

# Experimental Methods of Dietary Restriction

# General History of DR

## Pre-1930s

- reduced **tumor** incidence linked to reduced **growth**
- reduced **growth** rate enhances longevity
- low protein diet slows **growth** and extends lifespan of brook trout

## 1930s

- periods of **growth/no growth** enhanced lifespan
- conclusions were that reduced **growth** rate reduced food intake drastically decreases increased LS
- **McCay, et al. (1935)** generally credited with first demonstration that reduced dietary intake increases LS of rodents.

## 1940s – 60s

- a lot of focus on food reduction and **cancer**, other diseases
- protozoan and rotifer LS; a **widely occurring biological phenomenon?**
- decreased body **fat** was leading hypothesis for LS extension
- stronger support for **reduced calories**

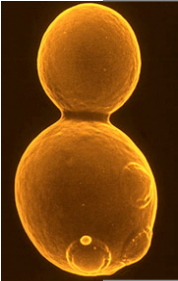
## 1988

- **Weindruch & Walford (1988)** generally credited with demonstration that DR lengthens the life in animals as diverse as worms, flies, rodents, and primates

# What is Dietary Restriction?

“eating fewer calories while maintaining adequate nutrition”

# Commonly used model organisms



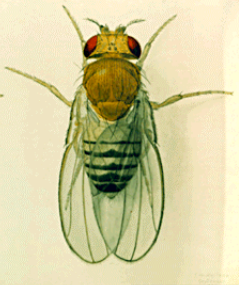
## **yeast (*S. cerevisiae*)**

replicative – number of buds one mother can produce  
chronological – amount of time a cell can remain viable in stationary phase



## **worms (*C. elegans*)**

amount of time the animal remains viable



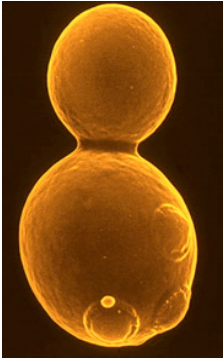
## **fruit flies (*D. melanogaster*)**

amount of time the animal remains viable

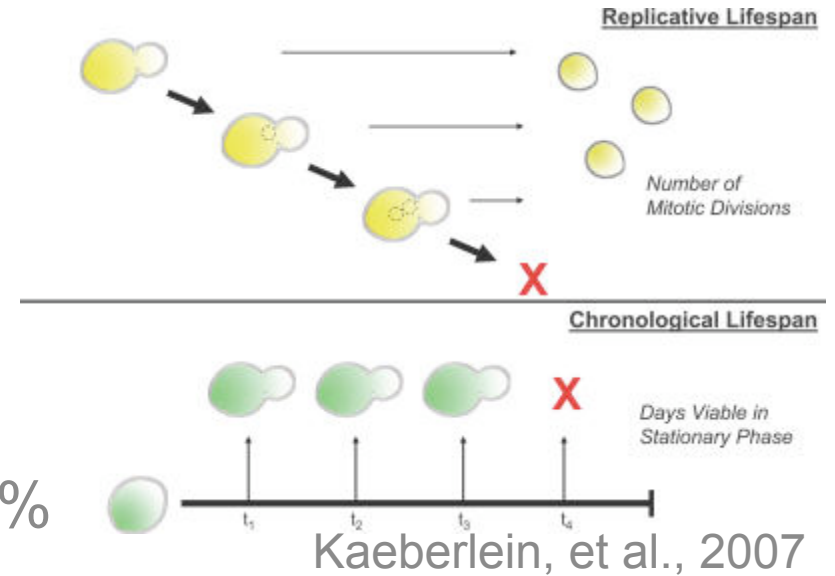


## **rodents**

amount of time the animal remains viable



# Yeast (*S. cerevisiae*)



## RLS

- reduced glucose 2% to 0.5%
- reduced glucose 2% to 0.05%
- reduced amino acids levels in media
- genetic mutations (e.g., *hvk2Δ*, *gpa2Δ*, *sch9Δ*, *tor1Δ*)

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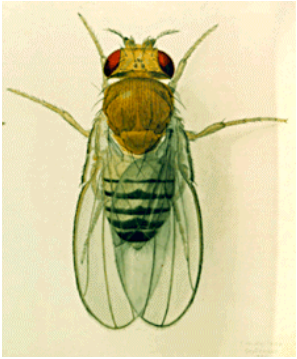
## CLS

- transfer of cells from medium to water
- reduced asparagine or glutamate levels in the media
- genetic mutations (e.g., *cyr1Δ*, *ras2Δ*, *sch9Δ*, *tor1Δ*)



## Worms (*C. elegans*)

- reduced bacterial food (solid medium)
- reduced bacterial food (liquid medium)
- complete removal of food (solid), “bacterial deprivation”
- axenic medium (liquid food removal)
- changing strain of bacterium used
- genetic mutations (e.g., *eat-2*)



## Fruit flies (*D. melanogaster*)

- dilution of agar-gelled food medium (present in excess)
- reduced yeast
- reduced availability of live yeast
- dilution of nutrients in food medium



# Rodents (mice and rats)

## works:

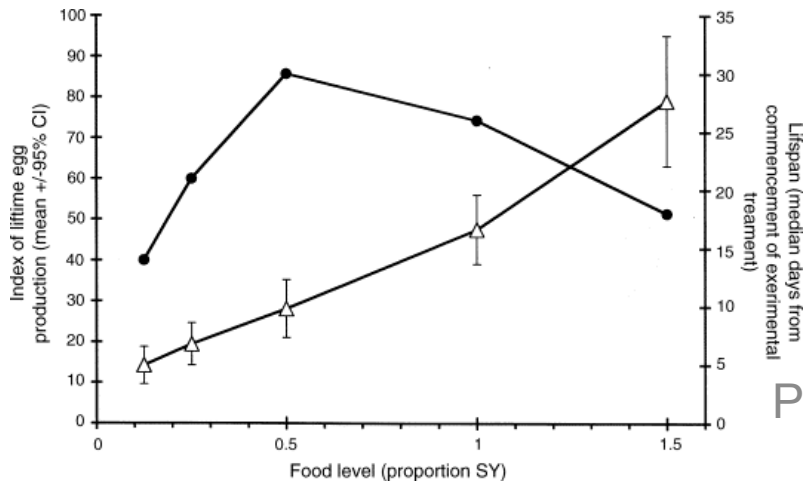
- reduced food
- reduced methionine
- reduced tryptophan
- alternate day feeding

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## doesn't work:

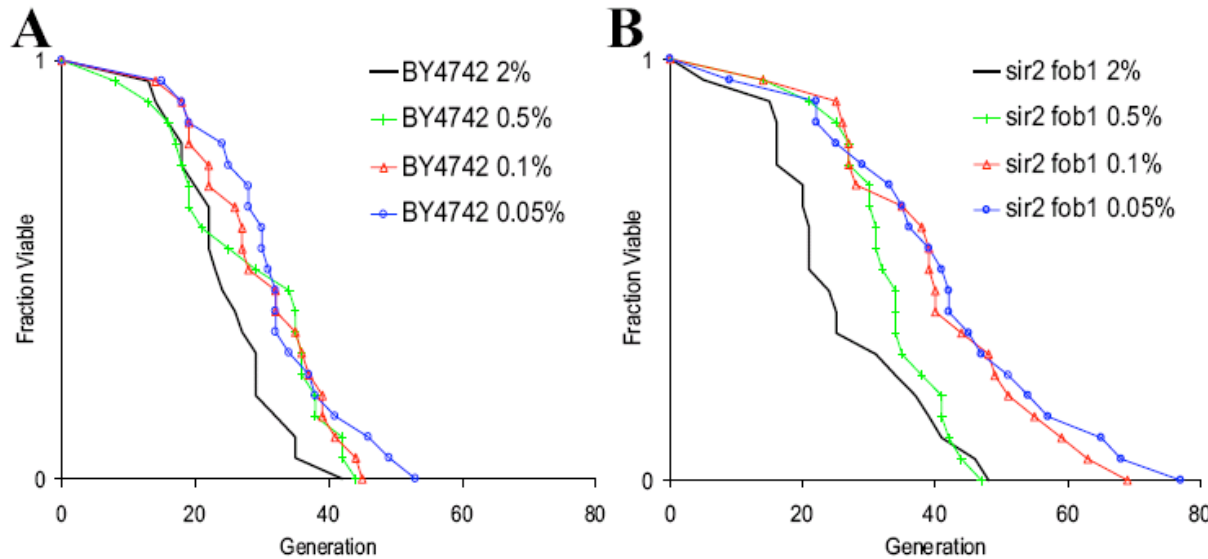
- reduced lipids, minerals, or vitamins
- reduce protein quantity or quality (very minimally works)

# DR protocol variance and resulting LS extension underlies the importance of optimizing DR conditions



Several studies in flies and worms have shown similar dome-shaped response curves

Partridge, et al., 2005



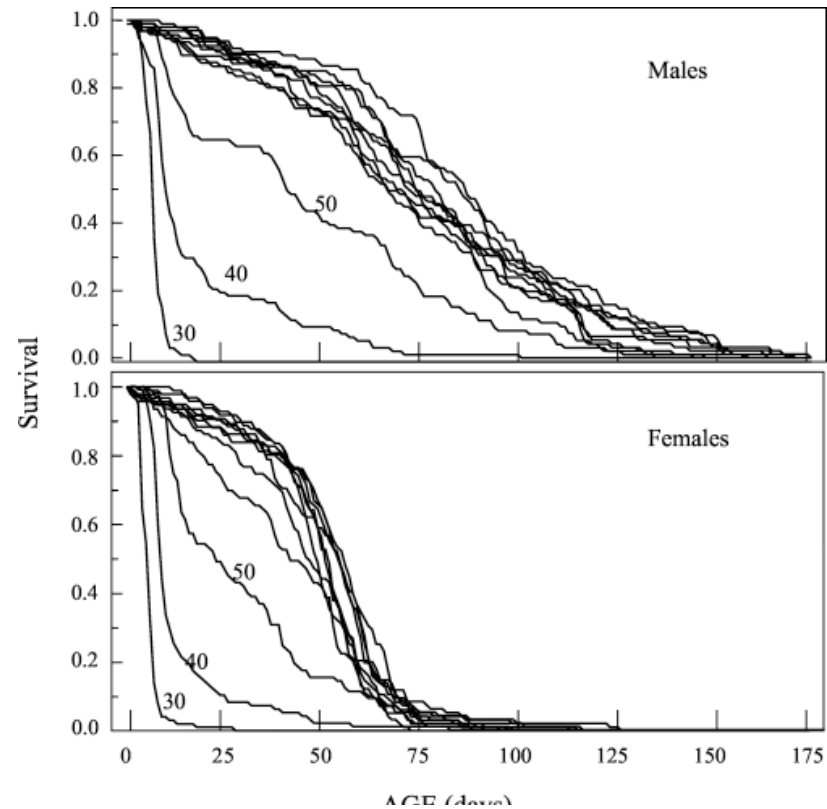
Different strains exhibit varying responses

Kaeberlein, et al., 2004

# If DR 'doesn't work', can we really conclude that it doesn't work?

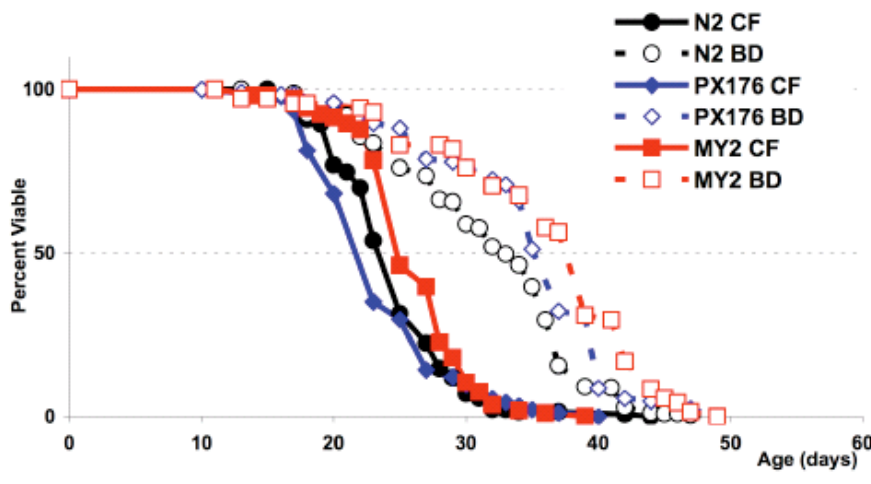
## Carey JR, et al., 2002 : medflies don't respond to DR

- 12 different diets ranging from *ad libitum* to 30% AL, none extended LS
- “diet was provided in a fixed volume of solution that was fully consumed each day, ensuring control of total nutrient consumption”
- flies were obtained from a medfly rearing facility days before hatching

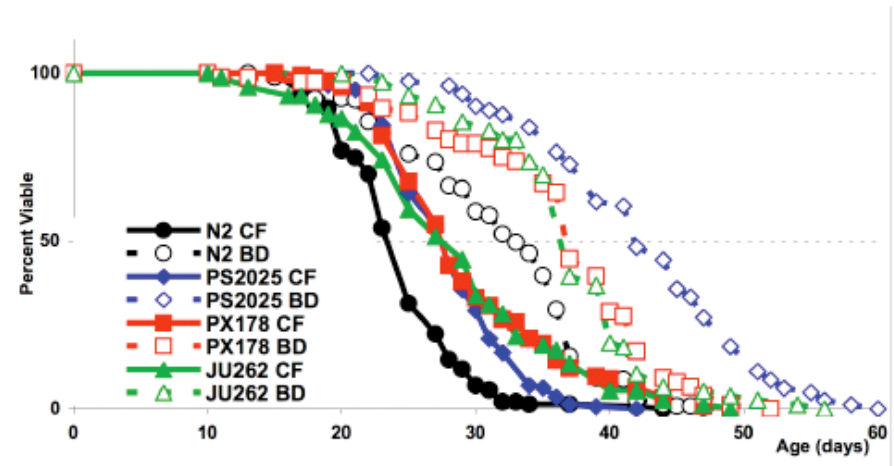


➤ Does DR only work in some organisms?

# Can DR work in wild animals?



- Wild-derived nematodes from Oregon (PX176) and Germany (MY2) respond to DR



- Wild-derived nematodes from California (PS2025), Oregon (PX178), and France (JU262) respond to DR.

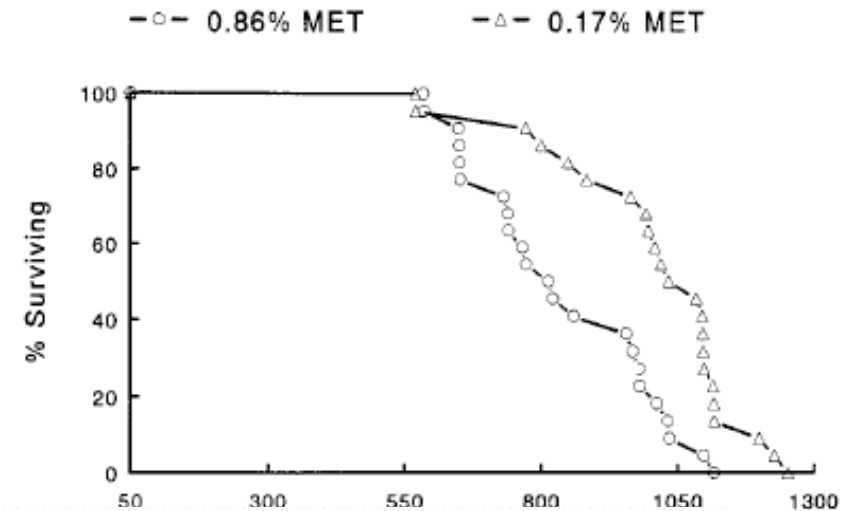
Why do wild worms respond to DR while wild mice do not?

# What about nutritional content?

Reduction of single amino acids in the media can extend lifespan

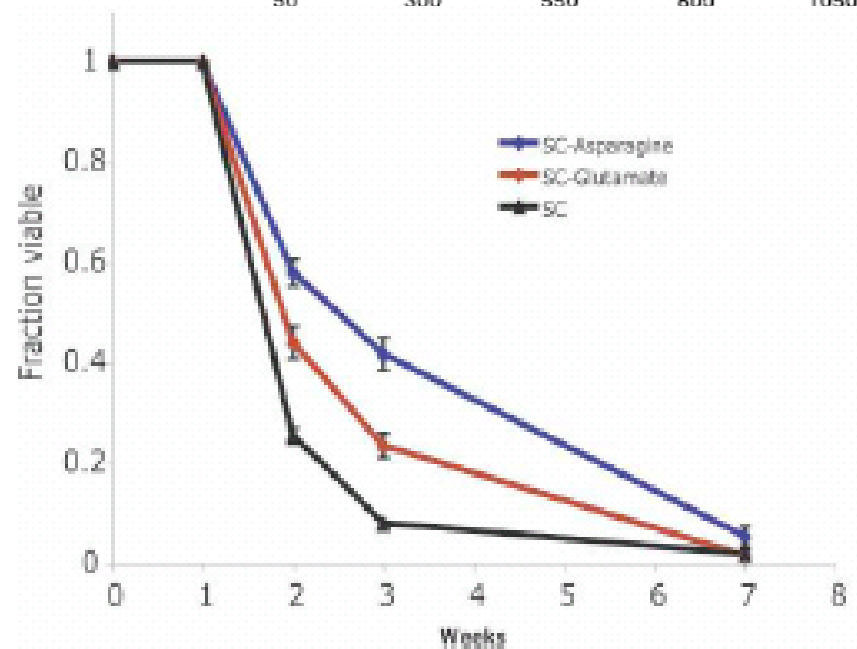
- Methionine restriction extends lifespan in rats

Orentreich, et al., 1993



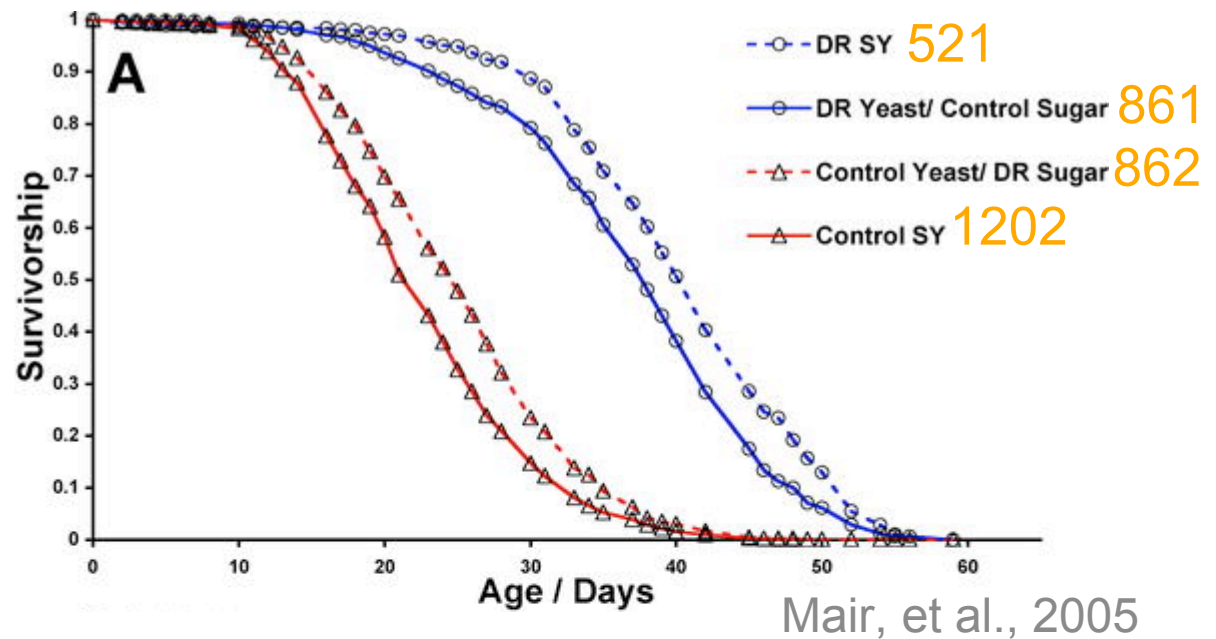
- Asparagine or glutamate restriction extends CLS in yeast

Powers, et al., 2006



# What about nutritional content?

Protein (yeast) has a greater effect on fly lifespan than sugar



## More things to consider:

- What about the timing at which DR is induced?
- What about exercise / activity?
- Do different DR protocols depend on the same molecular mechanisms for lifespan extension?

# Will DR work in humans?

“There are almost as many paths to Calorie Restriction as there are people practicing it...CR does not have rules and does not impute judgment to any particular diet or way of eating.” –  
calorierestriction.org

Common forms of human CR:

- Skip a meal every day
- Periodic fasting
- Many small meals
- Limit certain foods, unlimited amounts of others

To participate

-[http://calorierestriction.org/Human\\_CR\\_Study](http://calorierestriction.org/Human_CR_Study)

To donate

-<http://calorierestriction.org/ResearchOnAging>