Caloric restriction: mechanisms

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CR extends lifespan in every animal tested

Species	Mean lifespan	Max. lifespan	CR mean ls.	CR max. ls
Rat	23 months	33 months	33 months	47 months
Guppy	33 months	54 months	46 months	59 months
Bowl and doily spider	50 days	100 days	90 days	139 days
Protozoan	7 days	14 days	13 days	25 days
Yeast	21 generations	40 generations	26 generations	49 generations
Fly	25 days	47 days	46 days	78 days

CR phenotype

•Body temperature lower in mice but not in rats.

•If extreme CR started in juveniles, get reduced rate of reproduction in rats, cessation of reproduction in mice.

•Metabolic rate per cell falls initially, then recovers (More efficient use of oxygen?).

Is reduction in body fat critical for CR

- Typical lab mouse and rat strains become very lean on CR.
- Experiments using other lab strains including obese strains:
  - Leanness doesn't correlate with lifespan extension in mice/rats on CR.
  - Obese strains have a shorter lifespan. On a CR diet, they remain obese, but have a similar lifespan extension to standard strains.

Body fat reduction/leanness is NOT critical for CR.

CR phenotype

- Maintain youthful activity levels longer.
- Maintain immune function longer.
- Better performance in memory tests (water maze), retain memory abilities longer.
- Fewer tumors.
- More resistant to carcinogens.

- Lower mean blood glucose.

Primate NIA experiment

Findings in NIA Primate CR Study	Matches Rodent Data
(-) Body weight	Yes
(-) Fat and lean mass	Yes
(-) Time to sexual maturation	Yes
(-) Time to skeletal maturation	Yes
(-) Fasting glucose/insulin	Yes
(-) Metabolic rate (short-term)	Yes
(*) Metabolic rate (long-term)	Yes
(-) Body temperature	Yes
(*) or (+) Locomotion	Yes
(-) Triglycerides	Yes
(+) IGF-1/growth hormone	Yes
(-) Il-6	Yes
(*) Wound closure rate	Yes
(*) Clonal proliferation	Yes/?
(*) B-gal senescent cells	?
(-) Lymphocyte number	Yes
(*) Lymphocyte calcium response	No

NIA

(-) = decrease
(+) = increase
(*) = no change

Lane et al., 1999

Important characteristics of calorie restricted animals

- Maintenance of mitochondrial energy production
- Maintenance of a better daily balance of insulin and growth hormone that mirrors shifts in glucose vs fatty acid usage.
- Elevated sensitivity to hormonal stimulation, especially to insulin.
- Higher protein synthetic rates especially in old age
  - Ad Lib fed animals have a 40-70% decline over youthful levels

CR retards physiological effects of aging

- DNA repair rates decline with age.
  - CR retards this decline.
    - Mouse splenocytes (Licastro et al., 1988)
    - Mouse fibroblasts (Weraarchakul et al., 1989)
- CR effects particular types of DNA repair.
  - Regional differences seen in rat brain.

CR retards physiological effects of aging

DNA damage is reduced:

- Studies of damage at the HPRT locus show reduced damage in CR mice (Dempsey et al., 1993)

Mitochondria:

- DR started in middle age rats decreases mitochondrial deletions and muscle fiber loss (Aspnes et al., 1997)

CR and apoptosis

- CR promotes apoptosis in experiments on:
  - liver of old mice (Muskhelishvilli et al., 1995)
  - Small intestine and colon of rats (Holt et al., 1998)
- Apoptosis rate increased in pre-neoplastic cells in CR rats.

CR and protein damage

- Protein degradation declines with age
- Studies in rat liver show CR retards this decline (Ward, 1998).
- Not due to changes in proteome protein levels or activity.

Less oxidative damage in CR animals.

- Collagen crosslinks form slower (less AGEs).
- Lower rates of lipid peroxidation (free radical damage of lipids),
  - Indicated by lower levels of exhaled ethane and pentane (Matsuo et al., 1993)
- Oxidative damage to proteins reduced.
  - Lower levels of carbonylated proteins.
  - Age-associated loss of sulfhydryl groups reduced.

CR decreases mitochondrial free radical generation

- Rate of superoxide radicals and hydrogen peroxide in mitochondria reduced.
  - Brain, kidney, and heart of mice (Sohal and Dubey, 1994)

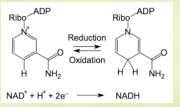
CR decreases free radical generation

- Plasma insulin levels were significantly lower in CR than in control rats.
- Hydrogen peroxide production rate significantly lower in CR (0.25 nmol/min/mg) than in fully-fed rats (0.60 nmol/min/mg)
- Decrease in hydrogen peroxide production rate was partially reversed (0.40 nmol/min/mg) by 2 weeks of 0.55 microL/hr insulin treatment of CR rats.

Mitochondria are central to CR's effects!

- Primary?
  - Effects of CR due to direct effects on mitochondrial activity or function.
- Or secondary?
  - Effects of CR coordinated by mitochondria.

Evidence from yeast



Glucose restricted yeast long-lived.

Pathway:

1. CR triggers switch from glycolysis to respiration (mitochondrial activity increased).
2. Less glycolysis -> more free NAD.
3. High NAD -> SIR2 is activated -> longevity.

CR doesn't activate known oxidative stress genes in yeast.

NAD=Nicotinamide adenine dinucleotide
SIR2 = yeast protein 'Silent information regulator 2'

Signaling from mitochondria to nuclear genome in yeast

Retrograde signaling from mitochondria to nucleus:

- Expression of nuclear genes RTG1, RTG2 depends on state of activity in mitochondria.
- Rtg1/Rtg2 complex with Rtg3 to form a transcription factor.

- Yeast without mitochondria live longer.
- This depends on RTG2 and RAS2 (another signaling gene).
- RTG2 activity depends on glutamate (produced by the Krebs cycle in mitochondria.
- The Rtg2 transcription factor controls mitochondrial and cytoplasmic genes.

Mitochondrial activity and CoQ

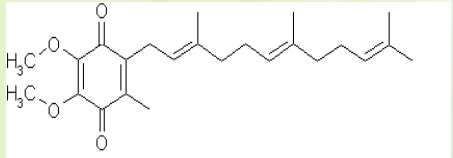
- Coenzyme Q is a carrier of electrons in the mitochondrial Electron Transport Chain.
- Electron transport in complexes I & III create a proton gradient across the inner membrane.
- This is coupled to the synthesis of ATP by complex V (Fo/F1 ATPase).

CoQ functions:

- antioxidant (scavenges electrons)
- prooxidant (generates superoxide)
- a redox-active component of plasma-membrane electron transport
- uridine synthesis
- a cofactor for proton-pumping activity in uncoupling proteins in mitochondria.

Q6, Q7, Q8, Q9, and Q10

- Coenzyme Q can have a variable length side chain, with typically 6 to 10 subunits, hence Q6, Q7, Q8, Q9, and Q10.
- Different species tend to produce Q with a particular length side chain
  - Q10 in human
  - Q9 in worm
  - Q8 in bacteria



Mitochondria and CR in worms

- *clk-1* mutants in worms lack endogenous Q9
  - relies instead on Q8 from bacterial diet.
- *clk-1* mutants live twice as long as wildtype worms.
- The missing *clk-1* gene encodes a di-iron carbolxylate enzyme:
  - Responsible for penultimate step in CoQ synthesis

Experiments in *C. elegans*

- Wild worms switched to Q-less diet during larval stage 4
  - To avoids developmental interference.
- Wildtype lifespan extended 59%.
- Lack of Q8 extends lifespan.

CR does not depend on the insulin-like signaling pathway

- Suppression tests were performed on the Age phenotype with *daf-16.*
- On a Q-replete diet, *daf-16* mutants live (slightly) shorter than wildtype.
- On a Q-less diet they live longer than wildtype.
- The lifespan extension produced by the Q-less diet is independent of *daf-16* and the insulin-like signaling pathway.
- *daf-2/clk-1* worms have a lifespan 5X (500%) of wild type worms (Lakowski and Hekimi, 1996), longer than either single mutation.
  - the effects of *clk-1* and the insulin-like signaling pathway are additive.

CR does not depend on the insulin-like signaling pathway

- Worms can be caloric restricted by reduced feeding or by mutations that reduce feeding such as *eat-2*, a mutation that reduces pharyngeal pumping.
- CR worms are long-lived (+29% to +153% of wildtype).
- Extent of lifespan extension depends on severity of the CR.
- *daf-2/eat-2* worms have a lifespan much longer than *daf-2* worms.
- Reduced feeding (CR) extends lifespan of *daf-2* worms.

CR acts through the same pathway as *clk-1* and a low CoQ diet

- Combining CR with *clk-1* or a low CoQ diet produces worms with no addition lifespan extension beyond the that found in the conditions separately.
- **This is evidence that reduced mitochondrial activity is part of the CR mechanism in worms.**

CoQ pathway mutants are long-lived.

- Using RNAi to knock down gene activity, 8 genes were identified that participate in Q9 biosynthesis in worms.
- RNA interference (RNAi) of Q9 biosynthesis genes extends lifespan.
- Worms treated with RNAi produce less superoxide anions (30-50% less).

Many mitochondrial mutants extend lifespan in *C. elegans*

- Genomic RNAi gene activity knock down screens identified many mitochondrial mutants that extend lifespan:
  - Complex I, II, III, and IV mutants.
- Not all mitochondrial mutants extend lifespan.
  - Some, like *mev-1* (ETC complex II), increase free radical production and shorten lifespan.

Mitochondrial Electron Transport Chain