Physiological Changes in Response to Skeletal Muscle Specific High-Expression of mu-Crystallin Following High- and Low-Fat Diets

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BACKGROUND

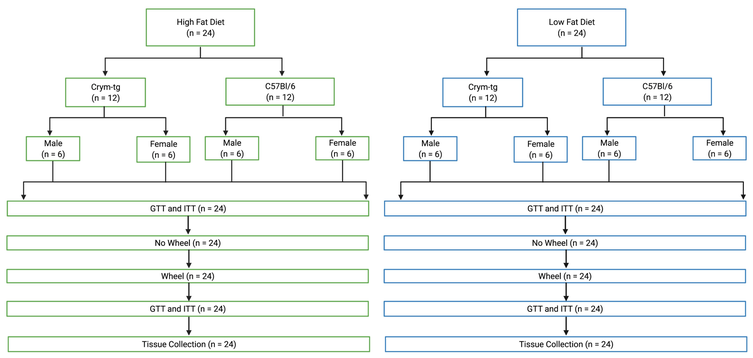
Obesity is epidemic in the United States and is associated with increased risk for type 2 diabetes mellitus, heart disease, stroke, and certain cancers. Healthcare costs associated with obesity-related conditions is over $147 billion annually. Traditional treatment includes changes in diet as well as increase in physical activity. For many individuals this does not offer enough benefit, however.

As potent regulators of metabolism, thyroid hormones are an attractive candidate for treating obesity. Directly targeting thyroid hormones, specifically T3, can be dangerous for non-hypothyroid individuals, so an indirect target is needed. The thyroid hormone binding protein, mu-crystallin, may play a role in controlling thyroid hormone levels and therefore provide an avenue for regulating metabolism.

Approximately 20 percent of the population expresses high levels of mu-crystallin in skeletal muscle, while the rest of the population expresses little to no mu-crystallin. Our studies focus on the consequences of skeletal muscle-specific high-expression (SM-HE) of mu-crystallin particularly as it relates to metabolism and muscle physiology. We hypothesize that SM-HE of mu-crystallin influences cellular and metabolic changes in a diet and sex specific manner by influencing changes in gene expression.

To evaluate this, we generated transgenic mice (*Crym-tg*) that highly-express mu-crystallin (CRYM) in skeletal muscle to the levels seen in human high expressers. The muscles accumulate T_3 to very high levels. We measured respiratory exchange ratio (RER) and found a small but significant increase in utilization of fat as an energy source when mice were on standard chow. The original transcriptomic and proteomic data showed increased expression of genes and proteins involved in fatty acid oxidation and decreased expression of genes involved in glycolytic metabolism as well as some members of the insulin signaling pathway. Here we explore the metabolic effects of SM-HE in male and female mice placed on high fat (HFD) and low fat (LFD) diets.

METHODS



CRYM-tg mice were bred at the University of Maryland, Baltimore. All mice were studied between 2 and 6 months of age. Homozygous and wild type littermates from breeding of C57Bl/6 heterozygous for the *Crym* transgene mice were compared. Center for Metabolism and Obesity Research, Johns Hopkins University School of Medicine determined daily body weight, food intake, physical activity, whole-body metabolic profile in an open-flow indirect calorimeter (Comprehensive Laboratory Animal Monitoring System, CLAMS; Columbus Instruments, Columbus, OH), body composition (by qMR, with an Echo-MRI-100), as well as glucose and insulin tolerance.

Glucose Tolerance Tests

Mice were fasted overnight (16 hours) before glucose injection, following standard procedures. Blood glucose was measured at 0, 15, 30, 60, and 120 min after glucose injection using a glucometer (NovaMax Plus, Billerica, MA).

Insulin Tolerance Tests

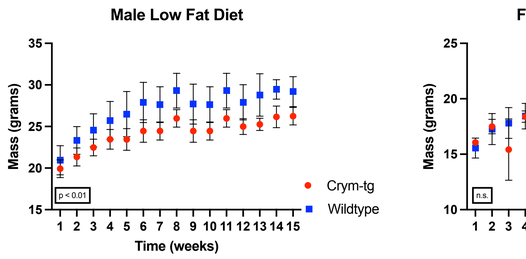
Food was removed 2 hours before insulin injection. Insulin was diluted in saline and injected at 1.0 U/kg body weight ip. Blood glucose was measured at 0, 15, 30, 60, and 90 min after insulin injection.

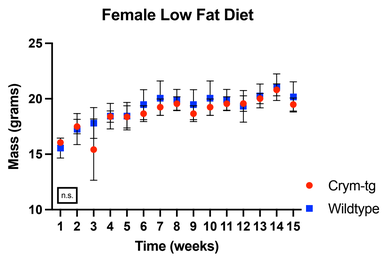
Indirect Calorimetry

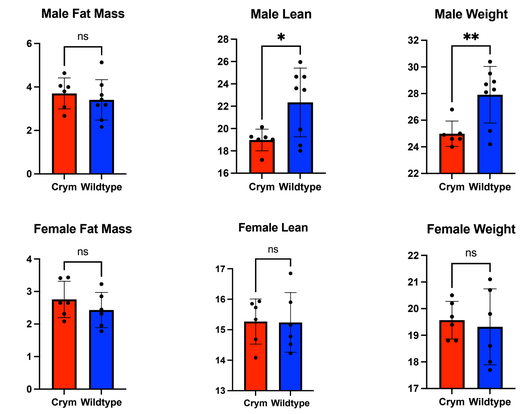
In brief, data were collected during ad libitum access to food and water for 3 days to allow mice to were acclimate. Data were analyzed from d4. Food was then removed for 24 h, then replaced. Next, running wheels were inserted into the chambers and mice were monitored for an additional 7 days. Oxygen consumption ($\dot{V}o_2$; $mL \cdot kg^{-1} \cdot h^{-1}$) and CO2 production ($\dot{V}co_2$; $mL \cdot kg^{-1} \cdot h^{-1}$) were measured every 24 min. Respiratory exchange ratio ($RER = \dot{V}co_2/\dot{V}o_2$) was calculated to estimate relative oxidation of carbohydrates (RER = 1.0) versus fats (RER = 0.7), not accounting for protein oxidation. Energy expenditure (EE) was calculated as $EE = \dot{V}o_2 \times [3.815 + (1.232 \times RER)]$. Physical activity was measured by IR beam breaks in the metabolic chamber, and by counting wheel rotations.

LOW FAT DIET RESULTS (LFD)

A. Male *Crym* tg mice on LFD diet weigh less than WT mice.
B. Male *Crym* tg mice on LFD have less lean muscle mass than WT mice.

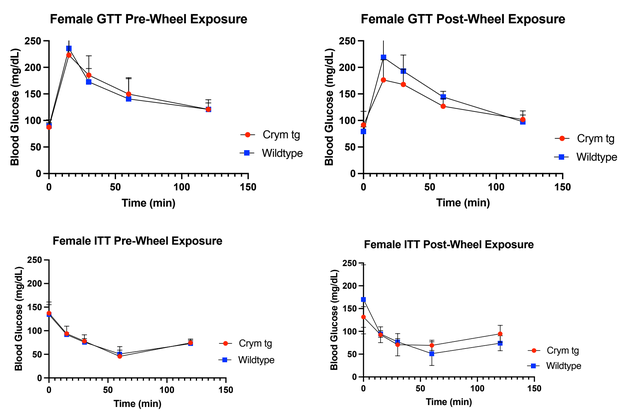






A. *Crym* tg male mice weigh approximately 3 grams less than WT on low-fat diet whereas there are no changes in the female mice.
B. Further investigation shows that the difference in body composition is a result of decreased lean muscle mass ($p < 0.05$).

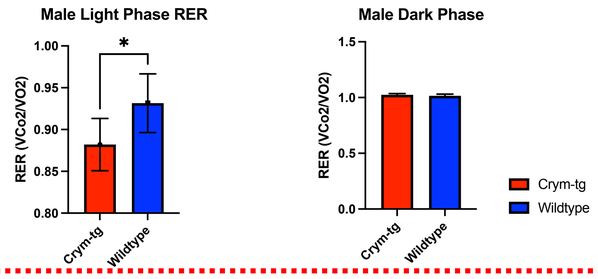
C. There are no significant changes in ITT or GTT in Females on LFD.



C. There were no significant differences in GTT or ITT in *Crym* tg females pre-CLAMS or post- exercise wheel exposure on LFD.

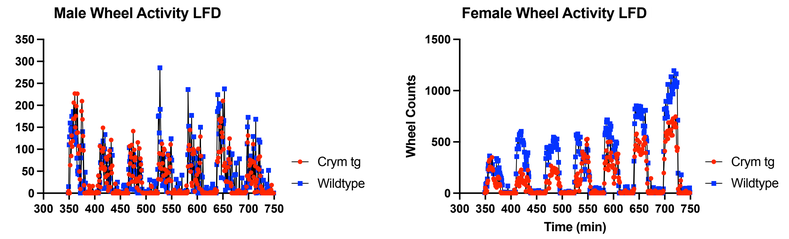
D. Respiratory Exchange Ratio is Lower in *Crym* tg Males on LFD During the Light Phase on LFD.

Respiratory Exchange Ratio (RER) = $(VCO_{2out}\text{-}VCO_{2in})/(VO_{2in}\text{-}VO_{2out})$



- RER is an indication of energy source
  - —RER = 0.707 = 100% fat as energy
  - —RER = 1.000 = 100% carbohydrates as energy

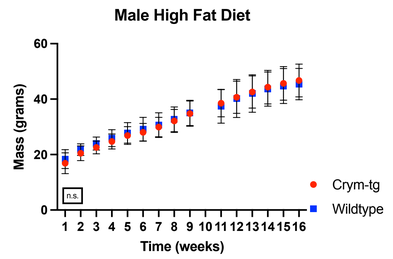
E. *Crym* tg Females on LFD Trended Towards Less Running Activity.

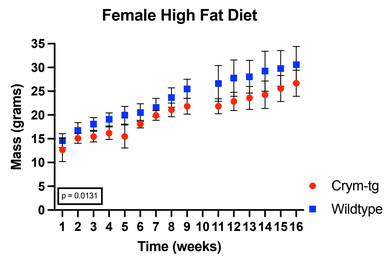


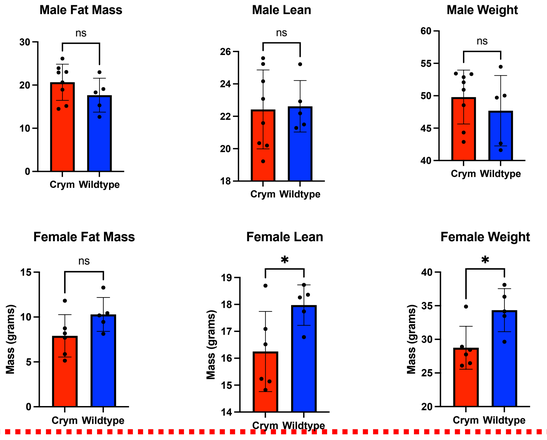
E. *Crym* tg females trended towards approximately half the running activity compared to wildtype controls ($p = 0.0945$) whereas there is no difference in males.

HIGH FAT DIET (HFD) RESULTS

F. Female *Crym* tg mice on LFD diet weigh less than WT mice.
G. Female *Crym* tg mice on HFD have lower lean and fat masses than WT.

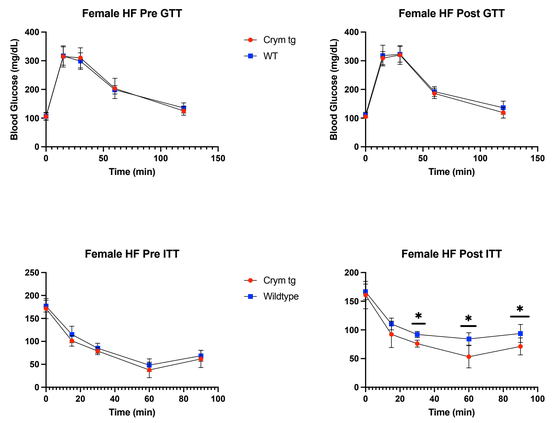






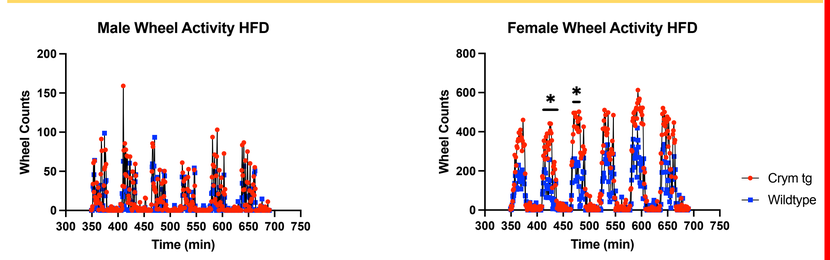
F. *Crym* tg female mice weigh approximately 5 grams less than WT on HFD whereas there are no changes in the male mice.
G. Further investigation shows that the difference in body composition is a result of decreased lean muscle mass ($p < 0.05$).

H. Exercised Female *Crym* tg on HFD Show Increased Insulin Sensitivity.



H. Unlike *Crym* tg females on LFD, *Crym* tg females on HFD show a significantly increased sensitivity to insulin bolus post-wheel activity exposure that is not evident pre-wheel exposure.

I. *Crym* tg Females on HFD Increased Wheel Utilization on HFD.



I. *Crym* tg females had higher running activity compared to wildtype controls ($p = 0.05$) whereas there is no difference in males.

CONCLUSIONS

- There are diet- and sex-specific differences in physiological responses to SM-HE of mu-crystallin when mice are challenged with either a LFD or HFD.
- *Crym* tg males show differences in mass and body composition on LFD that is not seen on HFD, as well as showing a lower RER on LFD not seen on HFD.
- *Crym* tg females show differences in mass and body composition on HFD that is not seen on LFD.
- *Crym* tg mice on HFD show a greater sensitivity to insulin bolus, after exercise, that is not seen on LFD.
- *Crym* tg female mice on LFD trended to run less than WT during wheel exposure, whereas on HFD *Crym* tg females increased wheel utilization, an outcome that may be secondary to their leaner body composition and decreased body weight.

FUNDING

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1950 70 years 2020

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