Caloric Restriction and Longevity
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People are living longer

In 2005 it was estimated that almost 500 million people are age $\geq$65, or 8% of the worldwide population.

By 2030 the world’s older population is estimated to be more than 1 billion or 13% of the total population.
Individuals of the Same Chronological Age can Appear to be of very Different Biological Age

But is this appearance a real biological phenomenon or merely superficial?
What is Aging?

- **Primary Aging** is the inevitable deterioration of cells and tissues structure and function that occur independent of disease and lifestyle
  - Determines maximal lifespan

- **Secondary Aging** is the decline in tissue structure and function that occurs as a result of external influences including non-communicable diseases and social behaviors such as smoking and physical activity habits
  - Determines mean lifespan
What is calorie restriction (CR)?

• CR is a diet that provides a reduced number of calories but maintains proper nutrition.

• CR is the only intervention known to date that consistently decreases the biological rate of aging and increases both average and maximal lifespan.

• The ability of CR to extend lifespan was first reported by Dr. Clive McCay in 1935. Observed in all other animal species studied to date including primates.

• The effects of CR in humans is not yet known.
Caloric Restriction, not Exercise, increases maximum lifespan
What is known in humans?

1. Okinawa, Japan

Has the highest number of living centenarians in the world. Life expectancy at birth is highest in Okinawa compared to its close neighbor Japan and other Westernized Countries.

Caloric restriction and human longevity: what can we learn from the Okinawans?

D. Craig Willcox · Bradley J. Willcox · Hidemi Todoriki · J. David Curb · Makoto Suzuki

Studies estimate the energy intake of Okinawans to be ~85% (15% CR) of their daily energy balance requirement.
2. Results from Biosphere 2

- The approximate 750 calorie per day deficit resulted in an average weight loss of 15%.

- The weight loss was associated with many beneficial physiological, hematological, biochemical and metabolic alterations consistent with CR in rodents and primates.
3. Self prescribed calorie restriction

The calorie restriction society is a group of self-selected individuals who engage CR. Studies have been performed on members in comparison to age- and BMI-matched controls (often runners).

Table 1. Body Composition

<table>
<thead>
<tr>
<th></th>
<th>CRONies</th>
<th>Age, and BMI-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 1</td>
<td>50 ± 1</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>60 ± 6</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>20 ± 2</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>7 ± 4</td>
<td>22 ± 7</td>
</tr>
</tbody>
</table>

Table 2. Dietary Intake Analysis

<table>
<thead>
<tr>
<th></th>
<th>CRONies</th>
<th>Age, and BMI-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Intake (calories per day)</td>
<td>1,112 - 1,958</td>
<td>1,976 - 3,537</td>
</tr>
<tr>
<td>% Fat</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>% Protein</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>% Carbohydrate</td>
<td>46</td>
<td>50</td>
</tr>
</tbody>
</table>

CR for 3-15 yr reduces risk factors for atherosclerosis.

The average serum total and LDL cholesterol concentrations is in the lowest 10% for people in their age group. Triglyceride levels, are similar to the 5th-%ile in 20-year-olds.

Blood pressure in the CR group was remarkably low, with values in the range found in 10-year olds.
Title = caloric restriction, calorie restriction, diet restriction, dietary restriction, energy restriction, food restriction
CR and Aging in Rhesus Monkeys

There are currently 3 colonies of primates undergoing CR
• University of Wisconsin (Dr Weindruch)
• National Institute of Aging, Baltimore
• Baltimore, Florida; Dr B. Hansen

**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 demeanour. His bloodwork shows he is as healthy as he looks.

**Human equivalent** Meals prepared by Mike Linksweyer, 36

**MONKEY MENU**
- **Daily calories**
  - 445

Monkeys also receive an apple each day.

**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 38**

**HUMAN MENU**
- **Daily calories**
  - 2,000

Beverages, snacks and desserts not shown. Diet varies according to body type, sex and activity level.
The University of Wisconsin Primate Study (Science, July 2009)

1. Effects on disease development

- Neoplasia
- Cardiovascular disease
- Glucoregulatory impairment

2. Effects on lifespan
LETTER

Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study

Julie A. Mattison¹, George S. Roth², T. Mark Beasley³, Edward M. Tilmont¹, April M. Handy¹,⁴, Richard L. Herbert⁵, Dan L. Longo⁶, David B. Allison⁷, Jennifer E. Young¹, Mark Bryant⁸, Dennis Barnard⁹, Walter F. Ward¹⁰, Wenbo Qi¹¹, Donald K. Ingram¹² & Rafael de Cabo¹³
Mixed results for dieting monkeys

According to previous studies, a low-calorie diet provides health benefits and increases lifespan in mammals, including primates. Yet a long-term investigation in rhesus monkeys finds no effect on longevity.
Supplementary Figure 1a, b. Kaplan-Meier Survival Curves for a) All-Cause and b) Age-Related Mortality for all (young- and old-onset) Monkeys. Both were analyzed using Cox regression with age of onset, age group, sex, and diet as predictors. a) Sex was the only significant predictor (p = 0.009), open circles represent monkeys that are still alive, b) none of the factors were statistically significant.
Onset of Age-Related Diseases

Cardiovascular disease, cancer, diabetes, arthritis, diverticulosis, amyloidosis

Nature, 2012
Age-Related Disease Incidence

Neoplasia
Control
CR

Diabetes
Control
CR

Cardiovascular Disease
Control
CR

Y = Young-onset
O = Old-onset

Nature, 2012
Primary Differences in CR Study Design

NIA compared to UW Study

1. Diet Composition
   - Protein source
   - Fat source
   - Vitamin and mineral supplementation
   - Sugar content

2. Feeding regimen
   - Twice daily vs. once
   - Controlled allotment vs. ad libitum

3. Medical care of the animals

4. Genetic origin—Chinese vs. Indian

5. Age of onset
Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy

**Aim:** determine whether calorie restriction causes a metabolic adaptation (decrease in energy expenditure larger than expected on the basis of weight loss) associated to reduced oxidative damage.
### Subjects & Methods

<table>
<thead>
<tr>
<th>Control</th>
<th>CR</th>
<th>CR+EX</th>
<th>LCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, Male/Female</td>
<td>5/7</td>
<td>6/6</td>
<td>5/7</td>
</tr>
<tr>
<td>Age, year</td>
<td>38 ± 8</td>
<td>39 ± 5</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.8 ± 9.3</td>
<td>80.9 ± 11.4</td>
<td>81.9 ± 10.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.6 ± 2.0</td>
<td>27.8 ± 1.4</td>
<td>27.5 ± 1.6</td>
</tr>
</tbody>
</table>

#### Randomization

- **5 wks Baseline (BL)**
- **TDEE1**
- **TDEE2**

#### Healthy Diet Control (Control)

- **25% CR (CR)**
- **12.5% CR + 12.5% EX (CR+EX)**
- **15% Wt. loss + Maintenance (LCD)**

#### Food Provided

- **M3**
- **Food at Home**
- **M6**

#### Measured Parameters

- **Body composition (DXA)**
- **Fat distribution (CT)**
- **Insulin Sensitivity (FSIGTT)**
- **VO₂ max & strength**
- **Biopsy’s (muscle & fat)**
- **TDEE (doubly labeled water)**
- **24h energy expenditure (chamber)**
- **24h core temperature**
- **Neuroendocrine function (24h blood sampling for GH & leptin)**
- **Fasting blood**
- **Psychological & Cognitive function**
Reduced body weight, FM and FFM

Body Mass Changes

Weeks

Weight change (kg)

Control

LCD

CR+EX

CR

Changes at week 24

Redman et al, JCEM, 2007
Rate of Living and Oxidative Stress Theory of Longevity

- **Hypothesis:** Free radicals produced during aerobic respiration cause cumulative oxidative damage resulting in aging (Harmon, 1957)

- Longevity among species inversely correlates with generation of $O_2^-$ and $H_2O_2$

- CR reduces ROS production

Potential Mechanism for anti-aging effects of CR
Methods for Metabolic Adaptation and Oxidative Stress

- **Metabolic rate was measured in a metabolic chamber for 23-h**
  - 24-h EE
  - Sleeping EE
  - Fat and CHO oxidation

**Oxidative damage:**
- Urinary Isoprostanes
- Protein Carbonyls
- Comet Assay
Sedentary Energy Expenditure is decreased by CR beyond that predicted by changes in body composition.

Similar results for sleeping metabolic rate.

Controls -22kcal/d, p=NS
CR -140kcal/d, p=0.002
CREX -121kcal/d, p=0.008
LCD -130kcal/d, p=0.006

24h-EE (kcal/d) = 596 + 26.8 * FFM, r² = 0.86, p<0.001

Heilbronn et al, JAMA, 2006
#1: CR decreases core body temperature

![Graph showing changes in core temperature over day and night with different mean, minimum, and maximum values for M0 and M6 groups.](graph.png)
DNA Fragmentation is decreased by CR

* Significantly different from baseline

Heilbronn et al, JAMA, 2006
Determinants of the Metabolic Adaptation

CR \downarrow \text{leptin} \downarrow \text{Gonadal axis} \downarrow \text{SNS} \downarrow \text{Thyroid hormones} \downarrow \text{Energy metabolism} = \text{metabolic adaptation}

Adapted from Leibel, 2002
Changes in Leptin Mesor Correlate with the Metabolic Adaptation Induced by CR

$L^2 = 0.14, p<0.001$

Lecoultre et al; JCEM 2011
**Vastus Lateralis Needle biopsy**

**Bergstrom Technique**

- **Muscle lipid metabolites content** (LC-MS, GC-MS)
  - 25 mg
- **Enzyme activities** (HPLC)
  - 50 mg
- **Gene and Protein expression** (PCR, Chips, WB)
  - 25 mg
- **Fiber typing** (IHC)
  - 25 mg
- **Lipid and glucose oxidation assay**
  - 50 mg
- **Primary culture of myoblasts**
  - 50 mg

**225 mg total**
Effects of Caloric Restriction on Skeletal Muscle Mitochondrial Biogenesis

Caloric restriction increases the nutrient sensors AMPK and SIRT1 mRNA

Civitarese et al, Plos Med 2007
Caloric restriction increases genes involved in the regulation of mitochondrial biogenesis

* Different from baseline within group

Civitarese et al, Plos Med 2007
Caloric restriction induces mitochondrial biogenesis in human skeletal muscle

**Graph:**
- **Y-axis:** % Change from baseline (mitDNA copy number normalized for nuclear DNA)
- **X-axis:** Control, CR, CREX
- **Legend:**
  - **Control:** Bar graph showing a slight increase.
  - **CR:** Bar graph showing a significant increase with a p-value of <0.05.
  - **CREX:** Bar graph showing a moderate increase.

*Civilarese et al, Plos Med 2007*
Methods: Culture of Human Primary Muscle Cells

1. Needle biopsy
2. Vastus Lateralis
3. Mixed cell population
4. Isolation
5. Amplification
6. Immunopurification
   - Anti-CD56 antibody (5.1H11)
7. Cell Sorting
8. Day 5 differentiated myotubes (20x)
9. Satellite cells
10. Differentiation
Globular Adiponectin Induces Mitochondrial Biogenesis in Primary Human Myotubes \( (n=5) \)

### mRNA/RPLPO mRNA

<table>
<thead>
<tr>
<th>Dose</th>
<th>COX II</th>
<th>mtDNA copy number normalized per nuclear genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="#" alt="Image" /></td>
<td><img src="#" alt="Image" /></td>
</tr>
<tr>
<td>0.25 µg</td>
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<td><img src="#" alt="Image" /></td>
</tr>
<tr>
<td>0.5 µg</td>
<td><img src="#" alt="Image" /></td>
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*\( p<0.05 \) compared to control

### COX II Protein Content

- Control
- 0.25 µg gAd

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Civitarese et al. Cell Metabolism 2006
Adiponectin Lowers Reactive Oxygen Species

![Graphs showing comparison of membrane potential (ΔΨm), superoxide indicator (MitoSoX Red), hydrogen peroxide production, and SOD activity.]

- Membrane ΔΨm
- Superoxide Indicator (MitoSoX Red)
- Hydrogen Peroxide
- SOD Activity

Civitarese et al. Cell Metabolism 2006
Mitochondria—A Nexus for Aging, Calorie Restriction, and Sirtuins?

Leonard Guarente1,*

Cell 132, January 25, 2008 © Elsevier Inc.

- Buffer to Aging
- Regulation of Sirtuins
- Regulation of ROS
- Reprogramming to Fat Oxidation
- Recycling of Damaged Mitochondria
Conclusions

Improved mitochondrial bioenergetics via SIRT1 and increased adiponectin signaling may partially explain the effect of CR on:

- insulin sensitivity in skeletal muscle
- metabolic adaptation (better P/O ratio)
- decreased oxidative stress to DNA
Acknowledgements

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Multi-site Study

Sponsor
National Institute on Aging

Clinical Sites

Tufts University
PI: Susan Roberts

PI: Eric Ravussin

Washington University in St. Louis
PI: John Holloszy

Coordinating Center
Duke University
PI: Jim Rochon
Calorie Restriction in Humans: Lessons from Mice and Rats

1) How much calorie restriction?  
The more the better

2) When should calorie restriction start?  
The earlier the better

3) Does long-term restriction cause adaptation with decreased hunger?  
Not sure!!!

Eric and Jacqueline Ravussin on July 4, 1975

CR of 15% for next 53 years

Benefit: 4.7 years
35 years later?

30% CR for 18 years

Benefit?

Two Months!
I prefer that…

… or take resveratrol possibly in a wine bottle
Calorically Restricted Resveratrol Metabolism in Humans

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A toast to Metabolic Health

A 30 day Resveratrol supplementation improves metabolic health in obese men

Cell Metabolism 2011
Calorie Restriction Mimetic Formula includes resveratrol, pterostilbene, quercetin, grape seed polyphenols, and black tea extract to provide even broader spectrum gene expression support in one nutritional compound. These nutrients have been shown to generate many of the same effects in the body as caloric restriction, without significant dietary modification. In particular, they help mimic caloric restriction’s favorable impact on genes that influence the aging process.