

# ENZYME ACTIVITY AND HIGH-FAT DIET: CITRATE SYNTHASE ACTIVITY IN MYOSTATIN PROPEPTIDE TRANSGENIC AND WILD-TYPE MICE

Diabetes is a serious and common health problem, with insulin resistance acting as a major pre-clinical condition. To investigate insulin resistance prevention, my host lab designed a myostatin propeptide transgenic mouse. Myostatin is a gene responsible for the inhibition of muscle growth and development. The myostatin propeptide transgenic mouse depressed myostatin function, therefore increasing muscle development. To better understand effects of the myostatin propeptide transgene, we designed an experiment to compare the citrate synthase (CS) activities of four groups of mice samples. CS is an exclusive marker of the mitochondrial matrix, taking part in the first step of the citric acid cycle. We expected that CS activity would be especially depressed in wild-type mice fed high-fat diets due to insulin resistance, suggesting mitochondrial dysfunction. Thirteen heart samples were taken from myostatin propeptide transgenic and wild-type mice with 45% kcal high-fat diet or normal diets (10% kcal fat). Enzyme activities showed that wild-type mice fed high-fat diets had depressed enzyme activity. These results indicate that depressing myostatin function can be a beneficial approach to preventing and understanding insulin resistance.

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## INTRODUCTION

The experimental design of the research was inspired by the growing number of individuals affected by diabetes and obesity. The two main types of diabetes, type 1 and type 2, are related to the body's reaction with insulin. Insulin controls the amount of sugar in the blood by moving it into the cells, where it can be used by the body for energy. Type 1 diabetes results from the body's failure to produce insulin. Insulin resistance, in which the body does not respond to insulin properly, is a precursor to type 2 diabetes. In previous studies conducted by my host lab, wild-type mice fed 45% kcal high-fat diets became insulin resistant, while myostatin propeptide transgenic mice fed the same 45% kcal high-fat diet remain healthy.

We hypothesized that insulin resistance suggests mitochondrial dysfunction, correlating with the body working improperly, resulting in depressed enzyme activities in the mitochondria. Citrate synthase (CS) is an enzyme that works in the first step of the citric acid cycle, catalyzing the reaction of oxaloacetate with acetyl CoA to form citrate. It is an important enzyme found exclusively in the mitochondrial matrix of cells. CS activity can therefore be indicative of mitochondrial function. High CS activity can suggest functional mitochondria and depressed CS activity can indicate dysfunctional mitochondria.

Myostatin is a muscle regulatory gene which can be thought of in two parts: one representing inhibition of muscle, the other promoting muscle growth (propeptide). Myostatin pro-

peptide transgenic mice were created by increasing the amount of propeptide in the myostatin gene, decreasing the myostatin (MSTN) function. Transgenic traits include increased muscle mass, maintained insulin sensitivity when fed high-fat diets, and less occurrence of obesity by high-fat diet. By comparing the CS activity within heart samples taken from wild-type mice fed 45% kcal fat diets, wild-type mice fed 10% kcal fat diets, transgenic mice fed 45% kcal fat diets and transgenic mice fed 10% kcal fat diets, mitochondrial function with each group can be compared and observed.

## METHODS

For the measurement of CS activity, we used protocols from Sigma (CS0720) and Animal Models of Diabetic Complications Consortium (AMDCC). Myostatin propeptide transgenic mice were generated by standard microinjection techniques. Heart samples were taken from myostatin propeptide transgenic and wild-type mice which were given free access to a normal fat diet (10% kcal fat) until 9 weeks of age. Male mice were then randomly assigned to two types of diet: high-fat (45% kcal); or normal (low) fat (10% kcal fat) diet. The 45% kcal high-fat diet contains more lard and maltodextrin, and less corn starch compared to normal fat diet. Mice were sacrificed at 5 months of age; hearts were taken and frozen at  $-80^{\circ}\text{C}$ . Ten mg (10  $\mu\text{g}$ ) heart samples were homogenized manually on ice with a Dounce homogenizer to prevent mitochondrial damage in a 20% ho-

mogenization buffer containing 20 mM HEPES, 10 mM EDTA, pH 7.4. Homogenized samples were frozen for 1 hour and diluted 1:10 before read in a spectrophotometer. The reaction buffer was made with 100 mM tris, 30 mM Acetyl CoA, 10 mM 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), and 10 mM OAA solutions. CS activity can be measured by the presence of DTNB which creates a yellow tint in the reaction buffer. Before adding 10 mM OAA (which acts as a substrate), absorbency of the baseline reaction was followed with a spectrophotometer at 412 nm for 1.5 minutes. The slope of the blank absorbencies was found and subtracted from the slope of the absorbencies read in the total sample activity. This number was then plugged into an equation representing the change in absorbency per minute, reaction volume, enzyme volume, dilution, extinction coefficient of TNB, and the path length of absorbance. Prior to finding absorbencies of actual heart sample, test trials were completed and standards were analyzed.

## RESULTS

### CS Activity Analysis

To prove results trustable, CS positive control (sigma C4741) was used as a standard. Saturation was observed in each of the standards by comparing the absorbencies. Spectrophotometer absorbency exhibited saturation in any amount of control 4  $\mu$ L and greater.

### CS activity in transgenic and wild-type mice

Heart sample absorbencies were observed in amounts of 7  $\mu$ L consistently on a spectrophotometer set to 412 nm for 1.5 minutes. This increment was found to be unsaturated and trustable. I was able to successfully find CS activity in all 13 samples representing myostatin propeptide transgenic mice and wild-type mice fed high-fat and normal diets. CS activity was found to be depressed in wild-type mice fed high-fat diets. Percent change by diet displayed wild-type mice enzyme activity as 56.8%. Transgenic diet change exhibited CS activity as almost unaffected at 12.1%, which is 4 times lower than the change by diet found in the wild-type mice group.

## DISCUSSION

Human myostatin is identical to mouse myostatin. Previous studies have demonstrated high-fat diets induce wild-type mice to become insulin resistant while myostatin propeptide transgenic mice remain insulin sensitive under similar conditions. My study shows that the high-fat diet significantly depressed CS activity in the wild-type mice group, while all other mice groups maintained healthy levels of enzyme activity. These results were proven trustable by comparing saturation found in the absorbencies of heart samples and standards, with standard enzyme activity falling in between the range of 2–16 units ( $\mu$ mol/mL/min). Results suggest mitochondrial damage

in wild-type mice fed high-fat diets. Data from this study also implicates insulin resistance in initiating mitochondrial dysfunction, depressing enzyme activity. The results support my hypothesis that wild-type mice fed high-fat diets have depressed enzyme activity, possibly as a result of insulin resistance. CS activity levels in wild-type mice and myostatin propeptide transgenic mice fed high-fat diets and normal diets directly correlate with previous assays on insulin tolerance, giving more beneficial evidence to the myostatin propeptide transgene. Further studies would be needed to support a direct connection between mitochondrial dysfunction and insulin resistance. To continue exploration of myostatin-deficient transgenic mice, CS activity should be observed in other organs such as the liver. Levels of reactive oxygen species (ROS) found in the mitochondria could also be found and compared in order to further suggest or disprove mitochondrial dysfunction in high-fat induced wild-type mice. Nonetheless, my findings provide evidence of the myostatin propeptide transgene maintaining healthy CS activity in mice fed high-fat diets.

## RESOURCES

1. Zhao B, Wall RJ, Yang J. Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. *Biochemical and Biophysical Research Communications*. 2005;337: 248–255.
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