Decreased fetal biometrics and impaired β-cell function in IUGR fetal sheep are improved by daily ω-3 PUFA infusion

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INTRODUCTION

Placental insufficiency-induced intrauterine growth restriction (IUGR) results in muscle-centric fetal programming that impairs glucose metabolism (Yates et al., 2019) and yields asymmetric growth that favors fat deposition (Gibbs et al., 2020). Decreased nutrients and O\textsubscript{2} impose fetal stress, initiating adaptations for survival that also increase the risk for hypertension, obesity, and diabetes after birth (Yates et al., 2011). Chronic exposure to inflammatory cytokines is a key culprit for the programming mechanisms that yield IUGR and life-long metabolic dysfunction (Yates et al., 2018; Posont et al., 2021). Thus, our objective was to study the effects of manipulating inflammatory activity brought on by fetal stress. Previous studies have found that ω-3 polyunsaturated fatty acids (PUFA) have anti-inflammatory actions and stimulate glucose metabolism (Kim et al., 2019). If we can effectively increase glucose metabolism and improve insulin sensitivity, then glucose homeostasis and metabolic efficiency in the IUGR fetus may be recovered. We hypothesized that manipulation of inflammatory pathways via daily infusion of IUGR fetuses with eicosapentaenoic acid (EPA) in late gestation would improve fetal biometrics and insulin secretion.

MATERIALS AND METHODS

Animals and Experimental Design

All studies were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln, which is AAALAC International-accredited. Time-mated Polypay ewes were used to produce control and placental insufficiency-induced IUGR fetuses as described previously (Yates et al., 2016). Briefly, ewes were exposed to ambient conditions of 40°C and 35% relative humidity from the 40th to the 95th dGA before returning to thermoneutral conditions (25°C). Ewes carrying control fetuses were pair-fed. At 118 dGA, partial cesarean surgeries were performed to place indwelling catheters in the femoral vein and artery of the fetus and an arterial blood flow probe as described previously (Cadaret et al., 2019). On dGA 121 ± 1, IUGR fetuses were randomly assigned to receive daily 1-h i.v. infusions of 0.25 mg/d EPA (i.e., IUGR + EPA; n = 4) or saline placebo (i.e., IUGR; n = 4) for 5 ± 1 d. Control fetuses (n = 12) also received a saline infusion.

Glucose-Stimulated Insulin Secretion

Pancreatic β-cell function was assessed by a square-wave hyperglycemic clamp at 125 ± 1 dGA as described previously (Cadaret et al., 2019). Three arterial samples were taken in 5-min intervals at baseline levels followed by a continuous variable-rate dextrose infusion to achieve...
steady-state hyperglycemia (2.5× baseline). Three additional arterial samples were then taken at 5-min intervals. Blood samples were analyzed using an ABL90 FLEX to measure blood gases and metabolites. Samples collected in EDTA syringes were centrifuged (14,000 × g, 2 min, 4°C) and plasma was isolated and stored at −80°C. Insulin concentrations were then determined using the Bovine Insulin ELISA (Alpco).

**Growth and Organ Metrics**

Ewes were euthanized at 126 ± 1 dGA and fetal body, hindlimb, semitendinosus (ST), flexor digitorum superficialis (FDS), soleus, longissimus dorsi (LD), heart, lung, liver, kidney, and brain masses were measured.

**Statistical Analysis**

All data were analyzed using the mixed procedure of SAS (SAS Institute) with the fetus as the experimental unit. Technical replicates were averaged, and data are presented as mean ± standard error. Significant differences were declared at \( \alpha \leq 0.05 \) and tendencies at \( \alpha \leq 0.10 \).

**RESULTS**

Experimental group x period interactions were observed \( (P < 0.05) \) for plasma insulin and blood lactate but not for blood glucose or glucose-to-insulin ratios. Blood glucose was less \( (P < 0.05) \) for IUGR and IUGR + EPA fetuses than for controls, regardless of period (Figure 1A). Plasma insulin was less \( (P < 0.05) \) for IUGR than controls at baseline and hyperglycemia but did not differ between IUGR + EPA and controls at baseline and was intermediate \( (P < 0.05) \) for IUGR + EPA between controls and IUGR at hyperglycemia (Figure 1B). Glucose-to-insulin ratios were greater \( (P < 0.05) \) for IUGR than controls and intermediate \( (P < 0.05) \) for IUGR + EPA, regardless of period. Blood lactate was greater \( (P < 0.05) \) for IUGR but not IUGR + EPA compared to controls during both periods (Figure 1C).

No group x period interactions were observed for \( \text{Na}^+, \text{K}^+, \text{Cl}^-, \) or \( \text{Ca}^{2+} \) concentrations. Blood \( \text{Na}^+ \) was greater \( (P < 0.05) \) for IUGR but not IUGR + EPA than for controls, regardless of period (Figure 2A). Blood \( \text{K}^+ \) did not differ among groups for either period (Figure 2B). Blood \( \text{Cl}^- \) was greater \( (P < 0.05) \) for IUGR but less \( (P < 0.05) \) for IUGR + EPA than for controls, regardless of period (Figure 3A). Blood \( \text{Ca}^{2+} \) did not differ among groups for either period (Figure 3B). Blood \( \text{Ca}^{2+} \) was less \( (P < 0.05) \) for IUGR but greater \( (P < 0.05) \) for IUGR + EPA than for controls, regardless of period (Figure 3C).

At necropsy, fetal mass was 22% less \( (P < 0.05) \) for IUGR but not IUGR + EPA than for controls.
Competition: $\omega$-3 PUFA and the IUGR fetus

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(Table 1). Fetal hindlimbs tended to be lighter ($P < 0.10$) for IUGR but not IUGR + EPA than for controls. ST muscle mass was less ($P < 0.05$) and soleus muscle mass tended to be less ($P < 0.10$) for IUGR and IUGR + EPA than for controls. LD muscle mass was less ($P < 0.05$) and FDS muscle mass tended to be less ($P < 0.05$) for IUGR but not IUGR + EPA than for controls. Heart and lung mass were less ($P < 0.05$) and kidney mass tended to be less ($P < 0.05$) for IUGR but not IUGR + EPA than for controls. Liver and brain weight did not differ among groups.

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**Table 1.** Fetal biometrics for \( \omega-3 \) PUFA-infused IUGR fetal sheep

| Variable | Experimental group | P-Value |
|----------|--------------------|---------|
|          | Control            | IUGR    | IUGR + EPA |
| Fetal weight, kg | 2.98 ± 0.14\(^{a}\) | 2.21 ± 0.19\(^{b}\) | 2.82 ± 0.17\(^{a}\) | 0.01 |
| Hindlimb, g    | 295 ± 15\(^{a}\)  | 229 ± 23\(^{a}\)  | 292 ± 26\(^{a}\)  | 0.07 |
| ST, g          | 6.21 ± 0.45\(^{b}\) | 4.6 ± 0.6\(^{a}\) | 4.33 ± 0.61\(^{a}\) | 0.04 |
| Soleus, g      | 0.86 ± 0.12\(^{a}\) | 0.5 ± 0.08\(^{b}\) | 0.46 ± 0.22\(^{a}\) | 0.08 |
| LD, g          | 60.6 ± 3.3\(^{a}\)  | 45.5 ± 3.8\(^{a}\)  | 60.4 ± 3.8\(^{a}\) | < 0.01 |
| FDS, g         | 5.5 ± 0.5\(^{a}\)  | 3.5 ± 0.5\(^{b}\)  | 4.8 ± 0.5\(^{a}\)  | 0.07 |
| Heart, g       | 26.3 ± 1.7\(^{a}\)  | 20.4 ± 0.8\(^{b}\)  | 23.1 ± 1.9\(^{a}\)  | 0.02 |
| Lungs, g       | 101.6 ± 5\(^{a}\)  | 76 ± 3.6\(^{a}\)  | 98.5 ± 4.2\(^{a}\) | < 0.001 |
| Liver, g       | 116.2 ± 10.5\(^{a}\) | 105.7 ± 31\(^{b}\) | 101.8 ± 7\(^{a}\) | NS |
| Kidneys, g     | 20.5 ± 1.6\(^{a}\)  | 15.6 ± 1.6\(^{b}\)  | 20.8 ± 2.5\(^{a}\) | 0.09 |
| Brain, g       | 45.2 ± 1.1\(^{a}\)  | 41.5 ± 1.5\(^{b}\)  | 43.4 ± 2.4\(^{a}\) | NS |

Data are presented as mean ± SE.

\(^{a,b}\)Means with different superscripts differ \((P < 0.05)\).

\(^{a,b}\)Means with different superscripts tend to differ \((P < 0.10)\).

NS, not significant.

**DISCUSSION**

In this study, we found that several metabolic and growth deficits caused by IUGR were mitigated by daily infusion of fetuses with the anti-inflammatory \( \omega-3 \) PUFA, EPA. Basal and glucose-stimulated insulin secretion, which was impaired by over 50% in the IUGR fetus, was improved by \( \omega-3 \) PUFA infusion, indicating \( \beta \)-cell function benefits from inflammatory mitigation. However, only partial recovery occurred under hyperglycemic conditions, indicating that factors other than inflammation also contribute to IUGR \( \beta \)-cell dysfunction. Our results may coincide with improved \( \beta \)-cell mass, as previous studies showed that \( \beta \)-cell mass reduced by placental insufficiency directly leads to poor insulin secretion (Gatford and Simmons, 2013). Fetal \( \beta \) cells require glucose to develop, and EPA has been shown to improve glucose uptake at least in fat cells (Figuers et al., 2011). Similar improvements in \( \beta \) cells might help explain better functionality. Furthermore, glucose-to-insulin ratios were improved in IUGR fetuses receiving \( \omega-3 \) PUFA treatment, despite a lack of differences in blood glucose. These results build upon our earlier studies by showing improved metabolic function in IUGR fetuses when fetal inflammation was controlled.

Glycolytic lactate production seemed to be favored over glucose oxidation in the IUGR fetus, as indicated by increases in basal blood lactate and even more profound increases under hyperglycemia. Under both conditions, however, high lactate was rectified by \( \omega-3 \) PUFA infusion. Increased lactate levels can increase insulin resistance in IUGR individuals (Kim et al., 2019), and previous work by us and others shows that limited capacity for glucose oxidation in favor of lactate production provides carbon substrates for hepatic glucose production in the IUGR fetus (Brown et al., 2015; Cadaret et al., 2019). Surprisingly, blood pH was increased in our IUGR fetuses, despite high lactate. However, greater blood HCO\( _3 \) presumably offset lactate concentration and increased pH. Controlling fetal inflammation with \( \omega-3 \) PUFA also improved CO\( _2 \) and O\( _2 \), which were increased and decreased by IUGR, respectively. Together, these findings further support our belief that glycolytic production of lactate is increased in IUGR fetuses as a nutrient-sparing mechanism.

Biometric deficits in the IUGR fetus were consistent with decreased muscle mass and asymmetric growth, as previously observed by our lab (Cadaret et al., 2019; Yates et al., 2019; Posont et al., 2021). However, controlling inflammation in these fetuses provided promising results that indicate improved total body and muscle-specific growth. The metabolic importance of skeletal muscle cannot be overstated, as it clears 65% of total glucose from circulation (Yates et al., 2018). Thus, improved muscle mass almost assuredly contributed to better glucose-to-insulin ratios, as it should increase the capacity for glucose oxidation independent of any other changes in metabolic function. Interestingly, soleus and ST muscle mass was not improved, indicating that the effect may be specific to certain muscle types.

Together, these findings allow us to conclude that multiple factors contributing to glucose homeostasis were improved by controlling inflammation in the IUGR fetus. Insulin secretion was recovered, and fetal biometrics indicate that muscle growth was at least partially recovered. We speculate that improvements observed represent mediation of fetal inflammation and its impact on developmental programming of IUGR.

**IMPLICATIONS**

Based on our results from this study, placental insufficiency-induced IUGR fetuses display deficits in metabolism and growth which are consequences of inflammation-induced fetal programming. Consequently, these mechanisms can be improved via daily infusion of the \( \omega-3 \) PUFA, EPA, during late gestation. Although not practical for commercial application, these findings provide a basis for further research that targets fetal inflammation through maternal delivery of the \( \omega-3 \) PUFA, as it is
placenta-soluble, in order to improve fetal growth and glucose homeostasis.

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