INTRAUTERINE GROWTH RESTRICTION ALTERS THE EPIGENETIC PROFILE AND DECREASES RNA OF STEAROYL COA DESATURASE IN NEWBORN RAT LUNG

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Intrauterine growth restriction (IUGR) is associated with impaired lung development and function. Lung function is impaired in IUGR rats, in part, by altered surfactant composition. The lipid profile in lungs determines surfactant lipid composition. Our IUGR rats have decreased lung palmitoleic acid (16:1). Stearoyl CoA Desaturase (SCD), the rate-limiting enzyme in synthesis of palmitoleic acid, is a target of transcription factor peroxisome proliferator activated receptor gamma (PPARγ) and is susceptible to transcriptional regulation through epigenetic modifications. There are a number of genes in the lung that show altered mRNA levels in association with decreased PPARγ levels and epigenetic modifications in the presence of IUGR. The effect of IUGR on the epigenetic profile of SCD as well as SCD mRNA and protein levels in the newborn rat lung is unknown. We hypothesize that IUGR will be associated with reduced SCD mRNA and protein levels consistent with the previously observed decrease in PPARγ levels in the lungs of d0 rat pups. We further hypothesize that there will be gender-specific changes in the epigenetic profile along the length of the SCD gene.

This hypothesis was tested using a uteroplacental insufficiency (UPI) model of IUGR in rats. Whole lung tissue was harvested at birth (d0) and used to assess SCD mRNA and protein levels by RT-PCR and western blot analysis. Epigenetic

modifications (H3K14Ac and H3K36Me3) were assessed along the SCD gene using chromatin immunoprecipitation (ChIP).

Results showed that, in control lungs females have 153% higher SCD mRNA than males ($p=0.005$). A consistent decrease in SCD mRNA levels was found in both male and female IUGR rats at birth relative to controls ($p=0.009$, $p=0.0023$). H3K14Ac was increased at SCD exon 4 in IUGR females to 210% of the level detected in gender-matched controls ($p=0.037$). H3K3Me3 was decreased at SCD exon 4 in IUGR males by 43% ($p=0.044$) and at SCD 3'UTR in IUGR females by 65% relative to gender-matched controls ($p=0.036$). There was no change in SCD protein levels in IUGR rats at birth. In conclusion IUGR is associated with decreased SCD mRNA levels and gender-specific changes to the epigenetic profile along the SCD gene.

TABLE OF CONTENTS

ABSTRACT iii

LIST OF FIGURES vii

Introduction 1

Intrauterine Growth Restriction 1
Lung Phenotype of IUGR 2
Preliminary Data of This Project 3
Role of Stearoyl CoA Desaturase in Lung Development and Function 4
Transcriptional Regulation of Stearoyl CoA Desaturase 5
Epigenetics 5
PPARγ 6
Hypothesis 7

Methods 8

Model 8
Real-Time RT PCR 9
Protein Quantification 9
Chromatin Immunoprecipitation (ChIP) 10
Statistical Analysis 12

Results 13

Gender-specific SCD mRNA levels in control rats 13
Epigenetic profile in male and female control rats 13
Epigenetic profile in male and female control rats 15
Epigenetic profile along the SCD gene 15
Effect of IUGR on H3K14Ac 15
Effect of IUGR on H3K36Me3 17
IUGR has no affect on SCD protein levels in lung tissue at d0 18

Discussion 19

Limitations 21
Future Research 22

Potential Impact 23
Conclusion 23

References 24

LIST OF FIGURES

Figure Page

1. Palmitoleic acid levels of d0 IUGR lungs relative to control lungs 4

2. Map of epigenetic modifications identified along the N-terminus of H3 6

3. SCD Gene Structure 11

4. SCD mRNA levels in male and female control rat lungs at d0 13

5. Epigenetic profile at 3 sites along the SCD gene in the lungs of male and female control rats at d0. 14

6. SCD mRNA levels in d0 IUGR lungs relative to controls 15

7. H3K14Ac along the SCD gene in d0 lungs of IUGR rats relative to controls 16

8. H3K36Me3 along the SCD gene in d0 lungs of IUGR rats relative to controls 17

9. Analysis of SCD protein levels in d0 lungs of IUGR rats relative to controls 18

INTRODUCTION

Intrauterine Growth Restriction

Intrauterine growth restriction (IUGR) is a condition affecting 5-12% of preterm infants born in the United States(1). These children not only fail to reach their genetic growth potential adjusted for gestational age but are also at an increased risk of premature delivery as well as postnatal morbidities and mortalities(2). Growth restricted infants have a 10-fold increased rate of death at term(3). IUGR can result from fetal development in an environment of uteroplacental insufficiency (UPI) caused by maternal malnutrition, restricted blood flow to the uterus or toxin exposure. In developed countries, IUGR is most often associated with conditions of restricted maternal blood flow such as preeclampsia(4). Preeclampsia is a significant occurrence in pregnant women affecting 5-10% of pregnancies in the United States (5). Regardless of the cause, the postnatal effects of IUGR persist throughout the lifecycle and impact a wide range of systems including lung development and function.

Lung Phenotype of IUGR

Although previous research has been heavily focused on the metabolic consequences of IUGR, recent attention has been drawn to the phenotypes observed in the pulmonary system. IUGR Infants, particularly those born prematurely, have an

increased risk of poor lung development and morbidity due to pulmonary complications(2). Population-based studies have concluded that IUGR infants, carried to term, require increased ventillary support (6) and pre-term IUGR infants are at an increased risk of developing respiratory distress syndrome (RDS)(7) as well as bronchopulmonary dysplasia (BPD) (8, 9) early in life. These implications of IUGR are seen in both male and female infants; however, studies have shown that males are more severely affected than females(10, 11). Symptoms of impaired lung function persist into adulthood in both term and preterm infants(12).

In animal models of IUGR, impaired alveolar development has been identified as a contributing factor to lung disease by impairing the efficiency of gas exchange in the lungs(13-15). IUGR alters characteristics of the developing lung structure that are crucial to proper alveolar formation (16). Such characteristics are also associated with the phenotype of BPD in animal models(17, 18).

Lung function and development is also disrupted in IUGR animals through impaired surfactant composition and synthesis. Phospholipids, the most significant lipid component of surfactant, have recently been identified as an additional target for IUGR induced changes in fetal phenotypes. The predominant phospholipid in surfactant is dipalmitoyl phosphatidyl choline (DPPC), which is a phosphatidyl choline molecule with two palmitic acid molecules attached. Changing surfactant composition changes its physiologic properties(19). Lungs of IUGR rat pups have significantly decreased compliance at birth, a phenotype that may be associated with altered surfactant(16).

In addition, the lipid environment can affect physiologic lung function beyond surfactant composition however the full scope of its effects has not been fully

characterized to date. Lipids are known to affect functional properties by way of cell membrane fluidity and structure (20). Fatty acids are additionally involved in many other cell functions important in pulmonary tissue such as binding of membrane-associated enzymes and hormone receptors, cell growth, cytotoxicity and second-messenger pathways(21, 22). Therefore, the fatty acid environment in lung tissue has the potential to affect not only lung development and function but also other physiological systems by way of communication mechanisms within cells and between cells. Understanding the effects of IUGR on lung lipid metabolism and composition will contribute to the evolving picture of the IUGR lung phenotype.

Prior to this study, little was known about the effects of IUGR on the lipid profile and fatty acid metabolism in the lungs. A study focusing on the effects of maternal nutrient restriction on fetal sheep demonstrated altered long chain polyunsaturated fatty acid (LCPUFA) levels in lung tissue(23). Furthermore, our previous research demonstrated a significant change in the phospholipid profile of lung surfactant in our uteroplacental insufficiency-induced IUGR rat model. Specifically the amount of DPPC present in the lungs of IUGR rats at birth is significantly lower than those of control rats(24). Based on these findings we measured lung phospholipid content of newborn IUGR rat pups.

Preliminary Data of this Project

We collected preliminary data demonstrating that the lungs of d0 IUGR rats had altered lipid profiles. IUGR altered the levels of palmitoleic acid found in whole lungs of both male and female rat pups at birth. Palmitoleic acid content was decreased in whole

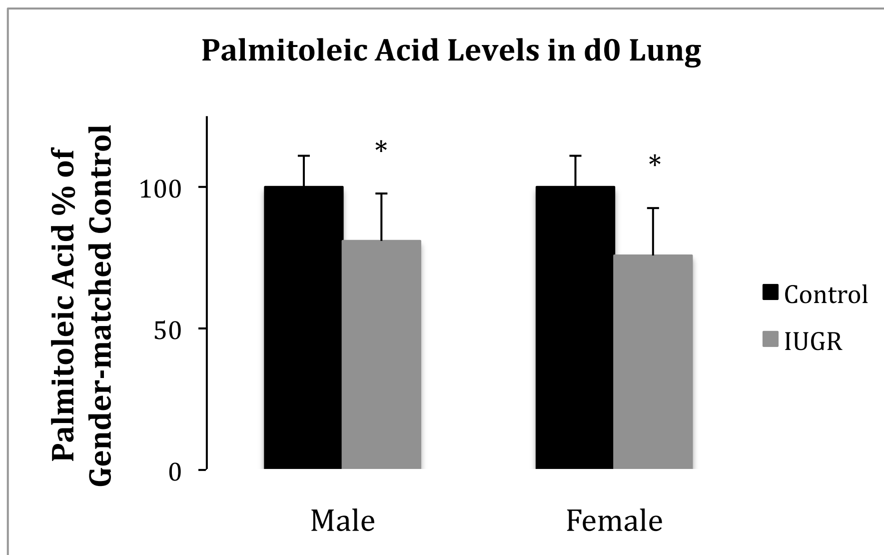


Figure 1. Palmitoleic acid levels of d0 IUGR lungs relative to control lungs. Data represented as a percent of controls ± standard deviation (*$p<0.05$).

lung tissue (80% of controls in males & 75% of controls in females $p<0.05$)(Figure 1).

Interestingly, no change was seen in the levels of the lipid precursor to palmitoleic acid, palmitic acid in the lungs of d0 IUGR rat pups (16:0). This data suggests that IUGR may interfere with the reaction of lipid metabolism responsible for the conversion of palmitic acid to palmitoleic acid. The effects of IUGR on the lipid profile and the mechanism of lipid metabolism in the lungs of newborn rats remain unknown.

Role of Stearoyl CoA Desaturase in Lung Development and Function

The profile of phospholipids present in lung tissue is predominately determined by the type and relative concentrations of intracellular fatty acids. The group of enzymes responsible for synthesizing many of the long chain fatty acids (LCFA) present in the lung is the desaturase enzymes. Delta-9 desaturase, also known as stearoyl CoA desaturase (encoded by the SCD gene) is an ideal target for studying an altered

pulmonary lipid environment because of the specificity inherent to its function. This enzyme catalyzes the rate-limiting step in the conversion of palmitic acid to palmitoleic acid, the two most abundant fatty acids found in surfactant and in lung tissue as a whole. SCD is therefore a clear target for further studies on the effects of IUGR on the lipid environment in the lungs as well as the physiologic properties of surfactant.

Transcriptional Regulation of Stearoyl CoA Desaturase

The reaction catalyzed by SCD is an opportunity for disruption of lipid metabolism in the developing lungs of newborn IUGR rats. One means of altering SCD levels in the lung is by changing the transcription of the SCD gene. Two ways that SCD transcription can be modified are changes in the epigenetic profile of the gene and altered levels of one of its transcription factors, PPARγ. IUGR has previously been associated with altered gene expression and epigenetic modification in lung tissue(24, 25).

Epigenetics

Epigenetic regulation of gene transcription is not static and responds to environmental cues. IUGR affects transcriptional regulation in part by influencing enzymes that alter the epigenetic profile of specific target genes. These enzymes induce phenotypic changes by placing specific epigenetic marks on the histone proteins associated with stretches of chromatin encoding target genes. Epigenetic marks either individually or together in concert alter interactions between the target gene and the transcription machinery. Our lab has previously shown that IUGR is associated with gender-specific changes in the epigenetic profile and subsequent mRNA levels of genes in the rat lung(25, 26).

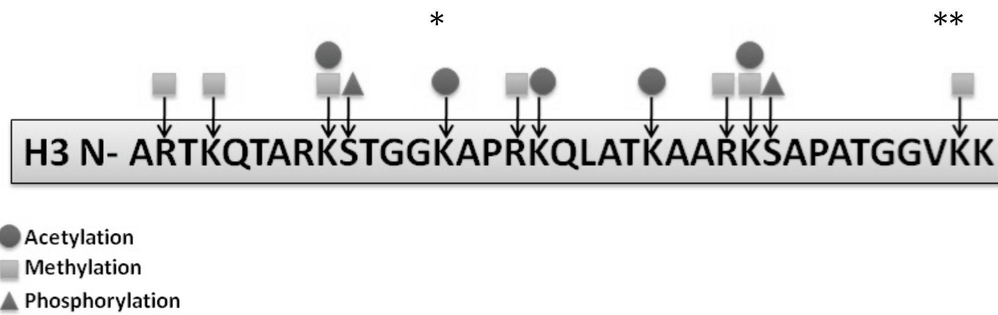


Figure 2. Map of epigenetic modifications identified along the N-terminus of H3, a histone protein. (* = H3K14Ac **= H3K36Me3)

Of the four histone proteins present in each nucleosome unit, H2A, H2B, H3, and H4 this project focused on modifications made to H3 (Figure 2). The two modifications detected in this project are tri-methylation at lysine 36 (H3K36Me3) and acetylation at lysine 14 (H3K14Ac). These epigenetic modifications exhibit gender-specific changes in our IUGR model and have been associated with altered mRNA levels of target genes (27).

PPARγ

In addition to epigenetic modifications, one opportunity for direct regulation of SCD transcription is through binding of the transcription factor, peroxisome proliferator activated receptor gamma (PPARγ). The SCD gene contains a PPARγ response element (PPRE) in its promoter region and is up regulated by elevated PPAR levels (α, β, and γ) in the liver of rats(28).

PPARγ plays a significant role in the phenotype of impaired lung development that is associated with IUGR. PPARγ-knockout mice have impaired lung development and function due to enlarged airspaces, increased lung volumes, and decreased tissue resistance(29). This phenotype has also been associated with altered transcription of

mesenchymal genes known to be targets of transcriptional regulation by PPARγ(29). Furthermore, PPARγ plays a crucial role in the parathyroid hormone related protein (PTHrP)-driven epithelial-mesenchymal paracrine loop that is critical to lung development(30). **IUGR decreases mRNA and protein levels of PPARγ in newborn rat lungs(25).** Furthermore, the transcription factor has been associated with altered transcriptional regulation of genes in the lungs of IUGR rats(25, 26). Despite our current knowledge that PPARγ mRNA and protein levels are decreased in IUGR and SCD activity is responsive to alterations in PPARγ concentration, we do not know the effects of IUGR on the desaturase enzyme or on lipid metabolism in lung tissue.

Hypothesis

In an effort to further characterize the effects of IUGR on fatty acid metabolism in the lung, this project focused measuring the levels of Δ9 desaturase mRNA and protein present in IUGR lung tissue compared to lung tissue from a non-IUGR controls as well as assessing the profile of epigenetic modifications that may be altering SCD transcription in the lungs of d0 IUGR rats. **We hypothesize that IUGR will decrease SCD mRNA and protein levels consistent with the previously observed decrease in PPARγ levels in the lungs of d0 rat pups. We further hypothesize that there will be gender-specific changes in the epigenetic profile along the length of the SCD gene.**

METHODS

Model

This project used the rat uteroplacental insufficiency model. This model is a well-established rat-model that induces IUGR by reducing fetal blood through uteral artery ligation on day 19 of gestation (full term is 21.5 days)(25, 31). The surgical procedures have been previously described in detail (32, 33). This procedure reduces fetal blood supply to a level that mimics the fetal conditions present during human UPI and induces IUGR in the pups(34). Our lab has extensive experience with this model, which provides insight into the effects of IUGR on alveolarization, a critical stage of pulmonary development that is typically occurring pre-birth in humans.

In this project, each experimental group originated from pregnant Sprague-Dawley rats that were housed individually under standard conditions with 12-hour light/dark cycles. The animals were randomly assigned to undergo UPI treatment or control. At d19 of gestation, the UPI intervention rats were anesthetized with intraperitoneal xylazine (8mg/kg) and ketamine (40mg/kg), and both inferior uterine arteries were ligated. The non-UPI control rats were subjected to an identical anesthesia without the surgical procedure.

One to two pups from six dams were assigned to each experimental group to assure that isolated tissues incorporate appropriate litter-to-litter variation. IUGR pups are asymmetrical growth restricted and approximately 25% smaller than control pups at

birth (31). On day zero (d0) the pups were delivered through caesarian section and euthanized using a sodium pentobarbital overdose (150 mg/kg). Serum was collected and lung tissue was immediately harvested and flash frozen in liquid nitrogen.

Real-Time RT PCR

This project utilized techniques of real-time reverse transcriptase PCR to quantify the amount of Δ9 desaturase mRNA present in lung tissue. Total mRNA was extracted from lung tissue using a "NucleoSpin RNA II" RNA extraction kit according to manufacturer's protocol. Total RNA was quantified and 1 μg was used to synthesize cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster CA). An assay-on-demand primer/probe set for SCD was used. GAPDH was utilized as the control.

All real-time PCR amplification, data acquisition and analysis was done using the 7900HT Real-time PCR system and SDS Enterprise Software (Applied Biosystems) using a 384-Well Optical Reaction Plate (Applied Biosystems) at the University of Utah genomics core facility. Taqman Universal PCR Mastermix (Applied Biosystems) was used in a 6 μL reaction, and performed in quadruplicate. Cycle parameters were: 50°C x 2 min, 95 °C x 10 min, followed by 40 cycles of 95°C x 15 sec and 60°C x 60 sec.

Protein Quantification

Lung tissue levels of Δ9 desaturase protein were quantified using western blotting. Lung tissue proteins were isolated using homogenization in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% Na-doxy-cholate, 1% NP-40 (Igepal), 0.1%

SDS) and protease inhibitor cocktail (Roche-Complete Mini), followed by centrifugation at 10,000g, 4°C, 15 minutes. Samples were assayed in triplicate for protein concentration using the bicinchoninic acid protein assay kit (Pierce). Western blotting was performed using standard procedures, with the following specifics; samples containing 100 μg total protein were run on 10% bis-tris XT Criterion gels (Bio-Rad Laboratories), PVDF membranes were blocked in 3% BSA-TBST and incubated with an anti-SCD primary antibody. Following incubation with anti-mouse secondary antibody, signal was determined using enhanced chemiluminescence (ECL) according to the manufacturer's instructions (Amersham, Little Chalfont, UK).

Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) was used to detect the presence of histone modifications at three sites along the length of the SCD gene: the promoter, exon 4, and 3' UTR (Figure 3). Experiments followed a revised protocol based on the methods of Farnham and Bomstztyk(35, 36). Chromatin was isolated from whole lungs of male and female rat pups as follows; tissue was fixed in 1% formaldehyde for 10 minutes at room temperature, followed by the addition of glycine to a concentration of 125 mM to stop the reaction. Samples were washed and re-suspended in PBS with protease inhibitor cocktail (PIC) at the concentration recommended by the manufacturer (Complete, Mini. Roche, Indianapolis, IN). After centrifugation, cell pellets were re-suspended in lysis buffer (5mM PIPES, pH 8.0, 85 mM KCL, 0.5% Igepal) with PIC and dounced on ice using a tight pestle. After centrifuging, pelleted nuclei were re-suspended in nuclei lysis buffer (50 mM Tris-Cl, pH 8.1, 10 mM EDTA, 1% SDS) with PIC. Incubated for 20 minutes on

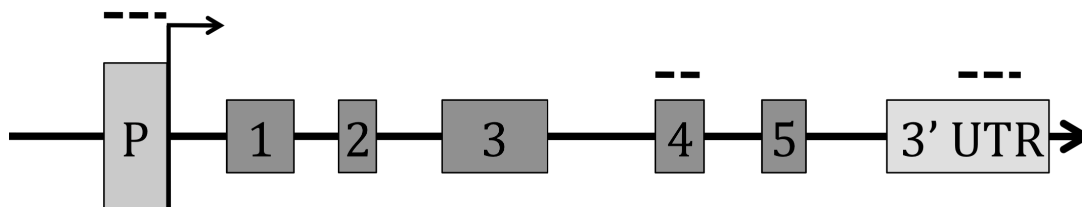


Figure 3. SCD Gene Structure. The 3 probe/primer sets (indicated by the dashed lines) used in the ChIP protocol were located at the promoter (P), exon 4 (4), and the 3' untranslated region (UTR) of the SCD gene.

ice and split into aliquots of approximately 200 ul in (1.5 mL tubes) for sonication. Sonication was performed on ice using a Fisher Scientific Moel 100 sonicator with micro tip attachment (Fisher Scientific, Pittsburg, PA). Each sample was pulsed 10 times with the microtip placed near the base of the tube and returned to ice, this was repeated a total of 10 times. After centrifuging, chromatin containing supernatants for each of the four experimental groups (male SHAM, male IUGR, female SHAM, and female IUGR) were frozen at -80°C.

Immunoprecipitation (IP) was performed as previously described using anti-$H3K36Me^3$ antibody (Abcam, ab9050-100), and anti-H3K14Ac (Milipore, DAM1548623)(25). Levels of the two epigenetic marks present at a non-transcribing intergenic region were used as a control (27). Table 1 contains primer and probe sequences for RT-PCR of ChIP DNA.

Table 1. Primer/probe sets for ChIP analysis.

Primer/Probe	Sequence
SCD Promoter	
Forward primer	TCCCACGCGCCTCTAGAA
Reverse primer	GCGCCGACCACTCACAA
Probe	TGCAAAGACCGGATGGA
SCD Exon 4	
Forward primer	CCGCCGTGGCTTTTTCT
Reverse primer	GGTGTTTGCGCACAAGCA
Probe	CTCTCACGRGGGTTGG
SCD 3’UTR	
Forward primer	TCAGTATTCTGTGAGACATGGGAT
Reverse primer	AAT
Probe	TTTGCTCATGCTCGCACCTA
	TTCCTGACCTACCTCCAC

Statistical Analysis

Data are presented as means ± standard deviation. Statistical significance was determined using ANOVA and student’s unpaired *t*-test using the Statistix 8 software package (Analytical Software, Tallahasse, FL) $p \leq 0.05$ was accepted for statistical significance.

RESULTS

Gender-specific SCD mRNA levels in control rats

There is a gender-specific difference in SCD mRNA levels of control rat lungs at d0 (Figure 4). SCD mRNA levels were approximately 153% higher in the lungs of female control rats when compared to the lungs of male control rats at birth ($p=0.005$).

Epigenetic profile in male and female control rats

H3K14Ac and H3K36Me3 were detectable across the length of the SCD gene. A similar epigenetic profile was detected in both modifications showing increased levels of both marks at exon 4 compared to the promoter or the 3' UTR. There was no significant gender-specific difference detected in the amount of H3K36Me3 or H3K14Ac in male and female control rats at d0 (Figure 5a and 5b).

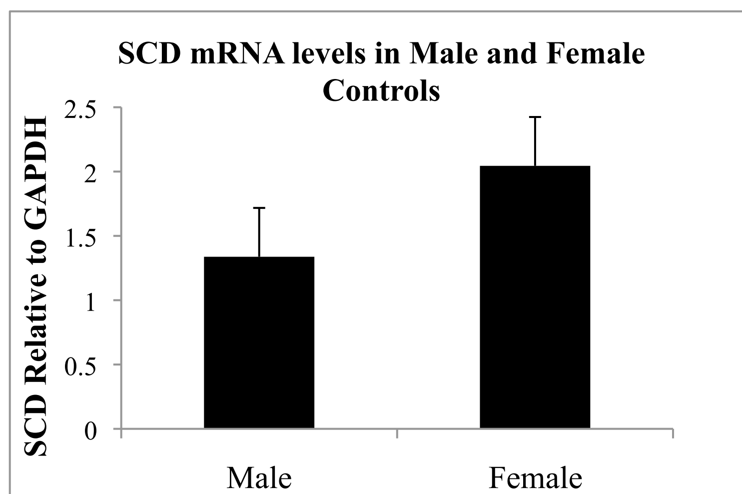
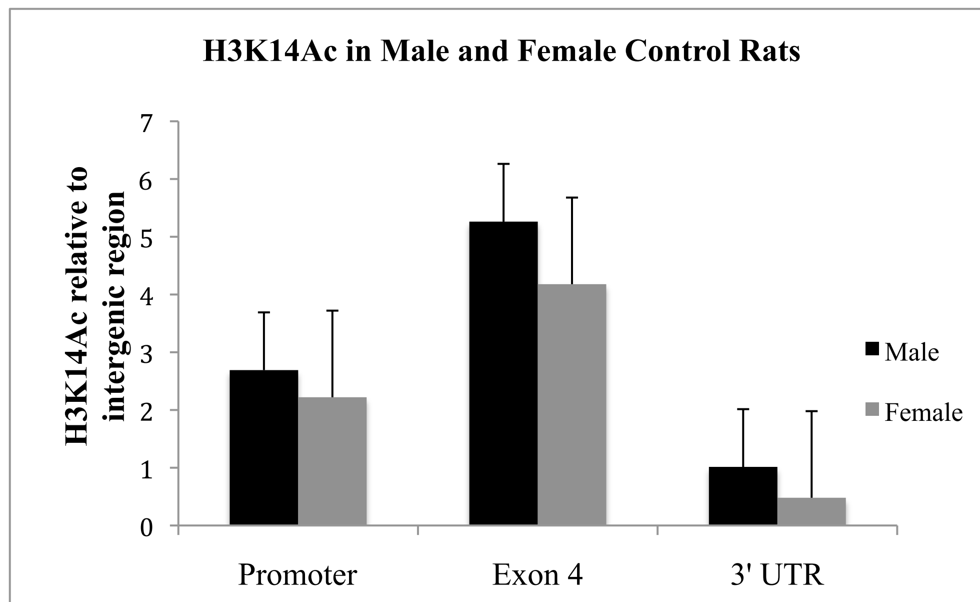


Figure 4. SCD mRNA levels in male and female control rat lungs at d0. (mean ± standard deviation, $p=0.005$)

a.



b.

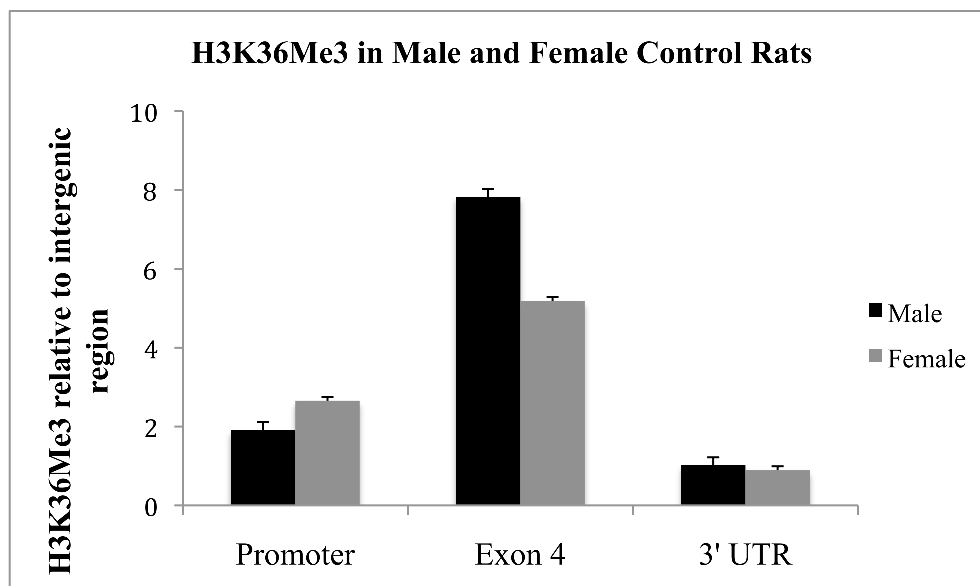


Figure 5. Epigenetic profile at 3 sites along the SCD gene in the lungs of male and female control rats at d0 (a. H3K14Ac, b. H3K36Me3). Data represented as mean ± standard deviation.

Effect of IUGR on SCD mRNA levels

IUGR decreased SCD mRNA levels at birth in males (p=0.0099) and females (p=0.0023) (Figure 6). There were no gender-specific differences in the effects of IUGR on SCD mRNA levels in d0 lungs.

Epigenetic profile along the SCD gene

Effect of IUGR on H3K14Ac

We used ChIP to determine the epigenetic profile along the SCD gene in lung tissue of male and female rats at d0. H3K14Ac was detectable across the length of the SCD gene at birth in male and female IUGR and control rat lungs (Figure 7a and 7b). IUGR had a gender-specific effect on H3K14Ac levels along the SCD gene. There was no change in H3K14Ac seen along the SCD gene in male rat pups, however IUGR increased levels of H3K14Ac in the 3'UTR region of SCD in the lungs of female rats at d0 to 211% of the level observed in controls (Figure 7b, p=0.037).

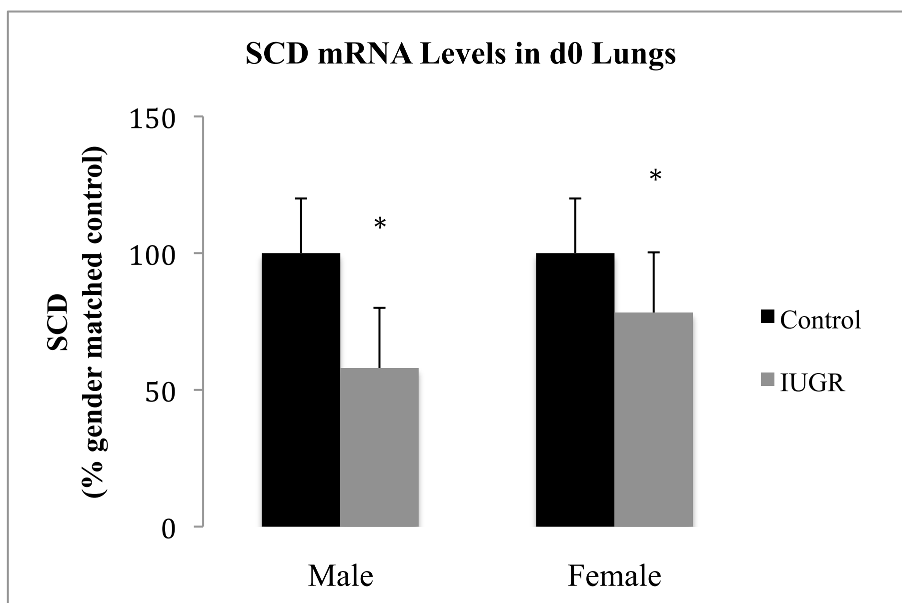
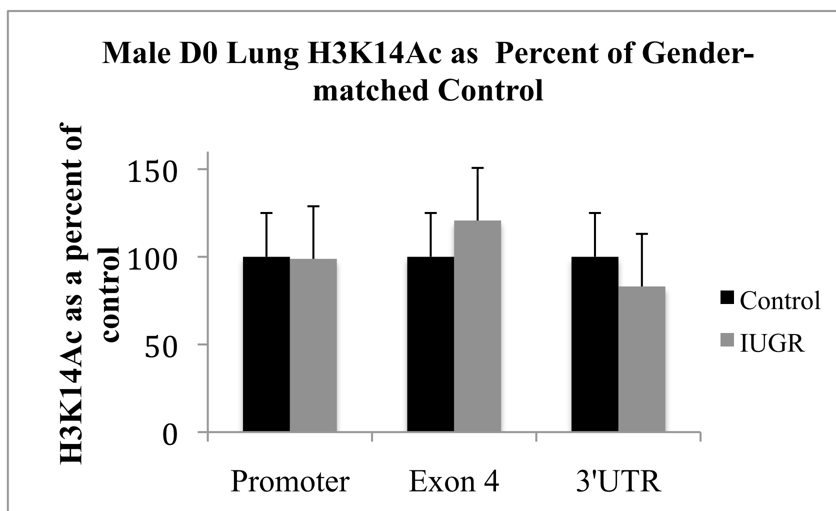


Figure 6. SCD mRNA levels in d0 IUGR lungs relative to controls. Data represented as percent of gender- and age-matched control ± standard deviation (*p<0.05).

a.



b.

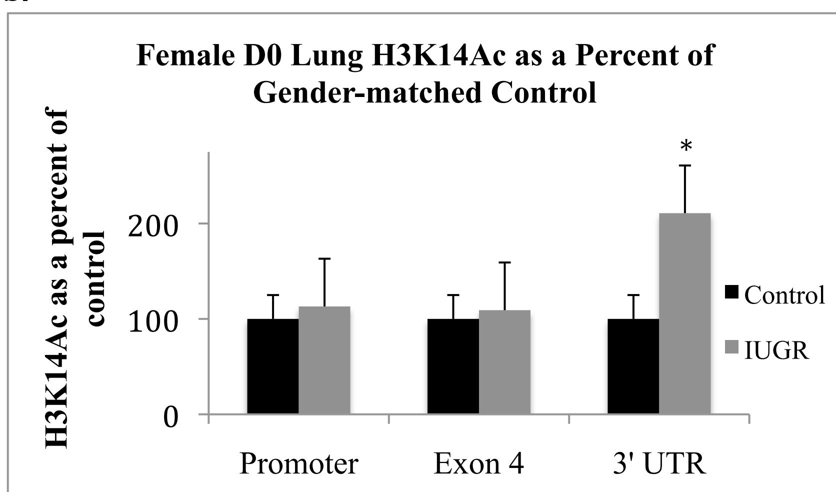
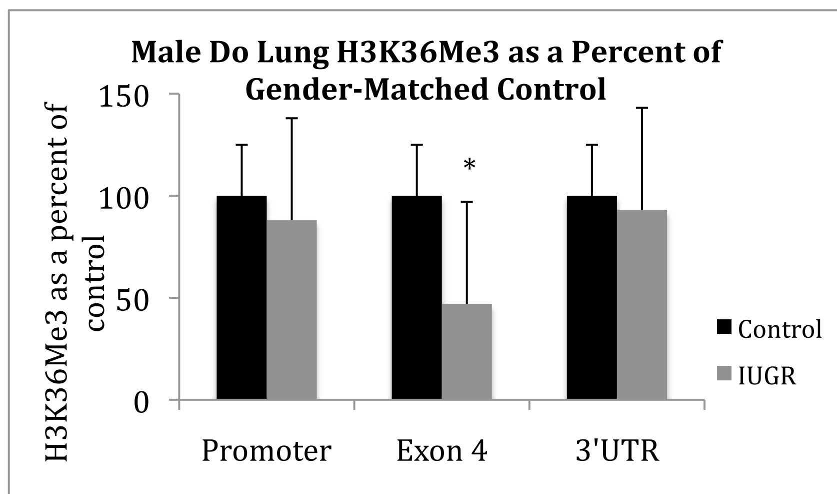


Figure 7. H3K14Ac along the SCD gene in d0 lungs of IUGR rats relative to controls (a. males, b. females). Data presented as H3K14Ac levels in IUGR as a percent of control levels at the promoter, exon 4, and 3' UTR ± standard deviation (*p=0.037).

Effect of IUGR on H3K36Me3

H3K36M3 was detectable across the length of the SCD gene at birth in the lungs of male and female control animals (Figure 8a and 8b). There was a gender-specific change in tri-methylation across the SCD gene associated with IUGR. IUGR decreased H3K36Me3 at the exon 4 of SCD in males by 43% ($p=0.036$) and at the 3'UTR of females by 35% ($p=0.044$) (Figure 8a and 8b) relative to control lungs at d0.

a.



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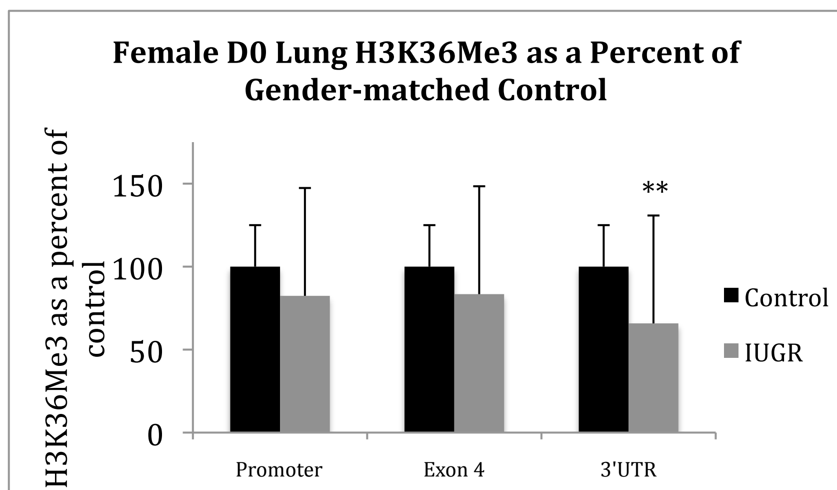
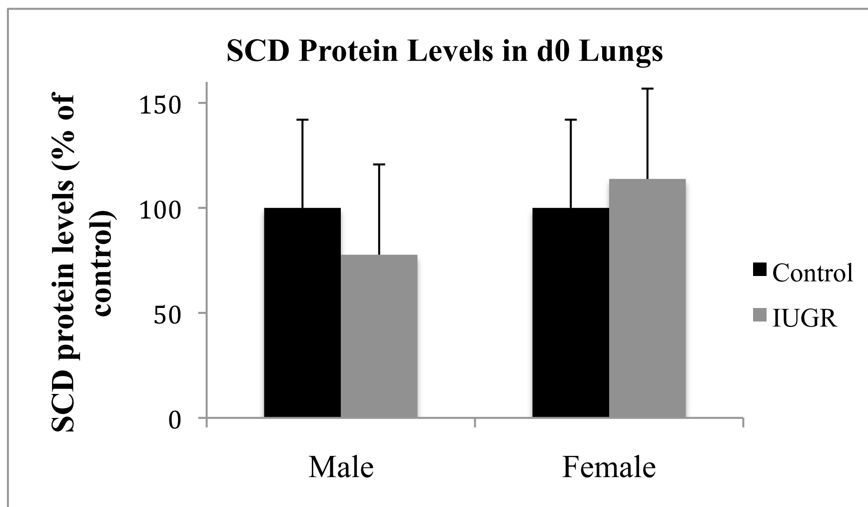


Figure 8. H3K36Me3 along the SCD gene in d0 lungs of IUGR rats relative to controls (a. males, b. females). Data presented as H3K36Me3 levels in IUGR as a percent of control levels at the promoter, exon 4, and 3' UTR ± standard deviation (*p=o.044, **p=0.036).

IUGR has no effect on SCD protein levels in lung tissue at d0

Contrary to SCD mRNA levels, there was no statistically significant difference in SCD protein levels detected between IUGR and control lungs in neither male (p=0.25) nor female (p=0.66) rat pups at d0 (Figure 9).

a.



b.

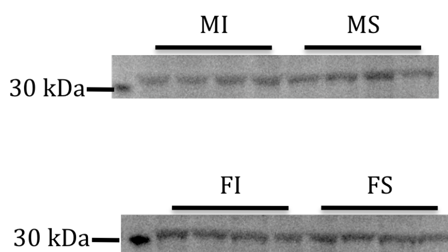


Figure 9. Analysis of SCD protein levels in d0 lungs of IUGR rats relative to controls (a. SCD protein levels represented as a percent of control ± standard deviation, b. western blot of SCD protein in d0 lungs of IUGR and control rats, MI = male IUGR, MS = male control, FI = female IUGR, FS = female control).

DISCUSSION

In this study we gained valuable insight into the potential mechanisms leading to altered lipid metabolism in the lungs of IUGR rats. The primary finding of this study is that IUGR alters the epigenetic profile along the SCD gene in a gender-specific manner. This is accompanied by a decrease in SCD mRNA levels in the lungs of newborn male and female IUGR rats compared to control rats. Despite decreased levels of SCD mRNA, we did not detect a statistically significant change in SCD protein levels in IUGR pups at d0 when compared to controls.

One striking aspect of IUGR seen in both human and animal models is the gender-specificity observed in many of the health implications throughout the lifespan. IUGR males most commonly fair worse than females(10, 11). The findings of this study were often, but not exclusively, gender-specific as well. The level of SCD mRNA detected in control rats at d0 was 153% higher in females than in males, however, this was not accompanied by any gender-specific difference in the epigenetic profile along the SCD gene. These findings lead us to speculate that this observation is associated with the differential timing of lung development seen in male and female rats. Specifically, female rat lungs are at a slightly advanced developmental stage when compared to males at birth and this has been noted to affect gene transcription and surfactant synthesis(37, 38).

A notable gender-specific effect of IUGR in this study, was alterations to the epigenetic profile along the SCD gene. We observed an increase in H3K14Ac at two different locations along the SCD gene in lungs of male and female rats at d0, exon 4 and in the 3'UTR respectively. Additionally, H3K36Me3 was only decreased at exon 4 in the SCD gene of IUGR males at d0. It is generally well accepted that increased H3K14Ac and H3K36Me3 is associated with increased gene expression (27, 39). Despite this association, our results demonstrated gender-specific increases in H3K14Ac and decreases in H3K36Me3 and a net decrease in SCD mRNA levels in the lungs of both male and female IUGR rats at birth. This finding highlights the importance of epigenetic modifications working in concert with each other to have a net effect on gene expression. Although there is an important association between altered epigenetic modifications and altered gene transcription in IUGR, it is unknown whether epigenetics is a causative factor in regulating these changes in gene expression or simply a marker of altered gene expression. Regardless of its role in regulating gene expression, studies such as this one have allowed us to deepen our understanding of the implications of IUGR on a molecular level.

Finally, our observed levels of SCD protein in IUGR lungs at birth compared to control lungs were contrary to what was expected. We expected to see a decrease in mRNA and protein levels consistent with our preliminary findings of decreased palmitoleic acid levels; however, we observed a decrease in SCD mRNA levels and no change in SCD protein levels in IUGR. This could be attributed, in part, to variability in the detection of protein levels through western blot analysis. Additionally, decreased palmitoleic acid levels may be attributed to decreased enzyme activity rather than

decreased protein abundance. An investigation of SCD enzymatic activity would be a valuable addition to the findings presented in this study.

Limitations

The primary limitation in this study was the inability to detect levels of PPARγ at the SCD promoter. Despite multiple attempts, technical difficulties prevented us from successfully completing ChIP with our anti-PPARγ antibody. This is an important piece of data that would contribute to the findings of this study by demonstrating an alteration in PPARγ occupancy of the SCD promoter.

Although transcriptional regulation, mRNA levels and protein levels are important indicators of altered SCD presence in the lungs, this study was not a comprehensive assessment of interference in the catalysis of the conversion of palmitic (16:0) acid to palmitoleic (16:1) acid because we did not include a measure of SCD enzyme activity. Enzymatic activity is a prominent opportunity for molecular interference in fatty acid metabolism and the subsequent lipid environment in the lung, however due to a limited timeframe it was not included in this project.

Additionally, total lung tissue was utilized in these experiments and therefore there will be no distinction of free fatty acids from phospholipid fatty acids. Given the strong correlation between levels of free fatty acids and the fatty acids incorporated into phospholipids, however, we do not perceive that this has greatly influenced our data. One final consideration is the inherent shortcomings of utilizing a rat model to study a human condition. This particular rat model, however, is well characterized and mimics the effects of UPI-induced IUGR on fetal development. Additionally, it allowed us to

study late stages of pulmonary development without the added complications of pre-term delivery.

Future Research

The findings of this study can be used to direct future research that will further elucidate the mechanisms of altered lung development and function in conditions of UPI-induced IUGR. Further elucidating the transcriptional regulation of SCD in the IUGR environment will shed light on potential interventions for the previously described pulmonary consequences of IUGR. Specifically, we plan to pursue investigating the effects of ligand-mediated activation of PPARγ on SCD levels and subsequent measures of lung function such as surfactant composition and compliance. Previous research has shown that polyunsaturated fatty acids (PUFA) can affect fatty acid metabolism by way of regulating gene expression. When supplemented in maternal diets, docosahexaenoic acid (DHA) induces a restorative effect that counters the consequences of IUGR on $PPAR_{\gamma}$ and Setd8 expression in lung tissue(25). Furthermore, studies in a tropical euyhaline fish model have shown that dietary PUFA's alter desaturase expression independent of development in a growth restricted environment (40). These studies indicate that there is strong rationale for observing the effects of DHA supplementation on desaturase expression in IUGR lung tissue. Based on previous findings that 0.1% DHA diet ameliorate the consequences of IUGR on PPARγ and Setd8 expression in lung tissue, I would propose that preliminary studies focus on the effects of 0.1% DHA diet on the lung lipid profile as well as SCD mRNA and protein levels in the lungs of newborn rats.

Potential Impact

The results of this study are a valuable addition to the body literature regarding fetal origins of adult disease. To date, researchers have elucidated only part of the phenotype associated with IUGR infants. By analyzing the levels of SCD mRNA and protein as well as the epigenetic modifications along the SCD gene, this study produced novel findings regarding the association between mRNA levels and epigenetic modifications in the presence of IUGR as well as the gender-specificity these implications. Further elucidating the regulation of lipid metabolism in lung tissue of IUGR infants has established a foundation for future research to deepen the understanding of the molecular mechanisms underlying the IUGR lung phenotype.

Conclusion

The findings of this study have further strengthened the proposition that IUGR impairs lung function in part by altering lung lipid metabolism. By identifying the novel findings of gender-specific histone modifications at different sites along the SCD gene, this study established that SCD is susceptible to transcriptional regulation through an environmentally responsive mechanism of epigenetics. We therefore speculate that the epigenetic modifications introduced along the SCD gene in IUGR rats create a new platform for the gene to respond to subsequent physiologic development, environmental changes and injury that is distinct from a response that would be seen in non-IUGR rats.

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