



The role of toll-like receptor 4 in mitochondrial function and reactive oxygen species (ROS) production in skeletal muscle

Yaru Wu, Madlyn Frisard, Elike Shabrokh, Kevin Voelker, Ryan P McMilian, Matthew W Hulver.

Department of Human Nutrition, Foods, and Exercise
Virginia Polytechnic Institute and State University
Blacksburg, VA



ABSTRACT

Toll-like receptor 4 (TLR4) is elevated in skeletal muscle of obese humans, and data from our lab showed that activation of TLR4 in skeletal muscle with lipopolysaccharide (LPS) results in decreased fatty acid oxidation. The purpose of this study was to determine if TLR4 activation in skeletal muscle alters mitochondrial function and increases the production of ROS. C2C12 and human primary myotubes, and mitochondria isolated from rodent gastrocnemius muscle were studied. TLR4 activation (LPS 50pg/ml for 2h) resulted in a significant reduction in maximal oxidative respiration in both C2C12 and human primary myotubes, which was observed in concert with an increase in production of ROS, and alterations in mitochondrial gene expression and protein content. These effects were blocked in the presence of the antioxidant, N-acetyl cysteine (NAC). Moreover, mitochondria isolated from skeletal muscle of mice 4 hours following an intraperitoneal injection of LPS (1mg/kg) displayed reduced respiratory control, ADP-stimulated respiration, and maximal respiration. These findings suggest that TLR4 activation causes mitochondrial dysfunction in skeletal muscle, which is partially dependent on the production of ROS.



CONTACT

Yaru Wu
Virginia Tech
yaru@vt.edu

Poster Design & Printing by Genographics® - 800.790.4001

INTRODUCTION

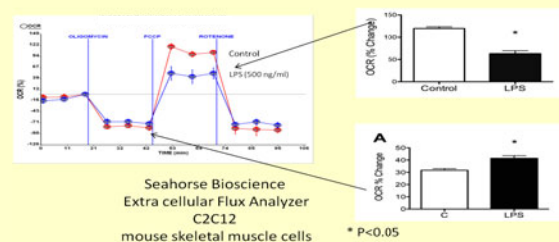
- TLR4 is a transmembrane receptor that, upon activation, plays an important role in the induction of inflammatory pathways.
- Growing evidence suggests a role of reactive oxygen species (ROS), a class of free radicals containing oxygen, in the development of many metabolic disorders.
- An elevated pro-inflammatory response in cells can further increase the level of oxidative stress.

PURPOSE

The purpose of this project is to test the hypothesis that TLR4 activation results in impaired mitochondrial function (reduced oxygen consumption), and whether it is partially dependent on the production of reactive oxygen species.

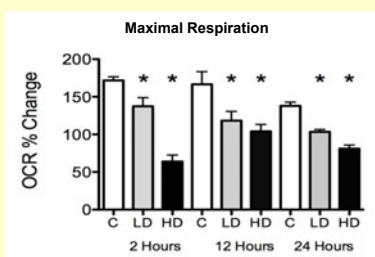
RESULTS

1 TLR4 reduces maximal oxygen consumption.



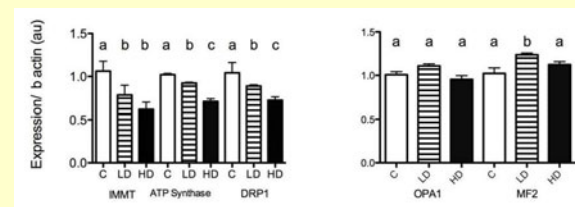
C2C12 muscle cells were grown to 90% confluency in DMEM plus 10% FBS and differentiated into multinucleated myotubes in DMEM plus 2% horse serum for 5 days. Myotubes were treated with 500ng/ml of LPS for 2 hours. Cellular respiration was measured using a Seahorse Bioscience extracellular flux analyzer. Oxygen consumption is measured under basal conditions and the administration of oligomycin (ATP synthase inhibitor), the uncoupler carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP) to assess maximal respiration, and rotenone (Complex I inhibitor). Values are expressed as mean \pm SEM. *P<0.05.

2 TLR4 reduces maximal respiration.



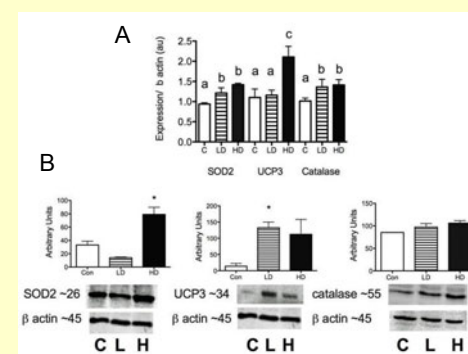
C2C12 muscle cells were grown and differentiated as described above. Myotubes were treated with either 50pg/ml or 500ng/ml of LPS for 2, 12, or 24 hours. Cellular respiration was measured as described above. Values are expressed as mean \pm SEM. *P<0.05.

3 TLR4 activation results in alterations in mitochondrial gene expression in skeletal muscle cell culture.



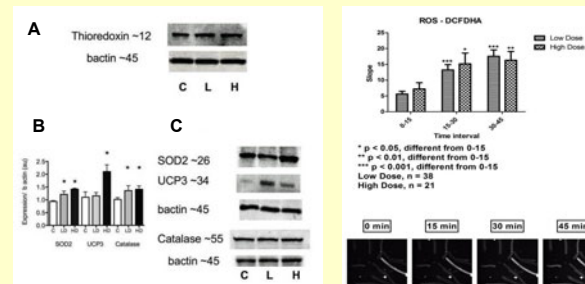
C2C12 muscle cells were grown and differentiated as described above. Myotubes were treated with either low or high dose LPS for 2 hours. The cells were harvested in Trizol, and RNA was extracted. mRNA levels for Mitochondrial inner membrane protein (IMMT), ATP Synthase, dynamin related protein 1 (DRP1), optic atrophy protein 1 (OPA1), and Mitofusin 2 (MF2) were measured and corrected for β actin mRNA levels. Values are expressed as mean \pm SEM. Different letters indicated significant differences.

4 TLR4 activation up regulates antioxidant mRNA in skeletal muscle cell culture.



C2C12 muscle cells were grown and differentiated as described above. Myotubes were treated with either low or high dose LPS for 2 hours. Immediately following treatment, cells were collected for assessment of mRNA (A) and protein (B) of Superoxide Dismutase 2 (SOD2), Uncoupling protein 3 (UCP3), and Catalase. Values are expressed as mean \pm SEM. *P<0.05.

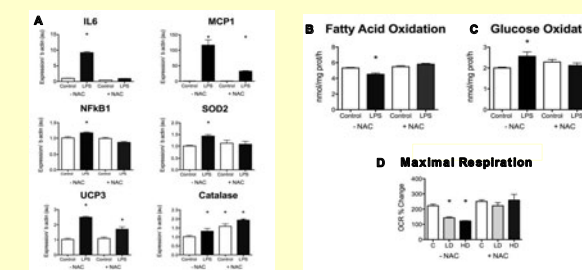
5 TLR4 increases reactive oxygen species (ROS) and oxidative damage.



Left Panel: Fully differentiated C2C12 myotubes were treated with either low or high dose LPS for 2 hours. Immediately following treatment, cells were collected for assessment of mRNA and protein. Treatment with LPS resulted in an increase in protein content of the antioxidant thioredoxin, a commonly used marker of oxidative stress (A). LPS treatment also resulted in significant increases in mRNA (B) and protein (C) of Superoxide Dismutase 2 (SOD2), Uncoupling protein 3 (UCP3), and Catalase.

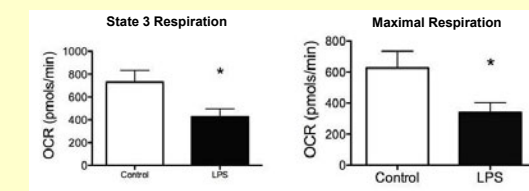
Right Panel: FDB muscle fibers were stained with carboxy-H2DFFDA, a reactive oxygen species detector, for 30 minutes at 37°C. Fibers were then exposed for 400 ms, and 1 image was recorded 15 minutes post exposure. Fibers were then treated with either low or high dose LPS, and imaged at 30 and 45-minute time points. LPS exposure resulted in a significant increase in ROS up to 45 minutes post exposure. Values are expressed as mean \pm SEM. *P<0.05.

6 The effects of TLR4 on skeletal muscle are dependent, in part, on reactive oxygen species.



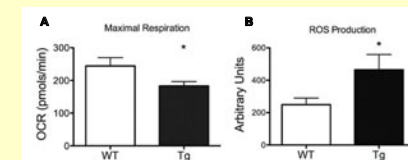
C2C12 muscle cells were grown and differentiated as described above. Myotubes were treated with high dose LPS in the absence and presence of 20 mM N-acetylcysteine (NAC) for 2 hours. Immediately following treatment, cells were collected for assessment of mRNA (A), Fatty acid oxidation (B), glucose oxidation (C), and cellular respiration (D) was immediately measured after treatment. Values are expressed as mean \pm SEM. *P<0.05.

7 TLR4 activation impairs mitochondrial function in isolated mitochondria from skeletal muscle.



Eight week old C57Bl/6 mice were injected with LPS at a dose of 25 μ g/mouse. The animals were sacrificed and gastrocnemius muscle was collected 4 hours post injection. Mitochondria were isolated and oxygen consumption was measured using a Seahorse Bioscience extracellular flux analyzer. Immediately following isolation and protein quantification, mitochondria were plated on Seahorse cell culture plates at a concentration of 5ug/ well in the presence of 10mM pyruvate and 5mM malate. Oxygen consumption was measured under basal conditions, ADP (5mM) stimulated state 3 respiration, oligomycin (2 μ M) induced state 4 respiration, and in the presence of FCCP (0.3 μ M) to assess maximal respiration. Values are expressed as mean \pm SEM. *P<0.05.

8 Muscle-specific over expression of TLR4 results in reduced maximal respiration and increased production of ROS.



C57Bl/6 mice with over expression of TLR4 and their wildtype littermates were sacrificed after 12 hour fast. Mitochondria were isolated and oxygen consumption (A) was measured using a Seahorse Bioscience extracellular flux analyzer. ROS production (B) was measured using Amplex Red Hydrogen Peroxide/ Peroxidase assay Kit. Values are expressed as mean \pm SEM. *P<0.05.

CONCLUSIONS

TLR4 activation in skeletal muscle results in alterations in mitochondrial function, i.e. reduced oxygen consumption, fatty acid oxidation, and mitochondrial mRNA and protein.

The TLR4 induced effects on mitochondrial function are partly due to the production of reactive oxygen species.

REFERENCE

Am J Physiol Endocrinol Metab 298:988-998, 2010.