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 – 1960s**
  - 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)

- reduced glucose 2% to 0.5%
- reduced glucose 2% to 0.05%
- reduced amino acids levels in media
- genetic mutations (e.g., hxk2Δ, gpa2Δ, sch9Δ, tor1Δ)

RLS

- transfer of cells from medium to water

CLS

- reduced asparagine or glutamate levels in the media
- genetic mutations (e.g., cyr1Δ, ras2Δ, sch9Δ, tor1Δ)

Kaeberlein, et al., 2007
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

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?

Sutphin and Kaeberlein, 2007
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?

Mair, et al., 2005
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