Excessive adiposity, calorie restriction and age-associated disease

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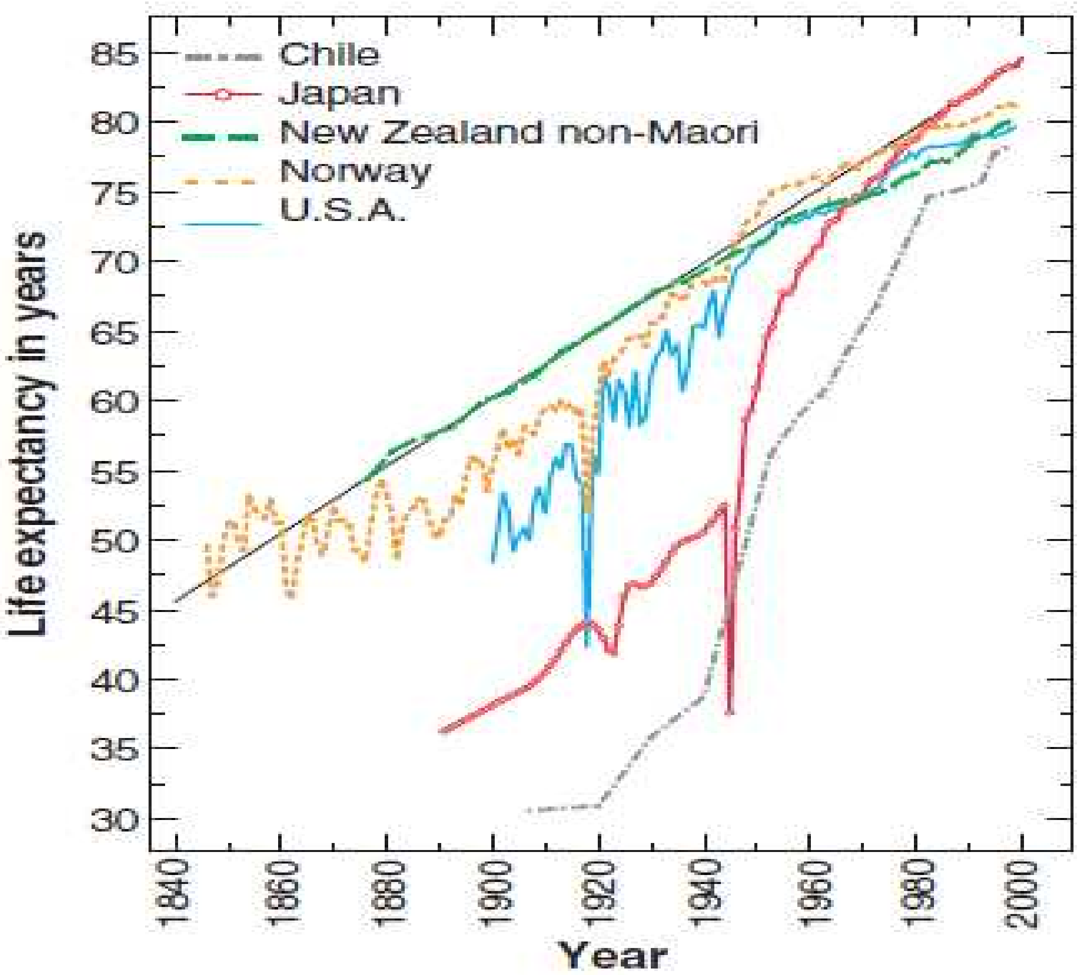
Life expectancy almost doubled between 1840 and 2007



Christensen et al. Lancet 2009

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

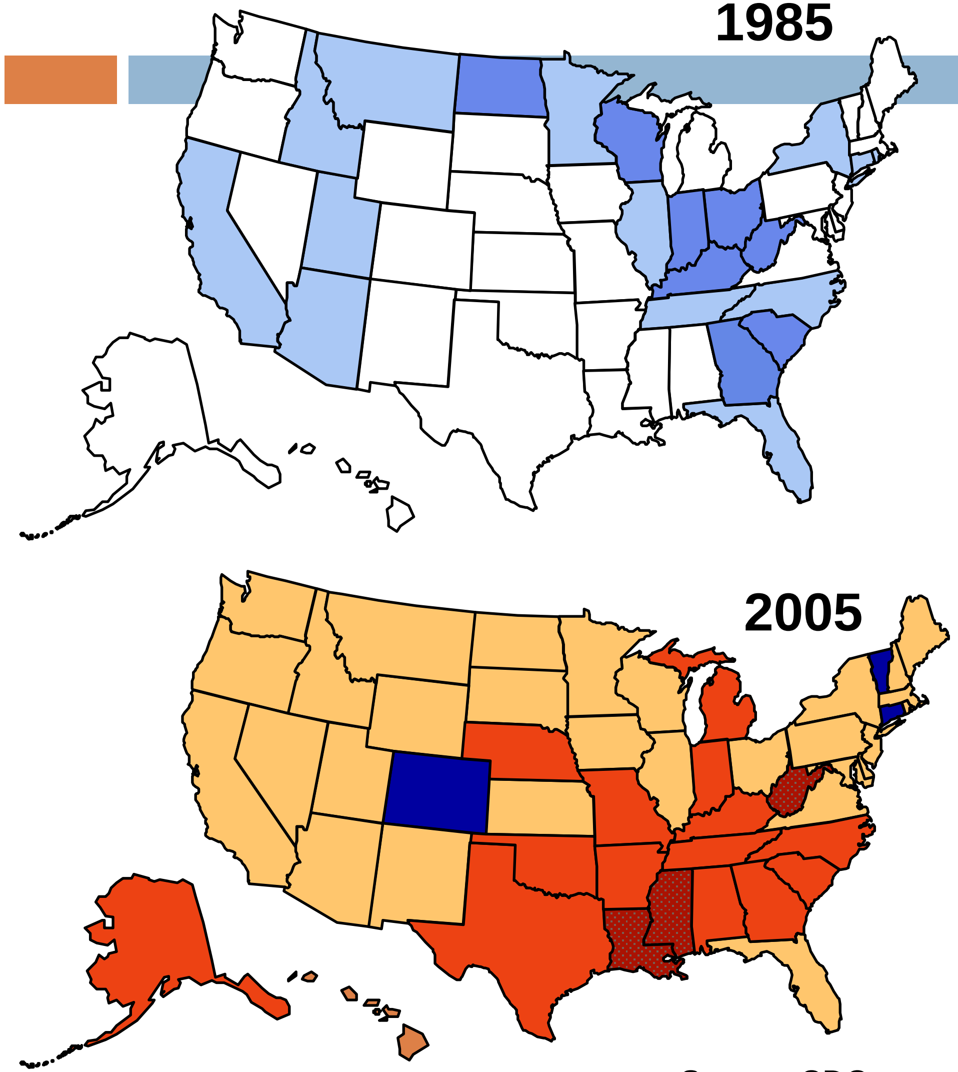
Years	Age distribution (%)			
	0-14	15-64	65+	80+
2001	14.4	67.4	18.2	4.1
2010	14.1	65.3	20.6	5.9
2030	11.6	60.4	28.0	9.4
2050	11.4	54.2	34.4	14.2

Prevalence of chronic disease

- About 80% of adults over 65 years of age have at least one chronic disease, and 50% have two or more chronic diseases.

- Cardiovascular disease, cancer, stroke and diabetes account for nearly 70% of the deaths in the United States and Europe

Epidemic of overweight/obesity in USA

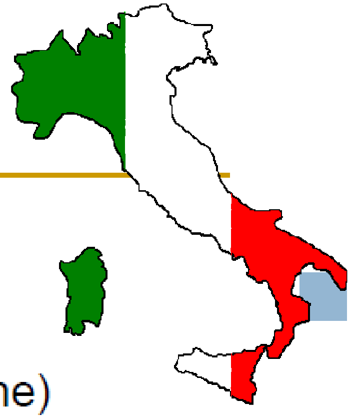


Source: CDC

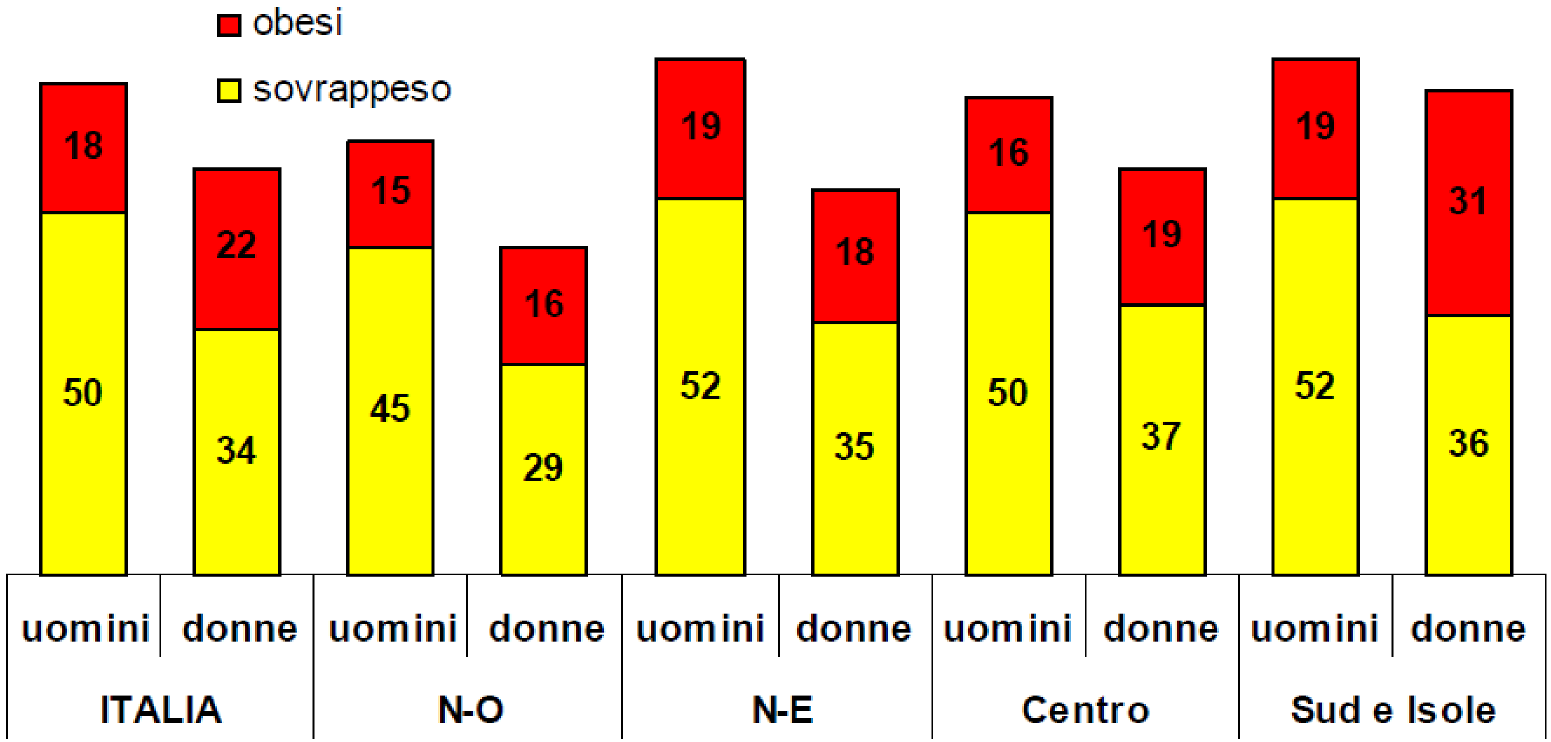
Botero: Una Familia

No Data <10 10%– % 5%–19% 20%–24% 25%–29%
≥30%

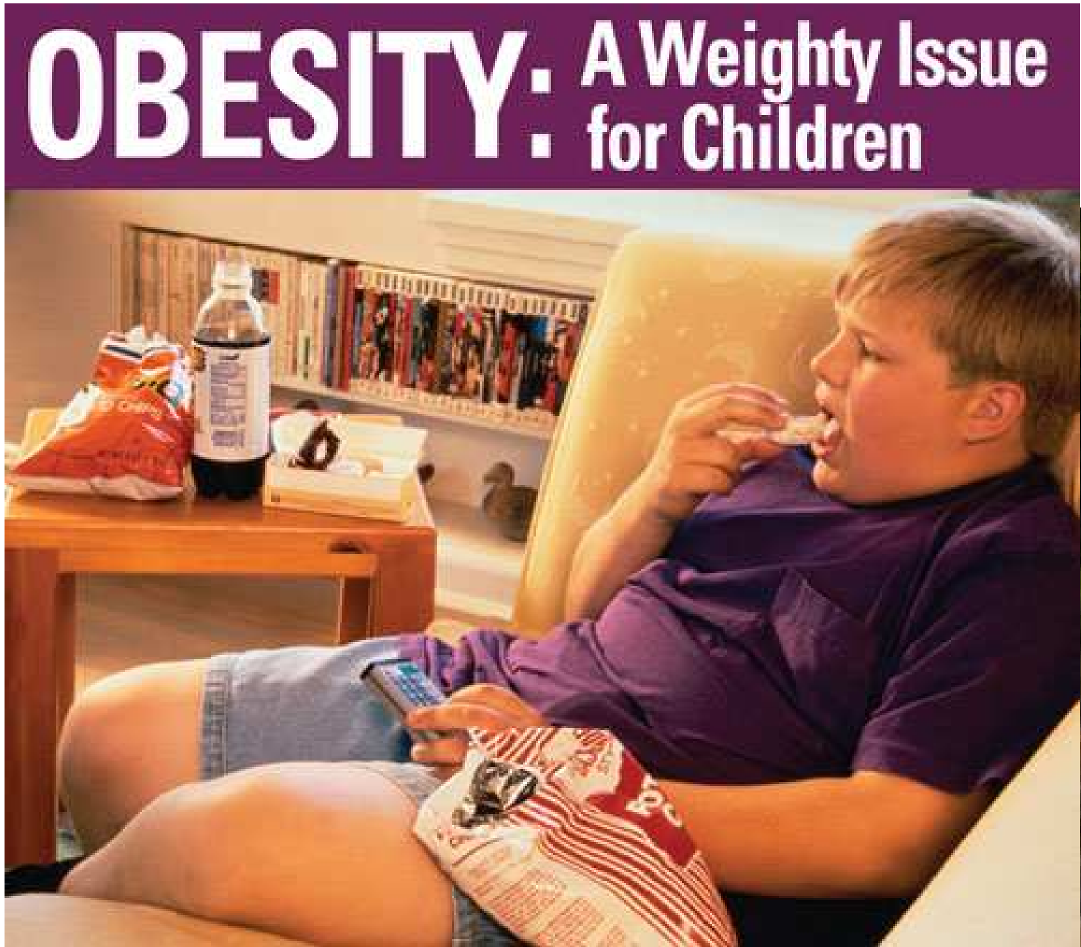
DATI EPIDEMIOLOGICI: ITALIA



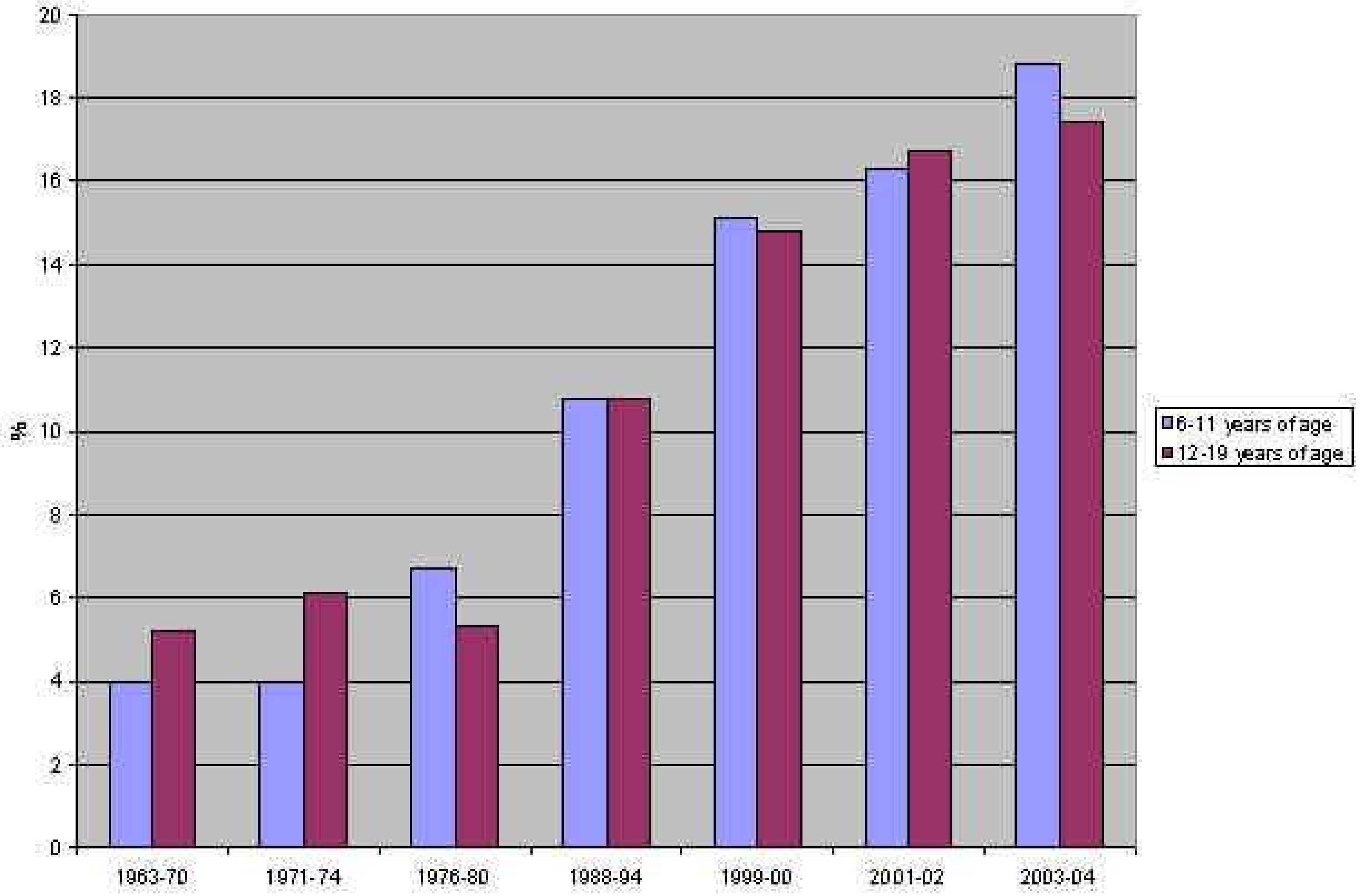
Percentuali di obesi e soprappeso
in campioni della popolazione italiana di età 35 - 74 anni
numerosità del campione: 9.712 soggetti (4.908 uomini e 4.804 donne)



Fonte: Ministero della Salute – ISS: Progetto "CUORE" – anni 1998-2002


OBESITY: A Weighty Issue for Children


Prevalence of obesity among children and adolescents in the USA



Medical Complications of Excessive Adiposity

Stroke

Pulmonary disease
abnormal function
obstructive sleep apnea
hypoventilation syndrome

Pancreatitis

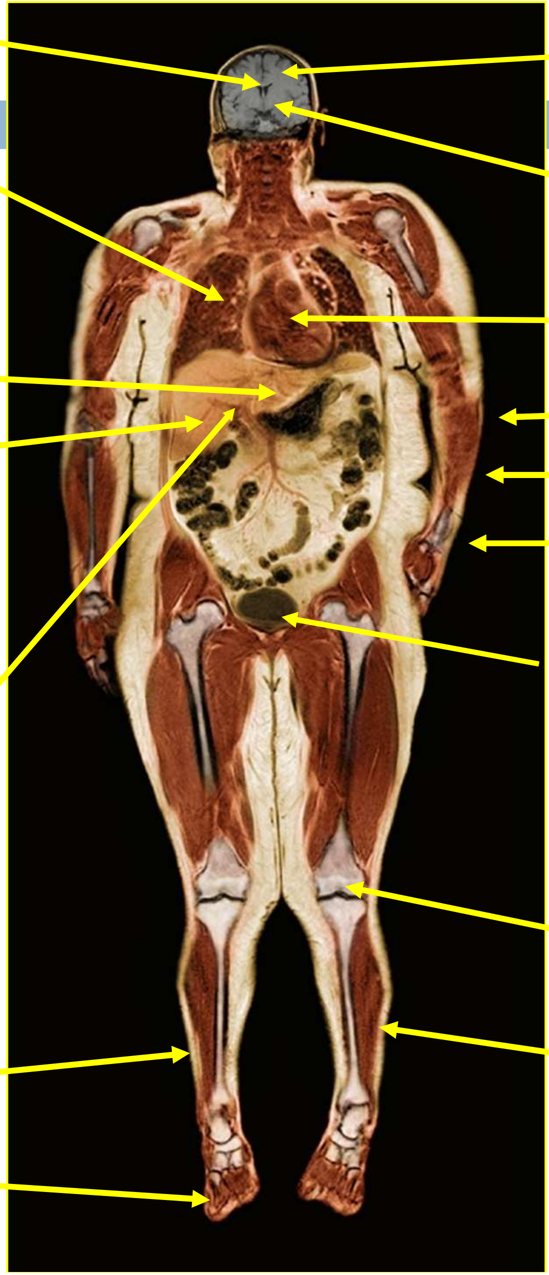
Nonalcoholic fatty liver disease
steatosis
steatohepatitis
cirrhosis

Gall bladder disease

Cancer
colon, breast, uterus, kidney, esophagus, pancreas, liver

Skin

Gout



Idiopathic intracranial hypertension

Cataracts

Coronary heart disease

Diabetes

Dyslipidemia

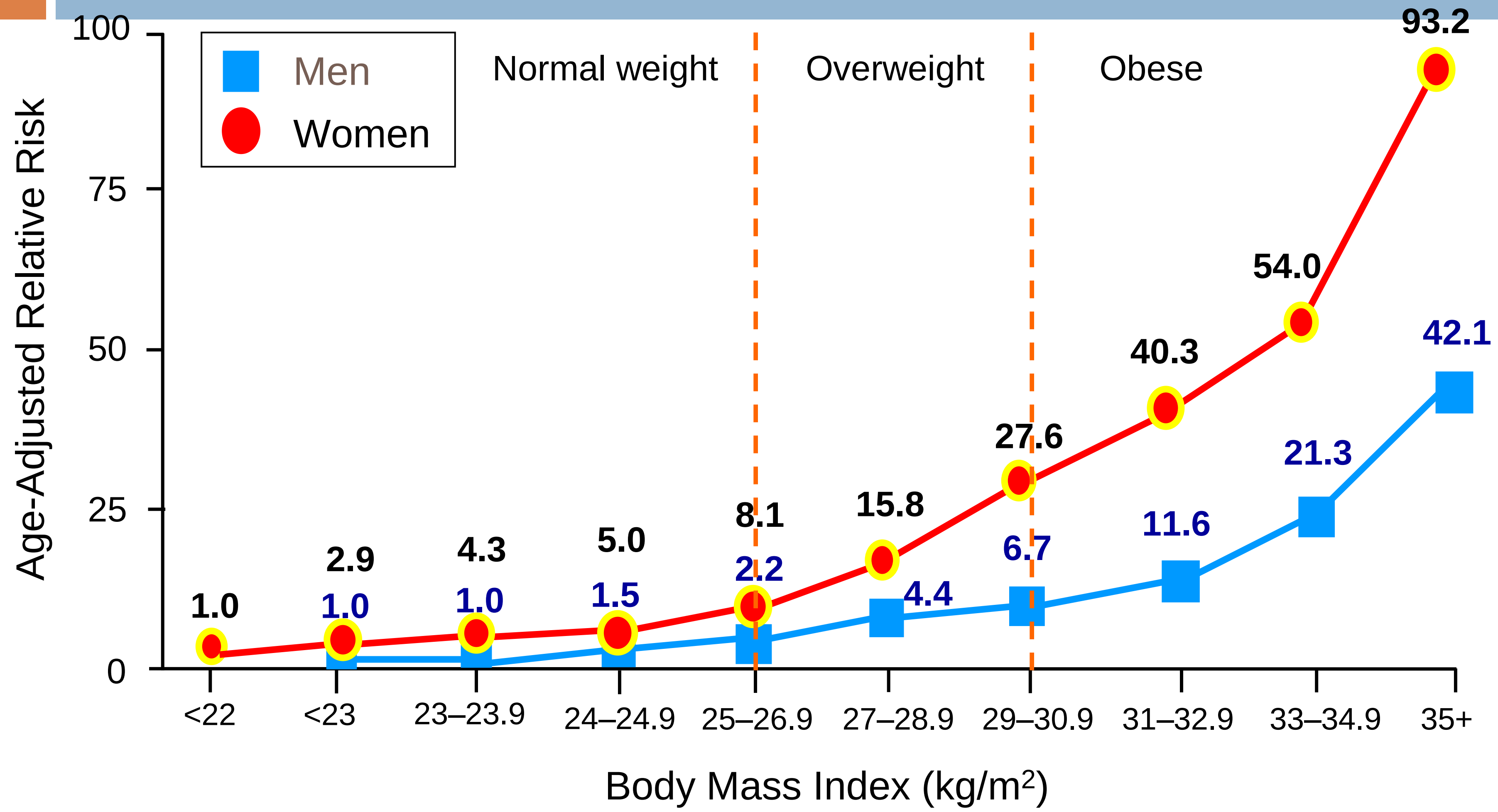
Hypertension

Gynecologic abnormalities
abnormal menses
infertility
polycystic ovarian syndrome

Osteoarthritis

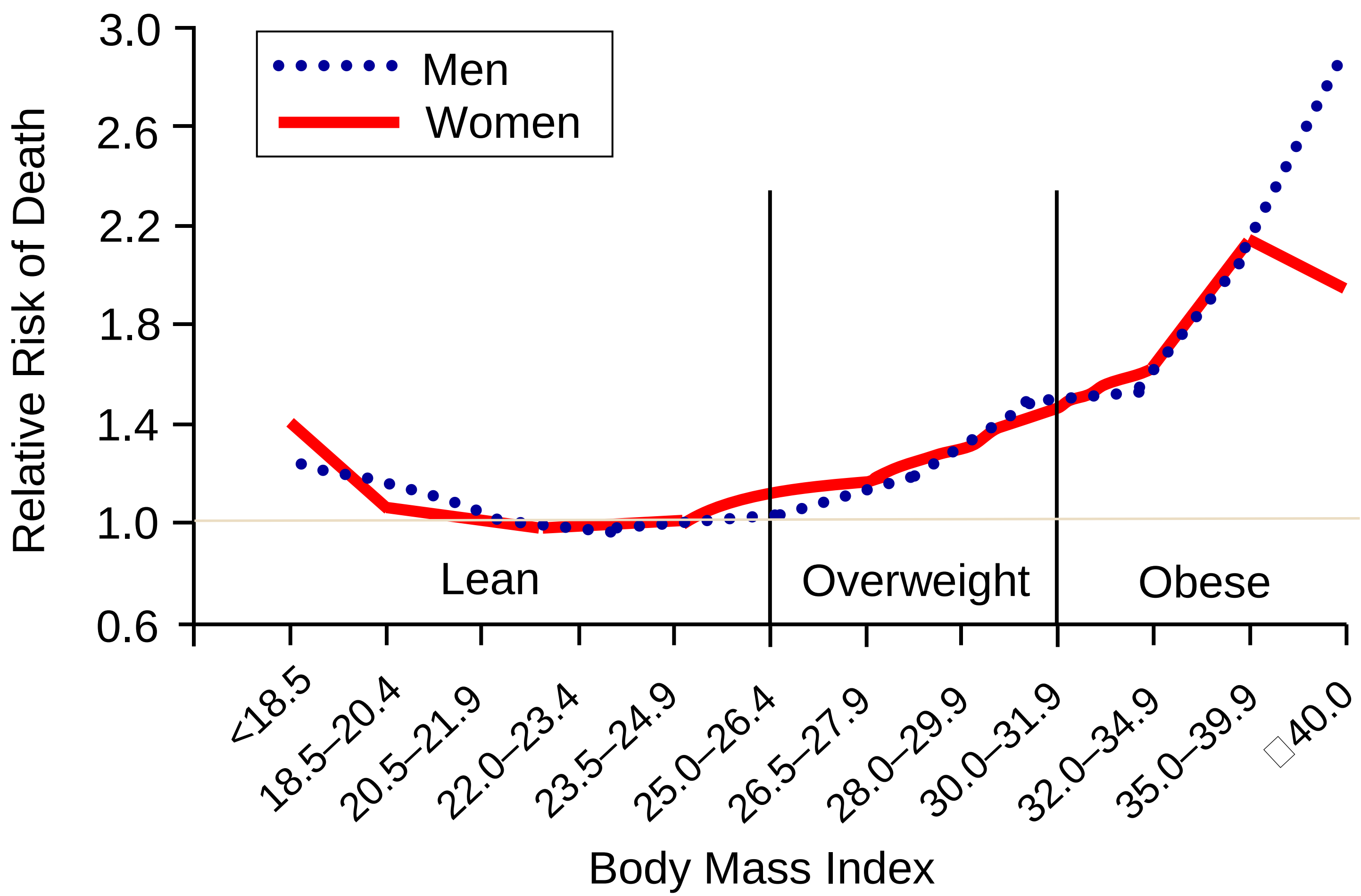
Phlebitis
venous stasis

Relationship Between BMI and Risk of Type 2 Diabetes Mellitus



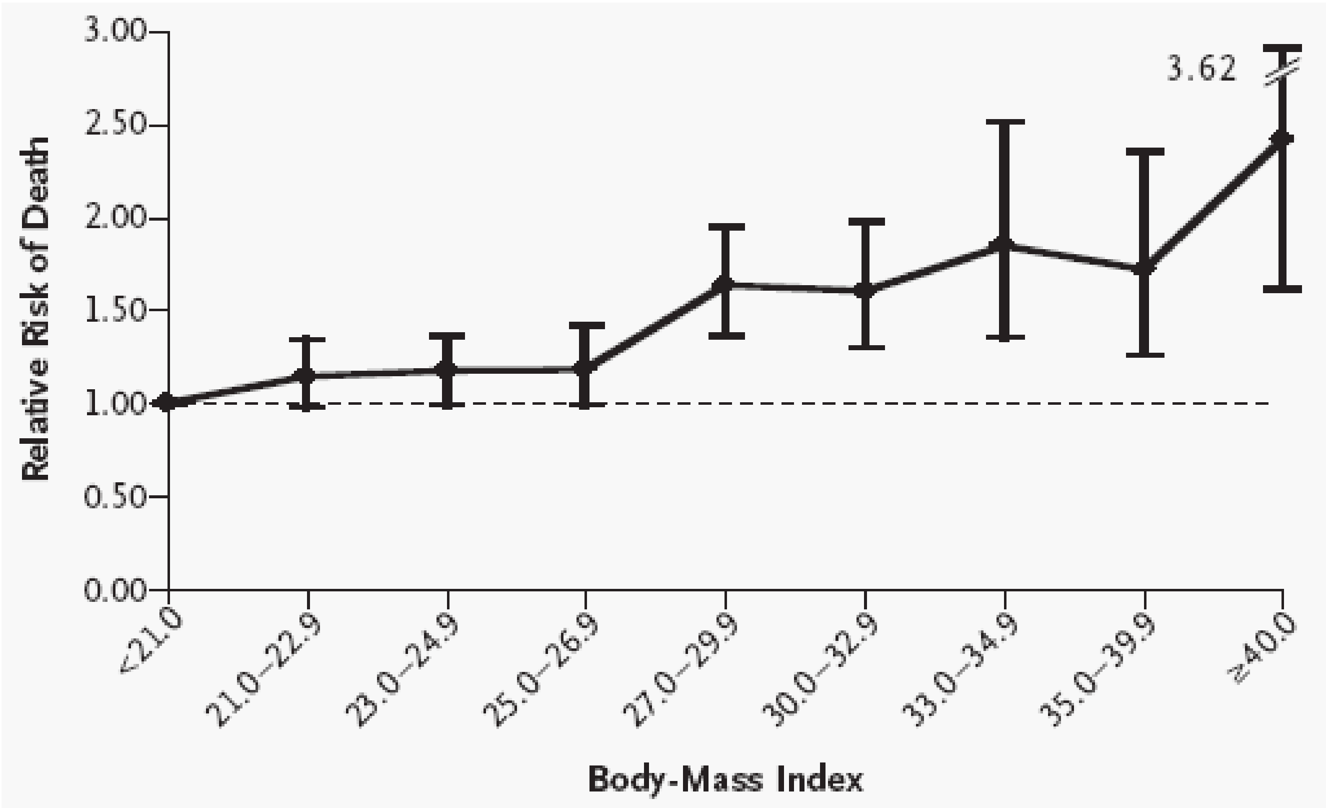
Chan J et al. *Diabetes Care* 1994;17:961.
Colditz G et al. *Ann Intern Med* 1995;122:481.

Relationship Between BMI and Cardiovascular Disease Mortality



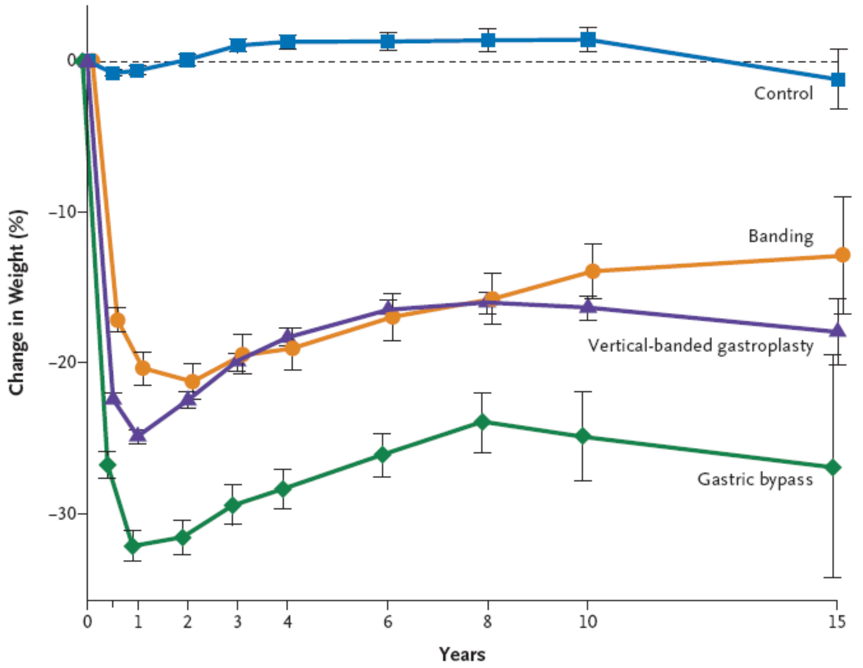
Calle et al. *N Engl J Med* 1999

Relationship Between BMI and Cancer Mortality in Women who never smoked



Hu FB et al., NEJM 2004

Effects of Bariatric Surgery on Mortality in Swedish Obese Subjects



No. Examined									
Control	2037	1768	1660	1553	1490	1281	982	886	190
Banding	376	363	357	328	333	298	267	237	52
Vertical-banded gastroplasty	1369	1298	1244	1121	1086	1004	899	746	108
Gastric bypass	265	245	245	211	209	166	92	58	10

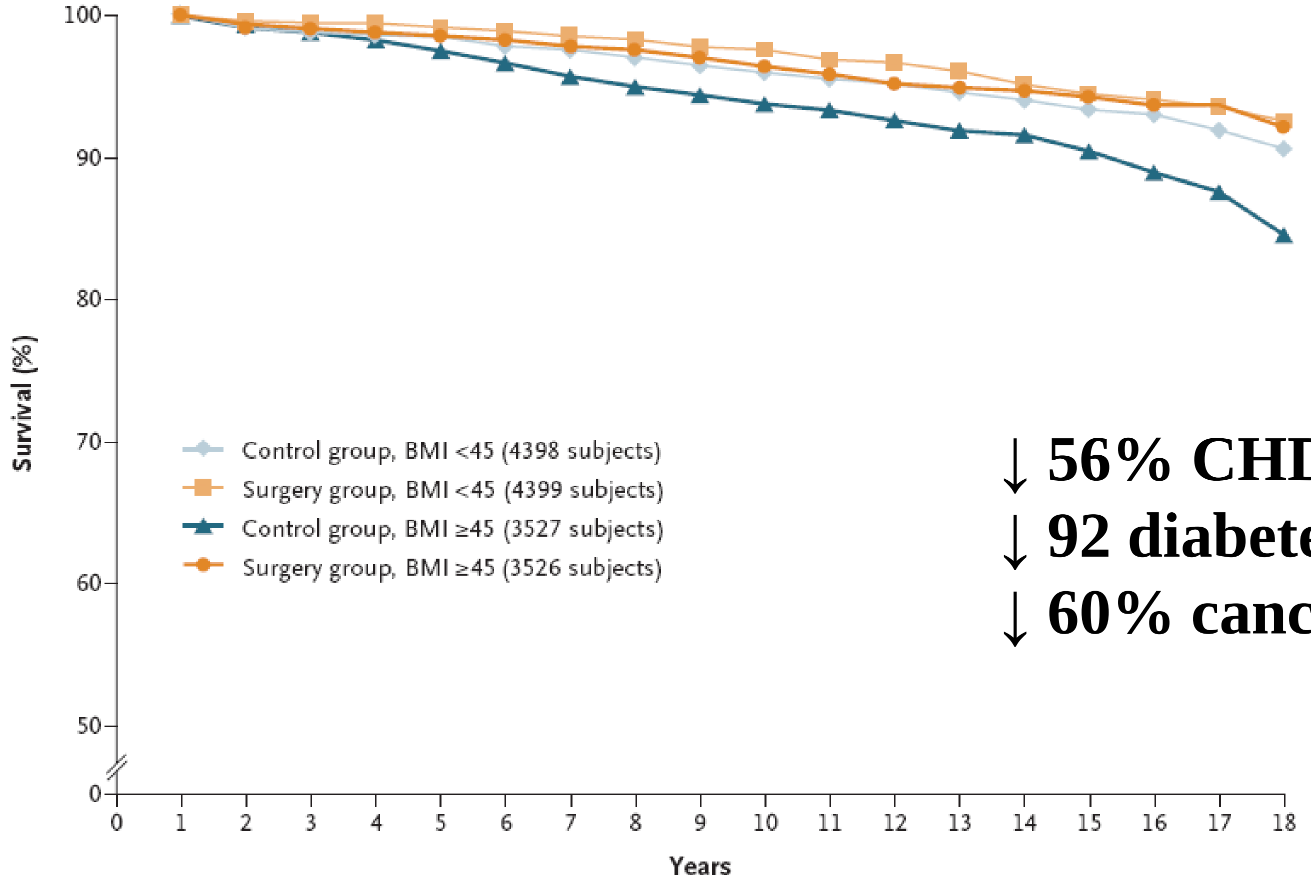
Variable	Surgery Group (N=2010)	Control Group (N=2037)
	no. of subjects	
Cardiovascular condition		
Any event	43	53
Cardiac	35	44
Myocardial infarction	13	25
Heart failure	2	5
Sudden death	20	14
Stroke	6	6
Intracerebral hemorrhage	2	4
Infarction	1	2
Noncardiovascular condition		
Any event	58	76
Tumor	29	48
Cancer	29	47
Total no. of deaths	101	129

↓ ~ 48%

↓ ~ 40%

SjÖström et al. N Engl J Med 2007

Long-Term Mortality after Gastric Bypass Surgery Reduced by 40%



↓ 56% CHD

↓ 92 diabetes

↓ 60% cancer

No. of Deaths	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Control group	41	66	85	117	153	176	199	219	234	244	259	271	281	294	302	310	318	327
Surgery group	42	54	62	74	86	102	113	132	141	159	169	182	192	202	206	210	213	213

Adams et al. N Engl J Med 2007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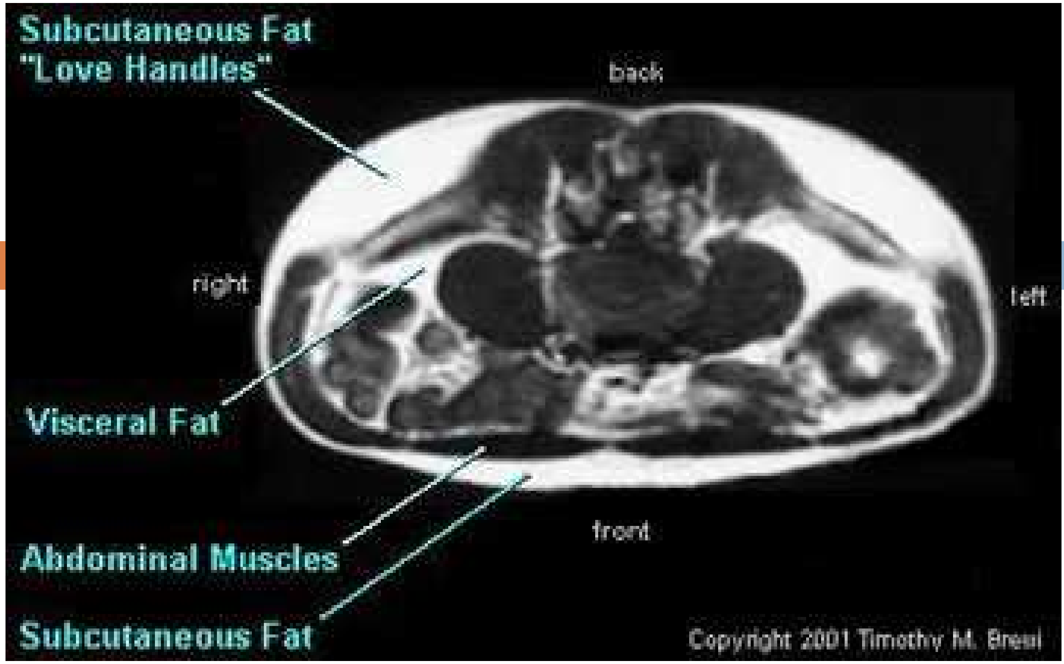
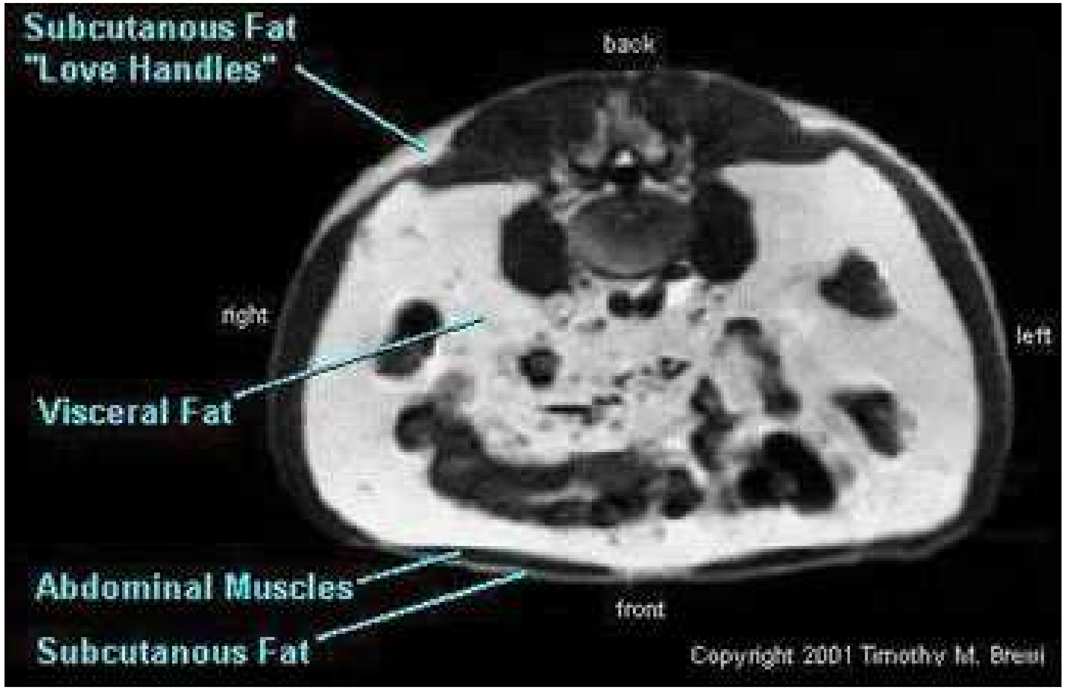
Lynn S. Ludwig

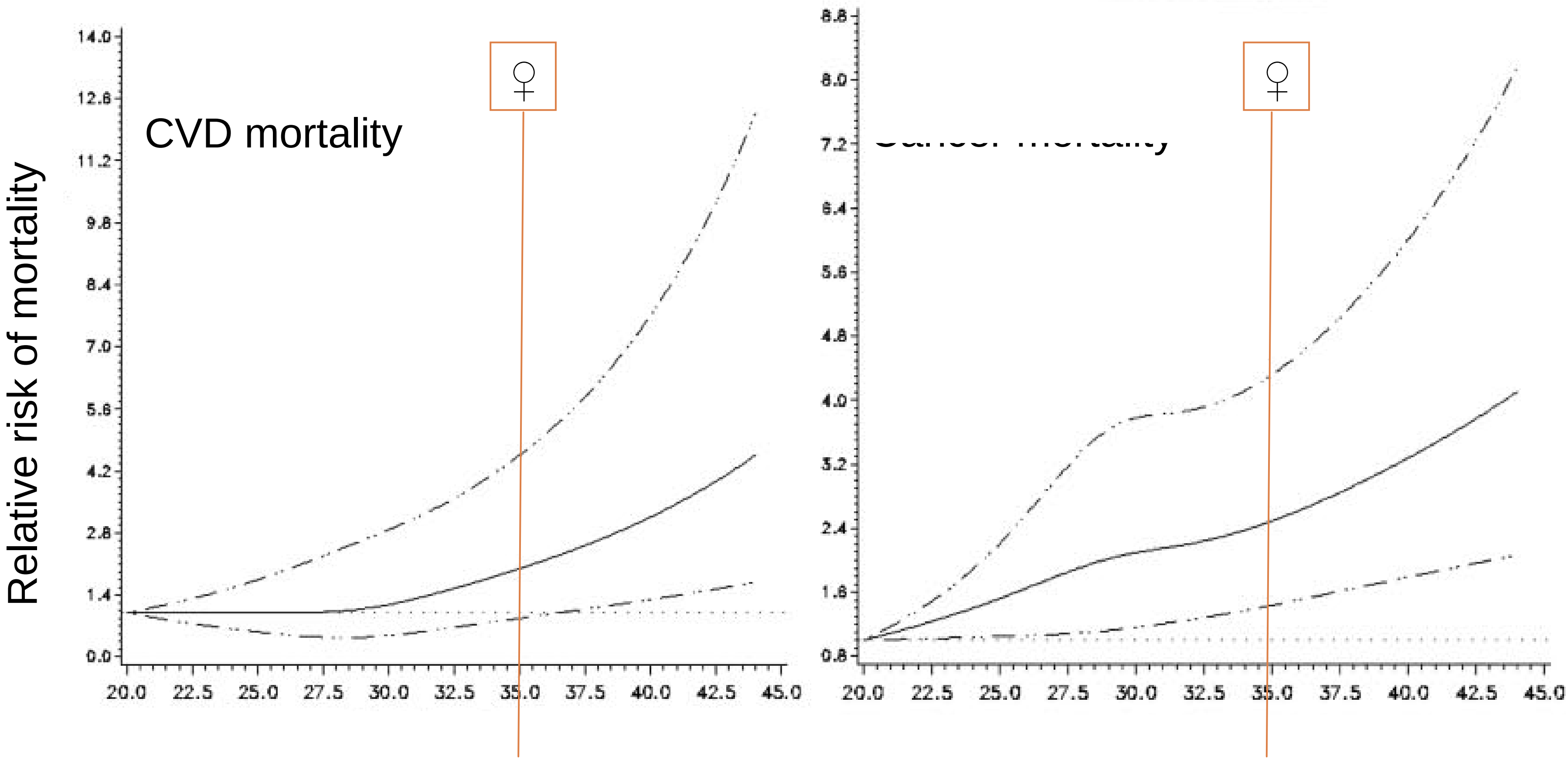


Normal



Visceral Obesity

Waist circumference and relative risk of CVD and cancer mortality



Zhang, C. et al. Circulation 2008

Ethnic specific values for waist circumference

Country/Ethnic group		Waist circumference*
Europids *In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes*	Male	≥ 94 cm
	Female	≥ 80 cm
South Asians *Based on a Chinese, Malay and Asian-Indian population*	Male	≥ 90 cm
	Female	≥ 80 cm
Chinese	Male	≥ 90 cm
	Female	≥ 80 cm
Japanese	Male	≥ 85 cm
	Female	≥ 90 cm
Ethnic South and Central Americans	*Use South Asian recommendations until more specific data are available*	
Sub-Saharan Africans	*Use European data until more specific data are available*	

Alberti KG et al. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006

Odds ratios of successful aging to age 70+ associated with mid-life waist circumferences in women

	Waist circumference				*P* for trend
	<71 cm	71-75 cm	76-80 cm	81-87 cm	
Case/No.	232/1081	304/1642	238/1683	211/2208	
Age-adjusted	1.0	0.87 (0.72 to 1.05)	0.66 (0.54 to 0.81)	0.45 (0.37 to 0.55)	<0.001
Multivariable‡	1.0	0.87 (0.71 to 1.07)	0.74 (0.58 to 0.93)	0.59 (0.45 to 0.77)	<0.001

Successful survival to age 70+ years was defined as having no history of 11 major chronic diseases and having no substantial cognitive, physical, or mental limitations.

Sun et al. BMJ 2009

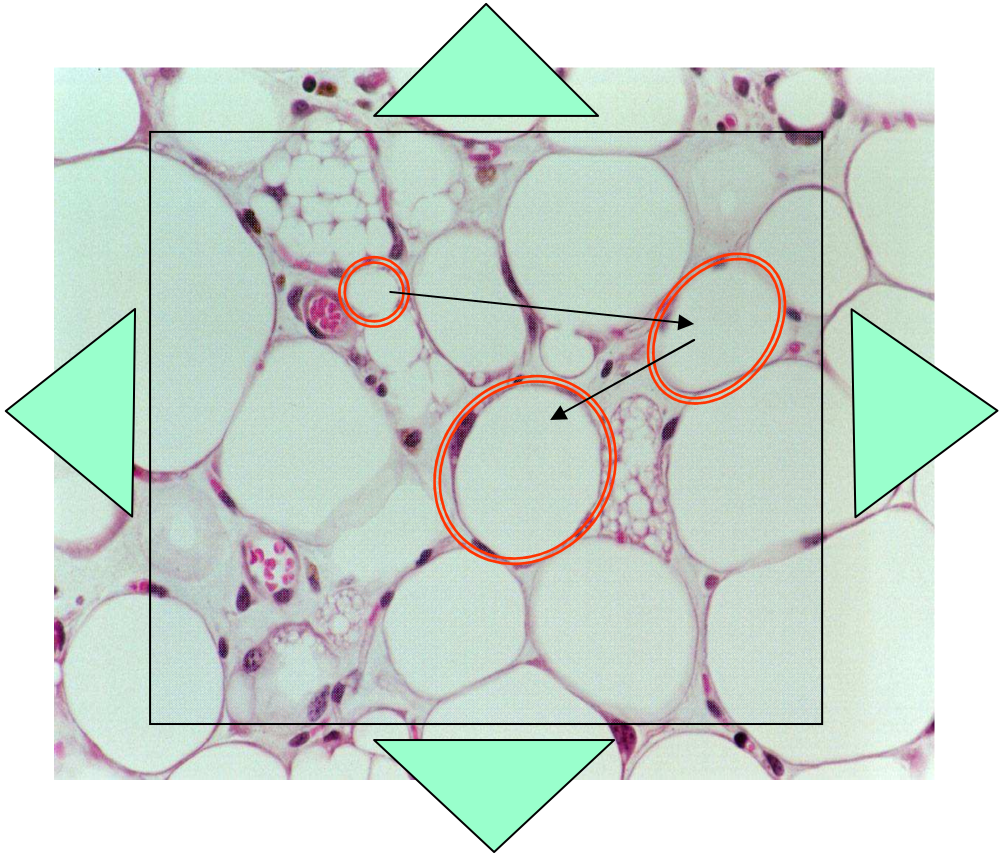
Adipose tissue as a secretory organ

↑ PAI-1, Angiotensinogen, IGF-1, TGF-β

↑ Leptin

↓ Adiponectin

↑ Resistin



↑ IL-6

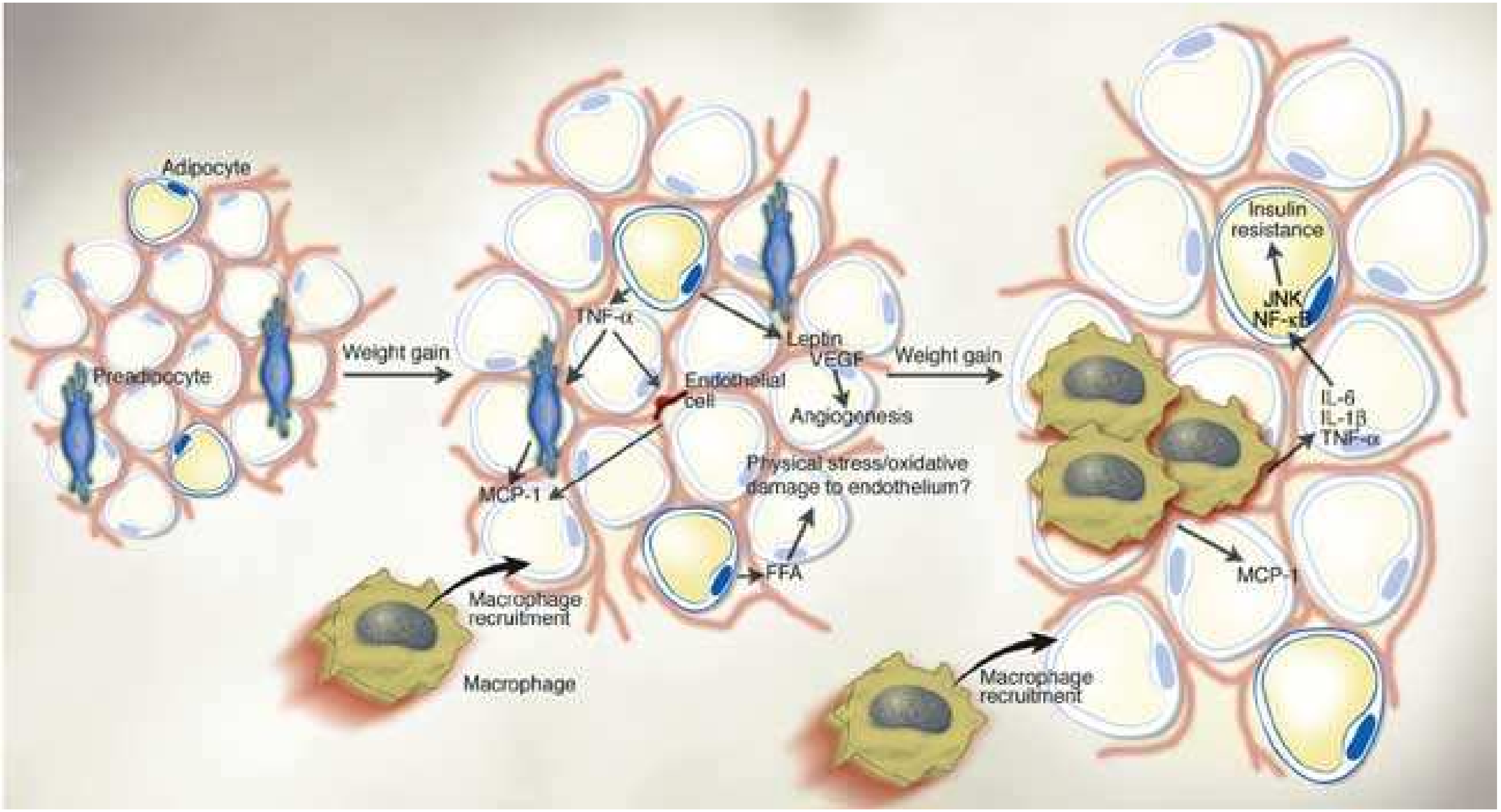
↑ TNF-α

↑ MCP-1

↑ MIF

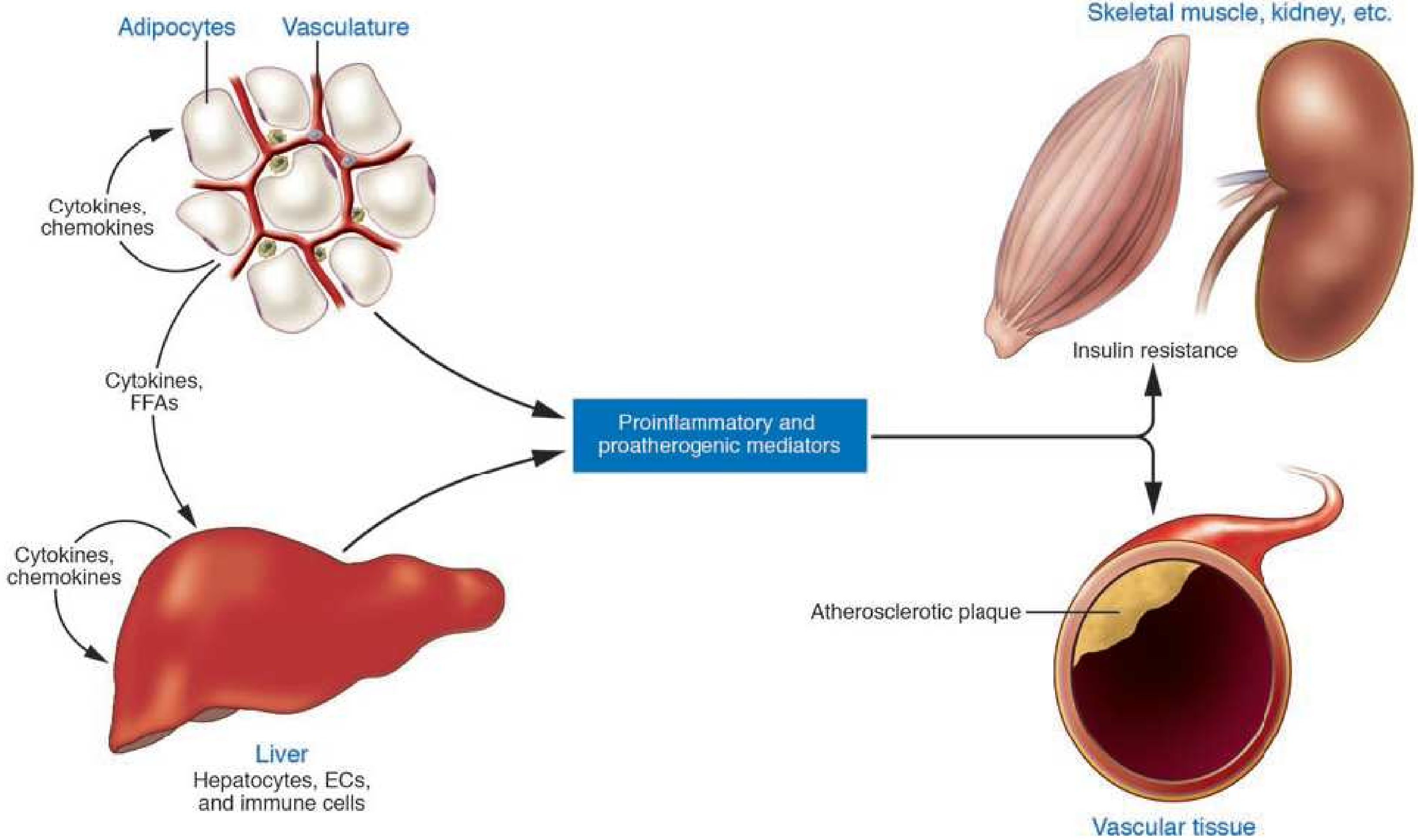
↑ Free fatty acids, Steroids, Prostaglandins, Complement factors

Adipose tissue a site of inflammation and cytokine production



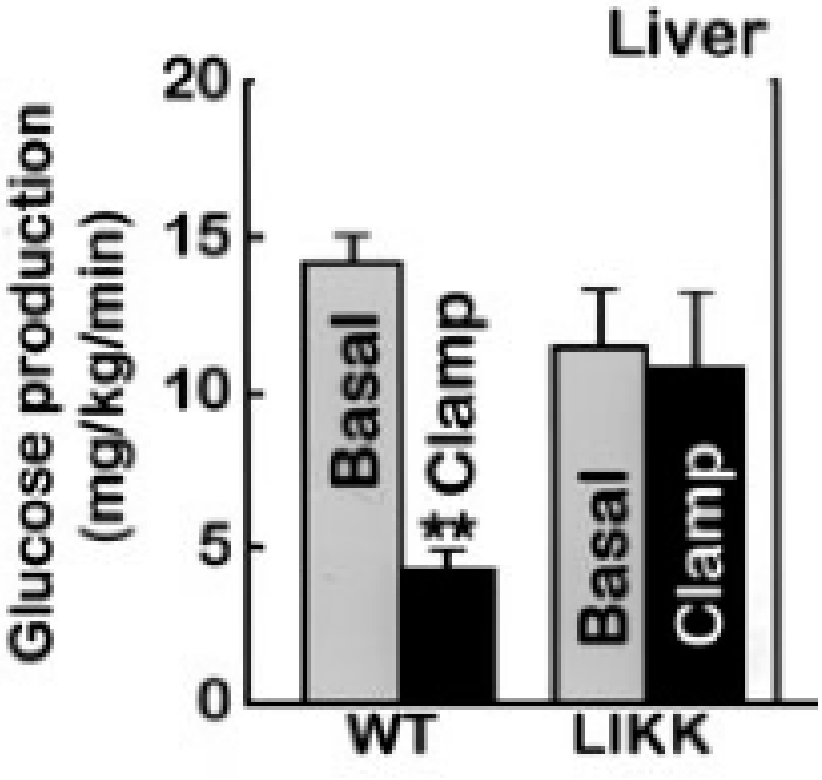
Wellen et al., JCI 2003

Inflammation, insulin resistance and atherogenesis

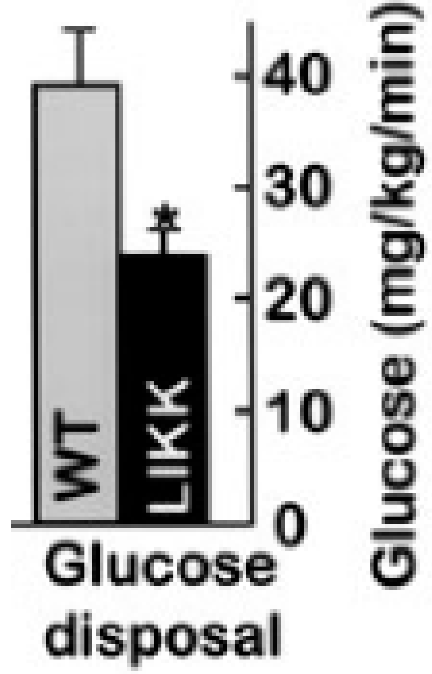


Shoelson et al. JCI 2006

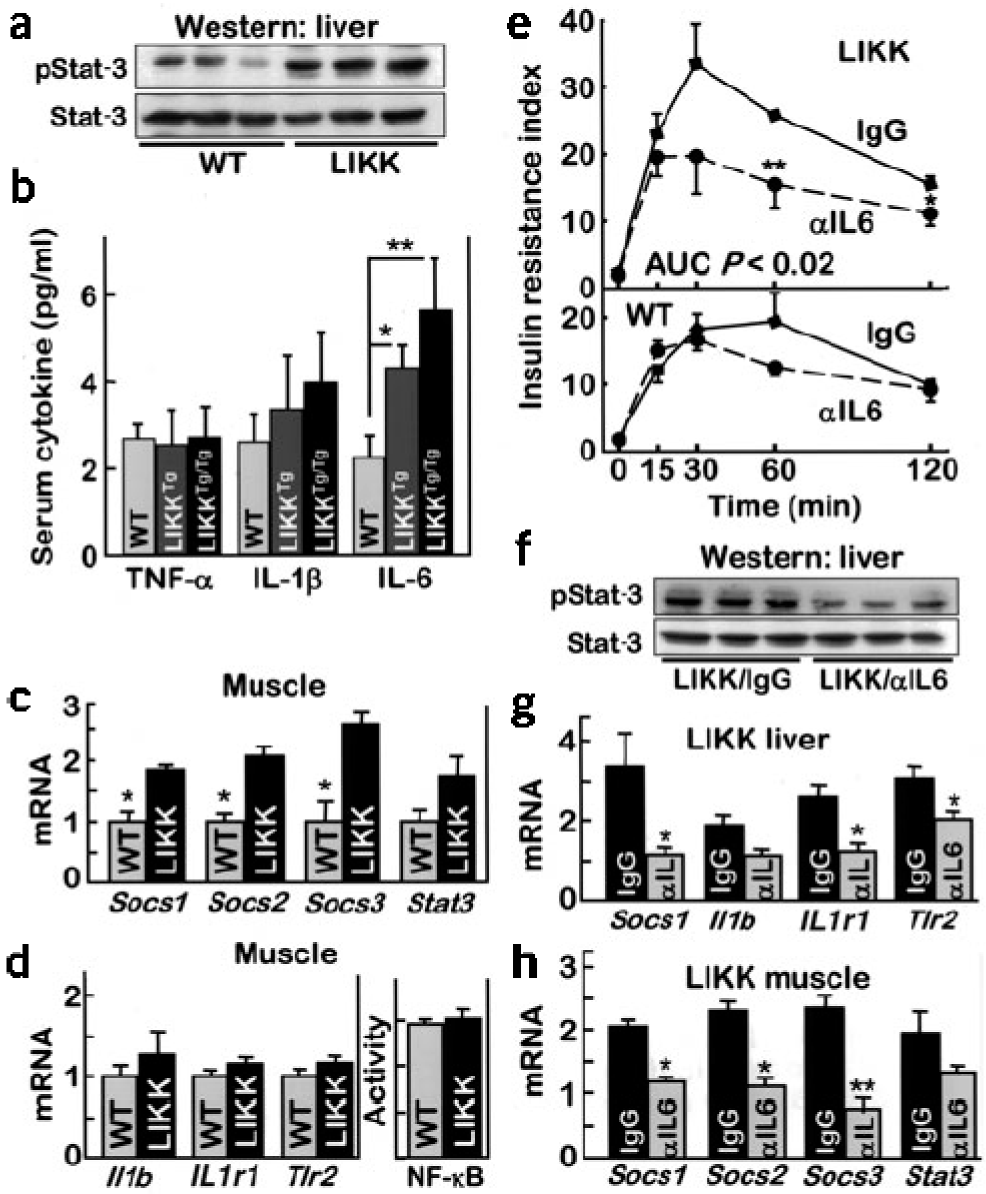
Hepatic "Inflammation" Causes Local and Systemic Insulin Resistance



↑IKK-β
↑NF-kB



Cai et al. Nat Med 2005;11:183



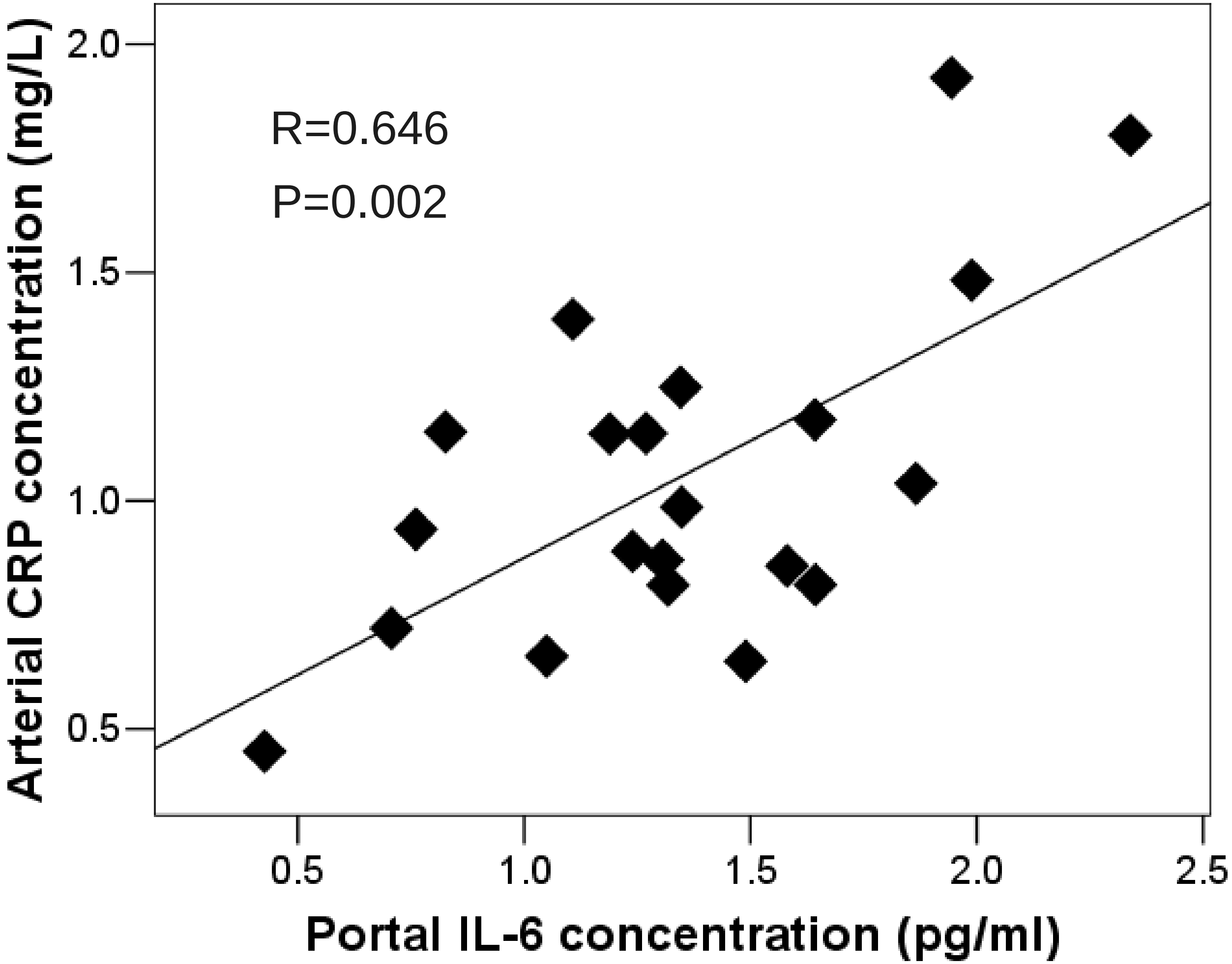
Cai D et al. Nat Med 2005;11:183

Visceral fat is an important endocrine organ that regulates systemic inflammation

	Radial artery	Portal vein
IL-6 (pg/ml)	28.5 ± 27.6	42.1 ± 41.8*
TNF-□ (pg/ml)	1.87 ± 0.8	1.93 ± 0.8
MCP-1 (pg/ml)	205 ± 88	190 ± 99
Resistin (pg/ml)	18.5 ± 11	18.1 ± 11
Leptin (ng/ml)	101 ± 51	81 ± 42**
Total adiponectin (□g/ml)	14.3 ± 10	14.7 ± 11
Insulin (□UI/ml)	15.2 ± 8	34.4 ± 21***

Fontana L et al. Diabetes 2007

Relationship between Portal Vein IL-6 and Systemic Inflammation (C-reactive protein)



Fontana L et al. Diabetes 2007

Cardiometabolic effects of weight loss in non-obese individuals

Fontana et al. JAMA 2007

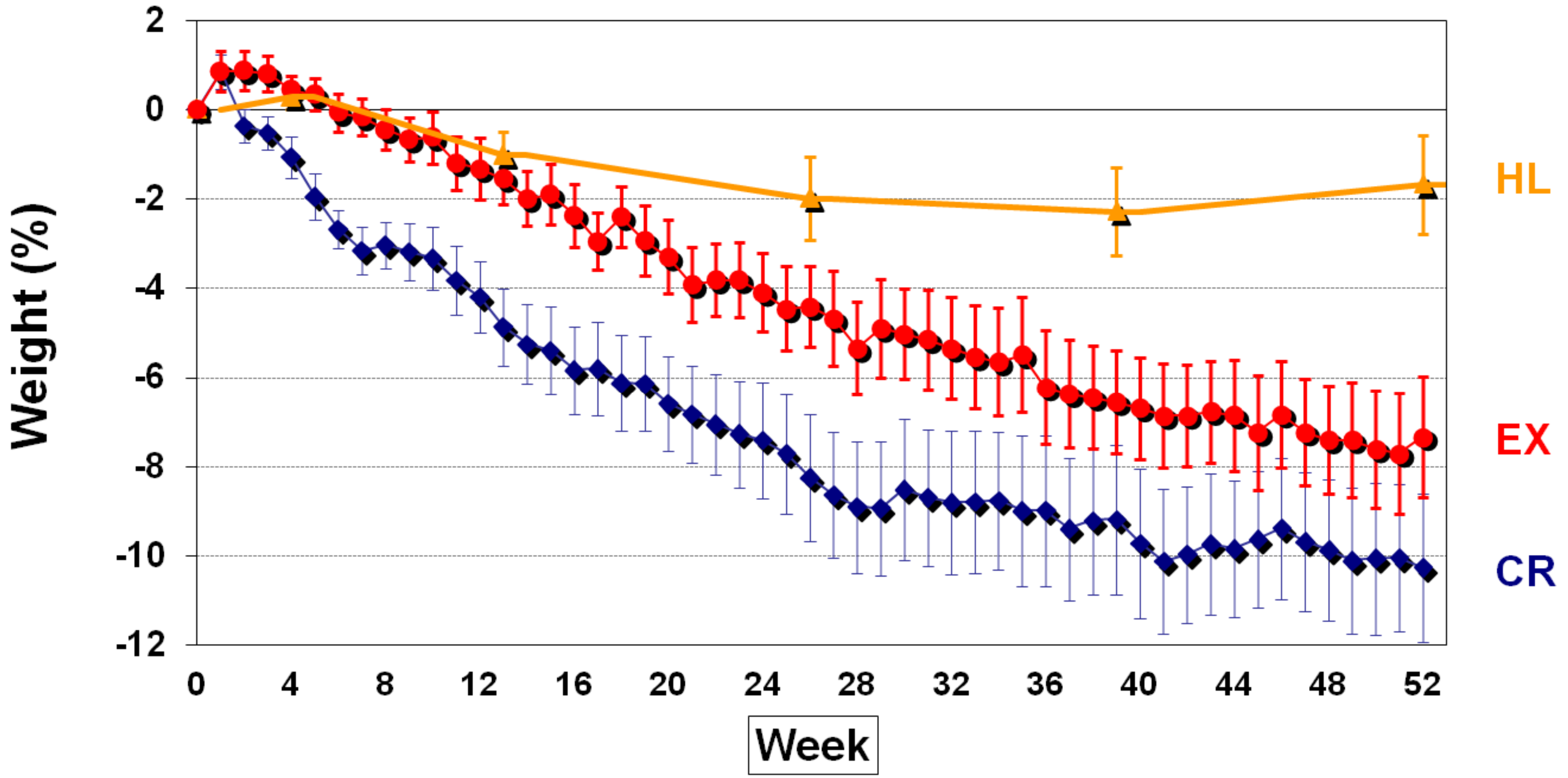
Washington University Calorie Restriction Randomized Clinical Trial

Subjects: 50-60 yrs, overweight, relatively healthy, sedentary

Randomized clinical trial

Groups:	**Interventions: 1 Year**
Healthy Lifestyle (HL)	nutrition education, yoga (n=10)
Caloric Restriction (CR)	20% ↓ in caloric intake (n=18)
Exercise (EX)	20% ↑ in energy expenditure (n=18)

Reduction of body weight Ds (%)



calerie

***Racette S. et al*. J Gerontol 2006**

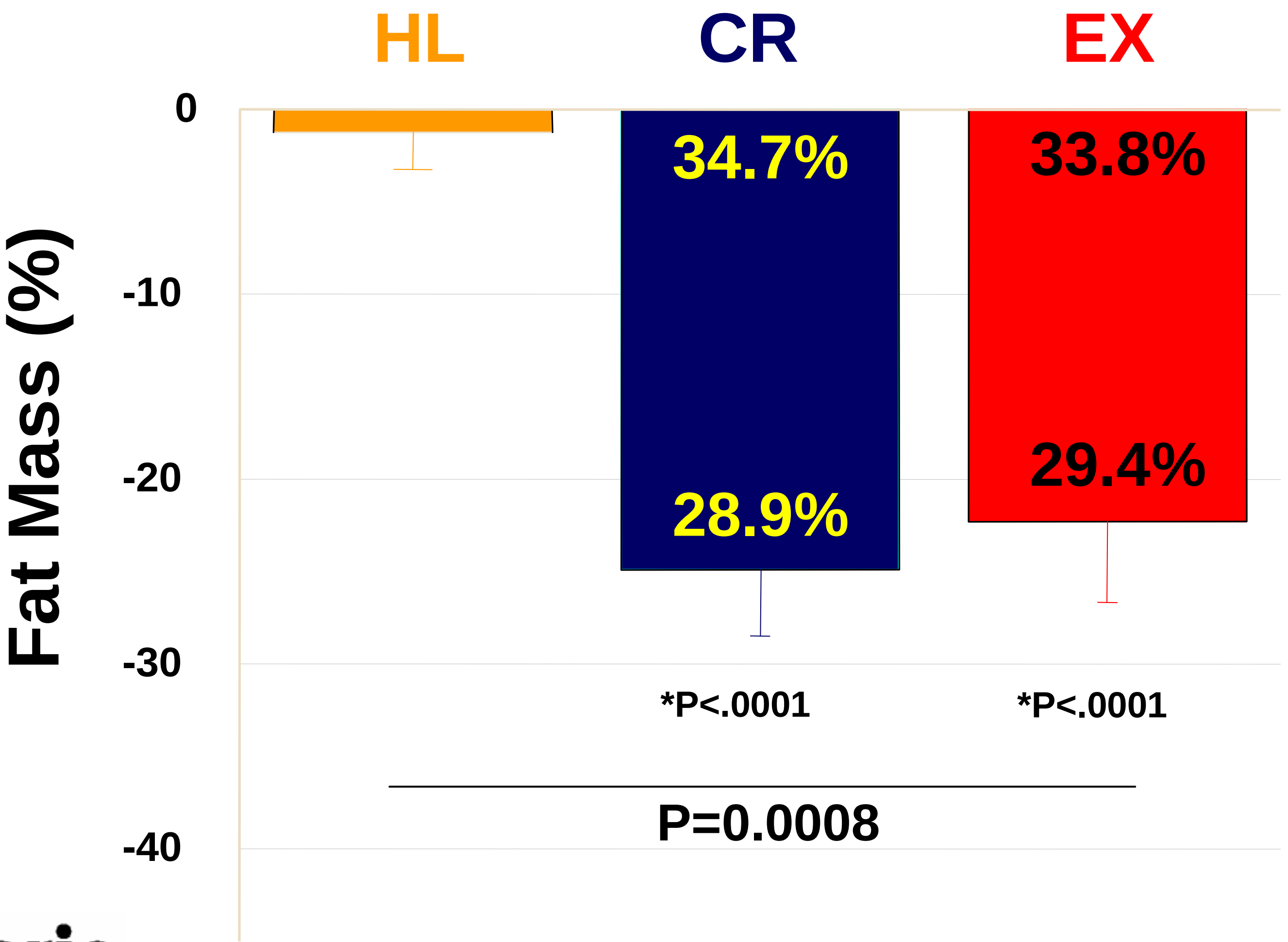
Body Mass Index - Absolute Values

	HL	CR	EX
0-mos	27.9±1	27.2±1	27.2±1
12-mos	27.4±1	24.2±1	24.8±1
		P=0.0001	P=0.0001

P=0.0003

Racette S. et al. J Gerontol 2006

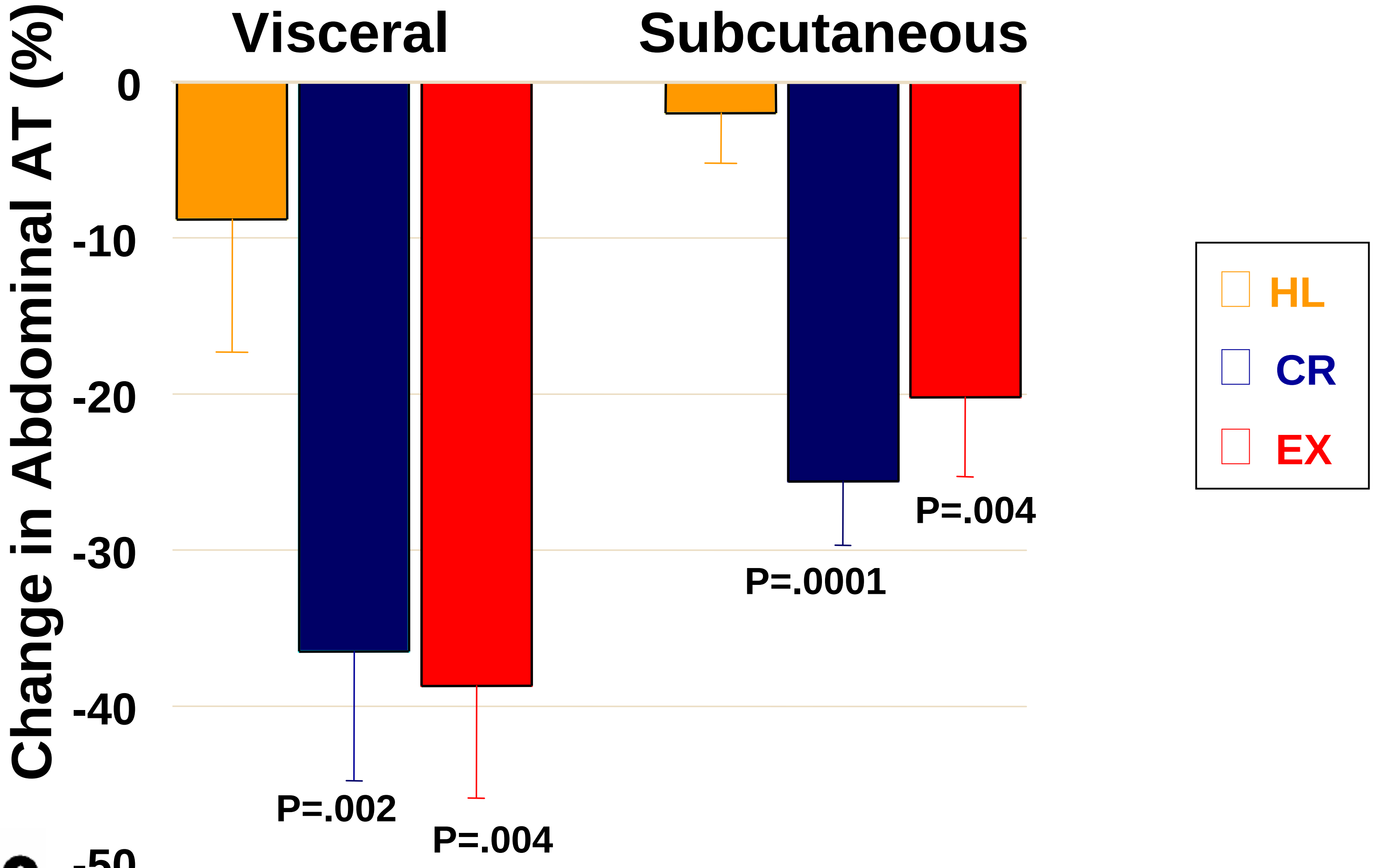
Reduction of % Fat Mass Ds



calerie

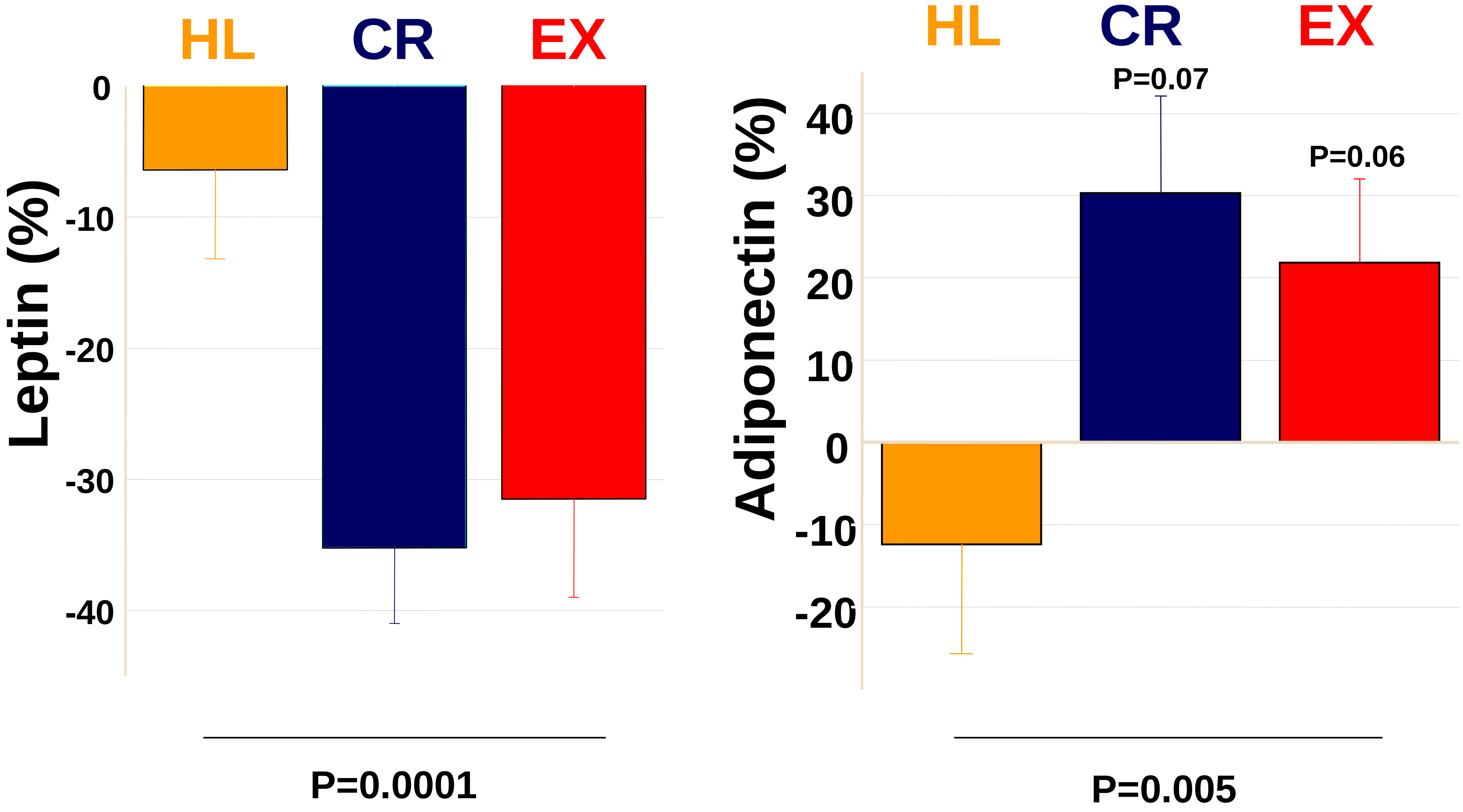
Racette S. et al. J Gerontol 2006

Reduction in Abdominal Adipose Tissue



Racette S. et al. J Gerontol 2006

Leptin and Adiponectin Ds



Weiss E. et al. Am J Clin Nutr 2006

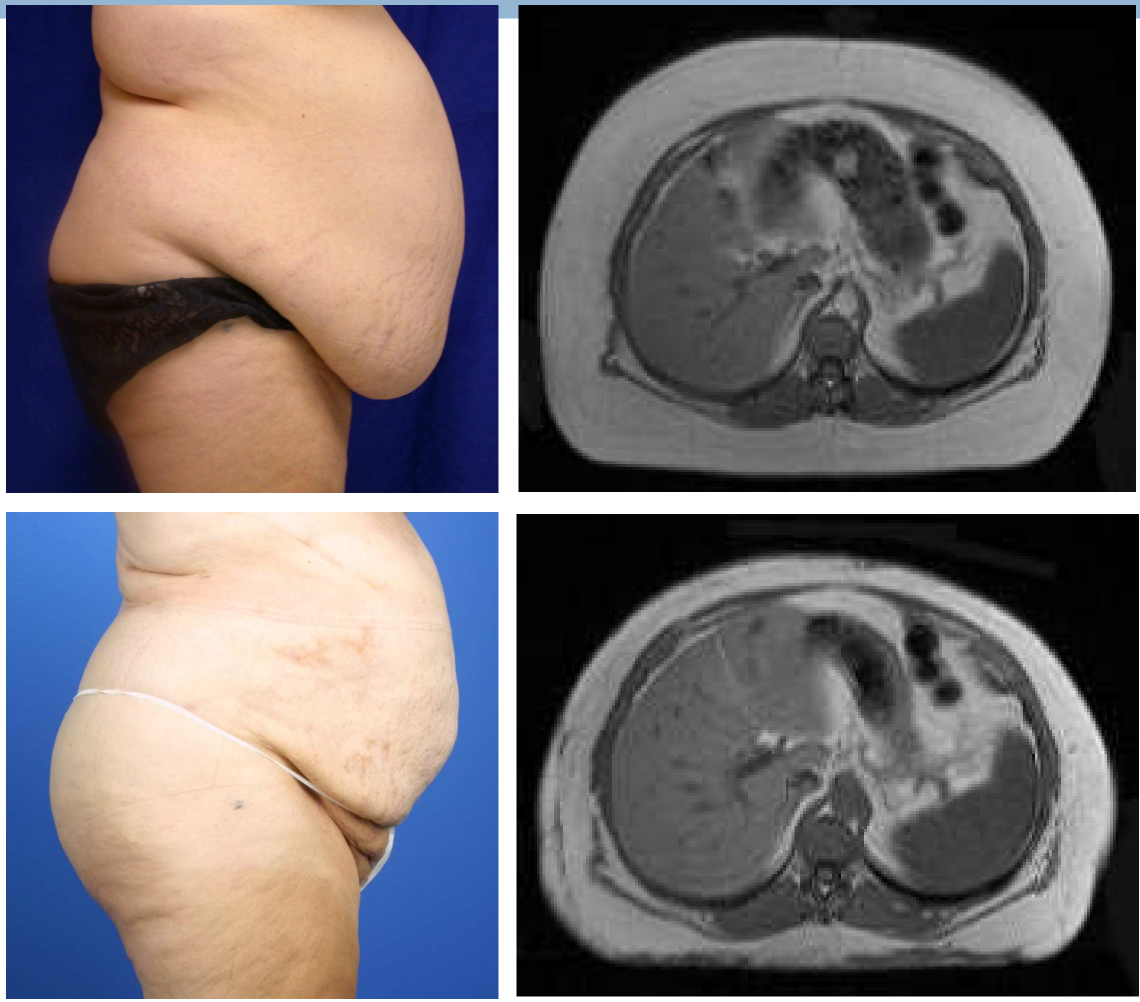
Improvement of cardiovascular risk

- Reduction of total and LDL cholesterol
- Increased HDL-cholesterol
- Improvement in insulin sensitivity
- Reduction of inflammation
- Improvement of left ventricular diastolic function

calerie

Fontana et al. AJP-EM 2007
Weiss EP et al. AJCN 2006
Riordan MM et al. AJP-HCP 2008

Metabolic effects of surgical fat removal in obese women



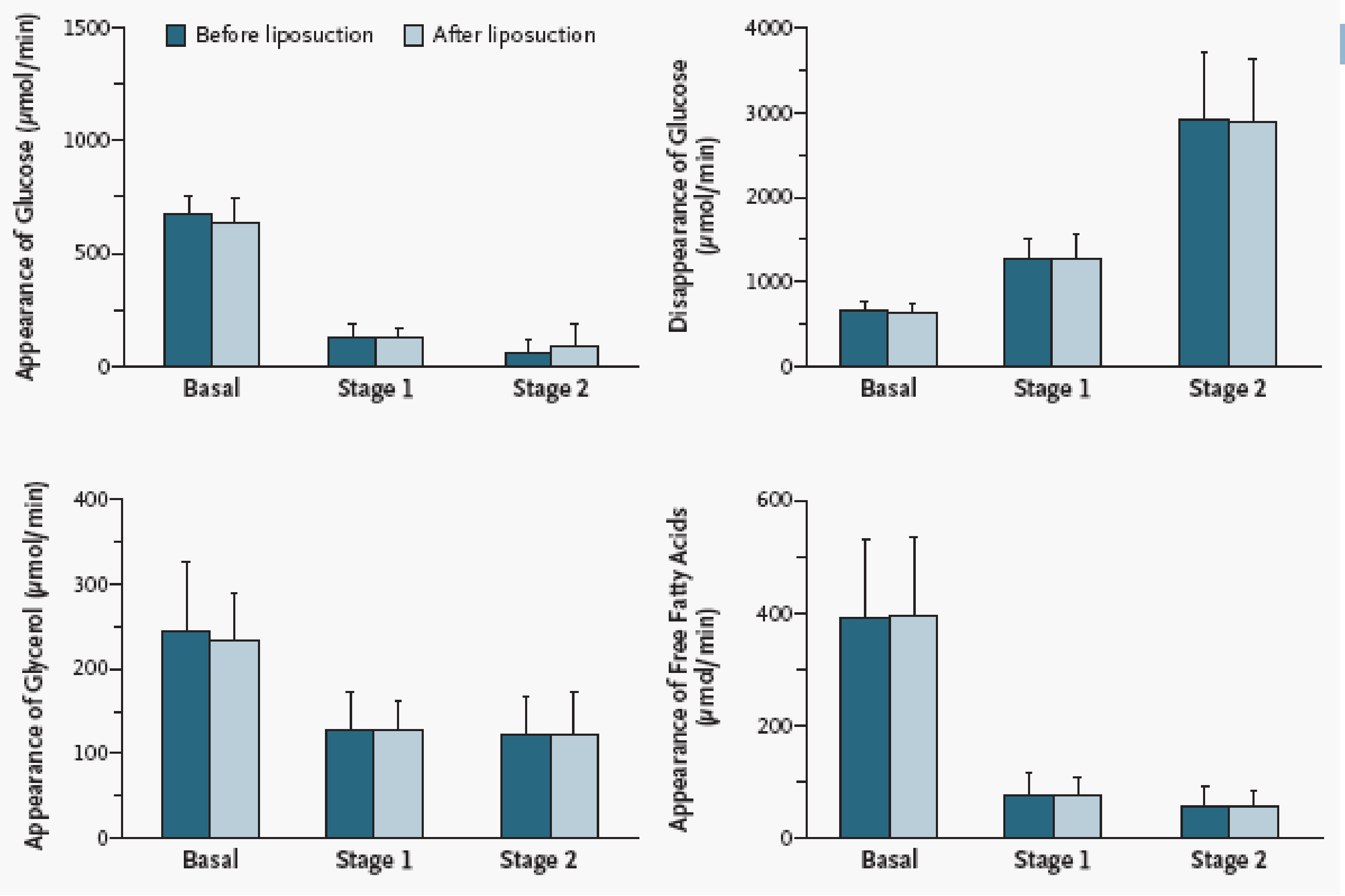
↓ ~19% total BF

=

↓ ~12% total BW

Klein S, Fontana L. et al. N Engl J Med 2004

Effect of Liposuction on Insulin Sensitivity



***Klein S, Fontana L. et al. N Engl J Med* 2004**

Effect of liposuction on CHD risk factors

	Obese normal OGT		Obese diabetes	
	Before	After	Before	After
Waist circumference	108±5	94±3**	119±4	107±3**
Systolic BP	119±5	124±4	132±4	137±6
Diastolic BP	70±3	65±4	73±3	68±4
Plasma glucose	89±1	90±2	121±15	123±15
Plasma insulin	11±3	9±2	15±2	14±3
Triglycerides	151±28	121±21	162±19	173±24
Total cholesterol	189±12	174±13	160±9	157±10
LDL cholesterol	113±9	110±11	82±7	80±11
HDL cholesterol	45±8	41±9	44±3	43±3

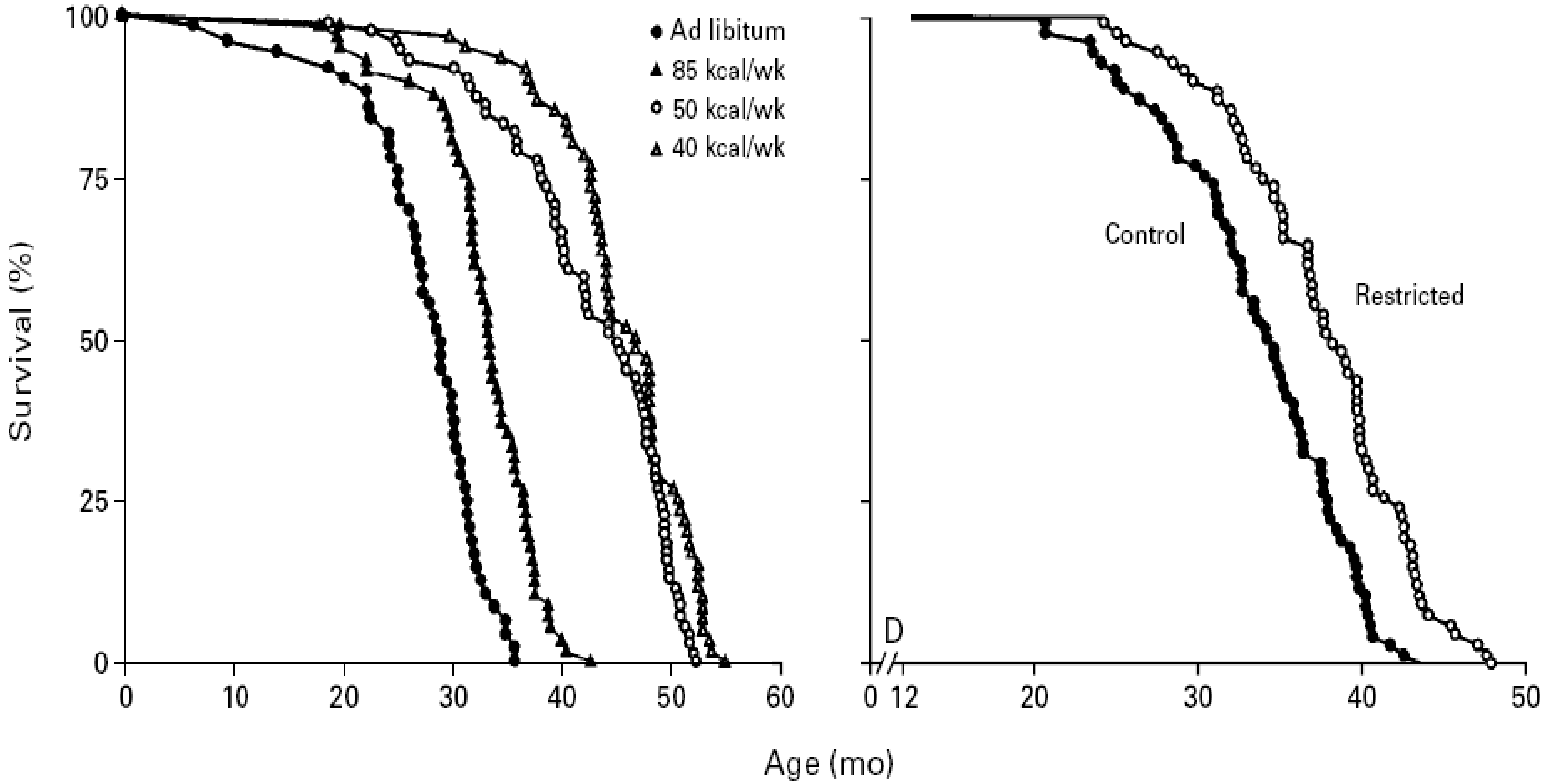
Klein S, Fontana L. et al. N Engl J Med 2004

Effect of liposuction on adipokines

	Obese normal OGT		Obese diabetes	
	Before	After	Before	After
Leptin (ng/ml)	32±12	23±5**	36±13	30±13**
Adiponectin (ng/ml)	5.0±2	4.5±2	4.3±2	3.6±2
TNF-α (pg/ml)	3.5±5	2.8±3	7.6±8	7.7±8
IL-6 (pg/ml)	1.5±1	2.4±1	3.8±4	3.2±2
CRP (mg/L)	6.9±6	6.7±6	8.2±7	7.7±7

Klein S, Fontana L. et al. N Engl J Med 2004

Calorie restriction without malnutrition increases healthspan and 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

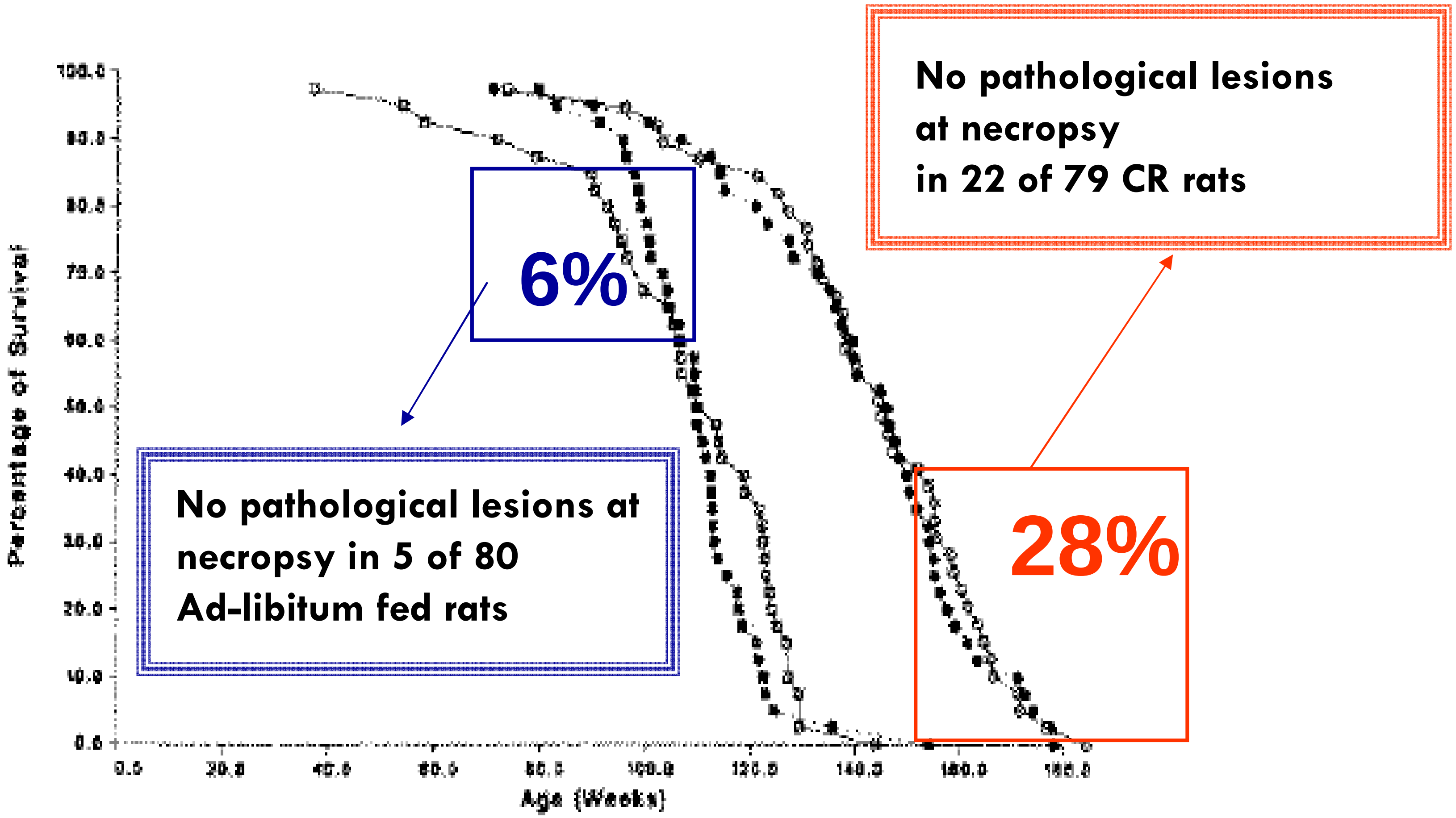
Number of experiments	Caloric restriction (%) Range	Caloric restriction (%) Mean (SE)	Tumor reduction (%) Mean (SE)
9	0	0 (1.5)	−9.5 (10.2)
18	7–20	15.3 (1.2)	20.2 (8.1)
22	21–30	25.9 (1.1)	49.6 (6.4)
17	31–40	37.0 (1.2)	52.5 (7.8)
16	41–58	52.9 (1.1)	62.2 (7.6)

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:

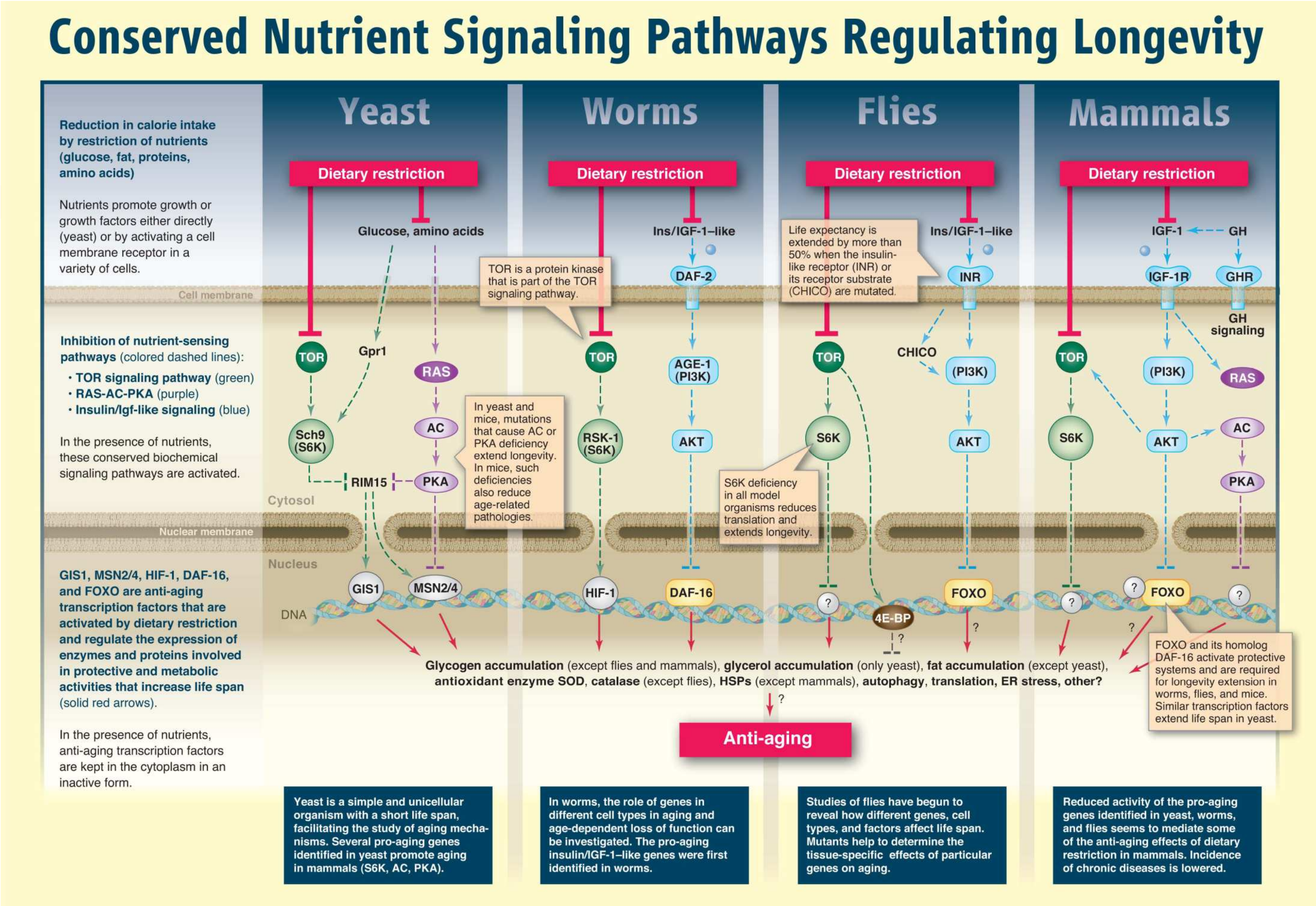
- 19% were ESCAPERS (= without common age-associated disease before 100 years of age)
- 43% were delayers (= age-associated disease after the age of 80 years)
- 38% were survivors (= age-associated disease before the age of 80 years)

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

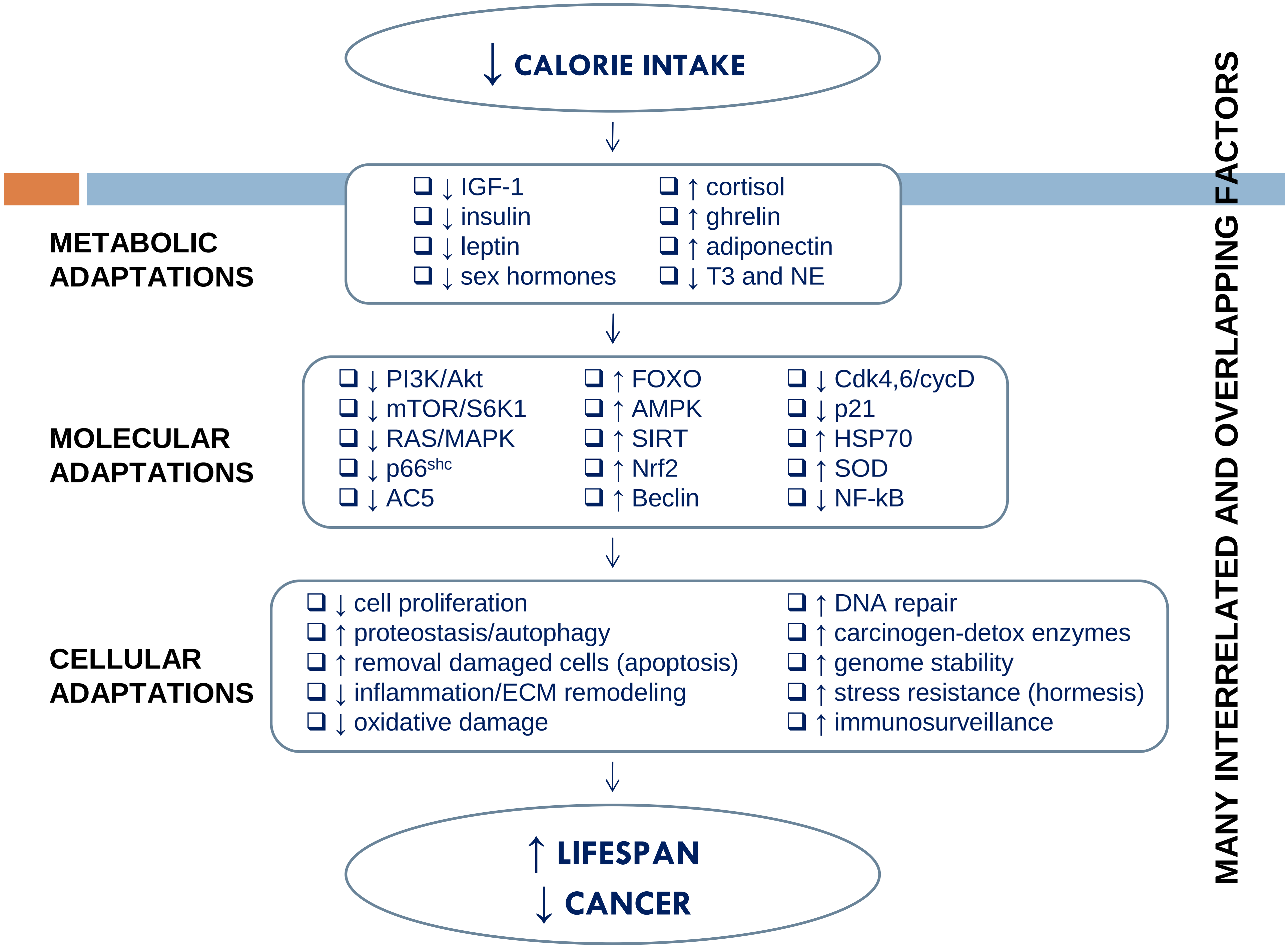
Mammalian animal models of longevity

- 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
- Rapamycin supplementation
- p66shc KO mice
- Type 5 Adenylyl Cyclase KO mice
- Angiotensin II type 1 receptor KO mice
- Mice overexpressing catalase targeted to mitochondria

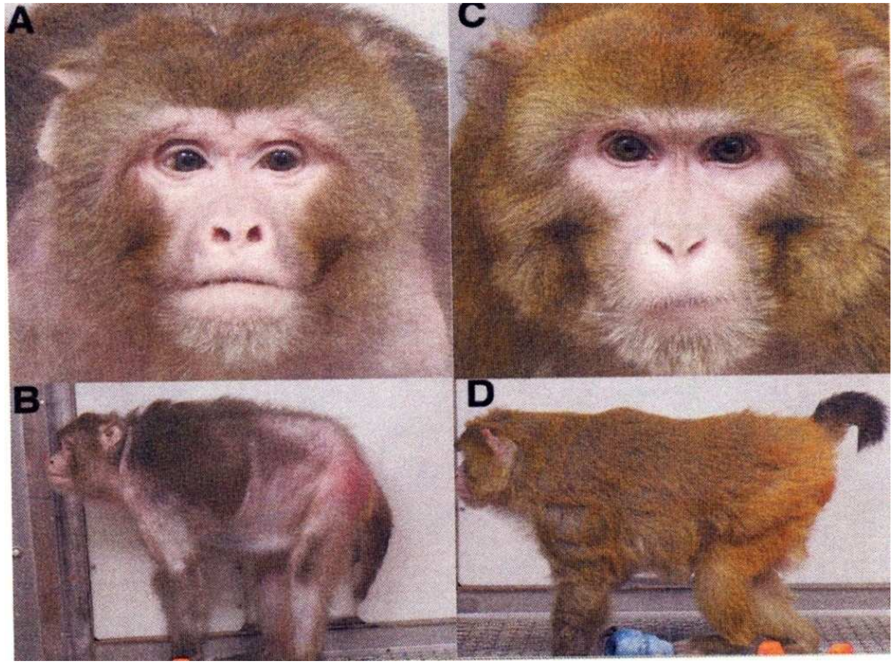
Down regulation Insulin/IGF-1/mTOR pathways
=
Nutrient –sensing signaling pathways



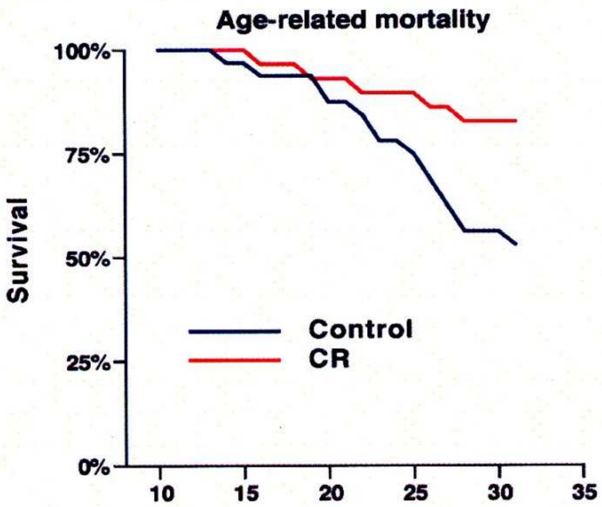
Fontana L et al. Science 2010


↓ CALORIE INTAKE
METABOLIC ADAPTATIONS
↓ IGF-1
↓ insulin
↓ leptin
↓ sex hormones
↑ cortisol
↑ ghrelin
↑ adiponectin
↓ T3 and NE
MOLECULAR ADAPTATIONS
↓ PI3K/Akt
↓ mTOR/S6K1
↓ RAS/MAPK
↓ p66shc
↓ AC5
↑ FOXO
↑ AMPK
↑ SIRT
↑ Nrf2
↑ Beclin
↓ Cdk4,6/cycD
↓ p21
↑ HSP70
↑ SOD
↓ NF-kB
CELLULAR ADAPTATIONS
↓ cell proliferation
↑ proteostasis/autophagy
↑ removal damaged cells (apoptosis)
↓ inflammation/ECM remodeling
↓ oxidative damage
↑ DNA repair
↑ carcinogen-detox enzymes
↑ genome stability
↑ stress resistance (hormesis)
↑ immunosurveillance
↑ LIFESPAN
↓ CANCER
MANY INTERRELATED AND OVERLAPPING FACTORS

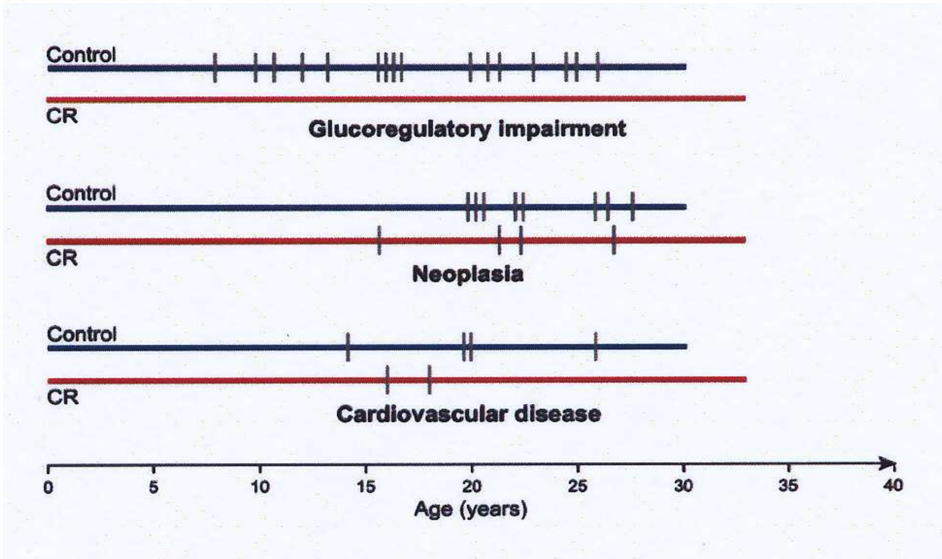
Calorie restriction reduces cardiovascular and cancer mortality by 50% in non-human primates



Ad libitum **CR**

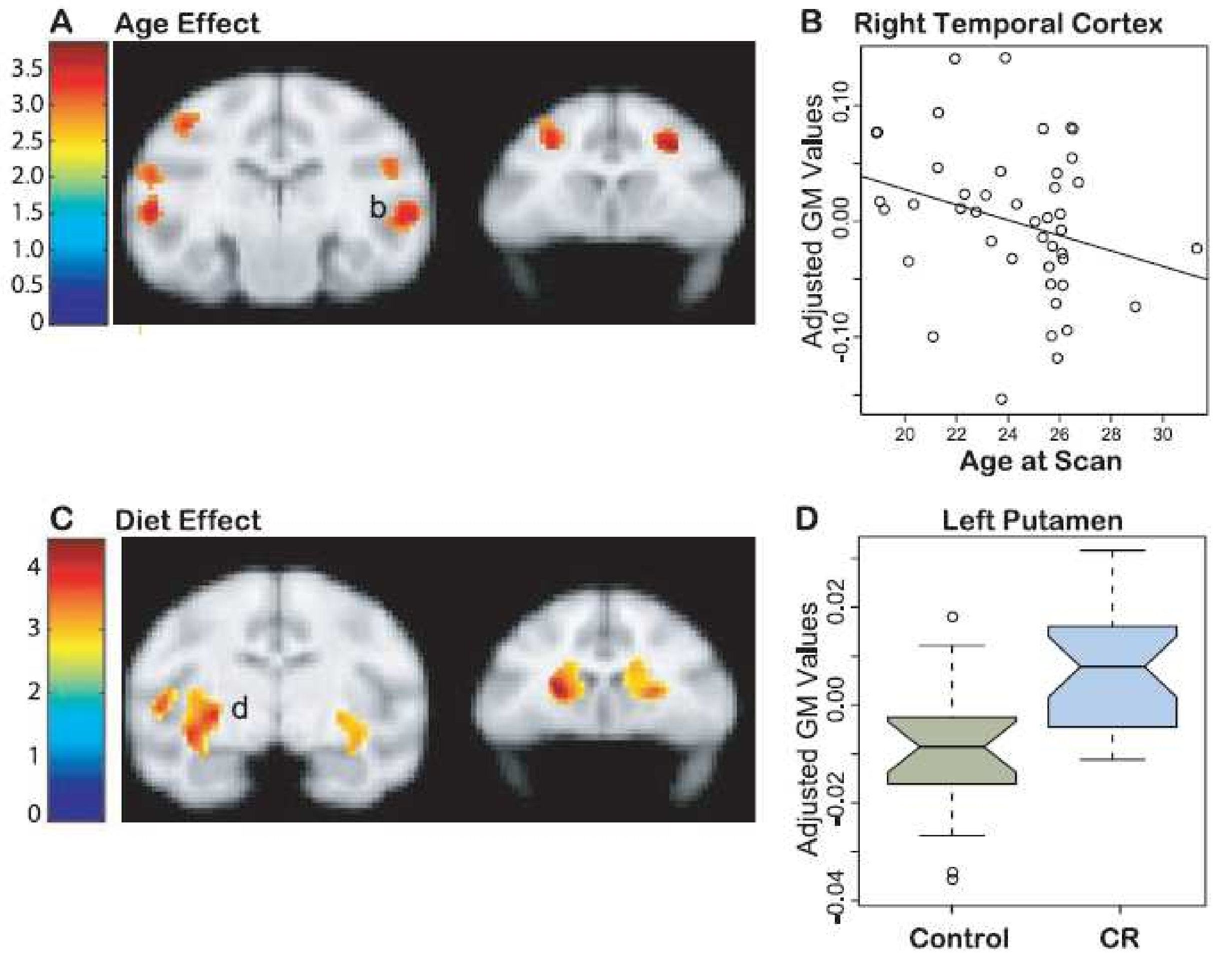


P = 0.03
HR = 3.0



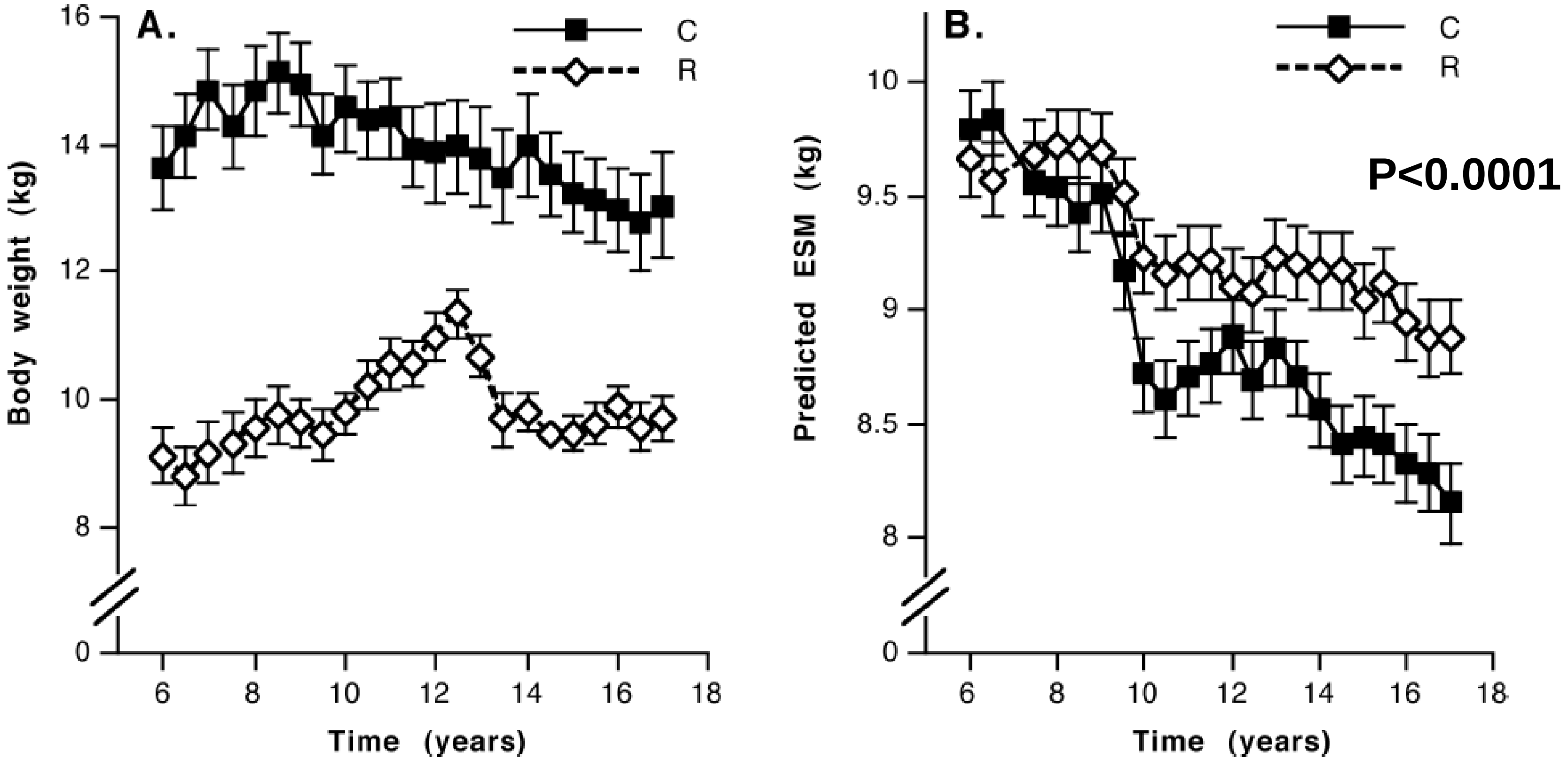
Colman et al. Science 2009

Calorie restriction reduces the age-associated brain atrophy in non-human primates



Colman et al. Science 2009

Attenuation of sarcopenia by CR in non-human primates



Colman et al. J Gerontol 2008

Effects of long-term CR in humans

	CR group (n=28)	EX group (n=28)	WD group (n=28)	Among group P
Age (years)	53.0±11	54.0±11	53.0±10	ns
Sex (M/F)	24/4	24/4	24/4	
Height (m)	1.73±0.1	1.75±0.1	1.76±0.1	ns
Weight (kg)	58.1±6.0*,**	68.0±7.6*	81.1±14.5	0.0001
BMI (kg/m^2)	19.5±1.7*,**	22.2±2.1*	26.0±3.0	0.0001
Total body fat (%)				
Men	9.7±4.6*	10.9±4.5*	23.2±6.2	0.0001
Women	20.5±9.9	20.1±1.7	32.0±7.8	0.085
Trunk fat (%)				
Men	7.0±5.0*	8.4±6.0*	25.2±8.4	0.0001
Women	14.1±8.8	13.2±2.6	27.5±10.4	0.056
Lean mass (kg)				
Men	51.7±4.8*,**	59.2±5.0	59.9±8.8	0.0001
Women	38.9±5.3	40.3±3.0	35.6±2.0	ns

Values are means ± SD

*$P \leq 0.0001$, significantly different from Western diet group; **$P \leq 0.001$, significantly different from EX group

Circulating adipokines and cytokines

	CR group (n=28)	EX group (n=28)	WD group (n=28)	Among group P
Adiponectin (μg/mL)	15.7±8.2*,**	11.1±5.5	9.5±4.3	0.001
Resistin (pg/mL)	7.0±2.2***	8.1±1.7	8.7±2.3	0.015
IL-6 (pg/ml)	0.73±0.3*	0.71±0.3*	1.21±0.8	0.001
s-TNF R-I (ng/mL)	1.05±0.33***	0.95±0.28*	1.30±0.27	0.0001
s-TNF R-II (ng/mL)	2.77±0.83***	2.81±0.69***	3.40±0.84	0.008
Fructosamine (μmol/L)	269±40**	241±17	262±34	0.005
sRAGE (μg/mL)	1.27±0.66	1.63±0.53***	1.11±0.69	0.01
Free fatty acids (mEq/L)	0.72±0.35***	0.59±0.18	0.51±0.20	0.015

All values are means ± SD

*$P \leq 0.003$, significantly different from Western diet group; **$P \leq 0.05$, significantly different from EX group; ***$P \leq 0.05$, significantly different from Western diet group

Glucose tolerance and insulin action

	CR	EX	WD
HOMA-IR index	0.3±0.1*	0.4±0.3*	1.6±1.3
ISI Matsuda index	18.5±6.7*	20.4±9.2*	7.0±3.6
Fasting glucose (mg/dl)	83±8*,†	91±8	95±8
Fasting Insulin (μU/ml)	1.4±0.7*	2.0±1.3*	6.9±5.6
2-hr glucose (mg/dl)	132±42†	103±28	116±28
2-hr insulin (μU/ml)	37.7±24†	16.8±11*	60.4±55
Glucose AUC (mg•min/dl)	16.1±3.2	14.9±2.6*	16.8±3.0
Insulin AUC (μU•min/dl)	3.5±1.7*	2.7±1.8*	6.2±3.6

Fontana et al. Age 2009

Cardiometabolic risk factors

	CR	EX	WD	P value
Total cholesterol (mg/dl)	162±36*	166±35*	202±36	0.0001
LDL cholesterol (mg/dl)	88±24*	92±26*	122±33	0.0001
HDL cholesterol (mg/dl)	63±19*	61±17*	50±11	0.004
T Chol/HDL Chol ratio	2.7±0.5*	2.8±0.6*	4.3±1.1	0.0001
Triglycerides (mg/dl)	58±18*	65±22*	159±94	0.0001
SBP (mm Hg)	103±9*,†	125±17	131±13	0.0001
DBP (mm Hg)	62±7*,†	72±8*	84±8	0.0001
Fasting glucose (mg/dl)	82±7*,†	90±7	95±9	0.0001
hsCRP (mg/L)	0.2±0.3*,†	0.8±1.1	1.1±1.1	0.004

Fontana et al., PNAS 2004

CR ameliorates the decline in diastolic function

	Parameter	Western Diet Mean±SD	CR 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

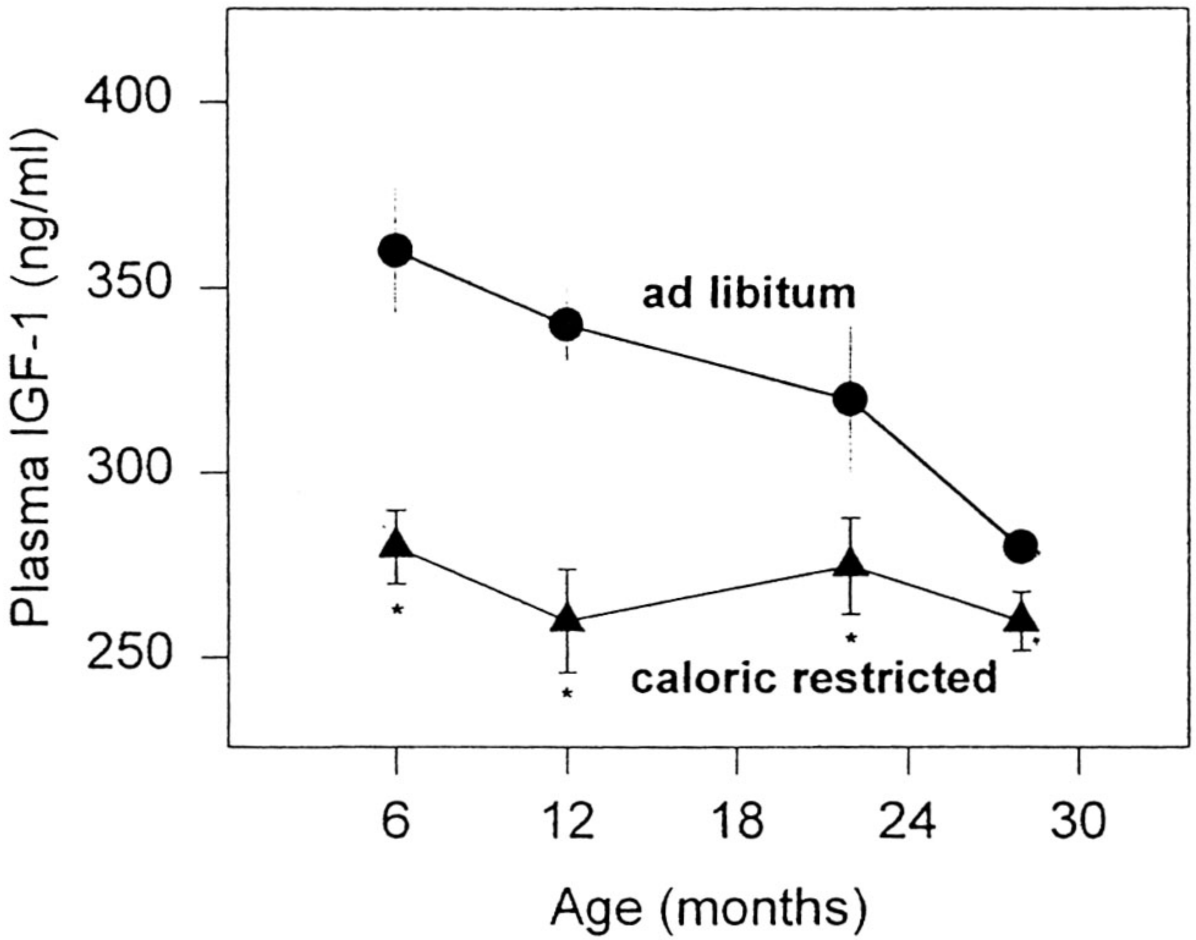
Meyer T et al. JACC 2006

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

- Reduces adiposity
- Reduces insulin
- Reduces growth factors such as IGF-1
  (if associated with lower protein intake)
- Reduces sex hormones
- Reduces inflammation
- Reduces oxidative stress

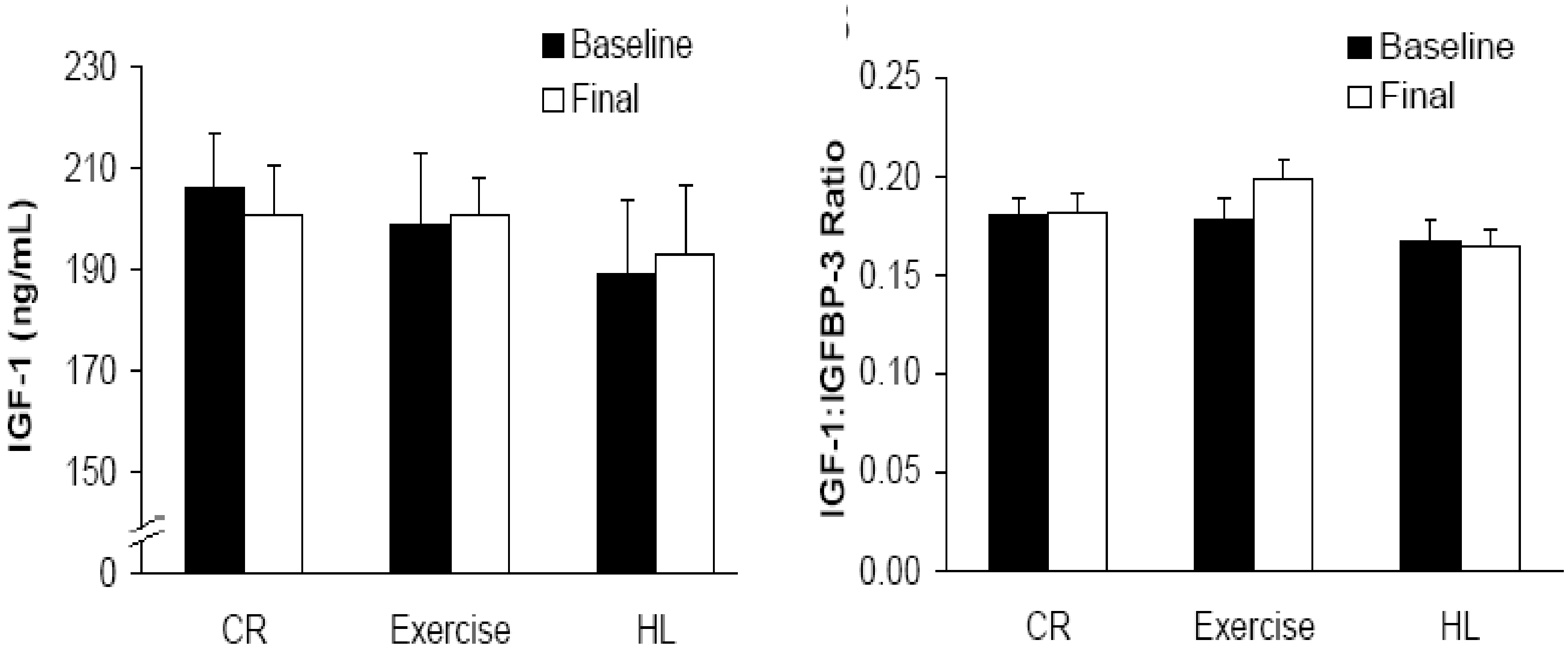
Longo and Fontana. Trends Pharmacol Sci

Long-term CR reduces plasma IGF-1 concentration by 30-40% in rats



Breese CR et al. J Gerontol Biol sci 1991

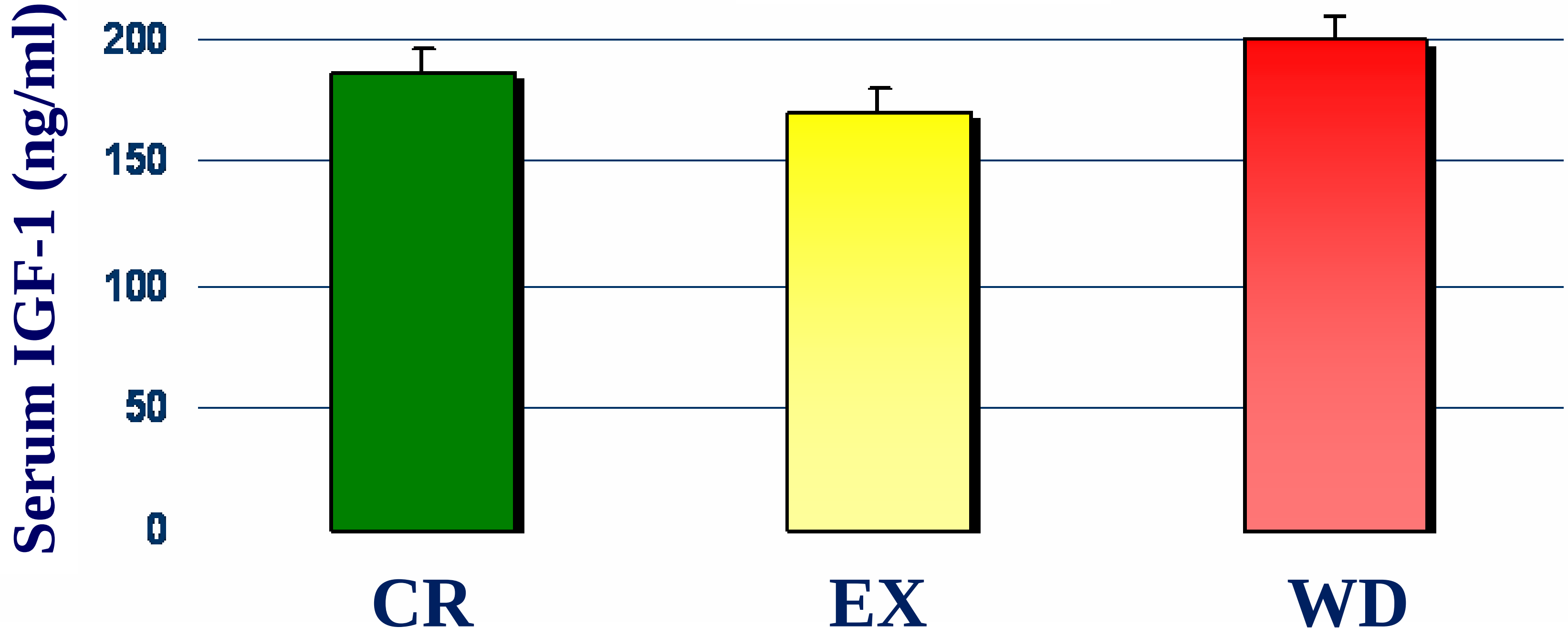
1-yr CR intervention does NOT reduce serum IGF-1 concentration



calerie

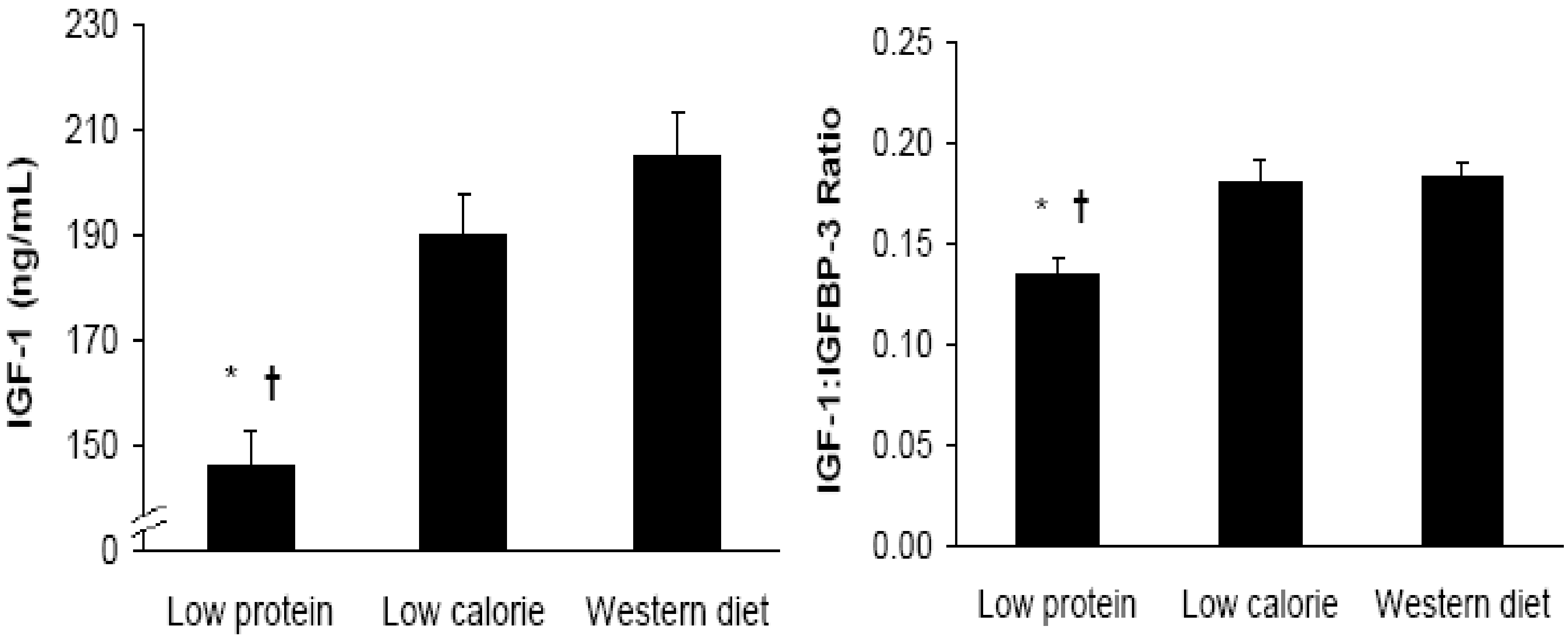
Fontana et al., Aging Cell 2008

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

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

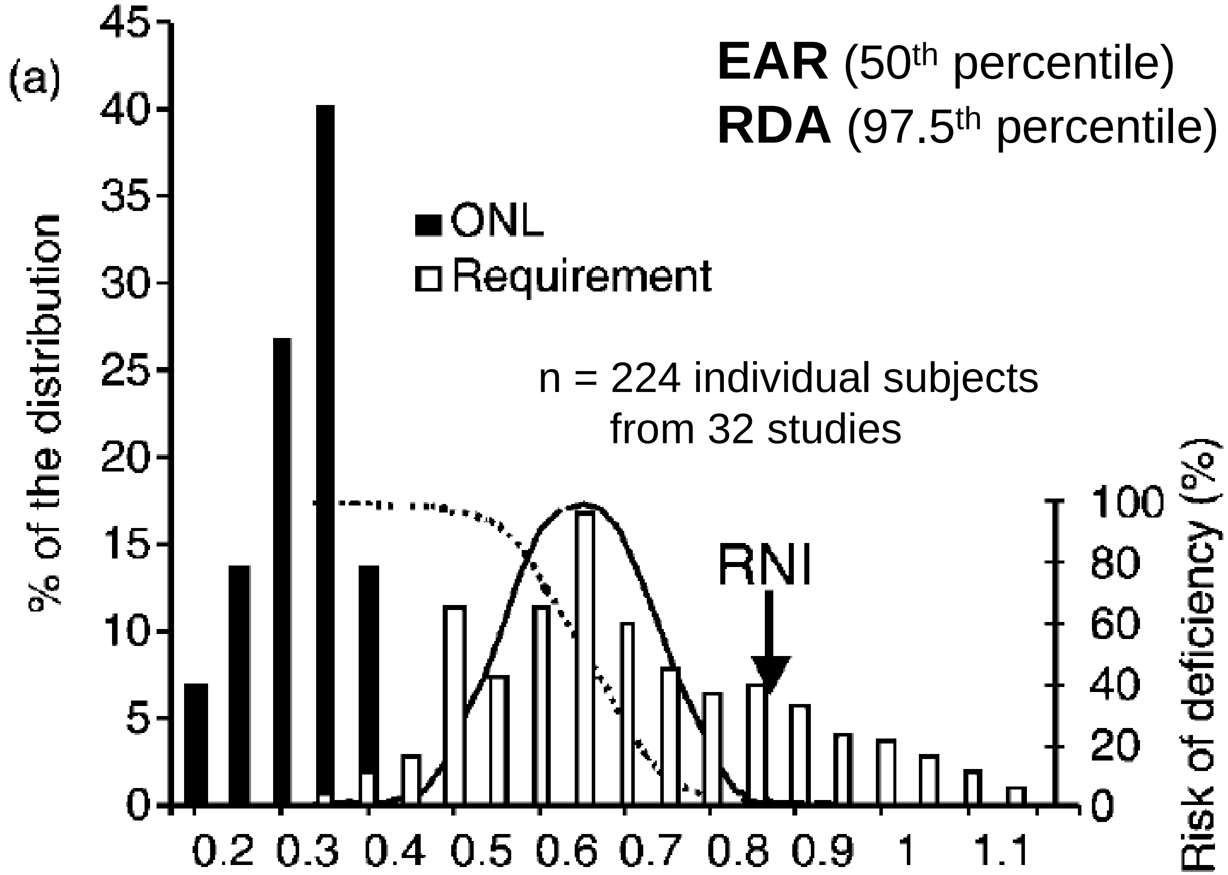
Plasma IGF	RR	RAR
Breast cancer (premenopausal, <50 years)		
<158 ng/mL	1·0	1·0
158–206 ng/mL	2·64	3·12
>207 ng/mL	4·58	7·28
Prostate cancer		
99–184 ng/mL	1·0	1·0
185–236 ng/mL	1·32	1·94
237–293 ng/mL	1·81	2·83
294–500 ng/mL	2·41	4·32

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

Hankinson SE, Lancet 1998 & Chan JM, Science 1998

Protein requirements for healthy adults

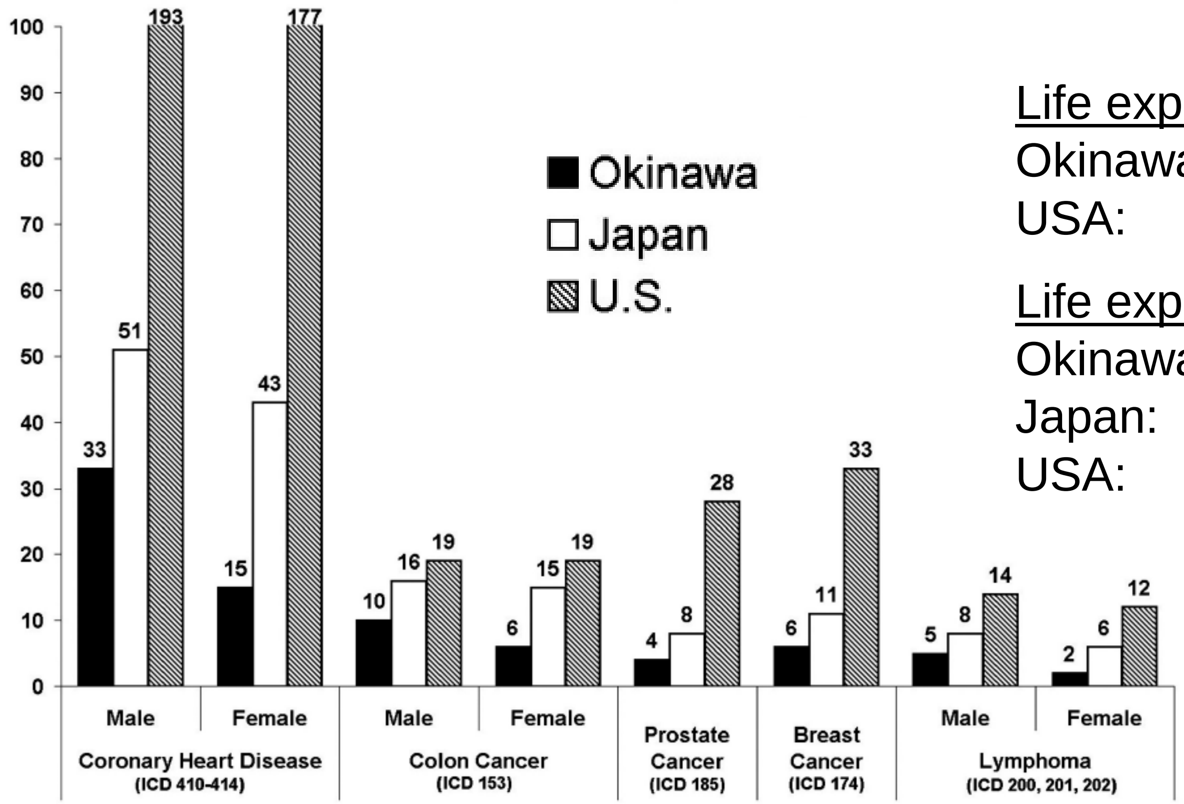
EAR (50th percentile) = **0.65 g/kg/d**
RDA (97.5th percentile) = **0.83 g/kg/d**



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)



<u>Life expectancy at birth</u>:

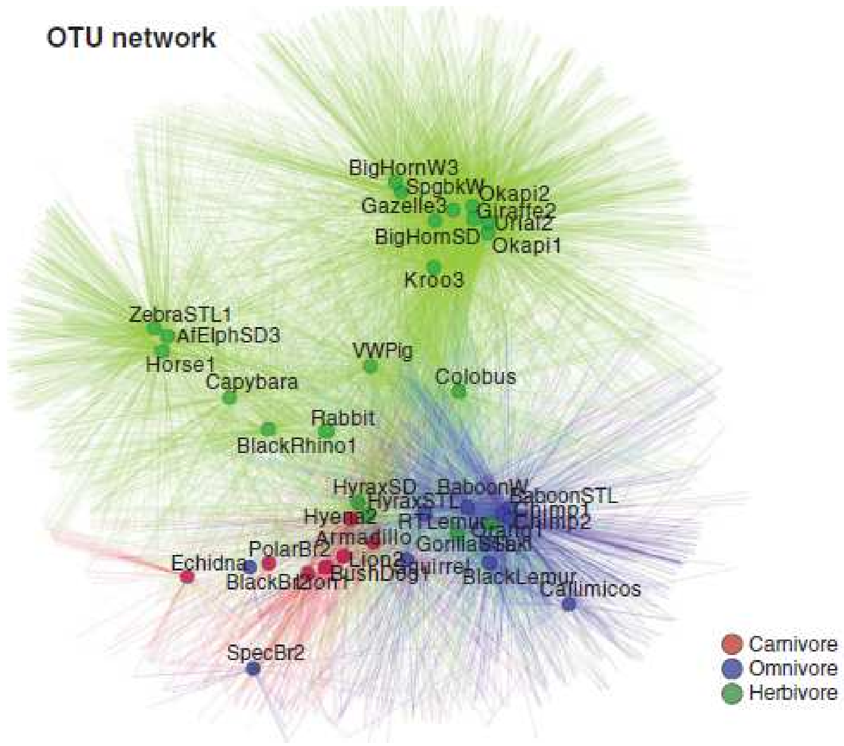
Okinawa:	86 y F; 77.6 y M
USA:	80 y F; 75 y M

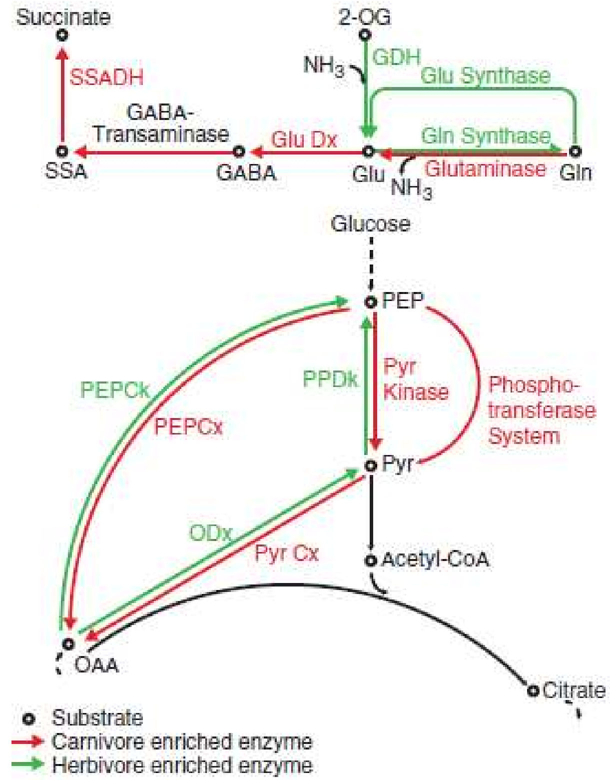
<u>Life expectancy at age 65</u>:

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

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





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

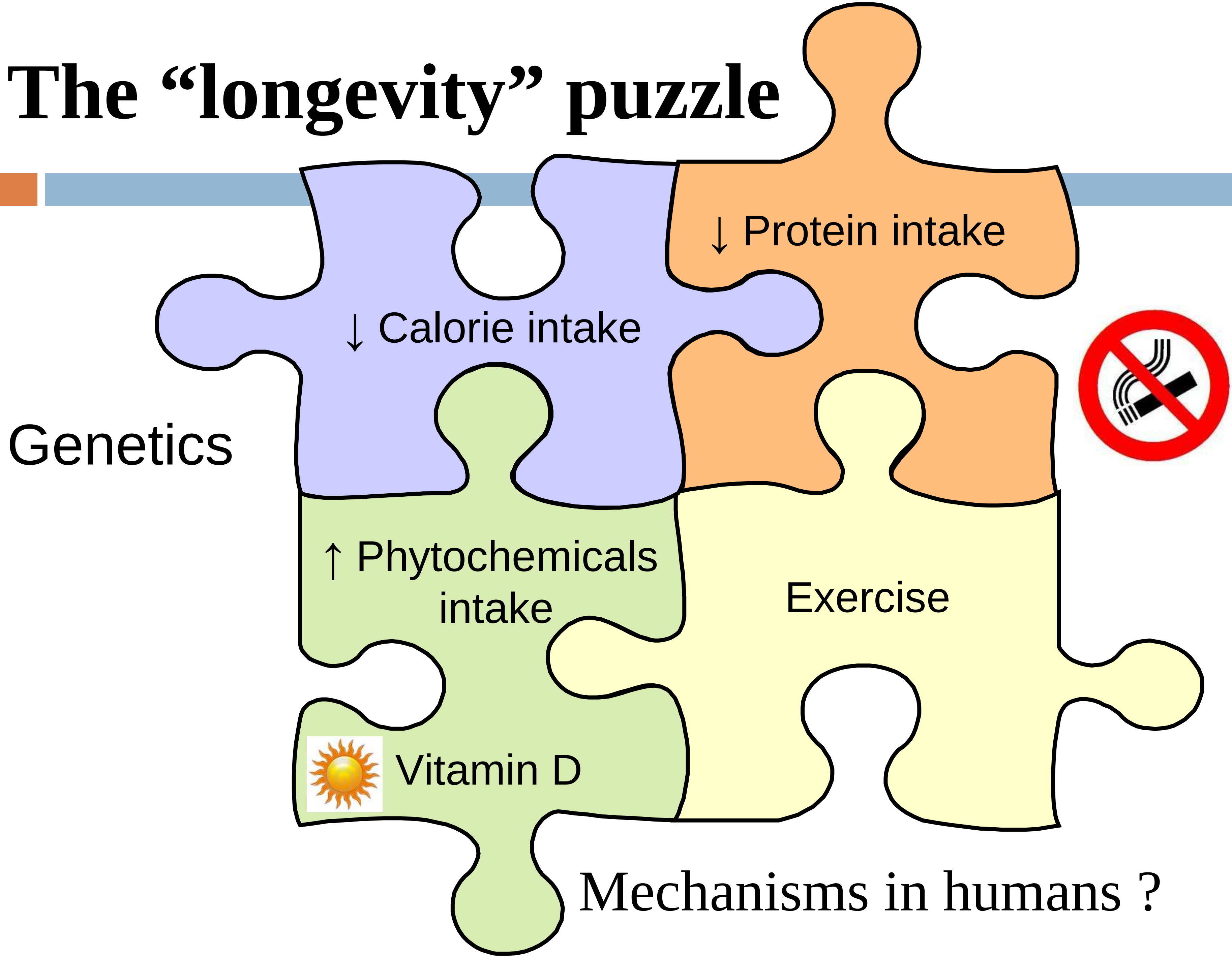
- ➢ Protein intake associated with KO data (R=0.307; adjusted p=0.030)
- ➢ Insoluble fiber associated with bacterial OTU (R=0.371; adjusted p=0.013

OTU = operational taxonomic units
KO = KEGG orthology groups

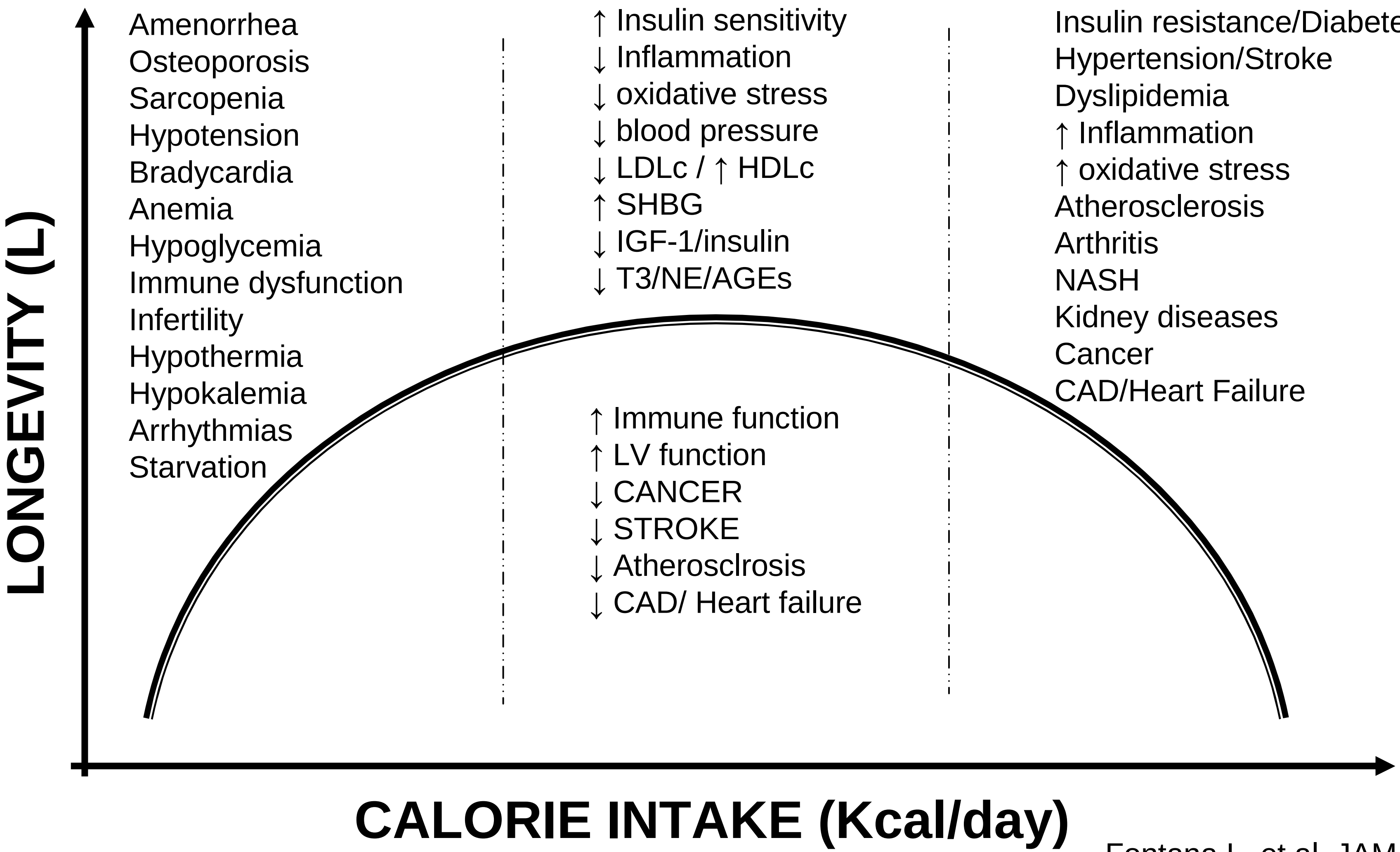
Muegge et al. Science 2011

CONCLUSIONS AND FUTURE DIRECTIONS

The "longevity" puzzle



OPTIMAL CALORIE INTAKE FOR SUCCESSFUL/HEALTHY AGING



Fontana L. et al. JAMA 2007

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