# Sex-dependent metabolic responses to protein restriction are ablated by ovariectomy


**Bailey Knopf**[1,2,3], Isaac Grunow[1,2], Michelle Sonsalla[1,2], Reji Babygirija[1,2,3], Mariah Calubag[1,2,3], Yang Liu[1,2], Chung-Yang Yeh[1,2], and Dudley Lamming[1,2]

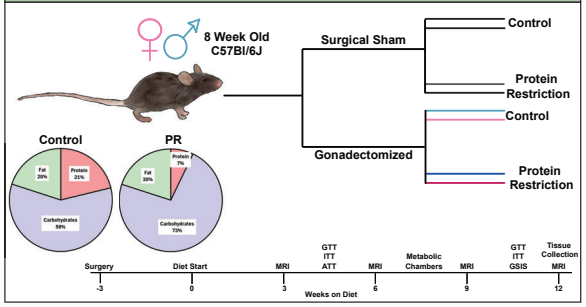
[1] University of Wisconsin-Madison, Madison, WI
[2] William S. Middleton Memorial Veterans Hospital, Madison, WI
[3] Cell and Molecular Biology Graduate Program, Madison, WI

Cellular and Molecular Biology Graduate Program
UNIVERSITY OF WISCONSIN–MADISON


## Introduction

- One method to increase overall health and longevity is to alter the diet. While calorie restriction is the gold standard for increasing longevity, recent studies have found that lifespan and dietary protein content are inversely related; decreasing dietary protein promotes longevity.
- Studies investigating protein restriction (PR) have found that lifelong restriction increases lifespan, decreases frailty, and improves metabolic health in a variety of model organisms. Conversely, a diet containing a high volume of protein has been associated with insulin resistance, obesity, diabetes, and mortality.
- In animal models, PR has been shown to decrease adiposity, increase energy expenditure, and improve glucose homeostasis. In mice, PR has sex-specific metabolic and molecular responses with females exhibiting a blunted response.
- This study aims to investigate the role that sex organs play in the sex-specific metabolic efffects of PR.

## Study Design




## PR reduces ovariectomy induced fat accumulation

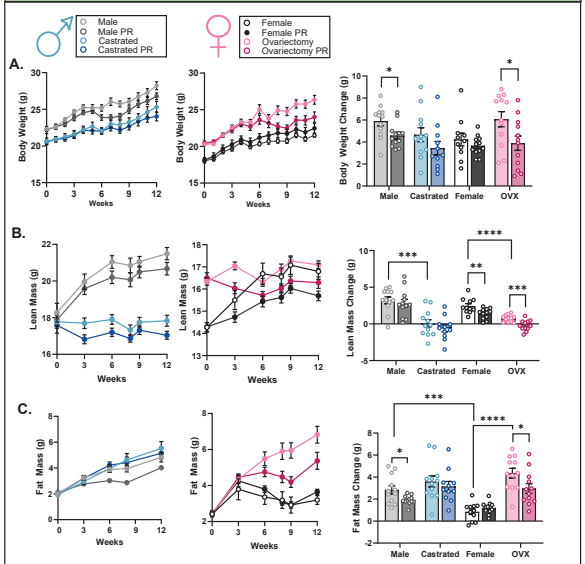



**Figure 1. PR decreases body weight via decreasing fat mass in male, castrated, and ovariectomized C57Bl/6J mice, but not in intact females.**
(A) Body weight change during 12 week dietary intervention shows that PR is a potent protetor against body weight gain in castrated and intact males. While PR has little effect on body weight in females, ovariectomy-PR mice show significantly decreased body weight gain. (B) The effects of PR on lean mass in male (left) and female (middle) mice, with lean mass change quantified on the right. Overall, PR decreases lean mass across all groups. (C) Fat mass accumulation is exacerbated by ovariectomy, but can be partially rescued by PR. (A,B,C) n=11-12 mice/group; one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data represented as mean ± SEM.

## PR improves ovariectomy associated metabolic dysfunction by improveming glucose handling

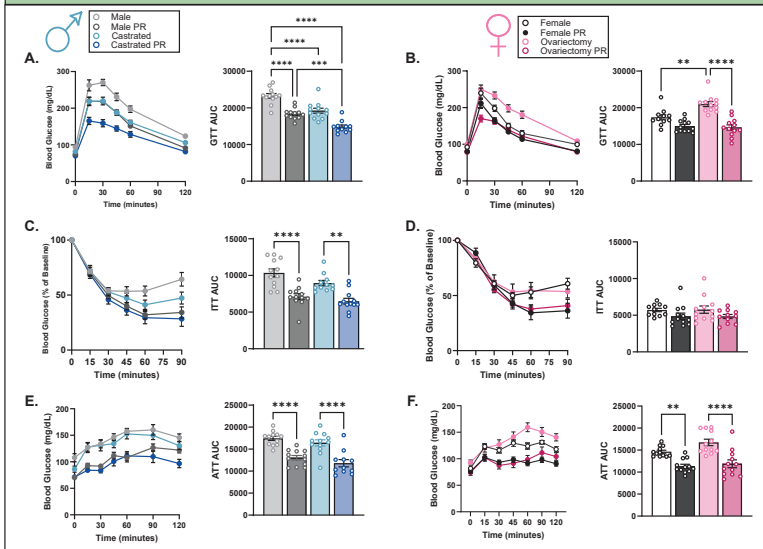



**Figure 2. Improvements in glucose handling are observed in male and ovariectomized female mice.**
(A) PR promotes improved glucose tolerance in both intact and castrated males. (B) Improved glucose tolerance is observed in ovariectomized females only, however ovariectomy itself promotes glucose intolerance. (C) Insulin sensitivity is improved in both male and castrated PR groups. (D). PR may improve insulin sensitivity in both intact and ovariectomized females. (E and F). Insulin sensitivity observed in the males and females is likely due to suppression of hepatic gluconeogenesis as observed by a reduction in AUC in ATT. (A,B,C) n=11-12 mice/group; one-way ANOVA, *p<0.05, **p<0.01, **p<0.001, ****p<0.0001. Data represented as mean ± SEM.

## Dietary PR improved metabolic health regardless of sex

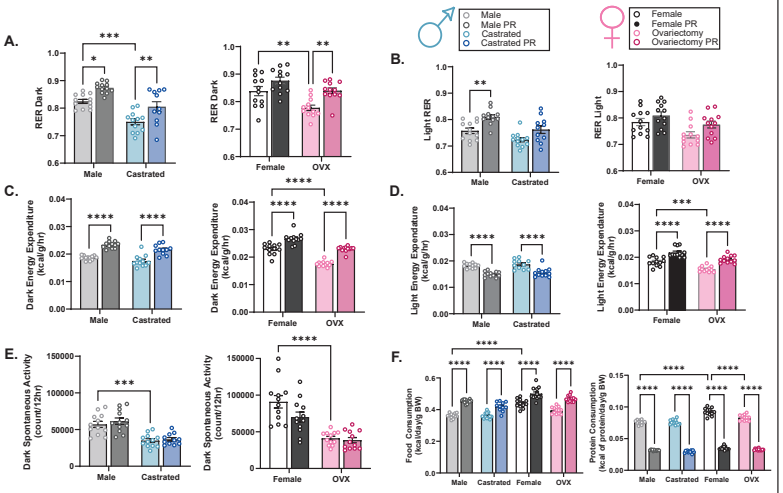



**Figure 3. Parameters to determine metabolic health are improved with PR across all sexes.**
(A) Respiratory Exchange Ratio (RER) across a 12-hour dark period was increased across PR groups regardless of sex. (B) RER during light period may be increased with PR. (C) Energy expenditure normalized to body weight over the dark cycle. (D) Energy expenditure is decreased in intact and castrated PR males during the light cycle, however the PR females exhibit increased expenditure as seen in the dark. (E) Spontaneous activity during the dark period exhibited a strong decrease in activity for gonadectomy mice indicating a role for sex organs and hormones in activty levels. (F) Average food consumption and protein consumption normalized to body weight throughout thr 12-week intervention. PR increases food consumption across all groups, however PR groups exhibit a significant decrease in overall protein consumption. (A-F) n=11-12 mice/group; one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data represented as mean ± SEM.

## PR increases lipid droplet size in the liver

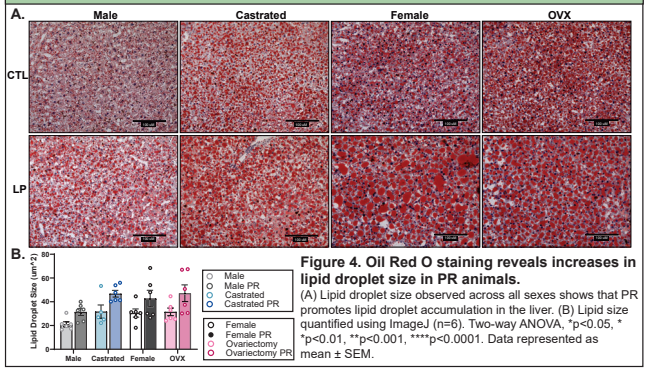



**Figure 4. Oil Red O staining reveals increases in lipid droplet size in PR animals.**
(A) Lipid droplet size observed across all sexes shows that PR promotes lipid droplet accumulation in the liver. (B) Lipid size quantified using ImageJ (n=6). Two-way ANOVA, *p<0.05, * *p<0.01, **p<0.001, ****p<0.0001. Data represented as mean ± SEM.

## Metabolic benefits induced by PR in ovariectomy may be due to decreased mTORc1 activity in skeletal muscle

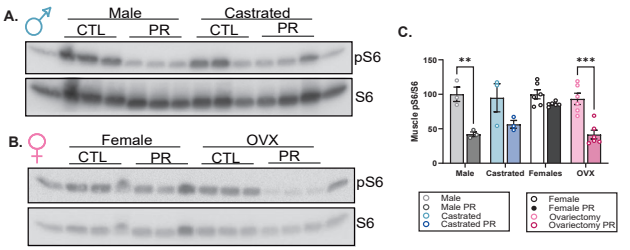



**Figure 5. Western Blot analysis of downstream mTORc1 proteins phospoS6 and S6 in skeletal muscle.**
(A) PR promotes decreased pS6/S6 in the skeletal muscle of both intact and castrated males (n=3). (B). PR has little effect on pS6/S6 in females however in ovariectomized mice pS6/S6 is significantly decreased indicating reduced mTOR activity in ovariectomized mice on PR (n=6) (C). Western blot quantification of pS6/S6 in skeletal muscle. (A&B) One-way ANOVA, *p<0.05, **p<0.01, **p<0.001, ****p<0.0001. Data represented as mean ± SEM.

## Conclusions and Future Directions

The present study highlights the important role that sex plays in response to longevity promoting dietary interventions. Here we determined that removal of the ovaries allows mice to respond robustly to the metabolic benefits of PR. We conclude this by discovering that PR promotes decreased weight gain, specifically fat, in ovariectomized mice. We also show that ovariectomized mice exhibit metabolic dysfunction that can be partially reversed with PR. Interestingly, we see an overall improvement in metabolic health across all sexes and surgical conditions. While the mechanism surrounding why ovariectomized mice are more senstivie to PR, we propose the metablic benefits may be driven by mTORc1 inhibition. Future studies aim to understand the exact mechanism by which PR imrpoves metabolic health in ovariectomized mice. We aim to look at mTORc1 activity across different tissue types and determine if FGF21- a potent hormone induced by PR- is promoting these benefits.

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