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### Regulator of G Protein Signaling 14 Disruption Affects the Gut Microbiota and Metabolome in Mice

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The gut microbiota is linked to brown adipose tissue (BAT), but the mechanisms and microbes facilitating BAT production or function are unknown. A novel mouse model containing a gene knock-out of regulator of G protein signaling 14 (RGS14KO) has increased BAT. Our early studies found a unique microbiota in RGS14KO mice and BAT-specific metabolites. **PURPOSE:** To identify key gut microbial species and uncover BAT-specific metabolites in RGS14KO mice. Further, we aim to identify and associate metabolites produced in specific tissue samples with these key gut microbes. **METHODS:** Twenty-two mice (N=13 RGS14KO, N=9 Wild type (WT)) were used to identify predominant microbes and metabolites. Gut microbiota profiles were obtained by sequencing bacterial ribosomal operons. Metabolomics used UHPLC to evaluate positive and negative untargeted metabolites in fecal, cecal, brain, and BAT samples. Microbiome analysis used Kulczynski distance to compare WT to RGS14KO reads in two-dimensional non-metric multidimensional scaling (NMDS) plots. Two-tailed t-tests were used to compare WT and RGS14KO metabolite means ( $p < 0.05$ ). MetaboAnalyst 5.0 was used to identify significant metabolite pathways in tissue samples and generate pathway plots. **RESULTS:** Approximately 500k rRNA reads post QA/QC ((84% identify; >1000 bp alignment) were obtained from all samples by MegaBlast. NMDS plots showed significant bacterial community differences (Genus:  $p = 0.035$ ; species:  $p = 0.028$ ; strain:  $p = 0.037$ ) between WT and RGS14KO mice. Specifically, RGS14KO mice housed two unique strains of *Akkermansia muciniphilia* (*A. muciniphilia* BIOML-A22 and *A. muciniphilia* AN78) while WT animals contained *A. muciniphilia* EB-AMDK-1. Untargeted metabolomics identified 82 significantly different ( $p < 0.05$ ) unique metabolites were between RGS14 KO and WT mice in one of the four samples. Specifically, RGS14KO animals had significantly higher levels of G6P, glycyl-l-proline, glycerophosphocholine, isoleucine, pipecolic acid, thymidine, UDP-D-glucose, malate, leucic acid, NADP<sup>+</sup>, and guanosine in BAT compared to WT animals. **CONCLUSION:** The gut microbiome differs between RGS14KO and WT mice from genus to strain level. Unique metabolites found in RGS14KO BAT may be linked to BAT function.