Role of Leptin and its Receptor in the Protective Effects of HSP70 against Diet-Induced Obesity

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Introduction

- The World Health Organization estimates that more than 650 million adults were obese in 2016. Obesity poses a significant threat to a person's health, as it is a risk factor for hypertension, hyperlipidemia, insulin resistance, and multiple types of cancer (WHO, 2018).
- One area of research to combat obesity is focused on the evolutionary conserved family of stress-inducible proteins called heat shock proteins (HSPs). Some studies have demonstrated that HSP70 may offer protection against metabolic disorders, such as insulin resistance (Henstridge, Molecular Metabolism, 2014).
- The Ciancio Lab at Midwestern University previously demonstrated that mice over-expressing HSP70 specifically in villin-expressing epithelial cells were protected against high fat, diet-induced obesity.
- Leptin has a well-recognized role in regulating calorie consumption and systemic inflammation, but studies have shown that leptin receptors have additional metabolic effects in peripheral organs (Anubhuti, Diabetes, Obesity, and Metabolism, 2008)

Hypothesis

Experiments conducted were designed to test the hypothesis that leptin receptor expression is elevated in the livers of mice on a high fat diet compared to expression levels in the livers of mice on a low fat diet, and that elevation is attenuated by the overexpression of HSP70.

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- Livers were removed from HSP70 transgenic (TG; n=14) and non-transgenic (NTG; n=14) littermates that received either a low fat diet (10 kcal% fat; 3.85 kcal/gm; Research Diets, Inc) or high fat diet (60 kcal% fat; 5.24 kcal/gm; Research Diets, Inc) for 14 weeks and were flash frozen in liquid nitrogen.

- RNA was isolated from each liver using the Trizol method, reverse transcribed into complementary DNA, and measured for relative levels of ObR expression using qPCR. GAPDH was used to normalize the results. qPCR results were calculated to determine the $2^{-\Delta\Delta Ct}$ for analysis of relative messenger RNA levels in each processed sample.

- Intracellular protein was extracted from frozen livers analyzed for concentration using the bicinchoninic assay method. Relative leptin receptor expression was determined by Western Blot. HSC70 was used to normalize relative protein loading per sample. Image Lab software was used to determine the relative signal intensity of the Western blots.

- GraphPad Prism was used for all statistical analysis. Data was analyzed using a 2-way ANOVA followed by a post-hoc test; $p \leq 0.05$ was determined to be statistically significant.

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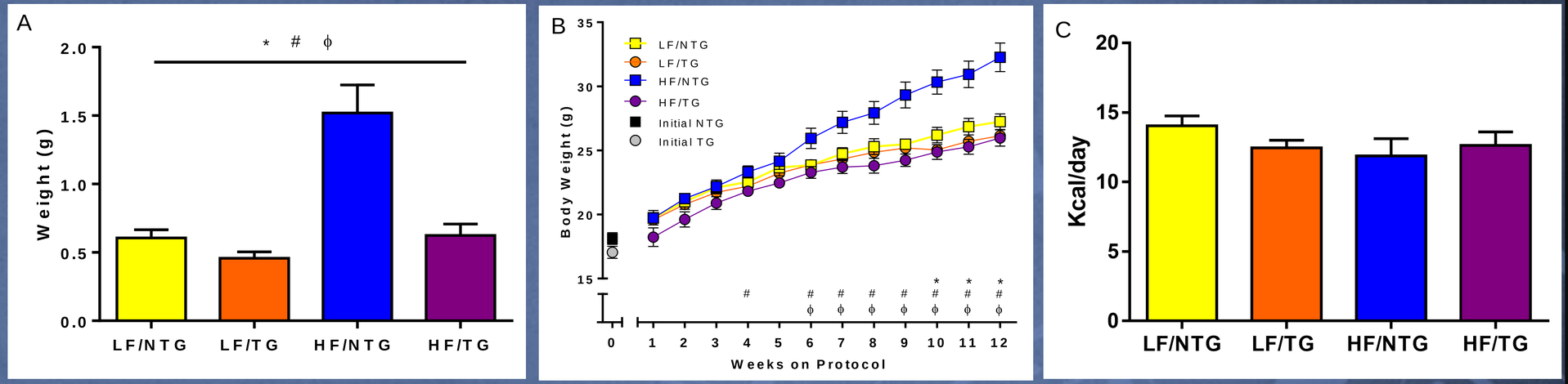

HSP 70 Transgenic Mice are Protected from High-fat, Diet-induced Obesity. A. HSP70 TG mice had significantly smaller epididymal fat pads compared to their non-transgenic (NTG) littermates on their respective diets. B. Diet-induced weight gain was significantly less in the HSP70 TG mice on a HF diet compared to the NTG mice on a HF diet. C. Calorie consumption in the TG mice was not significantly different from the consumption measured in the respective NTG mice, regardless of the diet's fat content. Abbreviations: low-fat/non-transgenic (LF/NTG); low-fat/transgenic (LF/TG); high-fat/non-transgenic (HF/NTG); high-fat/transgenic (HF/TG). Significant differences determined by 2 –way ANOVA followed by Sidak's post hoc test: "*" $p<0.05$ diet effect, "#" $p<0.05$ genotype effect, and "φ" $p<0.05$ interactive effect.

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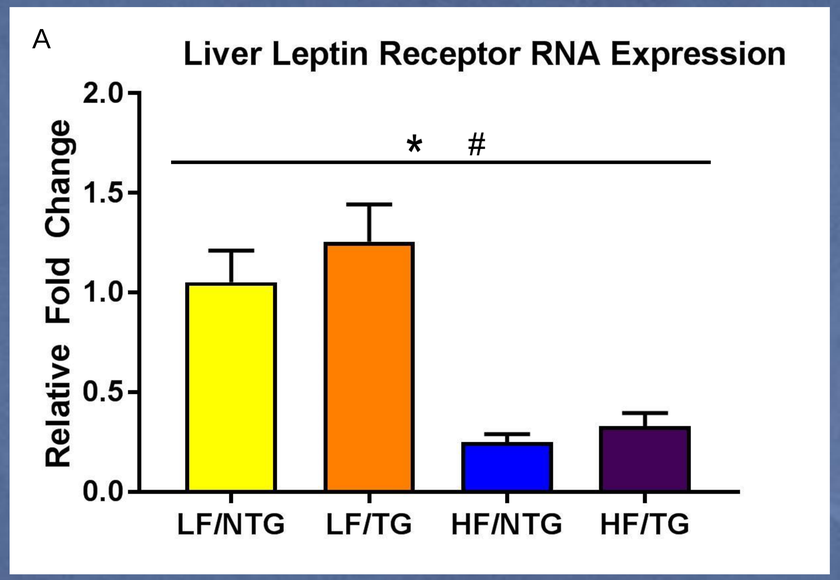

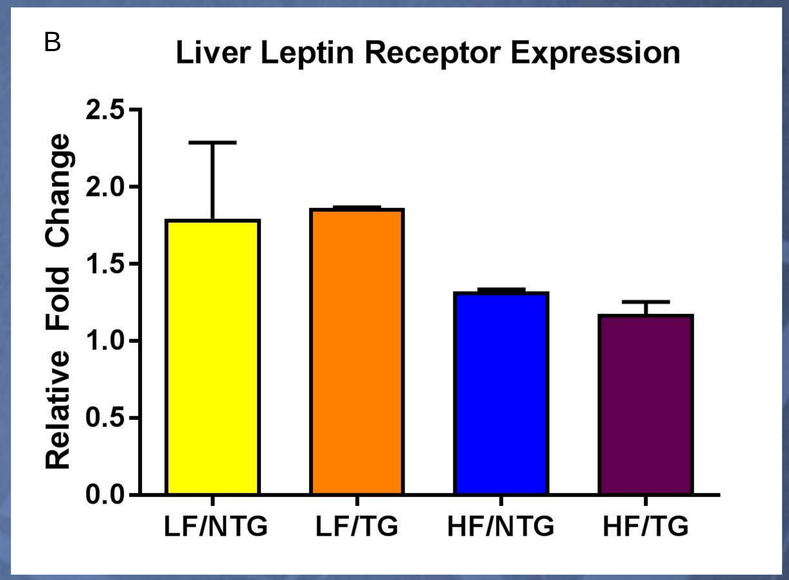

HSP 70 TG Mice had Significantly Greater Leptin Receptor (ObR) mRNA Expression than NTG Mice. A. Mice on the low-fat diet expressed significantly elevated levels of leptin receptor mRNA compared to the mice on a high fat diet for their respective genotypes. HSP70 TG mice expressed elevated levels of leptin receptor mRNA as a trend compared to their non-transgenic littermates for each respective diet. B. There was no significant differences in leptin receptor protein expression in the liver with our current data (n=2 per group). Abbreviations: low-fat/non-transgenic (LF/NTG); low-fat/transgenic (LF/TG); high-fat/non-transgenic (HF/NTG); high-fat/transgenic (HF/TG). Significant differences: "*" $p<0.05$ diet effect, "#" $0.05<p<0.1$ genotype effect.

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Ongoing Research

The relative leptin expression in the livers of HSP70 TG and NTG on either a LF or HF diet requires additional sample analysis before any statistically validated conclusions can be made. These experiments are currently in progress.

Measurement of circulating leptin receptors in the blood of the NTG and TG mice will also be performed. In a study by Lou et al. in 2010, mice exhibiting elevated levels of circulating soluble leptin receptor were found to also exhibit decreased body weight and increased metabolism. We plan to quantify the levels of circulating soluble leptin receptor in the HSP70 TG and NTG mice on a LF or HF diet. We hypothesize that HSP70 overexpression will significantly influence the levels of soluble leptin receptor, thereby modulating metabolism and weight in the TG mice.

Conclusions

This study examined the mRNA and relative protein expression of the leptin receptor in the livers of HSP70 TG and NTG mice on a LF or HF diet. Our results demonstrated a significant reduction of the liver's leptin receptor's mRNA expression in response to a HF diet, and that the HSP70 TG demonstrated a trend for a relative increase in receptor expression compared to their respective NTG littermates. Future studies designed to measure leptin receptor expression in the liver and blood will provide a clearer picture regarding the leptin receptor response in HSP70 TG mice. These results may help to clarify a potential therapeutic target for the prevention of obesity.

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