Endourology 2023 Summer Student Project Summary

Ascorbic Acid Turnover to Urinary Oxalate in the Gulonolactone Oxidase Deficient Mouse: Comparison of Aged and Obese Cohorts to Baseline Cohort

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Introduction

Kidney stones remain one of the most common diseases in urology, and kidney stone formers are at high risk of recurrence. It has recently been estimated that the prevalence of kidney stone disease is now over 11% in the United States and rising [1], with calcium oxalate being the composition in ~2/3 of cases [2]. As the rate of kidney stone disease is on the rise, there is an increased need for novel treatment options. Surgical treatments have advanced; proven pharmaceutical treatment options and dietary recommendations to prevent idiopathic calcium oxalate stone formation have remained stagnant [3]. Urinary oxalate excretion has shown to be a strong risk factor for stone formation [4], with at least half of the urinary oxalate excreted synthesized endogenously [5, 6]. Ascorbic acid (AsA) is an antioxidant that is endogenously degraded to oxalate non-enzymatically. Previous studies have failed to agree on the contribution of (AsA) to urinary oxalate excretion [7-10], though it has been shown that increased AsA intake is associated with increased urinary oxalate excretion and the risk of stone formation [11-16]. Most animals, unlike humans, can endogenously synthesize AsA which has made studying the conversion of AsA to oxalate in an animal model difficult. The Gulo-/- mouse, like humans, lacks gulonolactone oxidase and therefore cannot synthesize AsA endogenously. It must obtain AsA from the diet. A better understanding of the conversion of AsA to urinary oxalate and factors impacting this non-enzymatic breakdown may lead to more specific dietary recommendations and/or novel pharmaceutical targets to prevent calcium oxalate stone formation by decreasing endogenous oxalate production.

OBJECTIVES:

The primary objective of this study was to determine the impact of obesity and aging on the contribution of AsA to urinary oxalate in the Gulo-/- mouse to compare against the baseline contributions our lab has observed [17].

METHODS:

Obesity was induced through consumption of a 60% high fat diet and another cohort was allowed to age. Prior to beginning infusion studies, all Gulo-/-mice were provided ad libitum water containing 330mg/L AsA followed by a 5-day AsA depletion period (no AsA in the water) to eliminate tissue stores of AsA. Gulo-/- mice were then intravenously infused with the stable isotope of AsA, $^{13}C_6$-AsA, via a surgically implanted osmotic pump at a rate of 10 µmole/kg/h. After allowing 8 days for recovery and equilibration, 24-hour urines were collected for 3

consecutive days in metabolic cages. Urinary oxalate isotopomers, $^{12}C_2$-oxalate, and $^{13}C_2$-oxalate, were measured using anion chromatography/mass spectrometry to calculate the percent contribution of AsA to urinary oxalate. Each urine sample was measured in triplicate with the average of each value being averaged amongst cohorts for analysis. Statistical analysis was performed using one-way ANOVA with adjustment for multiple comparisons. Infusion studies were not performed on wild type mice as they maintain the ability to endogenously synthesize AsA.

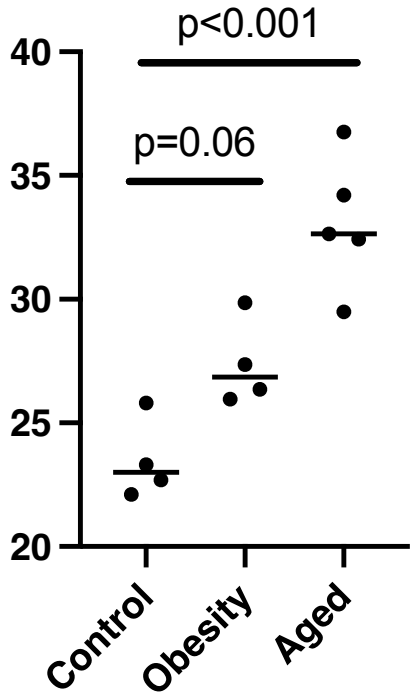
RESULTS:

We compared 3 cohorts of male Gulo-/- mice (n=4-5 per group): a baseline cohort (age 14-24 weeks), an obese cohort (age 23-31 weeks), and an aged cohort (age 79-101 weeks). We found that AsA contributes to 23.5% (standard deviation [SD] 1.6%) of urinary oxalate under baseline conditions, 27.4% (SD 1.7%) under obese conditions, and 33.1% (SD 2.7%) under aged conditions in the Gulo-/- mouse ($p=0.06$ between obese and baseline; $p<0.001$ between baseline and aged).

CONCLUSION:

Non-enzymatic conversion of ascorbic acid to oxalate appears to account for almost one quarter of urinary oxalate under baseline conditions in the Gulo-/- mouse, with a statistically significantly higher contribution in the aged cohort. It does appear that this conversion was greater in the obese cohort, though results were not statistically significant. In evaluation of the obese cohort, there is also the limitation of the difference in age compared to the baseline cohort. Further work is ongoing with samples from these mice to define the tissue sites of AsA turnover to oxalate and the mechanisms involved in this breakdown.

% Contribution of AsA to Urinary Oxalate in the Gulo -/- Mouse



	Baseline	Obese	Aged
Sample Size (N)	4	4	5
Age (weeks)	19.8 ± 5.9	25.7 ± 3.5	86.8 ± 8.1*
BW (g)	25.0 ± 1.6	31.7 ± 3.8*	29.2 ± 1.2
Urine Volume (ml)	4.0 ± 0.59	1.0 ± 0.4*	2.8 ± 0.9
Total Urinary oxalate (μG)	42.9 ± 2.2	40.79 ± 8.1	38.5 ± 1.1
$13C_2$ Urinary Oxalate (μG)	10.2 ± 1.0	11.4 ± 2.2	12.8 ± 1.3
$12C_2$ Urinary Oxalate(μG)	32.7 ± 2.3	29.6 ± 0.99	25.2 ± 4.3
$13C_2$ Urinary Oxalate enrichment (%)	23.5 ± 1.6	27.4 ± 1.7	33.1 ± 2.7*

Data expressed as mean ± SD. * indicates statistical significance vs baseline.

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