

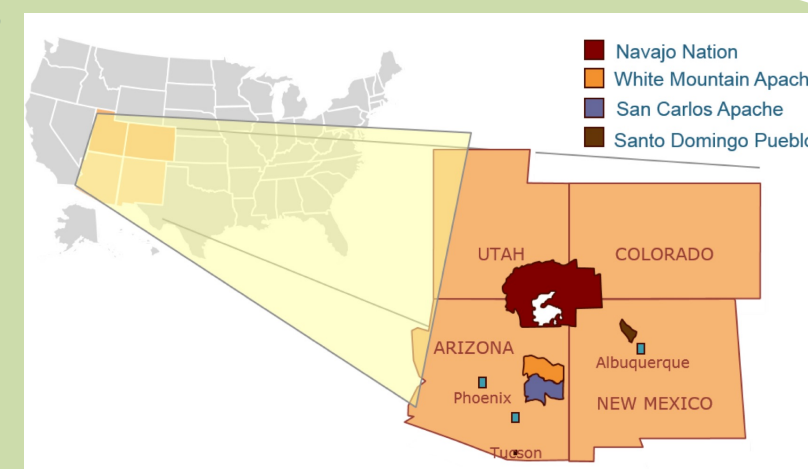
## Introduction

Early Childhood Caries (ECC) is one of the most prevalent, preventable childhood diseases and has long-term health consequences<sup>1,2</sup>. ECC impacts Indigenous children at an earlier age, with a higher prevalence, and with greater severity compared to the general US population due to early exposure to dietary sugar and previously correlated with limited access to clean water, health care, nutritious foods, and related early dietary sugar intake due to loss of healthy traditional diets and lifeways promulgated by colonization and ongoing historical and modern traumas<sup>3-5</sup>.

A recent sugar reduction educational program was implemented in the Shiprock community in the Navajo Nation<sup>6</sup>. Frequent sugar consumption can shift the oral microbiome composition towards more acid-producing, acid-tolerating species. However, the metabolic activity of the oral microbiome as it relates to ECC remains relatively unknown<sup>7</sup>.

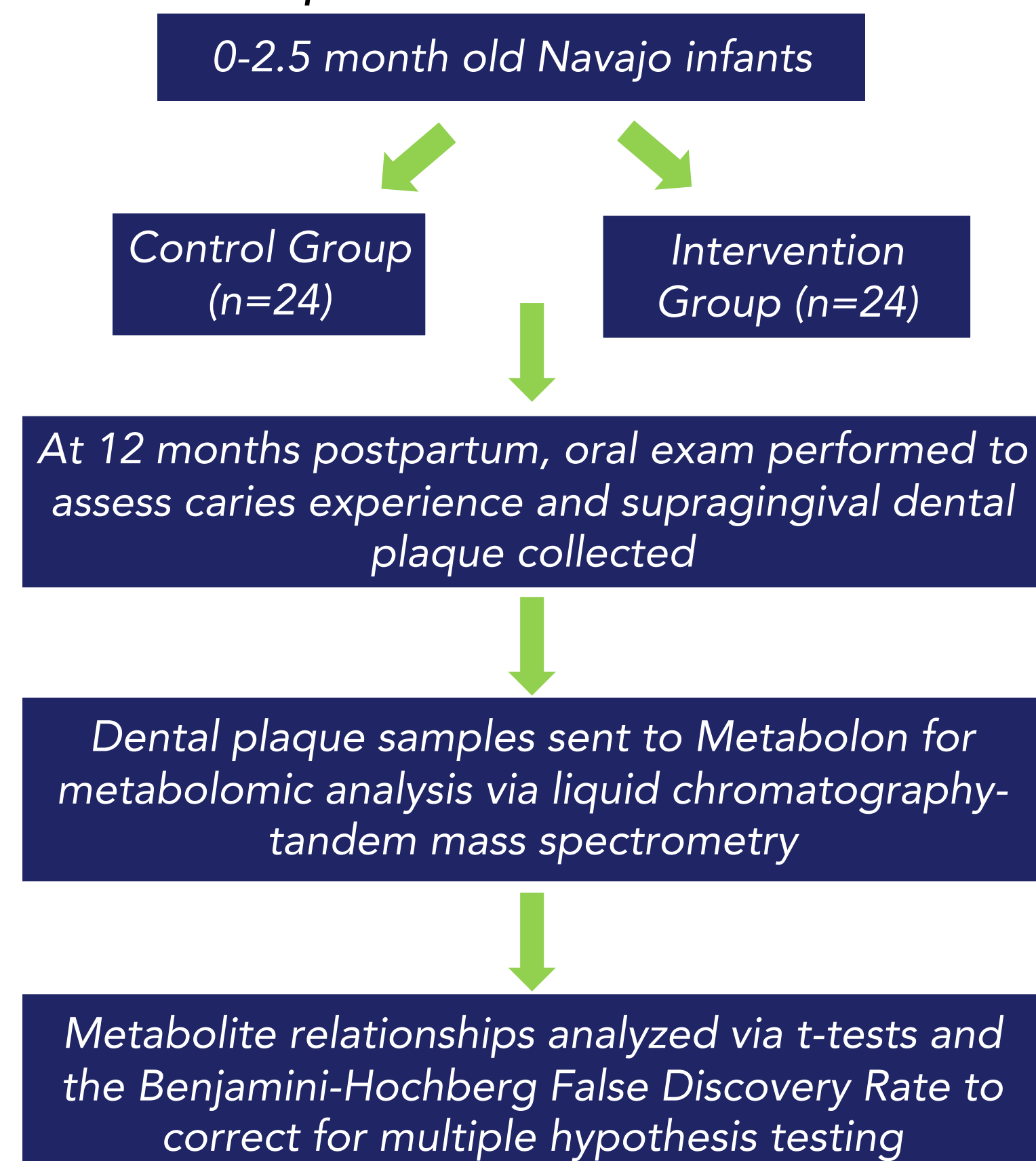
The purpose of this study is to evaluate the impact of a sugar reduction program on the metabolic function of the oral microbiome and to investigate the relationship between the oral metabolome and ECC among Navajo infants.

- The Navajo Nation is the largest reservation in the US, covering 27,673 mi<sup>2</sup>
- Median household income: \$27,389
- Poverty rate: 38%
- 44% of children <18 years of age live in poverty
- 13 grocery stores across the entire Navajo Nation
- Many community members report being food insecure<sup>8</sup>



## Materials & Methods

Navajo mothers and their infants aged 0-2.5 months postpartum living in the Northern Navajo Medical Center region were recruited. The study was nested in an early childhood home-visiting intervention trial to promote nutrition.



### Control Group

- 3 home-visiting lessons delivered by local Indigenous Family Health Coaches on home and child safety
- Clean water delivery

### Intervention Group

- 6 home-visiting lessons delivered by local Indigenous Family Health Coaches on: 1) avoid feeding children sugar sweetened beverages, 2) continued breast-feeding 3) responsive feeding, 4) optimal introduction of complementary foods
- Clean water delivery

Table 1. Study cohort characteristics.

Characteristics of study participants at 12m of age		
Study Group	A (n=24)	B (n=24)
<b>Gender</b>		
Female	16	10
Male	8	14
<b>Dentition at 12m</b>	mean ± SD	mean ± SD
Total Teeth	8.3±2.0	7.8±2.2
Anterior Teeth	7.9±1.6	7.3±1.7
Posterior Teeth	0.4±0.8	0.5±1.1
<b>Caries Experience</b>		
Caries-affected	15	16
Caries-free	9	8
dmft Index	2.9±3.3	2.8±3.1

Table 2. The number of statistically significant metabolites between groups.

Statistical Comparisons		
Two-Way ANOVA	Intervention (A vs B)	Caries/Caries-Free
<b>Metabolites p&lt;0.05</b>	10	288

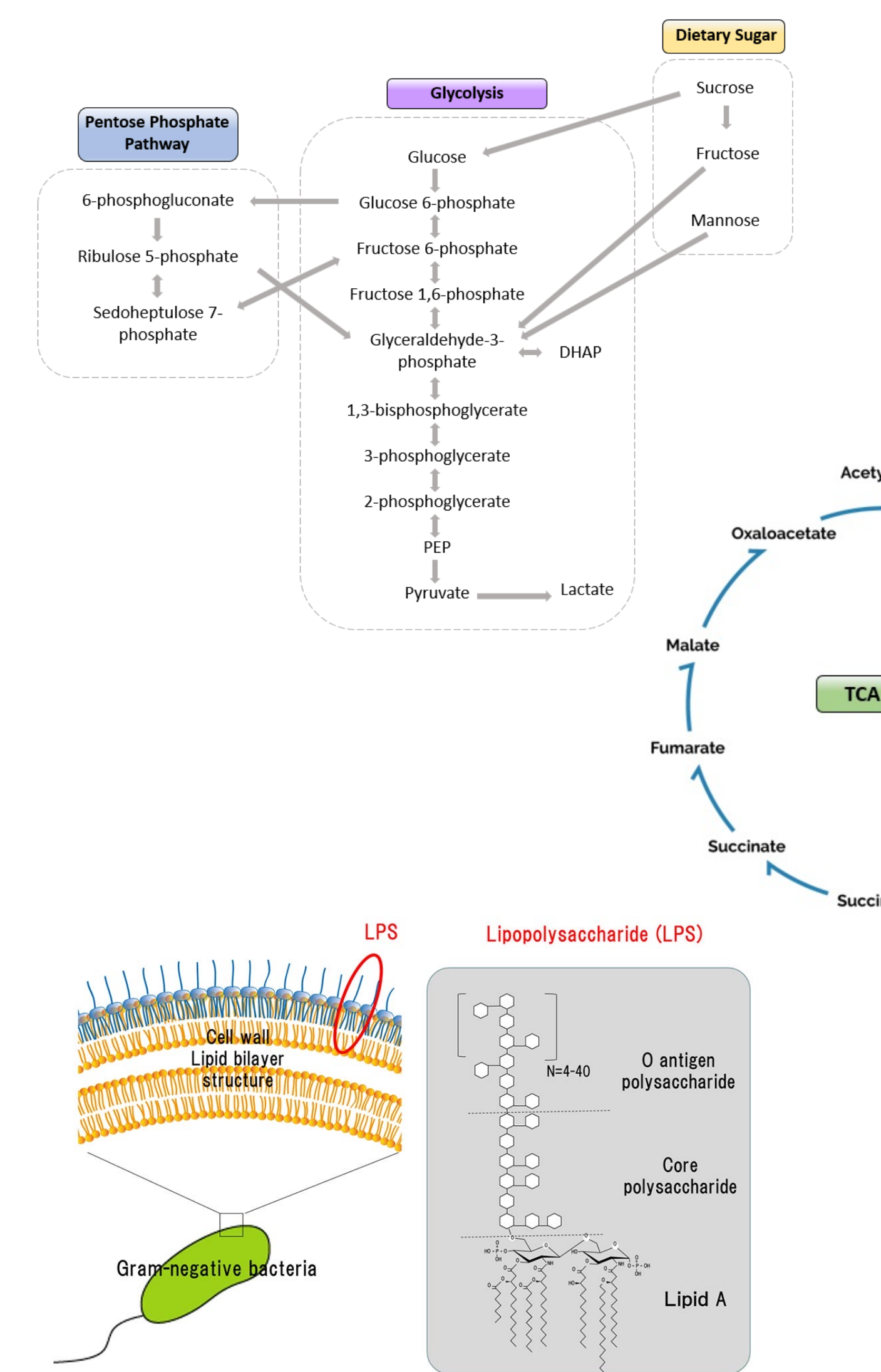


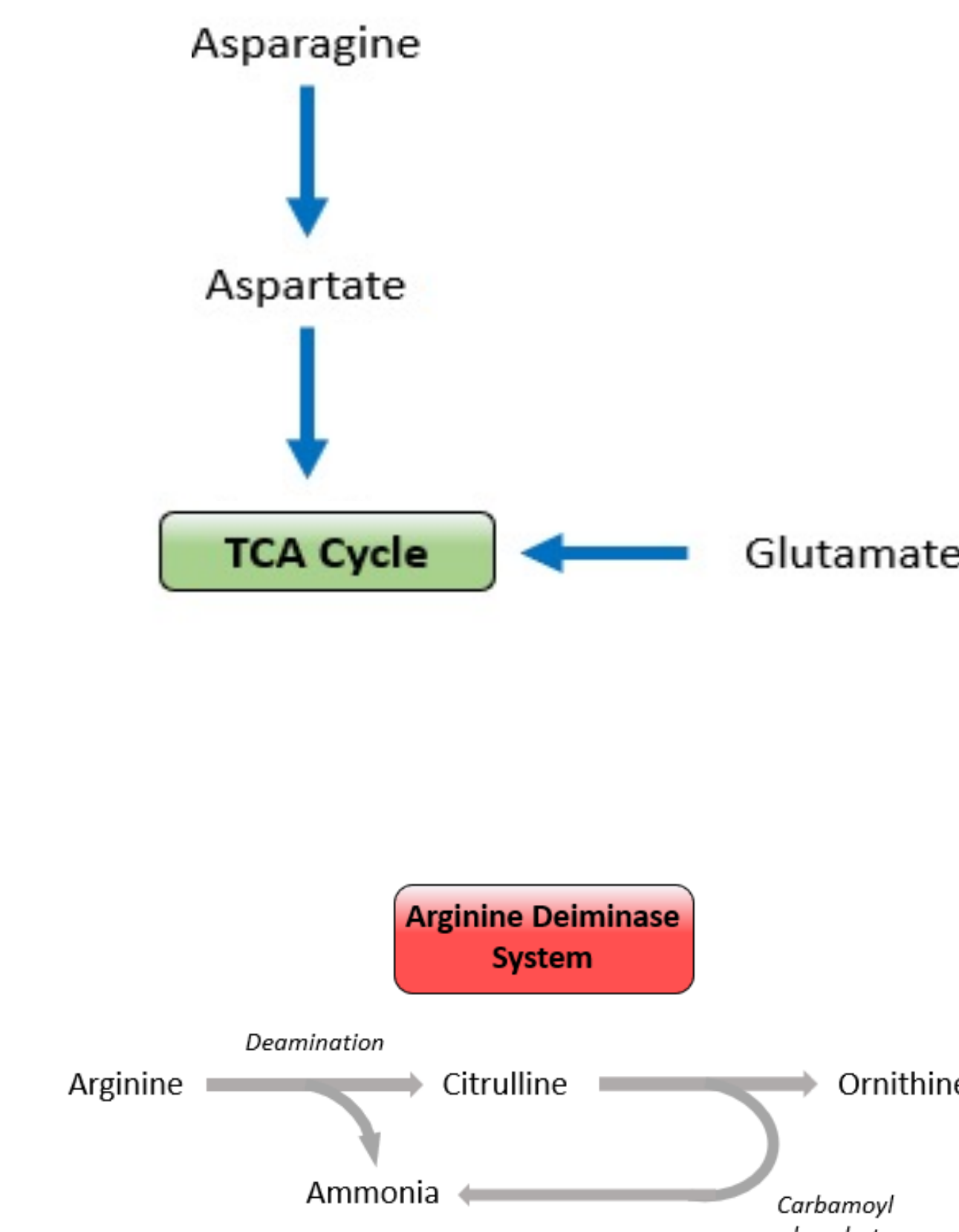
Figure 1: Targeted Metabolomic analysis of carbohydrate metabolism, Tricarboxylic Acid (TCA) cycle, and fatty acid metabolism related to lipopolysaccharide (LPS). The levels of metabolites were compared between caries-affected and caries-free infants. Only statistically significant metabolites are listed. P<0.05. Q-value accounts for the false discovery rate. Fold change indicates the ratio of mean scaled intensity between caries-affect and caries-free infants.

## Results

Table 3. Untargeted Metabolomics: Top 15 metabolites based on fold changes between caries-affected and caries-free infant subjects, sorted by type of pathway. P<0.05. Q-values account for the false discovery rate.

Pathway	Metabolite	p-value	q-value	Fold Change	Type
Food Component/Plant	Coumaroylquininate (3)	0.04	0.08	80.35	Exogenous
	Chlorogenate	0.03	0.07	68.50	Exogenous
	Coumaroylquininate (2)	0.02	0.06	48.38	Exogenous
	Coumaroylquininate (1)	0.00	0.04	39.17	Exogenous
	Coumaroylquininate (5)	0.03	0.07	22.89	Exogenous
Disaccharides and Oligosaccharides	Maltitol/lactitol/cellobiotol/palatinol	0.01	0.04	9.98	Exogenous
	Lysine Metabolism	0.00	0.03	9.40	Exogenous
Food Component/Plant	Quinate	0.01	0.04	8.24	Exogenous
	Acesulfame	0.03	0.07	7.03	Exogenous
Dipeptide	Fucitol	0.01	0.04	6.62	Exogenous
	Cyclo(leu-pro)	0.00	0.03	6.37	Endogenous
Food Component/Plant	Glucanate	0.00	0.03	5.53	Exogenous
Galactosyl Glycerolipids	1-linoleoyl-digalactosylglycerol (18:2)*	0.00	0.03	5.43	Exogenous
Tryptophan Metabolism	Kynurenate	0.01	0.04	5.29	Exogenous

2W ANOVA Main Effects (based on caries)				
Pathway	Biochemical Name	p-value	q-value	Fold Change
Dietary Sugar Sources	Glucose	0.0096	0.041	3
	Fructose	0.0045	0.036	3.4
	Mannose	0.012	0.076	3.4
	Arabitol/xylitol	0.0004	0.035	3.9
Sugar Substitutes	Maltitol/lactitol/cellobiotol/palatinol	0.0083	0.04	10
	Mannitol/sorbitol	0.0003	0.035	4.1
	Ribitol	0.0011	0.035	3.1
	Ribonate	0.0013	0.035	3.1
Pentose Sugars	Ribulose/xylulose	0.0049	0.036	2.9
	Xylose	0.0074	0.04	2.8
	Arabonate/xylonate	0.0037	0.035	2.7
	Sedoheptulose	0.0007	0.035	3
Glycolysis	Glucose 6-phosphate	0.013	0.063	1.9
	Lactate	0.0078	0.04	1.8
TCA Cycle	Aconitate [cis or trans]	0.02	0.059	1.9
	Isocitrate	0.048	0.1	2
	Succinate	0.0019	0.035	2.5
	Fumarate	0.0009	0.035	3
	Malate	0.0005	0.035	4.2
Fatty acid metabolism related to lipopolysaccharide (LPS)	2-hydroxyoctanoate	0.0048	0.036	2.5
	2-hydroxydecanoate	0.04	0.09	2.3
	2-hydroxylaurate	0.0079	0.04	2.1
	2-hydroxymyristate	0.0096	0.041	2.2
	2-hydroxypalmitate	0.0076	0.04	2.2
	2-hydroxystearate	0.011	0.044	2.4
	3-hydroxyhexanoate	0.018	0.056	2.3
	3-hydroxyoctanoate	0.0058	0.037	2.8
	3-hydroxylaurate	0.0043	0.036	2.4
	3-hydroxymyristate	0.003	0.035	2.6
	3-hydroxypalmitate	0.0028	0.035	2.6
	3-hydroxymargarate	0.0058	0.037	2.3
	3-hydroxystearate	0.0034	0.035	2.7
13-HODE + 9-HODE	0.0037	0.035	2.1	



2W ANOVA Main Effects (based on caries)				
Pathway	Biochemical Name	p-value	q-value	Fold Change
Alanine and Aspartate Metabolism	Alanine	0.0086	0.041	1.9
	N-acetylalanine	0.0012	0.035	2.2
	Aspartate	0.035	0.081	1.9
	N-acetylaspartate (NAA)	0.0003	0.035	2.3
Glutamate Metabolism	Asparagine	0.0018	0.035	4.2
	N-acetylasparagine	0.0029	0.035	2.8
	Alpha-ketoglutaramate*	0.0018	0.035	2.2
Lysine Metabolism	N-acetylglutamate	0.028	0.071	1.3
	N6-acetyllysine	0.033	0.078	2.4
	N6,N6,N6-trimethyllysine	0.038	0.085	3.2
	2-aminoadipate	0.021	0.061	2.2
Leucine, Isoleucine and Valine Metabolism	Pipecolate	0.0018	0.035	9.3
	6-oxopiperidine-2-Carboxylate	0.013	0.047	2.6
	N,N-dimethyl-5-Aminovalerate	0.0016	0.035	3.2
	4-methyl-2-oxopentanoate	0.013	0.048	1.9
	Isovalerate (i5:0)	0.019	0.057	1.8
	Isovalerylglycine	0.0072	0.04	3.1
	Beta-hydroxyisovalerate	0.019	0.057	1.9
	3-methylglutaconate	0.0022	0.035	2.2
	Isoleucine	0.026	0.068	1.8
	2-methylbutyrylcarnitine (CS)	0.018	0.056	2.5
Ethylmalonate	0.015	0.051	2.3	
Methylsuccinate	0.0084	0.04	2.1	
N-acetylvaline	0.023	0.064	2.1	
3-methyl-2-oxobutyrate	0.015	0.052	1.9	
Alpha-hydroxyisovalerate	0.0081	0.04	2.1	
Arginine Deiminase System	Arginine	0.026	0.026	3.1
	Citrulline	0.012	0.012	2.8
	N-acetylcitrulline	0.023	0.023	3.8
	N-delta-acetylornithine	0.0037	0.0037	2.6

Figure 2: Targeted Metabolomic analysis of significant amino acid metabolism and arginine deiminase system. The levels of metabolites were compared between caries-affected and caries-free infants. Only statistically significant metabolites are listed. P<0.05. Q-value gives the false discovery rate. Fold change indicates the ratio of mean scaled intensity between caries-affect and caries-free

## Conclusions

- Statistically significant differences between metabolite levels were based on the caries status, and not by the sugar reduction educational program.
- Several novel metabolites were found in significantly higher levels in Navajo infants with caries, which in combination, may serve as ECC biomarkers for this high risk population.
- Caries-affected subjects had significantly higher levels of metabolites related to food components, carbohydrate metabolism, fatty acid metabolism, amino acid metabolism, and the arginine deiminase system. These major pathways have been associated with ECC, confirming the differences in caries outcomes of Navajo infants.
- Additionally, these unique caries-related metabolite signatures can be used in developing ECC risk factors and therapeutic targets for future preventive strategies.

Future directions include evaluating the correlation between significant metabolites and caries experience, and between metabolomic and microbiome data.

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