Title

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# ABSTRACT

# INTRODUCTION

# METHODS

## Animal Husbandry

Mice were maintained in in ventilated cages at 70F at 40-60% humidity in a room with a 12-hour light/dark cycle (ZT0=6:00AM). Mice were provided *ad libitum* access to food and water unless otherwise noted.

For Muscle *Tsc1* knockout mice, wild-type mice were generated by crossing XXX. are defined as homozygous floxed *Tsc1*, absent the *Ckmm*-Cre transgene, while Muscle *Tsc1* knockout mice are defined as homozygous floxed *Tsc1*, with one copy of the *Ckmm*-Cre transgene. A/J mice (RRID:IMSR\_JAX:000646) were purchased at 8 weeks of age from The Jackson Laboratories.

## Rodent Diets

Mice were maintained on a normal chow diet (Lab Diet 5L0D; 5% of calories from fat, 24% from protein, 36% carbohydrate) unless otherwise specified. For ketogenic diet interventions mice were placed on either a ketogenic diet (Research Diets D17053002, 85% fat, 15% protein, 0% carbohydrates) or a matched synthetic control diet (Research Diets D1053001 10% fat, 15% protein, 75% carbohydrates). Both diets were in meal not pellet format and were provided in custom jars with holes to provide access.

## BHB Tolerance Test

Fed mice were intraperitoneally injected with 1 mg/kg (A/J ketogenic diet studies) or 1.5 mg/kg (muscle *Tsc1* knockout and diversity outbred studies) of beta-hydroxybutyrate dissolved in PBS at approximately ZT8. Prior to the injection, and then every 15 afterwards, a tail blood draw was collected and analyzed using a Precision Xtra Ketone Body Assay.

## Statistical Analysis

All statistical analyses were performed using R version 4.2.2 .We set statistical significance for this study at 0.05. For pairwise testing, we first tested for normality via Shapiro Wilk tests, and then

# RESULTS

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## Activation of MTOR promotes Ketone Disposal

To test whether activation of mTORC1 in muscle tissue alters disposal of ketone bodies, we performed a BHB tolerance test in *Tsc1* knockout mice. The *Ckmm*-Cre induced ablation of *Tsc1* causes activation of mTORC1 in muscle tissues. As shown in Figure 3A, both male and female knockout mice cleared the injected beta-hydroxybutyrate much more rapidly than their wild-type littermates. Using mixed-linear models and using sex as a covariate, and the animal as a random intercept, we found a significant reduction in BHB levels after the challenge (p=0.004). Similarly, when calculating the area under the curve from 0 to 60 minutes, there was a reduction in the knockouts, after adjusting for sex (25%; p=0.016). When stratifying by sex, knockouts had 41% lower AUC in males and 11% lower in females though sex differences did not reach statistical significance (p=0.20).

## MTORC regulates expression of Ketolysis genes

## KD Feeding Does not Improve BHB Disposal in A/J Mice

We hypothesized that prolonged exposure to elevated ketone body levels would result in physiological adaptations, resulting in improved disposal of ketone bodies. To test this hypothesis in male wild-type A/J mice we fed 10 week old A/J mice a control or a ketogenic diet for three weeks and then performed a BHB tolerance test. As expected, baseline ketone body levels were elevated from 0.43 mg/dL to 0.75 mg/dL in this assay (p=0.017). Upon injection we were surprised to observe that ketone body levels remain elevated more-so in the ketogenic diet-fed mice than the control mice (Figure 2A), even after subtracting for baseline differences (Figure 2B-C). These data suggest that ketone disposal is not improved after three weeks of a ketogenic relative to a control diet, and may actually be somewhat worsened in A/J mice (p=0.114 via linear mixed models).

## KD Increases Muscle MTOR activity

# DISCUSSION