vitro* models with human cells either lack the complexity of the epithelial gut barrier and immune system or the inclusion of the gut microbiome. Therefore, we developed an intestinal *in vitro* co-culture model to investigate the role of the microbiome and its possible modulatory capacity in the IBD disease course. The model includes an extracellular matrix, intestinal epithelial, goblet and macrophage-like cells and is co-cultured with bacterial samples to assess epithelial barrier integrity and inflammatory responses. The model consists of a Transwell setup with basally the different human cell lines and apically biofilms derived from pure bacterial cultures and/or natural microbial communities from fecal samples. Mucus production by host cells in the complex model was found to be thicker (83.2 µm) than in epithelial monolayers (64.6 µm), both without bacterial exposure. Moreover, results showed that the model is responding to a biochemically induced inflammation of TNFα by an increased IL-8 production (467 pg/mL) compared to a monolayer of epithelial cells exposed to the same pro-inflammatory conditions (64 pg/mL). Addition of *Lactobacillus rhamnosus* GG (LGG) to the model with human cells in an inflammatory state, induced a reduction in IL-8 production (300 pg/mL), while this reduction was limited in the monolayer of epithelial cells (47 pg/mL). Analysis of the effect of LGG on a biologically induced inflammation, coming from a complex microbial background (fecal sample) is still ongoing. Future research will cover a broader readout of human gene expression based on microarrays. Also, the model is amenable to *in vivo* samples from a disease background.

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Towards a microbiota-on-a-chip: Establishing a mucus-dependent cross-feeding network on a gut-on-a-chip

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Introduction
The colonic mucus layer lining the intestinal epithelium provides a nutrient source for commensal bacteria. A disrupted balance between microbial mucus degradation and production by the host has been associated with colitis, colorectal cancer and infection. To study host-microbe interactions at the mucosal layer, we currently rely on animal models, which are costly and poorly supported by society.
Approach
We aimed to develop a gut-on-a-chip in which intestinal microbes can be studied in an environment mimicking the human gut. We started by culturing mucus-producing human cell lines on a chip and analyzing metabolites in static versus dynamic conditions.
Results
HT29-MTX were seeded on a polycarbonate membrane in a gut-on-a-chip device. Static or dynamic culture conditions were applied by refreshing cell medium twice daily or, respectively, connecting the chip to a syringe pump in reverse at 60 µL/h. Outflow was collected after overnight incubation and analyzed by high-performance liquid chromatography, showing lactate production by the cells. Anaerobic conditions are required to obtain co-culture with intestinal bacteria, but the model could already be used to test the effect of bacterial products.
Discussion
The development of a gut-on-a-chip including a mucosal layer colonized by bacteria will increase our understanding of host-microbe interactions in health and disease, paving the way for therapeutic (pro- or prebiotic) targets in intestinal disorders. Eventually, this *in vitro* model may reduce the need for animal testing in biomedical research.

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A toolbox for the complete analysis of human intestinal content obtained by gastrointestinal sampling capsules

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Background: Detailed knowledge on the impact of dietary fibres inside the human intestinal tract is lacking. Access to this inner world of gut microbiota and fibres fermentation products such as short chain fatty acids (SCFAs) is now possible through novel human gastrointestinal capsules that sample lumen content in a non-invasive way. Due to the novelty of such devices, no methodology has been published to stabilize and analyse the resulting intestinal samples. Excretion of the capsule from the body may take days. Therefore a stabilisation reagent should be pre-loaded in the capsule to obtain a representative sample. Considering the small volume of recovered sample (200 µL), analytical methods must be optimized to collect as many information as possible from little material. We present a complete workflow for stabilising and analysing the fermentation of nutritional fibres in such samples, including microbiota composition, fibre composition, and fermentation products.
Methods: Fibre fermentation was mimicked through *in vitro* batch fermentations of dietary fibres (inulin, fructo- and galacto oligosaccharides) by human small intestine and faeces bacteria. Either control (Phosphate Buffered Saline) or the developed stabilizing reagent was added at the start. Incubations took place at 37°C for 48 hours, to mimic the situation in the capsule inside the gut. The remaining fibres (HPAEC-PAD), bacterial metabolites (GC-MS), and microbiota composition (16S rRNA sequencing) were measured at 0 and 48 hours.
Results: The SCFAs concentrations and the remainder of dietary fibres (galacto- and fructo-oligosaccharides/inulin) remained stable when human intestinal bacteria were incubated in the presence of stabilisation reagent for 48 hours. The microbiota composition at 48 hour was similar to the composition at start (Pearson Correlation>0.92) in the presence of stabilisation reagent. In contrast, in the controls fibres were broken down and SCFAs were produced by human intestinal bacteria. The sample extraction procedures were optimized, to measure in the same sample in one aliquot (100 µL) microbiota composition, and in the other aliquot (100 µL) in the organic phase the SCFAs, and in the aqueous phase dietary fibres.
Conclusions: Novel gastrointestinal sampling capsules aim to have a deeper understanding on processes taking place inside the human intestinal lumen. Our stabilisation reagent will provide a representative sample from the sampling location. Seen the small volumes of obtained samples and costs of these gastrointestinal sampling capsules, the developed combined protocol for analysing fibre substrates and fermentation products will be a major advantage for future gut health research.
Keywords: gastrointestinal sampling capsules, intestine, dietary fibres, microbiota, human
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Analytical methods to measure kinetics of fermentation of non-digestible carbohydrates inside the human gut using novel gastrointestinal sampling capsules

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Consumption of non-digestible carbohydrates (NDC) has been linked to many health benefits. However, detailed knowledge of the exact fate and impact of NDC in the intestinal tract is lacking. Gut microbiota use NDC as substrates to produce short chain fatty acids (SCFAs) and other metabolites. In mice after feeding NDC, only the uptake flux of SCFAs from the intestine correlated with improvements of the metabolic syndrome (1).
In humans, access to this inner world is becoming now possible by novel orally swallowable gastrointestinal capsules (2,3), that can deliver or sample at an exact location in the gastrointestinal tract. Specific challenges of using the gastrointestinal capsules are: 1) The small volumes of sample obtained. To overcome this, we optimized the analytical protocols to measure NDC breakdown, gut microbiota composition, and bacterial fermentation products (notably SCFAs) in the same small and complex samples as we expect to obtain from the capsule; and 2) the capsule with sample remains in the body until its excretion. Hence, we developed a stabilizing solution to be preloaded in the capsule that can efficiently stop fermentation for up to 2 days. This will allow us to collect a representative sample of the human colon. Furthermore, we verified that the quench solution was not a source of variation in the analytical analyses mentioned above.
This analytical toolbox to use gastrointestinal sampling capsule systems will allow us to study fibre intake, SCFAs uptake fluxes and improvements of the metabolic syndrome markers in human subjects.

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Nitrogen salvation by the early life gut microbiota: A metagenome study

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Abstract
When human life begins, a gut microbiota develops dynamically. Notably, this microbiota can aid with digestion and thus supports the infant's nutritional needs. To bolster up development of the body, the infant is in high demand of vitamins, amino acids and subsequent protein. For a large part, these nitrogenous compounds are products of microbial metabolism. However, it is unclear how the microbiota salvages the required nitrogen from breast milk. This complex bio-fluid holds several nitrogen sources, of which some are waste products of human metabolism. The aim of this study is to elucidate if the microbiota of the infant is equipped to utilize various sources of human milk nitrogen. This could lead to future insight on microbiota development and nutritional strategies**.** Recently, there has been an increase in infant gut shotgun metagenomics studies. These studies give insight into potential microbiota functionality. This study involves the screening of existing metagenome databases for genes involved with nitrogen salvation. Metadata on nutritional intake can be used to couple the outcome to dietary nitrogen sources. This study shows that diet has a profound effect on infant gut nitrogen metabolism.
Theme: Infant gut microbial ecology.

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Modelling the ecological network of the human microbiome

Susanne Pinto, Elisa Benincà and Hans Bogaards

Bacterial species can be in competition with each other for space and nutrients or they can be in a symbiotic relation because of metabolic processes such as crossfeeding. Knowledge about these ecological processes will learn us a lot more about the temporal and permanent changes of the microbial composition in our gut. Compositional changes are associated to multiple infectious and metabolic diseases, like inflammatory bowel disease, irritable bowel syndrome and obesity.
(Partial) correlation networks are often used to identify potential taxon-taxon interactions. Because of little knowledge of the 'wild' human gut microbiome the results are hypothetical and unverified. We simulated multi-species microbial communities with known interaction patterns and simple assumptions about growth behaviour. The generalized Lotka-Volterra equations can describe the microbial population dynamics and is therefore a popular tool in analyzing microbial communities. Using Lotka-Volterra dynamics we can simulate microbial time series that can be analysed in the same way as real-world microbiome data is currently analysed.
In contrast to real time series, the ecological interactions are now known and can be compared to the partial correlation network. The results show a higher precision and lower recall, which means that the change of missing an interaction is higher than finding an interaction that is not really there. We show how (small) differences in experimental and ecological parameter values can affect the network inference. Although it is risky to translate the presence of a correlation between bacteria to a biotic interaction, partial correlation networks can, under certain conditions, recapitulate the ecological interactions from microbial abundance data.

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Novel spin column and magnetic bead-based approaches for the isolation of host and bacterial DNA from human feces

Duddy Oyib, Marloes van der Zwalm

Gut flora has been identified as a key indicator of host health. As such, fecal samples are quickly becoming a frequently used sample type for genetic and microbial research. Fecal samples are abundant and can be collected non-invasively, making them an ideal specimen. Unfortunately, the presence of humic acid and other inhibitors can present challenges when isolating DNA from these samples. To purify DNA from these samples sufficiently, lengthy and hazardous purification protocols are often necessary. As an alternative, we present a magnetic bead based approach for DNA purification from feces, compatible with manual, small automated instruments, and robotic high through-put platforms. This magnetic bead based method offers a simple, non-hazardous, automatable alternative option for fecal DNA purification.

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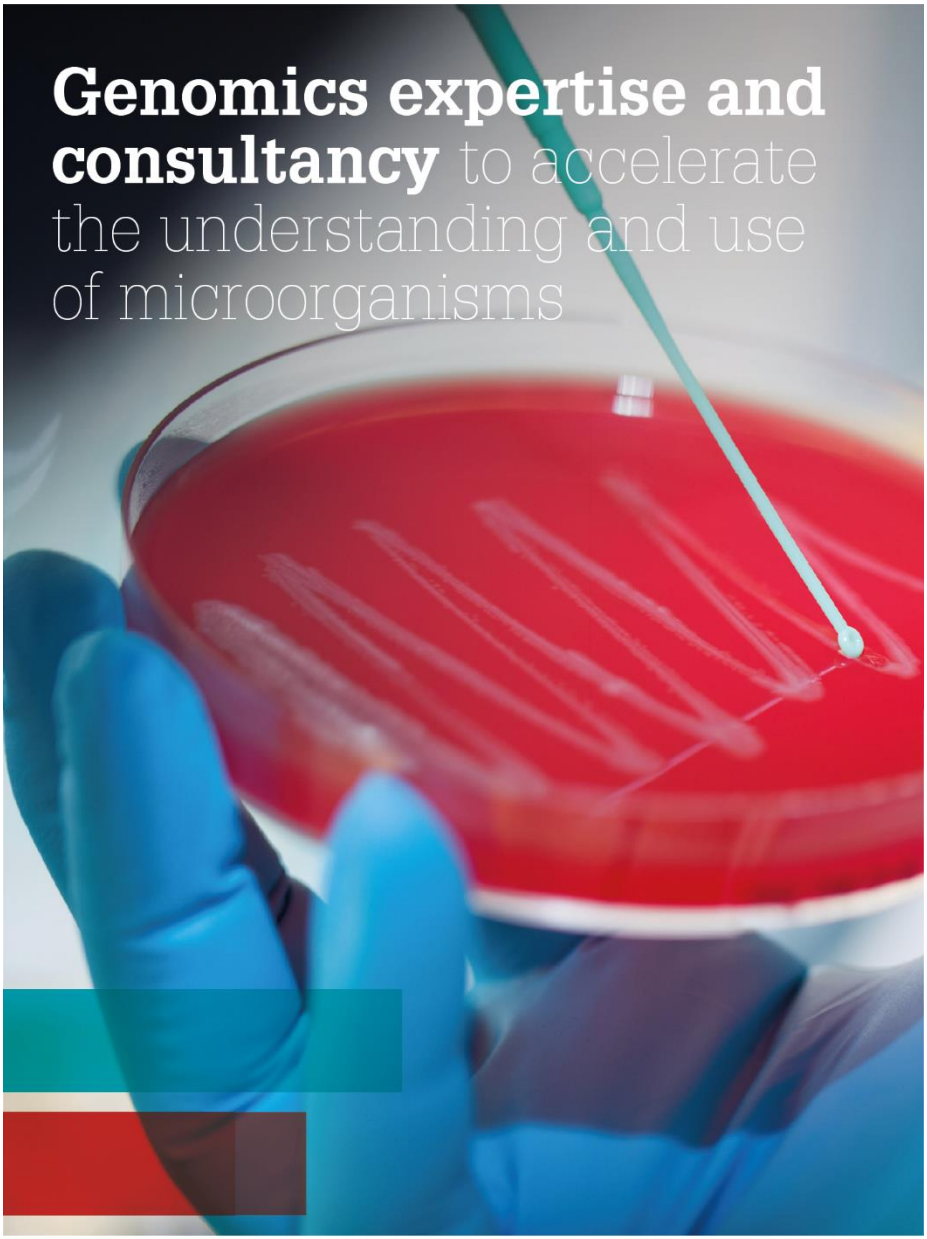

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