Lactosylceramide Promotes Cardiac and Mitochondrial Dysfunction in a Novel in vivo Heart Failure Model of Hypoplastic Left Heart Syndrome

Ashley E. Pietra[a]*, Mary E. Turner[a], Genevieve Sparagna[b], Anis Karimpour-Fard[b], and Anastacia M. Garcia[a]

[a]Department of Pediatrics, Section of Cardiology, University of Colorado Anschutz Medical Campus, Children's Hospital Colorado, Aurora, CO, United States,

[b]Department of Medicine, Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States,

*Presenting Author

Children's Hospital Colorado

University of Colorado Anschutz Medical Campus

Background

Single ventricle (SV) congenital heart disease (CHD) encompasses a heterogeneous group of severe abnormalities in cardiac structure where the abnormal development of the fetal heart results in only one functional ventricle.

The most common form of SV is Hypoplastic Left Heart Syndrome (HLHS). HLHS is characterized by "hypoplastic" or severe underdevelopment of the left ventricle, ascending aorta, and mitral valve. These cardiac anomalies generate a physiologically relevant hypoxic phenotype, where oxygen saturation can greatly fluctuate usually sitting at an average of around 70%.

A majority of HLHS patients undergo a series of three staged palliation surgeries to form a single systemic pumping chamber that results in passive flow to the pulmonary circuit (Figure 1). **Despite these remarkable advancements in surgical and post-operative care increasing the pre-transplant survival of HLHS patients, HLHS heart failure (HF) remains the leading indication for heart transplantation in infancy and is the most common cause of cardiovascular death in infants.**

The molecular mechanisms and progression to HF in HLHS are poorly understood, however increasing evidence suggests that both inflammation and mitochondrial dysfunction have pathogenic relevance in promoting or potentiating pathological cardiac remodeling and HF progression in HLHS. Specifically, our lab has determined that HLHS HF patients have significantly elevated levels of the glycosphingolipids (GSL), lactosylceramide (LacCer) (Figure 2), which may play a role in modulating cardiomyocyte and immune cell function and HLHS HF progression.

While there are currently no post-natal animal models of HLHS, this study aimed to assess whether exogenous LacCer can recapitulate *in vivo*, some of the salient cardiometabolic and inflammatory derangements seen in HLHS pathophysiology and ultimately identify novel therapeutic targets for this vulnerable population.

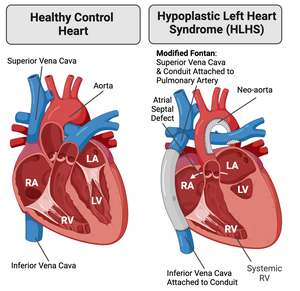

Figure 1. Anatomy of the Single Ventricle (SV) Heart. As compared to the healthy control heart, patients born with Hypoplastic Left Heart Syndrome (HLHS) have severe underdevelopment of the left ventricle (LV) and rely on a single systemic right ventricle (RV).

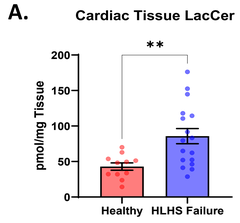

Figure 2: Elevated levels of Lactosylceramide (LacCer) in Cardiac tissue and Peripheral Blood Immune Cells (PBMCs) from patients with HLHS heart failure (HF). (A) Total RV myocardial LacCer, n=10 healthy and n=13 HF. (B) Total LacCer in purified PBMCs, n=6 healthy and n=7 HF. For all groups, bar equals mean±SEM; asterisk (*) denotes significant difference between groups, *p<0.05,**p<0.01; Welch's T-test.

Hypothesis

Aberrant LacCer-mediated signaling drives maladaptive cardiac, lung, and immune cell responses that predispose HLHS patients to progressive cardiac dysfunction and ultimately heart failure.

Methods

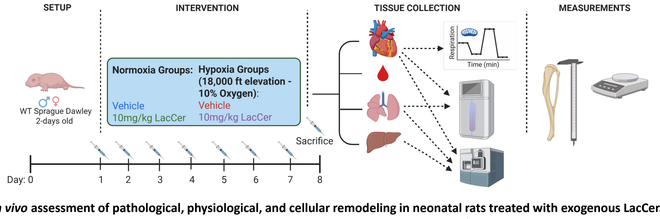

Figure 3. *in vivo* assessment of pathological, physiological, and cellular remodeling in neonatal rats treated with exogenous LacCer. Exogenous LacCer or Vehicle was delivered to two-day old neonatal WT Sprague Dawley rats by IP injection for 1 week. Animals were housed in normoxia (5,471ft elevation), or hypoxia (18,000ft elevation; 10% oxygen). After 1 week, animals were sacrificed and morphologic measurements were normalized to tibial length. Harvested organs were flash frozen, and serum was isolated from whole blood for downstream assays.

Results

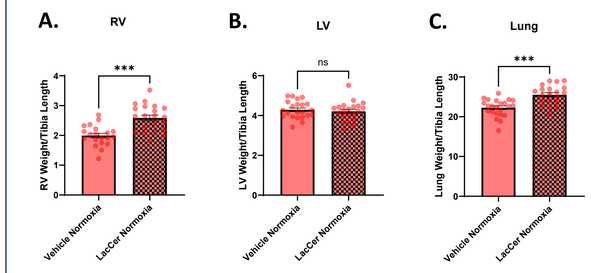

Figure 4. LacCer is Sufficient to Induce RV Hypertrophy and Significantly Enlarged Lung Mass *in vivo*. **(A)** RV size, **(B)** LV size, and **(C)** lung mass quantification, n = 22 vehicle normoxia, n=21 LacCer normoxia. For all groups, bar equals mean±SEM; asterisk (*) denotes significant difference between groups. ***p<0.001 Welch's T-test.

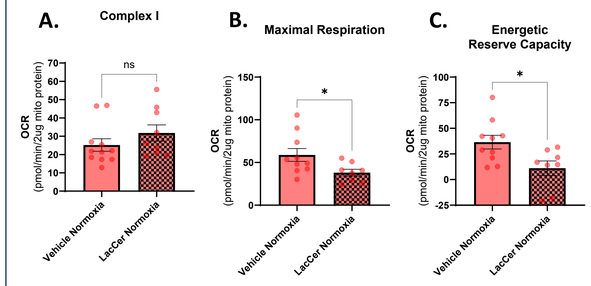

Figure 5. *in vivo* LacCer is Sufficient to Alter Myocardial Mitochondrial Bioenergetics. (A) Complex 1 Respiration, **(B)** Maximal Mitochondrial Respiration, and **(C)** Energetic Reserve Capacity in isolated cardiac mitochondria, n = 10 vehicle normoxia, n=9 LacCer normoxia. For all groups, bar equals mean±SEM; asterisk (*) denotes significant difference between groups. *p<0.05, Welch's T-test.

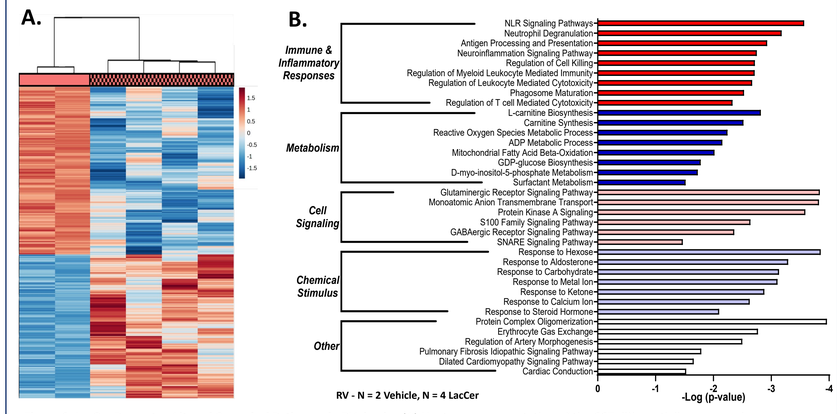

Figure 6. LacCer Promotes Altered Canonical Pathways in RV, *in vivo* (A) Heatmap representation of the 667 differentially expressed genes between exogenous LacCer treated RV and vehicle control RV **(B)** Significantly dysregulated pathways; canonical pathways related to Immune and Inflammatory Responses, Metabolism, Cell Signaling, Chemical Stimuli and Hormonal Responses, were identified with Ingenuity Pathway Analysis and Metaboanalyst using the 667 genes that changes significantly in response to LacCer in the RV; (n=2 Vehicle Control and n=4 LacCer; Fisher's exact test, -log10 (P-value) >1.3 or P<0.05.

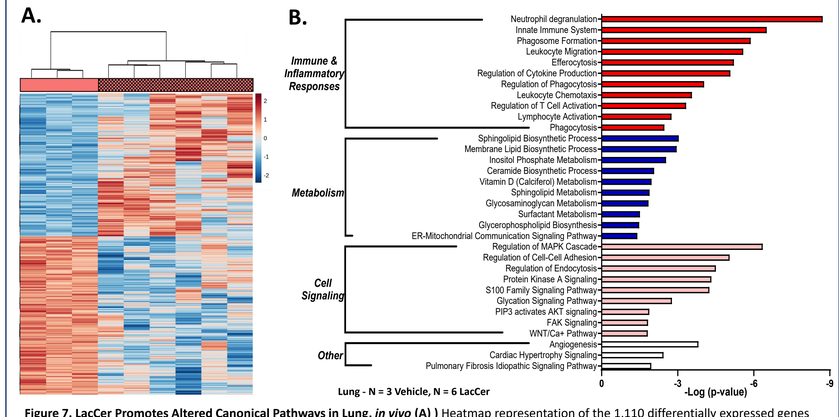

Figure 7. LacCer Promotes Altered Canonical Pathways in Lung, *in vivo* (A)) Heatmap representation of the 1,110 differentially expressed genes between exogenous LacCer treated lung and vehicle control lung **(B)** Significantly dysregulated pathways; canonical pathways related to Immune and Inflammatory Responses, Metabolism, and Cell Signaling, were identified with Ingenuity Pathway Analysis and Metaboanalyst using the 1,110 genes that changes significantly in response to LacCer in the lung (n=3 Vehicle Control and n=6 LacCer; Fisher's exact test, -log10 (P-value) >1.3 or P<0.05.

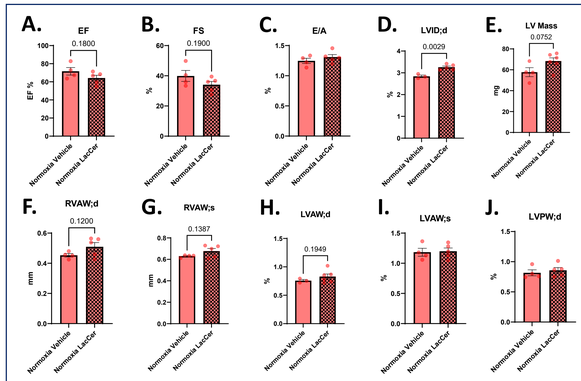

Figure 8. Exogenous LacCer *in vivo*, is Sufficient to Induce Changes in Cardiac Function as measured by Echocardiogram. Echocardiographic analysis was performed on the neonatal rats using a Vevo2100 system equipped with an MS400 18–38 mHz transducer (VisualSonics). **(A)** Ejection Fraction (EF), **(B)** Fractional Shortening (FS), **(C)** E Wave/ A Wave Ratio (E/A), **(D)** Left Ventricular Internal Dimension; End-Diastole (LVID;d), **(E)** corrected LV Mass, **(F)** Right Ventricle Anterior Wall in Diastole (RVAW;d), **(G)** Right Ventricle Anterior Wall in Systole (RVAW;s), **(H)** Left Ventricle Anterior Wall in Diastole (LVAW;d), **(I)** Left Ventricle Anterior Wall in Systole (LVAW;s), **(J)** Left Ventricle Posterior Wall in Diastole (LVAW;d), n = 4 vehicle normoxia, n=5 LacCer normoxia. For all groups, bar equals mean±SEM; P-value as indicated using Welch's T-test.

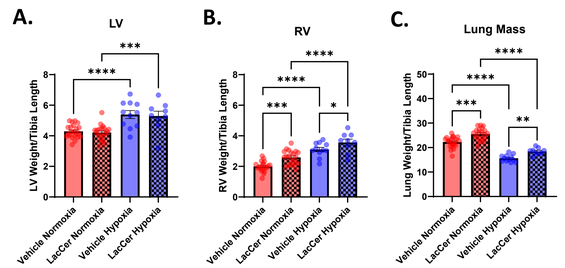

Figure 9. In hypoxic conditions, LV hypertrophy is induced independent of exogenous LacCer treatment, while RV hypertrophy is further exacerbated with exogenous LacCer treatment. (A) LV size, **(B)** RV size, and **(C)** lung mass quantification, n = 22 vehicle normoxia, n=21 LacCer normoxia, n=11 vehicle hypoxia, n=9 LacCer hypoxia. For all groups, bar equals mean±SEM; asterisk (*) denotes significant difference between groups. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 Ordinary one-way ANOVA, post-hoc Holm-Sidak's multiple comparisons test

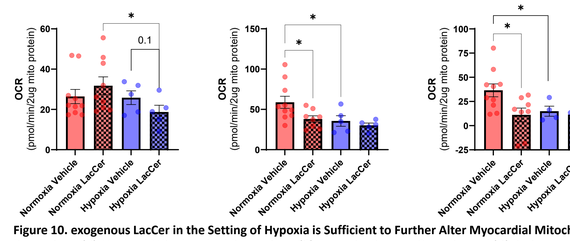

Figure 10. exogenous LacCer in the Setting of Hypoxia is Sufficient to Further Alter Myocardial Mitochondrial Function *in vivo* **(A)** Complex 1 Respiration, **(B)** Maximal Mitochondrial Respiration, **(C)** Energetic Reserve Capacity, and **(D)** Proton Leak in isolated cardiac mitochondria, n = 10 vehicle normoxia, n=9 LacCer normoxia, n= 5 vehicle hypoxia, n= 5 LacCer hypoxia. For all groups, bar equals mean±SEM; asterisk (*) denotes significant difference between groups. *p<0.05, **p<0.001, Ordinary one-way ANOVA, post-hoc Holm-Sidak's multiple comparisons test

Conclusions

- The failing HLHS myocardium is typified by impaired mitochondrial bioenergetics and altered immune and inflammatory signaling.
- Lactosylceramide (LacCer), is significantly increased in failing HLHS myocardium.

Here, we show that exogenous LacCer can recapitulate *in vivo*, some of the salient cardiometabolic and inflammatory derangements seen in HLHS pathophysiology, including:
(1) In the absence of hemodynamic stress, LacCer contributes to pathological cardiac remodeling and lung inflammation, marked by RV hypertrophy, increased lung mass, and altered inflammatory, metabolic, and signaling pathways. (2) Exogenous LacCer induces changes in cardiac function including decreased EF, as measured by echocardiogram. (3) In hypoxia, LV hypertrophy was induced, independent of the exogenous LacCer treatment, while RV hypertrophy was further exacerbated. (4) This novel *in vivo* model of HLHS heart failure can serve as a useful mechanistic platform to investigate signaling pathways and identify novel therapeutic targets for HLHS.
Future Directions include: Further analysis of *in vivo* molecular changes in hypoxia +/-LacCer, including tissue and single cell gene expression, identification of LacCer induced circulating factors, and heart and lung tissue histology to assess alterations in immune cell infiltration.

Acknowledgments

This work was supported by the **National Heart, Lung, and Blood Institute (NHLBI)** R01 Award to Anastacia M. Garcia, as well as by awards from the **American Heart Association** and the **Additional Ventures** Foundation.

Many thanks to the entire Garcia Lab Team, including Praveen Ramaswamy, Chase Bolding, Efren Montelongo, and Cristina Velasquez.