Investigating diet-induced metabolic syndrome in a typical American versus Mediterranean diet model in C57BL/6J mice

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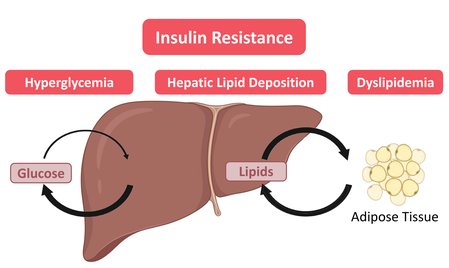
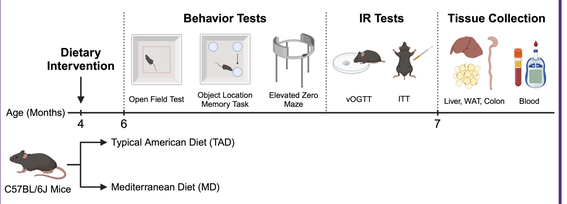
Neurobiology of Aging Research

More than 1 in 3 US adults have metabolic syndrome (MetS) and its development is multifaceted. Risk can be mitigated with lifestyle modifications, including improved nutrition. In the US, a typical American diet (TAD) is full of processed foods high in saturated fats and refined sugars and is associated with increased insulin resistance and obesity risk. In contrast, adherence to a plant-based Mediterranean diet (MD) rich in unsaturated fats, fiber, and non-refined carbohydrates has been found to reduce chronic disease risk. Despite the contrasting nutritional compositions, the average macronutrient distributions of these two human diet styles are similar (approximately 50% kcal carbohydrates, 15% kcal protein, and 35% kcal fat). There are few rodent studies in the literature that directly compare a TAD and MD. Further, studies often utilize a high-fat diet, consisting of 40-60% kcal fat, or individual nutrient supplements, such as olive oil, rather than comprehensive diet models. To address these limitations, our lab developed comprehensive, macronutrient-matched TAD and MD models that more closely mimic human diets in the U.S. and Mediterranean, respectively. A previous study in our lab found that six months of TAD consumption resulted in elevated body weight, increased inflammation, and excess hepatic lipid deposition, in comparison to the MD. Our current study looked to further characterize MetS under this diet model, specifically investigating obesity, insulin resistance, and dyslipidemia markers. Male and female C57BL/6J mice consumed either the TAD or MD from the age of 4 to 7 months. We found that three months on the TAD promoted hepatic steatosis and elevated serum cholesterol levels in both males and females. However, other findings suggest early signs of insulin resistance in TAD males, but not females. Future studies will investigate MetS after 6 months on diet to better elucidate insulin resistance development and these potential sex differences in health outcomes.

Study Overview

- Metabolic syndrome (MetS) is a cluster of concurrent cardiometabolic risk factors associated with an increased risk of diabetes, cardiovascular disease, and stroke
- MetS is defined by the presence of at least three of the following factors: abdominal obesity, hypertension, elevated triglycerides, low HDL cholesterol, and hyperglycemia
- A key pathophysiology of MetS is insulin resistance, which is a reduced sensitivity to insulin regulation of glucose uptake and metabolism
- The liver is a primary regulator of glucose homeostasis and lipid metabolism, closely intertwined with white adipose tissue function





	% kcal	MD	TAD
Carb	50	Brown rice & wheat starch	Corn starch
Fat	35	Olive oil, fish oil, & flaxseed oil	Safflower oil, beef fat, butter
Protein	15	Egg whites, soy, & fish protein	Casein (milk fat)

- A standard glucometer was used to measure blood glucose levels in the voluntary oral glucose tolerance test (vOGTT) and insulin tolerance test (ITT)
- Cholestech LDX Lipid Profile cassettes were used to assess serum lipid levels
- Metabolic hormones and inflammatory markers were quantified with a Meso Scale Discovery U-PLEX Metabolic Hormones Combo 1 (mouse) multiplex assay

Insulin Resistance Tests

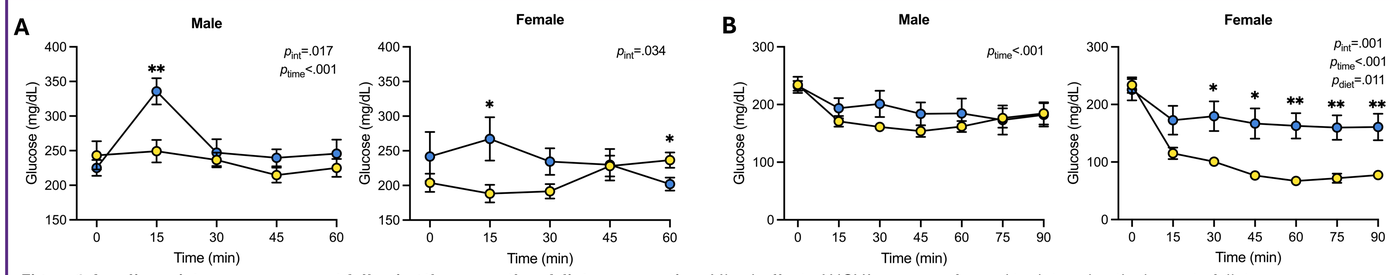


Figure 1. Insulin resistance assessment following three months of diet consumption. Mixed-effects ANOVAs were performed to determine the impact of diet and time on blood glucose response for each sex, followed by post hoc t-tests for multiple comparisons using Tukey's correction. (**A**) There was no main effect of diet but a significant interaction effect (sex x diet) on blood glucose response in the vOGTT for males and females. (**B**) There was a significant main effect of diet in the ITT for females, such that mice on the TAD were more responsive to insulin stimulation than the MD group. However, there was no main effect of diet in males. *p≤0.05, **p≤0.01, bars represent mean ± SEM.

Glucose Regulation

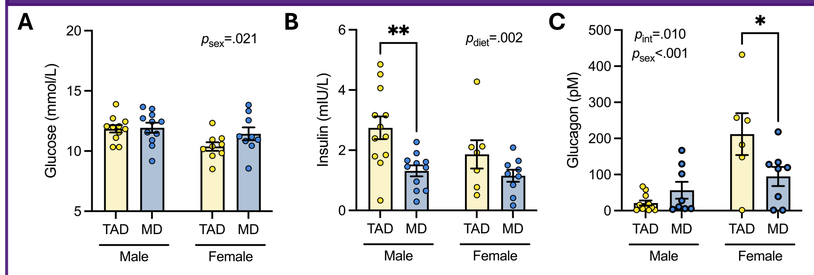


Figure 2. Sex differences in glucose regulatory hormones. Two-way ANOVAs were performed to determine the impact of diet and sex on glucose and regulatory hormones, followed by *post hoc* t-tests for multiple comparisons using Tukey's correction. (**A**) There was no main effect of diet on fasting blood glucose. (**B**) There was a significant main effect of diet on fasting serum insulin, with an increase in TAD males. (**C**) There was a significant interaction effect on fasting serum glucagon, with an increase in TAD females. *p≤0.05, **p≤0.01, bars represent mean ± SEM.

Adipose Tissue

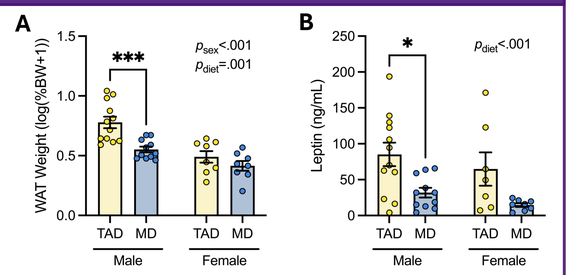


Figure 3. Increased adiposity in TAD males. Two-way ANOVAs were performed to determine the impact of diet and sex on adiposity, followed by *post hoc* t-tests for multiple comparisons using Tukey's correction. There was a significant main effect of diet on (**A**) gonadal WAT mass and (**B**) fasting serum leptin, with a significant elevation in TAD males but not females. *p≤0.05, ***p≤0.001, bars represent mean ± SEM.

Serum Lipid Levels

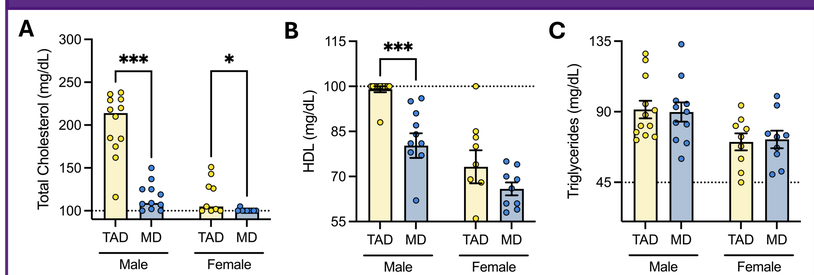


Figure 4. Elevated cholesterol levels following TAD consumption. Values on the dashed lines indicate measurements outside the machine's detectable range. (**A**) Mann-Whitney tests revealed significantly elevated total cholesterol for both males and females on the TAD. (**B**) HDL cholesterol was elevated in TAD males, but there was no difference in females. (**C**) There were no differences in triglycerides between diet conditions for both males and females. ***p≤0.001, bars represent mean ± SEM.

Inflammation

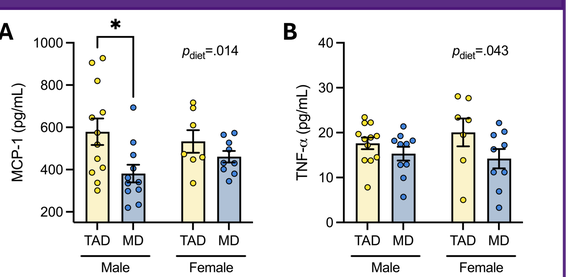


Figure 5. Increased inflammation in TAD males. Two-way ANOVAs were performed to determine the impact of diet and sex on adiposity, followed by *post hoc* t-tests for multiple comparisons using Tukey's correction. There was a significant main effect of diet on fasting serum (**A**) MCP-1, with an increase in TAD males, and (**B**) TNF-α. *p≤0.05. Bars represent mean ± SEM.

Conclusions

- Three months of TAD may promote an early transition towards an insulin-resistant state in males, as suggested by elevated insulin, cholesterol, and MCP-1. This was not observed in females.
- Male mice on the TAD had greater adiposity compared to the MD group. This was not observed in females.
- There were no significant differences in physiological insulin response between diet conditions in male mice. In females, mice on the MD had a significantly lower alternation in blood glucose following insulin administration, possibly suggesting a reduced insulin sensitivity.

Future Directions

- Repeat measures following six months of diet exposure.
- Explore the significance of early diet exposure during developmental years on disease risk.
- Investigate sex differences in health outcomes, possibly by exploring the role of estrogen in insulin-related signaling.

References

Braden-Kuhle, P.N., et al., A Mediterranean-style diet protects against cognitive and behavioral deficits, adiposity, and Alzheimer's disease-related markers, compared to a macronutrient-matched typical American diet in C57BL/6J mice. J Alzheimers Dis, 2025: p. 13872877251319467.

Kim, S.H. and G.M. Reaven, Insulin resistance and hyperinsulinemia: you can't have one without the other. Diabetes Care, 2008. 31(7): p. 1433-8

Kumashiro, N., et al., Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A, 2011. 108(39): p. 16381-5

Li, M., et al., Trends in insulin resistance: insights into mechanisms and therapeutic strategy. Signal Transduction and Targeted Therapy, 2022. 7(1): p.216

Reaven, G.M., Pathophysiology of insulin resistance in human disease. Physiol Rev, 1995. 75(3): p. 473-86.

Sartipy, P. and D.J. Loskutoff, Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proceedings of the National Academy of Sciences, 2003.100(12): p. 7265-7270

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