Pulmonary Hypertension Associated with Congenital Heart Disease: Translating Natural History into Pathobiology

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Abstract

The natural history of pulmonary hypertension (PH) associated with congenital heart disease (CHD) diverges depending upon hemodynamic parameters: patients exposed to increased pulmonary blood flow (PBF) and pulmonary pressure (PAP) develop PH more commonly than patients exposed to increased PBF alone. To investigate the hypothesis that differing mechanical forces drive differing physiologic and molecular responses, we developed two models of CHD utilizing fetal surgical techniques: 1) left pulmonary artery (LPA) ligation resulting in increased PBF alone; and 2) aortopulmonary shunt placement resulting in increased PBF as well as PAP. Hemodynamic and molecular studies were performed on control, LPA, and shunt lambs as well as pulmonary artery endothelial cells (PAECs) derived from them.

RNA Sequencing revealed excellent separation between groups via both principal components analysis and unsupervised hierarchial clustering. Physiologically, LPA lambs demonstrated an exaggerated increase in PAP following vasoconstricting stimuli compared to controls, but endothelium-dependent vasodilation remained intact; shunt lambs, however, demonstrated excessive vasoconstriction and impaired relaxation. These contrasting physiologic findings were associated with preserved and coupled NO signaling in the PAECs of LPA lambs, compared to shunt, as demonstrated by preserved bioavailable NO (NOx) and normalized reactive oxygen species (ROS) levels. In addition, we found hyperproliferation of PAECs from shunt but not LPA lambs at 72 hours (2.2±0.01-fold control, $p<0.05$.). This was associated with altered HIF-1α protein, which was increased modestly in LPA, but more robustly in shunt PAECs (5.2±0.4- and 8.7±0.1-fold control, respectively, $p<0.01$). Also, RNA seq datasets revealed highly enriched metabolic biologic processes in shunt PAECs. Differential proliferative phenotypes between LPA and shunt PAECs may be driven by synergy between HIF-1α and increased ROS driving a disequilibrium in antiproliferative NO signaling.

A further understanding of mechanical force-specific drivers of pulmonary vascular disease will enable development of precision therapeutics for PH associated with CHD.

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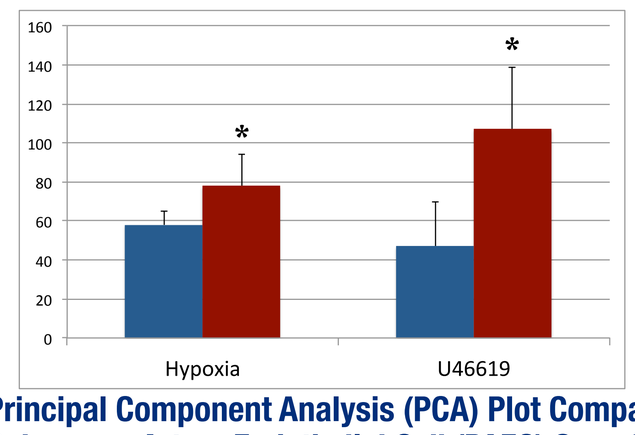
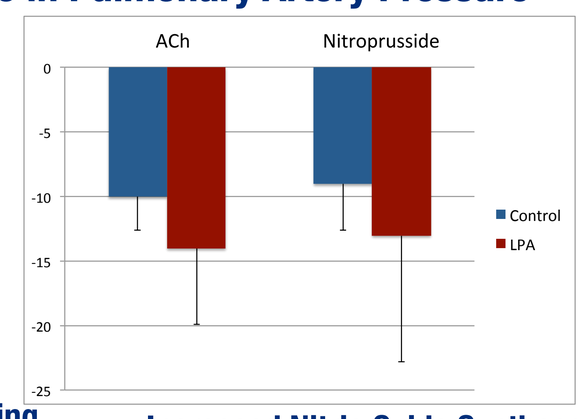
Baseline Hemodynamics in Control, LPA ligation, and Shunt Animals

	Control	LPA	Shunt
Pulmonary Artery Pressure (mmHg)	14.2±2.2	20.6±4.1	**42.5±15.1**
Pulmonary Blood Flow (RPA) (mL/min)	673±80	**1518±353**	**2272±801**
Pulmonary Vascular Resistance (mmHg/L per min)	9.2±0.6	12.0±3.8	18.2±17.7

Table: Baseline Hemodynamics in Control, LPA ligation, and Shunt animals (Shunt data were previously published Reddy In Utero Placement of Aortopulmonary Shunts *Circulation* 1995.) Values shown in **bold** were statistically significant with $p<0.05$

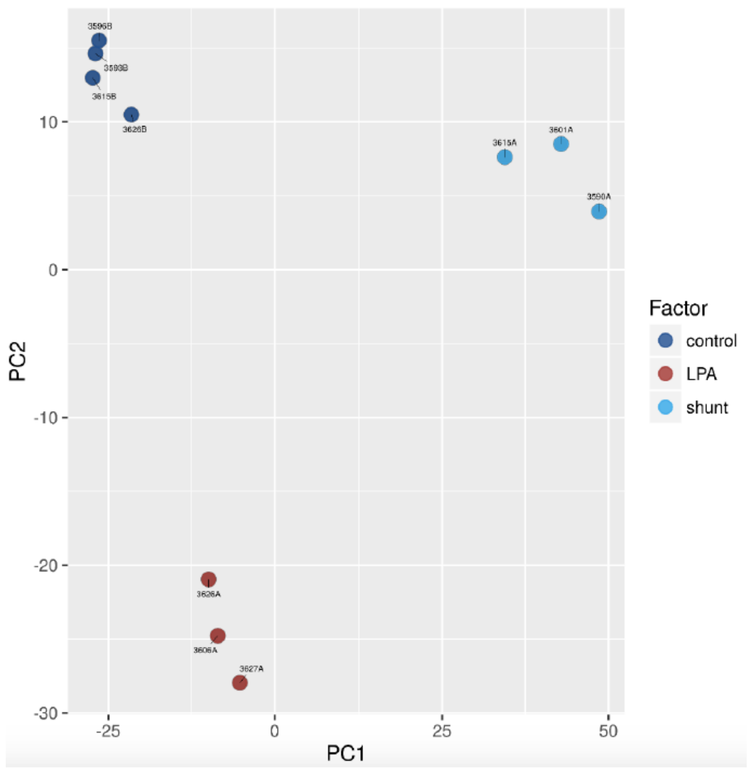
Response to Vasodilating and Vasoconstricting Stimuli in Control and LPA Ligation Animals Percent Change in Pulmonary Artery Pressure





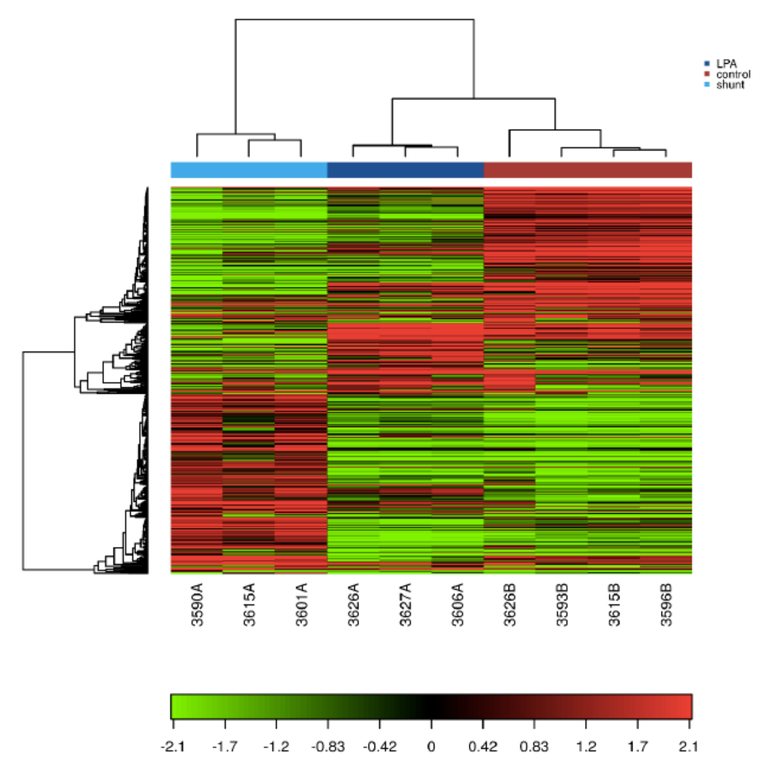
LPA ligation lambs have excessive pulmonary vasoconstriction in response to vasoconstricting stimuli: hypoxia (FiO_2 10%) and U46619 (1 μg/kg/min). In contrast, LPA ligation lambs have normal pulmonary vasodilation in response to both endothelium-dependent (acetylcholine, ACh) and endothelium-independent stimuli (nitroprusside). n = 5 in both groups. * denotes $p < 0.05$, compared to control.

Principal Component Analysis (PCA) Plot Comparing Pulmonary Artery Endothelial Cell (PAEC) Samples from Control, LPA ligation, and Shunt Lambs



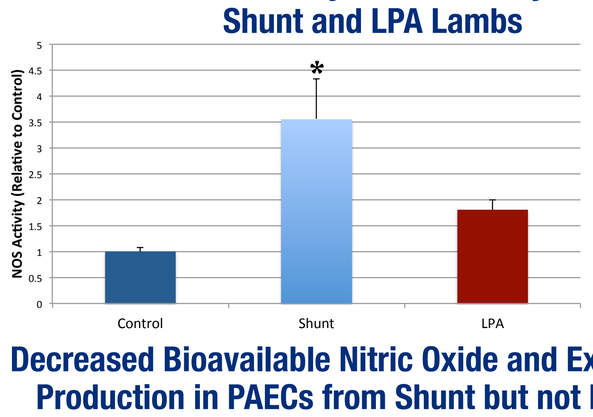
Principal component analysis (PCA) plot. The PCA was performed on all samples passing QC using the top 500 genes that have the largest coefficient of variation based on FPKM counts. Each dot represents a sample. Based on Normalized FPKM (abundance) for each gene for each sample.

Heat Map and Unsupervised Clustering Comparing PAEC Samples from Control, LPA ligation, and Shunt Lambs



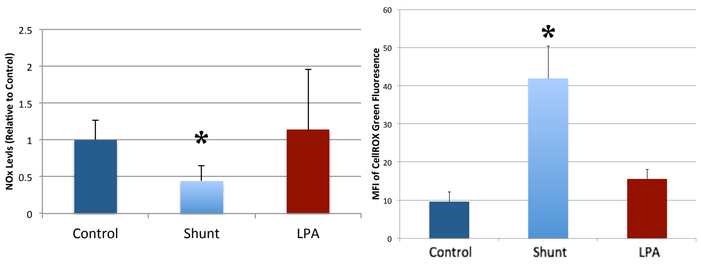
Heat Map and unsupervised hierarchical clustering by sample and transcripts was performed on all samples passing QC using the top 500 genes that have the largest coefficient of variation based on FPKM counts.

Increased Nitric Oxide Synthase Activity in PAECs from Shunt and LPA Lambs



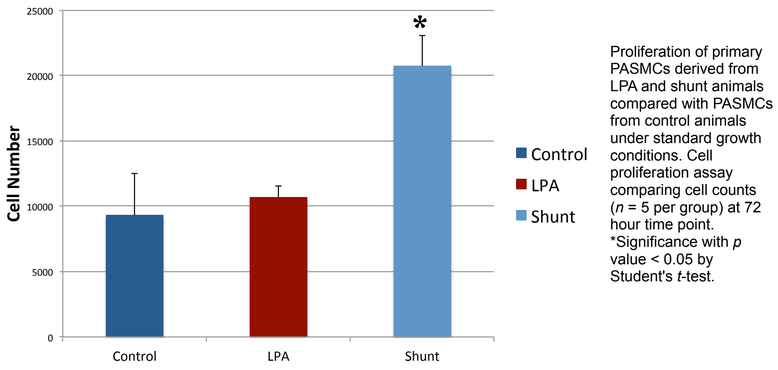
Total Nitric Oxide Synthase (NOS) activity is increased in PAECs Shunt, and, to a lesser extent, LPA lambs, compared to control animals. NOS activity was quantified using activity assay which distinguishes calcium-dependent and calcium-independent maximal NOS activity

Decreased Bioavailable Nitric Oxide and Excessive ROS Production in PAECs from Shunt but not LPA Lambs



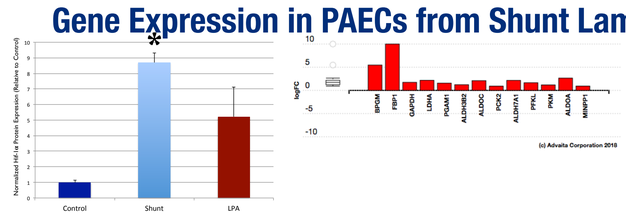
PAECs from shunt, but not LPA ligation lambs have decreased bioavailable nitric oxide (as quantified by NOx levels) and excessive intracellular reactive oxygen species (ROS) production compared to control lambs, as quantified by CellROX fluorescence $n = 3$-6 in all groups. * denotes $p < 0.05$, compared to control.

Increased Proliferation of PAECs from Shunt but not LPA Lambs



Proliferation of primary PASMCs derived from LPA and shunt animals compared with PASMCs from control animals under standard growth conditions. Cell proliferation assay comparing cell counts ($n = 5$ per group) at 72 hour time point. *Significance with p value < 0.05 by Student's t-test.

Increased Hif-1α Protein Expression and Glycolytic Gene Expression in PAECs from Shunt Lambs



Hif-1α Protein Expression is increased in PAECs from shunt and, to a lesser extent, LPA lambs. Glycolytic pathway gene targets are enriched in RNA Seq data from Shunt compared to LPA lambs. n=3-5 in each group, * $p < 0.05$. RNA Seq data shown with $q < 0.05$

Conclusions

- **Currently, PH associated with CHD is treated based on disease severity rather than mechanism of disease. Basic science models of disease may lead to more targeted application of therapy based on disparate mechanism**
- **The novel fetal surgical model of LPA ligation produces three distinct RNA expression pathways, reflective of the distinct mechanical forces associated with each model — increased PBF alone vs. increased PBF and PAP**
- **LPA lambs have intact endothelium-dependent pulmonary vasodilation and preserved bioavailable NO, while shunt lambs have decreased bioavailable NO and increased intracellular ROS, reflecting uncoupling of NOS**
- **Increased Hif-1α expression combined with increased ROS production may drive increased PAEC proliferation in shunt lambs, ultimately leading to more severe and early disease**