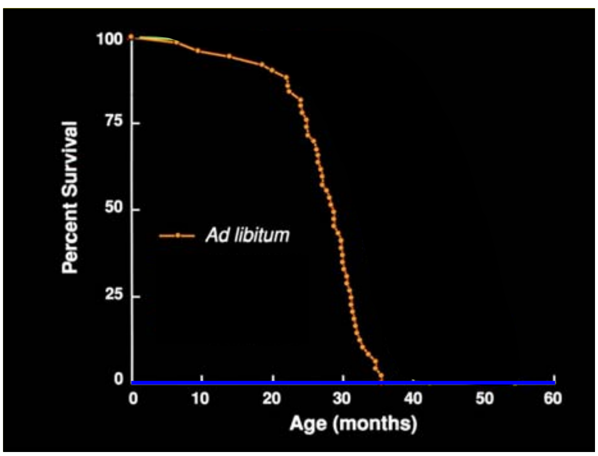
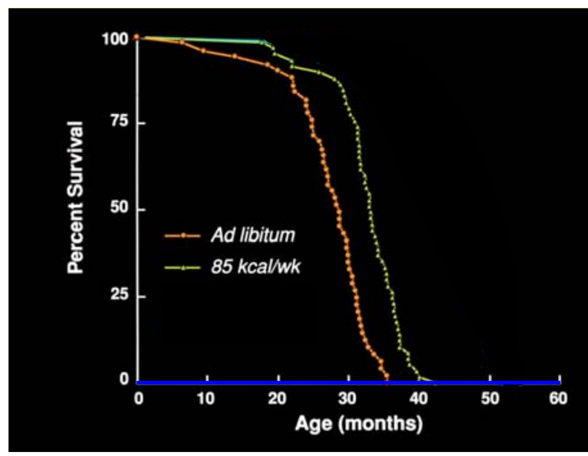
Aging and Life-prolonging interventions Lecture 4

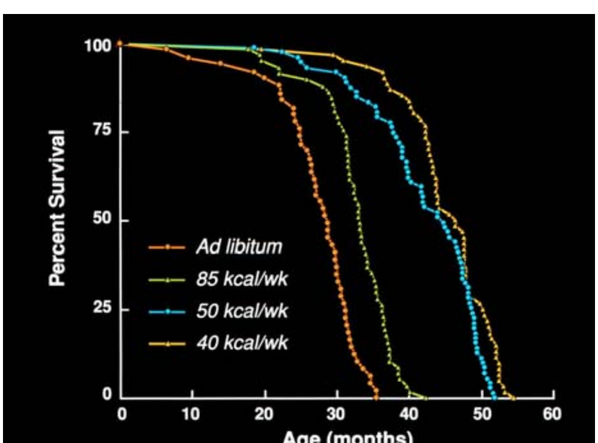
*slide contributions by Drs C. Leeuwenburgh T. Prolla and R. Weindruch

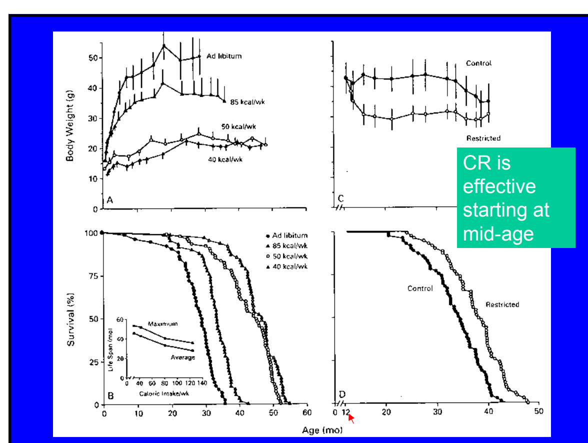
Aging and Caloric restriction

- 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



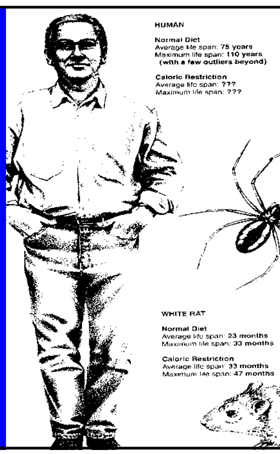






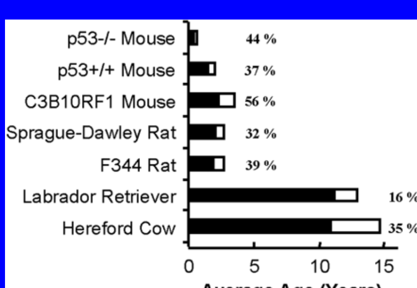
NIH-31/NIA Fortified Diet (CR)NIH31 Average Nutrient Composition (AL)

Average Nutrient Composition		NIH-31/NIA Fortified	NIH-31
Protein	%	18.74	18.42
Fat	%	4.41	4.47
Fiber	%	4.58	4.05
Ash	%	6.51	6.64
Nitrogen-Free Extract	%	55.04	55.91
Gross Energy	kcal/g	3.95	4.02
Digestible Energy	kcal.g	3.36	--
Metabolizable Energy	kcal.g	3.07	--
Linoleic Acid	%	1.79	--
Moisture	%	--	10.51
Amino Acids		NIH-31/NIA Fortified	NIH-31
Arginine	%	1.10	1.06
Methionine	%	0.36	0.39
Histidine	%	0.42	0.41
Leucine	%	1.50	1.61
Lysine	%	0.96	0.95
Tryptophan	%	0.22	0.24
Valine	%	0.88	0.96
Cystine	%	0.26	0.28
Isoleucine	%	0.76	0.90
Threonine	%	0.71	0.71
Pherylalanine +Tyrosine	%	1.53 Pherylalanine	0.92
		Tyrosine	0.70
Minerals		NIH-31/NIA Fortified	NIH-31
Calcium	%	1.03	1.06
Phosphorus	%	0.93	0.92
Sodium	%	0.30	0.26
Chlorine	%	0.48	0.42
Potassium	%	0.59	0.59
Magnesium	%	0.20	0.20
Iron	mg/Kg	336.41	300.20
Maganese	mg/Kg	156.01	152.80
Zinc	mg/Kg	48.41	50.40
Copper	mg/Kg	13.28	13.20
Iodine	mg/Kg	2.01	1.94
Cobalt	mg/Kg	0.53	0.53
Selenium	mg/Kg	0.30	--
Vitamins		NIH-31/NIA Fortified	NIH-31
Vitamin A	IU/g	40.49	--
Vitamin A3	IU/g	--	30.73
Vitamin D3	IU/g	7.00	4.19
Vitamin E	mg/Kg	52.15	38.30
Choline	mg/g	2.60	1.96
Niacin	mg/Kg	116.16	92.20
Pantothenic Acid	mg/Kg	55.07	39.50
Pyridoxine	mg/Kg	13.16	10.20
Riboflavin	mg/Kg	11.04	7.80
Thiamine	mg/Kg	123.44	77.30
Menadione	mg/Kg	111.01	22.00
Folic Acid	mg/Kg	2.13	1.70
Biotin	mg/Kg	0.38	0.13
Vitamin B12	mcg/Kg	93.80	53.00
Vitamin C	mg/g	--	--
Carotene	mg/Kg	--	--

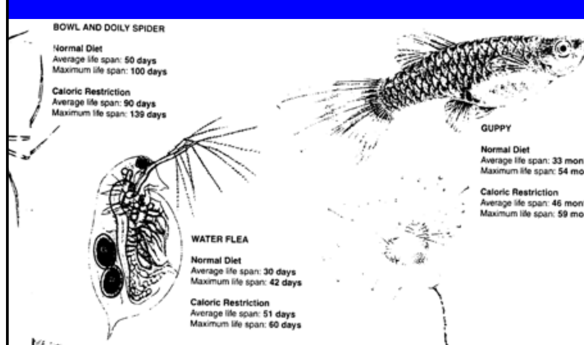


Caloric Restriction Increases Both Average and Maximal Life Span In Experimental Animals

- 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



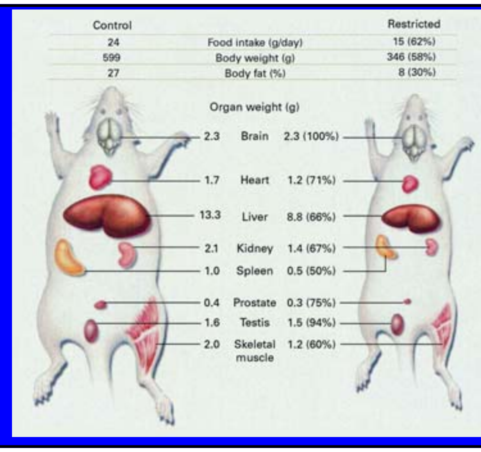
Adapted from Hursting et al, Annual Reviews of Medicine, 54:131-152,2003.



Continue

- 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)

Summary Continue

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. Calorie 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 22% 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 effect **MLSP** in some of the previously discussed studies

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

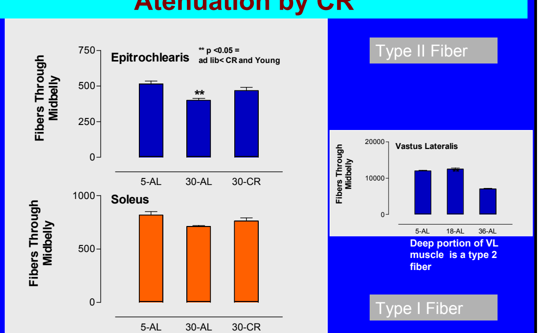
Is brain function improved with CR?

Improved responses to enclosed alleys

•Ingram, D. K., Weindruch, R., Spangler, E. L., Freeman, J. R., and Walford, R. L. (1987) Dietary restriction benefits learning and motor performance of aged mice. *J Gerontol* 42, 78-81.

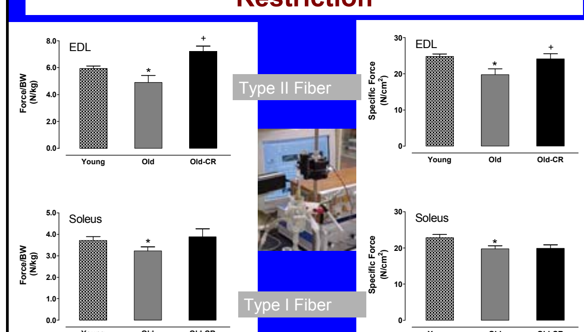
•Ingram, D. K., Chefer, S., Matochik, J., Moscrip, T. D., Weed, J., Roth, G. S., London, E. D., and Lane, M. A. (2001) Aging and caloric restriction in nonhuman primates: behavioral and in vivo brain imaging studies. *Ann N Y Acad Sci* 928, 316-326

Myocyte Cell loss During Aging and Atenuation by CR



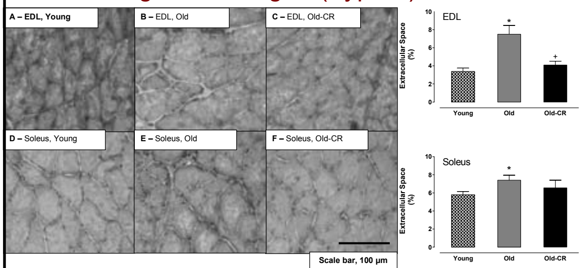
Lee, C.M., Aspnes, L.E., Chung, S.S., Weindruch, R., and Aiken, J.M. 1998. Influences of caloric restriction on age-associated skeletal muscle fiber characteristics and mitochondrial changes in rats and mice. *Ann N Y Acad Sci* 854:182-191.

Muscle Function with Age and Calorie Restriction



Anthony M. Payne, Stephen L. Dodd, and Christiaan Leeuwenburgh. Life-long calorie restriction in Fischer-344 rats attenuates age related loss in skeletal muscle specific force and extracellular space. *J Appl Physiol (September 12, 2003). 10.1152/japplphysiol.00758.2003.*

CR attennuated 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!!

NORMAL DIET

- **Food intake:** 688 calories per day
- **Body weight:** 31 pounds
- **Percent of weight from fat:** 25

MEASURES OF HEALTH

- **Blood pressure:** 129/60 (systole/diastole)
- **Glucose level:** 71 (milligrams per deciliter of blood)
- **Insulin level:** 93 (microunits per milliliter of blood)
- **Triglycerides:** 169 (milligrams per deciliter of blood)

REDUCED DIET

- **Food intake:** 477 calories per day
- **Body weight:** 21 pounds
- **Percent of weight from fat:** 10

MEASURES OF HEALTH

- **Blood pressure:** 121/51 (systole/diastole)
- **Glucose level:** 56 (milligrams per deciliter of blood)
- **Insulin level:** 29 (microunits per milliliter of blood)
- **Triglycerides:** 67 (milligrams per deciliter of blood)

Increased resistance to stress response

Table 6. Reduction of the Incidence of Tumors following Restriction of Food Intake

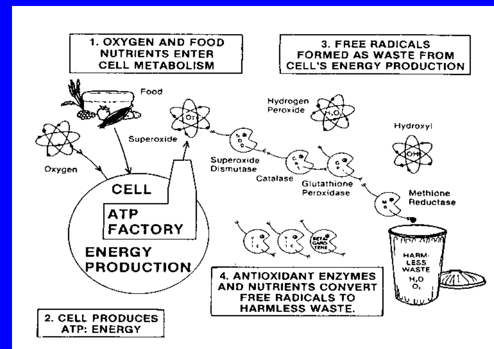
Treatment	No. Rats Observed	No. Rats with Tumor	%
Control	89	43	48
Restriction	77	0	0
Control/radiation	102	91	89
Restriction/radiation	128	29	23

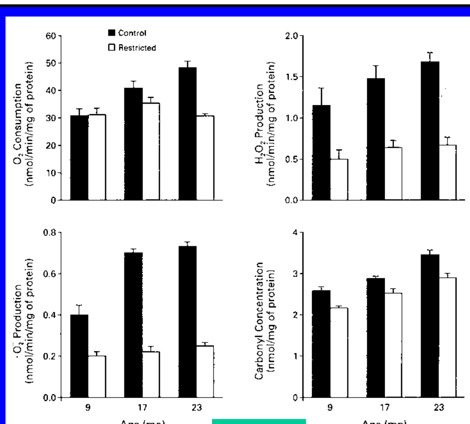
Table 7. The Effect of DR on Spontaneous Tumorigenesis

Type of Tumor	Onset	Progression	Reference
Leukemia	+	—	22
Pituitary adenoma	+	+	23
Pheochroma-cytoma	+	+	23
Testicular tumors	+	—	23
Pancreatic islet cell tumor	+	—	23
Mammory tumor	—	+	162, 163
Hepatoma	+	—	164
Prostate tumor	+	—	164

\+ Denotes a positive effect.
— Denotes no effect.

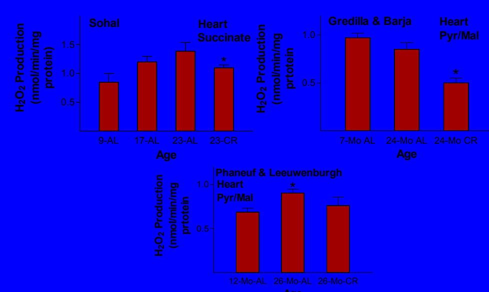
Mechanisms?





Raj Sohal

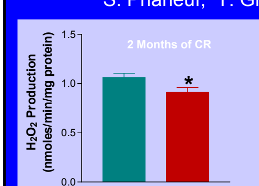
There is a chronic exposure to oxidants during a life-span



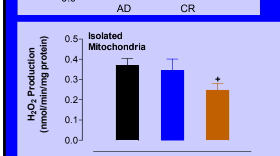
Sohal et al. Mech Ageing Dev 74 (1-2):121-33, **1994.**

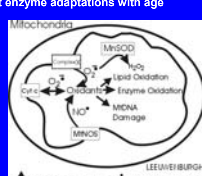
Gredilla R, Sanz A, Lopez-Torres M, and **Barja** G. FASEB J 15(9):1589-91, **2001.**

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



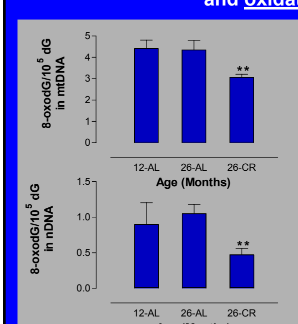
- **-Sub-mitochondrial populations (sub-sarcolemmal vs. intermyofibrillar)**
- **-Sub mitochondrial particles vs. intact isolated mitochondria**
- **-Antioxidant enzyme adaptations with age**



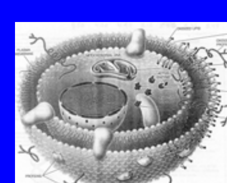


Drew, B., Phaneuf, S., Dirks, A., Selman, C., Gredilla, R., Lezza, A., Barja, G., and Leeuwenburgh, C. (2003) Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol Regul Integr Comp Physiol* 284, R474-R480

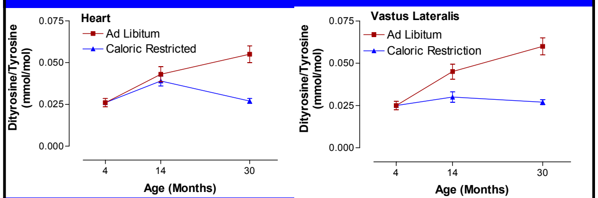
There is a <u>chronic exposure to oxidants</u> during a life-span and <u>life-long caloric restriction</u> reduces <u>oxidant production</u> and <u>oxidative stress</u>



Drew, B., Phaneuf, S., Dirks, A., Selman, C., Gredilla, R., Lezza, A., Barja, G., and Leeuwenburgh, C. (2003) Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol Regul Integr Comp Physiol* 284, R474-R480

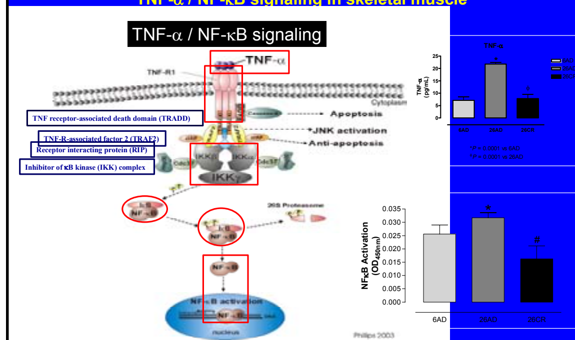


Oxidative Damage in Heart and Skeletal Muscle is Increased with Aging and is Reduced by Caloric Restriction



Leeuwenburgh, C. et al. Caloric restriction attenuates dityrosine cross-linking of cardiac and skeletal muscle proteins in aging mice. ***Arch Biochem Biophys*. 346:74-80. 1997.**

The effects of age and calorie restriction on TNF-α / NF-κB signaling in skeletal muscle



Phillips and Leeuwenburgh (unpublished)

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

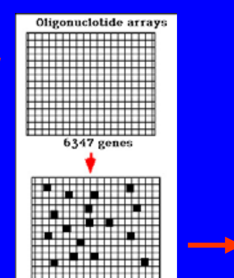
Calorie restriction decreases the inflammatory response

Lee et al., Gene Expression Profile of Aging and its Retardation by Caloric Restriction. Science, 285, 1390-3, 1999.

Aged Rats

Caloric Restricted Rats

Isolated RNA from Muscles



Locke

-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

Lee et al., Gene Expression Profile of Aging and its Retardation by Caloric Restriction. Science, 285, 1390-3, 1999.

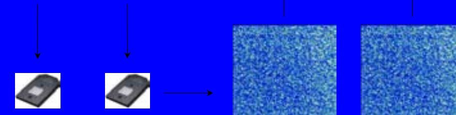
Summary of gene expression profiling procedure

Young tissue	Old tissue
mRNA	mRNA
cDNA	cDNA
cRNA	**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

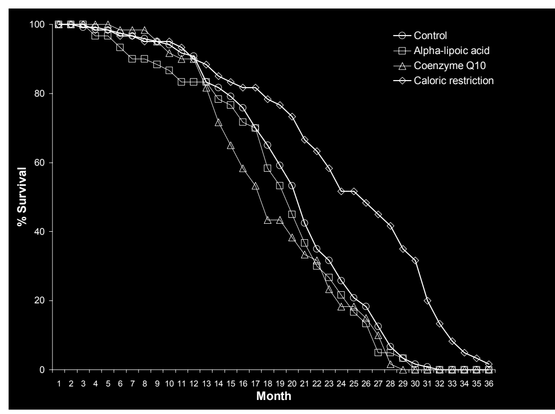
Prevents somatic mitochondrial DNA rearrangements associated with aging

Experimental design for heart study:

- ♦ Mouse B6C3F$_1$ male (5 month and 30 month)
- ♦ Dietary manipulation started at 14 months of age

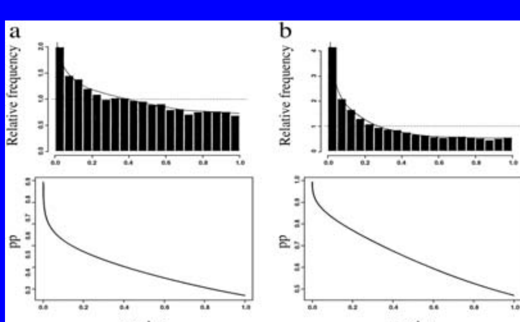
- CR group: 58 kcal/week
  (41% reduction from the control group)
- LA group: supplementation of α-lipoic acid
  (600 mg/kg) to control diet
- CQ group: supplementation of coenzyme Q10
  (100 mg/kg) to control diet

- ♦ Sample size: n = 5 per group
- ♦ Array: U74A 9,977 genes



Weindruch group data, unpublished.

P-value and Posterior Probability Distributions



AGING **CR**

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

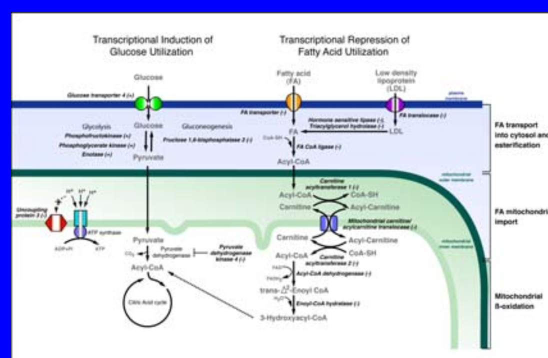
Gene	FC	CR effect(%)
Substrate selection		
Pyruvate dehydrogenase kinase	-8.5	139
Ucp 3	-2.6	146
Glycolysis		
Phosphofructokinase	1.5	105
Enolase	1.1	NC
Phosphoglycerate kinase	1.1	NC
Glut 4	1.4	NC

The aging heart is associated with alterations in fatty acid metabolism

Gene	FC	CR effect(%)
Mitochondrial b-oxidation		
Acyl-CoA thioesterase 1	-5.1	71
Lipase, hormone sensitive	-4.6	89
Acyl-Coenzyme A oxidase	-1.8	38
Carboxylesterase 3	-1.6	46
Peroxisomal delta3,	-1.6	182
delta2-enoyl-coenzyme A isomerase	-1.5	174
Enoyl coenzyme A hydratase 1	-1.5	13
Dodecenoyl-coenzyme A delta	-1.5	26
Alhpa-methylacyl-CoA racemase	4.5	32

The aging heart is associated with alterations in fatty acid metabolism

Gene	FC	CR effect(%)
Fatty acid transport into the cytosol		
Solute carrier family member 27	-2.0	25
CD36	-1.8	-14
Carnitine O-palmitoyl transferase	-3.3	46
Carnitine acetyltransferase	-1.6	-15
Mitochondrial carnititine translocase,	-1.5	73

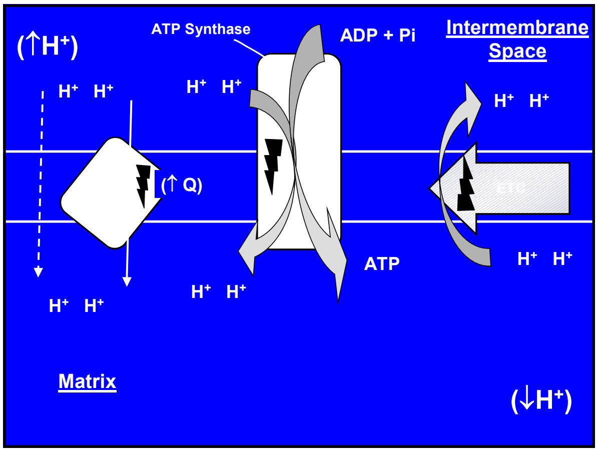


Best Explanations… That are Maybe TRUE

- It has been well established that caloric restriction slows **down metabolic rate**.
  - **<u>Maybe</u> 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)**

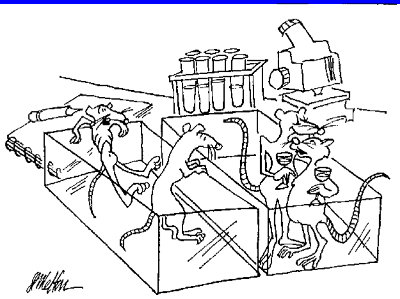
More importantly...

- Perhaps that due to long-term caloric restriction, mitochondria use **<u>oxygen more efficiently</u>** 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.



"Man, some rats have all the luck. Here we are in a nutritional deficiency study and Herb's doing the effects of alcohol on the libido!"

Control		Restricted
24	Food intake (g/day)	15 (62%)
599	Body weight (g)	346 (58%)
27	Body fat (%)	8 (30%)