Aurora

WHITE PAPER

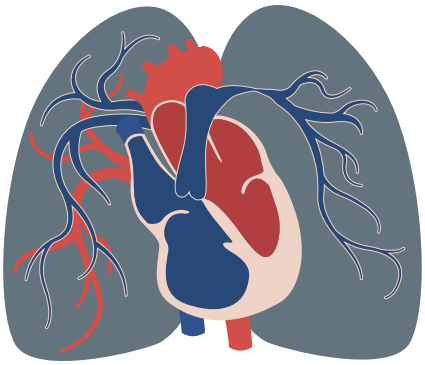
Significance of VERSA 1100 in Metabolic Profiling (John Hopkins All Children's Hospital)

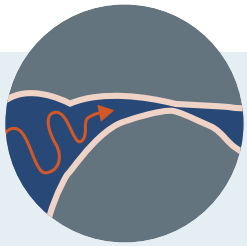
The paper "Spatial and temporal resolution of metabolic dysregulation in the Sugen hypoxia model of pulmonary hypertension (PAH)" explores the metabolic changes associated with the disease PAH, which is characterized by high blood pressure in the lungs and arteries leading to heart failure. Historically, other PAH studies have been conducted but they were limited to analyzing metabolites at a single point in time which doesn't dive into the shift in their concentrations to represent this complex condition. Recognizing this gap, John Hopkin's scientists utilized a mouse model that closely mimics the human PAH condition to examine how metabolic disturbances develop overtime across different tissues like heart, lungs and liver.

The study involved treating rodent models to develop symptoms similar to human PAH, allowing for investigating disease progression in a controlled environment. In addition, the researchers have also employed Aurora Biomed's VERSA 1100 in the study to filter and prepare the tissue samples before they were analyzed by LC-MS. The precision in sample handling by the VERSA 1100 was vital for ensuring consistent metabolic profiling, which is essential when studying metabolic changes across different tissues. By using this approach, researchers were able to identify early biomarkers of PAH and understand the metabolic pathways involved. This further allows for targeted therapies and early interventions to treat the disease.

Male Wistar rats were treated with VEGFR2 inhibitor SU5416 and exposed to hypoxia to develop PAH characteristics (SuHx models). The diseased rats' tissues were collected from heart, lungs and liver tissue after 7, 14 and 21 days post-induction providing a timeline to analyze how metabolic changes correlate with the development of the PAH disease. Control rats were also injected with the SU5416 vehicle but kept in normoxic (normal oxygen level) conditions to serve as a baseline for comparison with SuHx rats. Results showed that control rats did not exhibit the same metabolic and physical changes as the SuHx rats as they maintained normal metabolite levels and ventricular sizes throughout the experiment.

For tissue collection, the sample underwent preparation using Aurora's VERSA 1100 via solid phase extraction in a 96-well plate format. More specifically the Impact Protein Precipitation Plates from Phenomenex. Aurora's liquid handler deck layout allowed for the automation of this sample preparation protocol prior to LC-MS analysis. The data obtained was subjected to statistical analysis including PCA and PLS-DA to showcase the differences in metabolic profiles between the control and PAH-affected rats.





The study involved treating rodent models to develop symptoms similar to human PAH.

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Results and Discussion

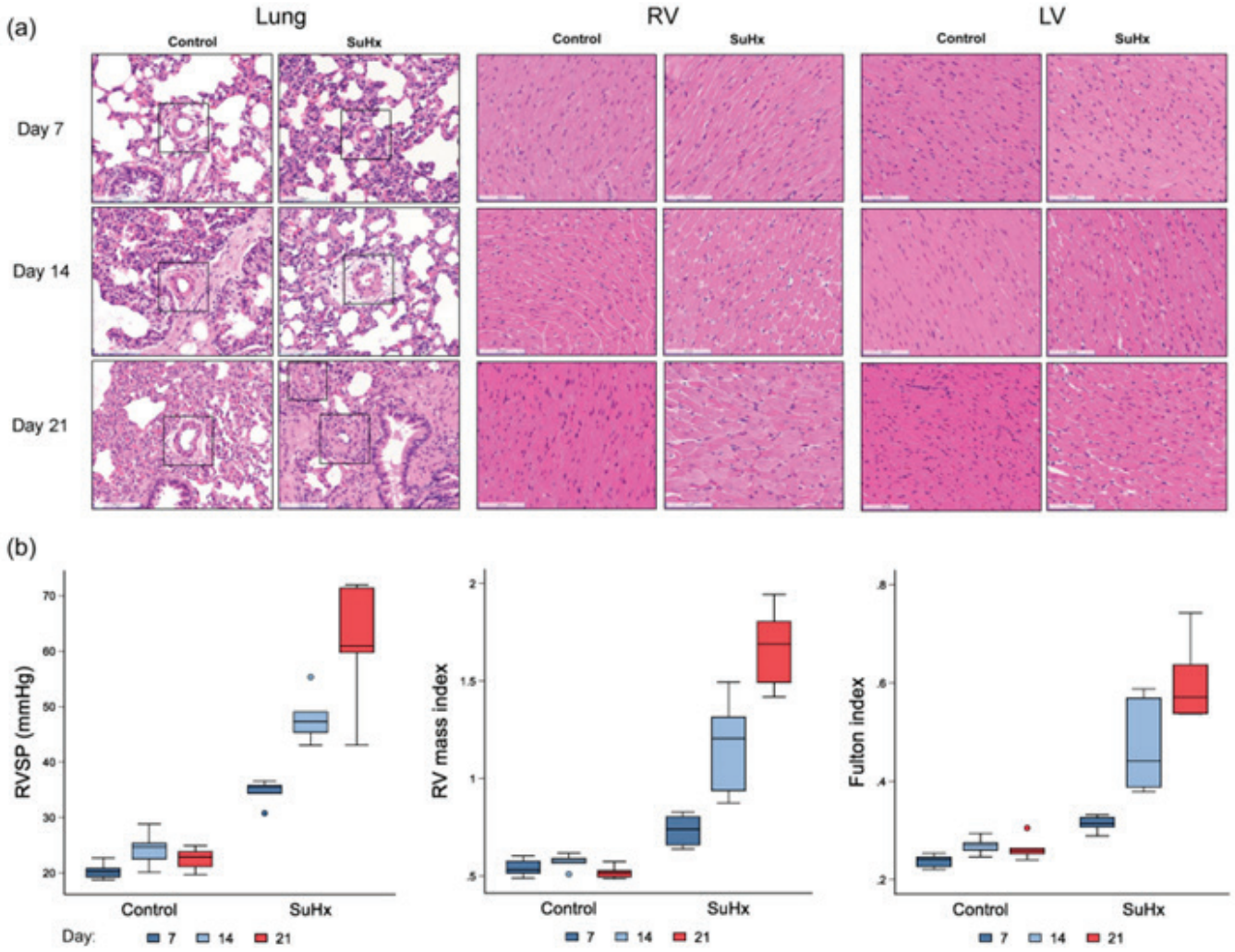

FIGURE 1. (a) H&E-stained images of lungs, right ventricle and left ventricle from control and SuHx rats at Days 7. 14 and 21. (b) Box and whisker plots demonstrating changes in RVSP, RV mass index and Fulton Index for SuHx versus control rats at Weeks 1, 2 and 3 post-inductions with Sugen.

Figure 1 is a visual format clarifying the progression of pulmonary hypertension, with rising RVSP and RV mass index indicating a worsening heart strain due to an increase in lung pressure. The Fulton index, a measure of heart remodeling, also increases which reflects the physical change in the heart's structure as the disease progresses with time.

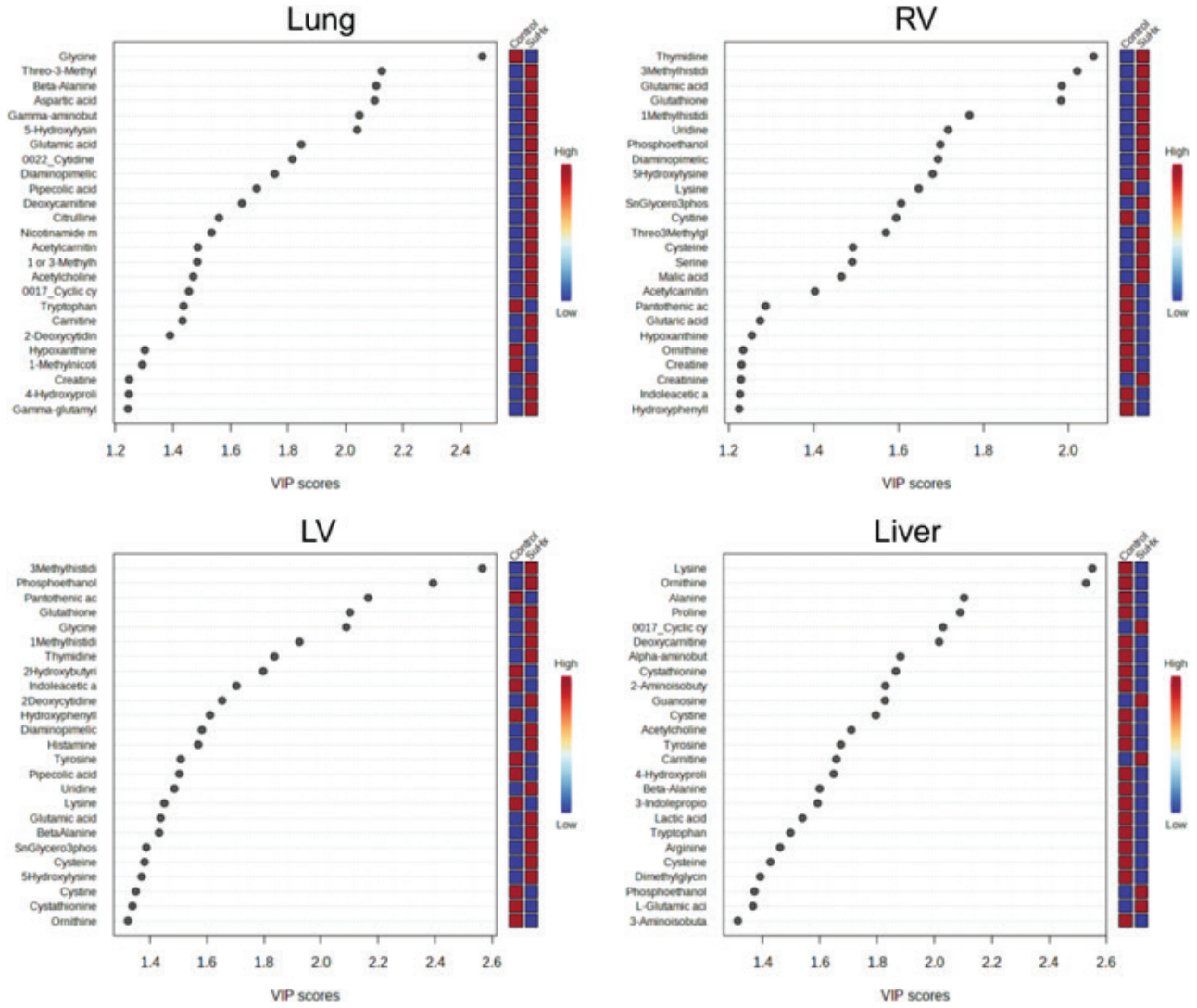

FIGURE 2. Projection Plots showing VIP scores for individual metabolites resulting from the PLS-DA models in the heart, lungs and liver. The bars to the right of the dot plots showcase relative metabolite abundance in SuHx versus control.

The variable importance in projection (VIP) scores show the degree to which each metabolite differentiates the metabolic profiles of SuHx and control rats across various tissues (heart, lungs and liver). Each bar represents a metabolite with its height indicating the VIP score. Metabolites with higher scores are more critical for separating diseased samples from controls, suggesting their significant role. The bar colors vary to show metabolite concentration differences between SuHx and control groups, providing insights into which metabolites are upregulated or downregulated when the disease progresses. This can potentially highlight biomarkers for early detection of PAH.

Following the experiment, it was concluded that metabolic disturbances in the SuHx model of PAH develop as early as one-week post-induction. This is followed by phenotypic changes which were also observed in the different types of tissue.

Findings from this study also showcase the vital role that the VERSA 1100 liquid handler played in the analysis of metabolic dysregulation in the SuHx model of pulmonary hypertension. By enabling precise and efficient sample filtration and preparation, the integrity of the metabolites was maintained and consistent throughout the experiment. The ability to accommodate high-throughput allowed for the analysis of metabolic profiles of different types of tissue which gave insights into metabolic changes that occur as the disease progresses.

In addition, the "high throughput" analysis that was made possible by the VERSA 1100 allowed for the identification of potential targets and biomarkers. This is crucial for early intervention of pulmonary hypertension, illustrating the huge impact of the VERSA 1100 in driving forward metabolic research and its applications in disease modeling and therapeutic development.