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VESSEL REACTIVITY AND BLOOD FLOW IN RATS EXPOSED TO NEONATAL SUPPLEMENTAL
OXYGEN

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A thesis submitted in partial fulfillment of the requirements
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All requirements for graduation with Honors in the
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Vessel Reactivity and Blood Flow in Rats Exposed to Neonatal Supplemental Oxygen

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ABSTRACT

Premature babies make up 12.8% of live births per year. Because their lungs are poorly developed, supplemental oxygen is a necessary treatment. Recent studies in our laboratory, in a rat model of prematurity, show that aortic pulse wave velocities were higher in rats exposed to neonatal supplemental oxygen. This is an indicator of significant aortic stiffening. This study aims to determine if supplemental oxygen also affects the downstream vasculature reactivity. We hypothesized that exposure to supplemental oxygen during the neonatal period will decrease vessel reactivity and we will observe smaller changes in blood flow with hypoxic and carbon dioxide challenges. Twelve month old rats exposed to 80% and 21% oxygen for eight days during the neonatal period were ventilated with hypoxic (12% O₂), hypercapnic (5% CO₂), and room air conditions. Each exposure lasted 10 minutes and followed with different 10 μm neutron-activated BioPAL microspheres injections into the left ventricle. The microspheres were allowed to circulate for 300-400 cardiac cycles. Microspheres lodged in the tissues were used to quantify changes in visceral blood flow. 80% O₂ exposed rats showed a decreased baseline cardiac output to tissues compared to controls. In hypoxic and carbon dioxide conditions, 80% O₂ exposed rats showed decreased changes in blood flow to tissues compared to controls, but results were not significant. Some tissues showed decreased blood flows when the rats were exposed to hypoxia and carbon dioxide challenges suggesting some vasoconstrictive effects had also occurred.

INTRODUCTION

Infants born premature have an increasing survival rate since 1991 with the introduction of surfactant therapy. Surfactant therapy has been successfully reducing mortality from respiratory distress syndrome (RDS), decreased pulmonary air leak, and lowers the risk of chronic lung disease (Polin 2014). Even though surfactant therapy has increased preterm infant survival rate, supplemental oxygen therapy has also been an essential treatment for premature infants to deliver adequate oxygen to tissues until the infant's lungs are fully developed (Tin 2007). The amount of oxygen in room air (21%) is insufficient for underdeveloped lungs to oxygenate blood to the necessary levels making higher concentrations of oxygen a necessary treatment to compensate.

While supplemental oxygen is an essential respiratory support, it has also been found to cause problems in the premature infant. Some of the long term effects include increased risks of bronchopulmonary dysplasia and retinopathy of prematurity (Jobe 2010). Continued respiratory problems into childhood have been reported including lower pulmonary function

and increased airway resistance. Further studies have shown that adults born premature have inefficient gas exchange and lower power output during exercise (Farrell 2015). In addition, neonatal supplemental oxygen induces abnormal ventilatory responses to hypoxia and hyperoxia in both infants and adults born prematurely and could possibly be a result of dysfunctional carotid chemoreceptors (Bates 2014). Recent studies in our laboratory, in a rat model of prematurity, show that aortic pulse wave velocities were higher in rats exposed to neonatal supplemental oxygen, indicating significant aortic stiffening (Bates, unpublished). Premature infants make up about 13% of live births each year and the long term effects are not yet fully understood; thus making further investigation of neonatal supplemental oxygen necessary.

This study aims to determine if supplemental oxygen affects the downstream vasculature, causing the vessels to be less reactive. We used hypoxia and hypercapnia as physiological challenges. In hypoxia and hypercapnia, vasodilation is expected to increase blood flow to splanchnic and brain vascular beds. We hypothesize that exposure to supplemental oxygen during the neonatal period will decrease vessel reactivity and we will observe smaller changes in blood flow with hypoxic and hypercapnic change. In this study, microspheres were used to quantify changes in blood flow. Based on the total microspheres injected into the blood stream, the percent of microspheres that are lodged in the precapillary arterioles of each tissue is proportional to the percent of cardiac output that perfuses that tissue.

The use of microspheres to examine blood flow has been a useful technique since 1967 due to its potential to measure cardiac output and blood flow without causing hemodynamic problems (Prinzen 2000). The microsphere technique was essential in our experimental study of determining differences in vessel reactivity.

METHODS

Animal model: A rat model of prematurity was used to test the physiological effects of neonatal oxygen exposure. The rats themselves are term-born, but their cardiopulmonary anatomy and physiology are similar to a premature infant. Because these animals are born at term, this model can be specifically used to isolate the effect of supplemental oxygen from other confounders, including growth restriction and steroid administration.

In-vivo measurements: Twelve month old rats exposed to 80% and 21% oxygen for eight days during the neonatal period made up the OXY and CON groups respectively. The OXY group modeled premature infants and the CON group modeled term born infants. Both groups of rats were anesthetized with urethane (1.3 g/kg i.p.), mechanically ventilated, and a fluid filled catheter was placed in the left ventricle. Rats were then ventilated with hypoxia (12% O₂), hypercapnia (5% CO₂), and room air in the lab. After 10 minutes of exposure, 10 μm neutron-activated BioPAL microspheres were injected into the left ventricle and allowed to circulate for at least a minute to allow 300-400 cardiac cycles. The microspheres were small enough that they could reach the precapillary arterioles but not too small that they would pass through back into circulation.

Quantification of blood flow: Once the rat had been euthanized, the brain, liver, kidneys, spleen, heart, soleus and gastrocnemius were dissected and separated to prevent cross contamination of microspheres between tissues. The tissues were separated into 15 mL conical

vials containing 5 M NaOH solution and placed in a hot bath for 24 hours. This causes hydrolysis of the tissue.

After 24 hours had passed, tissues were individually vacuum filtered through filters with 8 μm pores. These filters allow the dissolved tissue and NaOH mixture to flow through but not the microspheres within the tissue. The filters, containing the microspheres, were sent out to be counted by the manufacturer. Positive and negative controls were used to ensure no microspheres were lost or transferred into other tissues. For the positive control, a known amount of microspheres was filtered randomly between tissues. For the negative control, a blank was filtered.

RESULTS

Cardiac Output in room air conditions (21% O₂)

The effect of neonatal supplemental oxygen on baseline cardiac output tissues is shown in Figure 1 below. The data represents the percent of total cardiac output that flows to each tissue for both 80% O₂ exposed rats and 21% O₂ control rats in room air (21% O₂) conditions. Rats exposed to 80% neonatal supplemental oxygen had lower percent cardiac output to tissues when compared to controls (Figure 1). Differences between 21% and 80% oxygen exposed rats were not significant however, except in two of the tissues studied. Significant differences of percent cardiac output to tissues between 80% oxygen exposed rats and control rats are seen in the gastrocnemius ($p=0.085$) and right kidney ($p=0.021$) (Figure 1).

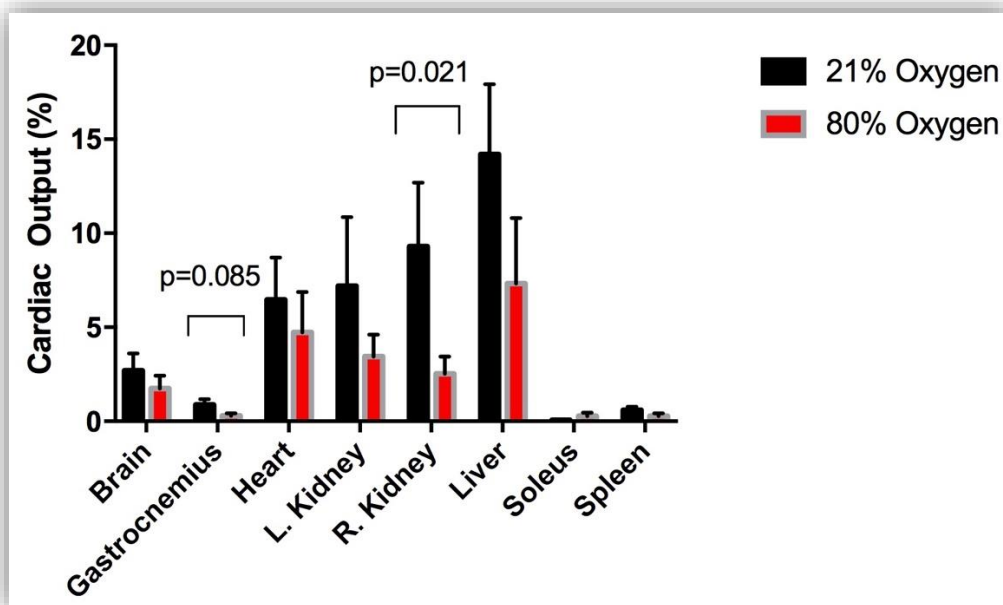


Figure 1. Neonatal supplemental oxygen exposed rats have decreased baseline cardiac output to tissues compared to control rats. The gastrocnemius and R. Kidney show a significant

difference. These cardiac output values show the baseline blood flow to tissues for both 21% and 80% neonatal oxygen exposed rats

Change in Cardiac Output with hypoxia (12% O₂).

The difference in cardiac output to tissues from 21% O₂ to 12% O₂ is shown in Figure 2. The data represents the percent change in cardiac output to each tissue for both 80% O₂ exposed rats and 21% O₂ control rats in hypoxic (12% O₂) conditions. Negative changes in cardiac output as seen in the graph indicate smaller hypoxic cardiac output than cardiac output in room air conditions. Positive changes in cardiac output indicate greater hypoxic cardiac output than cardiac output in room air conditions. All tissues show negative changes in cardiac output for both 80% O₂ exposed rats and control rats except in the heart tissue. The brain, gastrocnemius, left kidney, right kidney, liver and spleen all show less of a decrease in blood flow under hypoxic conditions for the 80% O₂ rats than control. Differences seen between control and supplemental oxygen exposed rats are not significant however (Figure 2).

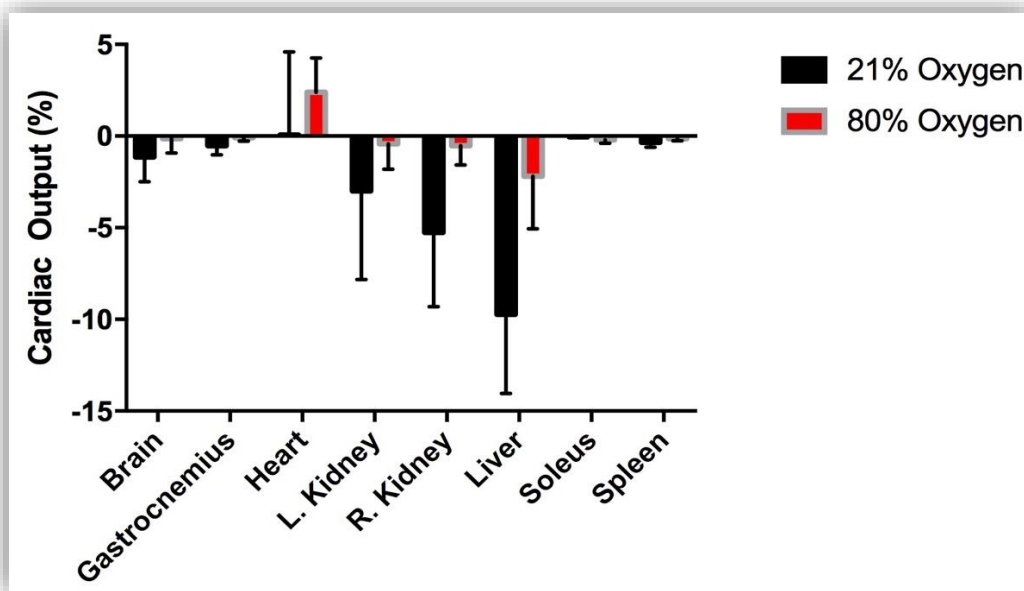


Figure 2. Neonatal supplemental oxygen exposed rats and control rats increase cardiac output to the heart in 12% O₂ hypoxic conditions, but decreased cardiac output to all other tissues. This suggests heart tissue has a greater oxygen need than other tissues during hypoxic stress. Differences in cardiac output to 80% and 21% O₂ exposed rats' tissues were not significant, but data show that hypoxia may have some vasoconstrictor effects.

Change in Cardiac Output with Carbon Dioxide Challenge (5% CO₂).

The difference in cardiac output to tissues between 21% O₂ and 5% CO₂ are shown in Figure 3 below. Negative changes in cardiac output as seen in the graph indicate smaller cardiac output

in carbon dioxide challenge than cardiac output in room air conditions. Positive changes in cardiac output indicate greater cardiac output in carbon dioxide challenge than cardiac output in room air conditions. In the 80% oxygen exposed rats, most of the tissues have an increase in cardiac output after being exposed to 5% CO₂. In 21% O₂ control rats, most of the tissues had decreased cardiac output after being exposed to 5% CO₂ except for the heart tissue (Figure 3).

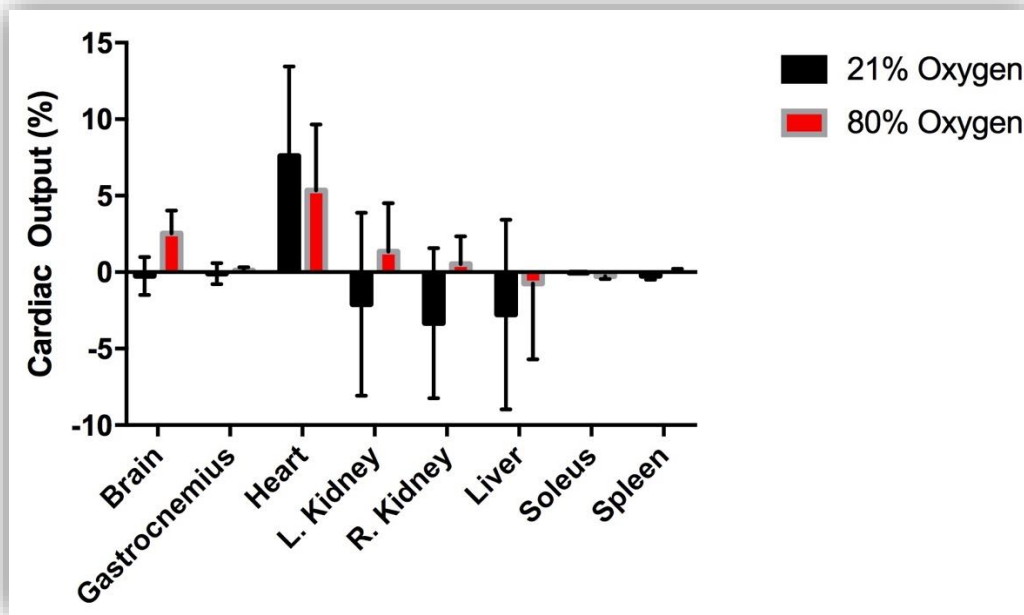


Figure 3. Carbon Dioxide Challenge (5% CO₂) greatly increases blood flow to heart tissue. This increase is seen in both supplemental oxygen exposed and control rats, but are not significantly different. Some vasoconstrictive effects are seen in the control rats when exposed to 5% CO₂. In brain tissue, hypercapnic stress has a greater impact on neonatal supplemental oxygen exposed rats than control rats.

DISCUSSION

Effect of Neonatal Supplemental Oxygen On Baseline Cardiac Output Tissues.

Neonatal supplemental oxygen exposed rats have a decreased baseline cardiac output to tissues, but the difference was only found to be significant in the gastrocnemius and R. Kidney (Figure 1). This indicates that rats exposed to supplemental oxygen may have poor tissue perfusion in room air conditions where no oxygen or carbon dioxide challenges were present.

Effect of Neonatal Supplemental Oxygen On Change in Cardiac Output with Hypoxia (12% O₂)

From the data shown in Figure 2, heart tissue seems to be more prone to the vasodilator effects than other tissues suggesting the blood flow is preferentially directed to the heart over other tissues especially in the 80% oxygen exposed rats. The negative changes in cardiac output indicate a decrease in blood flow which suggest hypoxia may have vasoconstrictive effects on some tissues while vasodilator effects on the heart. Changes in cardiac output are still greater

in control rats over rats exposed to supplemental oxygen for the other tissues (Figure 2) which would indicate that supplemental oxygen exposure decreases vessel reactivity, but the differences were not significant.

Effect of Neonatal Supplemental Oxygen On Change in Cardiac Output with Carbon Dioxide Challenge (5% CO₂)

Rats exposed to supplemental oxygen mostly increased blood flow to tissues while control rats decreased blood flow to tissues except the heart. This could indicate that rats exposed to supplemental oxygen are not able to preferentially direct blood flow to heart as well as control rats in carbon dioxide stress. The changes in blood flow are smaller for supplemental oxygen exposed rats as well. This would also suggest decreased vessel reactivity, but the differences were not significant.

Differences in cardiac output to 80% and 21% O₂ exposed rats' tissues were not significant suggesting hypoxic stress and carbon dioxide challenges may not be enough to expose differences in vessel reactivity. To further this study, the use of nitric oxide as a vasodilator may show a significant difference in the vessel reactivity of rats exposed to neonatal supplemental oxygen compared to controls.

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