

# Selective over expression of toll-like receptor 4 in skeletal muscle causes impaired adaptation to high fat feeding

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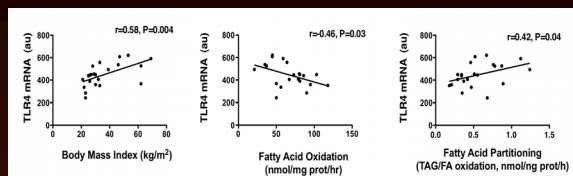


## Abstract

Our laboratory has shown that toll-like receptor 4 (TLR4) is elevated in skeletal muscle of obese humans and its activation results in a partitioning of fatty acids toward storage at the expense of oxidative pathways. To better understand this phenomenon, we developed a mouse model, on C57Bl/6 background, with selective over expression of TLR4 in skeletal muscle (mTLR4). Mice were metabolically characterized on chow and a 45% high fat (HF) diet. Skeletal muscle from mTLR4 mice displayed heightened activation of pro-inflammatory pathways as evidenced by increased protein levels of interleukin-6 and tumor necrosis factor-alpha. On a chow diet, fasting palmitate oxidation was 27% lower in red skeletal muscle from mTLR4 mice compared to wild-type (WT); no differences were observed in white muscle. Following 16 weeks of HF diet, fatty acid oxidation was significantly increased in WT mice (+17.8%,  $p < 0.05$ ) while this adaptation was not evident in the mTLR4 mice (-5.2%). mTLR4 mice tended to gain more weight (+17.2±1.2 vs. +15.1±0.9g,  $P=0.09$ ) and became more glucose intolerant (GTT AUC, 35.3±2.6 vs. 29.7±1.2, mmol,  $p < 0.05$ ) on HF diet compared to WT mice. Energy expenditure (kJ/h/kg FFM) was differentially affected by diet between mTLR4 and WT mice; no differences were observed in response to low fat diet (103.1±4.2 vs. 104.5±4.7) but values were significantly lower in mTLR4 mice following HF diet (98.7±2.2 vs. 108.6±3.4,  $p < 0.05$ ). In conclusion, mTLR4 mice possess a heightened pro-inflammatory milieu and blunted oxidative capacity in skeletal muscle, which are associated with impaired adaptation to high fat feeding. These data suggest that increased TLR4 expression in skeletal muscle may play a role in the metabolic dysregulation associated with obese and diabetic states.

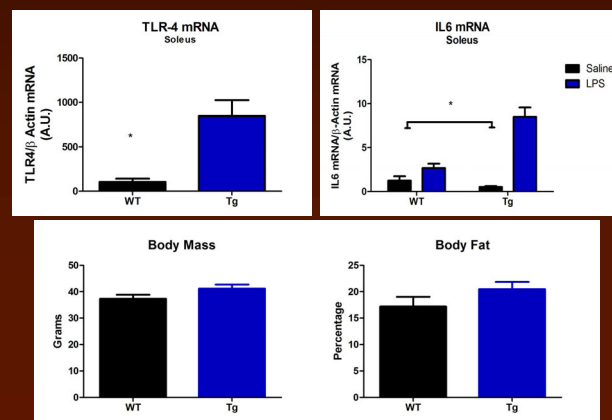
## Introduction

TLR4 expression is elevated with obesity and associated with intramuscular TAG synthesis.



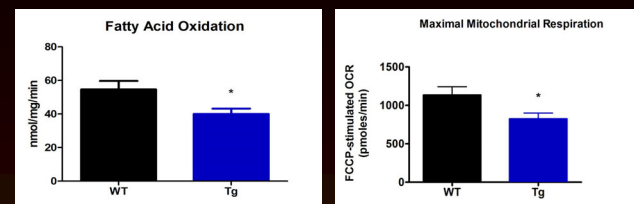
Rectus abdominus muscle was obtained from nonobese and obese humans and TLR4 expression and [1-<sup>14</sup>C]-palmitic acid metabolism were assessed.

Skeletal muscle-specific over expression (mTLR4) of TLR4 results in elevated LPS-induced IL6 response



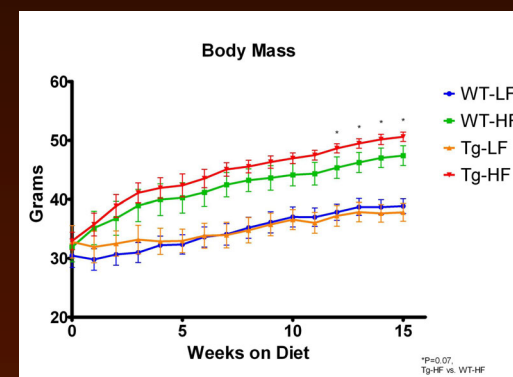
C57Bl/6 mice with muscle specific TLR-4 over expression were generated via the muscle creatine kinase promoter. IL-6 gene expression was assessed following 500ng/mL LPS injection. Body composition was assessed at 12 weeks of age.

## 1 mTLR4 mice have suppressed skeletal muscle fatty acid oxidation and mitochondrial respiration.

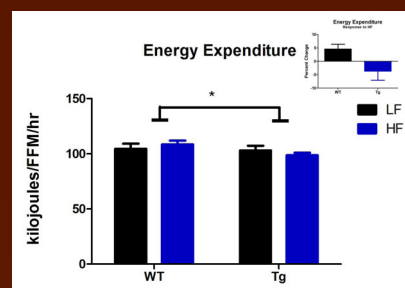
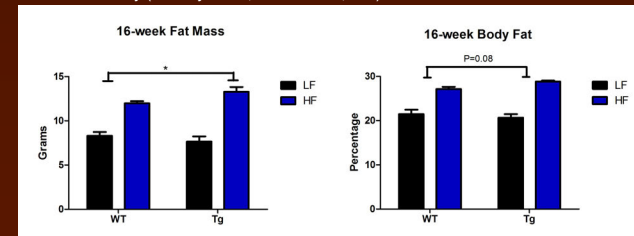


Eight week old mice were fasted overnight, sacrificed and tissue was harvested for ex-vivo metabolism measurements. Mitochondria were isolated from red gastrocnemius and quadriceps muscle. Fatty acid oxidation was measured in isolated mitochondria by assessing [1-<sup>14</sup>C]-palmitic acid metabolism to <sup>14</sup>CO<sub>2</sub>. Maximal mitochondrial oxygen consumption was measured in response to FCCP (0.3 μM) using a Seahorse Bioscience extracellular flux analyzer. Data expressed as mean ± SEM, \*  $p < 0.05$ .

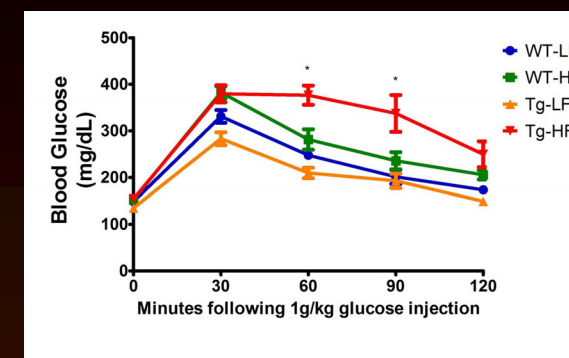
## 2 mTLR4 mice gain more weight on a high fat diet compared to wild-type littermates



Mice were maintained on 12hr light-dark cycles and fed either a 45% high fat (Teklad TD.10505) or control (TD.10453) diet for 16 weeks. Body composition was assessed via the Bruker mini spec LF90. Energy expenditure was measured over 48-hours using indirect calorimetry (TSE Systems, Chesterfield, MO)

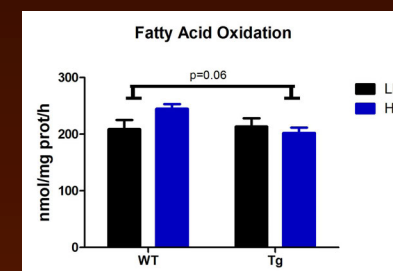


## 3 mTLR4 mice are glucose intolerant on a high fat diet compared to WT



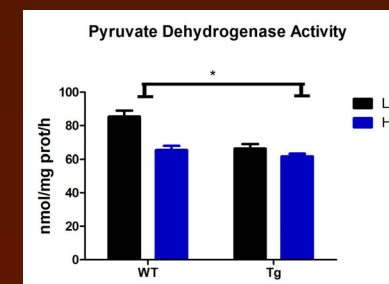
Following an overnight fast, mice were injected with 1g/kg glucose and blood was measured via the tail vein every 30 minutes for 2 hours. Data is expressed as mean ± SEM. \*  $P < 0.05$ .

## 4 Adaptive response of mitochondrial fatty acid oxidation in response to high fat diet is absent in mTLR4 mice



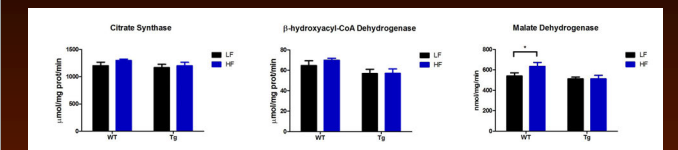
[1-<sup>14</sup>C]-palmitic acid oxidation to <sup>14</sup>CO<sub>2</sub> was assessed at 16-weeks of high fat feeding. Isolated mitochondria from red skeletal muscle was incubated in 1 μCi/mL of [1-<sup>14</sup>C]-palmitic acid for 3 hours. Data presented at means ± SEM. \* $p < 0.05$ .

## 5 Pyruvate Dehydrogenase Activity is reduced in WT in response to high fat diet; no change in mTLR4



[1-<sup>14</sup>C]-pyruvic acid oxidation to <sup>14</sup>CO<sub>2</sub> was assessed at 16-weeks of high fat feeding. Isolated mitochondria from red skeletal muscle was incubated in 1.5 μCi/mL of [1-<sup>14</sup>C]-pyruvic acid for 3 hours. Data presented at means ± SEM. \* $p < 0.05$ .

## 6 Changes in oxidative capacity are associated with mitochondrial enzyme activities.



Enzyme activities were measured in isolated mitochondria from red skeletal muscle. Citrate synthase activity was determined spectrophotometrically by the rate of DNTB reduction upon exposure to acetyl coA (412nm). BHAD and MDH activities were determined by the rate of NADH oxidation in the presence of acetoacetyl coA or oxaloacetate, respectively (340nm). Data presented at means ± SEM. \* $p < 0.05$ .

## Conclusions

Mice with skeletal muscle-specific over expression of TLR4 display a blunted oxidative capacity on a chow diet

When challenged with a high fat diet, mTLR4 Tg mice fail to adapt, compared to WT mice, as evidenced by:

- A propensity to gain more weight
- Glucose intolerance
- An inability to increase muscle oxidative capacity

These studies suggest that increased muscle TLR4 may play a role in the metabolic dysregulation associated with obese and diabetic states

## Funding

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