Seeking the Fountain of Youth: Is Dietary Restriction the Key to a Longer and Healthier Life?

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My goal is to study and implement strategies for the promotion of HEALTHY LONGEVITY

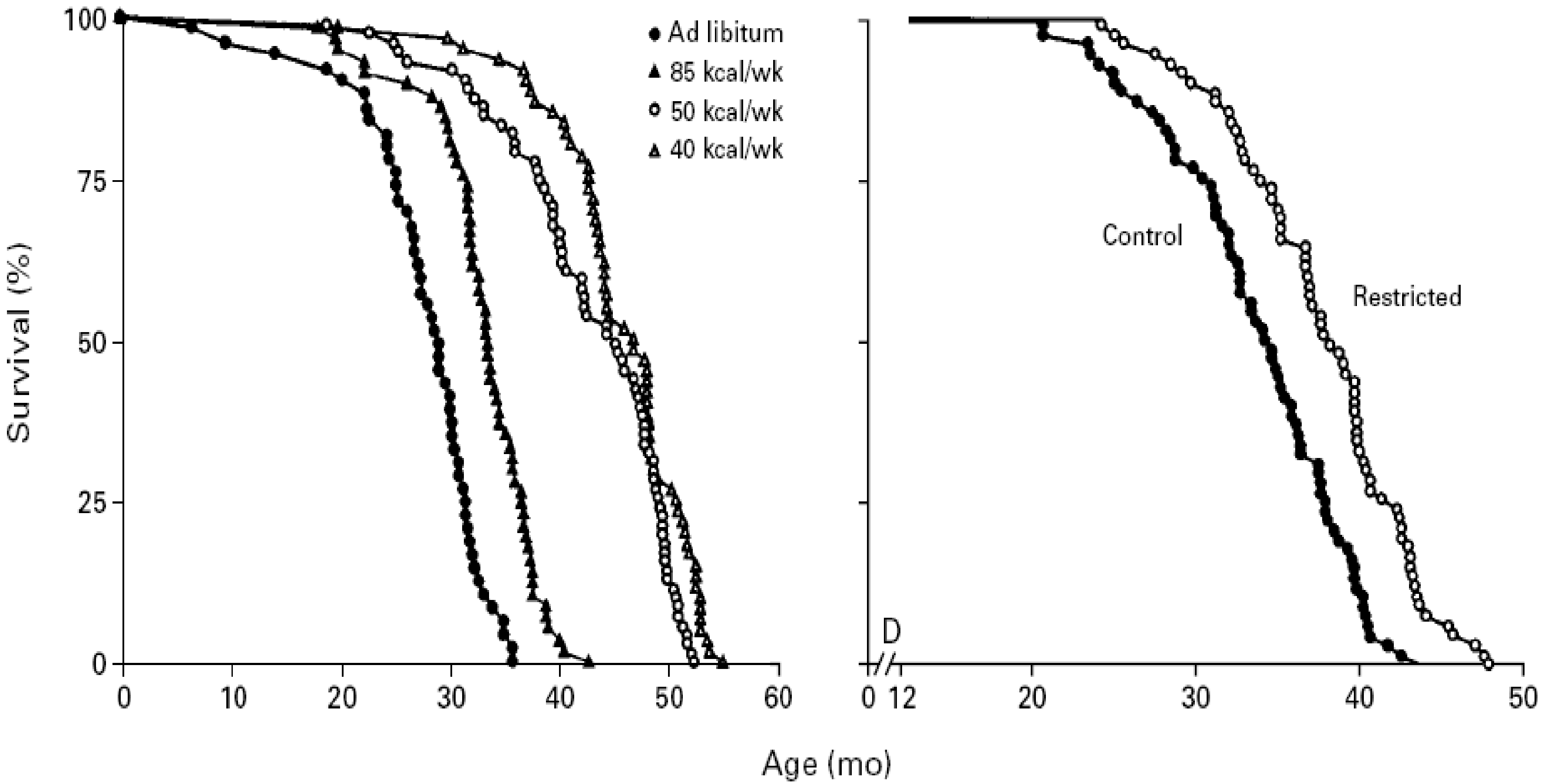
HEALTHY LONGEVITY defined as
the ability of human beings to AVOID DISEASE and DISABILITY and remain:

- **physically and cognitively healthy**
- **happy and creative**
- **enpowered**
- **contributing to social and productive activities**
- **active & independent**

..... for as long as possible

.... ideally for the entire life.

Calorie restriction without malnutrition increases maximal lifespan up to 50% in rodents



Masoro EJ. Mech Ageing Dev. 2005

Weindruch R. N Engl J Med 1999

Calorie restriction protects against spontaneous, radiation- and chemical- induced tumors

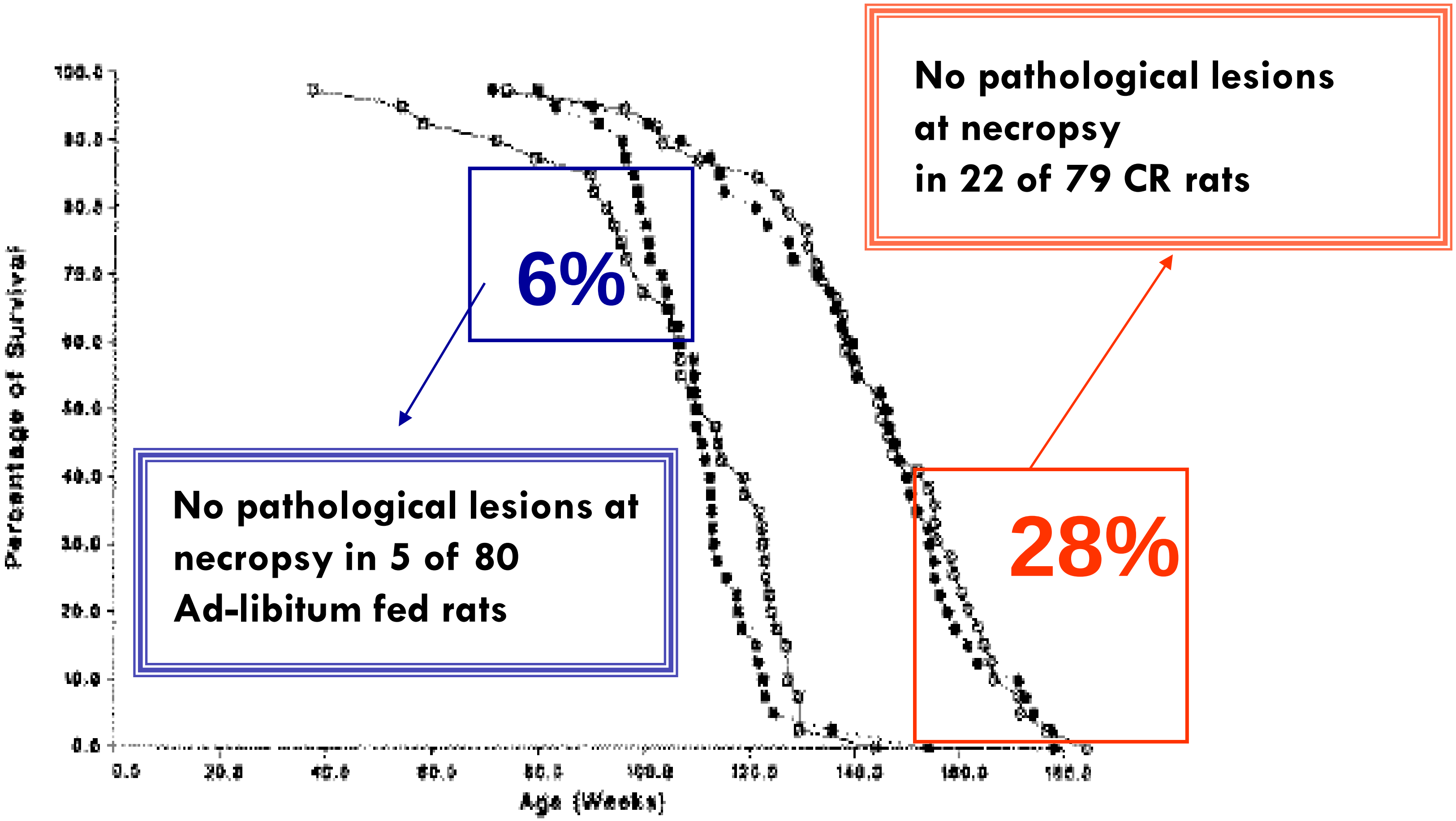
Number of experiments	Caloric restriction (%)		Tumor reduction (%)
	Range	Mean (SE)	Mean (SE)
9	0	0 (1.5)	−9.5 (10.2)
18	7–20	15.3 (1.2)	20.2 (8.1)
22	21–30	25.9 (1.1)	49.6 (6.4)
17	31–40	37.0 (1.2)	52.5 (7.8)
16	41–58	52.9 (1.1)	62.2 (7.6)

Site- and fat-adjusted means ± SE, weighted by number of animals per experimental group.

Data from 82 published experiments involving several tumor sites in mice

Albanes D. Cancer Research 1987

~30% of the CR rodents dies without any gross pathological lesion



Shimokawa I et al. J Gerontol 1993

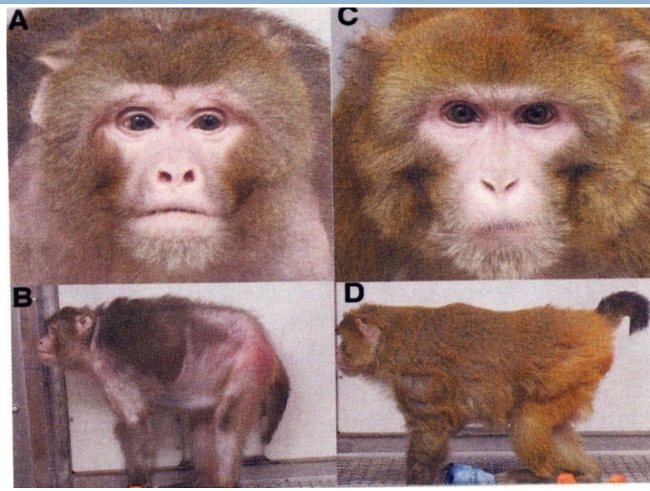
~20% of centenarians are escapers

In a longitudinal study of the 424 centenarians:

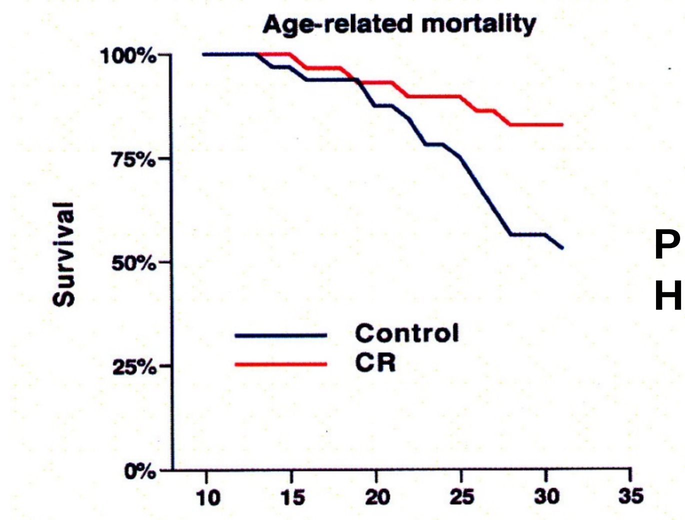
- 19% were ESCAPERS (= without common age-associated disease before 100 years of age)
- 43% were delayers (= age-associated disease after the age of 80 years)
- 38% were survivors (= age-associated disease before the age of 80 years)

Evert *et al., J. Gerontol. A Biol. Sci. Med. Sci 2003*

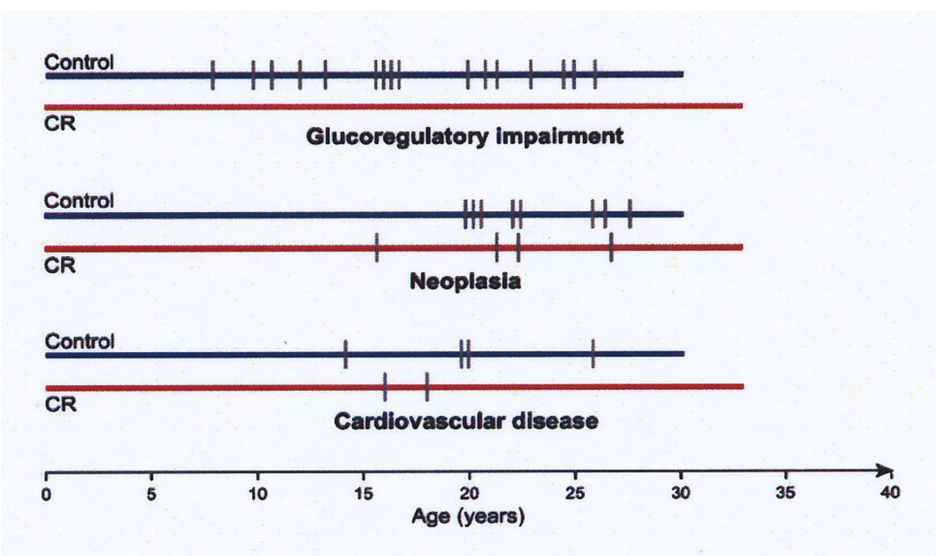
CR reduces cardiovascular and cancer mortality by 50% in non-human primates (WNPRC study)

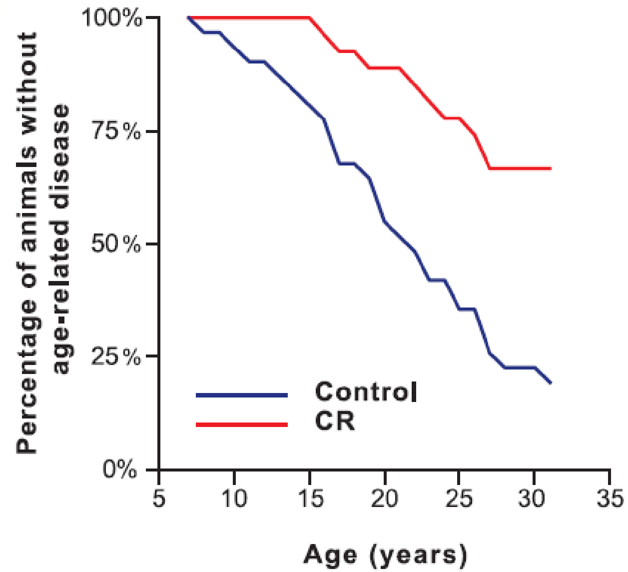


Ad libitum **CR**



P = 0.03
HR = 3.0

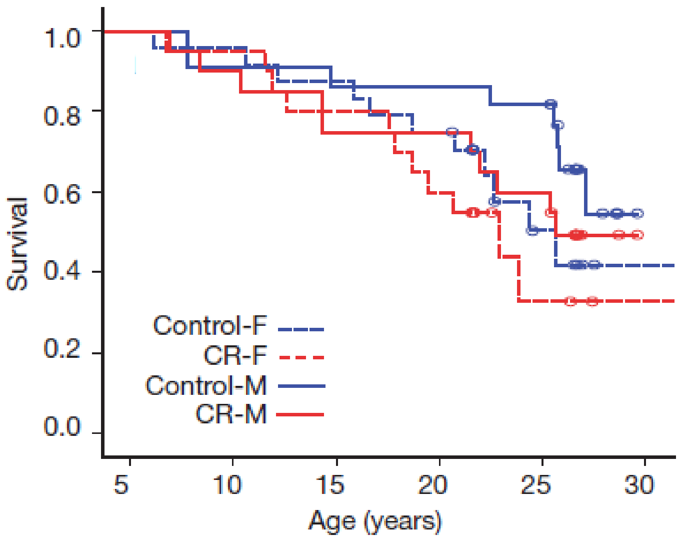


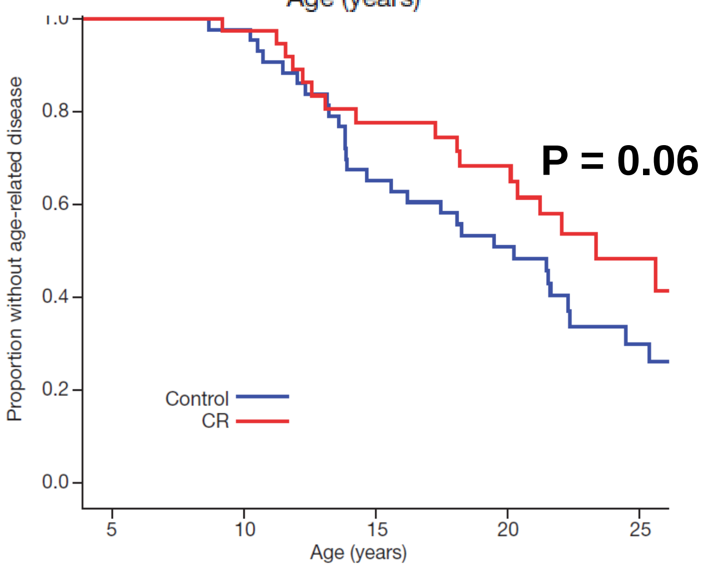


P = 0.008
HR = 2.9

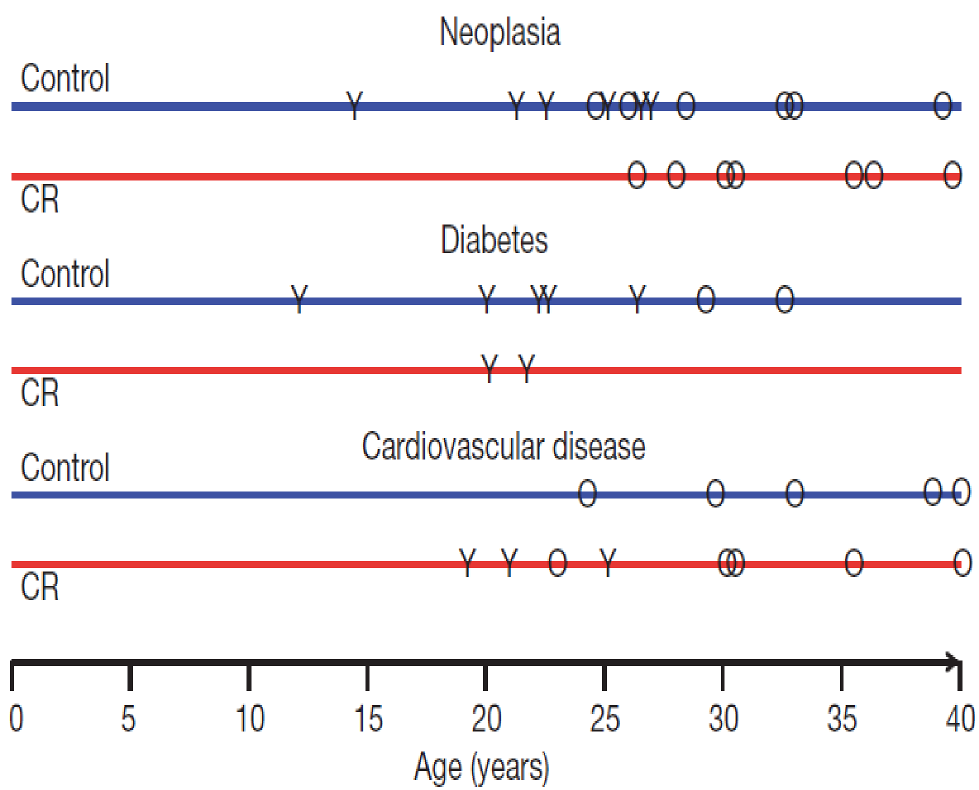
Colman et al.
Science 2009

CR prevents cancer and diabetes, but does not extend lifespan in non-human primates





NIA Primate Study



Mattison et al. Nature 2012

NIA CR monkey study

- No cancer in 30% CR, but 6 cases in control monkeys
- 50% reduction in diabetes in 30% CR
- 37% of the control monkeys had died from age-related causes vs only 13% in the CR group.
- In both the NIA control and CR male monkeys average lifespan for was ~45% longer than in Rhesus monkeys kept in captivity (35.4 yrs vs 27 yrs)
- 4 CR and 1 control monkeys have lived more than 40 years, which is an extremely long life (> 120 yrs for a human being)

Mattison et al. Nature 2012

	NIA monkeys	WNPRC monkeys
Control monkeys	Controlled allotment of food each day	Fed ad libitum
Diet composition & nutrients	Natural ingredients 3.77 kcal/gr 17,3% protein (fish, soybean, wheat, corn) 5% fat (fish, soy oil, wheat, corn, alfalfa) 56.9% CHO (ground wheat and corn) 3.9% from sucrose Vitamin supplementation +40% of RDA for both CON and CR; all had same diet	Semipurified pellets 3.9 kg/gr 15% protein (lactalbumin) 10% fat (corn oil) 65% CHO (cornstarch, sucrose) 28.5% from Sucrose Vitamin supplementation beyond RDA only for CR monkeys
	Pesco-vegetarian diet	**Semipurified diet rich in refined foods**

Effects of long-term CR in humans

Subjects: 35-82 y, healthy volunteers

Cross-sectional study

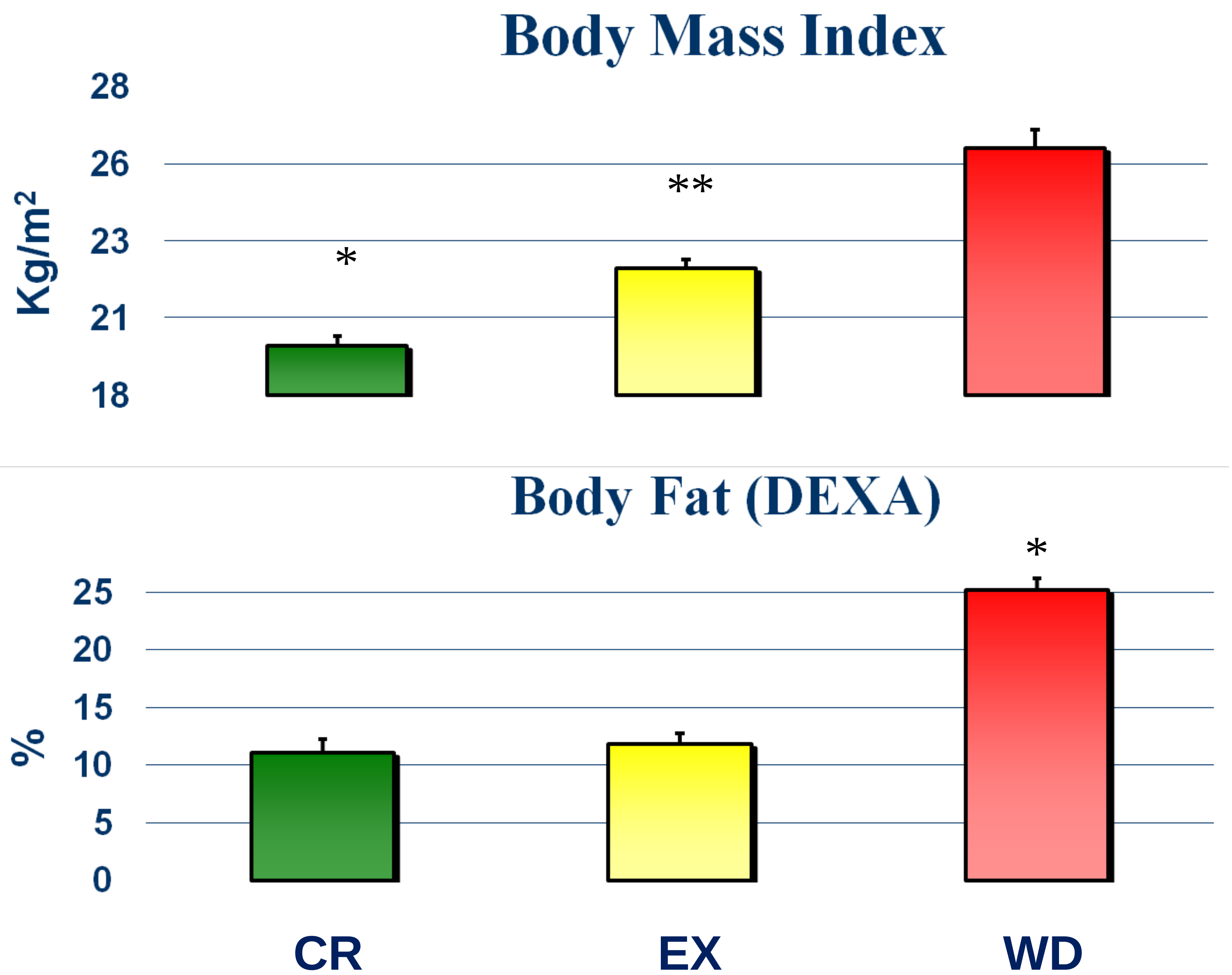
	CR (n=32)	EX (n=32)	WD (n=32)
Age	52.2±1.8	53.0±1.8	52.5±1.6
Sex (M/F)	27/5	27/5	27/5
Kcal/d	1781±75	2768±144	2581±99
Years (range)	8 (3-15)	21 (5-38)	52.5

EX: average miles/wk → 48.4 (range 26-90)

CR practitioner before starting CR and after 7 years of CR

Body weight	180 lb or 81.6 kg (BMI 26.0 kg/m^2)	134 lb, or 60.8 kg (BMI 19.4 kg/m^2)
T-chol and LDL-c	244 mg/dl and 176 mg/dl	165 mg/dl and 97 mg/dl
Fasting glucose	87 mg/dl	74 mg/dl
Blood pressure	145/85 mmHg	95/60 mmHg

Fontana L et al. Science 2010



Body Mass Index
Kg/m2
28
26
23
21
18
*
**
Body Fat (DEXA)
%
25
20
15
10
5
0
*
CR
EX
WD

Cardiometabolic risk factors

	Value		
Parameter	CR (n = 18)	Controls (n = 18)	P value
Tchol, mg/dl	158 ± 39	205 ± 40	0.001
LDL-C, mg/dl	86 ± 28	127 ± 35	0.0001
HDL-C, mg/dl	63 ± 19	48 ± 11	0.006
Tchol/HDL-C ratio	2.6 ± 0.5	4.5 ± 1.3	0.0001
TG, mg/dl	48 ± 15	147 ± 89	0.0001
TG/HDL-C ratio	0.8 ± 0.3	3.5 ± 2.8	0.0001
Systolic BP, mmHg	99 ± 10	129 ± 13	0.0001
Diastolic BP, mmHg	61 ± 6	79 ± 7	0.0001
Fasting glucose, mg/dl	81 ± 7	95 ± 8	0.0001
Fasting insulin, mIU/ml	1.4 ± 0.8	5.1 ± 2	0.0001
Hs-CRP, μg/ml	0.3 ± 0.2	1.6 ± 2.2	0.001

Values are means ± SD. IU, international unit; Hs-CRP, high-sensitivity CRP; 1 mmHg = 133 Pa.

Fontana et al., PNAS 2004

Absence of established risk factors at 50 yrs of age is associated with very low lifetime risk for CVD and markedly longer survival

	Men		
	Lifetime Risk for CVD (95% CI), %		
Risk Stratum*	To 75 y	To 95 y	Median Survival (IQR), y
Overall	35.0 (32.9–37.2)	51.7 (49.3–54.2)	30 (22–37)
All optimal risk factors	5.2 (0–12.2)	5.2 (0–12.2)	>39 (32–>45)
≥1 Not optimal risk factor	17.6 (10.9–24.4)	36.4 (23.1–49.6)	36 (29–42)
≥1 Elevated risk factor	26.0 (21.0–31.0)	45.5 (38.0–53.1)	35 (26–42)
1 Major risk factor	37.6 (33.8–41.5)	50.4 (46.2–54.5)	30 (23–36)
≥2 Major risk factors	53.2 (47.1–59.3)	68.9 (61.7–73.2)	28 (18–35)

Framingham Heart Study

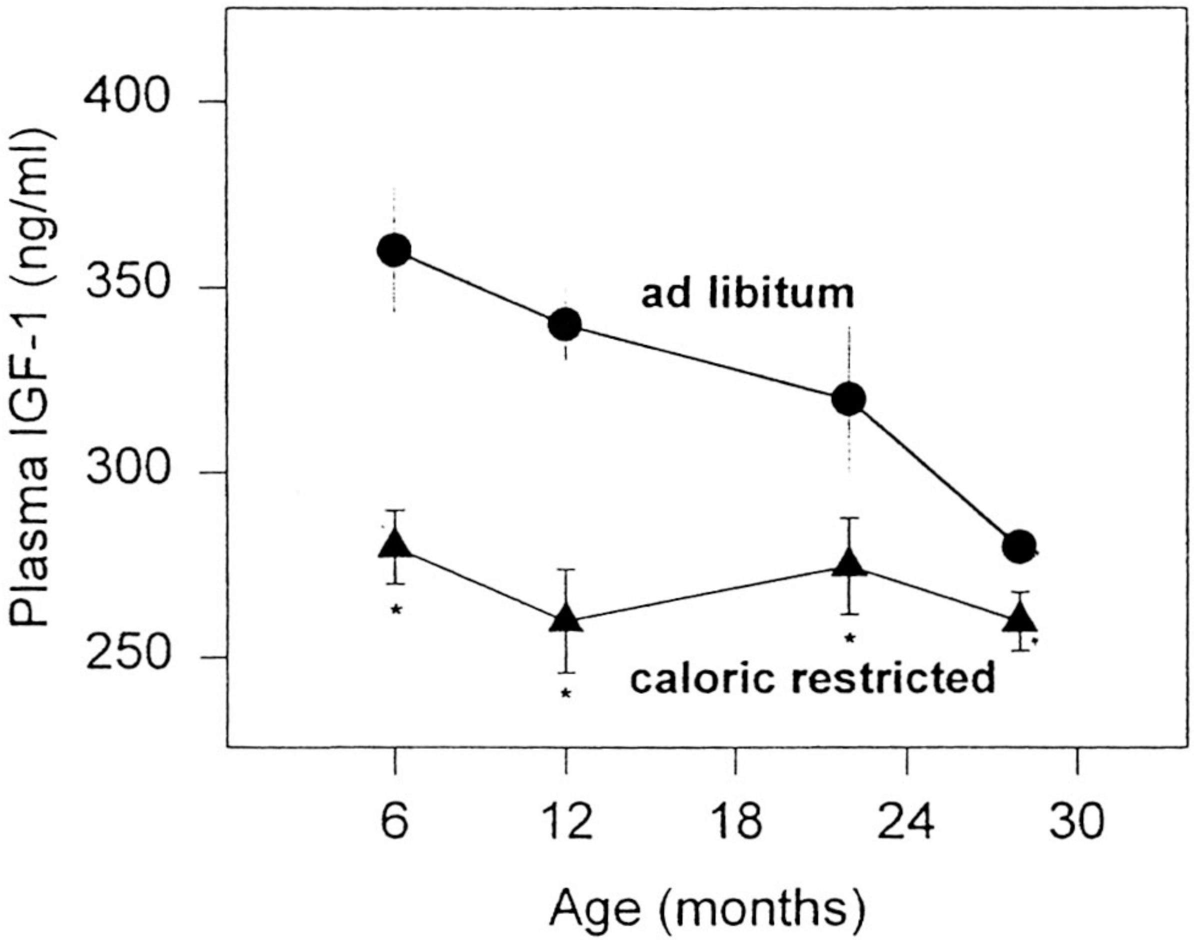
Lloyd-Jones et al., Circulation 2006

Long-term CR reduces metabolic factors associated with cancer in humans

- **Reduces adiposity** (Fontana et al. PNAS 2004)
- **Reduces insulin** (Fontana et al. Age 2010)
- **Increases adiponectin** (Fontana et al. Age 2010)
- **Reduces sex hormones** (Cangemi et al. Aging Cell 2010)
- **Reduces inflammation** (Meyer et al. JACC 2006)
- **Reduces oxidative stress** (Hofer et al. Rejuv Res 2008)
  (Omodei et al. Aging 2013)

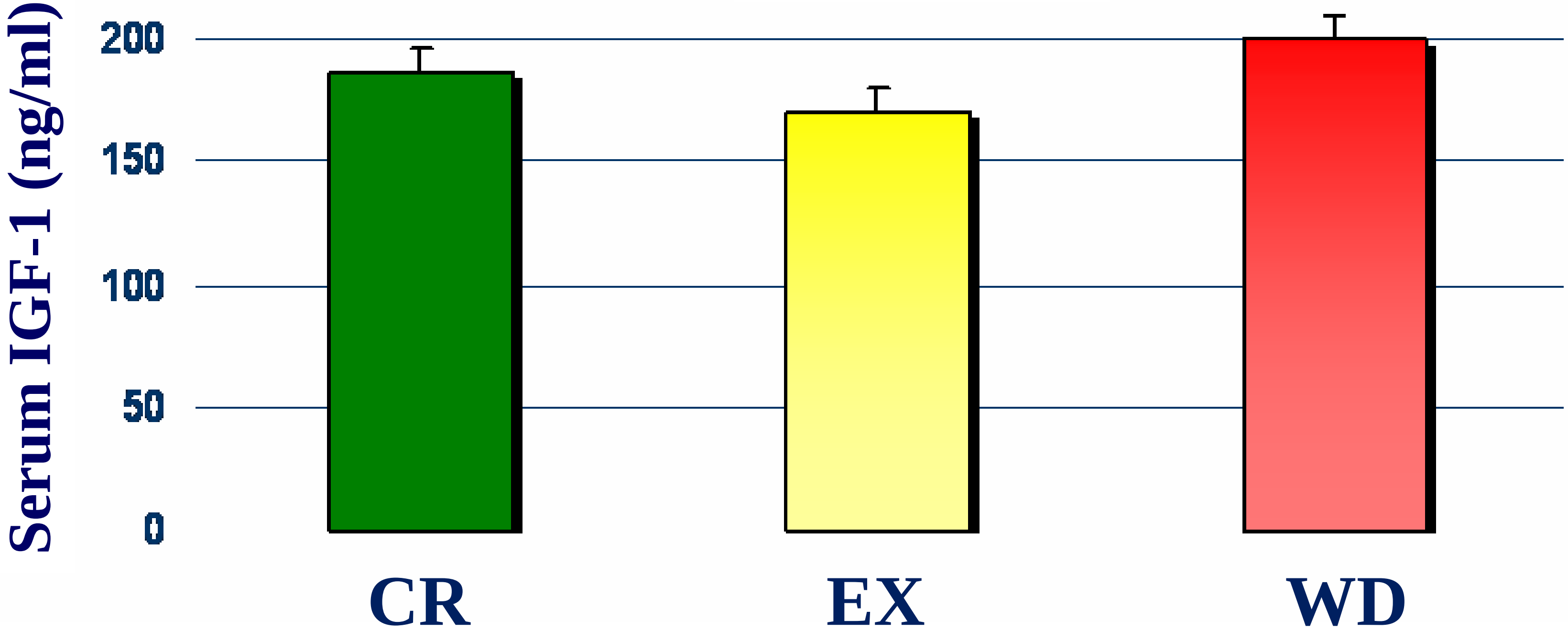
Longo and Fontana. Trends Pharmacol Sci 2010

Long-term CR reduces plasma IGF-1 concentration by 20-40% in rats



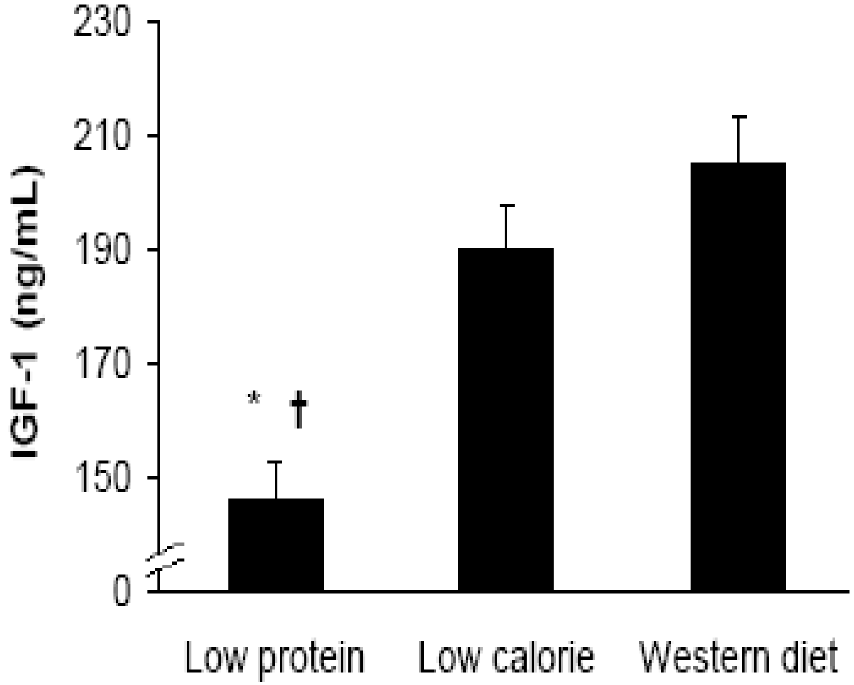
Breese CR et al. J Gerontol Biol sci 1991

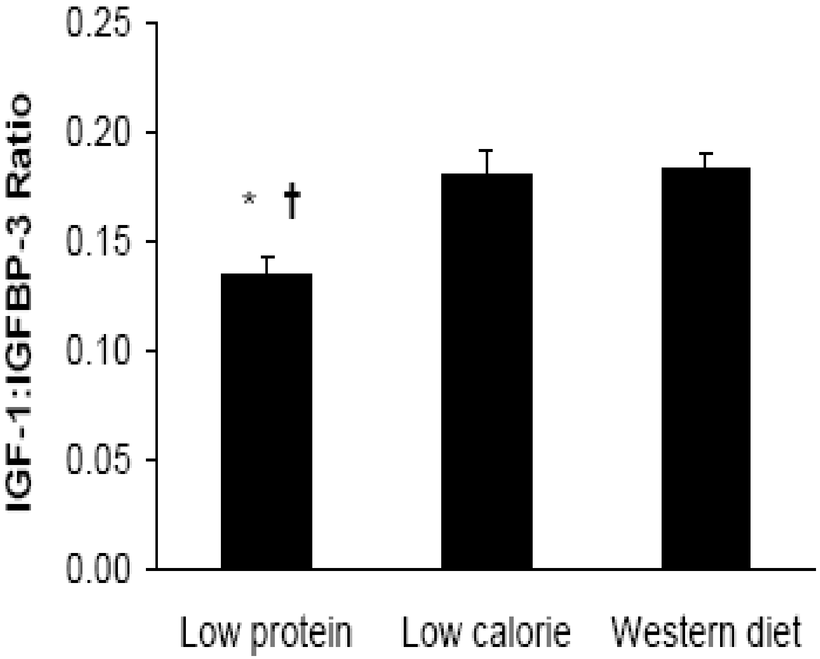
Long-term CR does NOT reduce serum IGF-1 concentration



Fontana et al., Aging Cell 2008

Moderate protein restriction reduces serum IGF-1 concentration





Fontana et al., Aging Cell 2008

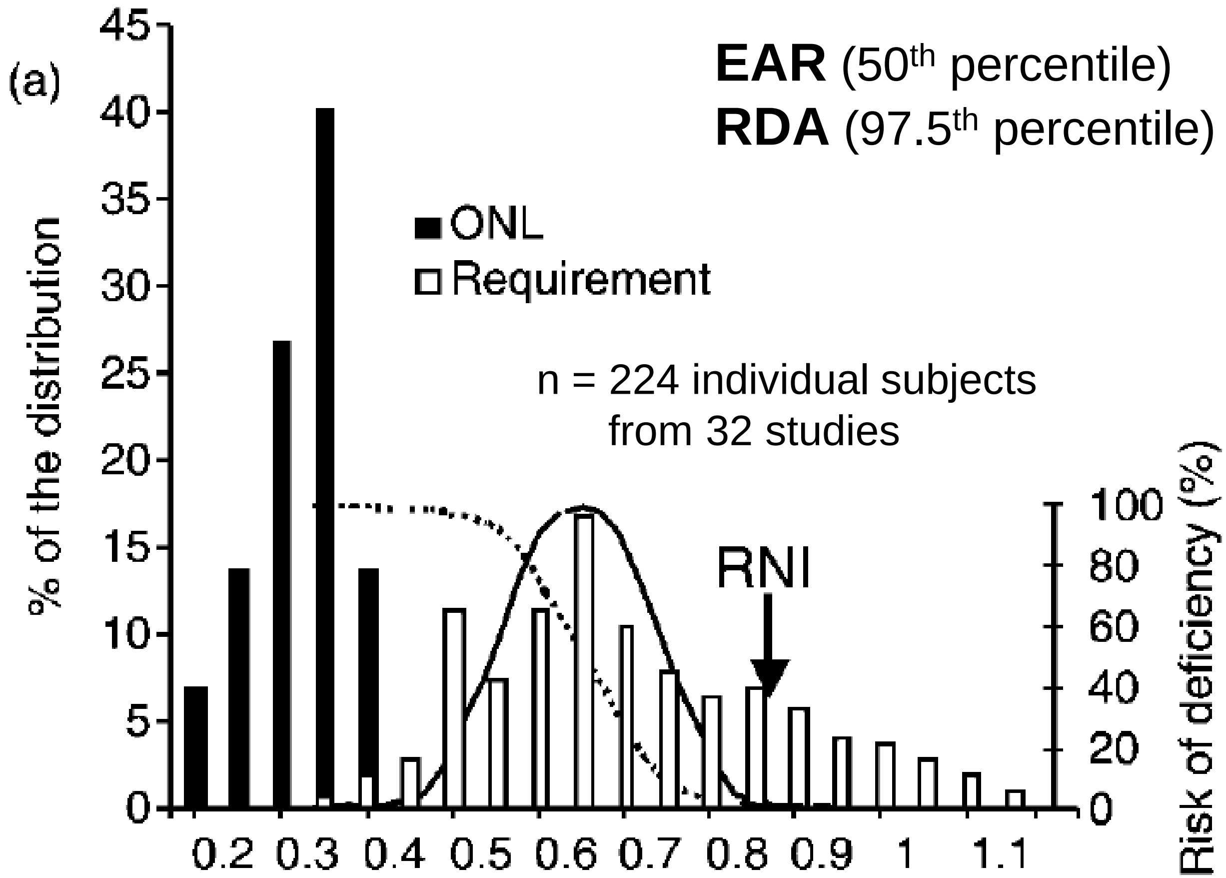
Diet composition: protein restricted vegan diet versus CR diet

	PR vegan (n=28)	CR diet (n=28)	WD (n=28)
Age (yrs)	53.4±11	52.2±12	53.7±8.2
Body fat (%)			
men	15.2±5.4*,†	7.1±4.6*	23.6±6.5
women	25.8±7.7*	20.5±9.9*	36.9±3.9
Calorie intake (kcal/d)	1980±535*	1772±351*	2505±522
Protein intake			
(%)	9.6±3.3*,†	23.5±5.7*	15.9±3.0
(g/Kg/day)	0.76±0.2*,†	1.73±0.4*	1.24±0.3
Fat intake (%)	41.3±10*,†	28.1±9*	33.6±6

Fontana et al., Aging Cell 2008

Protein requirements for healthy adults

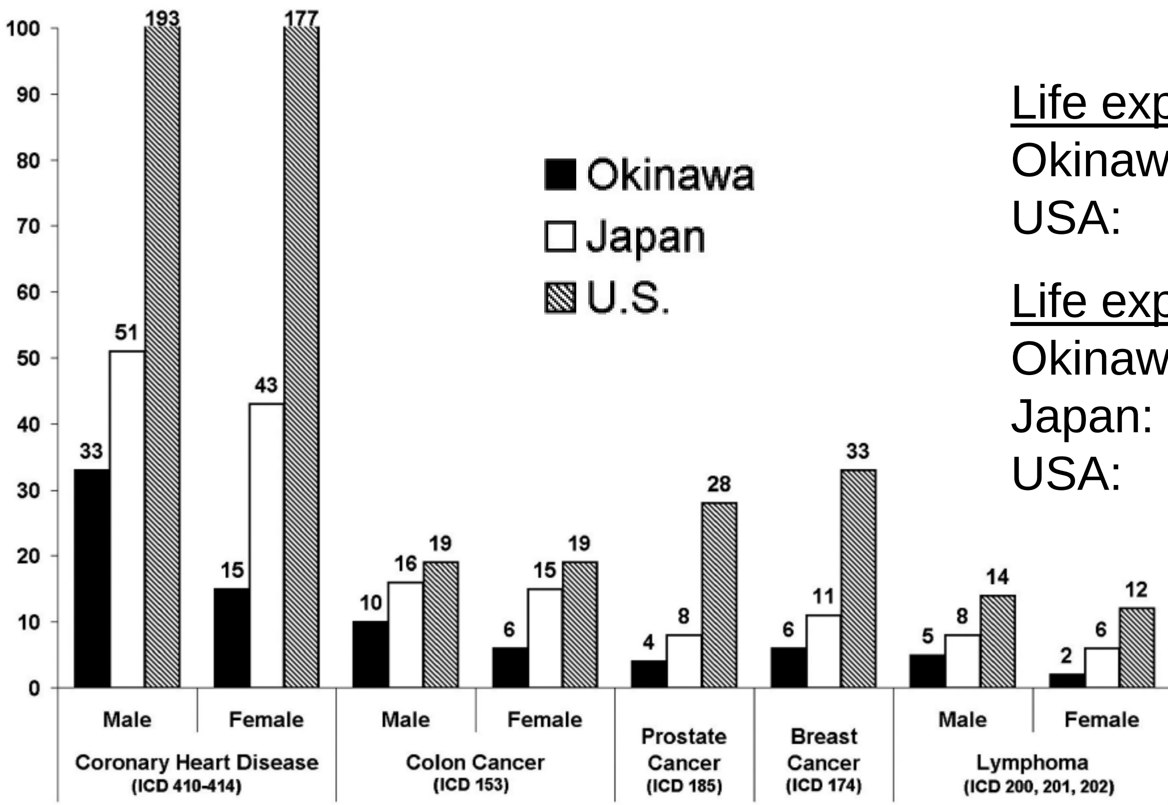
EAR (50th percentile) = **0.65 g/kg/d**
RDA (97.5th percentile) = **0.83 g/kg/d**



Rand WM et al. AJCN 2003

Traditional dietary intake of Okinawans and Japanese in 1950

	Okinawa, 1949[a]	Japan, 1950[b]
Total calories	1785[c]	2068
Total weight (grams)	1262	1057
Caloric density (calories/gram)	1.4	2.0
Total protein in grams (% total calories)	39 (9)	68 (13)
Total carbohydrate in grams (% total calories)	382 (85)	409 (79)
Total fat in grams (% total calories)	12 (6)	18 (8)



Life expectancy at birth:

Okinawa: 86 y F; 77.6 y M

USA: 80 y F; 75 y M

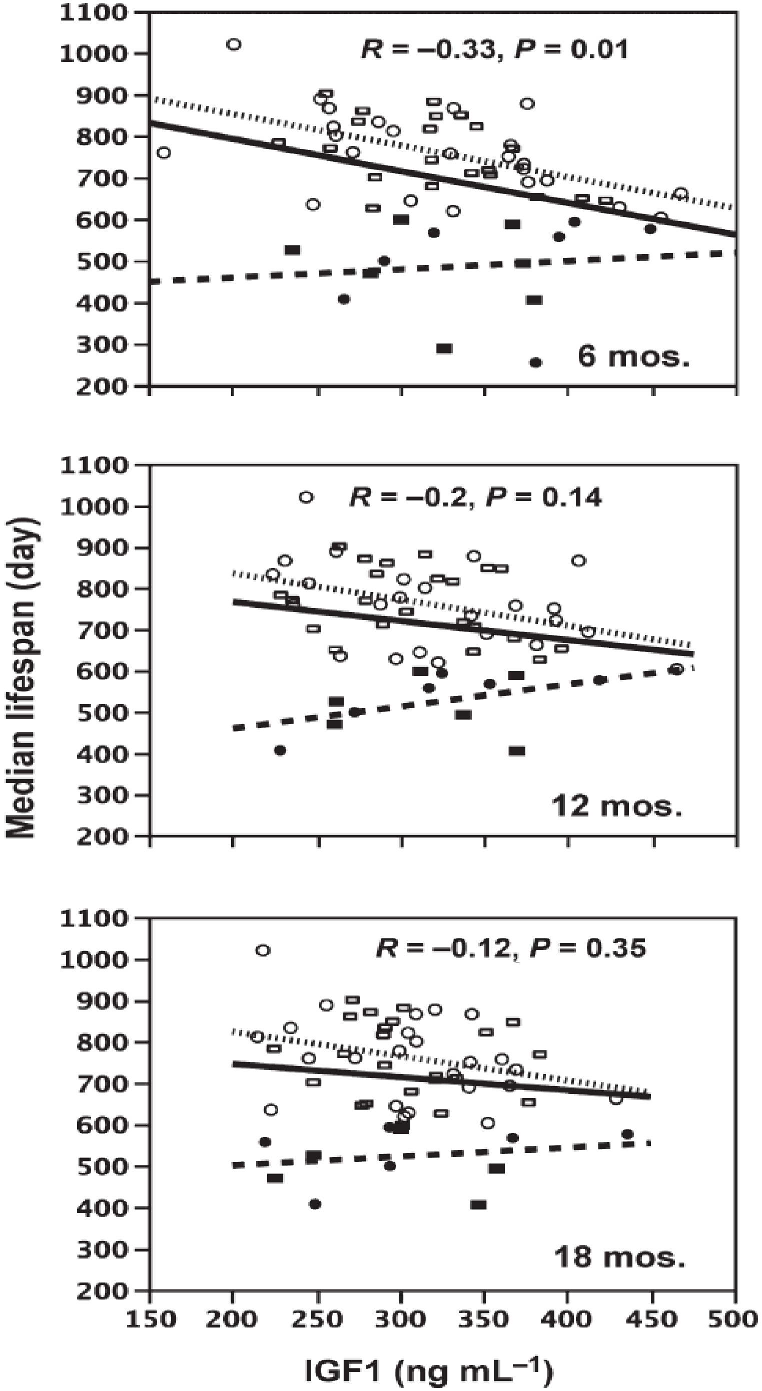
Life expectancy at age 65:

Okinawa: 24.1 y F; 18.5 y M

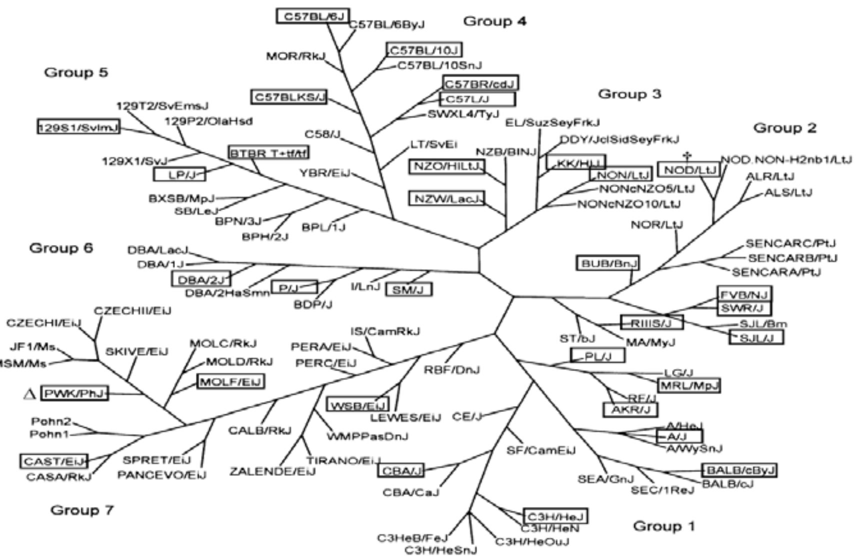
Japan: 22.5 y F; 17.6 y M

USA: 19.3 y F; 16.2 y M

Willcox BJ et al. Ann NY Acad Sci 2007



Plasma IGF-1 levels are negatively correlated with median lifespan in mice



31 genetically-diverse inbred mouse strains
(median lifespan: 251-964 days)

For the longer-lived strains (>600 days), the negative correlation between lifespan and IGF-1 is stronger:
6 mos R=-0.53, P<0.01; 12 mos R=-0.39, P<0.01; 18 mos R=-0.3, P<0.05.

Yuan et al., Aging Cell 2009

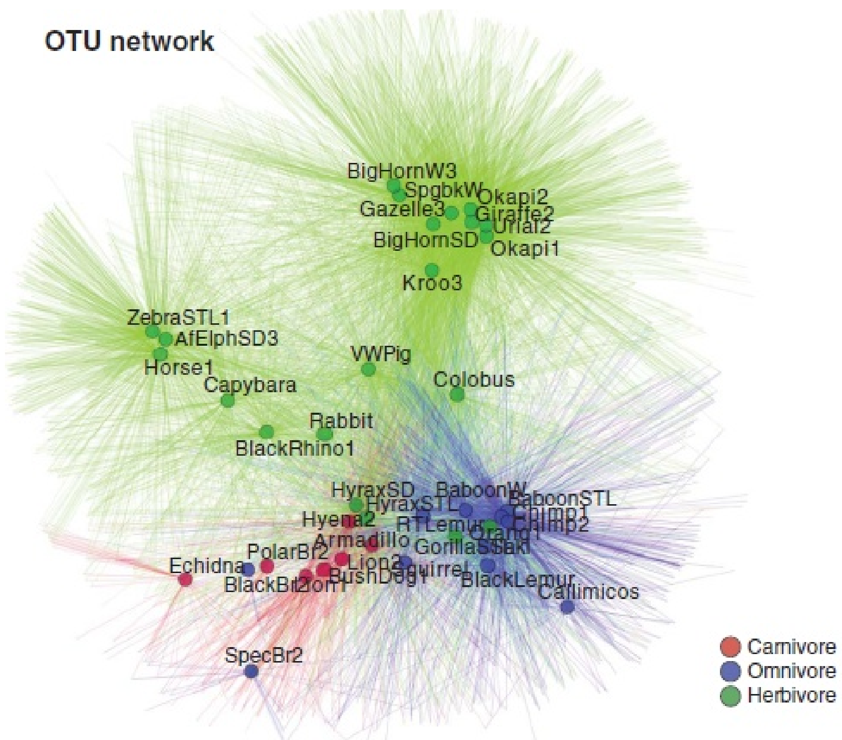
Serum IGF-1 is associated with increased risk of breast and prostate cancer

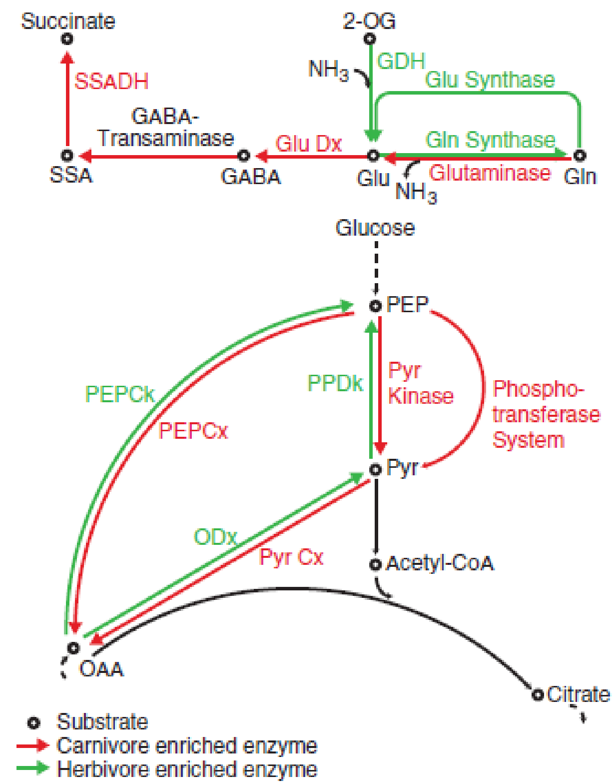
Plasma IGF	RR	RAR
Breast cancer (premenopausal, <50 years)		
<158 ng/mL	1·0	1·0
158–206 ng/mL	2·64	3·12
>207 ng/mL	4·58	7·28
Prostate cancer		
99–184 ng/mL	1·0	1·0
185–236 ng/mL	1·32	1·94
237–293 ng/mL	1·81	2·83
294–500 ng/mL	2·41	4·32

RR, relative risk; RAR, risk adjusted for IGFBP3.

Hankinson SE, Lancet 1998 & Chan JM, Science 1998

Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans





<u>In 18 CR individuals</u>:

- Protein intake associated with KO data (R=0.307; adjusted p=0.030)
- Insoluble fiber associated with bacterial OTU (R=0.371; adjusted p=0.013

OTU = operational taxonomic units
KO = KEGG orthology groups

Muegge et al. Science 2011

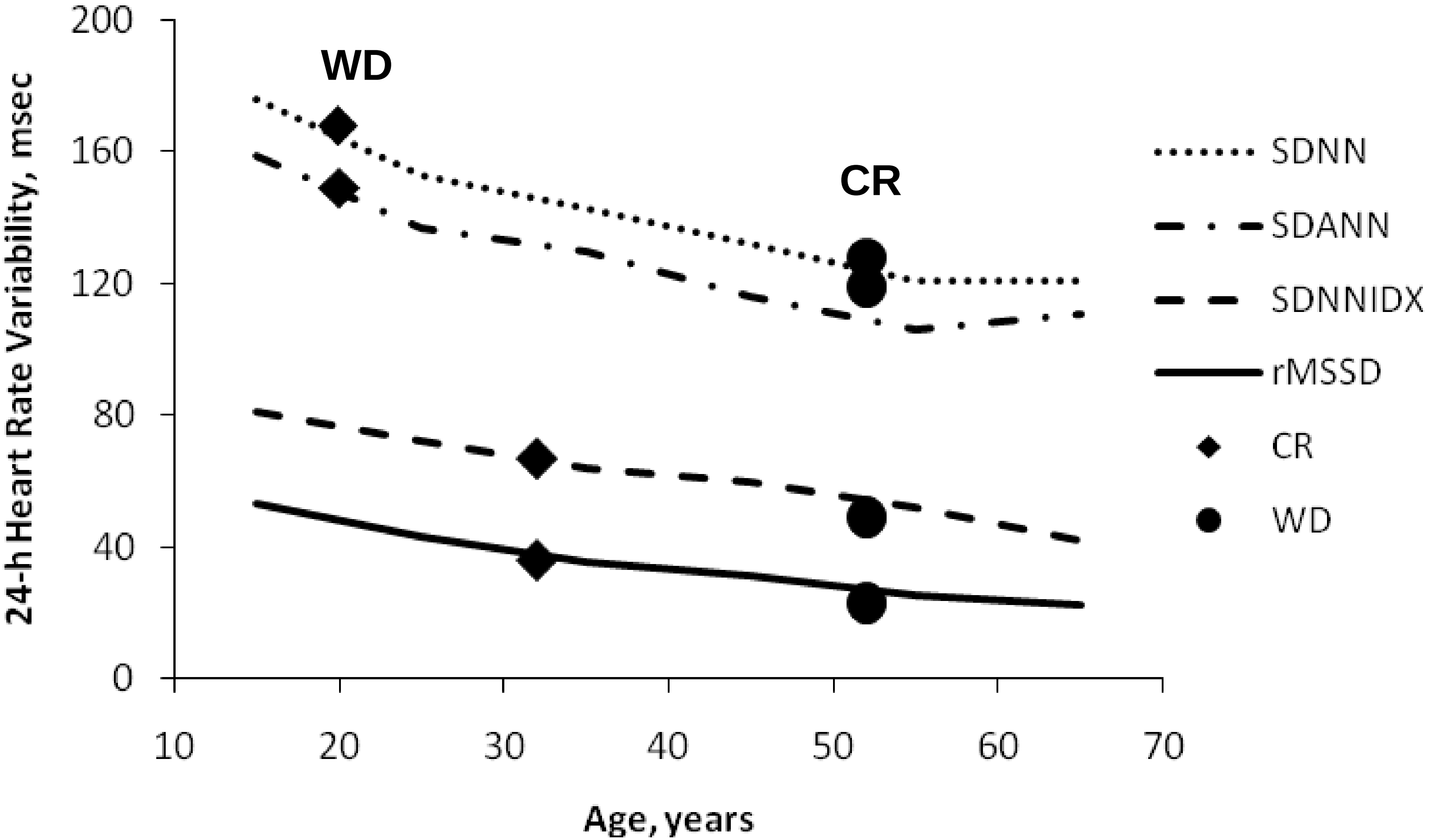
Long-term CR ameliorates biomarkers of aging

CR ameliorates the decline in diastolic function

		Western Diet	CR	
	Parameter	Mean±SD	Mean±SD	p value
Diastolic Function				
	E_{peak} (cm/sec)	64.3 ± 12.6	70.8 ± 13.4	ns
	A_{peak} (cm/sec)	53.0 ± 10.2	45.7 ± 9.0	0.011
	E/A	1.24 ± 0.28	1.61 ± 0.44	0.001
	Atrial filling fraction	0.35 ± 0.05	0.29 ± 0.06	0.0001
Tissue Doppler Imaging				
	$E'_{Lateral}$ (cm/sec)	10.2 ± 2.8	14.3 ± 3.0	0.001
Model Derived Parameters				
	c (g/sec)	19.6 ± 3.6	14.9 ± 5.0	0.001
	k (g/sec^2)	218.9 ± 44.6	180.1 ± 41.6	0.003

Meyer T et al. JACC 2006

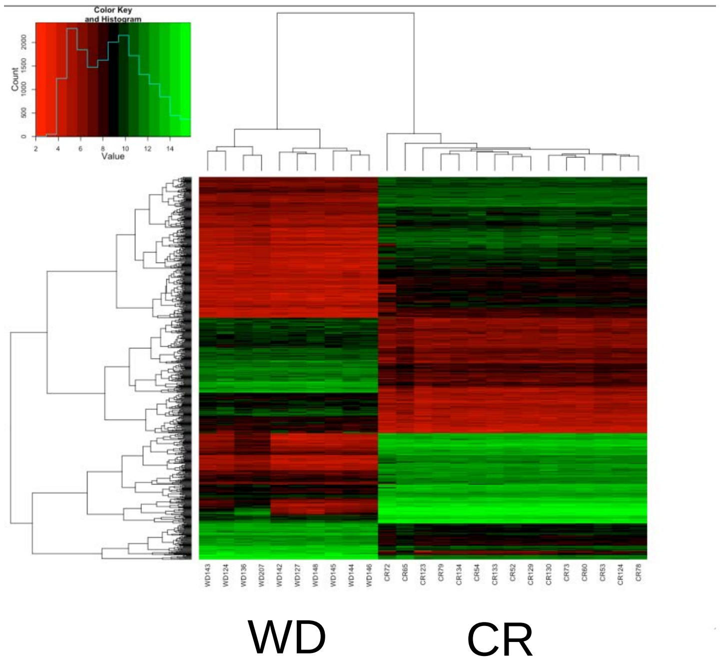
Long-term CR opposes the age-associated impairment of heart rate variability

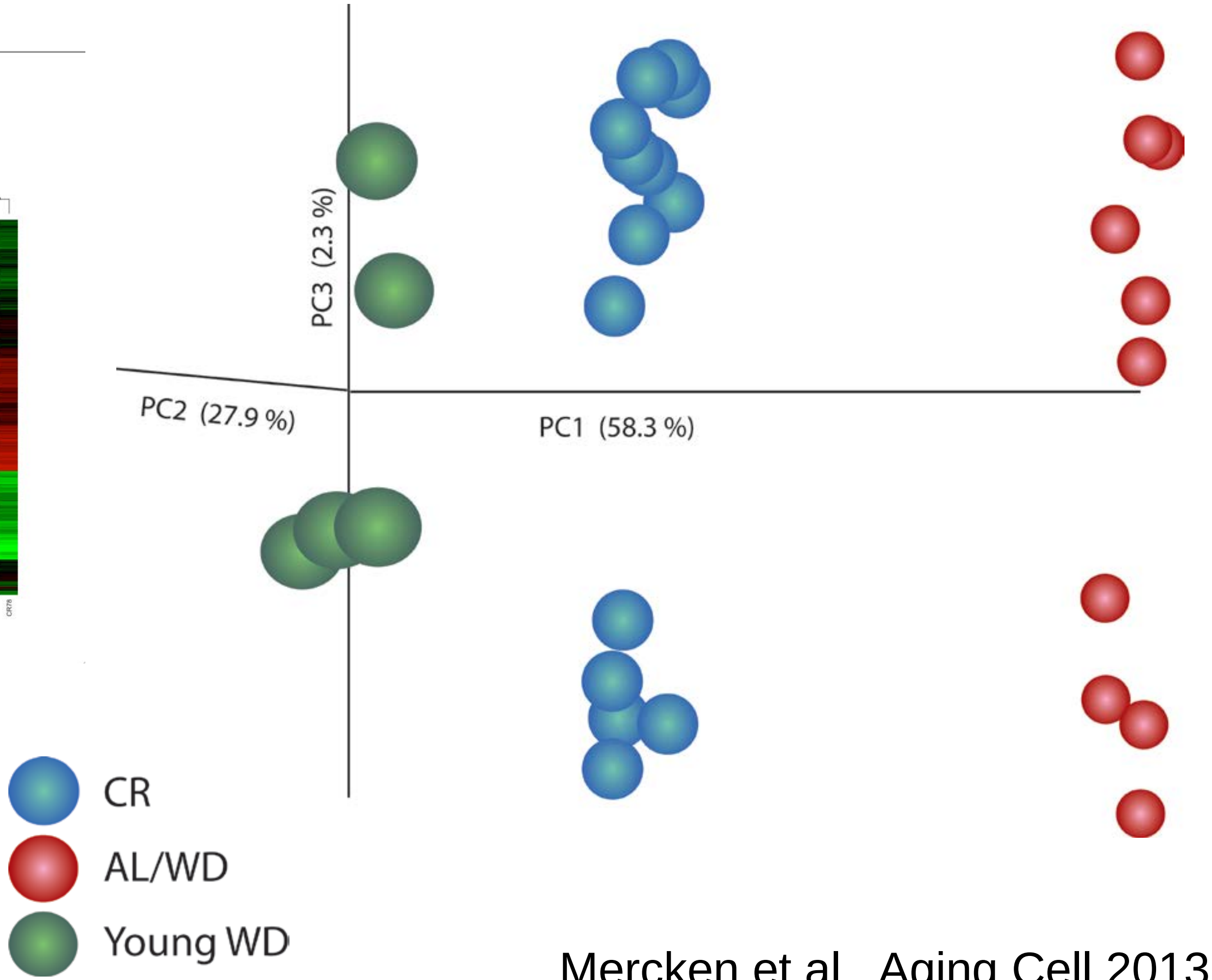


Umetani et al., JACC 1998

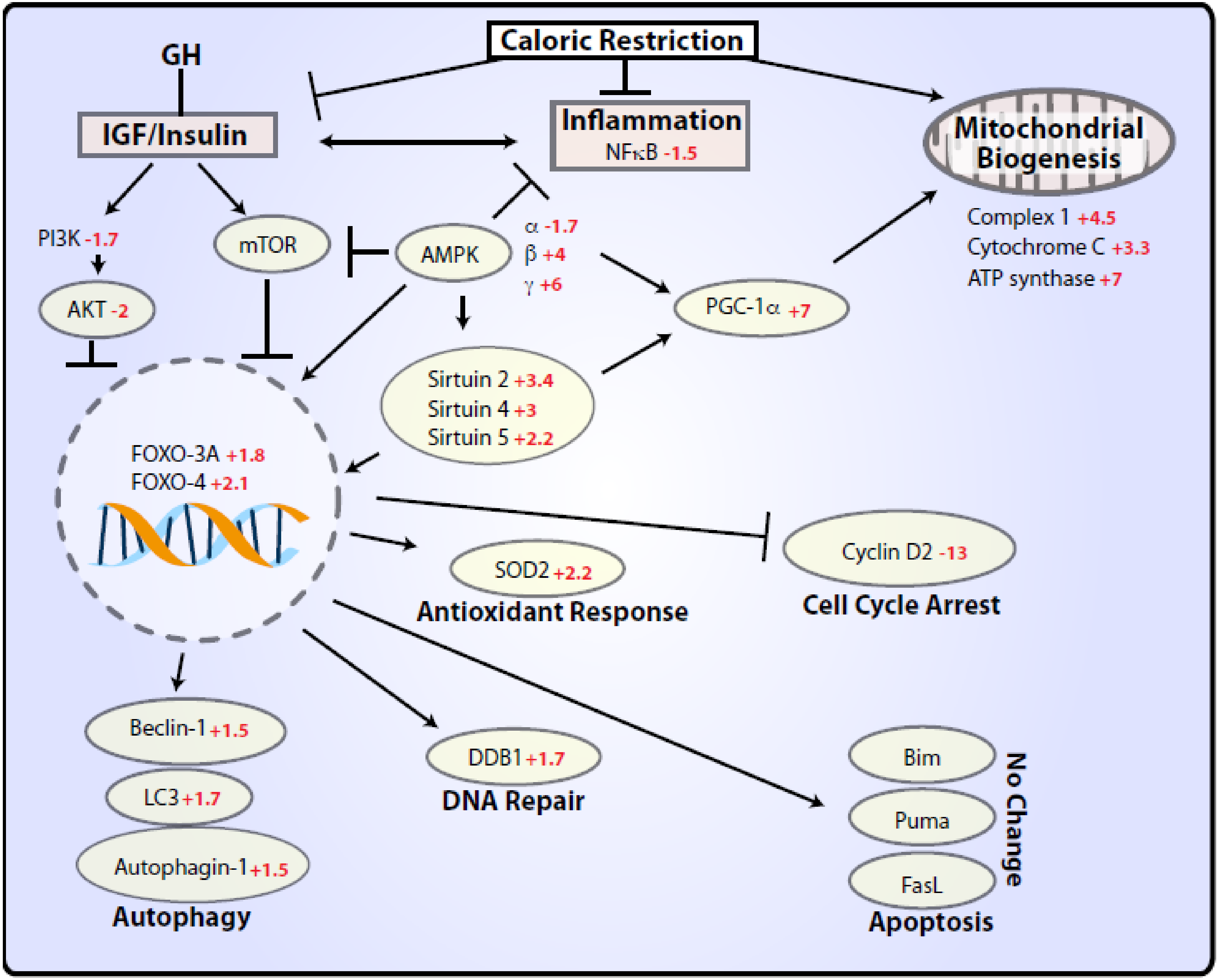
Stein PK et al. Aging Cell 2012

CR induces dramatic changes of the skeletal muscle transcriptional profile that resemble those of younger individuals



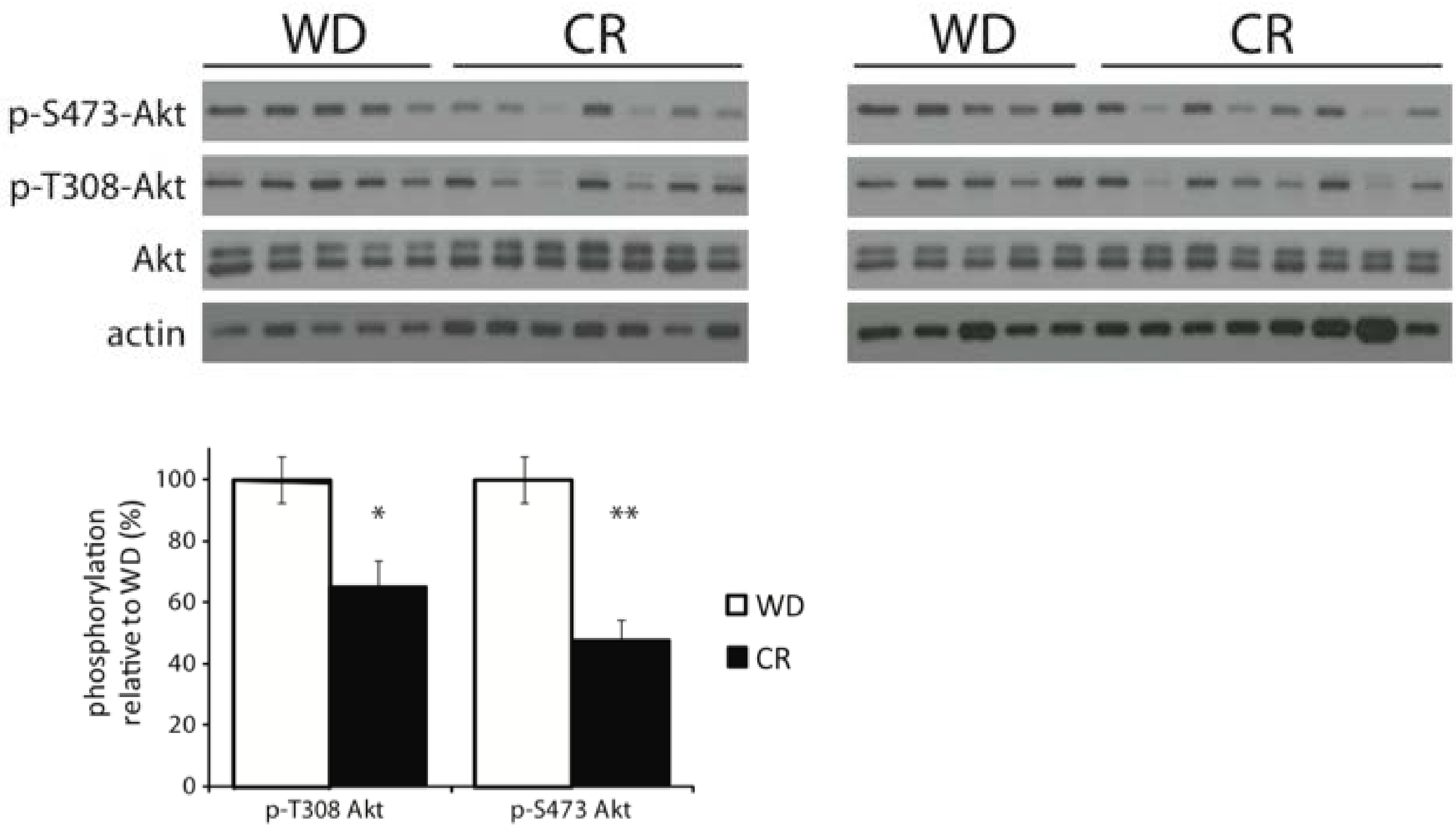


Mercken et al., Aging Cell 2013



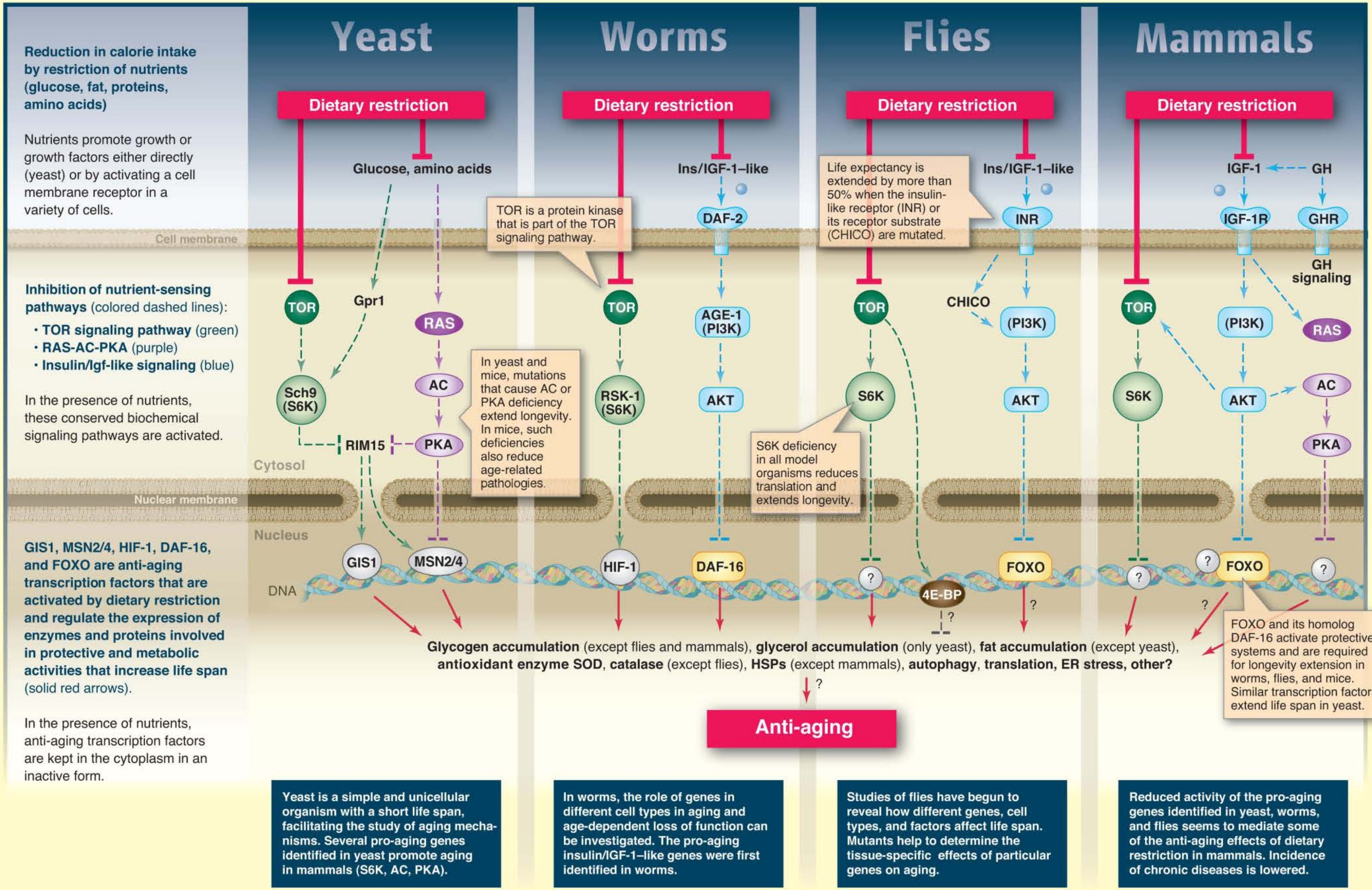
Mercken et al., Aging Cell 2013

Long-term CR decreases AKT phosphorylation at both serine and threonine residues in muscle



Mercken et al., Aging Cell 2013

Conserved Nutrient Signaling Pathways Regulating Longevity



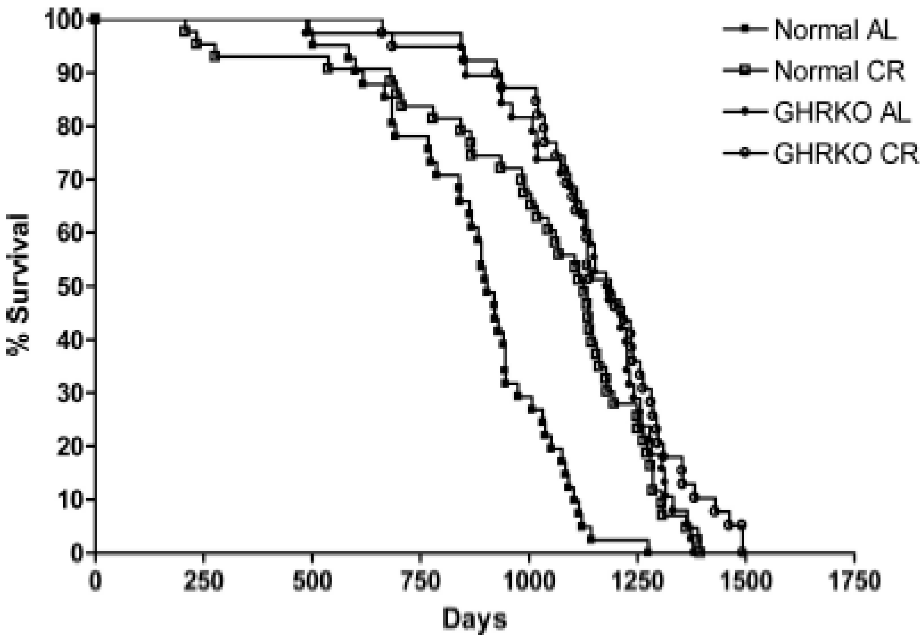
Fontana L et al. Science 2010

Mammalian animal models of longevity

- Calorie restriction and intermittent fasting
- Methionine restriction
- Ames and Snell dwarf mice
- Growth hormone receptor KO mice
- IGF-1 receptor deficient mice
- Klotho overexpressing mice
- Fat Insulin Receptor KO (FIRKO) mice
- Insulin Receptor Substrate 1 KO mice
- Brain IRS-2 KO mice
- PAPP-A KO mice
- Ribosomal S6 protein kinase-1 KO mice
- Transgenic overexpression of FGF-21
- Rapamycin supplementation
- SIRT6 overexpressing mice
- p66shc KO mice
- Type 5 Adenylyl Cyclase KO mice
- Angiotensin II type 1 receptor KO mice
- Mice overexpressing catalase targeted to mitochondria

Down regulation Insulin/IGF-1/mTOR pathway = Nutrient –sensing signaling pathways

GH receptor KO mice live 40-50% longer than WT mice

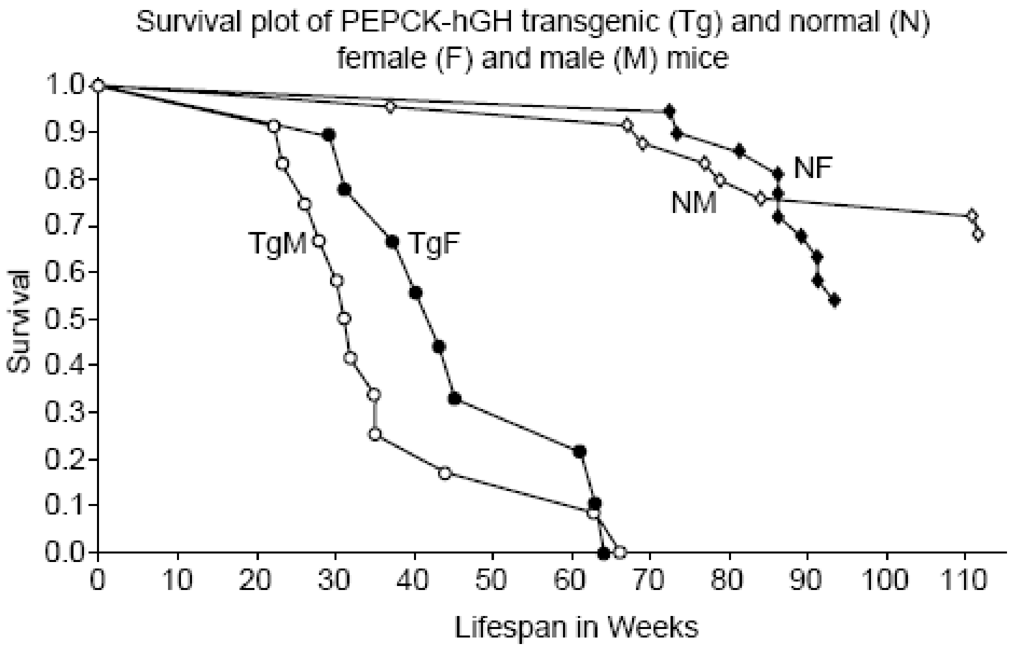


	N-AL	N-CR	KO-AL	KO-CR
Body weight (g)	36.7 ± 1.6[a]	29.5 ± 0.5[b]	15.8 ± 1.1[c]	13 ± 0.3[c]
Glucose (mg/dl)	189.4 ± 9.6[a]	139.7 ± 14.1[b]	155 ± 14.1[a,b]	74.8 ± 11.5[c]
Insulin (ng/ml)	4.2 ± 0.7[a]	1.4 ± 0.2[b]	0.9 ± 0.1[b]	0.4 ± 0.1[c]
IGF-I (ng/ml)	315 ± 29[a]	194 ± 33[b]	ND	ND
Corticosterone (ng/ml)	29.0 ± 8.1[a]	44.3 ± 11.2[a]	83.6 ± 18.6[b]	86.3 ± 11.6[b]
Glucagon (ng/ml)	62.8 ± 8.4	66.4 ± 11.6	75.8 ± 5.6	78.5 ± 9.8
Leptin (ng/ml)	5.9 ± 1.0[a]	2.3 ± 0.4[b]	9.9 ± 1.8[c]	4.7 ± 0.6[a,b]
Adiponectin (μg/ml)	5.5 ± 0.4[a]	5.1 ± 0.3[a]	7.7 ± 0.3[b]	8.6 ± 0.8[b]

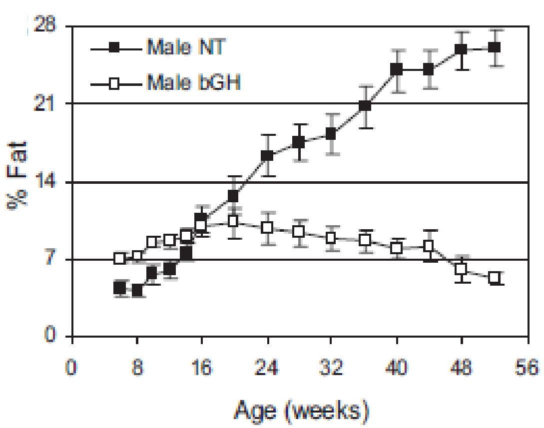
Bonkowski et al., PNAS 2006

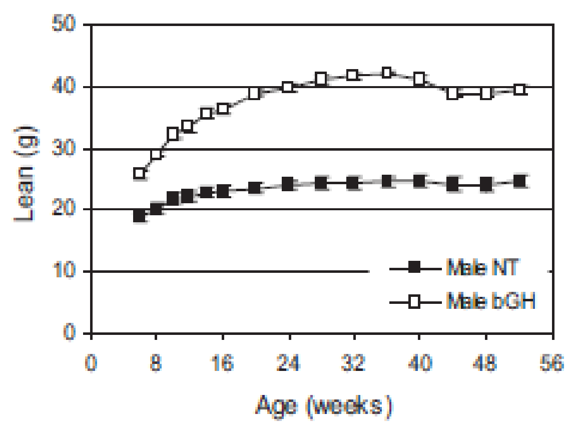
COSCHIGANO et al., Endocr 2000

Overexpression of GH receptor accelerate aging in mice



	NT	bGH
GH (ng/ml)	1.6 ± 0.5	636.3 ± 136[a]
IGF-I (ng/ml)	492.5 ± 20.1	868.4 ± 25.1[a]

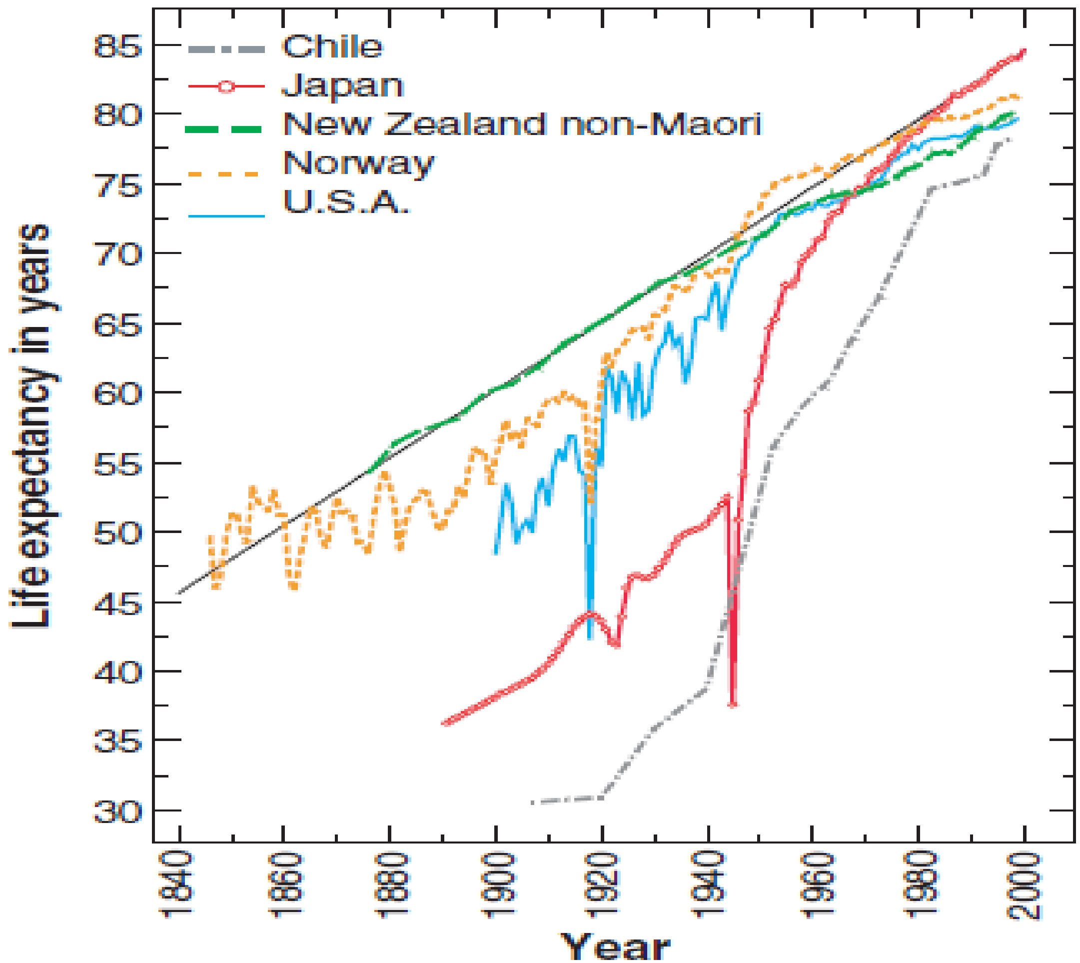




Bartke et al. Neuropeptides 2002

CONCLUSIONS
AND FUTURE DIRECTIONS

Life expectancy almost doubled between 1840 and 2007

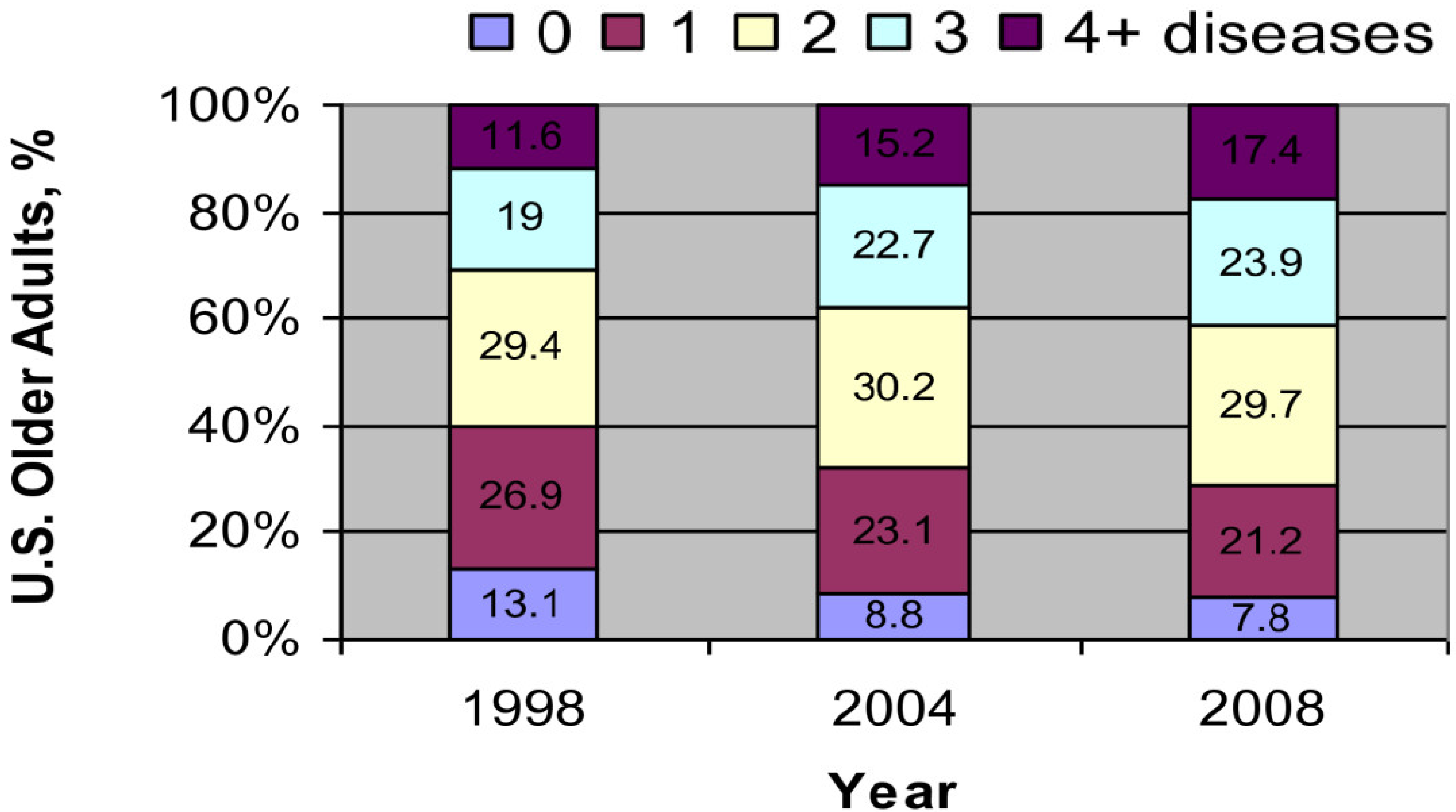


Christensen et al. Lancet 2009

Demographics:
older adults (65 yrs or older) in Italy

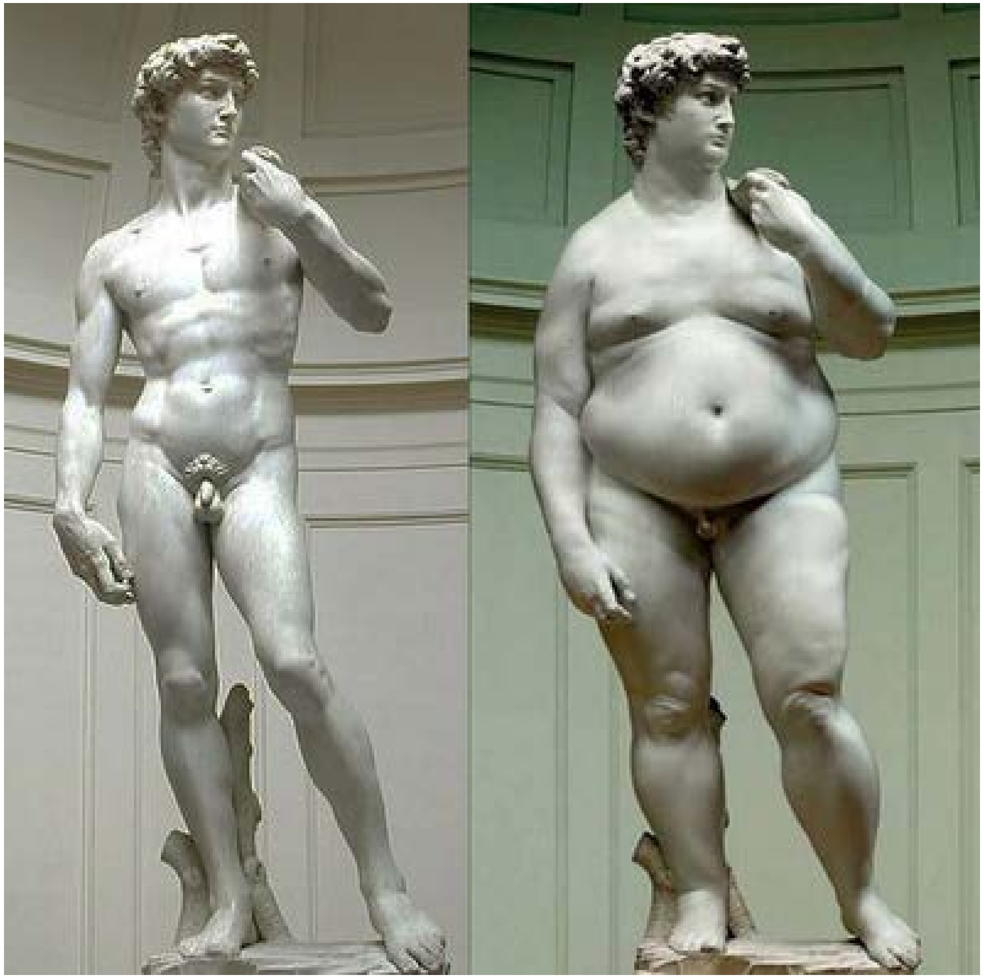
Years	Age distribution (%)			
	0-14	15-64	65+	80+
2001	14.4	67.4	18.2	4.1
2010	14.1	65.3	20.6	5.9
2030	11.6	60.4	28.0	9.4
2050	11.4	54.2	34.4	14.2

Prevalence of chronic disease in individuals older than 65 years

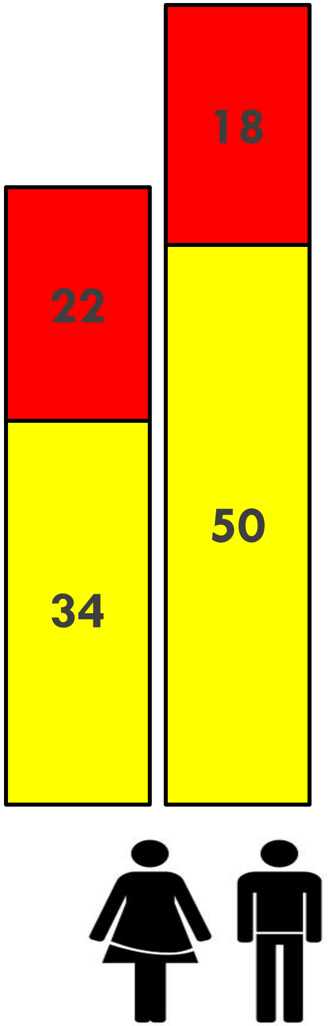


Hung *et al. BMC Geriatrics* 2011 **11**:47

Epidemic of overweight & obesity

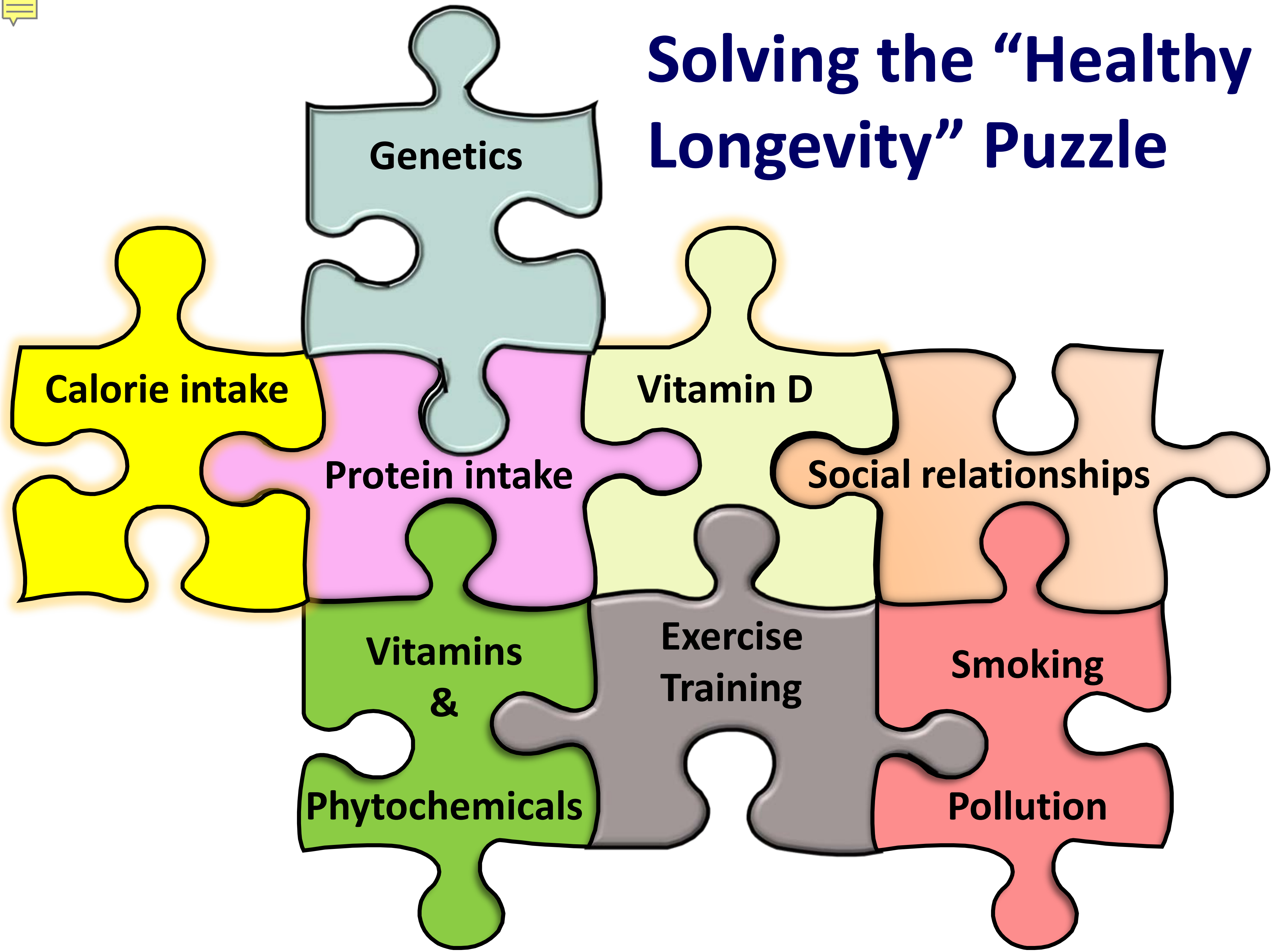


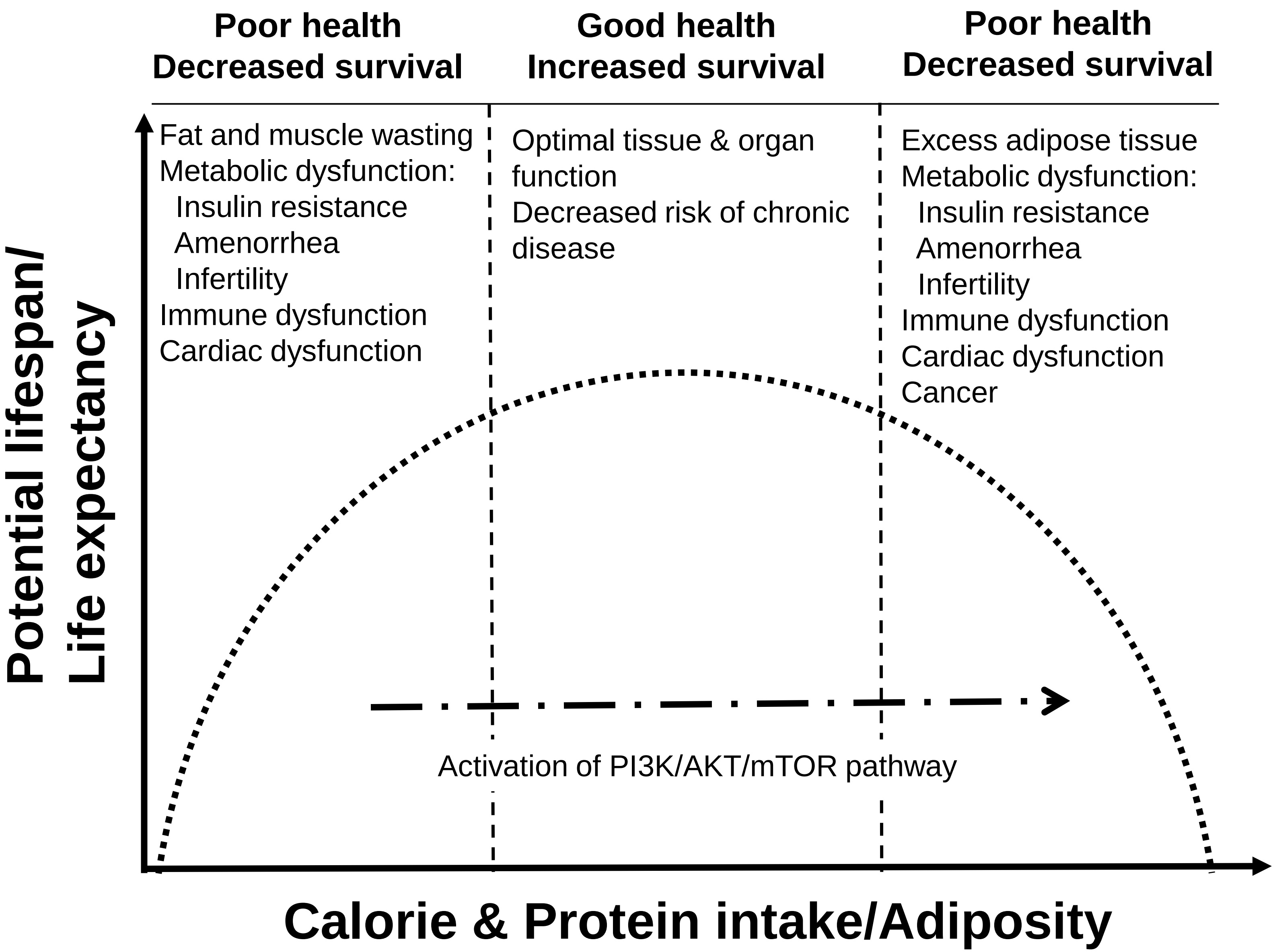
Source: “Progetto Cuore” ISS – 1998-2002



Adults (35-74 yrs)

Solving the "Healthy Longevity" Puzzle





Fontana et al. JAMA 2007

Acknowledgments

Washington University in St. Louis
SCHOOL OF MEDICINE

Division of Geriatrics and Nutritional Science, WUSTL, USA

- Timothy Meyer & Ted Weiss
- Dennis Villareal & Roberto Cangemi
- Kathie Obert & John Holloszy

Cardiovascular Biophysics Laboratory, WUSTL, USA

- Sandor Kovacs

FUNDING

Bakewell Foundation
AFAR
Glenn Foundation
Longer Life Foundation
Scott and Annie Appleby Charitable Trust
NIH

Department of Medicine, Salerno University Medical School, Italy

CEINGE Biotecnologie Avanzate, Naples, Italy

- Daniela Omodei & Domenico Martone
- Pasqualina Buono & Lucia Sacchetti
- Franco Salvatore

Massachusetts Institute of Technology (MIT), Cambridge, MA, USA

- David Sabatini

Lab of Experimental Gerontology, NIA, NIH, Baltimore, USA

- Rafael De Cabo

Rosewell Park Cancer Institute, Buffalo, NY, USA

- Roberto Pili