# Muscle mTORC1 Activation Causes Reduced Fat Mass

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# Abstract

# Introduction

* Dynamic Nature of Energy Expenditure
* High protein and weight loss
* mTORC1 and body composition
* Muscle TSC KO papers

# Methods and Materials

## Animal Husbandry

All mice were purchased from The Jackson Laboratory. For high protein diet (HPD) feeding studies, a custom high protein diet and a matched control diet (CD) were purchased from Research Diets (see Table 1). Male C57BL/6J mice were purchased at 8 weeks of age, and allowed to acclimate to the animal facility. Animals were placed on control or HPD at 10 weeks of age.

For High For muscle specific knockouts, FVB-Tg(Ckmm-cre)5Khn/J transgenic mice (stock 006405) were crossed with floxed *Tsc1tm1Djk*/J mice (stock 005680). To generate F1 mice that were heterozygous for the floxed allele, and either had or lacked the *Ckmm-Cre* transgene. These parents were intercrossed to generate knockout mice (*Tsc1fl*/fl, *Ckmm-Cre*Tg/+), wild type mice(*Tsc1+/+*, *Ckmm-Cre*+/+) and controls containing the transgene only (*Tsc1+/+*, *Ckmm-Cre*Tg/+)or the floxed allele only (*Tsc1fl*/fl, *Ckmm-Cre*+/+). All four genotypes were evaluated for all experiments. If there were no significant differences between the three control strains, these were combined and labeled as Controls. Animals were kept on a 12h light/dark cycle. The UTHSC Institutional Animal Care and Use Committee approved all animal procedures.

## Food Intake and Body Composition

Food intake was determined throughout the CD/HPD feeding studies by weighing the food in the cages (4 mice per cage) throughout the study and calculated based on the caloric content of the food. Food intake was therefore the average food eaten by a cage of mice divided by the number of mice in that cage. Body composition was determined using an echoMRI 1100 (echoMRI , Houston, TX). Body weights were determined using a standard scale. Tissue weights were determined for both the left and right hand side tissue and combined as total weights.

## Cell Culture

## CLAMS

## Western Blotting

## Statistical Analyses

All statistical analyses were performed using the R package, version 3.2.2 [1]. For longitudinal measurements (body weights, fat mass and lean mass), the data were analyzed by mixed linear models using uncorrelated random slopes and intercepts using the lme4 package version 1.1-8 [2]. Statistical significance was determined via χ2 tests of models containing or missing the genotype or diet term. Pairwise comparisons were tested first for normality via a Shapiro-Wilk test, then for equal variance via Levene’s test. Based on these, appropriate pairwise tests were performed as indicated in the figure legends. Corrections for testing of multiple hypotheses were done using the method of Benjamini and Hochberg [3]. All raw data and reproducible statistical analyses for this manuscript are available at <http://bridgeslab.github.io/TissueSpecificTscKnockouts>

# Results

## High Protein Diet Feeding Results in Lean Mice With Increased Energy Expenditure

To test the effects of a high protein diet, we fed 10-week old C57B/6J mice a diet containing either 10% protein or 40% protein (Table 1). We observed a modest decrease in body weight in these animals (29% reduction, p=0.036, Figure 1A). This was not due to a decrease in lean mass (Figure 1B) but rather was due to reduced accumulation of fat mass (44% reduction, p=0.057, Figure 1C). This corresponded to reduced mass of both subcutaneous and epididymal fat pads at the end of the 14 weeks of diet. Skeletal and cardiac muscle mass were slightly increased at the end of the diet (Supplementary Figure 1A).

In order to identify changes in energy balance in these animals, we monitored food intake of HPD and CD-fed animals throughout the study. As shown in Figures 1E-F there was no significant difference between caloric consumption either weekly or cumulatively between the diets. We next evaluated energy expenditure and substrate preference in these animals at the end of the dietary treatment. We observed an increase in energy expenditure (Figure 1E without a corresponding increase in physical movement (Figure 1F).

## High Protein Diet Feeding Results in mTORC1 Activation

Since mTORC1 is a major regulator of metabolism and is activated by elevated amino acids, we next evaluated whether mTORC1 activity is increased in C2C12 myotubes. As shown in Figure 2A supplementation of cultured myotubes with protein results in increased phosphorylation of S6K, in a rapamycin sensitive mannerTo test this in vivo, we evaluated quadriceps lysates from HPD fed animals. To avoid the confounding effects of acute protein feeding, animals were starved for 16h prior to sacrifice. We blotted lysates from quadriceps for the mTORC1 target S6K and found increased phosphorylation of S6K, indicating increased mTORC1 activity in muscle tissue. This is consistent with previous reports of high protein feeding and mTORC1 activity.

## Muscle Tsc1 Deletion Elevates Fasting Glycogen and Lipid Levels

To test whether activation of mTORC1 in muscle caused the reduced fat mass observed in the high protein diet fed animals, we generated muscle specific *Tsc1* knockout mice using a floxed *Tsc1* allele [4] and the *Ckmm-Cre* transgene (muscle creating kinase; [5]). As shown in Figure 3A, we observed efficient knockout of TSC1 and TSC2 proteins, corresponding to an increase in mTORC1 activity in quadriceps lysates from these animals.

We next evaluated the cell autonomous changes in muscle tissue associated with *Tsc1* ablation. We found that both fasting triglycerides and fasting glycogen levels were substantially elevated in *Tsc1* knockout muscles (Figures 3B-C). The increase in fasting glycogen was correlated with increases in both processed SREPB1c protein and PTG protein (Figure 3A). This is consistent with our previous report of a mTORC1 -> SREBP1c -> PTG pathway that regulates fasting glycogen in the liver [6]. To determine whether this increased muscle glycogen content correlates with improved muscle function in these animals we evaluated both grip strength (Figure 3D) and amount of cycles spent on a running wheel (Figure 3E). This is consistent with previous reports of reduced muscle strength in *Tsc1* knockout muscles [7]. Our findings support the hypothesis that while short-term contractile function is reduced, overall muscle fitness is improved, potentially related to increased nutrient storage in these muscles.

## Deletion of *Tsc1* in Muscle Results in Reduced Fat Mass

We followed the body weights and composition of the muscle *Tsc1* knockout animals over the course of XX months. While we did not observe any reductions in lean mass, we did observe a striking lack of fat mass accumulation as these animals grew in size (Figures 4A-C). A previous study using a *HSA-Cre* mediated knockout of *Tsc1* observed dramatic reductions in both lean mass and fat mass [7].

## Activation of mTORC1 in Muscle Does Not Result in Insulin Resistance

## Ablation of Muscle Tsc1 Results in Increased Expression of Fatty Acid Uptake Genes

# Discussion

* Athlete’s paradox
* Differences between KO models
* Anabolic Signaling and Energy Expenditure

# References

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# Tables

Table 1: Composition of Control and High Protein Diets.

# Figure Legends

Figure 1: Effects of high protein diet on body composition and energy expenditure. Body composition was determined weekly with body weight (A), lean mass (B) and fat mass (C). D) Weights of fat pad depots at the end of the 14 week dietary intervention. Statistical significance (p<0.05, n=6) was denoted if reached via asterisks based on χ2 test (A-C) or Welch’s *t-*test (D).

Figure 2: Regulation of mTORC1 by amino acids and protein feeding

Figure 3: Knockout of *Tsc1* in muscle leads to increased fasted glycogen and triglyceride levels in quadriceps.

Supplementary Figure Legends

Supplementary Figure 1: Supplementary data related to high protein diet feeding studies. A) Muscle weights at the end of the 14 weeks of diet.