Title

DIETARY-INDUCED BIOTIN DEFICIENCY AND TAMOXIFEN-INDUCED, INTESTINE-SPECIFIC DELETION OF THE BIOTIN TRANSPORTER IN ADULT MICE LEAD TO GUT MICROBIOME PERTURBATIONS

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Methods: 10-15 week-old germ-free C57BL/6 mice were colonized with fecal microbiota from a healthy control ("HC mice", n=13) or from a patient with UC experiencing a flare ("UC mice", n=13). For each group, half of the mice were kept on a control diet (7004, Teklad) and the other half were fed a HSD (7004 supplemented with 4% NaCl) plus 1% NaCl in drinking water for 3 weeks. At sacrifice, colon tissue was collected for histological analysis, RNA sequencing, and flow cytometry. Fecal samples were collected for microbiota analysis by 16S rRNA sequencing and lipocalin-2 determination.
Results: Colonic polymorphonuclear (PMN) cells were higher in UC compared with HC mice, regardless of diet ($p<0.01$). HSD in UC mice led to higher PMN cell counts versus HC mice ($p<0.001$). HSD also increased the proportion of $\alpha4^+$IFNg-producing T cells in the colonic lamina propria in UC, but not HC mice ($p<0.01$). This was paralleled by higher fecal lipocalin-2 in UC mice fed HSD compared with control diet ($p<0.05$). The major driver of microbial profile clustering was the type of colonization. UC mice displayed increased known proinflammatory taxa such as *Prevotella* and decreased beneficial taxa such as *Bifidobacterium* ($p < 0.05$). Targeted analysis of RNA sequencing data revealed that 7 genes, mainly related to the JAK/STAT pathway (i.e., *Stat2, Tyk2, Tlr7*) were differentially expressed ($p\leq0.05$) between HC and UC mice, fed the control diet. A different gene expression signature was found in mice fed HSD compared with control diet, with an increase in proinflammatory genes in UC compared to HC mice, such as *Il12a* and *Il6* ($p<0.05$).
Conclusions: Immunocompetent mice colonized with microbiota from a UC patient in flare spontaneously developed a proinflammatory immune tone, which was exacerbated by HSD. The different pattern of gene induction observed in mice colonized with the microbiota from the same UC donor, but fed HSD, suggests independent and synergistic pathways conducive to inflammation. Future studies will explore the preclinical therapeutic effect of novel drug candidates for the inhibition of such pathways.

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DIETARY-INDUCED BIOTIN DEFICIENCY AND TAMOXIFEN-INDUCED, INTESTINE-SPECIFIC DELETION OF THE BIOTIN TRANSPORTER IN ADULT MICE LEAD TO GUT MICROBIOME PERTURBATIONS

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Background and Aims: We recently demonstrated that dietary-induced biotin deficiency (BD) and tamoxifen-induced, intestine-specific deletion of the biotin transporter (SMVT-icKO) in adult mice induce signs and symptoms of inflammatory bowel diseases. Furthermore, broad-spectrum antibiotic treatment in SMVT-icKO mice ameliorates colitis, demonstrating a role for the gut microbiota. Here, we investigate microbiome shifts accompanying colitis induction in both SMVT-icKO and BD mice.
Methods: Stool samples were collected at weeks 0, 4, 8, and 12 from controls, BD mice, and BD mice supplemented with 1 mM biotin (BD_supp). For SMVT-icKO mice and their age/sex-matched control littermates, we collected stool samples at day 0 and day 7 after tamoxifen treatment, with additional colon tissue samples on day 7. 16S rRNA gene sequencing was performed and the data was processed using DADA2 in QIIME2. Alpha and beta diversity were assessed using Shannon and Chao1 indices and robust Aitchison principal component analysis. Significance was evaluated using linear mixed-effects models and repeated measures PERMANOVA, respectively. Differential taxa abundance testing was performed using zero-inflated negative binomial mixed-effects models.
Results: BD mice exhibited a significantly altered microbiome composition compared to controls and BD_supp mice, and they trended towards reduced species evenness. BD_supp mice had similar microbiome composition as controls. Relative to controls and BD_supp mice, BD mice demonstrated increased abundance of 10 members of family Lachnospiraceae and reduced abundance of 6 members of family Ruminococcaceae and 2 members of order Bacteroidales. Notably, BD mice had increased abundance of *Bacteroides thetaiotaomicron*, a species which had an average relative abundance of 40%. SMVT-icKO mice had a distinct microbiome composition from their control littermates and reduced species evenness and richness that approached significance. Compared to controls, SMVT-icKO mice had increased abundance of inflammation-associated Erysipelotrichaceae species and 2 members of order Bacteroidales, and reduced abundance of 7 members of order Bacteroidales as well as 3 members of order Clostridiales.
Conclusion: Biotin-deficiency in both BD and SMVT-icKO mice is accompanied by altered microbiome composition and reduced microbial diversity. Differences in the specific patterns of differentially abundant microbes between the two biotin deficiency mouse models could reflect differences in biotin accessibility for the microbes, where more biotin is available to the microbes under SMVT-icKO conditions compared to BD. This study paves the way for further investigations into the role of the biotin deficiency-associated microbiome in mediating intestinal inflammation.
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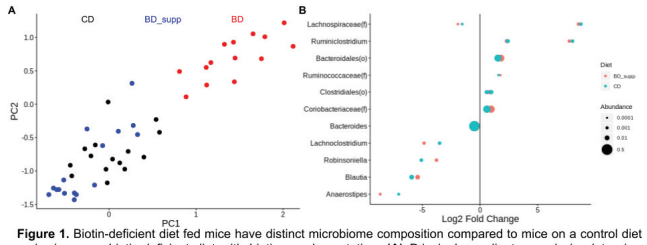

Figure 1. Biotin-deficient diet fed mice have distinct microbiome composition compared to mice on a control diet and mice on a biotin-deficient diet with biotin supplementation. **(A)** Principal coordinates analysis plot using Robust Aitchison distances. Samples from Weeks 4, 8, and 12 are shown, with each dot representing one sample, colored by diet. Significance determined by repeated measures PERMANOVA. **(B)** Amplicon sequence variants (ASVs) identified as significantly differentially abundant in BD_supp and CD compared to BD, with effect size indicated by the log2 fold change. Dot size indicates relative abundance of one ASV, with color of the dot representing diet. ASVs lacking genera assignments or family assignments are grouped by family (f) or order (o), respectively.

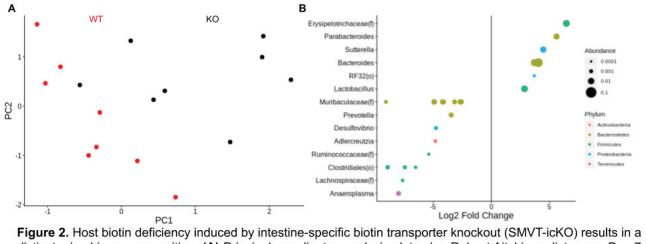

Figure 2. Host biotin deficiency induced by intestine-specific biotin transporter knockout (SMVT-icKO) results in a distinct microbiome composition. **(A)** Principal coordinates analysis plot using Robust Aitchison distances. Day 7 samples are shown, with each dot representing one sample, colored by genotype. Significance determined by PERMANOVA. **(B)** ASVs identified as significantly differentially abundant in KO compared to WT, with effect size indicated by the log2 fold change. Dot size indicates relative abundance of one ASV, with color of the dot representing phylum. ASVs lacking genera assignments or family assignments are grouped by family (f) or order (o), respectively.

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THE GUT MICROBIOME MODULATES BODY WEIGHT LOSS IN RESPONSE TO PHYSICAL ACTIVITY

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Background: The gut microbiome plays an important role in host energy balance. However, how the gut microbiome modulates the effect of physical activity (PA) on long-term body weight change remains unknown. The current study analyzed the gut microbiomes profiled by shotgun metagenomics sequencing in relation to recent and long-term PA and body weight change across the adulthood from age 21 to 65. **Methods:** We collected data on PA type and intensity and body weight using the validated biennial questionnaires since 1986 from 51,529 men enrolled in the Health Professionals Follow-up Study to determine the long-term average PA level up to age 65, recent PA level reported at age 65, and body weight change from age 21 to 65. In a subcohort of 307 healthy men, we profiled 929 stool metagenomes in 2012-2013. We first assessed the associations of recent and long-term PAs and body weight change with the gut microbiome composition and function. We then examined how the microbial species may modify the associations between PA and body weight change from age 21 to 65. We defined body weight responders to PA as those having a high PA level and a low weight change or having a low PA level and a high weight change (based on the median cutoffs). **Results:** Among the 307 healthy men, mean age (standard deviation) at stool collection was 70 (4) years and mean body weight change (standard deviation) from age 21 to 65 was 10 (11) kg or 13% (15%). 61% of participants were body weight responders to PA, with 30% in the high-PA-low-weight-gain group. A higher level of PA, particularly vigorous and aerobic PA, was associated with a higher Shannon index. A higher Shannon index was significantly associated with a less body weight change in both high- and low-PA groups. The joint classification of PA and body weight change explained a higher proportion of variation in the species community (R^2=1.10%) compared to PA (R^2= 0.76%) or body weight change (R^2=0.29%) alone. Higher PA, particularly vigorous and aerobic PA, was associated with shifts in gut microbiome composition and function, including higher abundance of *Clostridium spp.* and higher potential for short-chain fatty acid production. *Eubacterium rectale* modified individuals' response in weight change to PA: each 15-MET-hours/week increment in PA (corresponding to 33 minutes/day of brisk/very brisk walking) over the adulthood was associated with an average of 1.35 kg and 0.45 kg weight loss during age 21 to 65 years in those with and without *Eubacterium rectale*, respectively (P for interaction=0.003). **Conclusions:** Our findings support the importance of the gut microbiome for host energy balance and suggest the modulating role of *Eubacterium rectale* in body weight loss in response to physical activity.