Aging and Life-prolonging interventions
Lecture 4

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T. Prolla and R. Weindruch

Aging and Caloric restriction

- Reduction in Caloric Intake without malnutrition
- Characteristics
  - Increases mean and maximum life span
  - Sixty years ago scientist at Cornell University made the extraordinary discovery - McCay
  - Reduces tumors, renal disease, cardiomyopathy

CR is effective starting at mid-age
Caloric Restriction Increases Both Average and Maximal Life Span In Experimental Animals

- **White Rat**
  - On a normal diet, average life span is 23 months with a max of 33 months
  - When intake is restricted (by about 1/3), average life span is 33 months and maximal life span is 47 months

Adapted from Hursting et al, Annual Reviews of Medicine, 54:131-152, 2003.
• Relationship has also been demonstrated with: spiders, insects, fish, and even protozoa.
• Monkey Studies are ongoing

(from Weindruch, R. Scientific American, Jan. 1996)

Summary 1
Caloric Restriction (CR) and Aging
CR (30 - 50% ↓ caloric intake w/o malnutrition) is the only intervention shown in mammals to extend maximum lifespan and retard the development of a broad spectrum of age-associated pathophysiological changes. Three topics are being actively studied:

Mechanisms by which CR retards aging in rodents
Effects of CR on aging and diseases in primates
Development of CR mimetics (e.g., nutraceuticals)

Animals = Caloric Restriction increases maximum life span, it is thought to slow the aging process itself
Monkeys = effects on “health” parameters, but we may predict that it will not effect longevity per se (MLSP), but only MLP.
Humans = Unclear if it would work. Specifically in this “lazy-convenient” and “toxic-food” western society we live in.
And/or Simply due to stringent-complex evolutionary genetic programs ingrained for millions of years, which make it impossible to alter maximum life-span. In other words, any alteration made to a human cell will likely have an negative impact on MLSP.

Restriction of one Macronutrient
• Restriction of one macronutrient (fat, carbohydrate or protein) without a reduction in caloric intake does not increase maximal life span. Caloric intake is the key.
• Total calories same in Ad lib vs. ONE nutrient restricted. Studies showed no difference in MLSP
• Still more studies are needed to come to firmer conclusions

Degree of adiposity does not seem to be important
When the bodyweight of genetically obese mice is kept normal by caloric restriction, maximal life span increases by 50%.
– They live longer than genetically normal control mice despite the fact their body fat level is over twice as high (48% vs. 22%).
– They live about as long as calorie restricted normal mice (who have only 13% body fat). From Harrison et al. 1984.
– In other words, when comparing mice with 48% body fat (after CR) there was no difference in maximum live span as compared to mice with 25% and 13% fat.
Caloric restriction, not bodyweight reduction, is the key to improved longevity

- Rats that had only a mildly restricted intake (92% of controls) but that were kept lean with exercise (weighing 40% less than controls) had an increase in average life span but not maximal life span (Holloszy 1997) compared to sedentary.
  - 8% CR (-40% BW mostly fat) had similar MLSP as control rats.

General confounding factor with CR

- Animals may become more active when caloric restricted.
  - This has been observed in caloric restricted animals, they may be "searching for food".
  - More active during the feeding process
- You may be comparing obese mice with healthy lean mice?
  - Maybe not too big of an issue, since fat % per se does not seem to affect MLSP in some of the previously discussed studies

What are some of the basic functional beneficial changes following CR?

Is brain function improved with CR?

Improved responses to enclosed alleys


Myocyte Cell loss During Aging and Atenuation by CR

Muscle Function with Age and Calorie Restriction
CR attenuated the age associated rise in extracellular space in the fast extensor digitorum longus (Type 2) muscle

Clinical Health Parameters and CR on Monkey’s

Primate Data,
A few studies with rhesus monkeys are in progress, but longevity data will not be available for many years

- Blood insulin and glucose levels drop
- Body temperature decreases in CR monkeys just as is observed in rodents.
- Both observed in humans during temporary caloric restriction (Biosphere)
- However, these are normal adaptations to food deprivation -- tells us nothing of mechanism
  - Think of set-point!!

Increased resistance to stress response

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Rat</th>
<th>No. Rat with Tumor</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89</td>
<td>43</td>
<td>48</td>
</tr>
<tr>
<td>Restriction</td>
<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control/radiation</td>
<td>102</td>
<td>95</td>
<td>89</td>
</tr>
<tr>
<td>Restriction/radiation</td>
<td>128</td>
<td>29</td>
<td>23</td>
</tr>
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<table>
<thead>
<tr>
<th>Type of Tumor</th>
<th>Ossisy</th>
<th>Progression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>+</td>
<td>+</td>
<td>33</td>
</tr>
<tr>
<td>Renal adenomas</td>
<td>+</td>
<td>+</td>
<td>33</td>
</tr>
<tr>
<td>Prostatic adenomas</td>
<td>+</td>
<td>+</td>
<td>33</td>
</tr>
<tr>
<td>Testicular tumors</td>
<td>+</td>
<td>+</td>
<td>33</td>
</tr>
<tr>
<td>Pancreatic/test cell tumor</td>
<td>—</td>
<td>+</td>
<td>102, 163</td>
</tr>
<tr>
<td>Mammmary tumor</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>+</td>
<td>+</td>
<td>166</td>
</tr>
<tr>
<td>Prostate tumor</td>
<td>+</td>
<td>+</td>
<td>166</td>
</tr>
</tbody>
</table>

+ Denotes a positive effect.
— Denotes no effect.
Mechanisms?

There is a chronic exposure to oxidants during a life-span and life-long caloric restriction reduces oxidant production and oxidative stress.


Caloric Restriction reduces oxidant production in mitochondria
S. Phaneuf, T. Grune and C. Leeuwenburgh


Oxidative Damage in Heart and Skeletal Muscle is Increased with Aging and is Reduced by Caloric Restriction

Caloric Restriction decreases the concentrations of the products of oxidative damage to DNA, proteins and lipids in brain, heart, and skeletal muscle

Calorie restriction decreases the inflammatory response

-55 genes decreased expression with age
-13% were involved with energy metabolism
-58 genes increased expression with age
-16% were mediators of the stress response

- lower expression of metabolic and biosynthetic genes
- a marked stress response


Summary of gene expression profiling procedure

- Young tissue mRNA → cRNA
- Old tissue mRNA → cRNA

Biological data analysis

Statistical data analysis

Image/Data analysis using Affymetrix algorithm
Gene Expression Profile of Skeletal Muscle from Old Mice

- Stress Response:
  - Heat shock response genes, DNA damage response genes, Oxidative stress inducible genes
- Energy Metabolism:
  - Reduced glycolysis, mitochondrial dysfunction
- Neuronal Injury:
  - Reinnervation induced genes, muscle injury induced genes, neurite extension and sprouting

Most alterations (~80%) were completely or partially prevented by caloric restriction.

Caloric Restriction and the Aging Heart

CR reduces the incidence of spontaneous cardiomyopathy in Sprague-Dawley rats

CR prevents age-associated alterations in late diastolic function in B6D2F1 mice

CR reduces the concentration of 8-hydroxyguanine in DNA and dityrosine cross-linking of proteins in heart of aged mice

Prevents somatic mitochondrial DNA rearrangements associated with aging

Experimental design for heart study:

- Mouse B6C3F1, male (5 month and 30 month)
- Dietary manipulation started at 14 months of age
  - CR group: 58 kcal/week (41% reduction from the control group)
  - LA group: supplementation of α-lipoic acid (600 mg/kg) to control diet
  - CQ group: supplementation of coenzyme Q10 (100 mg/kg) to control diet
- Sample size: n = 5 per group
- Array: U74A 9,977 genes

Conclusion: CR induces a marked transcriptional reprogramming in the heart

Overview of Aging and CR Induced Gene Expression

- 9,977 genes studied
- 5701 genes expressed in the heart
- 996 (10%) of transcripts changed in expression with aging
- 2,075 (21%) of transcripts changed in expression with CR

Conclusion: CR induces a marked transcriptional reprogramming in the heart.
The aging heart is associated with alterations in carbohydrate metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>CR effect(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate selection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate dehydrogenase kinase</td>
<td>-8.5</td>
<td>139</td>
</tr>
<tr>
<td>Ucp 3</td>
<td>-2.6</td>
<td>146</td>
</tr>
<tr>
<td>Mitochondria</td>
<td></td>
<td></td>
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<tr>
<td>Phosphofructokinase</td>
<td>1.5</td>
<td>105</td>
</tr>
<tr>
<td>Enolase</td>
<td>1.1</td>
<td>NC</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>1.1</td>
<td>NC</td>
</tr>
<tr>
<td>Glk 4</td>
<td>1.4</td>
<td>NC</td>
</tr>
</tbody>
</table>

The aging heart is associated with alterations in fatty acid metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>CR effect(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial bet-oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acyl-CoA thioesterase 1</td>
<td>-5.1</td>
<td>71</td>
</tr>
<tr>
<td>Lipase, hormone sensitive</td>
<td>-4.6</td>
<td>89</td>
</tr>
<tr>
<td>Acyl-Coenzyme A oxidase</td>
<td>-1.6</td>
<td>38</td>
</tr>
<tr>
<td>Carboxylesterase 3</td>
<td>-1.6</td>
<td>46</td>
</tr>
<tr>
<td>Peroxisomal delta3, -4.6</td>
<td>-1.6</td>
<td>182</td>
</tr>
<tr>
<td>delta2-eneoyl-coenzyme A isomerase</td>
<td>-1.5</td>
<td>174</td>
</tr>
<tr>
<td>Enoyl coenzyme A hydratase 1</td>
<td>-1.5</td>
<td>13</td>
</tr>
<tr>
<td>Dodecenoyl-coenzyme A delta</td>
<td>-1.5</td>
<td>26</td>
</tr>
<tr>
<td>Althpa-methylacyl-CoA racemase</td>
<td>4.5</td>
<td>32</td>
</tr>
</tbody>
</table>

The aging heart is associated with alterations in fatty acid metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>CR effect(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid transport into the cytosol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solute carrier family member 27</td>
<td>-2.0</td>
<td>25</td>
</tr>
<tr>
<td>CD36</td>
<td>-1.8</td>
<td>14</td>
</tr>
<tr>
<td>Carnitine O-palmitoyl transferase</td>
<td>-3.3</td>
<td>46</td>
</tr>
<tr>
<td>Carnitine acetyltransferase</td>
<td>-1.6</td>
<td>-15</td>
</tr>
<tr>
<td>Mitochondrial carnitine translocase</td>
<td>-1.5</td>
<td>73</td>
</tr>
</tbody>
</table>

Best Explanations… That are Maybe TRUE

- It has been well established that caloric restriction slows down metabolic rate.
  - **Maybe TRUE** (depends on study you read)
  - In our groups there is no reduction in metabolism
  - There may be variations in tissues (lean body mass and fat mass may show differences).
- Perhaps lower oxygen consumption leads to less oxygen radical production
  - not always a direct relationship

More importantly...

- Perhaps that due to long-term caloric restriction, mitochondria use **oxygen more efficiently** such that less superoxide anion is produced per liter of oxygen consumed
- Adaptations to phosholipids (cardiolipin) and a reduction in “proton leak”
Most Important

- Genes more “youthful” (Science 1999)
- Reduction in oxidant production and oxidative stress. Over time less oxidative damage to different complexes, therefore less of a “electron leak” less damage to DNA, lipids, proteins.

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“Mark some not today all the lack.  Here we are not a numerical deficiency..."