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NEONATAL SUPPLEMENTAL OXYGEN EXPOSURE PROMOTES THE DEVELOPMENT OF METABOLIC DISEASE IN ADULT RATS

by

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All requirements for graduation with Honors in the
Health and Human Physiology have been completed.

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Background

The survival rate for prematurely born (<37 weeks gestation) babies has increased dramatically in the past 25 years, resulting in a large population of prematurely born adults. Since then, these preterm babies have been commonly treated with supplemental oxygen (>80%) immediately after birth, during the critical window of development. Supplemental oxygen treatment facilitates oxygen diffusion in the undeveloped lungs of the prematurely born infant, and assists synthesized surfactant to allow for survival.

The long-term effects of this early life exposure are poorly understood, and are becoming increasingly relevant with the growth in the affected population. While it is well-established that oxygen exposure causes damage to the eyes and lungs of the premature infant, there is a need for information about the effects of supplemental oxygen on other physiological processes, including metabolism. Adults born prematurely are at an increased risk of obesity and diabetes (4).

Emily Farrel, et. al collected additional supporting information in the study titled, *Pulmonary Gas Exchange and Exercise Capacity in Adults Born Preterm* (3). This study provided information about the subclinical disadvantage experienced by adults born prematurely under exercise conditions. Adults born prematurely consumed the same amount of oxygen, but had a lower power output during a high-intensity exercise session.

The goal of this study was to understand the impact of supplemental oxygen exposure in the neonatal period on metabolic function. There were several ways to investigate this question,

but the most effective and informative experiment at this juncture was to focus on the development of a diabetic phenotype. To answer our questions, we used a well-established rat model of premature birth in which newborn rats were exposed to supplemental oxygen for the first 14 days after birth. We hypothesized that exposure to supplemental oxygen immediately after birth caused impairments in the response to a glucose challenge, and that these rats developed a diabetes phenotype earlier than controls when offered a high fat diet. As secondary endpoints, we also evaluated weight gain and food intake in this model.

Methods

Pregnant Sprague-Dawley rats were obtained in pairs from Charles River. At the time of delivery, one dam was placed in a plexiglass environmental cabinet containing 80% O2 (OXY) and the other was placed in a cabinet containing 21% O2, as a control (CON). Pups were maintained in the chambers for 14 days. Dams were offered standard chow and water, as well as water ad libitum, and rotated daily to prevent hyperoxic lung injury. Post exposure, all rats were returned to standard housing.

Experiment 1 – Baseline Glucose Tolerance: At 14 days and 12 months post-exposure, glucose tolerance was evaluated in OXY and CON rats. These rats were fasted for eight hours, then baseline glucose was measured using a standard, clinical glucose meter. Blood was taken from the tails of OXY and CON rats. An intraperitoneal injection of glucose was administered (3g/kg, time = 0 minutes), followed by glucose monitoring at set time points. Specifically, these time points were: 15 minutes, 30 minutes, 60 minutes, and 120 minutes post-administration. Then, rats were returned to normal housing.

Experiment 2 – High Fat Diet: Eight-week-old OXY and CON rats were randomly assigned to a high fat diet, with 60% of calories from animal fat, or to a control diet for nine weeks. Total body

weight and food intake was measured 5 days a week for all subjects. Once per week for eight weeks, fasting blood-glucose measurement was measured, followed by a glucose tolerance test, as described above. Plasma insulin concentration was measured every three weeks, 30 minutes after glucose injection.

Data Analysis: Results were analyzed with a repeated measures, nested general linear model (Minitab, Stat College PA) where the animal was nested in the condition. Post hoc comparisons were completed with a Dunnett test where the 21% control chow group was designated as the control. Significance was considered when $p<0.05$.

Results

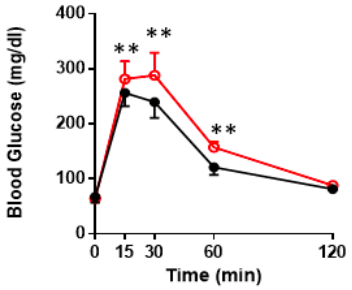

Figure 1: Blood glucose concentration versus time points during glucose tolerance tests. This data has been analyzed using a repeated measures ANOVA test, considering age, oxygen exposure groups, and time. Age groups were combined.

Groups with 21% oxygen exposure exhibit an expected blood-glucose concentration curve, rising after glucose administration and returning to baseline around 120 minutes (3). Analysis of blood glucose concentration at each time point during the glucose tolerance tests shows statistically significant difference at 15 minutes, 30 minutes, and 60 minutes between groups of different oxygen exposure. Concentration is significantly higher in the group with 80% supplemental oxygen exposure during the glucose tolerance test. Baseline and final concentrations are similar between oxygen exposure groups on the same diet.

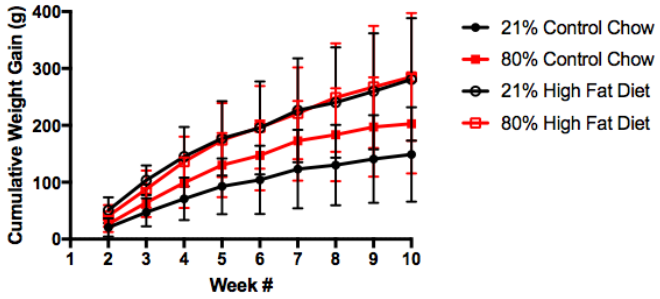

Figure 2: Average cumulative weight gain of rats over the course of the nine-week protocol. Groups are divided by oxygen exposure and diet.

Figure 2 displays the cumulative weight gain for all groups, in which both high fat diet groups follow a similar pattern. Rats under the 80% control chow condition also gained more weight than their 21% exposed control chow counterparts. Statistical analysis of all groups revealed significance in all groups compared to the 21% control chow group.

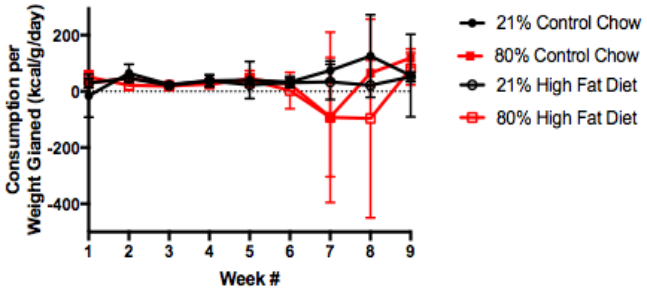

Figure 3: Comparison of caloric consumption per weight gained on a daily basis averaged across each week of the protocol. Each group is defined by oxygen exposure and diet.

Weight gain efficiency is plotted for the duration of the nine-week protocol in Figure 3, where weight gain efficiency is determined in kilocalories consumed per gram of total body weight each day. All treatment groups exhibit similar patterns for all nine weeks, and no significance was found (*p>0.05).*

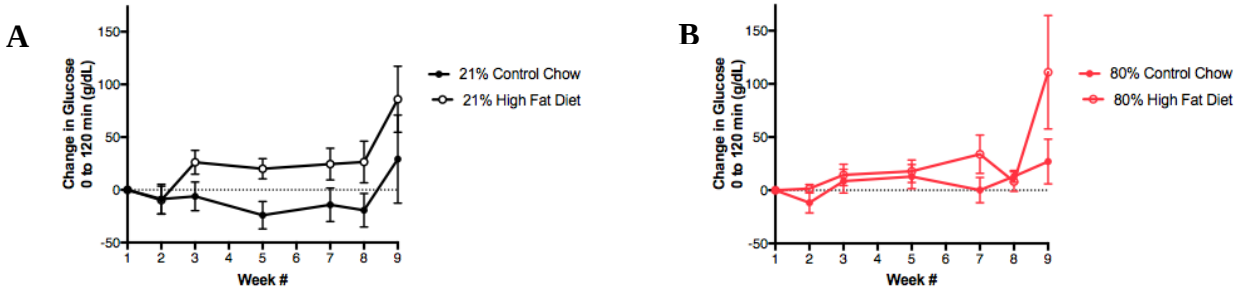

Figure 4: Average blood-glucose concentration difference between the 120-minute time point and the baseline measurements per week for rats exposed to 21% (*Figure 2.A*) and 80% (*Figure 2.B)* neonatal oxygen as compared to the value calculated for the first week of the protocol. Groups are divided by assigned diet.

In Figure 4, the difference in blood-glucose concentration at time = 0min and time = 120min during a standard, fasted glucose tolerance test as compared to the value calculated for the first week increased significantly in the 80% exposed high fat diet group (*p=0.003*) when compared to the control group (21% exposed, control chow). The 120min-0min difference of the 21% exposed high fat diet group is trending toward being increased from the control, but is not significant. The 80% exposed control chow group is not significantly different from the control.

Discussion

The results of this study have illuminated the way supplemental oxygen exposure in the neonatal period impacts metabolic function through discovery of differences in glucose handling and weight gain. One portion of the study analyzed the difference between 21% and 80% oxygen-exposed rats of different ages. Another part of the study was the effects of a high fat diet in combination with 21% and 80% oxygen exposure. Finally, weight gain was recorded and displayed in Figure 2 as an additional measure of metabolism. These findings are an important part of understanding the health and well being of a new population of people that have only recently begun to reach adulthood, and could have an impact on how adults born prematurely are treated for metabolic diseases such as diabetes.

How does neonatal supplemental oxygen impact glucose tolerance? Because a statistically significant increase in blood-glucose concentration was observed at the 15, 30, and 60 minute time points, evaluation of glucose tolerance curves in Figure 1 suggests that neonatal 80% supplemental oxygen affects the response to a glucose challenge in rats regardless of age. While this fact alone does not constitute a diabetic phenotype, it does indicate improper handling of glucose and hyperglycemia at these points, which can cause damage to internal organs if sustained. The fact that there was no statistical difference between the 14-day-old and 12-month-

old rats indicated that supplemental oxygen exposure causes immediate and sustained metabolic impairments. These results also justified further inquiry into the effects of a high fat diet in addition to oxygen exposure on the development of a diabetic phenotype, to see if this diet would exacerbate the improper handling of glucose.

How does the combination of high fat diet and supplemental oxygen affect weight gain? All non-control (21% high fat diet, 80% high fat diet, and 80% control chow) groups in the protocol experienced statistically significant weight gain as compared to the 21% control chow group. Cumulative weight data is provided in Figure 2 and it shows that the high fat diet groups were similar between the two oxygen exposure conditions. However, there is a significant difference between the 80% and 21% control chow groups, indicating that supplemental oxygen can cause additional weight gain between subjects on the same diet. In order to understand the factors that contribute to this excessive weight gain, we evaluated the kilocalories consumed per gram of body weight per day between the four groups in Figure 3. This weight gain efficiency was not significantly elevated or depressed in any one group, leaving the conclusion that the 80% exposed control diet group was hyperphagic compared to the control chow 21% exposed group. Other studies, such as that performed by Archer et al. with adult Sprague-Dawley rats on a high fat diet show that the rats gained a maximum average total body weight of 600 to 700 grams, which is consistent with our findings (1). It is therefore possible that the two high fat diet groups have such similar cumulative weight gain because the rats were physically incapable of gaining more weight than they did during the protocol.

How does a high fat diet in addition to neonatal oxygen exposure impact glucose handling? Impaired glucose tolerance is expressed by the failure of blood-glucose concentration to return to a similar level as the baseline measurement (2). In Figures 4.A and 4.B, the

difference between concentration at the 120 minute time point and the baseline measurement is compared to the value calculated for the first week of the study to express changes across the nine week protocol. Increased changes from the initial 120min-0min calculation suggest that glucose handling is becoming more impaired as the blood-glucose concentration fails to return to original levels by larger margins after a glucose challenge, ultimately leading to hyperglycemia. As seen in Figure 4, rats exposed to 80% oxygen and put on a high fat diet began to display significant improper glucose handling after four weeks, as compared to the control group. The 80% control chow group and 21% exposed high fat diet both did not exhibit significantly higher 120-0min blood-glucose change across the nine-week protocol. However, the 80% oxygen exposed rats given the high fat diet group did display significant hyperglycemia by the end of the protocol. It is therefore reasonable to conclude that the relation between the supplemental oxygen exposure and the high fat diet is responsible for the improper glucose response.

What can be done in the future to further this study? As of now, hyperglycemia has been established in subjects exposed to supplemental oxygen during the critical developmental window, but a true diabetic phenotype will require analysis of plasma insulin activity. Examination of GLUT4 receptors will expose the effectiveness of glucose uptake at the receptor level in subjects with oxygen-exacerbated impaired glucose, as well. Pancreatic RNA-sequencing will illuminate the protein synthesis in rats with impaired glucose response as well, which will provide understanding of pancreatic function. This study has also established the effect of supplemental oxygen on weight gain, but further investigation into the body composition of subjects under these conditions will confirm or deny the presence of enhanced adiposity.

What does this study mean from a clinical perspective? Neonatal supplemental oxygen clearly creates adverse effects on glucose handling and weight gain, but it is also necessary for facilitating oxygen diffusion for infants born before 37 weeks gestational age. The aim of our study has been to determine the development of a diabetic phenotype, and going forward is to continue this pursuit and to determine the mechanism by which hyperglycemia and enhanced adiposity occurs in the face of supplemental oxygen and a high fat diet. From there, prematurely born adults will not only be able to make informed decisions about their health, but we will be much closer to finding potential additional treatments to reduce the impairments caused by supplemental oxygen exposure.

References

1. Archer, Z. A., Rayner, D. V., Rozman, J., Klingenspor, M. and Mercer, J. G. (2003), Normal Distribution of Body Weight Gain in Male Sprague-Dawley Rats Fed a High-Energy Diet. Obesity Research, 11: 1376–1383. doi:10.1038/oby.2003.186

2. Ernsberger P and Koletsky RJ. The glucose tolerance test as a laboratory tool with clinical implications. *Research Gate* 2012.

3. Farrell ET, Bates ML, Pegelow DF, Palta M, Eickhoff JC, O'Brien MJ, and Eldridge MW. Pulmonary Gas Exchange and Exercise Capacity in Adults Born Preterm. *Annals of the American Thoracic Society* 2015.

4. Thomas EL, Al Saud NB, Durighel G, Frost G, and Bell JD. The effect of preterm birth on adiposity and metabolic pathways and the implications for later life. Clinical Lipidology 7: 275-288, 2012.