Endourology Summer Student Scholarship 2024 Project Summary

Examining the Impacts of Metabolic Dysfunction-Associated Steatotic Liver Disease Upon Endogenous Oxalate Synthesis

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects 25% of US adults and has been shown to positively associate with calcium oxalate (CaOx) kidney stone formation[1]. Dietary oxalate intake and endogenous oxalate synthesis are the main contributors to the urinary oxalate pool, but their relative contributions to urinary oxalate excretion in those with MASLD have yet to be defined. There is growing evidence that MASLD is linked to other conditions commonly associated with an elevated body mass index such as diabetes and hypertension[2-6]. Further, previous studies have suggested alterations to the endogenous oxalate synthesis pathway in patients with elevated BMIs with data revealing statistically significant positive associations body mass index and levels of glycolate, an oxalate precursor, and overall urinary oxalate excretion[7-9]. In studying patients with MASLD, a positive association has been discovered between the degree of hepatic steatosis and urinary oxalate excretion, suggesting possible amplification to the endogenous oxalate synthesis pathway[10].

The endogenous oxalate synthesis pathway is thought to occur in the liver, and the enzymes involved are well known. The final step of the pathway is the conversion of glyoxylate to oxalate by lactate dehydrogenase. However, glyoxylate can be diverted from oxidation by alanine- glyoxylate aminotransferase, a liver peroxisomal enzyme encoded by the AGXT gene. In those afflicted with type 1 primary hyperoxaluria, a condition associated with extremely high oxalate excretion, CaOx kidney stone formation and renal damage including kidney failure, AGT is absent, defective or mistargeted to mitochondria[11]. A former study discovered hypermethylation of the AGXT gene in patients with MASLD, leading to gene downregulation associated with increased oxalate synthesis[10].

Another known source of urinary oxalate excretion is the non-enzymatic conversion of ascorbic acid (AscA) to oxalate[12]. Previous work in which carbon-13 AscA was administered to humans has demonstrated that AscA turnover contributes 40% to endogenous oxalate synthesis[13]. AscA conversion to oxalate is thought to be promoted by oxidative stress[12]. Hence, there is evidence that this pathway may be heightened in MASLD as MASLD progression is hallmarked by increased inflammation and oxidative stress[14,15]. We hypothesize that endogenous oxalate synthesis increases with the severity of MASLD due to both a deficiency in enzymes involved in glyoxylate

metabolism and increased ascorbic acid turnover, resulting in increased urinary oxalate excretion.

Objectives/Hypothesis: The primary objective of this study was to demonstrate that endogenous oxalate synthesis increases with the severity of MASLD. Our working hypothesis is that this phenomenon occurs due to increased ascorbic acid turnover and deficiencies in enzymes involved in glyoxylate metabolism, including hepatic alanine-glyoxylate aminotransferase (AGT), resulting in increased urinary oxalate excretion.

Approach: The contribution of endogenous oxalate synthesis to urinary oxalate excretion in adults with MASLD, based on guideline-recommended diagnostic criteria, will be assessed by collecting hourly and 24-hour urine specimens from participants consuming a low oxalate-controlled diet for 4 days. Both an oral load of $^{13}C_6$ -ascorbic acid and $^{13}C_2$-glycolate will be given to participants at different time points throughout the 4 days, and urine samples and breath tests will be utilized to approximate the contributions of ascorbic acid and glycolate to the urinary oxalate pool and estimate alanine-glyoxylate aminotransferase activity.

Results: In participants with severe MASLD, we expect to see a significantly greater hourly and 24-hour urine oxalate excretion compared with participants with milder MASLD (simple steatosis with no evidence of fibrosis). We also anticipate the contribution of ascorbic acid and glycolate metabolism to the urinary oxalate pool will be greater in those with more severe MASLD. For the assessment of AGT activity, we anticipate that breath $^{13}CO_2$ levels will be diminished in those with severe MASLD, which would suggest lower AGT activity. In mild MASLD, increased conversion of glycolate to CO_2 was observed in our preliminary analysis, compared with healthy volunteers whereas urinary oxalate excretion is similar. This suggests that the metabolic pathway of glycolate to glycine conversion is enhanced, contrary to our initial assumptions, at least in mild MASLD. This is compatible with recent data obtained in mice. A more detailed analysis of the glycolate dosing is in progress. Moving forward, our expectation and hypothesis is that individuals with more severe forms of MASLD will have a functional deficiency of AGT, thus resulting in increased production of oxalate and possibly explaining why those with more severe forms of MASLD have worsening stone disease.

Summary / Conclusions: The successful completion of this project has the potential to generate data supporting the working hypothesis that endogenous oxalate synthesis and urinary oxalate excretion increase with severity of MASLD while also highlighting the effects of oxalate precursors upon the synthesis process. Results obtained from this study will provide baseline data for future work examining endogenous oxalate synthesis in kidney stone formers with and without MASLD and open a door to the development of potential therapeutic interventions reducing steatotic liver and/or endogenous oxalate synthesis that could be beneficial in the MASLD population.

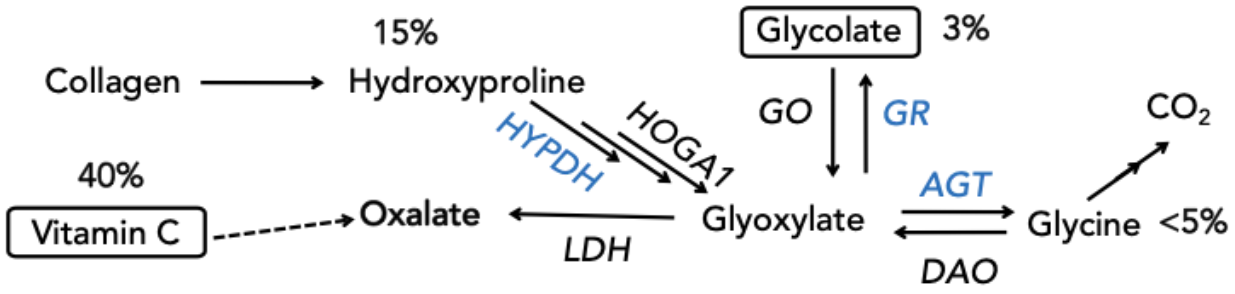

Fig. 1. Metabolic pathways of endogenous oxalate synthesis. AGT: alanine:glyoxylate aminotransferase, LDH: lactate dehydrogenase, GO: glycolate oxidase, GR: glyoxylate reductase, HOGA1: 4-hydroxy-2-oxoglutarate aldolase, HYPDH: hydroxyproline dehydrogenase. DAO: D-amino oxidase. Vitamin C to oxalate is non enzymatic. %: contribution of precursors in healthy non-stone forming human subjects. Enzymes putatively decreased in NAFLD are shown in blue.

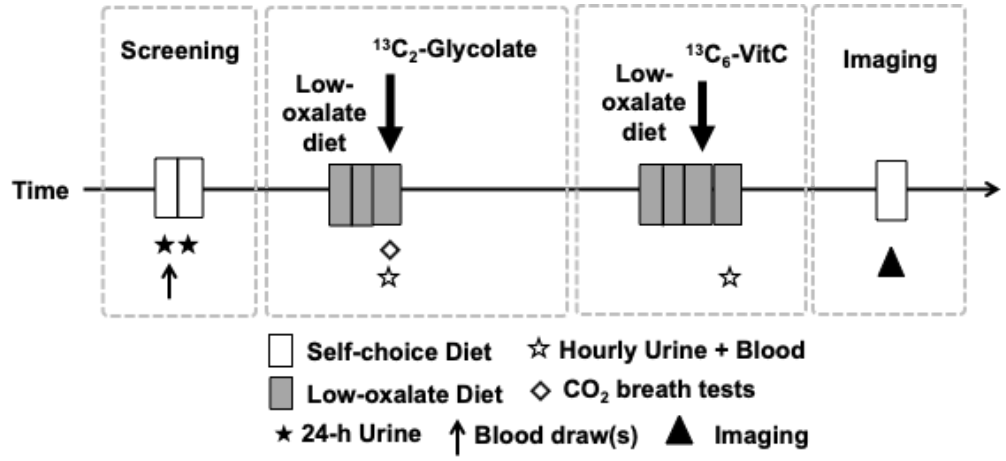

Fig 2. Study Protocol.

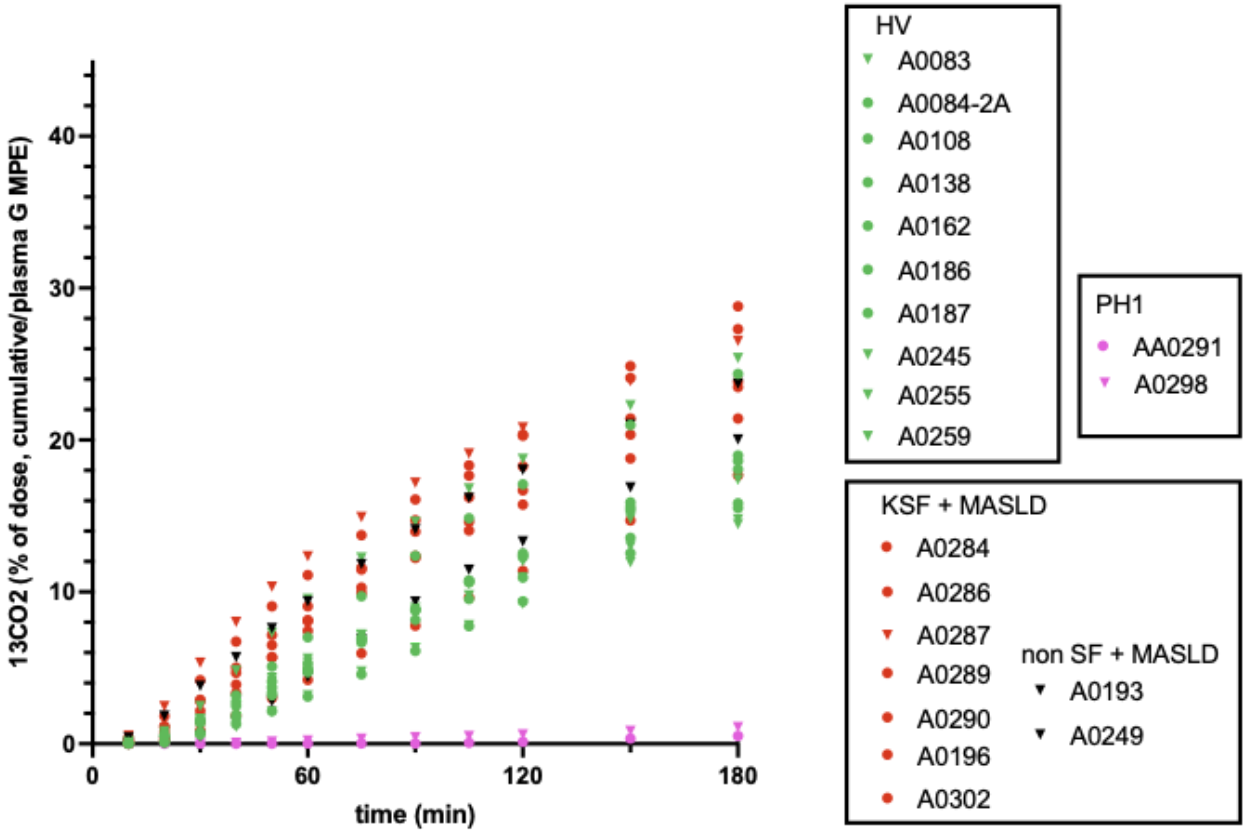

Fig. 3. Time course of breath $^{13}CO_2$ following oral dosing with $^{13}C_2$-Glycolate in participants with mild MASLD and controls. Nine individuals with mild MASLD, 10 healthy volunteers and 2 patients with primary hyperoxaluria type 1 (PH1) received $^{13}C_2$-Glycolate (0.5 mg/kg) followed by serial breath, plasma and urine collections over 6-hrs. Males. (▼), females (●), results as $^{13}CO_2$ generated relative to dose given, first 3-hrs post-dosing shown.

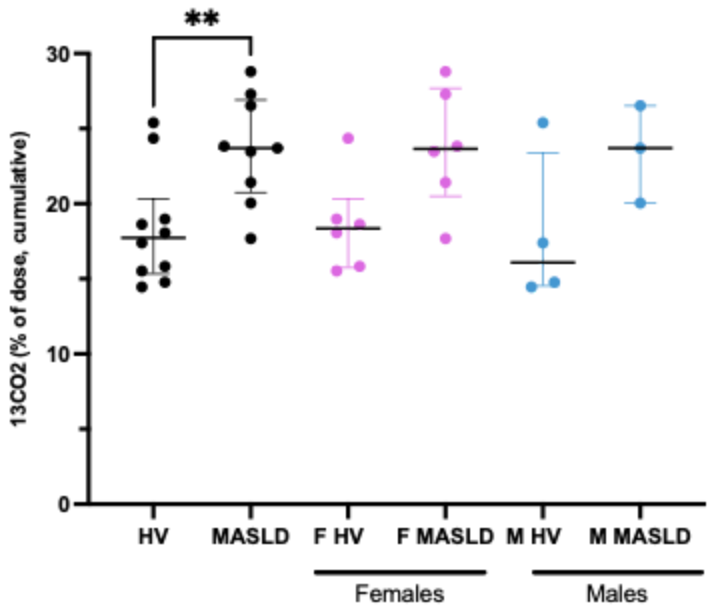

Fig. 4. Cumulative $^{13}CO_2$ in breath following $^{13}C_2$-Glycolate oral dosing at the 3-hr mark in participants with and without MASLD. Nine individuals with mild MASLD and 10 healthy volunteers were dosed with $^{13}C_2$-Glycolate. There was a significant difference in $^{13}CO_2$ levels after dosing in MASLD patients versus healthy volunteers. There was no significant difference between genders. (p: 0.006, unpaired t-test).

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