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BRIEF REPORT

# The effect of maternal protein restriction during perinatal life on the inflammatory response in pediatric rats

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**Abstract:** Fetal growth restriction can affect health outcomes in postnatal life. This study tested the hypothesis that the response to an inflammatory pulmonary insult is altered in pediatric fetal growth restricted rats. Using a low-protein diet during gestation and postnatal life, growth-restricted male and female rats and healthy control rats were exposed to an inflammatory insult via the intratracheal instillation of heat-killed bacteria. After 6 h, animal lungs were examined for lung inflammation and status of the surfactant system. The results showed that in response to an inflammatory insult, neutrophil infiltration was decreased in both male and female rats in the growth-restricted animals compared with the control rats. The amount of surfactant was increased in the growth-restricted animals compared with the control rats, regardless of the inflammatory insult. It is concluded that fetal growth restriction results in increased surfactant and altered neutrophil responses following pulmonary insult.

*Key words:* fetal growth restriction, lung inflammation, neutrophils, surfactant, low-protein diet.

**Résumé :** Le retard de croissance intra-utérin peut affecter les résultats cliniques au cours de la vie postnatale. Cette étude visait à confronter l'hypothèse selon laquelle la réaction à des lésions pulmonaires inflammatoires est altérée chez des rats pédiatriques présentant un retard de croissance intra-utérin. À l'aide d'un régime alimentaire à faible teneur en protéines au cours de la gestation et de la vie postnatale, nous avons exposé des rats mâles et femelles avec retard de croissance intra-utérin, ainsi que des rats témoins en bonne santé à des lésions inflammatoires provoquées par l'instillation intratrachéale de bactéries tuées par la chaleur. Après 6 h, nous avons examiné le poumon des animaux quant à l'inflammation pulmonaire et à l'état du système du surfactant. Les résultats ont montré que dans la réaction à des lésions inflammatoires, l'infiltration des neutrophiles diminuait de façon plus marquée chez les rats mâles comme femelles avec retard de croissance intra-utérin que chez les témoins. La quantité de surfactant était plus élevée chez les animaux avec retard de croissance intra-utérin que chez les témoins, sans égard aux lésions inflammatoires. Nous en arrivons à la conclusion que le retard de croissance intra-utérin entraînait une hausse du surfactant avec une réaction des neutrophiles altérée à la suite de lésions pulmonaires. [Traduit par la Rédaction]

*Mots-clés :* retard de croissance intra-utérin, inflammation pulmonaire, neutrophiles, surfactant, régime alimentaire à faible teneur en protéines.

## Introduction

Numerous studies have demonstrated the significant impact of fetal growth restriction (FGR) on health outcomes later in life (Harding and Maritz 2012). In general, FGR is defined as an infant with a birth weight below the 10th percentile for its gestational age, and there is strong evidence linking this condition with greater susceptibility to develop chronic diseases such as diabetes, hypertension, and dyslipidemia later in life (El Hajj et al. 2014; Smith and Ryckman 2015). Furthermore, a potentially contributing factor to the development of these chronic conditions is the altered responses to various insults, injuries, and illnesses occurring in this population throughout life (El Hajj et al. 2014). Thus, it is important to understand the impact of FGR on inflammatory responses during various stages of life.

Our lab and others have been interested in the effects of FGR on lung development and disease susceptibility (Harding and Maritz 2012; Khazaee et al. 2019). For example, FGR has been implicated as a risk factor for the development of asthma (Pike et al. 2012). In addition, animal studies using multiple species have demonstrated that FGR impairs lung development, in part, through alterations of the pulmonary surfactant system (Harding and Maritz 2012; Joyce et al. 2001; Khazaee et al. 2019). Surfactant is a phospholipid–protein mixture that lines the alveolar surface, where it has the dual role of allowing for breathing with minimal effort and providing an innate host-defense mechanism (Goerke

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1998). We previously observed that FGR in rats impacts both lung mechanics and the surfactant system in the postnatal period of female offspring, with no differences observed in the male offspring (Khazaee et al. 2019). These observations were recorded until weaning (e.g., 21 days); however, it is unknown how these alterations impact the response to a lung insult in the subsequent pediatric period.

Based on the above information, it was hypothesized that the response to an inflammatory pulmonary insult is altered in pediatric FGR rats. To test this hypothesis, we utilized our diet-induced rat model of FGR and exposed male and female offspring after weaning (e.g., days 25–30) to an inflammatory insult delivered via an intratracheal instillation of heat-killed bacteria (HKB). Inflammation was assessed through the analysis of neutrophil infiltration, inflammatory mediators, and the status of the pulmonary surfactant system.

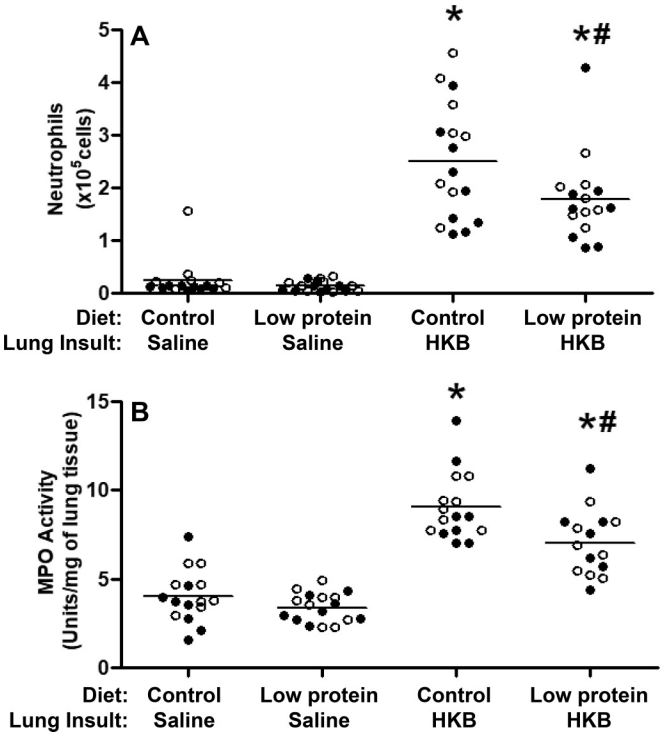
## Methods

### Animal procedures

Animals were treated in accordance with the Canadian Council on Animal Care (CCAC) guidelines, and the procedures were approved by the Animal Care Committee at the University of Western Ontario. The breeding protocol for maternal protein restriction has been previously reported (Khazaee et al. 2019). Briefly, after the acclimatization period and mating (gestation day 0), pregnant Wistar rats were housed individually and randomized to one of two dietary conditions: a 20% protein (control) or an low-protein (8% ) diet (LP, lower casein) (Bio-Serv, Frenchtown, New Jersey) (Khazaee et al. 2019; Sohi et al. 2013). Immediately after birth, the litters were culled to 10 pups to limit the effect of litter size on outcomes. Birth weights of the culled animals in the LP group were lower than those in the control group, consistent with previous studies in this model (Khazaee et al. 2019; Sohi et al. 2015). Mothers were kept on the same dietary regimes until the pups were weaned at postnatal day 21. Both male and female pups were maintained on the same diet until utilized for the experimental procedures at day 25 ($\pm$1.5 days) for the control animals and day 26 ($\pm$2 days) for the LP animals, with no differences between males or females among experimental groups (mean $\pm$ standard deviation (SD), $p > 0.05$).

To assess the response to an inflammatory insult, animals in both diet groups were randomized to receive an intratracheal bolus of either saline or heat-killed bacteria (HKB). To minimize the potential effects of distinct litters on the outcomes, only one or two animals per litter were randomized to any individual experimental group. HKB were prepared as previously reported using *Pseudomonas aeruginosa* (ATCC 27853, Sigma–Aldrich, Oakville, Ont., Canada) (Coorens et al. 2017). Briefly, $3 \times 10^6$ colony-forming units (CFU)·mL$^{-1}$ saline were heated at 90 °C for 15 min, and complete killing of the bacteria was verified by colony counting. Prior to the intratracheal instillation, all experimental animals were weighed and were given ketamine (100 mg·kg$^{-1}$) + dexmedetomidine (1.0 mg·kg$^{-1}$). Once anesthetized, rats were placed on an intubation stand to allow for placement of the endotracheal tube (18–20 gauge) to administer either 2 mL·kg$^{-1}$ of saline (control) or 2 mL·kg$^{-1}$ of $3 \times 10^6$ CFU·mL$^{-1}$ HKB. Rats were extubated, received 1.5 mL of warm saline subcutaneously plus the dexmedetomidine reversal agent antisedan (atipamezole hydrochloride, 1 mg·kg$^{-1}$) by intraperitoneal injection, and were moved to a cage placed on a slight incline with a heat lamp for 15 min while receiving 100% $O_2$ passively to ensure adequate oxygenation. Animals were then monitored for 6 h before being euthanized by intraperitoneal injection of sodium pentobarbital and exsanguination by severing the descending aorta.

**Fig. 1.** The effect of diet and instillation of heat-killed bacteria (HKB) on (A) the number of neutrophils in the bronchoalveolar lavage (BAL) and (B) myeloperoxidase (MPO) activity. Solid circles indicate data from individual female rats; open circles represent individual male rats. Statistical significance: *, $p < 0.05$ versus saline group with the same diet; #, $p < 0.05$ versus the control diet HKB group.



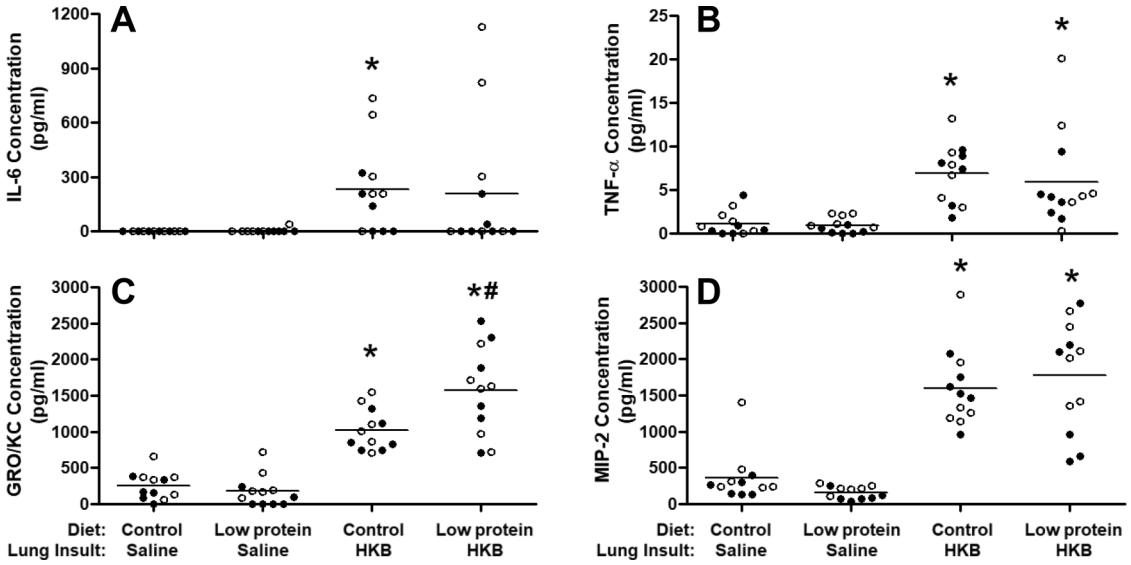

### Outcome analyses

After exsanguination, a bronchoalveolar lavage (BAL) was performed and processed as previously described (Khazaee et al. 2019). Briefly, lavage volume was recorded and centrifuged at 150*g* for 10 min to obtain a cell pellet. This pellet was resuspended and used for cell counting and differential cell analysis to obtain the number of neutrophils in the lavage fluid. An aliquot of the supernatant of the 150*g* centrifugation was stored and utilized to measure the inflammatory mediators (TNF-α, IL-6, MIP-2, and GRO/KC) using a multiplex immunoassay kit (R&D Systems, Minneapolis, Minnesota). These mediators were selected based on previous observations using this model of lung inflammation (Coorens et al. 2017). An aliquot of the 150*g* supernatant was also utilized to analyze the total amount of surfactant in the lavage by the measurement of phospholipid phosphorus (Bligh and Dyer 1959; Duck-Chong 1979). The remainder of the supernatant was centrifuged at 40 000*g* for 15 min to obtain a pellet of the active form of the surfactant, the large aggregates (LA). This resuspended pellet, as well as the 40 000*g* supernatant containing the small aggregates (SA), was also analyzed for phospholipid phosphorus (Bligh and Dyer 1959; Duck-Chong 1979). Following the lavage procedure, the remaining lung tissue was excised, divided into four pieces, and snap frozen in liquid nitrogen to be stored at –80 °C. This tissue was used for the measurement of myeloperoxidase (MPO) as previously reported (Tyml et al. 2017).

### Statistics

Statistical significance was determined using Graphpad Prism software (www.graphpad.com) by two-way analysis of variance (ANOVA) followed by a Tukey–Kramer post hoc test to determine

**Fig. 2.** The effect of diet and instillation of heat-killed bacteria (HKB) on the concentration of inflammatory mediators in the bronchoalveolar lavage (BAL) fluid: (A) IL-6, (B) TNF-α, (C) GRO/KC, and (D) MIP-2. Solid circles indicate data from individual female rats; open circles represent individual male rats. Statistical significance: *, $p < 0.05$ versus saline group with the same diet, #, $p < 0.05$ versus the control diet HKB group.




differences among experimental groups. Results with a $p$ value <0.05 were considered statistically significant.

## Results

### Animal cohorts

A total of 74 animals were utilized in our study. Body weight (mean ± SD) of the male animals was 93 g ± 8 g and 60 g ± 9 g for control and LP diet, respectively, and for female animals, it was 87 g ± 9 g and 61 g ± 13 g for control and LP diet, respectively. These body weights were significantly different for the two different diets for each sex ($p < 0.001$), but there was no statistical difference between males and females for either diet group.

### Inflammatory responses

To evaluate the response to an inflammatory insult, neutrophil infiltration into the lungs of our various experimental groups was assessed by two measurements (Fig. 1). The number of neutrophils obtained in the BAL fluid was quantified and shown to be increased in animals receiving HKB compared with animals receiving saline. Comparison between the two diets revealed no difference in the saline groups but a significantly lower number of neutrophils in the BAL from the group with the low-protein diet compared with the control in the HKB groups (Fig. 1A). The MPO activity data showed a similar pattern to the neutrophil counts (Fig. 1B). Most notably, MPO activity was lower in the group with the low-protein diet that had received HKB compared with the corresponding control diet group. There were no significant differences between male and female responses in either neutrophil counts or MPO activity in any of the experimental groups.

As the spleen is an important reservoir of neutrophils and monocytes in various models of bacterial inflammation and injury, we used an established flow cytometry protocol to characterize splenocytes for effects of low-protein diet and response to inflammatory lung insult (Supplementary Fig. S1[1]) (Barnett-Vanes et al. 2016). The results showed no significant differences in the proportion of splenic neutrophils among the experimental groups (Supplementary Fig. S1[1]).

The results of the inflammatory mediator measurements in BAL fluid are shown in Fig. 2. In general, all four mediators (IL-6, TNF-α, GRO/KC, and MIP-2) were significantly increased in response to HKB compared with saline, although this did not reach statistical significance for IL-6 in the low-protein group ($p = 0.089$). There were no significant differences between the diet groups except for the concentrations of GRO/KC, which was significantly higher in the low-protein diet group that received HKB compared with the corresponding control diet group. There were no significant differences between males and females.

### Surfactant analysis

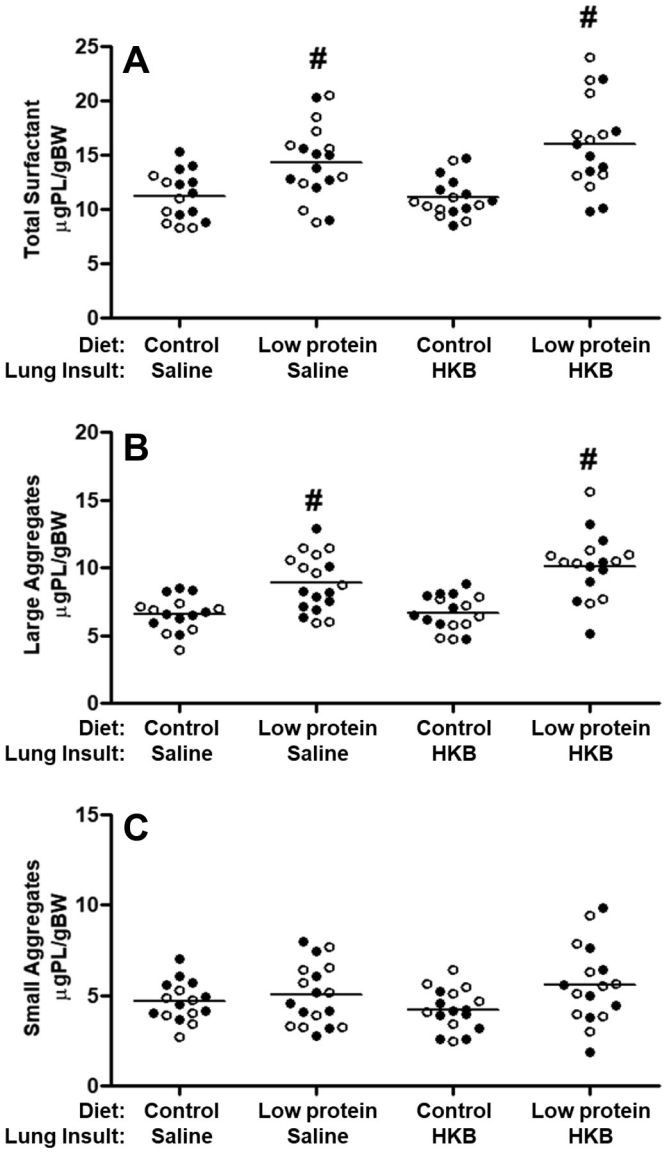
Figure 3A shows the amounts of surfactant recovered from the BAL. Significantly more surfactant was obtained from the low-protein group compared with the control. This was observed in both the animals receiving saline and those receiving HKB and was not different between males and females. As shown in Figs. 3B and 3C, analysis of the surfactant subfractions LA and SA revealed that the low-protein diet resulted in significantly higher amounts of LA in saline and HKB groups, but that diet did not significantly affect the amounts of SA recovered.

## Discussion

This study tested the hypothesis that the response to an inflammatory pulmonary insult is altered in pediatric FGR rats. Using an established diet model of FGR (Khazaee et al. 2019; Sohi et al. 2014) together with an inflammatory insult induced by intratracheal instillation of HKB (Coorens et al. 2017), it was found that the FGR animals had less neutrophil infiltration in their lungs in response to the HKB compared with normal birth weight offspring. This was observed by both differential cell count and measurement of MPO activity in the tissue; however, this finding did not correlate with altered levels of inflammatory mediators or surfactant due to HKB. Furthermore, there were no significant differences between male and female animals in any of the outcomes. Collectively, these results support the hypothesis, and it is concluded that in this experimental model, poor in utero and

[1]Supplementary data are available with the article at https://doi.org/10.1139/cjpp-2020-0431.

**Fig. 3.** The effect of diet and instillation of heat-killed bacteria (HKB) on the amounts of surfactant recovered from the bronchoalveolar lavage (BAL) fluid: (A) total amount; (B) the large aggregate subfraction; and (C) the small aggregate subfraction. Solid circles indicate data from individual female rats; open circles represent individual male rats. Statistical significance: #, $p < 0.05$ versus the control diet group with the same lung insult.




postnatal environments result in altered neutrophil responses following a pulmonary insult early in life.

The premise of the current study was that the long-term detrimental health effects reported for people with low birth weights (El Hajj et al. 2014; Smith and Ryckman 2015) are likely attributed to the accumulation of altered responses to metabolic and inflammatory challenges experienced during one's lifespan. To address this further, we utilized a maternal protein restricted model of FGR to test the effect of an initial insult experienced at a young pediatric age. This model does not affect maternal food intake, pregnancy weight gain, or postnatal food intake but does generate offspring with low birth weight (Sohi et al. 2015). To verify this model, we confirmed the lower birth weights in culled litter mates, as well as lower body weights at the time of study, in our animals. The rationale for utilizing HKB as an intrapulmonary challenge in these animals was that the respiratory system is a common route for inflammatory insults (Ather et al. 2020). Further, the stimulus of HKB is a more robust insult than the more commonly used administration of lipopolysaccharide, as it contains multiple bacterial products capable of stimulating the immune system through multiple toll-like receptors. In our study, the inflammatory insult was verified by significant increases in neutrophils and inflammatory mediators in animals receiving the HKB compared with the controls. Together, these experimental procedures provided a reliable approach to test our hypothesis.

The main finding of our study was a decrease in neutrophils within the lungs of both male and female FGR animals in response to HKB. Although the physiological or long-term effect of this response was not investigated, this result indicates that even at this early age, FGR animals have altered responses to an inflammatory insult. Unlike the results from the lungs, we found no difference in the proportion of splenic neutrophils in FGR animals, suggesting that FGR-induced alterations to the spleen were not responsible for the altered pulmonary responses observed. Similarly, the altered neutrophil infiltration was unrelated to sex differences despite previous observations of divergent male–female differences with respect to inflammatory responses and in FGR outcomes (Casimir et al. 2013). For example, utilizing the same experimental model, we previously observed sex differences in lung mechanics and surfactant levels in neonatal animals (Khazaee et al. 2019). As sex hormones play a role in inflammatory responses (Casimir et al. 2013), the lack of differences may be related to the young, sexually immature age of the animals. Consistent with this notion are previous reports of sexually dimorphic changes in glucose intolerance and cholesterol homeostasis in 4-month-old offspring, but not earlier (Chamson-Reig et al. 2009; Sohi et al. 2011). Future studies should examine the effect of inflammatory insults at older ages and different time points following insult, as well as examine a repetitive exposure at multiple ages to determine the life-long consequences of FGR on pulmonary inflammation in males and females.

The second aspect that our study focused on was the pulmonary surfactant system. The observation that surfactant levels were increased in the FGR group was surprising as our previous study, which analyzed animals up 21 days old, did not show such an effect (Khazaee et al. 2019). The metabolism of surfactant is complex and involves production, secretion by the alveolar type II cell, turnover of subfractions, and reuptake by type II cells, or degradation by alveolar macrophages (Goerke 1998). Although deviations in each of these metabolic steps have contributed to the altered amounts observed in our study, we speculate that altered degradation by alveolar macrophages due to the low-protein diet contributes to this observation. This requires further study. Functionally, as the first line of host defense encountered at the alveolar surface, pulmonary surfactant has the potential to mitigate the response to an inflammatory insult (Pison et al. 1994). The results tentatively support such an effect as the lower neutrophil infiltration was associated with the relatively higher amounts of surfactant in the FGR groups.

Overall, our study demonstrated a decreased infiltration of neutrophils in the lungs of FGR animals following an inflammatory insult. Limitations include a lack of physiological measurements and the use of only a single time point. In addition, our experimental model included protein restriction during gestation, as well as postnatally, and therefore precludes specific assessment of only the low birth weight component of this model. Nevertheless, the data support the concept of altered lung immune responses in FGR offspring at an early age. In extrapolating the data to the human population, we speculate that multiple inflammatory exposures over time will have cumulative, long-term consequences for lung health of infants born at a low birth weight.

### Disclosure

The animals in the control group of this study were also utilized in a separate study exploring the effects of glucocorticoids treatment (doi:10.1007/s00408-020-00399-2).

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## References

Ather, B., Mirza, T., and Edemekong, P.F. 2020. Airborne precautions. StatPearls Publishing. Available from https://www.ncbi.nlm.nih.gov/books/NBK531468/.

Barnett-Vanes, A., Sharrock, A., Birrell, M.A., and Rankin, S. 2016. A single 9-colour flow cytometric method to characterise major leukocyte populations in the rat: Validation in a model of LPS-induced pulmonary inflammation. PLoS ONE, **11**(1): e0142520. doi:10.1371/journal.pone.0142520. PMID:26764486.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911–917. doi:10.1139/o59-099. PMID:13671378.

Casimir, G.J., Lefèvre, N., Corazza, F., and Duchateau, J. 2013. Sex and inflammation in respiratory diseases: A clinical viewpoint. Biol. Sex Differ. **4**(1): 16. doi:10.1186/2042-6410-4-16. PMID:24128344.

Chamson-Reig, A., Thyssen, S.M., Hill, D.J., and Arany, E. 2009. Exposure of the pregnant rat to low protein diet causes impaired glucose homeostasis in the young adult offspring by different mechanisms in males and females. Exp. Biol. Med. **234**(12): 1425–1436. doi:10.3181/0902-RM-69. PMID:19657071.

Coorens, M., Banaschewski, B.J.H., Baer, B.J., Yamashita, C., van Dijk, A., Haagsman, H.P., et al. 2017. Killing of *Pseudomonas aeruginosa* by chicken cathelicidin-2 is immunogenically silent, preventing lung inflammation in vivo. Infect. Immun. **85**(12): e00546-17. doi:10.1128/IAI.00546-17. PMID:28947647.

Duck-Chong, C.G. 1979. A rapid sensitive method for determining phospholipid phosphorus involving digestion with magnesium nitrate. Lipids, **14**: 492–497. doi:10.1007/BF02533467.

El Hajj, N., Schneider, E., Lehnen, H., and Haaf, T. 2014. Epigenetics and lifelong consequences of an adverse nutritional and diabetic intrauterine environment. Reproduction, **148**(6): R111–R120. doi:10.1530/REP-14-0334. PMID:25187623.

Goerke, J. 1998. Pulmonary surfactant: functions and molecular composition. Biochim. Biophys. Acta, **1408**(2–3): 79–89. doi:10.1016/S0925-4439(98)00060-X. PMID:9813251.

Harding, R., and Maritz, G. 2012. Maternal and fetal origins of lung disease in adulthood. Semin. Fetal Neonatal Med. **17**: 67–72. doi:10.1016/j.siny.2012.01.005. PMID:22277111.

Joyce, B.J., Louey, S., Davey, M.G., Cock, M.L., Hooper, S.B., and Harding, R. 2001. Compromised respiratory function in postnatal lambs after placental insufficiency and intrauterine growth restriction. Pediatr. Res. **50**(5): 641–649. doi:10.1203/00006450-200111000-00018. PMID:11641461.

Khazaee, R., McCaig, L.A., Yamashita, C., Hardy, D.B., and Veldhuizen, R.A.W. 2019. Maternal protein restriction during perinatal life affects lung mechanics and the surfactant system during early postnatal life in female rats. PLoS ONE, **14**(4): e0215611. doi:10.1371/journal.pone.0215611. PMID:31002676.

Pike, K., Jane Pillow, J., and Lucas, J.S. 2012. Long term respiratory consequences of intrauterine growth restriction. Semin. Fetal Neonatal Med. **17**(2): 92–98. doi:10.1016/j.siny.2012.01.003. PMID:22277109.

Pison, U., Max, M., Neuendank, A., Weißbach, S., and Pietschmann, S. 1994. Host defence capacities of pulmonary surfactant: Evidence for "non-surfactant" functions of the surfactant system. Eur. J. Clin. Invest. **24**(9): 586–599. doi:10.1111/j.1365-2362.1994.tb01110.x. PMID:7828630.

Smith, C.J., and Ryckman, K.K. 2015. Epigenetic and developmental influences on the risk of obesity, diabetes, and metabolic syndrome. Diabetes Metab. Syndr. Obes. **8**: 295–302. doi:10.2147/DMSO.S61296. PMID:26170704.

Sohi, G., Marchand, K., Revesz, A., Arany, E., and Hardy, D.B. 2011. Maternal protein restriction elevates cholesterol in adult rat offspring due to repressive changes in histone modifications at the cholesterol 7α-hydroxylase promoter. Mol. Endocrinol. **25**(5): 785–798. doi:10.1210/me.2010-0395. PMID:21372147.

Sohi, G., Revesz, A., and Hardy, D.B. 2013. Nutritional mismatch in postnatal life of low birth weight rat offspring leads to increased phosphorylation of hepatic eukaryotic initiation factor 2α in adulthood. Metabolism, **62**(10): 1367–1374. doi:10.1016/j.metabol.2013.05.002. PMID:23768545.

Sohi, G., Barry, E.J., Velenosi, T.J., Urquhart, B.L., and Hardy, D.B. 2014. Protein restoration in low-birth-weight rat offspring derived from maternal low-protein diet leads to elevated hepatic CYP3A and CYP2C11 activity in adulthood. Drug Metab. Dispos. **42**(2): 221–228. doi:10.1124/dmd.113.053538. PMID:24212381.

Sohi, G., Revesz, A., Ramkumar, J., and Hardy, D.B. 2015. Higher hepatic MIR-29 expression in undernourished male rats during the postnatal period targets the long-term repression of IGF-1. Endocrinology, **156**(9): 3069–3076. doi:10.1210/EN.2015-1058. PMID:26151354.

Tyml, K., Swarbreck, S., Pape, C., Secor, D., Koropatnick, J., Feng, Q., et al. 2017. Voluntary running exercise protects against sepsis-induced early inflammatory and pro-coagulant responses in aged mice. Crit. Care, **21**(1): 210. doi:10.1186/s13054-017-1783-1. PMID:28789683.