



# Poster #4: RNA INTERFERENCE OF HEPATIC LACTATE DEHYDROGENASE AND LIVER METABOLOMIC EFFECTS

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## Introduction

Endogenous oxalate synthesis primarily occurs via lactate dehydrogenase (LDH) activity in the liver. Liver specific RNAi LDH therapeutics are currently in clinical trials. Previous work using RNAi against liver lactate dehydrogenase demonstrated reduction of urinary oxalate in the Agxt knock out mouse (Agxt KO), a model for primary hyperoxaluria Type 1 (PH1). Our objective was to evaluate the effects of siRNA knock down of liver LDHA on liver metabolomics

## Methods

Agxt KO mice (n=4 for each group) were placed on an ultra-low oxalate diet. The mice were subcutaneously administered various doses (0, 0.3, 1, 3, 10 mg/kg) of LDHA siRNA (Anylam Pharmaceuticals). Liver tissue was collected at 4 weeks post single siRNA dose. Tissue metabolites were measured using ion chromatography/mass spectrometry and high pressure liquid chromatography. Statistical analysis was performed utilizing one way ANOVA.

## Results

Statistically significant decreases in liver lactate (p=0.012) were noted with increased dosage. Statistically significant increases in liver pyruvate (p=0.0002) and liver alanine (p=0.03) were noted with increased dosage. Certain KREB cycle amino acids were altered; liver citrate (p=0.025), liver oxaloacetate (p=0.006), and liver malate (p=0.0003) levels were increased following dose escalation.

## Discussion

These results demonstrate an alteration in liver metabolism following increasing dosage of RNA interference of hepatic LDH. Further studies are needed to determine the long term consequences of these findings especially since clinic trials are ongoing.

Plasma ( $\mu\text{M}$ )	Saline Control		3 mg/kg LDHA siRNA		P Value
	Mean	SEM	Mean	SEM	
Lactate	5396	290	5202	180	0.590
<b>Pyruvate</b>	<b>87</b>	<b>9</b>	<b>137</b>	<b>17</b>	<b>0.040</b>
Lac:Pyr Ratio	62	7	42	6	0.070
Glycolate	116	6	196	43	0.110
Liver Glycolytic Intermediates (nmoles/g)	Saline Control		3 mg/kg LDHA siRNA		P Value
	Mean	SEM	Mean	SEM	
Lactate	4575	777	2898	470	0.110
<b>Pyruvate</b>	<b>54</b>	<b>5</b>	<b>228</b>	<b>25</b>	<b>0.0005</b>
<b>Lac:Pyr Ratio</b>	<b>93</b>	<b>27</b>	<b>13</b>	<b>3</b>	<b>0.030</b>
Liver TCA Cycle Intermediates (nmoles/g)	Saline Control		3 mg/kg LDHA siRNA		P Value
	Mean	SEM	Mean	SEM	
<b>Citrate</b>	<b>169</b>	<b>7</b>	<b>230</b>	<b>17</b>	<b>0.015</b>
<b>Isocitrate</b>	<b>75</b>	<b>2</b>	<b>84</b>	<b>2</b>	<b>0.017</b>
2-oxoglutarate	58	6	67	6	0.323
Succinate	388	87	387	79	0.996
<b>Fumarate</b>	<b>127</b>	<b>8</b>	<b>218</b>	<b>17</b>	<b>0.003</b>
<b>Malate</b>	<b>350</b>	<b>19</b>	<b>534</b>	<b>30</b>	<b>0.001</b>
<b>Oxaloacetate</b>	<b>26</b>	<b>0</b>	<b>29</b>	<b>1</b>	<b>0.009</b>
Other Liver Metabolites (nmoles/g)	Saline Control		3 mg/kg LDHA siRNA		P Value
	Mean	SEM	Mean	SEM	
NAD:NADH ratio	2.00	0.35	1.90	0.05	0.950
Glycerol- 3- Phosphate	1145	253	1800	295	0.070
Glycolate	144	26	231	45	0.140
Glyoxylate	2.50	0.05	2.61	0.13	0.540
<b>Alanine</b>	<b>1836</b>	<b>218</b>	<b>2657</b>	<b>247</b>	<b>0.047</b>
<b>ATP</b>	<b>2692</b>	<b>45</b>	<b>1955</b>	<b>271</b>	<b>0.036</b>
AMP	1645	153	2061	388	0.320
AMP:ATP Ratio	0.61	0.06	1.21	0.39	0.180