# 5'SL mutant mice show improved diastolic function following 28 weeks of a high fat/high sucrose diet

Tara M. McDaid[1], Jacob J. Russell[1], Francisco I Ramirez-Perez[2,4], Makenzie Woodford[3,4], David A. Brenner[6], Vincent G. DeMarco[5,7], Luis Martinez-Lemus[2,4,7], Jaume Padilla[3,4,5], Bysani Chandrasekar[4,5,7], Shawn B. Bender[1,4,5]
Departments of [1]Biomedical Sciences, [2]Medical Pharmacology & Physiology, [3]Nutrition & Exercise Physiology, and [4]Dalton Cardiovascular Research Center, University of Missouri; [5]Truman Veterans Hospital; [6]School of Medicine, University of California-San Diego; [7]Department of Medicine, University of Missouri

## Background:

Obesity and chronic hypertension result in cardiac diastolic dysfunction via mechanisms including metabolic dysfunction, fibrosis, inflammation, and microvascular dysfunction. The multifunctional ribonucleoprotein Larp6 (La Ribonucleoprotein 6, Translational Regulator) binds the 5' stem loop (SL) region of type I collagen mRNA, thereby increasing its half-life, translation, and deposition upon upregulation in disease states. Thus, we **hypothesized** that preventing Larp6 binding to the 5'SL region of collagen mRNA would decrease collagen deposition and improve cardiac dysfunction in obese, hypertensive mice.

## Methods:

### Animals

Male wild-type (WT) and mice (C57BL/6) with knock-in mutation (MT) of the 5' SL region of collagen mRNA were fed High Fat/High Sucrose (HFHS) diet (5.06 kcal/g, 60.01% fat, 25.0% carbohydrate) or control diet (3.35 kcal/g; 13.4% fat, 56.7 carbohydrate) for 28 weeks starting at 8 weeks of age.

### Cardiac Function

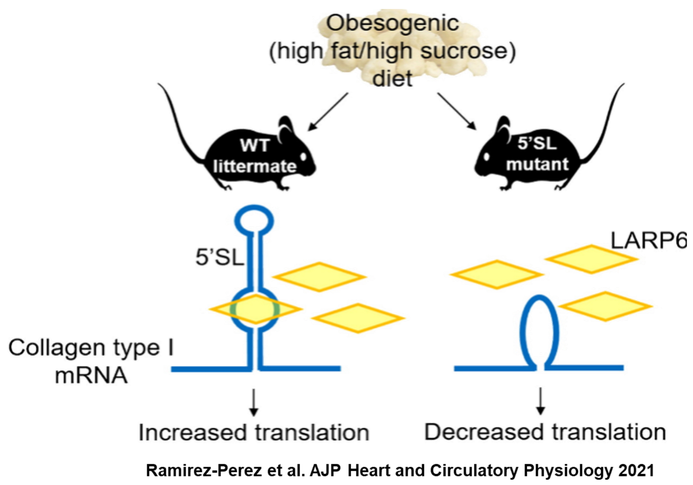
Echocardiography was performed with a Vevo 2100 small animal ultrasound system

### Immunofluorescence and Staining

Fibrosis assessed was by picrosirius red staining; capillary density by CD31 staining; and macrophages by CD68 staining in sections of left ventricular free wall.

### RNA Sequencing

Total mRNA was isolated from the left ventricular free wall and subjected to Illumina RNA sequencing followed by Ingenuity Pathway Analysis.




Ramirez-Perez et al. AJP Heart and Circulatory Physiology 2021

**Image 1. The mechanism of Larp6.** Larp6 binds to the 5' SL of the collagen mRNA. Binding increases the half-life, translation, and deposition of collagen mRNA. This causes an increase in fibrosis.

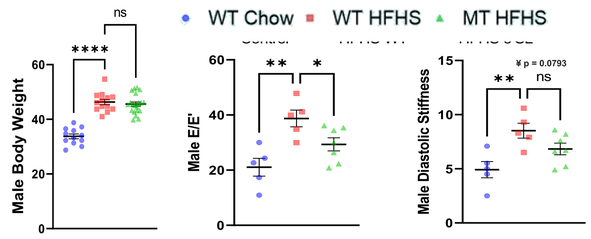



**Figure 1. HFHS induced obesity is associated with diastolic impairment and attenuated in MT mice.** Male mice had increased body weight following HFHS feeding in both wild-type and 5'SL mutant mice. HFHS feeding lead to diastolic dysfunction as measured by elevated E/E' and diastolic stiffness in male mice, both of which were attenuated by the 5'SL mutation. *P < 0.05, **P < 0.01, ****P < 0.0001

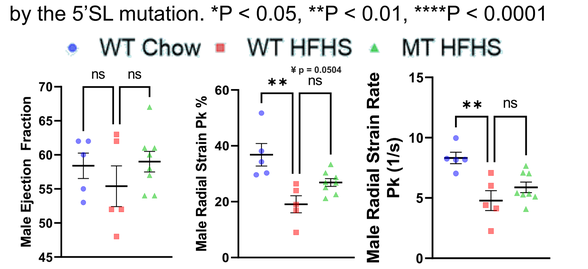



**Figure 2. MT mice are protected from obesity-associated cardiac systolic impairment.** HFHS feeding did not impact ejection fraction but reduced radial strain and radial strain rate in male mice. The latter was not impacted in MT mice.
**P < 0.01.

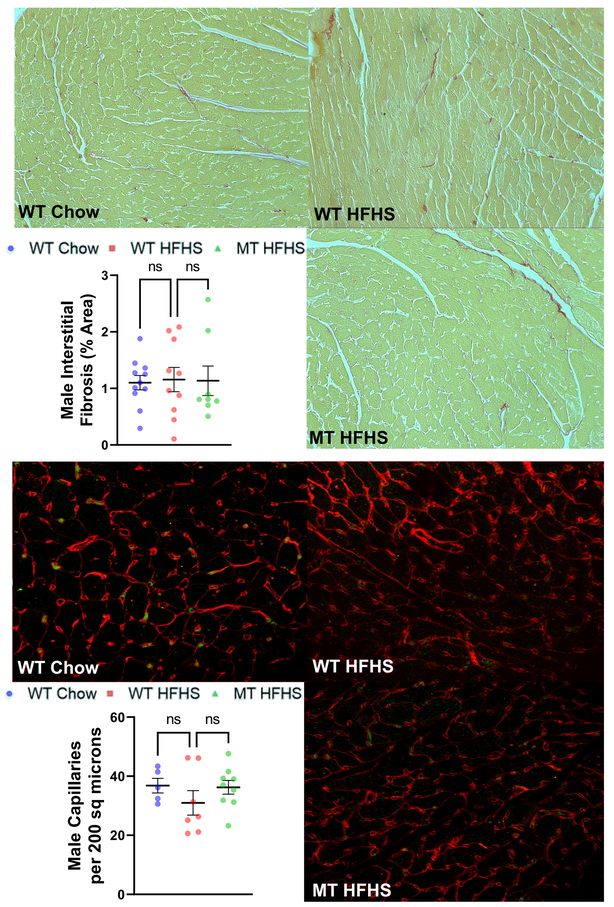



**Figure 3. Obesity had no effect on cardiac fibrosis or capillary density.** HFHS feeding did not change cardiac collagen (top) or capillary density (bottom) in male wild-type or MT mice.

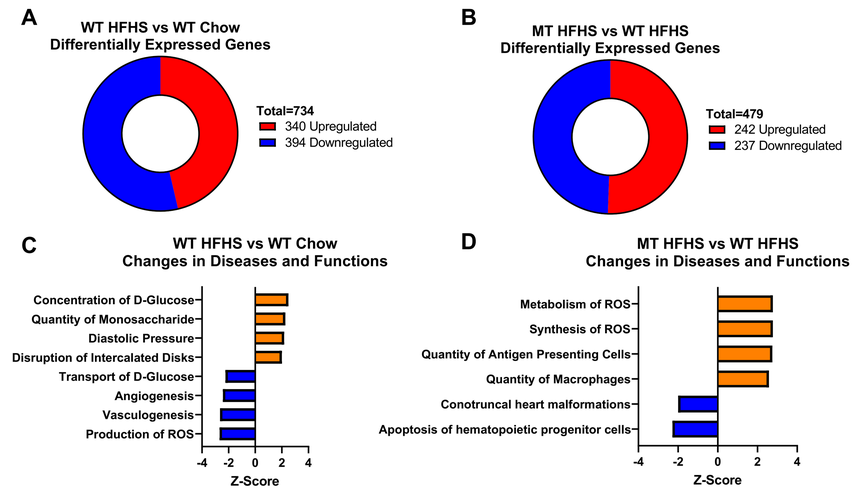



**Figure 4. Obesity altered the cardiac transcriptome and this is modulated in MT mice.** **A.** HFHS feeding in WT mice led to 734 differentially expressed genes compared to WT Chow animals. **B.** HFHS feeding in MT mice led to 479 differentially expressed genes compared with WT HFHS mice. **C.** Activated/inhibited diseases and functions in the WT mice fed HFHS diet. **D.** Activated/inhibited diseases and functions in the MT mice fed HFHS diet, compared to WT HFHS mice. Group size for sequencing is n=6.

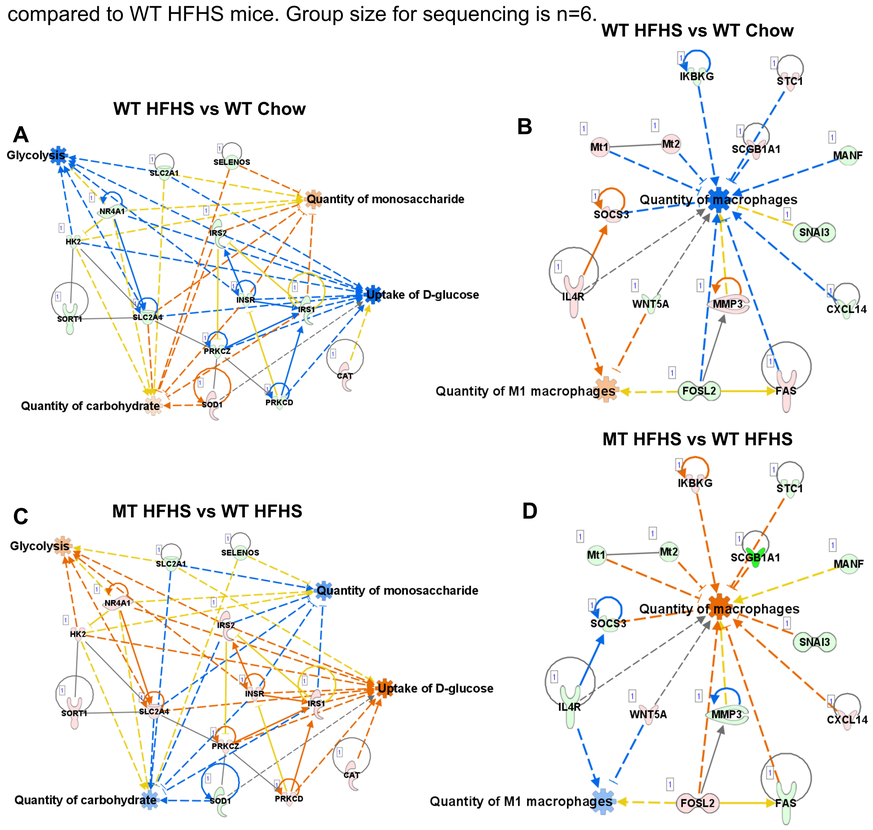



**Figure 5. Obesity induced gene networks associated with derangements of cardiac glucose handling and increased M1 macrophages that is reversed in obese MT mice.** **A.** HFHS feeding in WT mice led gene changes associated with decreased glycolysis and uptake of glucose, as well as increased quantities of monosaccharides/carbohydrates. **B.** WT HFHS mice showed gene signatures associated with increased activation/presence of M1 macrophages compared to WT Chow mice. **C.** MT HFHS mice showed attenuation of gene signatures associated with impairments in cardiac glucose handling, and **D.** gene signatures associated with reduced M1 macrophages. Blue arrows, predicted inhibition; Orange arrows, predicted activation. Group size for sequencing is n=6.

## Results and Conclusions:

- Obesity induced cardiac diastolic and mild systolic was blunted in mutant animals (**Figs 1 & 2**), independent of cardiac fibrosis and capillary density **(Fig 3)**.
- Transcriptomic changes in WT obese hearts were consistent with alterations in cardiac glucose handling and macrophages. These were generally reversed in obese MT mice **(Fig 4&5)**.
- **Prevention of Larp6 binding to collagen mRNA in 5'SL mutant mice blunted obesity-associated diastolic dysfunction independent of fibrosis in male mice highlighting novel actions of this multifunctional protein that warrant further investigation.**

**Funded by:** NHLBI R01 HL088105, R01 HL136386, R01 HL142770, NIDDK DK130243, SRCS (IK6BX004016), and merit BX005845.
Stipend for Tara M. McDaid is supported by the University of Missouri College of Veterinary Medicine Office of Research

Veterinary Research Scholars Program
University of Missouri