### **RE-EVALUATING CALORIES AND FASTING** IN A DIETARY RESTRICTION PARADIGM

Heidi H. Pak, Ph.D.

**UW-Madison** 

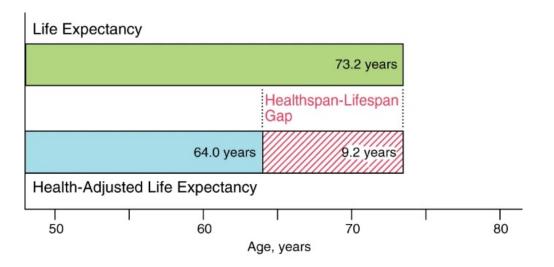






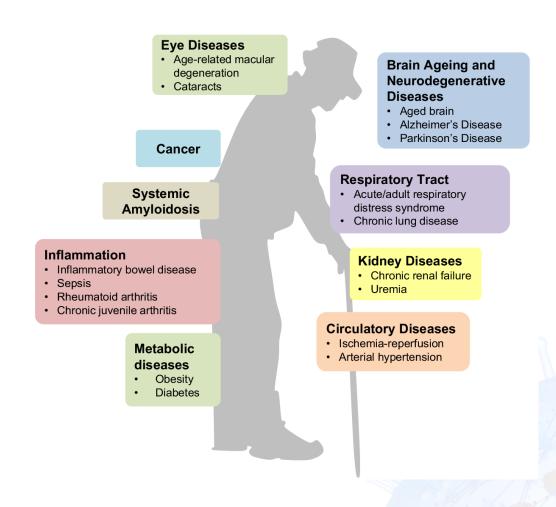
### Why Study Aging?

Lifespan: Total number of years lived by an individual



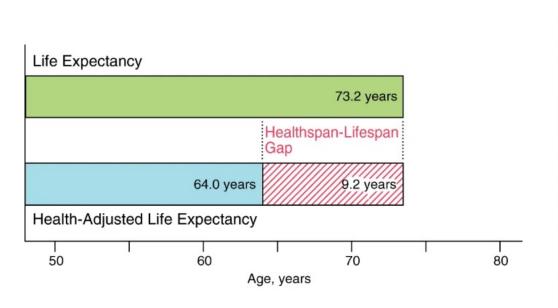
Healthspan: Number of disease-free years lived

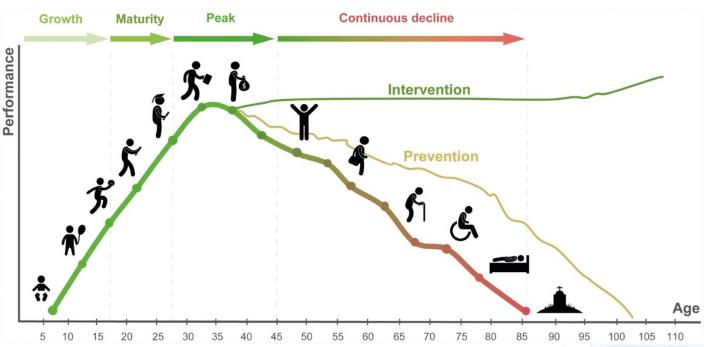
Garmany et al. npj Regenerative Medicine 2021



### Why Study Aging?

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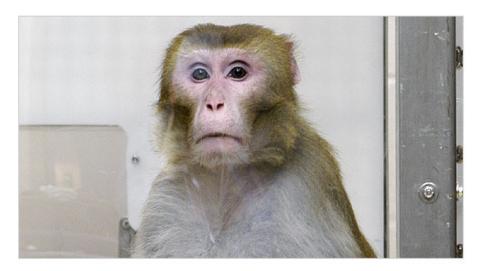
**Healthspan:** Number of disease-free years lived

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#### **UW-Madison Rhesus Monkey Calorie Restriction Study**





#### Canto, 25

Although a senior citizen — the average rhesus monkey lifespan in captivity is 27 — Canto, above, is aging fairly well. Outwardly, he has a nice coat, elastic skin, a smooth gait, upright posture and an energetic demeanor. His bloodwork shows he is as healthy as he looks.

Human equivalent Meals prepared by Mike Linksvayer, 36



Breakfast fermented soybeans and garlic



Lunch tofu, konyakku and carrots



445 885

Monkeys also receive an apple each day.

2,000

MONKEY MENU

HUMAN MENU

Daily calories

Beverages, snacks and desserts not shown. Diet varies according to body type, sex and activity level.

3,000



Dinner vegan sausage, kale, tomato sauce and salad



NORMAL DIET

#### Owen, 26

He gets more food, but Owen, above, isn't aging as well. His posture has been affected by arthritis. His skin is wrinkled and his hair is falling out. Owen is frail and moves slowly. His bloodwork shows unhealthy levels of glucose and triglycerides.

Diet of an average, active human male of 36

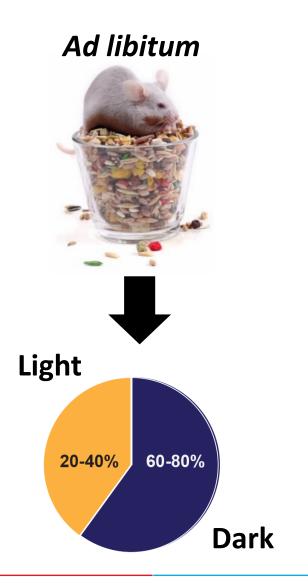






Photos by Jim Wilson and Tony Cenicola/The New York Times and Lars Klove for The New York Times

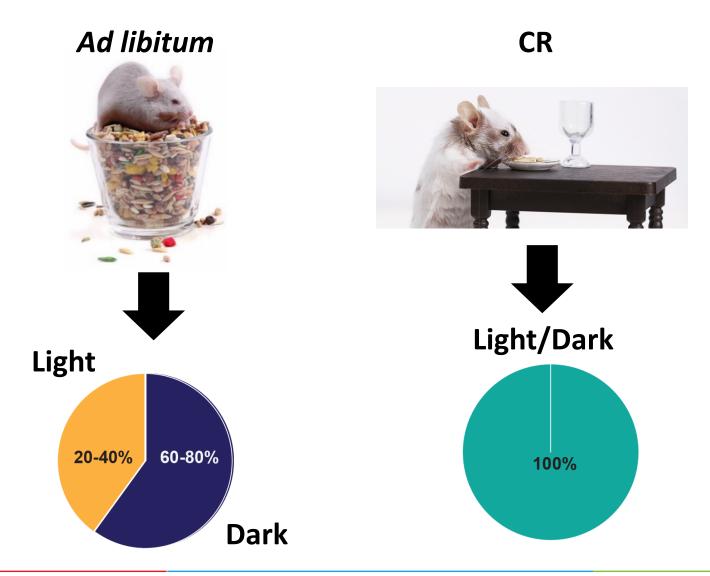
### **Overlooked feature in Calorie Restriction**



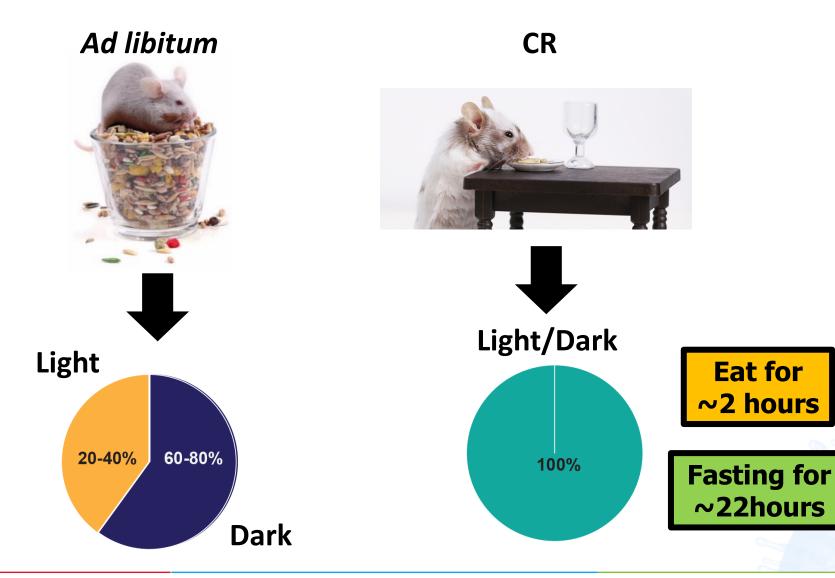
CR



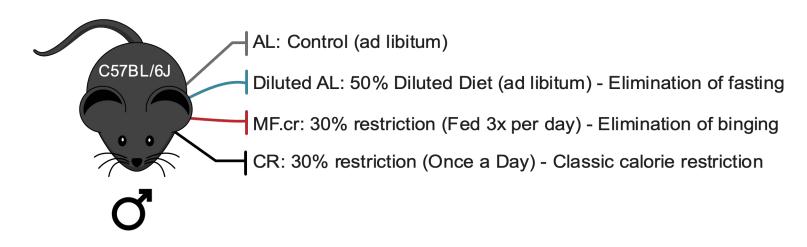
### **Overlooked feature in Calorie Restriction**

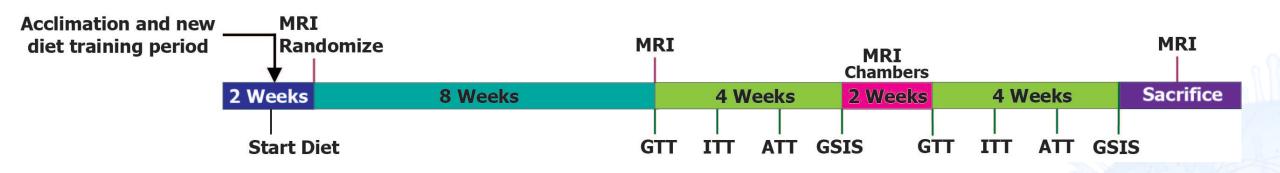


### **Overlooked feature in Calorie Restriction**

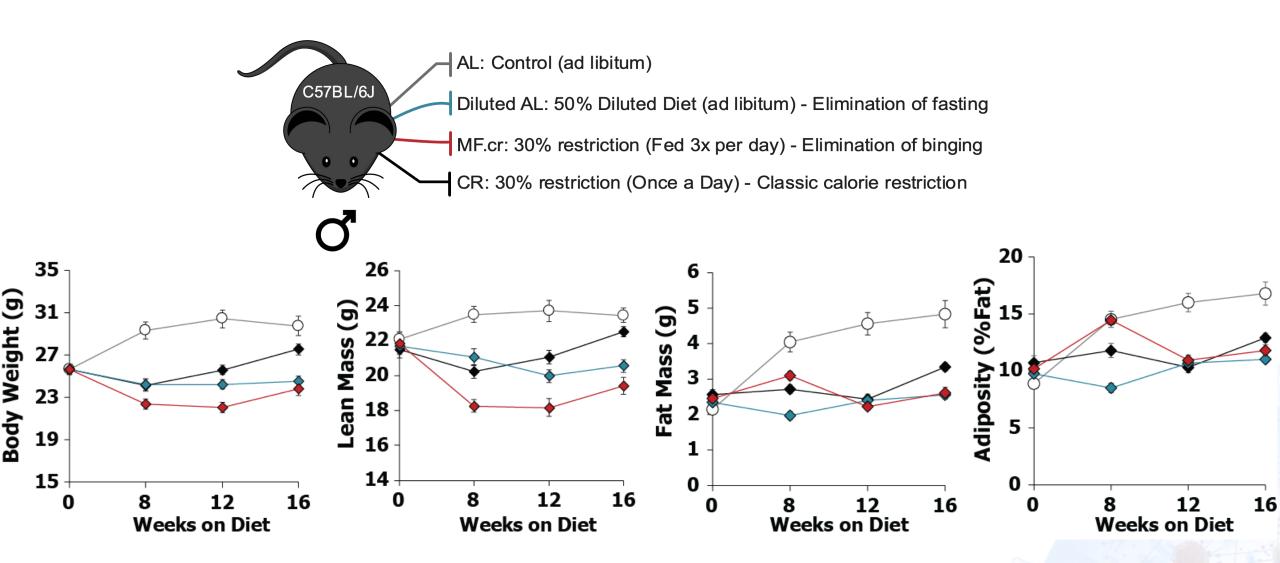


### Distinguish the fasting-dependent and calorie-dependent effects of CR on metabolic health, fitness, and longevity



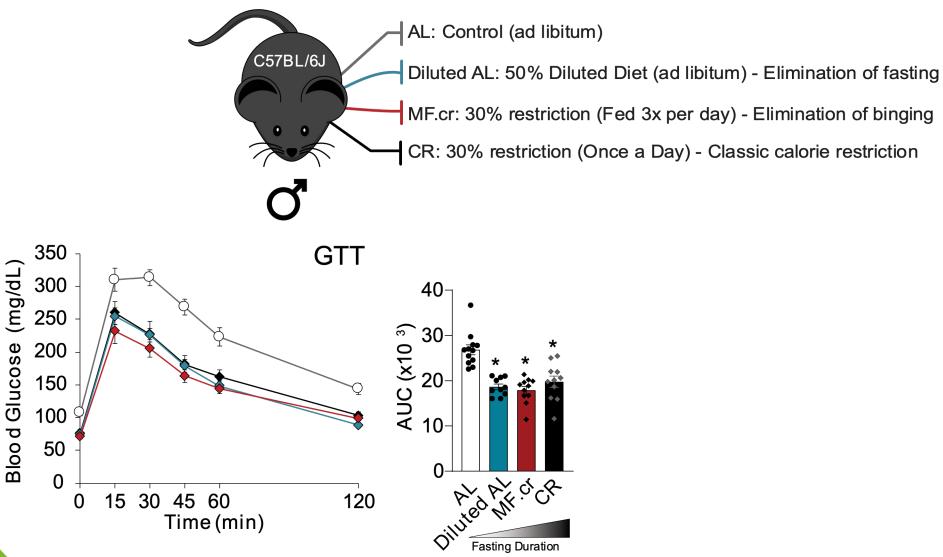


#### Body composition outcome of three modes of calorie restriction

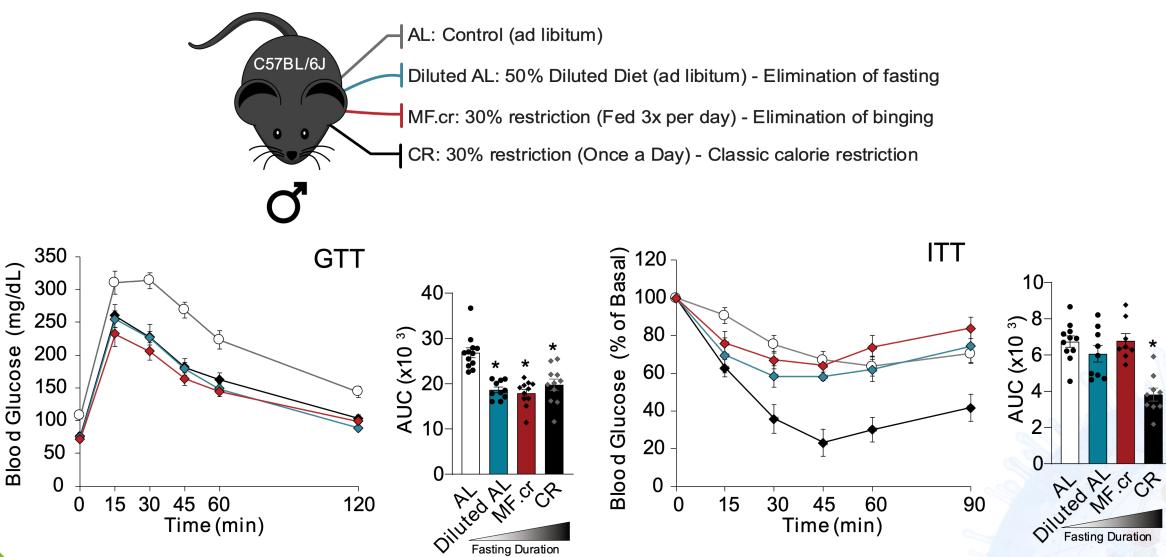




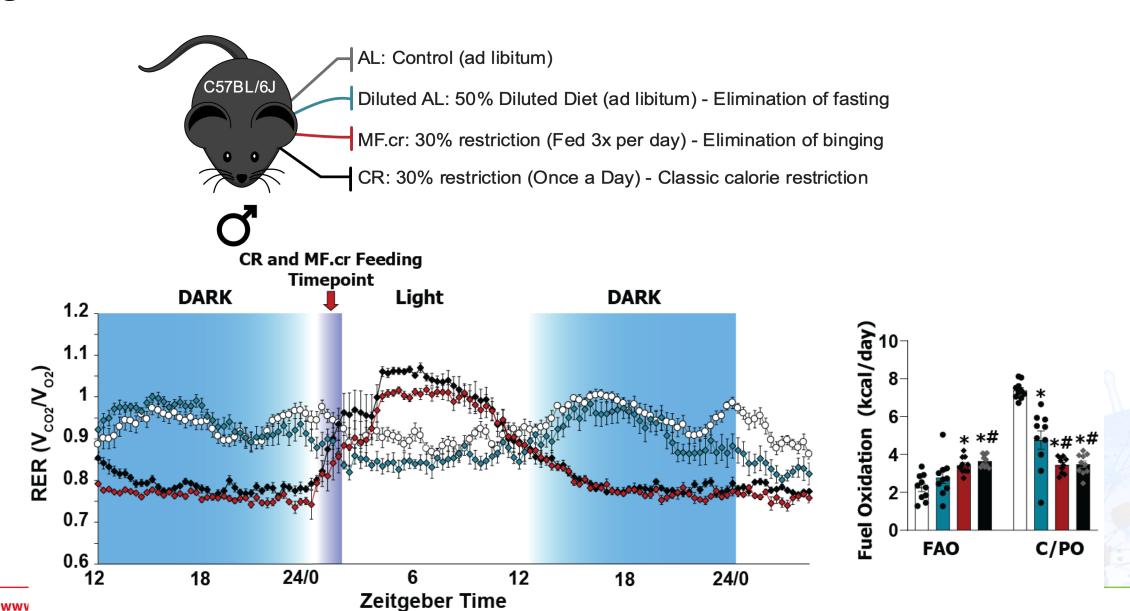
#### Glucose tolerance is improved in all CR models



#### Fasting is required to improve insulin sensitivity

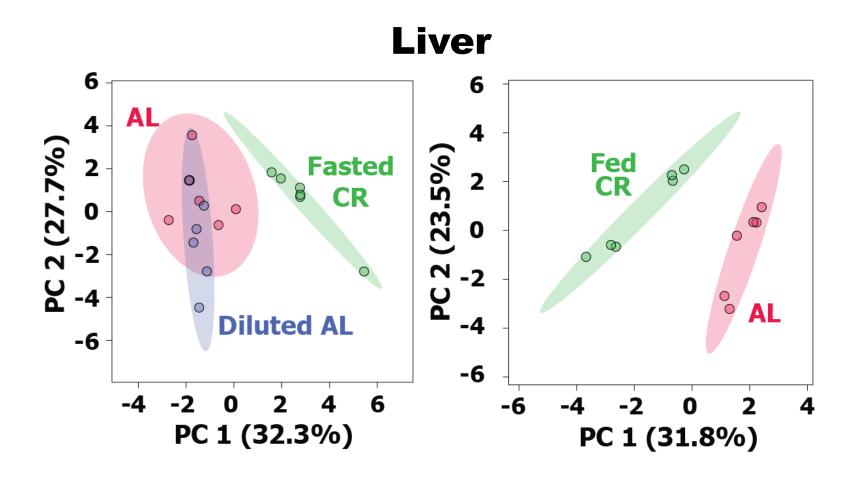


### Fasting is required for the distinct fuel utilization pattern in CR-fed mice

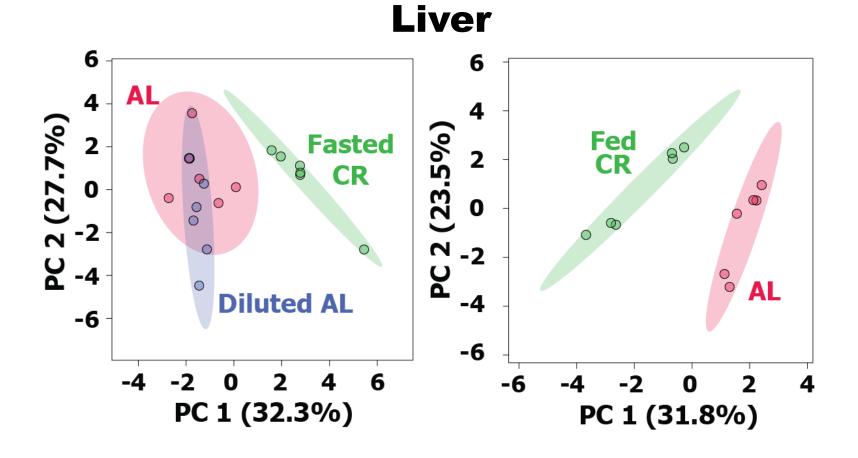


**ICoMI** 

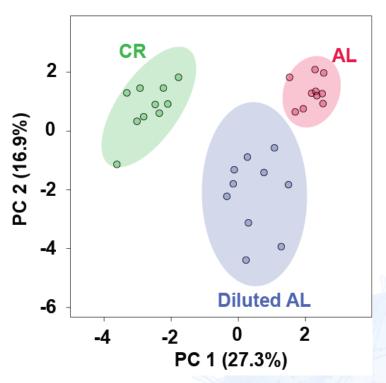
#### Fasting promotes the unique metabolomic signature in CR mice



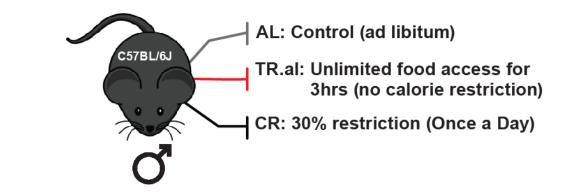
#### Fasting promotes the unique metabolomic signature in CR mice



#### **Skeletal Muscle**



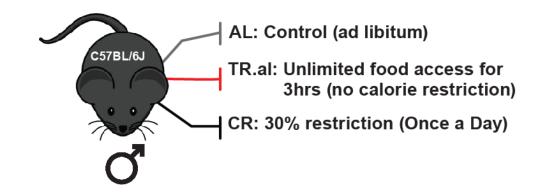
#### Fasting alone recapitulates the CR response

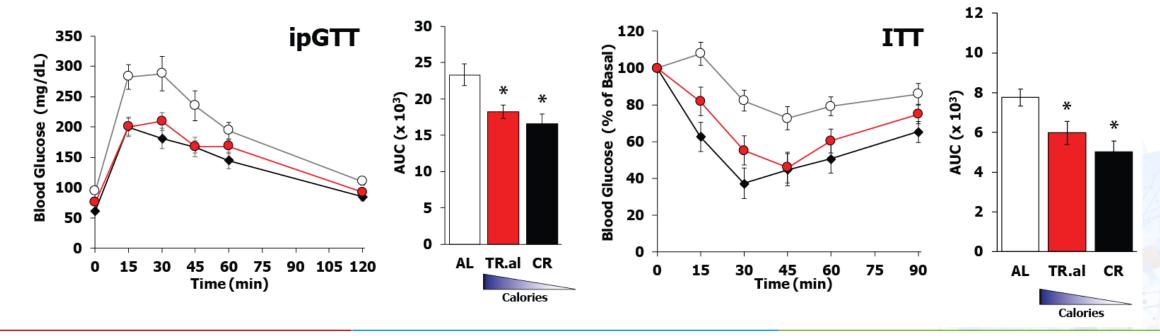






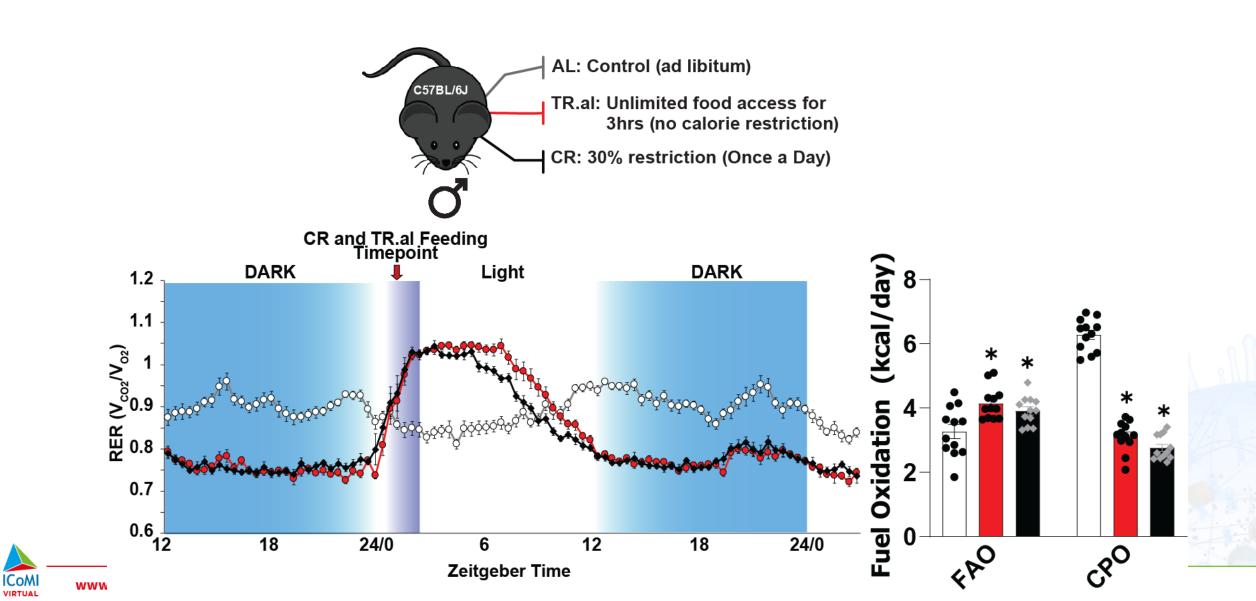
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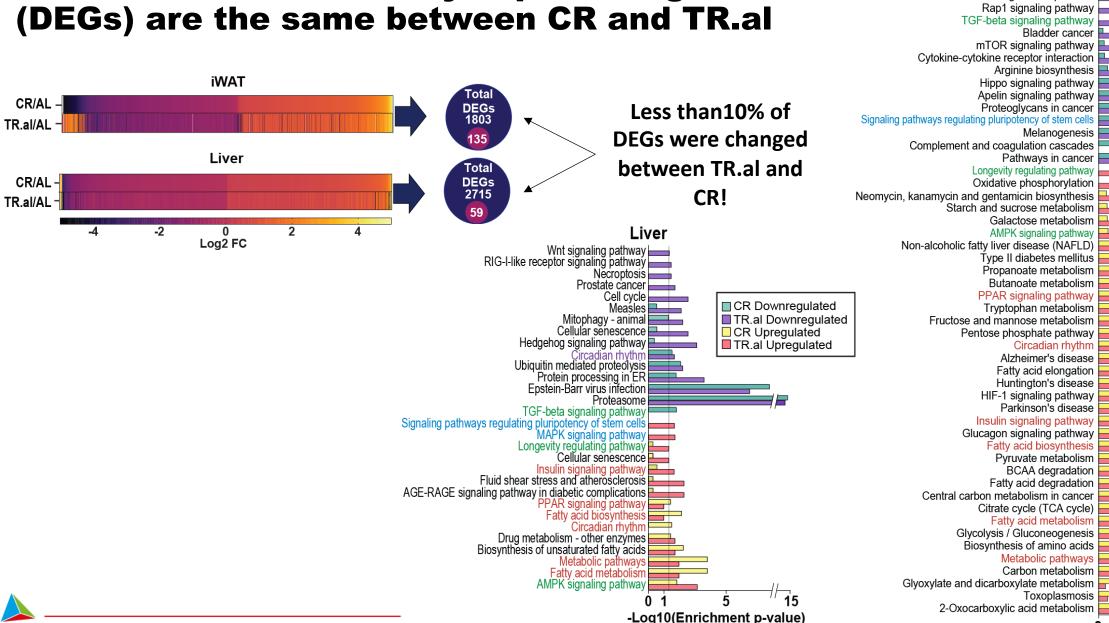


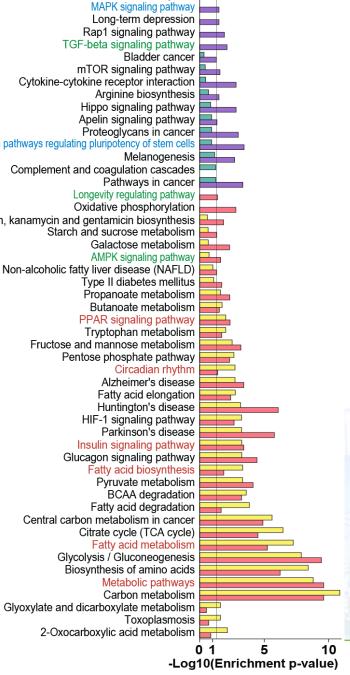


#### Fasting alone recapitulates the CR response



### Over 90% of differentially expressed genes

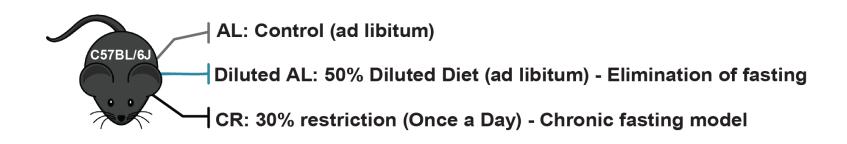


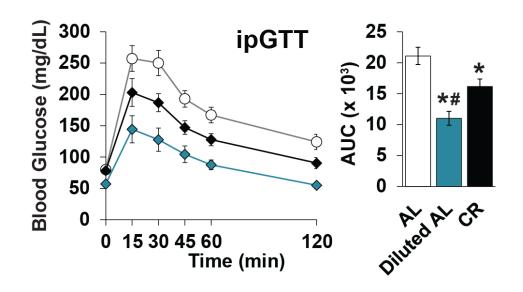


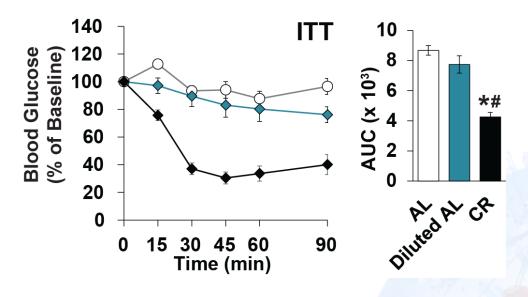
**iWAT** 

Adherens junction Endocvtosis —

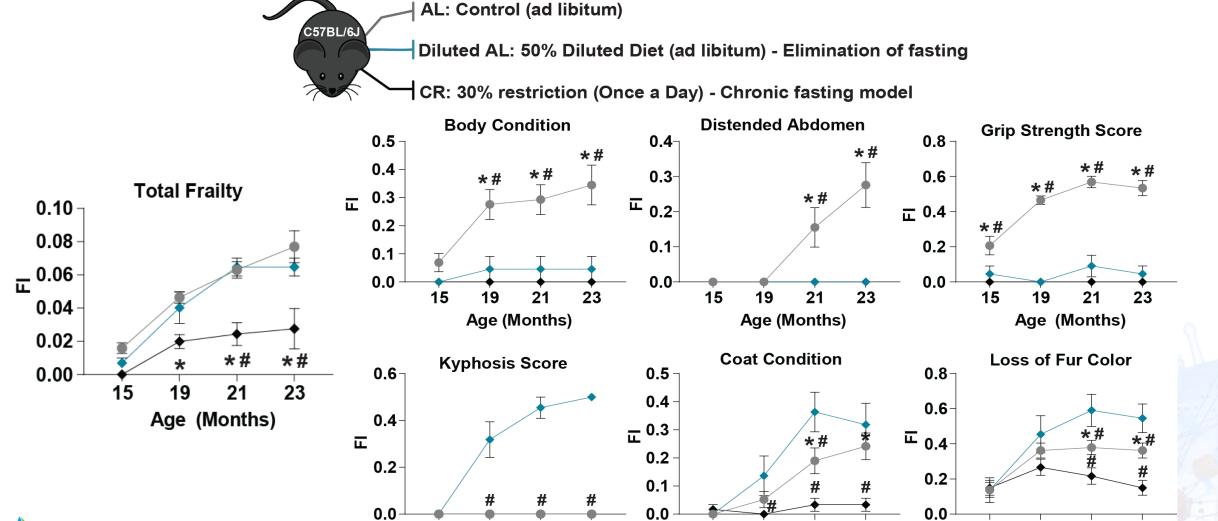
# Fasting is necessary for CR-induced improvement in insulin sensitivity







#### Fasting is necessary for CR-induced improvements in frailty



Age (Months)

Age (Months)

Age (Months)

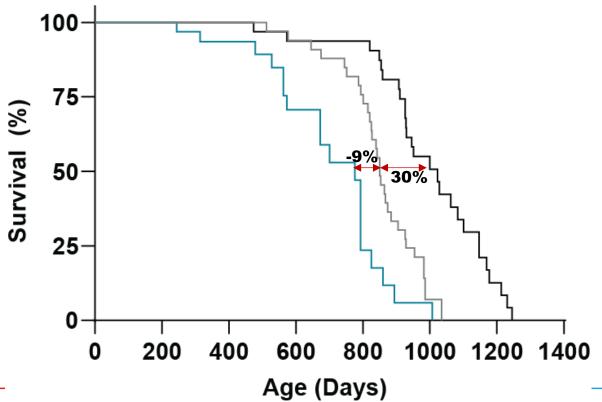


#### Fasting is necessary for CR-induced improvements in lifespan



Diluted AL: 50% Diluted Diet (ad libitum) - Elimination of fasting Median Lifespan = 776 days

CR: 30% restriction (Once a Day) - Chronic fasting model Median Lifespan = 1022 days

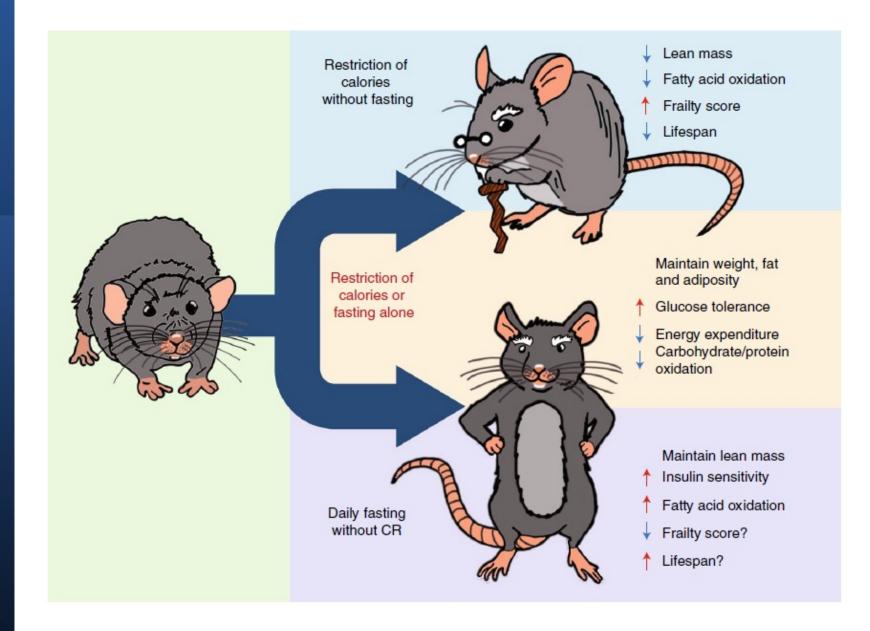


# Cancer Observed at Necropsy 700 800 900 1000 1100 Age (Days)

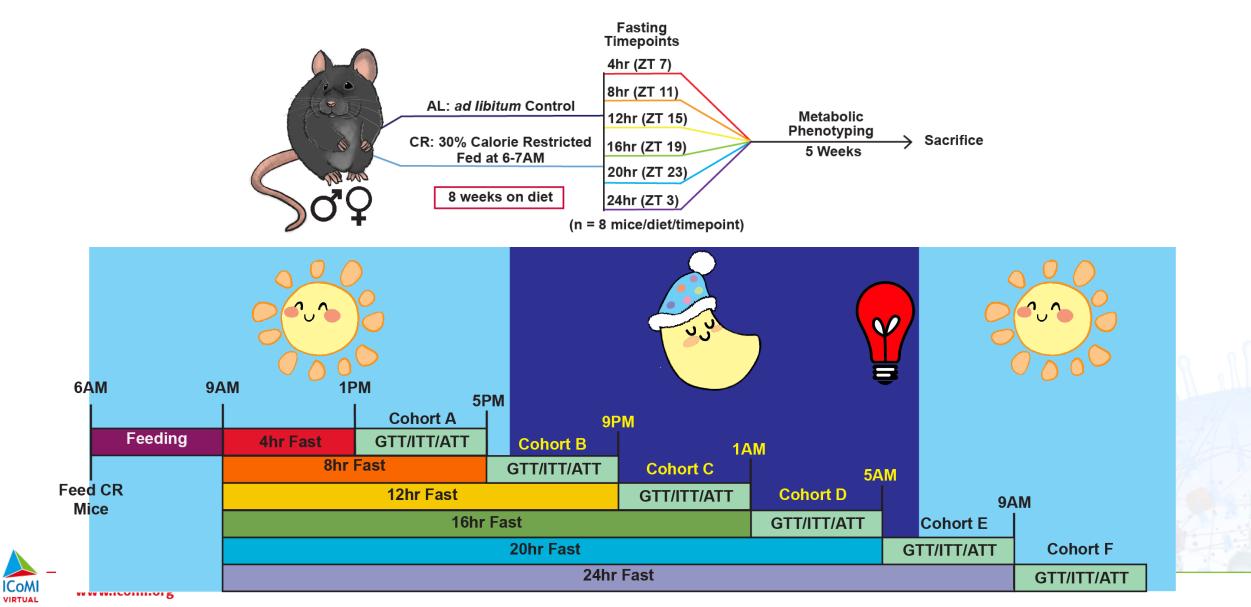


C57BL/6J

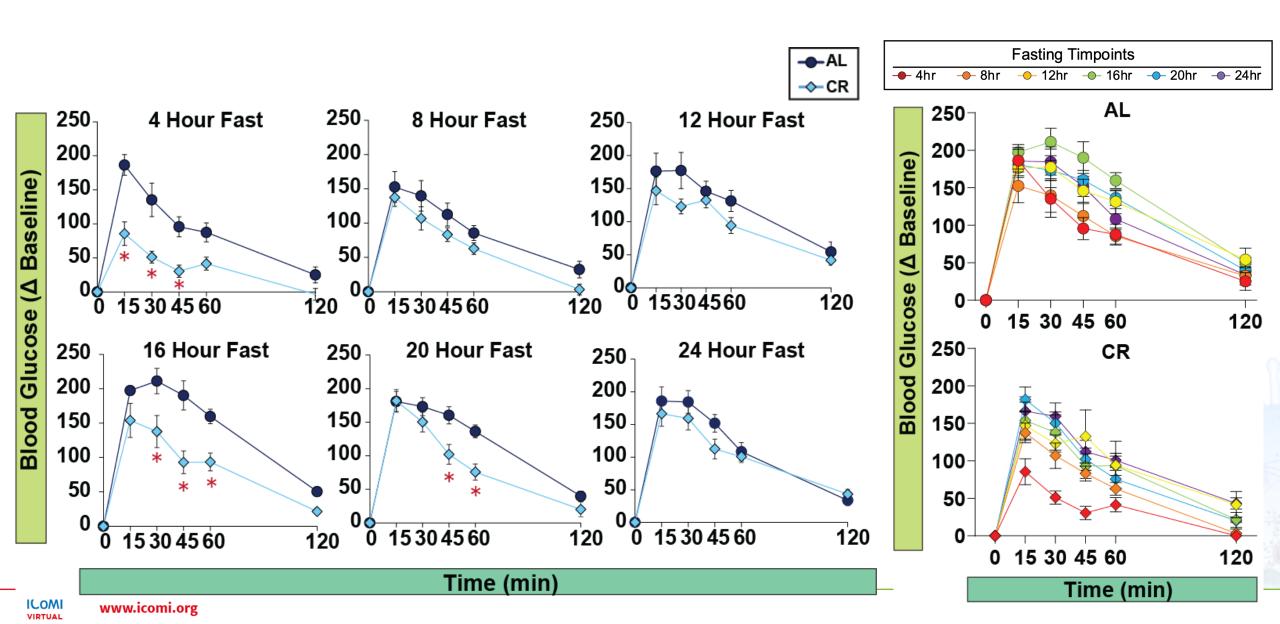
### Summary



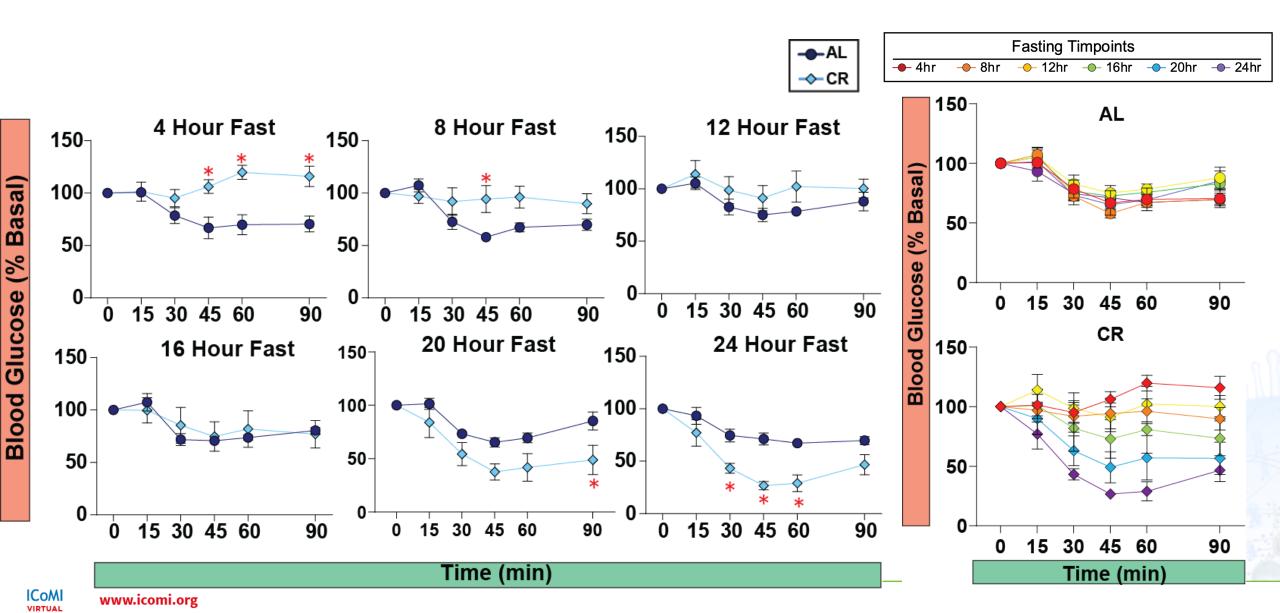
### **Chronic Fasting model vs Non-fasting model**



### Glucose tolerance is affected by fasting duration



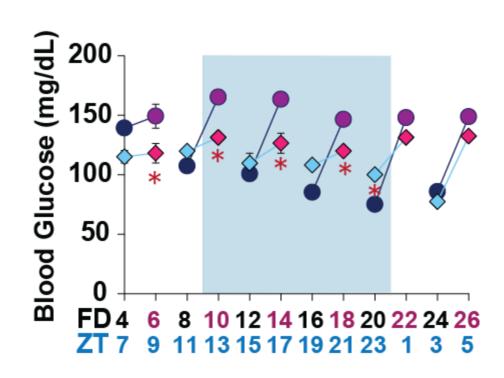
## Insulin sensitivity is only observed after a prolonged fast in CR mice

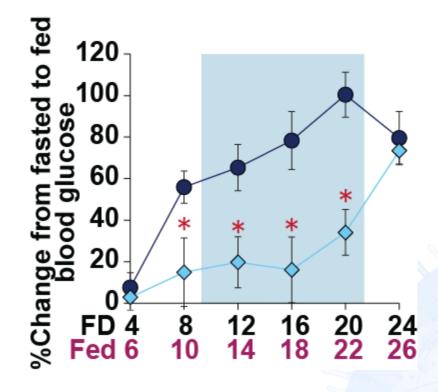


# CR animals are adapted to maintain tight blood levels during a prolonged fast







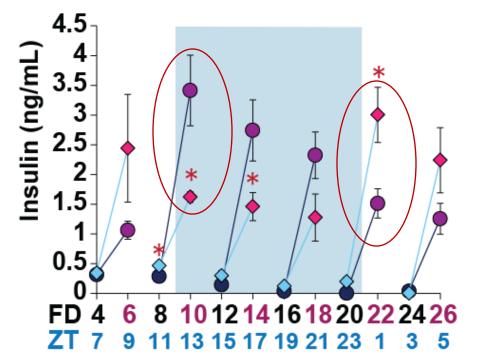




#### Insulin levels may be dependent on time of feeding

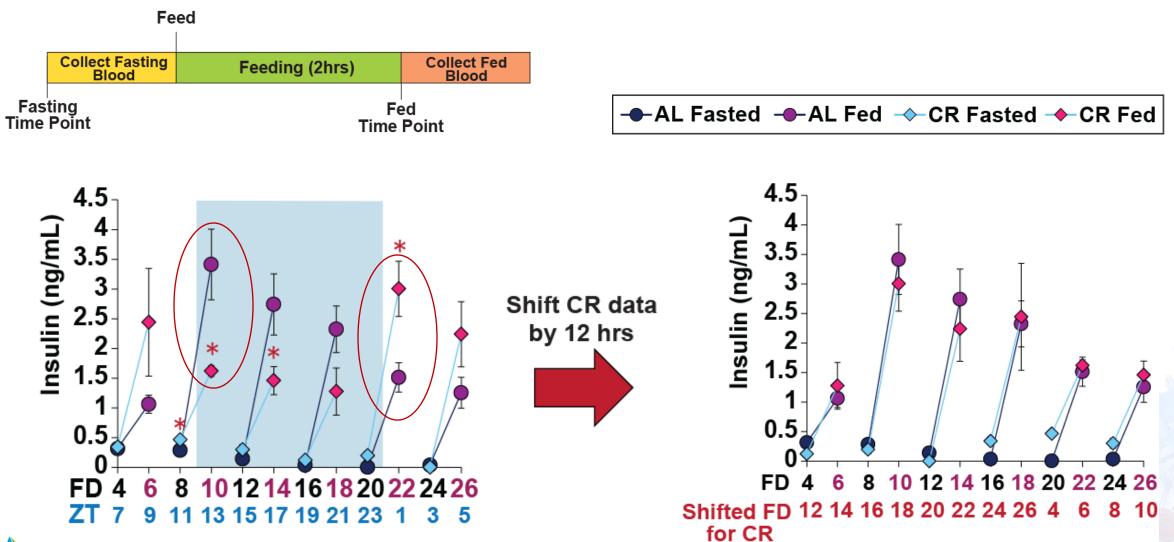




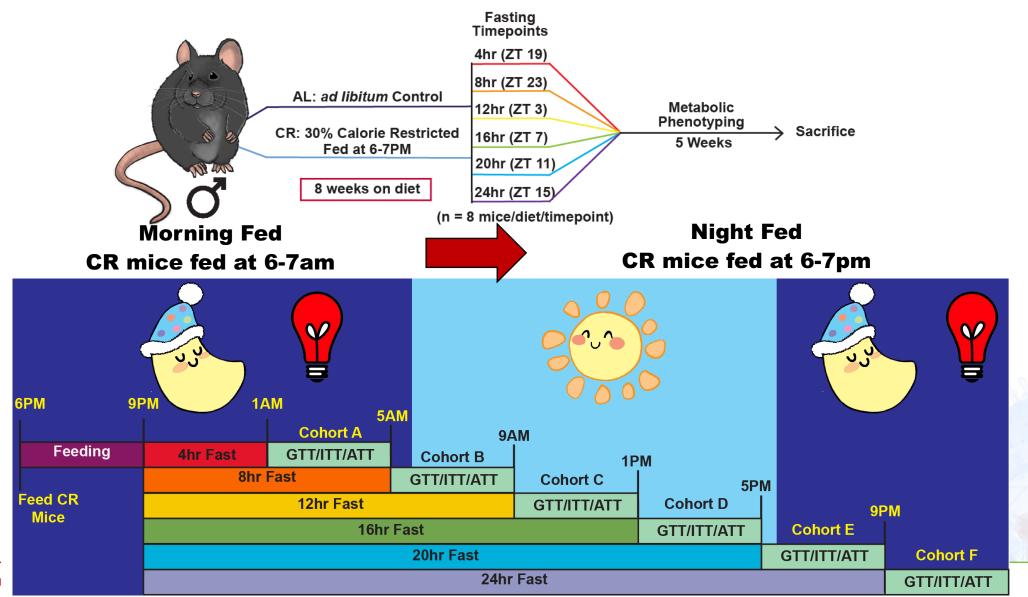




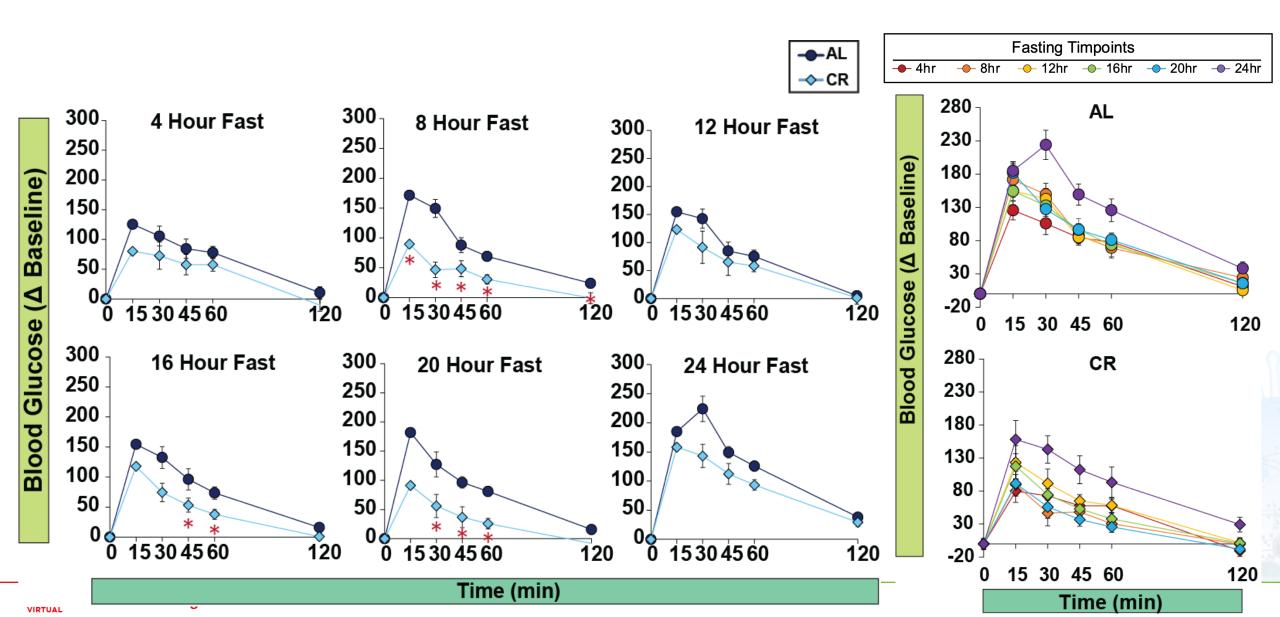
#### Insulin levels may be dependent on time of feeding



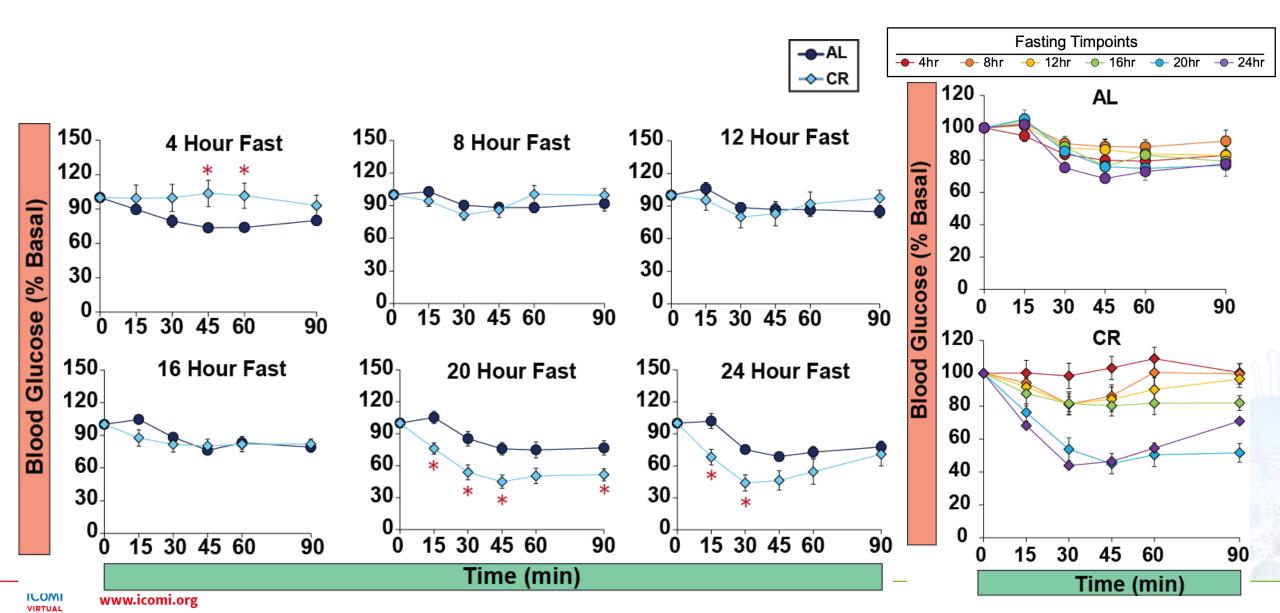
#### Is circulating insulin level dependent on the time of first feeding?



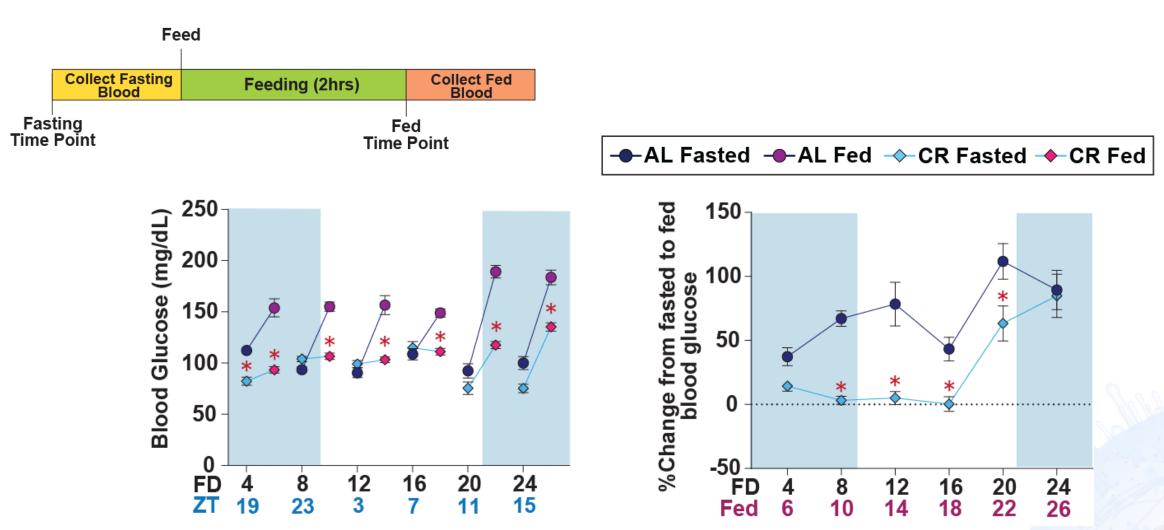
# Night-fed CR mice had similar improvements in glucose tolerance as morning fed CR mice



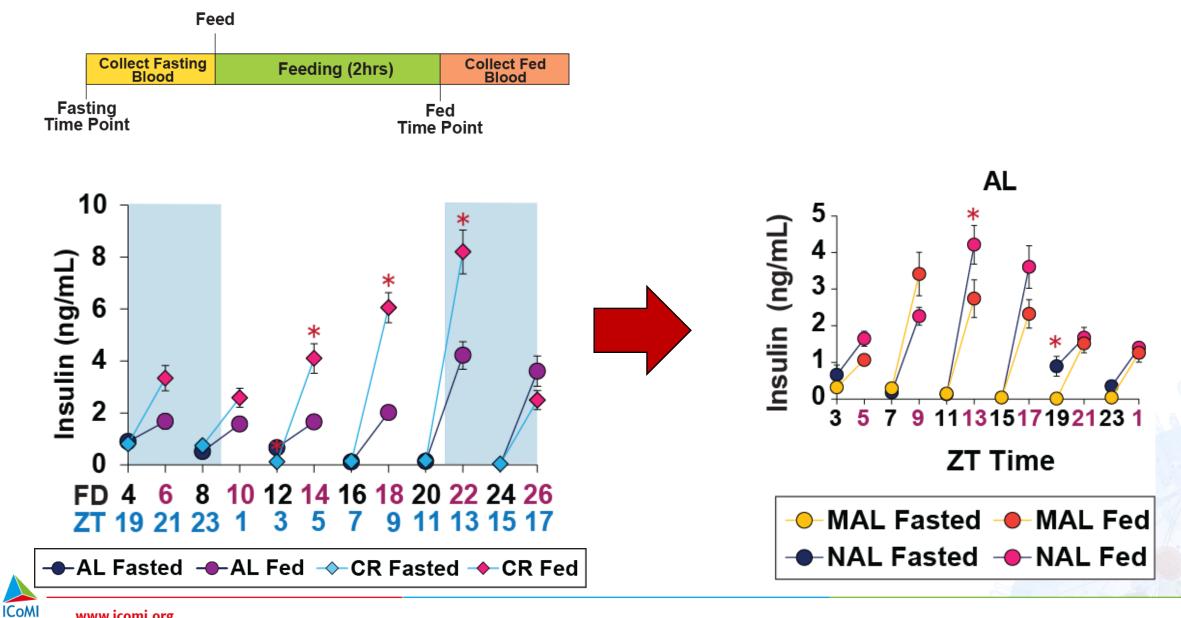
# Insulin sensitivity was also only observed after a 20hr of fast



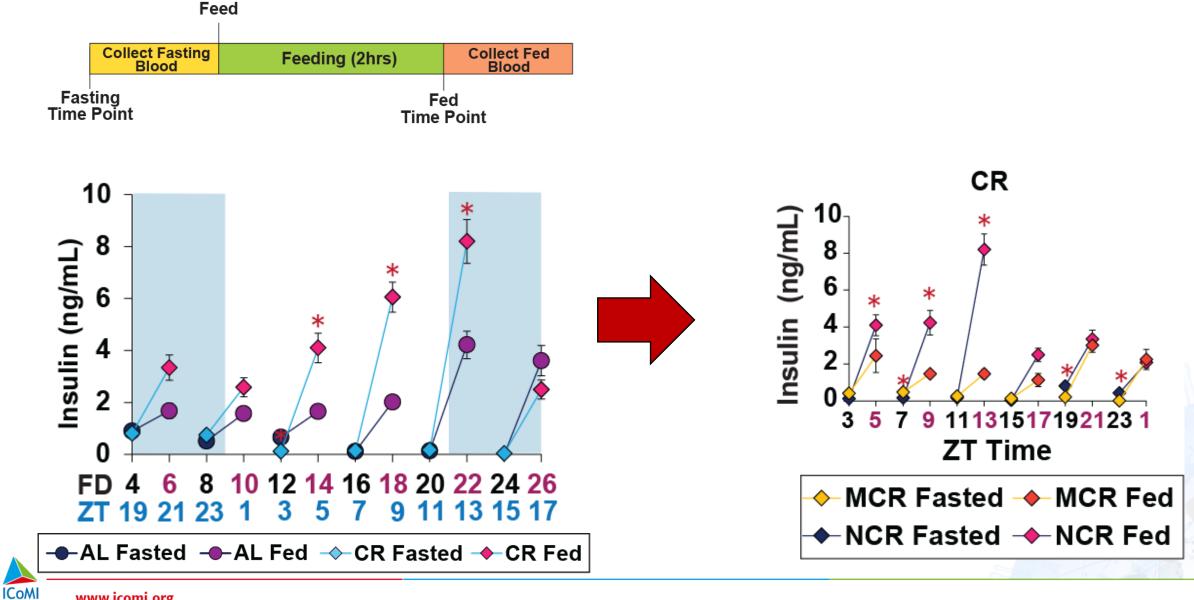
# CR animals have adapted to maintain tight blood glucose levels during a prolonged fast



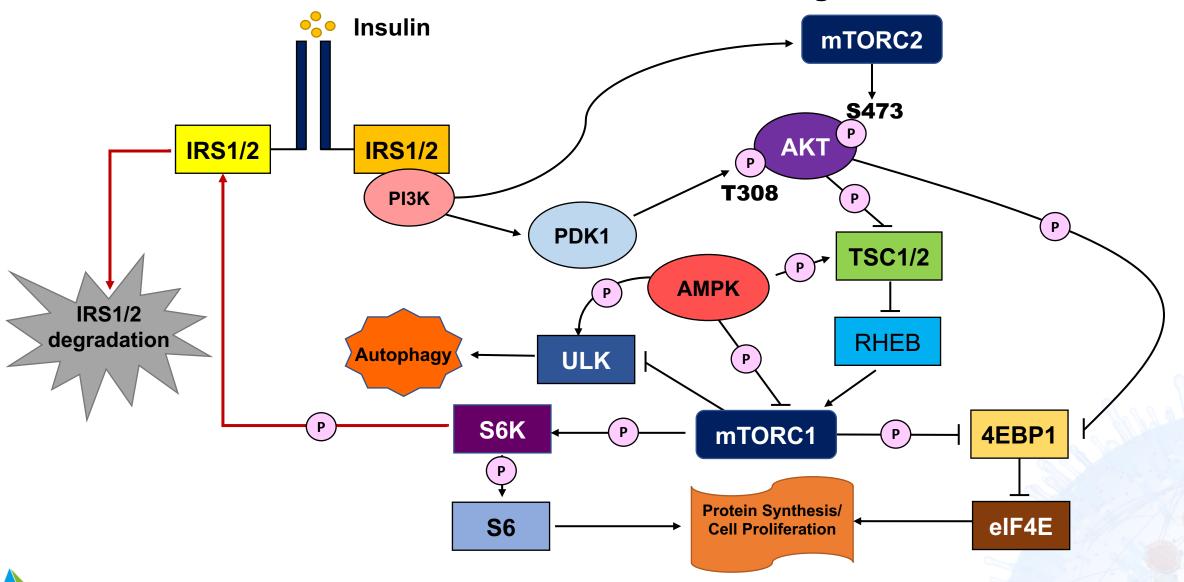
#### Insulin levels are dependent on time of day for AL mice



### Insulin levels are dependent on time of day and time of feeding for CR mice



#### PI3K/AKT/mTOR Pathway



# Genetic/pharmacological inhibition of mTORC1 extends lifespan

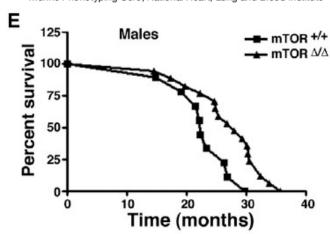
Reports Report

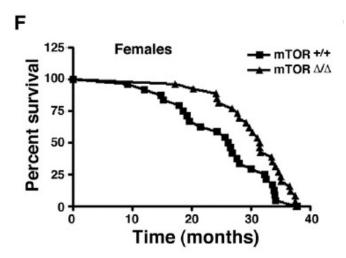


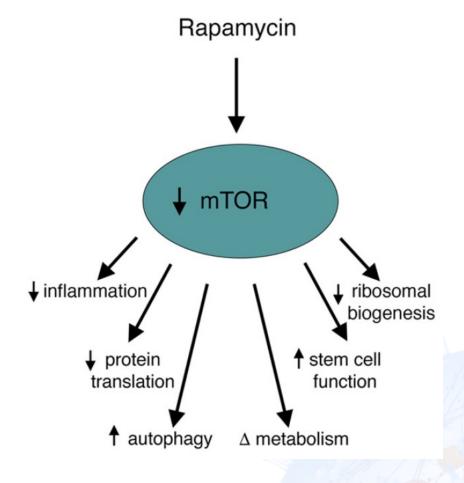
# Increased Mammalian Lifespan and a Segmental and Tissue-Specific Slowing of Aging after Genetic Reduction of mTOR Expression

J. Julie Wu, <sup>1</sup> Jie Liu, <sup>1</sup> Edmund B. Chen, <sup>1</sup> Jennifer J. Wang, <sup>1</sup> Liu Cao, <sup>5</sup> Nisha Narayan, <sup>1</sup> Marie M. Fergusson, <sup>1</sup> Ilsa I. Rovira, <sup>1</sup> Michele Allen, <sup>2</sup> Danielle A. Springer, <sup>2</sup> Cory U. Lago, <sup>1</sup> Shuling Zhang, <sup>3</sup> Wendy DuBois, <sup>3</sup> Theresa Ward, <sup>6</sup> Rafael deCabo, <sup>6</sup> Oksana Gavrilova, <sup>4</sup> Beverly Mock, <sup>3</sup> and Toren Finkel<sup>1,\*</sup>

<sup>&</sup>lt;sup>2</sup>Murine Phenotyping Core, National Heart, Lung and Blood Institute





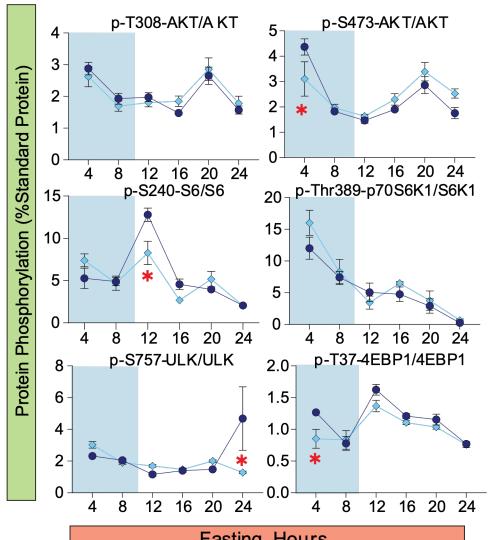


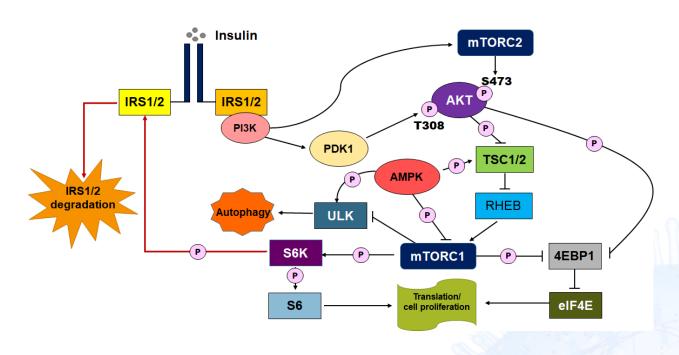
Pan and Finkel, JBC 2017 Review



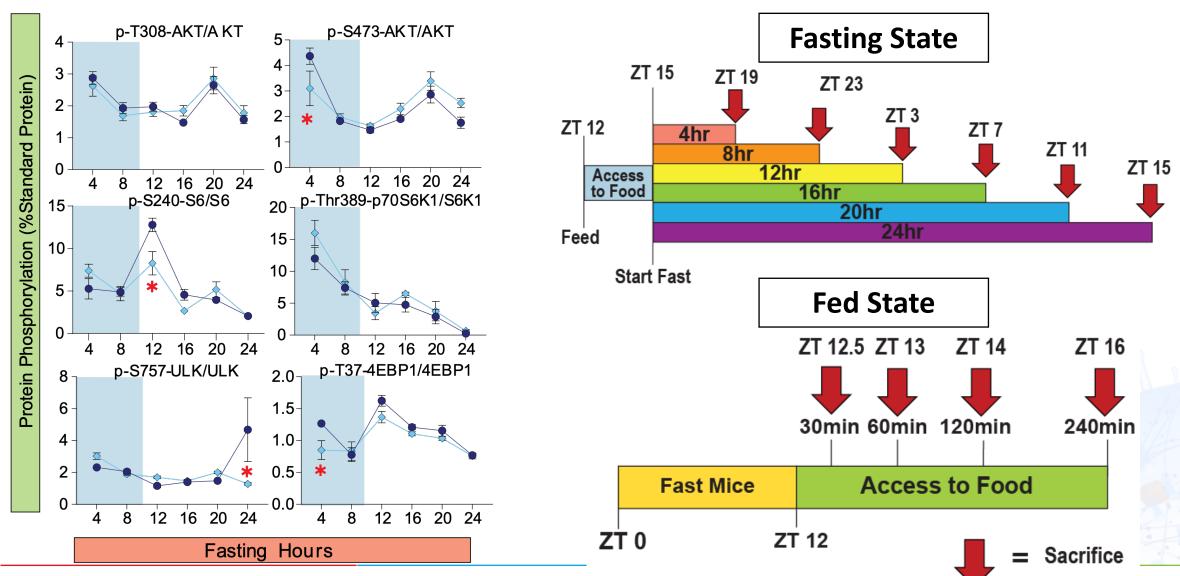
<sup>&</sup>lt;sup>1</sup>Center for Molecular Medicine, National Heart, Lung and Blood Institute

#### Hepatic mTORC1 activity is regulated by the fed/fasted state

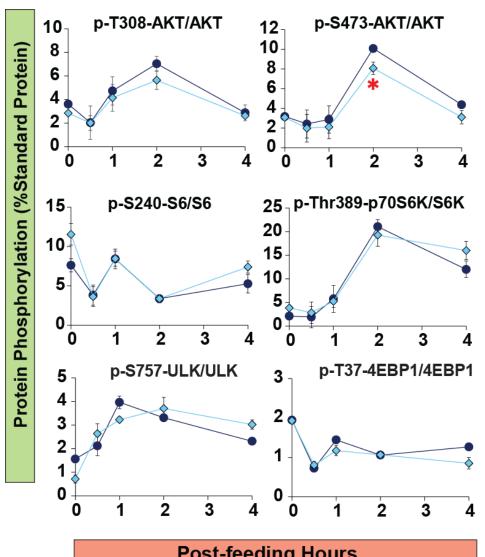


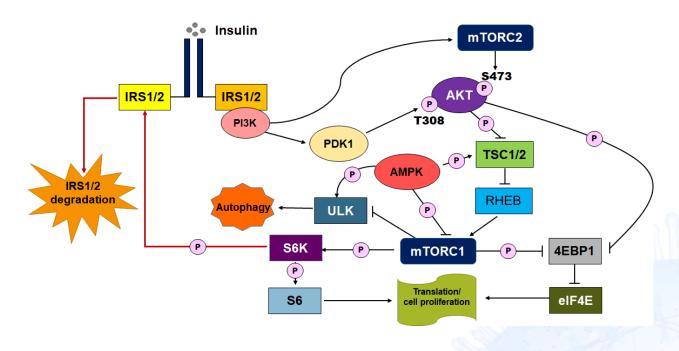


## Hepatic mTORC1 activity is regulated by the fed and fasted state



#### Hepatic mTORC1 activity is regulated by the fed and fasted state







### Summary

#### CR mice maintain tight blood glucose levels during a prolonged fast

- 1. CR mice have improved glucose tolerance compared to AL mice during a 24hr fast
- 2. CR mice have a unique response to insulin at different fasting durations
- 3. CR mice suppress hepatic glucose production during a fast

#### Insulin is required for the tight regulation of glucose level in CR mice

- 1. CR mice do not have improved insulin sensitivity until a 20hr fast
- 2. Insulin is required for the tight regulation of glucose homeostasis in CR mice
- 3. Insulin secretion/clearance is shifted to match that of time of day and feeding schedule

#### mTORC1 activity is mediated by the fed and fasted stated

- 1. AL and CR mice have the same hepatic mTORC1 when feeding status is the same
- 2. Suppression of hepatic mTORC1 activity is not required to elicit the CR effect



#### LAB MEMBERS

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Mariah Calubag
Michelle Sonsalla
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