ANDES PEDIATRICA
REVISTA CHILENA DE PEDIATRÍA
www.scielo.cl

Andes pediatr. 2022;93(3):392-399
DOI: 10.32641/andespediatr.v93i3.3895

ORIGINAL ARTICLE

Histomorphological changes in newborn rat lung induced by maternal sugar intake

Cambios histomorfológicos en pulmón de rata recién nacida inducidos por ingesta materna de azúcar

Héctor Javier Nava Reyes[a], Luis Felipe Domínguez Robert[a], Ramiro Pardo Ramírez[b], Alberto Pizaña Venegas[b], Guadalupe Hiriart Valencia[c], Erika Liliana Monterrubio Flores[c], Juan Francisco Nava Aguirre[a], Elías Parra Hernández[d], Armando Cristian Sánchez Lara[e], Pedro Zamudio Cortés[a]

[a]Laboratorio De Farmacología, Instituto Nacional de Enfermedades Respiratorias. "Ismael Cosío Villegas" (INER). Ciudad de México, México.
[b]Oficina De Bioterio, Instituto Nacional de Enfermedades Respiratorias. "Ismael Cosío Villegas" (INER). Ciudad de México, México.
[c]Laboratorio De Morfología, Instituto Nacional de Enfermedades Respiratorias, "Ismael Cosío Villegas" (INER). Ciudad de México, México.
[d]Comisión Federal para la Protección contra Riesgos Sanitarios (COFEPRIS). Ciudad de México, México.
[e]The Queen´s Veterinary School Hospital. Universidad de Sheffield. Reino Unido.

Received: Jul 2, 2021; Approved: December 14, 2021

What do we know about the subject matter of this study?

Maternal nutritional status before and during gestation is fundamental for fetal growth and newborn weight. However, there is still no information that relates fetal lung development to sucrose consumption.

What does this study contribute to what is already known?

These results highlight the influence of sugar consumption in the maternal diet on neonatal lung development and maturity, specifically on elastic fibers and collagen essential for alveolarization and lung function.

Abstract

Respiratory conditions are the most common reason for admission to the neonatal unit for both term and premature newborns. It is known that nutritional imbalances during pregnancy affect the maturation and functional capacity of organs. **Objective:** to describe the pulmonary histomorphology of newborn rats due to maternal sugar intake by light microscopy. **Material and Method:** Twenty 4-week-old female Wistar rats divided into control and experimental groups with sucrose before and during pregnancy were used. At week 15, the females mated with males overnight. We recorded values from the body and lung weight of the newborns. The lungs were stained using Hematoxylin and Eosin, Masson's trichrome, Periodic acid-Schiff, and Verhoeff. **Results:** Newborns from the experimental group presented significantly lower body and lung weight (6.980 ± 0.493* g, 0.164 ± 0.022* g; *p < 0,05) compared with controls (7.854 ± 0.497 g, 0.189 ± 0.005 g). The lungs of the experimental

Keywords:
Hypercaloric Diet;
Lung Immaturity;
Rat;
Newborn;
Sucrose

Correspondence:
Héctor Javier Nava Reyes
hectornavamx1@gmail.com

Edited by:
Paul Harris Diez

How to cite this article: Andes pediatr. 2022;93(3):392-399. DOI: 10.32641/andespediatr.v93i3.3895

group showed structural alterations in the lung parenchyma, as well as changes in glycogen deposits, collagen fibers, and elastin compared with the control group. **Conclusion:** Alterations in newborn lung growth and development are associated with maternal sucrose intake. It is important to remember that interventions on the maternal diet have beneficial effects for both the mother and the newborn.

Introduction

In newborns, respiratory diseases are the main causes of morbidity and mortality[1]. Therefore, in recent decades, there has been considerably increased interest in the different ways the environment can influence children's health[2], specifically on the impact of environmental exposures on lung growth and function[3].

Lung development is a multi-stage process based on biochemical, mechanical, and anatomical events that span all gestational ages[4]. It is important to mention that the lung has a limited potential to recover from prenatal aggressions and present long-term consequences[5]. The evidence establishes that adverse environments such as poor maternal nutrition trigger structural and functional changes in different fetal organs[6], especially the lung, which is susceptible to this stimulus and[7], as a result, the fetus tries to adapt to changes in its physiology and metabolism in response to adverse microenvironments with limited or exaggerated nutrient and oxygen supply[8]. The consumption of sugar-rich foods before and during pregnancy increases the risk of gestational diabetes, preeclampsia[9], and preterm delivery[10]. It is also stated that a maternal fat-rich diet causes placental insufficiency, fetal growth restriction, and inhibition of pulmonary development[11]. The objective of this study was to describe the morphological changes in the lung of newborns born to mothers who consume sugar during pregnancy.

Material and Method

The study was performed on Wistar rats from the Laboratory Animal Facility of the National Institute of Respiratory Diseases. The animals were maintained under conventional conditions of temperature, humidity, a light cycle of 12 hours, and allowed free access to rat food (Harlan 2018 Tecklad Global, 18% protein rodent diet) and drinking water. The animals were handled according to the technical specifications for the care and use of laboratory animals of the Norma Oficial Mexicana[12] and the USA Guide for Care and Use of Laboratory Animals 8th edition (NRS 2011)[13].

Twenty female Wistar rats weighing approximately 65-80 gr and 12 young-adult males of the same breed and origin were used for mating. After one week of acclimatization, the females were randomly assigned to a control group that had free access to a commercial laboratory rat diet + drinking water (n = 10); and to an experimental group, which had free access to a commercial diet + high sucrose diet with 30% sucrose solution (30 g of sucrose in 100 ml of drinking water as the only drink) (n = 10). Females remained on this diet before mating and during gestation.

After 15 weeks on their respective diets, females from both groups were placed at a 2:1 female/male ratio overnight for mating. The following day, the presence of spermatozoa was verified by vaginal smear and was defined as day zero of gestation. On day 22 of gestation, the mothers were kept fasting, and body weight was recorded (Ohaus PA4101 Pioneer Plus digital precision scale). After delivery, the newborns were weighed on a precision scale (Denver instrument MXX model-123, Denver Instrument Inc, NY, USA). Of the neonates, nine from each group were randomly selected and were euthanized by decapitation.

Subsequently, the lungs were removed, weighed, and fixed by immersion with 10% buffered formalin. After 48 hours of fixation, nine slices between 5-7 µm thick were made (Reichert HistoStat rotary microtome) for each lung sample, and then histological staining was performed. Hematoxylin and eosin (H&E) staining was used to evaluate the morphology of the lung parenchyma; periodic acid-Schiff (PAS) for glycogen detection by color intensity; Masson's trichrome stain for collagen analysis; and Verhoeff's staining was used for elastin visualization.

The nine lung tissue sections were analyzed by light microscopy for morphological evaluation, and the findings of the experimental group were compared with the control group. Blind image analysis was performed by the same investigator, and five fields of view were randomly selected for photography. Images were digitized using a high-resolution digital camera (Hitachi KP-D580) placed on a microscope (Zeiss Axioskop) equipped with 2x/0.10, 10x/0.52, 40x/0.65, and 100x/1.25 immersion oil objectives.

Statistical analysis

Descriptive statistics were performed for quantitative variables (body and lung weight); arithmetic mean

was used to measure central tendency and standard deviation of the mean as a measure of dispersion. The results were expressed as mean ± SD. Subsequently, SPSS version 21 was used for the statistical analysis. Regarding inferential statistics, the unpaired Student's t-test was used to compare the parametric quantitative variables between the two groups. The significance level was established with a value ($p < 0.05$).

Results

Table 1 shows the body and lung weights of the offspring whose mothers were subjected to sucrose ingestion.

Mammalian lung development has well-defined stages that chronologically describe the morphological changes from the embryonic stage to maturity[14]. In the lung samples of the control group stained with H&E, normal architecture was observed with the presence of alveoli, terminal bronchioles, and alveolar ducts ending in alveolar sacs. The surrounding mesenchymal tissue condensed forming primary septa with cells in their interstitium, and multiple secondary rete ridges were observed that form the primitive alveoli (Figure 1A).

Regarding the lung morphology of the experimental group, there was a collapsed lung structure and massive widening and elongation of airspace, resulting in a reduction of mesenchymal tissue. However, alveolar wall thickening was still present, as well as irregular and small air spaces (Figure 1B).

In the control group, PAS reaction showed lower lung glycogen content, with dilated air spaces and a thin alveolar wall lined by more differentiated epithelium (Figure 2A). In contrast, in the experimental group, glycogen deposits were observed with greater intensity in alveolar wall cells in newborn lungs (Figure 2B).

Regarding the evaluation of essential collagen for maintaining lung structure, the control group showed normal deposition in the alveolar interstitium and lung parenchyma (Figure 3A). The experimental group showed more significant accumulation in the pulmonary artery and parenchyma (Figure 3B).

On the other hand, elastin fibers in the lung of the control group were mainly located at the apex of the secondary rete ridges and in the mesenchyme surrounding the distal airways developing before alveolarization (Figure 4A); in contrast, the lung of the experimental group showed reduced expression of elastin fibers and was limited to the mesenchyme surrounding the distal airways (Figure 4B).

Discussion

Respiratory disorders are the most frequent cause of admission to the neonatal intensive care unit in both term and preterm neonates[15]. Thus, during prenatal lung development, maternal nutrition plays a key role in altering lung growth mechanisms[16]; however, there is little information that relates newborn lung development to maternal sucrose intake.

This study demonstrated that abnormal lung development is associated with maternal sucrose intake. We noted a lower mean body weight of newborns in the experimental group compared with those of the control group, which is consistent with previous studies[17]. Likewise, experimental results associate maternal carbohydrate intake with fetal and placental size[18]. On the other hand, maternal malnutrition is known to cause dysregulation of placental glucose transporters, consequently limiting fetal nutrient availability and causing impaired growth[19]. It is important to emphasize that poor fetal growth and low birth weight are associated with an increased risk of glucose intolerance, type 2 diabetes, metabolic syndrome, and cardiovascular disease in adulthood[20].

It is also relevant to mention that lower lung weight was observed in the experimental group compared with the control group. This suggests the possible loss of stimuli to lung growth and differentiation during prenatal and early postnatal development. Specifically, caloric restriction during cellular hyperplasia produces alterations in lung growth which can be identified by a lower number of cells in some organs during the neonatal period[21].

Regarding the pulmonary morphological evaluation of the newborns of the experimental group, they showed alveolar collapse, alveolar wall thickening, and cellularity increase. In relation to this, experimental studies indicate that maternal hyperinsulinemia causes a delay in fetal pulmonary maturation[22] and a deficiency in the synthesis and secretion of surfactant[23]. It is worth mentioning that, in experimental models and humans, sucrose intake causes insulin resistance[24], hyperinsulinemia, and hyperlipidemia[25].

On the other hand, arterial hypertension secondary to sugar intake is[26] strongly related to the risk of preeclampsia, consequently altering vascular growth in the maternal-fetal pair and preventing proper airway de-

Table 1. Newborn lung and body weight

Groups	Body weight (g)	Lung weight (g)
Control	7.854 ± 0.497	0.189 ± 0.005
Experimental	6.980 ± 0.493*	0.164 ± 0.022*

Values are expressed as mean ± SD, *$p < 0.05$ compared to group I control, n = 9.

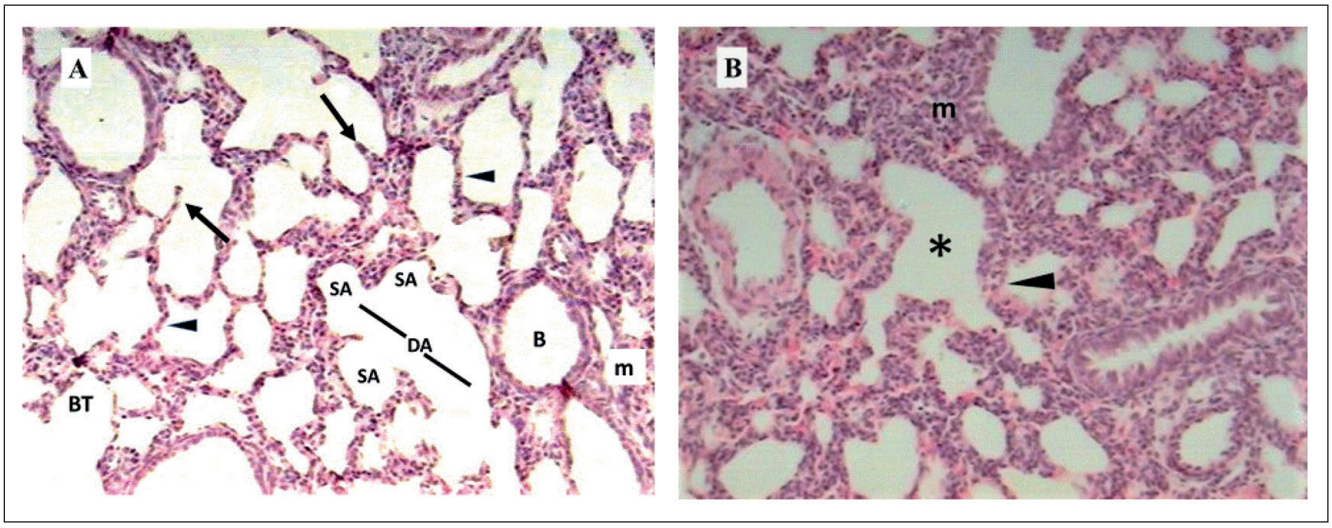

Figure 1. A: Photomicrograph of lung tissue section of the control group. Alveolus (A), alveolar sac (SA), terminal bronchiole (BT), bronchiole (B), alveolar duct (DA), mesenchyme (m), secondary ridges (arrowhead) and thin alveolar wall (arrow).10x, H-E staining. **B:** Photomicrograph of lung tissue section of the experimental group. Compact lung structure, thickened alveolar wall (arrowhead), mesenchyme (m), widening and lengthening of airspace (asterisk). 10x, H-E stain.

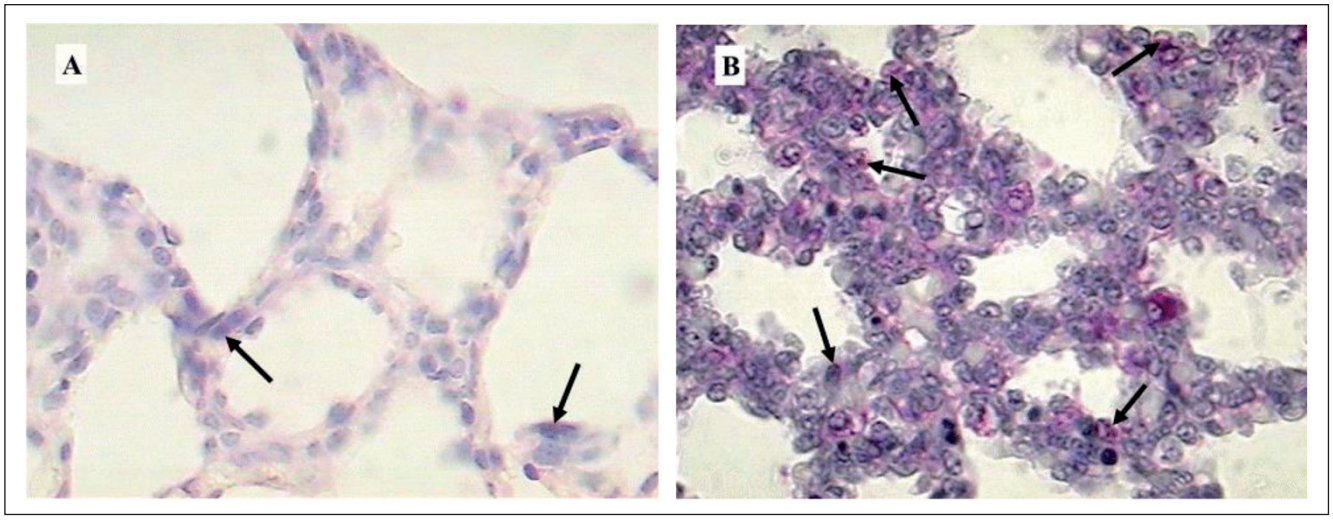

Figure 2. A: Photomicrograph of lung tissue section from the control group. Normal glycogen is observed in lung parenchyma (arrows). 40x, PAS stain. **B:** Photomicrograph of lung tissue section from the experimental group. Increased glycogen in epithelial cells (arrows). 40x, PAS stain.

velopment of the neonate[27]. Therefore, we believe that the metabolic and hormonal changes suffered by the mother due to sucrose intake delay the pulmonary development of the newborn.

Another important finding was the presence of pulmonary glycogen in the newborns of the experimental group. It is known that glycogen is the source of energy, and its synthesis begins in the ninth week of gestation in the human embryo, but the levels slowly decrease as the fetus approaches term. This indicates the relationship between lung glycogen and its functional maturation in the newborn[28]. It is clear that, under physiological conditions, the pulmonary glycogen content decreases, while in pathological conditions, its presence becomes more evident[29]. Therefore, we assume that glycogen accumulation has a negative impact on lung maturation and surfactant production since the degradation of glycogen to glucose generates the glycerol chains required for the surfactant synthesis.

mbnMasson's trichrome staining showed an increase in pulmonary collagen deposition in the newborns of the experimental group. This result is relevant

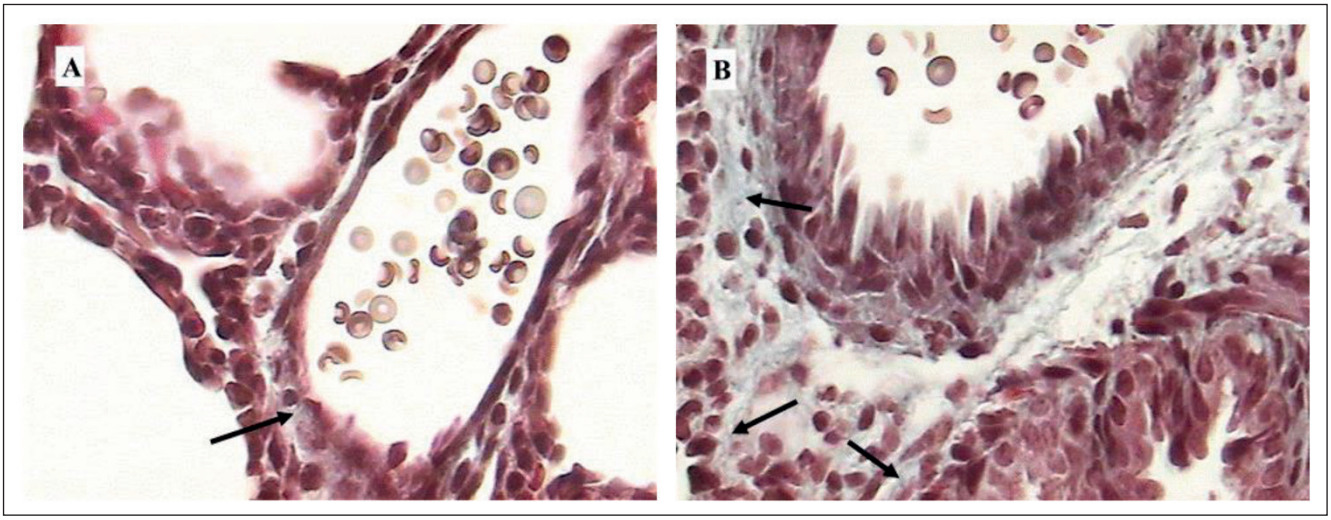

Figure 3. A: Photomicrograph of lung tissue section from the control group. Normal collagen deposition in lung parenchyma (arrow). 40x, Masson's trichrome stain. **B:** Photomicrograph of lung tissue section from the experimental group. Abnormal accumulation of collagen in parenchyma and pulmonary artery (arrows). 40x, Masson's trichrome stain.

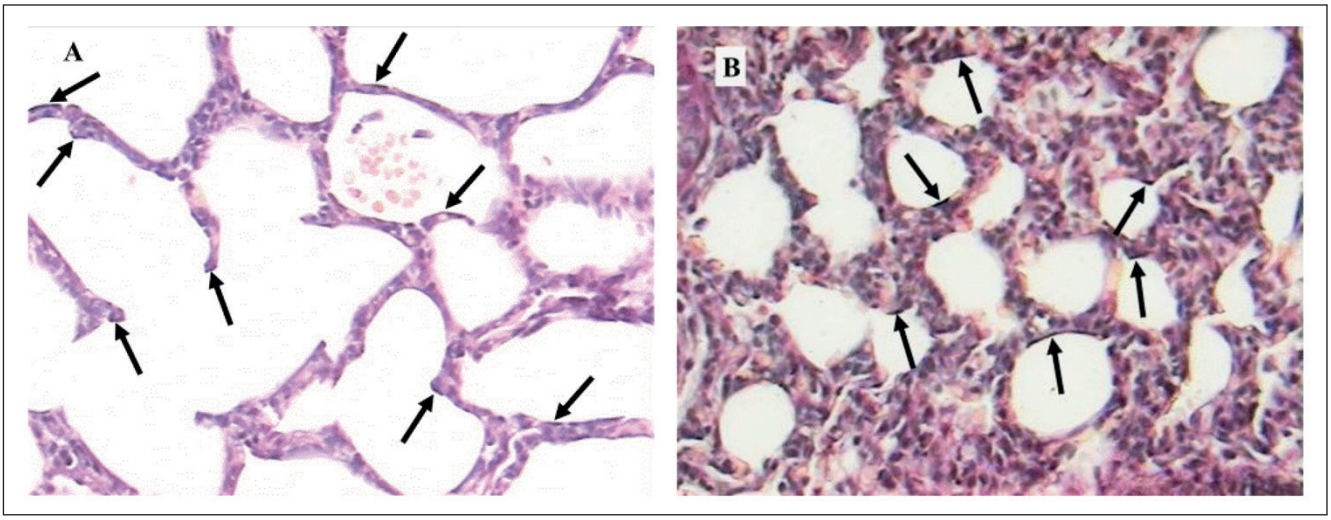

Figure 4. A: Photomicrograph of lung tissue section from the control group. Elastins organises in the secondary alveolar septa (arrows). 20x, Verhoeff´s stain. **B:** Photomicrograph of lung tissue section from the experimental group. Evident disorganisation and abnormal elastin deposition in the lung interstitium (arrows). 20x, Verhoeff´s stain.

since collagen is a component of the pulmonary extracellular matrix[30] and provides mechanical stability during the physiological function of the lung[31]. In this case, increased collagen deposition may lead to stiff and fibrotic airways in the neonatal lung[32]. In addition, studies in several animal models have shown increased collagen production and lung stiffness associated with bronchopulmonary dysplasia[33].

We believe that transforming growth factor-beta (TGF-β) is implicated in this outcome since it is an inducer of collagen expression and several respiratory diseases involving inflammation and remodeling[34]. On the other hand, maternal exposure to a hypercaloric diet during pregnancy imposes a high intake of advanced glycation end products generated through non-enzymatic reactions[35]. Such molecules cause alveolitis, fibroblast proliferation, abnormal interstitial collagen deposition, and pulmonary fibrosis[36].

Elastin synthesis begins at the pseudoglandular stage and is associated with areas where airways branch throughout lung development and subsequently increase during the following periods of fetal develop-

ment and peaks during alveolarization in the neonatal stage. It is also known that vascular compartments, conducting airways, and alveoli require elastic symmetry to undergo repeated distension and recoil throughout the life of the lung[37].

Our results show a reduced expression and disorganized deposition of elastin in the lung of the newborn in the experimental group. Alterations in elastin deposition are known to affect alveolarization and lung function. This was confirmed in a model of postnatal rat hyperoxia showing reduced elastin expression, elastin fiber breakage, and lung maturation arrest[38,39]. It is important to mention that elastin is a vital component of lung structure that allows expansion and recoil of the parenchyma. This elastic fiber is mainly deposited in the apex of the secondary rete ridges during alveolarization and in the mesenchyme surrounding the distal airways before lung development. It should also be considered that insufficient elastin affects the growth of blood vessels associated with growth in new or existing tissues leading to lung growth arrest and impaired respiratory function[40]. Therefore, these findings suggest that pulmonary elastin deposition in the newborn is compromised by maternal sucrose intake, implying alveolar reduction and delayed lung development.

This study has limitations that should be considered. First, we had a small number of newborns in our experiment, although it should be noted that this is a preliminary study testing the feasibility, equipment, and methods for our larger-scale research design. However, an increase in the number of animals could be established to obtain more data and achieve the research objectives. Investing in planning and experimental design guarantees that the information obtained will be sufficient and of high quality. Second, despite the basic staining techniques used in this work, we found significant findings. Therefore, we believe it is convenient to compare the relevance of our results with other experimental techniques. Our working group intends to approach the determination of pulmonary surfactant by molecular testing since adequate production of pulmonary surfactant is important for newborn survival.

In the prematurely developed newborn lung, pulmonary surfactant deficiency is thought to cause respiratory distress syndrome, considering the study of the placenta as a transient organ that exchanges a wide range of nutrients, endocrine signals, cytokines, and growth factors to ensure fetal growth and development. In addition, it is our interest to determine mitochondrial functionality as a fundamental mechanism of organ maturation failure in premature infants and intend to implement image analysis by fractal dimension to quantify and characterize the extracellular matrix in the lung of the newborn rat.

In conclusion, according to lung histomorphology, we demonstrate that alterations in newborn lung growth and development are associated with maternal sucrose intake. In this sense, we know that lung architecture is established early in life, and nutritional status has the potential to induce alterations in lung structure and function throughout life. It should be mentioned that maternal nutrition has important consequences on neonatal lung maturation through its influence on placental development and fetal growth.

It is worth noting that this model can be used in future studies to explore the development of respiratory disorders in early life and adulthood due to the influence of sugars. Although more research is needed, we believe that maternal sugar intake is a health hazard for developing newborns. Therefore, it is crucial to promote public health interventions to control the excessive intake of high fructose beverages and flours and encourage healthy habits. Special attention is also needed for one of the most vulnerable groups of the population, namely women in the reproductive stage and pregnant women.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

Aknowledgments

We thank all staff working in the INER biotherium office for their support in the care of the animals. We thank Mtra. María Angélica Velázquez González for her contribution in the revision of the manuscript.

References

1. Baseer K, Mohamed M, Abd-Elmawgood E. Risk Factors of Respiratory Diseases Among Neonates in Neonatal Intensive Care Unit of Qena University Hospital, Egypt. Ann Glob Health. 2020;86(1):22-30.
2. Perlroth N, Branco C. Current knowledge of environmental exposure in children during the sensitive developmental periods. J Pediatr. 2017;93(1):17-27.
3. Miller M, Marty M. Impact of Environmental Chemicals on Lung Development. Environ Health Perspect. 2010;118:1155-64.
4. Stocks J, Hislop A, Sonnappa S. Early lung development: Lifelong effect on respiratory health and disease. Lancet Respir. Med. 2013;1(9):728-42.
5. Bui D, Lodge C, Burgess J, et al. Childhood predictors of lung function trajectories and future COPD risk: A prospective cohort study from the first to the sixth decade of life. Lancet Respir. Med. 2018;6(7):535-44.
6. Calkins K, Devaskar S. Fetal origins of adult disease. Curr Probl Pediatr Adolesc Health Care. 2011;41(6):158-76.
7. Joss-Moore L, Albertine K, Lane R. Epigenetics and the Developmental Origins of Lung Disease. Mol Genet Metab. 2011;104(1-2):61-6.
8. Lucas A. Programming by early nutrition in man. Ciba Foundation Symposium. 1991;156:38-50.
9. Samuelsson A, Matthews P, Jansen E, et al. Sucrose feeding in mouse pregnancy leads to hypertension, and sex-linked obesity and insulin resistance in female offspring. Front Physiol. 2013;4(14):1-11.
10. Englund-Ögge L, Brantsæter A, Haugen M, et al. Association between intake of artificially sweetened and sugar-sweetened beverages and preterm delivery: a large prospective cohort study. Am J Clin Nutr. 2012;96(3):552-59.
11. Mayor R, Finch K, Zehr J, et al. Maternal high-fat diet is associated with impaired fetal lung development. Am J Physiol Lung Cell Mol Physiol. 2015;309(4):360-8.
12. Especificaciones Técnicas para la Producción, Cuidado y Uso de Animales de Laboratorio de la Norma Oficial Mexicana NOM-062-ZOO-1999. Diario Oficial de la Federación 6 dic, 1999. Estados Unidos Mexicanos.
13. Guide for Care and Use of Laboratory Animals. U.S. Eighth Edition. The Guide NRS, 2011.
14. Tschanz S, Salm L, Roth-Kleiner M, et al. Rat lungs show a biphasic formation of new alveoli during postnatal development. J Appl Physiol. 2014;117(1):89-95.
15. Pramanik A, Rangaswamy N, Gates T. Neonatal Respiratory Distress. Pediatr Clin North Am. 2015;62(2):453-69.
16. Hanson C, Brigham E. Maternal nutrition and child respiratory outcomes: paradigms of lung health and disease. Eur Respir J. 2020;55(3):1-4.
17. Soria A, Chicco A, Mocchiutti N, et al. A sucrose-rich diet affects triglyceride metabolism differently in pregnant and nonpregnant rats and has negative effects on fetal growth. J Nutr. 1996;126(10):2481-6.
18. Godfrey K, Robinson S, Barker D, et al. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. BMJ. 1996;312(7028):410-4.
19. Jansson T, Powell T. Role of placental nutrient sensing in developmental programming. Clin Obstet Gynecol. 2013;56(3):591-601.
20. Cox A, Gottheil S, Arany E, et al. The effects of low protein during gestation on mouse pancreatic development and beta cell regeneration. Pediatr Res. 2010;68(1):16-22.
21. Winick M, Noble A. Cellular response with increased feeding in neonatal rats. J Nutr. 1967;91(2):179-82.
22. Pignol B, Bourbon J, Ktorza A, et al. Lung maturation in the hyperinsulinemic rat fetus. Pediatr Res. 1987;21(5):436-41.
23. Gewolb I, Unger M, Merdian W, et al. High glucose causes delayed fetal lung maturation as measured by fluorescence anisotropy. Biochem Biophys Res Commun. 1993;193(2):794-800.
24. Cao L, Liu X, Cao H, et al. Modified high-sucrose diet-induced abdominally obese and normal-weight rats developed high plasma free fatty acid and insulin resistance. Oxid Med Cell Longev. 2012;2012(8):1-9.
25. Oliveira L, Santos D, Barbosa-da-Silva S, et al. The inflammatory profile and liver damage of a sucrose-rich diet in mice. J Nutr Biochem. 2014;25(2):193-200.
26. Hwang I, Ho H, Hoffman B, et al. Fructose-induced insulin resistance and hypertension in rats. Hypertension. 1987;10(5):512-6.
27. Hansen A, Barnés C, Folkman J, et al. Maternal preeclampsia predicts the development of bronchopulmonary dysplasia. J Pediatr. 2010;156(4):532-6.
28. Maniscalco W, Wilson C, Gross I, et al. Development of glycogen and phospholipid metabolism in fetal and newborn rat lung. Biochim Biophys Acta. 1978;530(3):333-46.
29. Ridsdale R, Post M. Surfactant lipid synthesis and lamellar body formation in glycogen-laden type II cells. Am J Physiol Lung Cell Mol Physiol. 2004;287(4):743-51.
30. Suki B, Ito S, Stamenović D, et al. Biomechanics of the lung parenchyma: critical roles of collagen and mechanical forces. J Appl Physiol. 2005;98(5):1892-9.
31. Burgstaller G, Oehrle B, Gerckens M, et al. The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. Eur Respir J. 2017;50(1):1601805
32. Vogel E, Britt R, Faksh A, et al. Moderate hyperoxia induces extracellular matrix remodeling by human fetal airway smooth muscle cells. Pediatr Res. 2016;81(2):376-83.
33. Mižíková I, Morty R. The Extracellular Matrix in Bronchopulmonary Dysplasia: Target and Source. Front. Med. 2015;2(91):1-20.
34. Ojiaku C, Yoo E, Panettieri R. Transforming Growth Factor β1 Function in Airway Remodeling and Hyperresponsiveness: The Missing Link? Am J Respir Cell Mol Biol. 2017;56(4):432-42.
35. Csongová M, Gurecká R, Koborová I, et al. The effects of a maternal advanced glycation end product-rich diet on somatic features, reflex ontogeny and metabolic parameters of offspring mice. Food Funct. 2018;9(6):3432-46.
36. Machahua C, Montes-Worboys A, Llatjos R, et al. Increased AGE-RAGE ratio in idiopathic pulmonary fibrosis. Respiratory Research. 2016;17(1):144-55.
37. Barry CS. Lung Elastin and Matrix. CHEST. 2000;117:229S-34S.
38. Joss-Moore LA, Wang Y, Yu X, et al. IUGR decreases elastin mRNA expression in the developing rat lung and alters elastin content and lung compliance in the mature rat lung. Physiological Genomics. 2011;43(9):499-505.
39. Mychaliska GB, Officer SM, Heintz CK, et al. Pulmonary elastin expression is decreased in the nitrofen-induced rat model of congenital diaphragmatic hernia. Journal of Pediatric Surgery. 2004;39(5):666-71.
40. Wu CS, Chen CM, Chou HC. Pulmonary Hypoplasia Induced by Oligohydramnios: Findings from Animal Models and a Population-Based Study. Pediatrics & Neonatology. 2017;58(1):3-7.