Acetate as metabolic target in the prevention of overweight-related chronic metabolic disorders

Gerben D. A. Hermes[1], Emanuel E. Canfora[2], Koen Venema[3], Hauke Smidt[1], Ellen E. Blaak[2], Erwin G. Zoetendal[1]

[1]Laboratory of Microbiology, Wageningen University & Research; [2]Department of Human Biology, and [3]Campus Venlo, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre +

BACKGROUND

The gut microbiota has been suggested to be involved in the etiology of obesity and type 2 diabetes mellitus. Gut-derived short chain fatty acids (SCFA), formed by microbial fermentation of indigestible carbohydrates, might be involved in the control of body weight and insulin sensitivity. Recently, it was shown that acute infusions of the SCFA acetate in the amount achieved by a high fibre diet, in the distal, but not in the proximal part, of the colon increased circulating acetate, fasting fat oxidation, circulating peptide YY and slightly decreased tumour necrosis factor alpha. Based on this study, we will further investigate the effects of a dietary fiber mixture leading to high distal colonic acetate in relation to effects on the human metabolism and insulin sensitivity.

Data on metabolic benefits of fermentable dietary fibres are inconsistent. This may relate to 1. the characteristics of the dietary fibre including the site/amount/type of SCFA produced and 2. the microbes involved in the degradation. This poster will focus on the first in vitro part of the project where we define a dietary fibre giving lead to high distal colonic acetate and will report the data on microbial composition

METHODS

To design a fibre with the desired properties and to study which microbes are involved in the degradation, a screening was performed in an *in vitro* model (TIM-2, figure 1) that mimics the conditions in the human colon. The model was inoculated with a pooled microbiota of 5 metabolically healthy and 5 obese, prediabetic subjects individuals and 4 single dietary fibres (T1,T2,T3,T5) and a control (C) in combinations with resistant starch (RS). Before these substrates are added, the model is run with the microbiota for 48h to stabilize. At t=0 and after 24,25,26,28,30,32 and 48h samples were taken. 16S rRNA gene amplicon sequencing of the V4 region was performed on an Illumina Hiseq machine and NG-Tax an in-house pipeline was used to demultiplex the raw data, pick OTUs and assign taxonomy.

Figure 1: TIM-2, an *In vitro* model that mimics the conditions in the human colon

RESULTS & DISCUSSION

We generated 164,735 +/-SD 64597 reads/sample and quality control by sequencing of positive controls (synthetic communities of known composition) demonstrated high quality sequencing results.

Beta diversity & composition

NMDS ordination of Bray-Curtis dissimilarities showed clear clustering based of the lean and obese microbiota. The final composition at t48 is mainly determined by the starting composition (figure 2A), while the differences in composition at t0 and during the fermentation, are largely driven by the high relative abundance of *Prevotella 7* at t0 in the obese microbiota and the large increase of *Lachnospiraceae* NK4Aat t0136 group in the obese microbiota in time, compared to the lean microbiota (figure 2B).

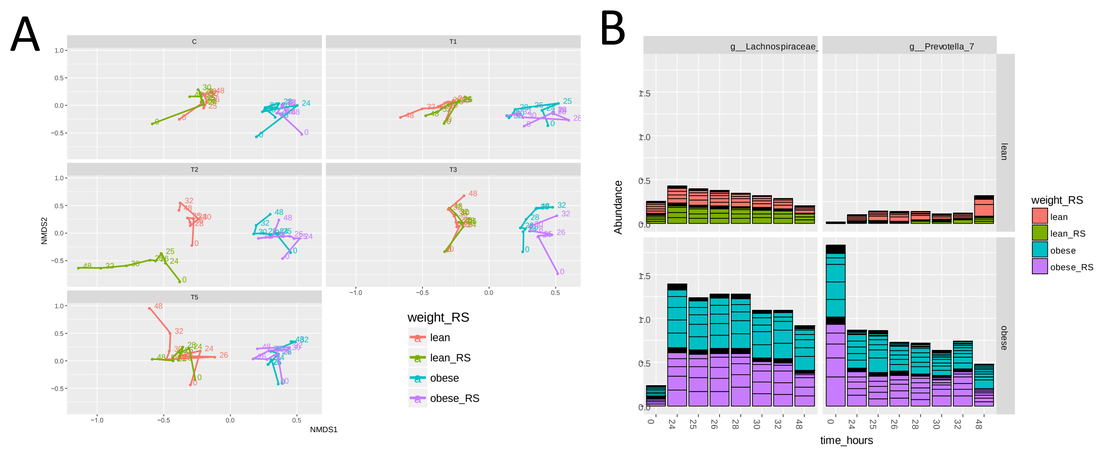

Figure 2: A: Non-metric Multi Dimensional Scaling of Bray_Curtis dissimilarities, faceted by fibre. **B:** Stacked relative abundance barplots of two important microbial drivers of these clusters.

The heatmap in figure 3 shows the composition of samples based on bacteria that are important for the clustering. The overall composition of most samples after addition of the fibres is very similar, however some fibres show distinct microbial signatures (closer clustering of samples).

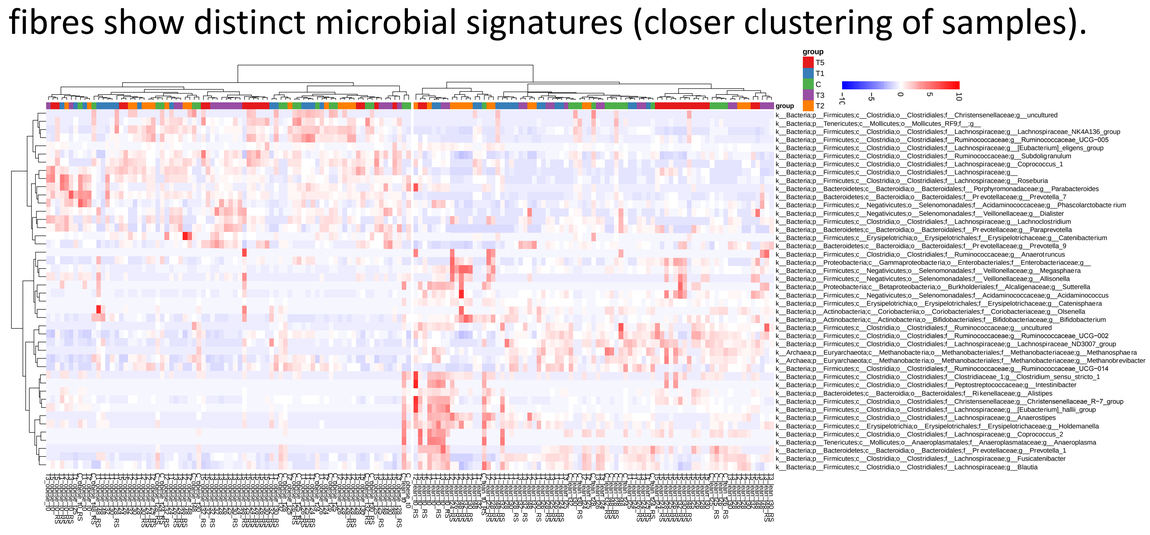

Figure 3: Heatmap of bacteria whose abundance significantly differed between t0 & t48 between the two clusters (FDR <0.05). Color value show bacterial abundance centered and scaled over the rows. Samples are hierarchically clustered using the Ward.D2 algorithm on a Bray-Curtis dissimilarities matrix.

Alpha diversity

Within sample diversity was calculated using the Inverse Simpson and phylogenetic diversity metrics. Generally the diversity of the samples at t0 in the obese microbiota are lower, partially due to the higher relative abundance of *Prevotella* 7 in these samples. The diversity of samples from lean or obese individuals follow similar trends and for all fibres the Inv Simpson diversity shows a downward trend in time (Figure 3)

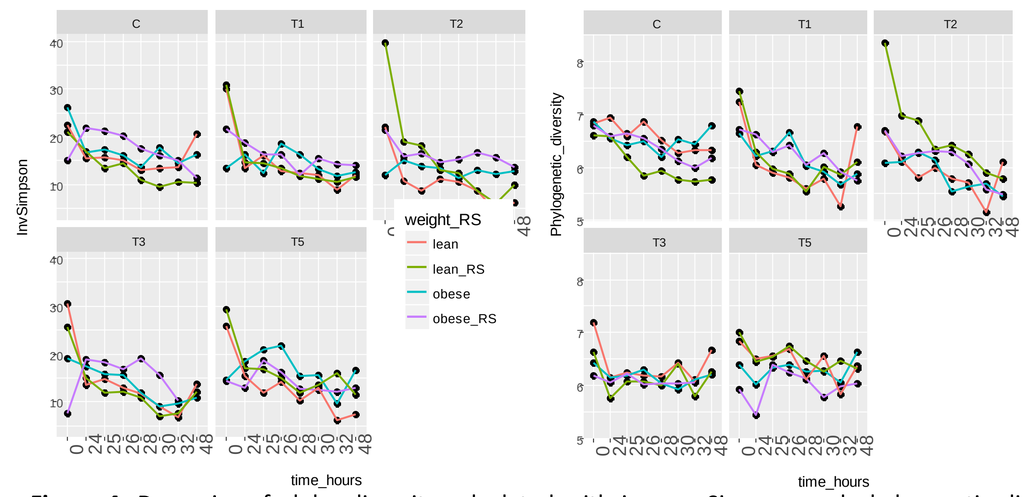

Figure 4: Dynamics of alpha diversity calculated with inverse Simpson and phylogenetic diversity metrics.

Conclusion

The starting microbiota composition (lean or obese) drove the population dynamics during fermentation of the different fibres.

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