Duodenal infusion of *Anaerobutyricum soehngenii* ameliorates glycemic control and postprandial GLP-1 responses and alters the transcriptional profile of small intestine in subjects with metabolic syndrome.

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Background

The gut microbiota constitutes an important modulator of metabolic heath and an unbalanced microbiota is increasingly recognized as an important risk factor for metabolic disorders, such as obesity, insulin resistance and type 2 diabetes. However, insights into the role and mode of action of specific gut microbes in host metabolism regulation and proofs of causality in humans are scarce.
In a previous clinical study, we identified the butyrate-producing gut commensal *Anaerobutyricum soehngenii* to be associated with improved insulin sensitivity in subjects with Metabolic Syndrome (MetS).

Aim

Seeking for novel treatments to counteract insulin resistance and metabolic disturbances in obese subjects, we explore whether delivering a protective commensal bacteria *(Anaerobutyricum soehngenii* L2-7) directly into the small intestine of treatment-naïve subjects with metabolic syndrome (MetS) would ameliorate glucose metabolism (*NTR NL6630)*.

Experimental Design

In this randomized double-blind placebo-controlled cross-over study, 12 MetS male subjects received 10^{11} *A. soehngenii* L2-7 cells or placebo by duodenal-tube infusion; all subjects received both treatments, with a washout period of 4 weeks in between, as depicted in **Figure 1.** The order of administration was randomized and blinded.
Systemic metabolic responses were assessed by performing a 2-hour mixed meal test (at 6 hours post-infusion) and continuous glucose monitoring (with FreeStyle Libre technology, for up to 24 hours post-infusion). Whereas the impact of *A. soehngenii* L2-7 on duodenal transcriptome profiles was determined by RNA-seq of duodenal biopsies obtained 6 hours after infusion.

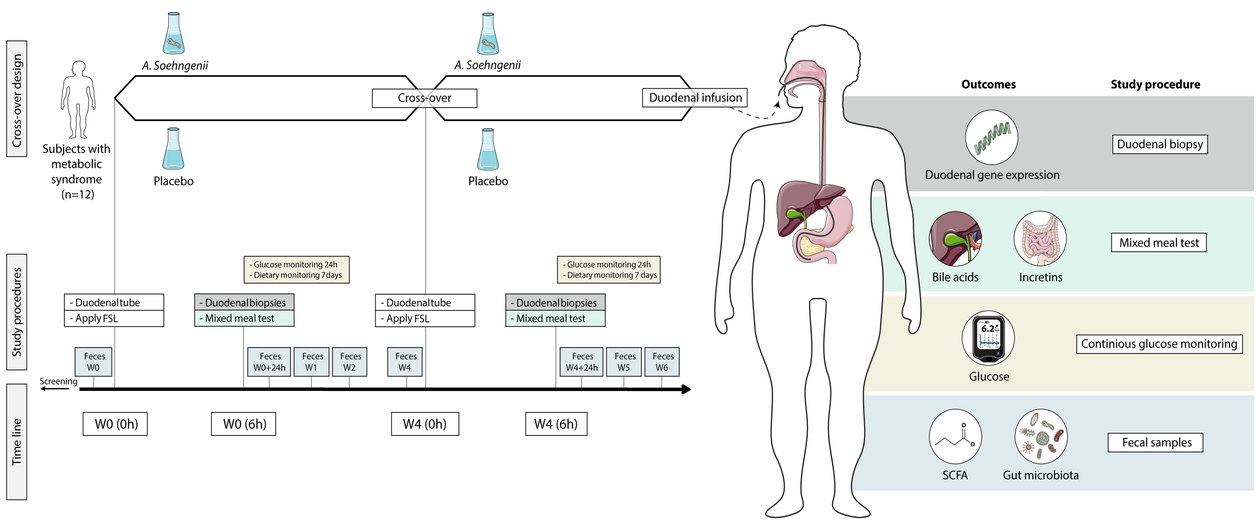

Figure 1: Study overview
Schematic representation of the study design showing the time-points of interventions and of biological samplings: all 12 subjects received placebo (10%glycerol in PBS) or treatment (*A. soehngenii* L2-7) at week 0 or week 4 (time of intervention cross-over).
FSL: FreeStyle Libre system for continuous glucose measurements (CGM).

Results

A single-dose of duodenal infusion containing ***A. soehngenii*** **improves peripheral glucose control** as shown by a significant decrease in the glucose excursions: the median absolute deviations (MAD) of continuous glucose measurements were significantly diminished after the *A. soehngenii* L2-7 intake compared to placebo infusion (**Figure 2A**, p=0.045). Moreover, duodenal *A. soehngenii* L2-7 specifically stimulates the postprandial secretion of the insulinotropic hormone GLP-1 (glucagon-like peptide 1) (**Figure 2B, 2C**, p=0.021), which is produced by intestinal enteroendocrine L cells and has been reported to positively act on both insulin secretion and sensitivity.

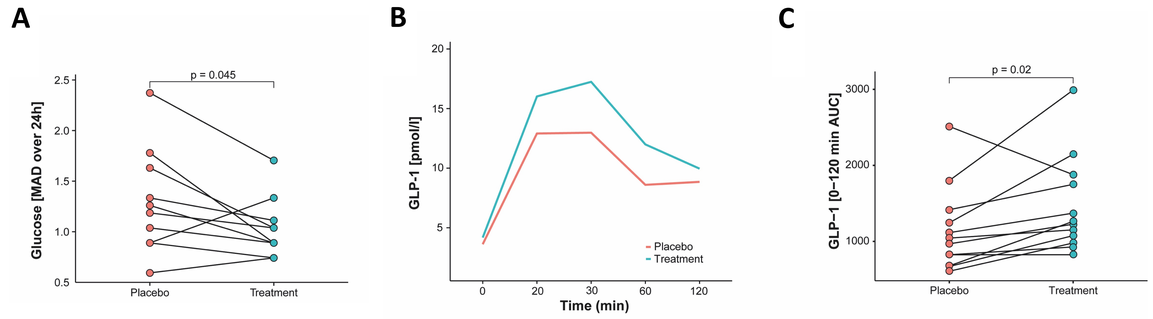

Figure 2: (**A**) Median absolute deviation (MAD) of continuous glucose measurements (CGM) over the first 24 hours after placebo/treatment-intervention, (**B**) Plasma GLP-1 levels (pmol/l) at 0, 20, 30, 120 minutes during mixed meal test (MMT), (**C**) Plasma GLP-1 levels during MMT as total area under the curve (AUC).

To unbiasedly dissect the changes in the transcriptional profile of duodenal mucosa induced by *A. soehngenii* infusion, we employed RNA-sequencing technology using RNA isolated from duodenum biopsies. Using 3 distinct analysis pipelines, Sleuth, EdgeR and DESeq2, we found, respectively, **380, 323 and 217 differentially expressed (DE) genes** (**Figure 3A**). Of these 73 DE genes were significantly up- or down-regulated upon *A. soehngenii* duodenal infusion in all 3 analysis pipelines (**Figure 3A, 3B**). The most up-regulated or down-regulated genes after bacteria administration encode for proteins involved in metabolite transport, cholesterol metabolism or cytokine signaling (**Figure 3B**). However, the most remarkable effect was the *A. soehngenii*-induced expression of ***REG1B***, which encodes for the Regenerating Islet-Derived 1 Beta protein (**Figure 3B, 3C**). Importantly, Reg family members are small secreted proteins that have been reported to promote proliferation, β-cell mass expansion and exert anti-diabetogenic activities.

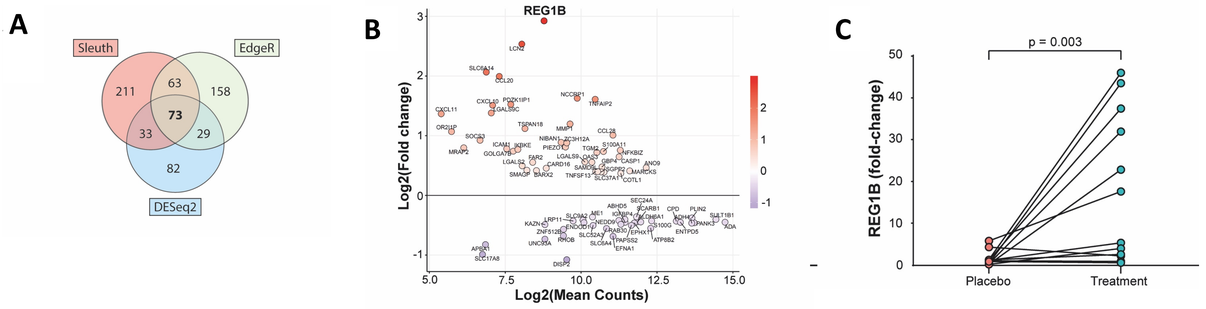

Figure 3: (**A**) RNA sequencing data sets analyzed by 3 digital gene expression (DGE) technologies: Sleuth, EdgeR and DESeq2, (**B**) MA (ratio intensity) plot visualizing the gene expression ratios (fold-changes treatment versus placebo). (**C**) Gene expression measured by qPCR in duodenal biopsies of *REG1B.*

Strikingly, the levels of the 3 most upregulated genes *REG1B*, *LCN2* and *SLC6A14* negatively correlate with glucose variation rates (MAD) within the first 24 hours after a single-dose of bacteria, hinting to a protective function in glycemic control:
REG1B **(rho=-0.48, p=0.025),** ***LCN2*** **(rho=-0.43, p=0.045) and** ***SLC6A14*** **(rho=-0.61, p=0.002).**

Conclusions & Future Prospectives

- **Altogether our findings disclose that a single-dose of *A. soehngenii* is sufficient to greatly impact the duodenal transcriptional profile and ameliorate glucose metabolism in metabolic syndrome individuals, likely through induction of intestinal GLP-1 production. Nonetheless, further studies are needed to delineate the specific pathways involved in induction of REG1B and GLP-1.**
- **Since duodenal administration of *A. soehngenii* was safe and well-tolerated, duodenal engraftment by multiple *A. soehngenii* administrations may be a novel treatment against insulin resistance in obesity and type 2 diabetes.**