Maternal inflammation at ***mid-gestation*** in pregnant rats impairs fetal muscle growth & development ***at term***

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Abstract

Intrauterine growth restriction is linked to lifelong decreases in muscle mass and increased risk for metabolic disease. The poor intrauterine environment resulting from maternal stress increases fetal loss and causes growth-restricting adaptations in fetal muscle. **Our objective was to determine the effects of sustained maternal inflammation at mid-gestation on fetal mortality, muscle growth, and development at term.** Pregnant rats were treated daily with bacterial endotoxin (LPS) on days 9-11 of gestation to induce maternal inflammation. On day 20, the number of fetuses did not differ between groups, but total fetal mass was lower ($P < 0.05$) in LPS-treated rats. Fetal plasma TNFα tended to be greater ($P < 0.1$) and fetal skeletal muscle TNFR1 and IL6R mRNA tended to be decreased ($P < 0.1$) in LPS-treated rats compared to controls. RNA markers of total macrophages (CD68) and M2 macrophages (CD163) tended to be decreased ($P < 0.1$) in fetal hindlimb muscle after maternal inflammation. CD68-positive nuclei were also decreased ($P < 0.05$) but CD163-positive nuclei were not different. Moreover, there was a tendency for less ($P < 0.1$) myoD-positive cells and greater ($P < 0.1$) myogenin-positive cells in fetal hindlimb after maternal inflammation. Decreased resident macrophages combined with increased circulating TNFα indicate that fetal macrophages were more productive after maternal inflammation despite reduced prevalence in muscle. Moreover, decreased muscle cytokine receptor expression is likely a compensatory response that reduces muscle cytokine sensitivity in response to greater circulating cytokines. Lastly, reduced myoD-positive nuclei and increased in myogenin-positive nuclei indicates impaired myoblast function, which is likely responsible for decreased fetal mass. Together, our findings demonstrate that maternal inflammation at mid-gestation causes fetal adaptations that impair subsequent muscle development and growth.

Introduction

- Skeletal muscle makes up less than 50% of total body mass but greater than 80% of insulin-stimulated glucose utilization.
- Uninterrupted myogenesis during gestation is critical for postnatal skeletal muscle growth, repair, and metabolism.
- Inflammation impairs myoblast function and muscle metabolism.
- We **hypothesized** that sustained maternal inflammation impairs normal fetal muscle growth and development and thus metabolic homeostasis.

Materials and Methods

- On **days 9-11** of gestation, timed-pregnant rats were given daily IP injections of saline or LPS (100µg/kg BW; *E. coli* O55:B5) and monitored:
  - Maternal blood & BT
  - Blood glucose concentration
  - Plasma TNFα concentrations
- On **day 20** of gestation, maternal & fetal tissues were collected:
  - Fetal weight
  - Maternal & fetal blood samples
  - Fetal hindlimbs for IHC & ddPCR analysis

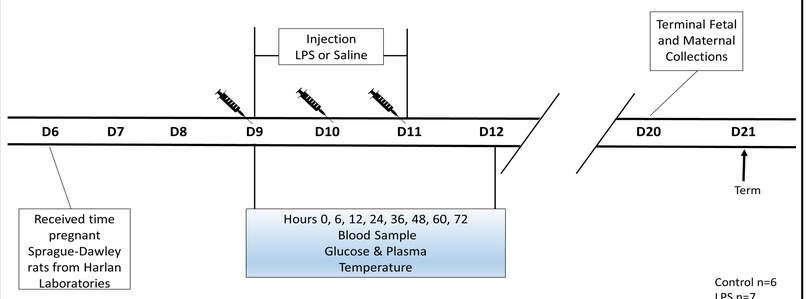

Figure 1: Experimental timeline in gestational days.

Results

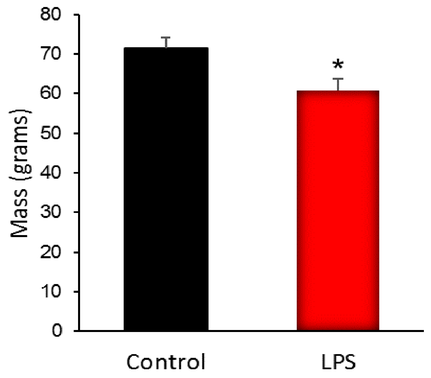

Figure 2: Fetal mass at term.

Mater. Inflam. ↓ subsequent fetal growth

- ND in fetuses / litter
- ND in fetal loss (reabsorption sites)

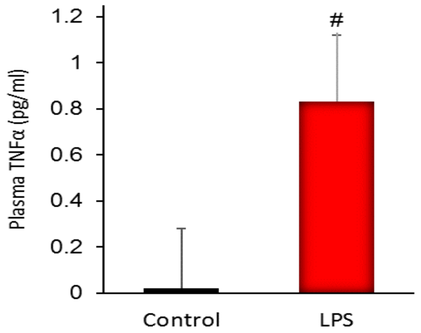

Figure 3: Fetal plasma TNFα at term.

Mater. Inflam. ↑ subsequent fetal TNFα

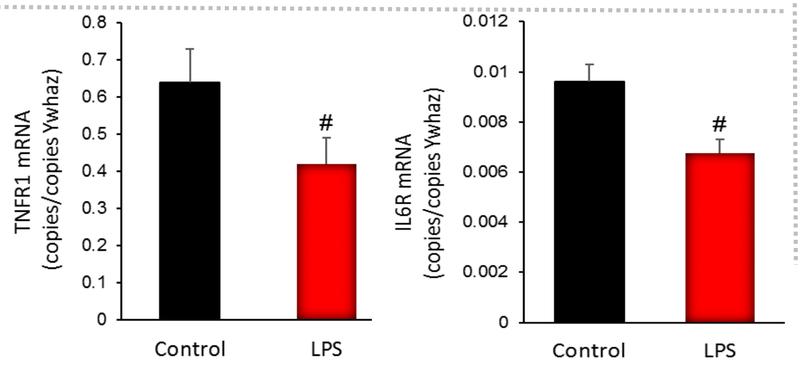

Figure 4: Fetal muscle cytokine receptors.

Mater. Inflam. ↓ fetal muscle TNFR1 & IL6R

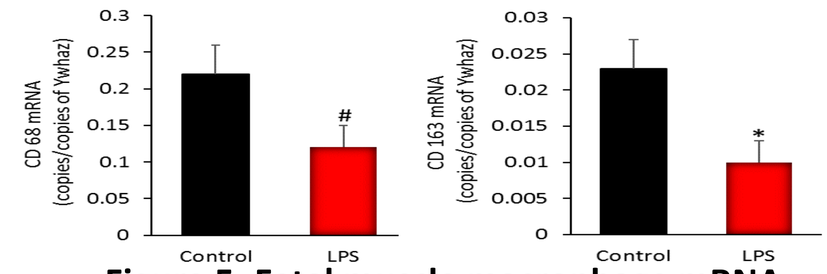

Figure 5: Fetal muscle macrophage mRNA

Mater. Inflam. ↓ muscle Pan & M2 macrophage RNA

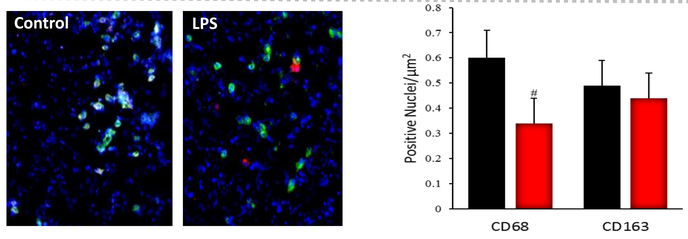

Figure 6: Fetal muscle M1 & M2 macrophages

Mater. Inflam. ↓ total resident muscle macrophages

- ND in M2 (anti-inflam.) macrophages

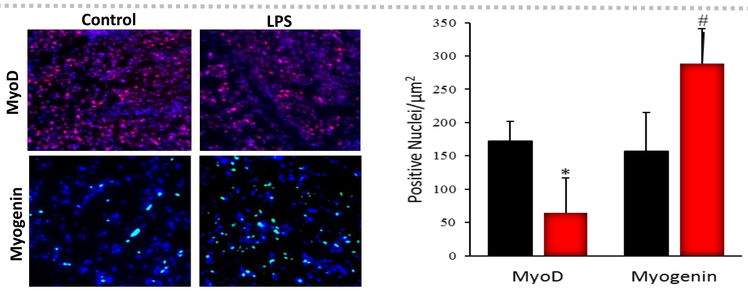

Figure 7: Fetal hindlimb myoD & myogenin

Mater. Inflam. ↓ fetal myoblast function

- Allows precocious myoblast differentiation (↓myoD/↑ myogenin)
- ND in myf5

Implications

Maternal inflammation at mid-gestation induces fetal adaptations that disrupt cytokine-regulated myoblast function and thus skeletal muscle development & growth capacity.

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