Absence of fumaric acid as a fecal biomarker for non-alcoholic fatty liver disease

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INTRODUCTION

- The global rise in the prevalence of non-alcoholic fatty liver disease (NAFLD) poses a huge health burden worldwide.
- Metabolomics-based studies are increasingly emerging as a new direction for understanding disease pathogenesis and in discovering non-invasive biomarkers.
- However, fecal biomarkers for NAFLD are still lacking.

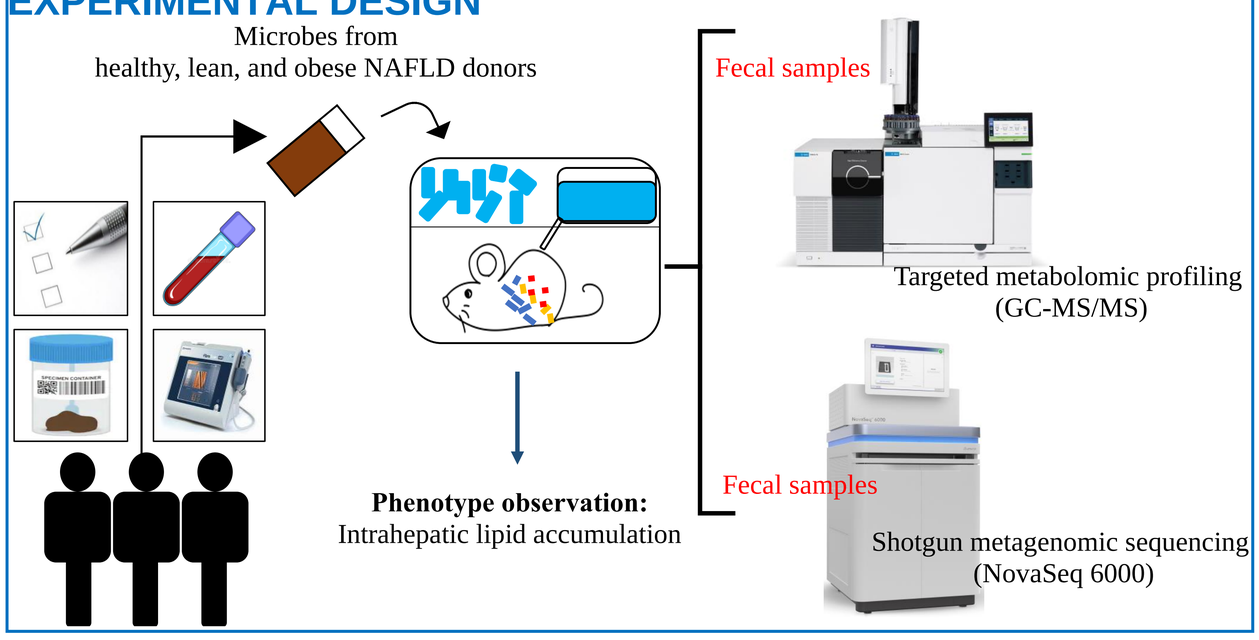
AIM

- To discover a fecal biomarker for NAFLD in a human microbiota-associated murine model

MATERIAL & METHODS

Fecal microbiota transplantation (FMT) was performed individually in a total of 24 male C57BL/6J mice (n=8 each in 3 groups) using fecal slurry from the same number of healthy human donors, and donors with lean NAFLD (BMI <25 kg/m^2) and obese NAFLD (BMI ≥30 kg/m^2), with liver steatosis quantified with controlled attenuation parameter measurements by vibration-controlled transient elastography. Targeted metabolites such as short-chain fatty acids (SCFAs), amino acids, and tricarboxylic acid (TCA) cycle-related metabolites were measured in feces from post-FMT mice using gas chromatography 7890B and 7010 triple quadrupole mass spectrometer.

EXPERIMENTAL DESIGN



Result: Distinct functional shifts of microbial metabolites in post-FMT mice

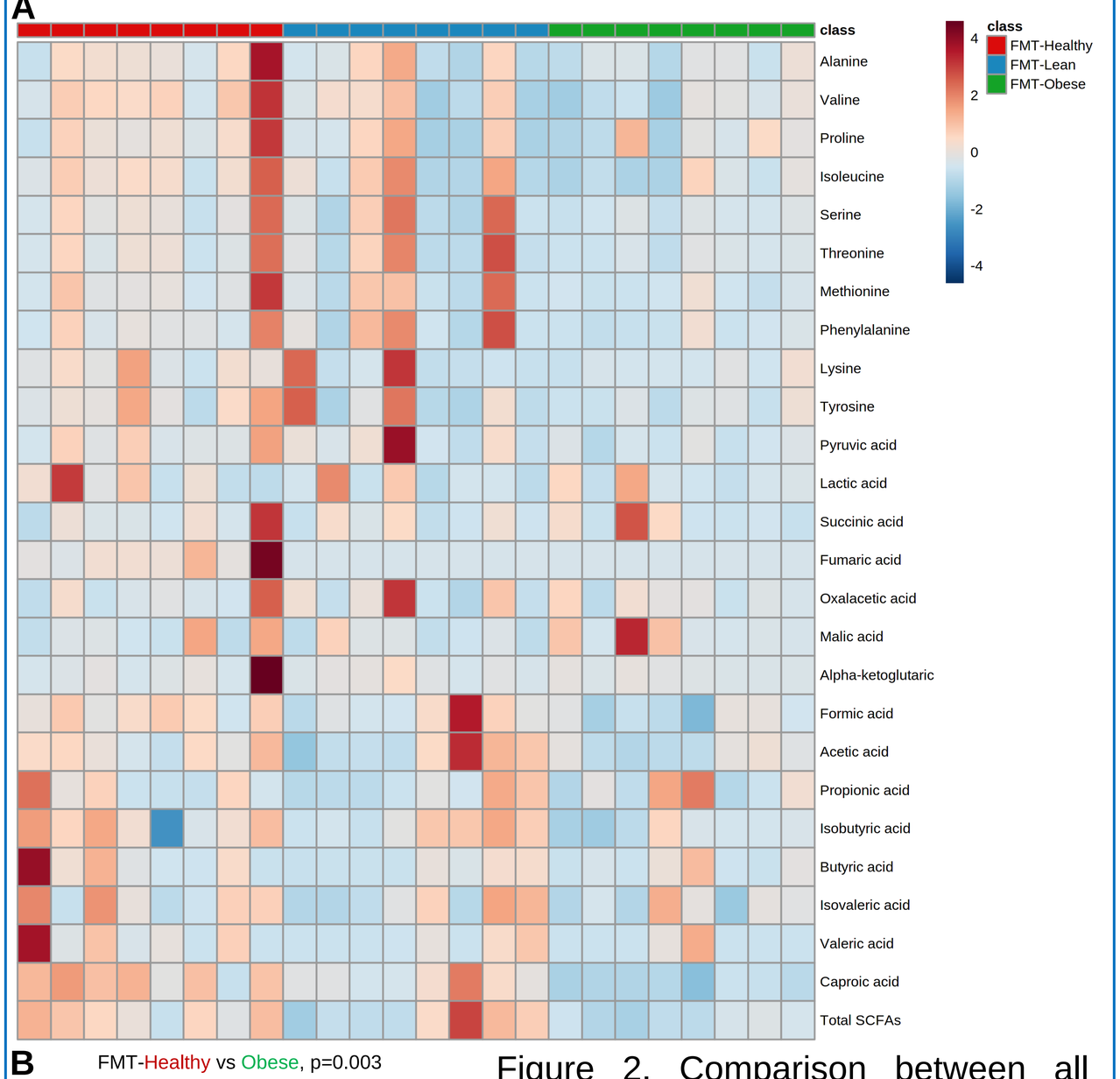


Figure 2. Comparison between all metabolomic data of stool samples from post-FMT mice. **(A)** Heatmap showing the microbial metabolite profiles. The colors changing from blue to red indicate higher abundance. **(B)** Principal coordinates analysis of all microbial metabolites based on Bray-Curtis distance and significance was calculated by permutational multivariate analysis of variance test with 9999 permutations.

Result: The effects of stool microbes from human donors on intrahepatic lipid accumulation

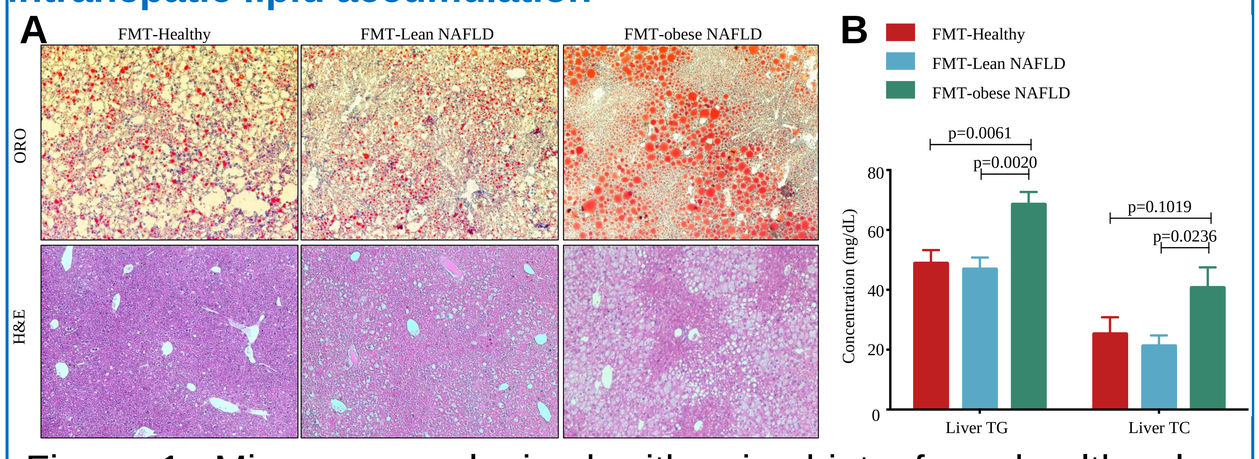


Figure 1. Mice were colonized with microbiota from healthy, lean NAFLD and obese NAFLD donors (n=8 each group). **(A)** Representative images of Oil red O stained (upper) and H&E stained sections (lower) of liver tissue with x100 magnification; **(B)** Hepatic triglycerides (TG) and total cholesterol (TC) content. Data were shown as mean ± SEM.

CONCLUSION

Unique metabolomic signatures were noted in mice colonized with microbiota from human NAFLD patients. With its complete undetectability in FMT-Lean and FMT-Obese mice, fecal fumaric acid may have a potential biomarker role for both lean and obese NAFLD.

ACKNOWLEDGMENT

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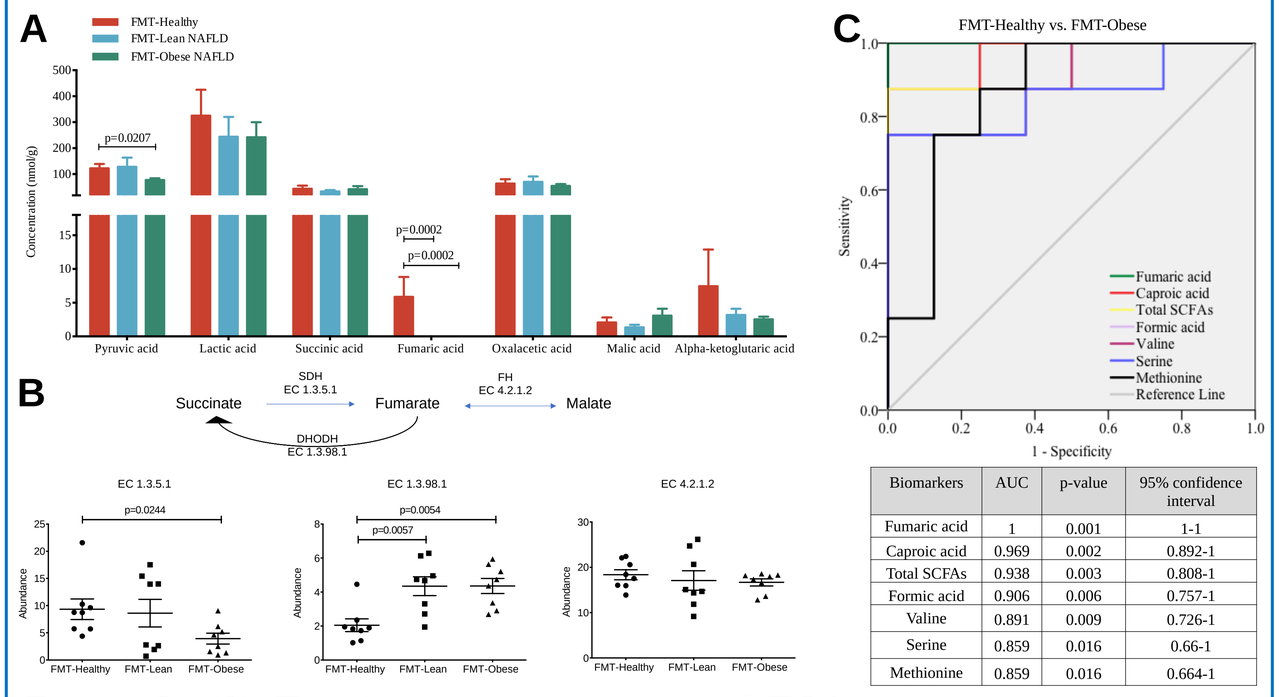
Result: Identification of fecal metabolites as potential biomarkers for NAFLD



Biomarkers	AUC	p-value	95% confidence interval
Fumaric acid	1	0.001	1-1
Caproic acid	0.969	0.002	0.892-1
Total SCFAs	0.938	0.003	0.808-1
Formic acid	0.906	0.006	0.757-1
Valine	0.891	0.009	0.726-1
Serine	0.859	0.016	0.66-1
Methionine	0.859	0.016	0.664-1

Figure 3. **(A)** The fecal concentration of TCA-related metabolites in post-FMT mice. **(B)** The alterations in microbial genes involving fumarate metabolism, which were obtained from shotgun metagenomic sequencing data. **(C)** Discrimination ability between FMT-Healthy and FMT-Obese mice by receiver operating characteristic (ROC) analysis for all identified metabolites. Data were shown as mean ± SEM.
Enzyme Commission (EC) number 1.3.5.1: succinate dehydrogenase (SDH); EC 4.2.1.2: fumarate hydratase (FH); EC 1.3.98.1: dihydroorotate dehydrogenase (DHODH).