Metabolic Regulation by Stress Mediators in Adult Myoblasts and Fetal Skeletal Muscle

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ABSTRACT

Stress factors including catecholamines and cytokines regulate glucose metabolism. However, the underlying mechanisms and impact on developing muscle are not known. **Our objective** was to determine how β adrenergic agonists and inflammatory cytokines impact glucose metabolism in myoblasts and fetal skeletal muscle. In Exp. 1, glucose uptake/oxidation were determined in primary bovine myoblasts differentiated for 4 days with insulin, β1 agonist, or β2 agonist. In Exp. 2, chronic (2 wk) inflammation was induced in pregnant ewes by repeated LPS injection in the early 3rd trimester. Ten days later, ewes were euthanized and fetal soleus muscle was used to determine glucose uptake/ oxidation when incubated with insulin or TNFα. In myoblasts, β2 agonist (but not β1 agonist) increased glucose uptake and oxidation. Both β agonists slightly reduced insulin-stimulated glucose oxidation but not insulin-stimulated uptake. In fetal muscle, insulin-stimulated and TNFα-stimulated glucose oxidation were greatly decreased subsequent to chronic maternal inflammation. Our findings in Exp. 1 show that adrenergic-stimulated glucose oxidation is mediated by the β2 receptor and is independent of insulin action or glucose uptake rates. We previously found that insulin and TNFα directly stimulate glucose oxidation in muscle ex vivo, but in Exp. 2 we show that chronic inflammation in utero reduces the subsequent stimulatory abilities of both. Together, these results show that stress mediators promote glucose oxidation in skeletal muscle, but this effect is diminished by over-exposure.

INTRODUCTION

- Skeletal muscle accounts for **>80%** of insulin-stimulated glucose metabolism.
- β adrenergic agonists enhance muscle growth efficiency in food animals when used as a feed additive.
- **Obj. 1** was to determine whether β adrenergic agonists have similar effects on glucose metabolism in bovine myoblasts as previously found in adult skeletal muscle.
- We previously found that TNFa and IL-6 directly stimulate glucose oxidation in skeletal muscle
- **Obj.** 2 was to determine if chronic inflammation alters metabolic responsiveness to insulin and/or cytokines in fetal muscle, thus reducing their capacity to stimulate glucose oxidation.

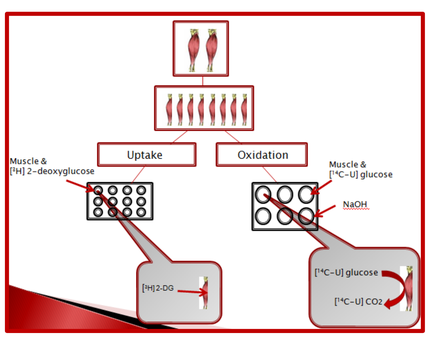
MATERIALS AND METHODS

Experiment 1:

- Bovine myoblasts were differentiated for 4 days with 5mU/ml insulin, 1.0 μM β1 (ractopamine HCl) adrenergic agonist, or 0.05 μM β2 (zilpaterol HCl) adrenergic agonist.
- Glucose uptake rates were determined by incubating myoblasts in treatment spiked KHB media with 1 mM [^{3}H] 2-deoxyglucose and 39 mM [U-^{14}C] mannitol for 20 min as previously described (Cadaret et al. 2017).
- Glucose oxidation rates were determined by incubating myoblasts in treatment spiked KHB media with 5 mM [^{14}C-U] D-glucose for 120 min as previously described (Cadaret et al. 2017).

Experiment 2:

- Timed-pregnant ewes were injected with saline (controls) or bacterial endotoxin (LPS) every 3rd day between day 100-112 of gestation to induce chronic maternal inflammation.
- On day 122, ewes were euthanized and fetal soleus muscle was isolated and split longitudinally.
- Glucose uptake rates were determined by incubating muscle strips in KHB media that was un-spiked (basal) or spiked with either insulin or TNFα.
- Glucose oxidation rates were determined by incubating muscle strips in KHB media that was un-spiked (basal) or spiked with either insulin or TNFα.



RESULTS

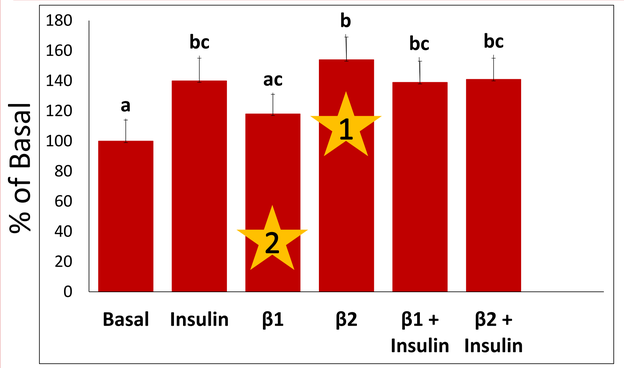


Figure 1. Glucose Uptake in Diff'd Bovine Myoblasts

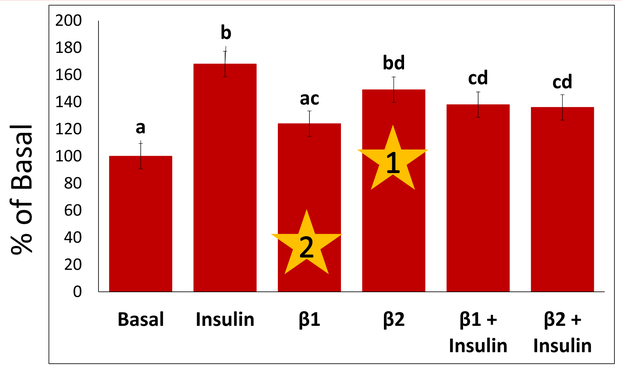


Figure 2. Glucose Oxidation in Diff'd Bovine Myoblasts

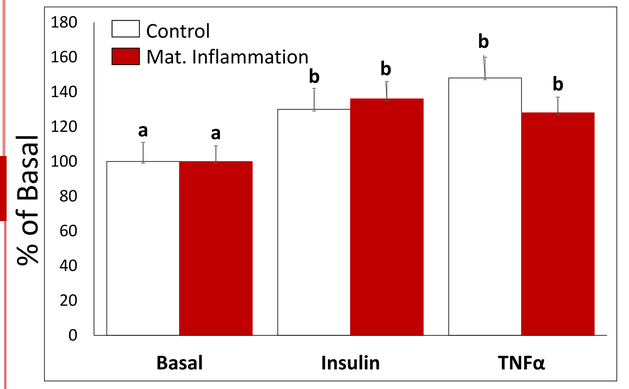


Figure 3. Glucose Uptake in Primary Fetal Muscle

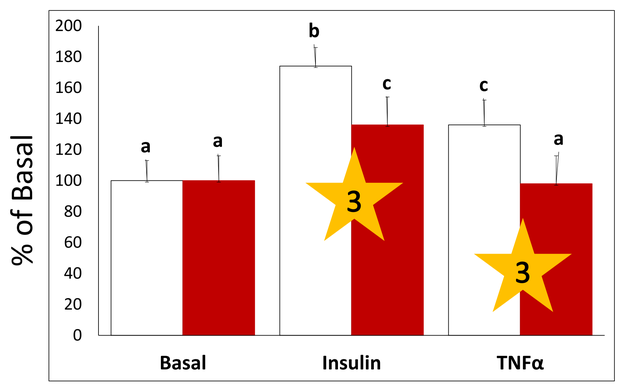


Figure 4. Glucose Oxidation in Primary Fetal Muscle

1. In myoblasts, **β2 adrenergic agonist increased glucose uptake and oxidation**
2. **β1 had no effect on glucose uptake or oxidation** in myoblasts
3. In fetal muscle, **maternal inflammation decreased subsequent insulin-stimulated & TNFa-stimulated glucose oxidation**

IMPLICATIONS

- Both β1 and β2 adrenergic agonists (trade names Ractopamine HCl and Zilpaterol HCl, respectively) are FDA-approved feed additives for food animals, but this study demonstrates that **β2 agonist is more effective in increasing metabolic efficiency** in muscle stem cells.
- Adrenergic and inflammatory mediators **regulate glucose metabolism** in developing skeletal muscle.
- The **response to stress mediators is diminished by chronic exposure**, even after exposure is alleviated.

ACKNOWLEDGEMENTS

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