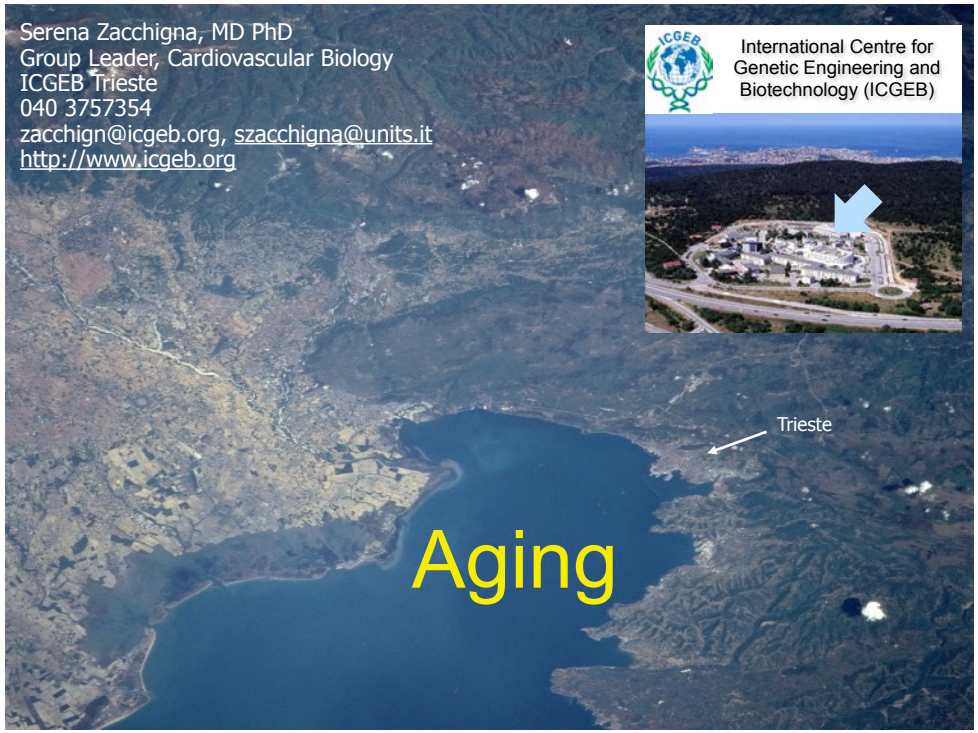


Terapia Genica e Medicina Rigenerativa

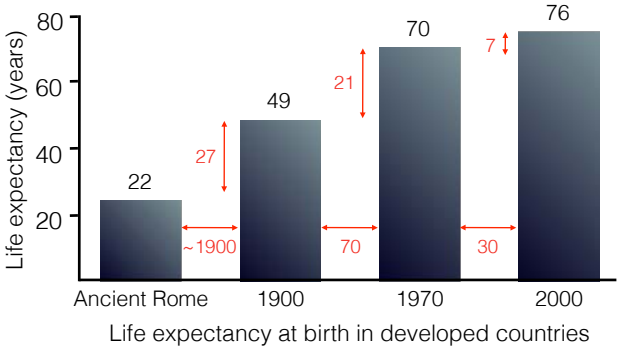
- Biologia dell'invecchiamento
- Introduzione all'ingegneria genetica e alla terapia genica
- Vettori retrovirali e terapia genica delle malattie del sistema ematopoietico
- Vettori AAV e terapia genica delle malattie del muscolo e dell'emofilia
- Nuove terapie per la malattie oculari
- Terapia genica delle malattie neurodegenerative
- Terapia genica delle malattie cardiovascolari
- Vettori adenovirali e terapia genica dei tumori
- Tecniche di silenziamento genico e applicazioni
- Cellule staminali
- Terapia cellulare e genica per la rigenerazione della pelle
- Rigenerazione cardiaca
- Terapia cellulare del sistema nervoso
- Gene editing
- Ingegneria tessutale

Aging

Aging is commonly characterized as a progressive, generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease and death. It is also usually accompanied by a decline in fertility. Thus, aging is associated with major age-related losses in Darwinian fitness, posing the puzzle of why it has not been more effectively opposed by natural selection.

"*It is remarkable that after a seemingly miraculous feat of morphogenesis, a complex metazoan should be unable to perform the much simpler task of merely maintaining what is already formed*" (Williams, 1957)

How long shall we live?



- US Census Bureau Middle Series: life expectancy in 2050 will be ~82 years for both sexes in the US
- US Social Security Administration: life expectancy of 78.1, 80.4 and 83.5 years for both sexes in 2066 on three alternative assumptions
- G7 Industrialized Countries: life expectancy in 2050 with a maximum of 90.9 in Japan and a minimum of 82.9 years in USA

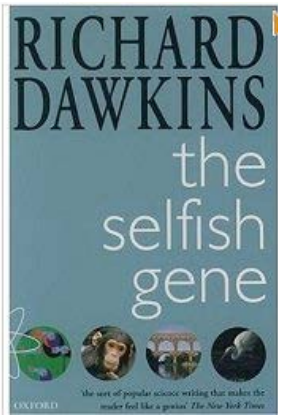
Why do we age?

Final part of the developmental program
(aging selected because provides advantage to the species?)

How do we age?

Exhaustion of the proliferative or functional capacity of all or some somatic cells (eg. in stem cells?)

Changes in biochemical composition of tissues (increased adipose tissue, lipofuscin deposit, increased ECM component cross-linking, increased glycation products)



Shall we live forever?

Maximum life span for the human species (unchanged in the last 100,000 years): 125 years

The longest-lived human being is Jeanne Calment (122.5 years), died in France, in August 1997

Maximum life span in other species:

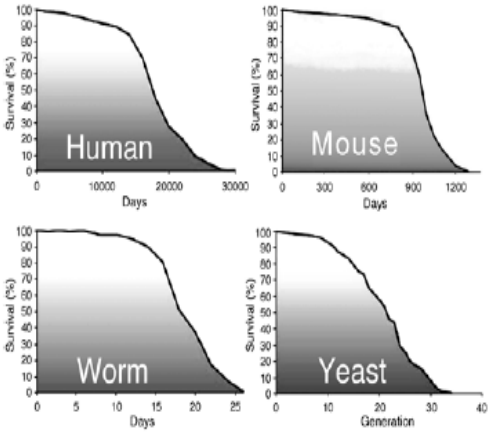
Rat: 3 years
Squirrel: 25 years
Sheep: 12 years
Turtle: 150 years
Dog: 15-30 years
Fly: 3 months
Canary 15 years
Bat 50 years

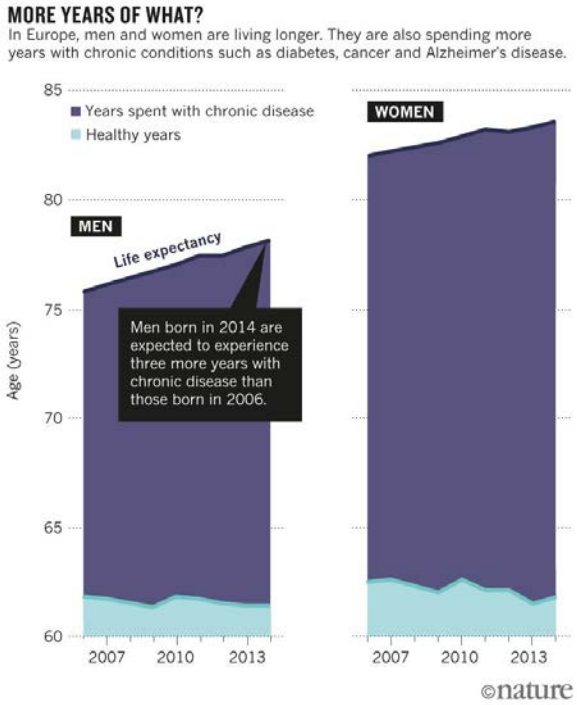
In animal studies, **maximum life span** is often taken to be the mean life span of the most long-lived 10% of a given cohort. By another definition, however, maximum life span corresponds to the age at which the oldest known member of a species or experimental group has died. Calculation of the maximum life span in the latter sense depends upon initial sample size.

EXISTENCE OF A BIOLOGICAL CLOCK?

Age-related diseases are degenerative in nature and compressed at the end of our life

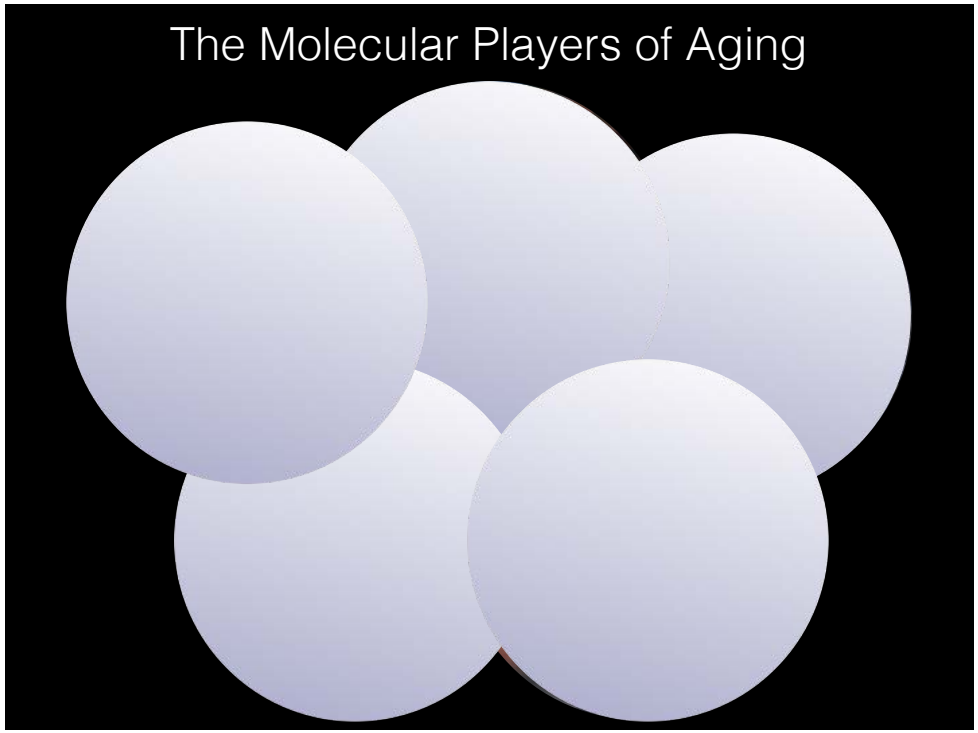
- Increased mortality with age maturation
- Increased susceptibility and vulnerability to disease (centenarians live >90% of their lives in very good health and with high level of independence - marked morbidity compression toward the end of life)





Nature 2018

The Molecular Players of Aging



Theories of Aging

1. **Somatic mutation theory or Loose cannon theory or Free radical theory of aging.** Damage produced by free radicals, glucose, or other agents slowly disrupt cellular macromolecules. This causes an age-related increase in somatic mutation and other forms of DNA damage
2. **Telomere loss theory.** A decline in cellular division capacity with age linked to the progressive shortening of telomeres as cells divide
3. **Mitochondrial theory.** Accumulation of mitochondrial DNA mutations with age
4. **Altered proteins theory and waste accumulation theory.** Accumulation over time of damaged proteins (e.g. Alzheimer's disease, Parkinson's disease, cataract, etc.). Linked to functional declines of proteasomes and chaperones
5. **Antagonistic pleiotropic theory.** Pleiotropic genes exist having opposite effects on fitness at different ages: they are beneficial in early life, when natural selection is strong, but harmful at later ages, when selection is weak
6. **Mutation accumulation theory.** Since late-acting alleles, arising by de novo germline mutation, are not efficiently selected by natural selection, over successive generations they accumulate within the genome.
7. **Rate of living theory.** Metabolic rate is inversely correlated with longevity. Smaller mammals tend to have high metabolic rates and thus tend to die at an earlier age than larger mammals
8. **Weak link theory.** A specific physiologic system (e.g. the neuroendocrine or the immune system) is particularly vulnerable over time and its dysfunction accelerates senescence of the whole organism

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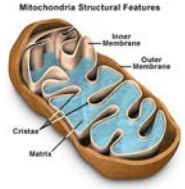
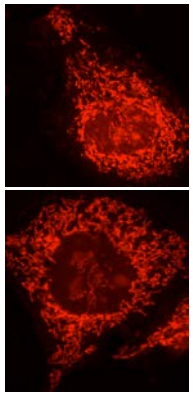
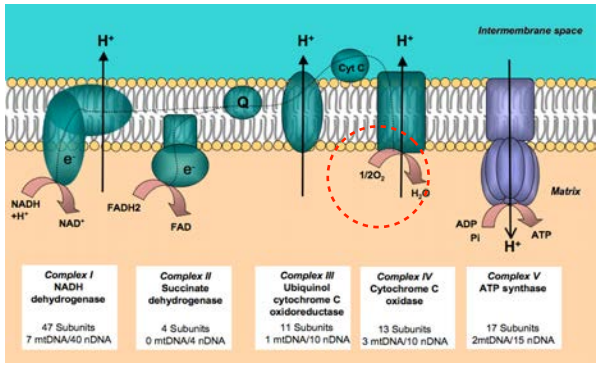
9. **Error catastrophe theory.** Errors in DNA transcription or RNA translation eventually lead to genetic errors that promote senescence
10. **Master clock theory.** Aging is under genetic control (gene that controls telomere shortening? or cell division? or DNA repair?
11. **Disposable soma theory.** Since the metabolic resources of an organism are limited (chiefly: energy), the organism should optimally allocate them between the maintenance and repair of its soma and the other functions that it must carry out in order to maximise its Darwinian fitness (growth, reproduction,...)
12. **Combined network theories** of aging. Multiplicity of aging mechanisms (e.g.: a gradual accumulation of mtDNA mutations might lead to a steady increase in the production of ROS and a gradual decline in energy production





red: mitochondria

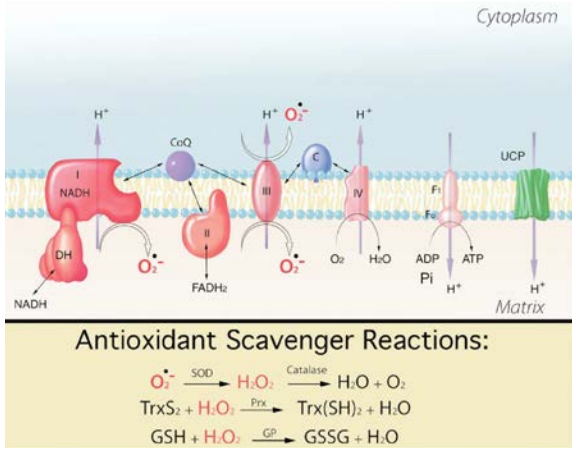
We use oxygen to generate energy!



Oxidative phosphorylation:
The metabolic pathway in mitochondria in which energy released by the oxidation of nutrients is used to reform ATP

In a cardiomyocyte, the are ~10,000 mitochondria, which occupy ~30% of the cytoplasm

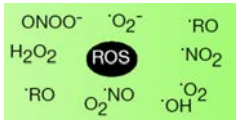
Reactive oxygen species (ROS)



ROS are produced in multiple compartments:

- mitochondria (90%)
- NADPH oxidases on the plasma membrane
- lipid metabolism in the peroxisomes
- cytosolic enzymes such as cyclooxygenases
- cytochrome P450 enzymes

0.2-2% of total oxygen consumption is funneled to ROS generation



superoxide anion	O_2^-
hydroxyl radical	·OH
hydrogen peroxide	H_2O_2
nitric oxide	NO

The "free radical theory of aging" (Harman, 1956)

Aging and its associated degenerative diseases can be attributed to deleterious effects of free radicals on various cell components

Now better called "Oxidative stress theory of aging" (many ROS are not free radicals)

Mitochondria are the main source of ROS

Mitochondrial mutations and aging

- The **mutation rate** in mitochondria is 10-20 times faster than the nuclear DNA mutation rate
- Specific mutations in mitochondria could lead to **defects in energy production** and production of ROS by faulty electron transport
- **Age-dependent declines in mitochondrial function** are seen in many species including **humans**
- **Inherited mitochondrial DNA variants** are associated with aging and longevity (the J haplogroup is more represented in centenarians in **Northern Italy** than in younger subjects)
- **Knock-in mice** expressing a proof-reading deficient form of a nuclear-encoded mitochondrial DNA polymerase exhibit an increased mitochondrial mutation rate, appearance of a number of age-related phenotypes - including **hair loss, kyphosis, and reduced fertility -, and shortened life span**

C. elegans mutants, oxidative stress and aging

isp-1 mutants are **long-lived** (missense mutation in a component of complex III of the respiratory chain in mitochondria)

A **systematic RNAi** screen sought to inactivate over 5600 random C. elegans genes screening for long-lived animals: ~15% of the identified genes regulate mitochondrial activity

mev-1 mutants (mutation in a subunit of complex II) have increased ROS generation and are **short lived**; mice heterozygous for mitochondrial SOD2: increased incidence of nuclear DNA damage and tumor formation

clk-1 mutants are **long-lived** (lack an enzyme required in the biosynthesis of ubiquinone (coenzyme Q), an electron acceptor for both complex I and II-dependent respiration - *NB: although coenzyme Q is sold as a life-extending anti-oxidant, its withdrawal from the diet of wt worms increases life span by 60%!!!*

You can live longer if you have mutations that makes the mitochondrion less functional and thus able to generate lower amount of ROS

Indy (I'm not dead yet): **50%** increase in life span. Indy encodes a protein with sequence homology to mammalian sodium dicarbocylate cotransporters, which import Krebs cycle intermediates into cells. Indy is expressed in the midgut and the fat body, the fly functional equivalent of mammalian liver and white adipose tissue. Indy mutations create a metabolic state similar to that found in dietary restriction.

Mth (methuselah): 25% increase in life span. Family of the seven transmembrane spanning GTP-binding protein-coupled receptors (GPRC). The cognate ligand is the product of the stunted gene, encoding for a subunit of the F_1F_0-ATP synthase of the electron transport chain (!)

Oxidants and antioxidant therapies in aging

Box 1 Table **Oxidants, antioxidants and diseases of ageing**

Disease system	Laboratory/animal studies	Clinical data
Cardiovascular	Pre-atherosclerotic blood vessels have increased levels of ROS[86]	PHS I: no overall benefit of beta-carotene on CVD? Benefit in high-risk subgroup[99]
	Vitamin E protects against development of atherosclerosis[87]	CHAOS trail: vitamin E reduces rate of non-fatal myocardial infarct[100]
	Disruption of SOD leads to heart failure[88,89] and overexpression protects against injury[90]	ATBC study: no overall benefit on CVD rate with vitamin E or beta-carotene?[1] Increase in CVD deaths with beta-carotene[10]
Ophthalmological	Offspring of pregnant mice depleted of glutathione develop cataracts[91]	PHS I: non-significant reduction in cataracts and macular degeneration with vitamin E and multivitamins[102]
	Retinal pigments produce ROS after light exposure[92]	NHS: carotenoids intake may decrease risk of cataracts[103]
	Retinal degeneration in primates with vitamin A or E deficiencies[93]	
Neurological	Mutations in SOD1 result in human ALS[94] and transgenic animal models rescued by antioxidants[95,96]	Vitamin E not protective in early Parkinson's disease[104]
	NMDA-receptor stimulation produces superoxide[97]	Vitamin E beneficial in Alzheimer's disease[105]
	Defects in the function of complex 1 seen in Parkinson's disease[98]	*N*-acetylcysteine does not effect survival in ALS[106]

The references cited above should be viewed as only representative examples derived from a much larger, relevant body of literature, which owing to space constraints cannot be fully presented. Acronyms and abbreviations: PHS I, Physicians' Health Study I; CHAOS, Cambridge Heart Antioxidant Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; NHS, Nurses' Health Study; CVD, cardiovascular disease; ALS, amyotrophic lateral sclerosis; NMDA, *N*-methyl-d-aspartate glutamate receptors.

In humans, meta-analysis of randomized controlled trials showed that selenium and vitamin C have no effect while standard antioxidant supplementation (vitamins A and E and beta-carotene) actually increases mortality

BIOMEDICAL RESEARCH

Antioxidants Could Spur Tumors by Acting on Cancer Gene

Many people take vitamins such as A, E, and C thinking that their antioxidant properties will ward off cancer. But some clinical trials have suggested that such antioxidants, which sop up DNA-damaging molecules called free radicals, have the opposite effect and raise cancer risk in certain people. Now, in a provocative study that raises unsettling questions about the widespread use of vitamin supplements, Swedish researchers have showed that moderate doses of two widely used antioxidants spur the growth of early lung tumors in mice.

Some cancer specialists caution against basing public health advice on the study, published online this week in *Science Translational Medicine*. "You can't extrapolate from this study to make a recommendation to people," says Barry Kramer, director of the Division of Cancer Prevention at the National Cancer Institute in Bethesda, Maryland. He notes that the science of antioxidants is complicated and that the results of mice studies often don't apply to humans. Still, Kramer and others say the new findings demand further exploration.

The observation decades ago that people who consumed lots of fruits and vegetables had less cancer suggested that the antioxidants in these foods might be protecting them. But in the 1980s, researchers launched two large clinical trials to test whether the antioxidants β carotene (a vitamin A precursor), vitamin A, and vitamin E protect smokers from lung cancer—and found more cases of lung cancer in volunteers taking β carotene, leading one trial to end early. A more recent trial testing vitamin E and selenium to prevent prostate cancer was stopped when prostate cancer turned out to be more common in the vitamin E group.

The Swedish researchers, led by Per Lindahl and Martin Bergö of the University of Gothenburg, studied two antioxidants: n-acetylcysteine (NAC), a water-soluble drug used to thin mucus in people with lung disease, and fat-soluble vitamin E. They gave mice genetically engineered to develop lung tumors a dose of NAC comparable to what a patient would receive or chow containing about 10 times more vitamin E than is in ordinary mouse food. "A lot of vitamin pills contain a lot more than that. It's a conservative dose," Bergö says.

Compared with mice on a normal diet, the mice consuming the antioxidants developed more lung tumors, their tumors were more aggressive, and they lived only half as long. Follow-up studies suggested that by reducing reactive oxidative species and DNA damage in the cell, the antioxidants turn down a gene, *p53*, that is key to keeping cell growth in check and is often inactivated in cancer. For example, *p53*'s protein stops the cell cycle so enzymes can repair damaged DNA and triggers apoptosis, or self-destruction, in severely damaged cells. In cancer cells in which *p53* had been turned off, Lindahl and Bergö found, the antioxidants had no effect on cell proliferation.

The implication, Bergö suggests, is that people at high risk of cancer—such as smokers—and others who have incipient tumors should avoid taking extra antioxidants. "In a normal cell an antioxidant might be very good. But if you have a small tumor that might become a cancer, it will reduce *p53* and the tumor will grow," Bergö says.

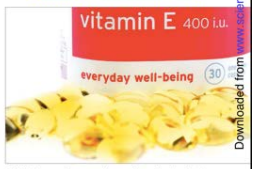
A clinical researcher involved with the aborted trials that tested antioxidants to prevent lung and prostate cancer says he is "thrilled" by the study. "It's the first paper I've seen that goes into some of the molecular biology to explain what we saw," says medical oncologist Gary Goodman of the Swedish Cancer Institute in Seattle, Washington. "This really shows that high doses of vitamins can be harmful."

Others are more restrained. "It's a provocative study," says cancer biologist David Tuveson of Cold Spring Harbor Laboratory in New York. "Perhaps we should look more carefully at what's available over the counter." But he would like to see a more detailed explanation of how the cell's sensing of reactive species controls *p53* activity. Lung disease researcher Shyam Biswal of Johns Hopkins University in Baltimore, Maryland, wonders if the results would be the same in mice with cancer sparked by a carcinogen, rather than an existing mutation. "The model is great, but it's a very aggressive model," Biswal says.

Another huge caveat, Kramer adds, is that in the earlier lung cancer prevention trials, only the participants taking β carotene had a higher risk of lung cancer, not those on vitamin E alone. "It's not likely that all antioxidants are exactly the same," he says. He and others also emphasize that the study does not suggest that people should eat less fruit and vegetables, which provide smaller doses of antioxidants and likely have other benefits.

Bergö and Lindahl now plan to extend their mouse studies to tests of β carotene and vitamin C and to other cancer types. They also plan to comb through medical records in Sweden to see if lung disease patients receiving NAC are at higher risk for lung cancer.

–JOCELYN KAISER



Risky? Consuming a moderate dose of vitamin E spurred lung tumor growth in cancer-prone mice.

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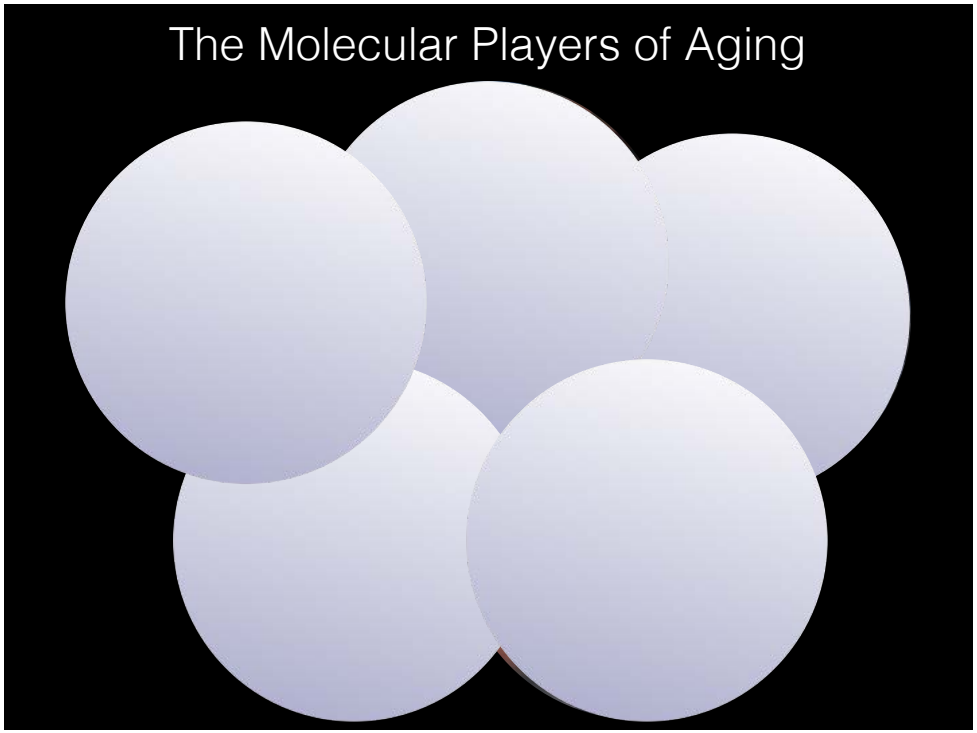
The Molecular Players of Aging



Potential targets for ROS in aging

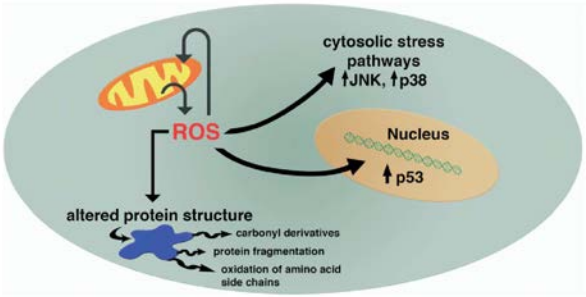
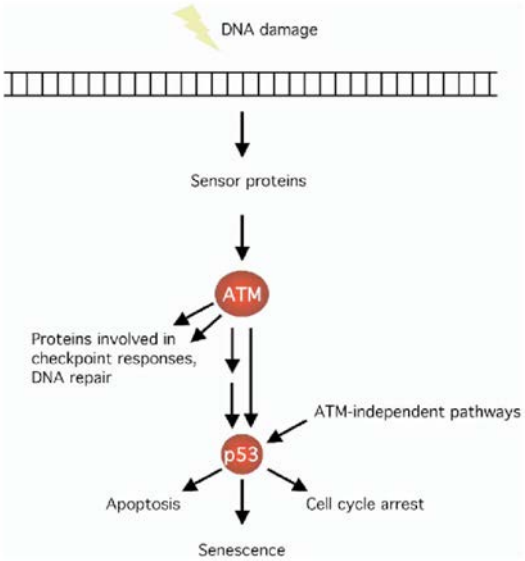


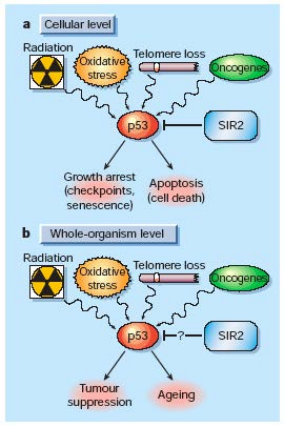
Figure 3. Potential Targets of ROS within Cells that May Determine the Rate of Aging

ROS generated within the mitochondria can potentially feed back on the organelle and directly damage mitochondrial DNA and other components in a putative vicious cycle. Similarly, mitochondrial oxidants can damage nuclear DNA leading to activation of p53 and other DNA damage pathways. Cytosolic elements including stress-activated kinases such as JNK and p38 may be potential targets. Finally, direct oxidative modification of proteins may be an important element of aging (see Berlett and Stadtman [1997]).

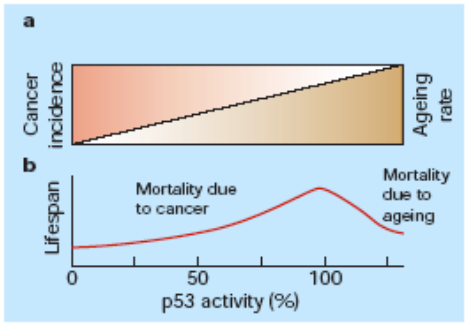
The DNA Damage Response:
senescence, checkpoints, cell proliferation and cancer



Aging: the price for tumor suppression?



Cellular senescence might have evolved as a mechanism of tumor suppression. Therefore, ageing would be an antagonistically pleiotropic manifestation of evolutionary pressure to prevent malignant transformation



A fine equilibrium between the anti-neoplastic and pro-aging effects of p53 may lead to the optimal lifespan for an organism

... but turtles can live up to 150 years

... do they form more tumors than other species?

Double stranded DNA break repair by non-homologous end-joining

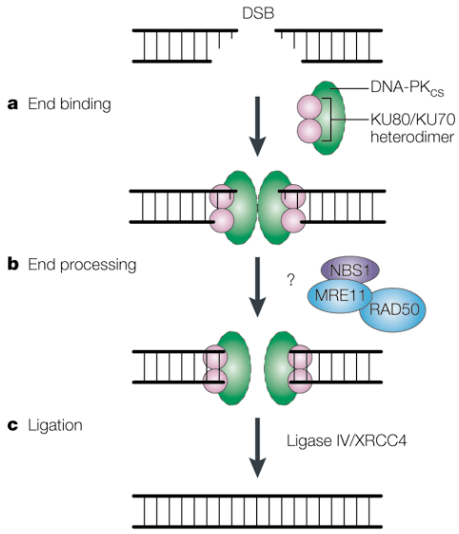
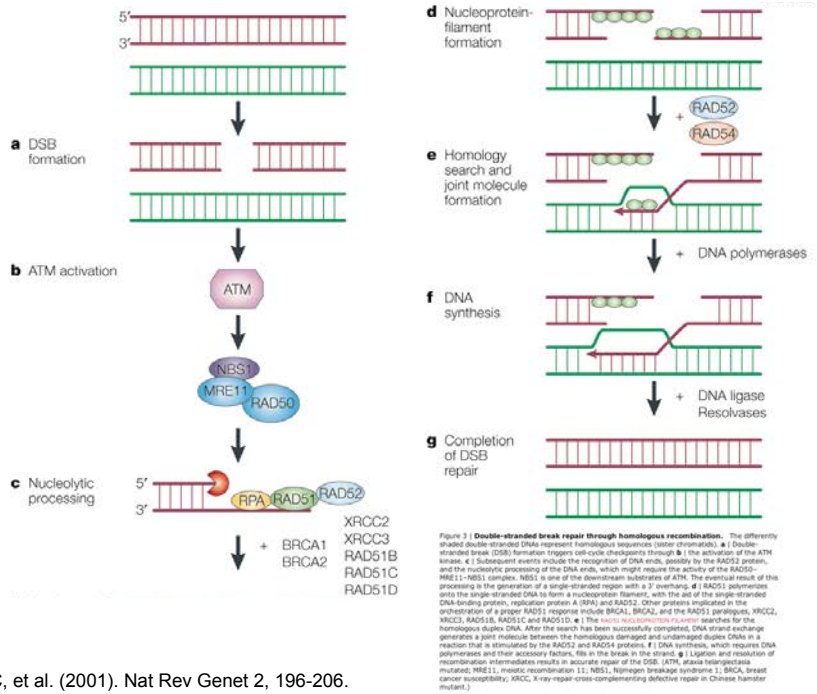


Figure 5 | **Double-stranded break repair by non-homologous end-joining.** **a** | After double-stranded break (DSB) formation, the KU–DNA-PK $_{CS}$ complex is probably involved in the initial recognition of the DSB and in the juxtaposition of the DNA ends. **b** | The ends might be processed, which results in the removal or addition of a few base pairs. **c** | This is followed by end-to-end ligation by the DNA ligase IV–XRCC4 complex. The role of the RAD50–MRE11–NBS1 complex is not yet clear. It might be involved in the unwinding and/or nucleolytic processing of the ends. Non-homologous end-joining does not make use of a template for repair and, therefore, this DSB-repair pathway is intrinsically error prone. (DNA-PK $_{CS}$ catalytic subunit DNA-dependent protein kinase; XRCC4, X-ray-repair-cross-complementing defective repair in Chinese hamster mutant 4; MRE11, meiotic recombination 11; NBS1, Nijmegen breakage syndrome 1.)

van Gent, DC, et al. (2001). Nat Rev Genet 2, 196-206.



van Gent, DC, et al. (2001). Nat Rev Genet 2, 196-206.

Human progeroid syndromes

Table 1 **Genetic instability syndromes**

Disease*	OMIM†	Gene	Function	Major phenotypes
Werner syndrome	277,700	*WRN* (ref. 6)	Helicase/exonuclease	Skin atrophy, cataracts, diabetes mellitus, osteoporosis, hypogonadism, atherosclerosis, cancer predisposition[2,9]
Rothmund–Thomson syndrome	268,400	*RecQ4* (ref. 50)	Helicase	Poikiloderma, photosensitivity, skeletal abnormality, cataracts, cancer predisposition (osteosarcoma)[51]
Cockayne syndrome, type A	216,400	*CKN1* (ref. 52)	WD repeat protein	Neurodegeneration, skeletal abnormality (widened face), impaired sexual development, photosensitivity[54]
Cockayne syndrome, type B	133,540	*ERCC6* (ref. 53)	Helicase	
Ataxia telangiectasia	208,900	*ATM* (ref. 55)	Kinase	Cerebellar dysfunction, sensitivity to ionizing radiation, cancer predisposition[56]
Nijmegen breakage syndrome (Ataxia-telangiectasia variant)	251,260	*NSB1* (ref. 57)	Unknown	Microcephaly, growth retardation, immunodeficiency, cancer predisposition; sensitivity to ionizing radiation[58]

*The listed disorders are all autosomal recessives.
†OMIM, Online Mendelian Inheritance in Man (ref. 49).

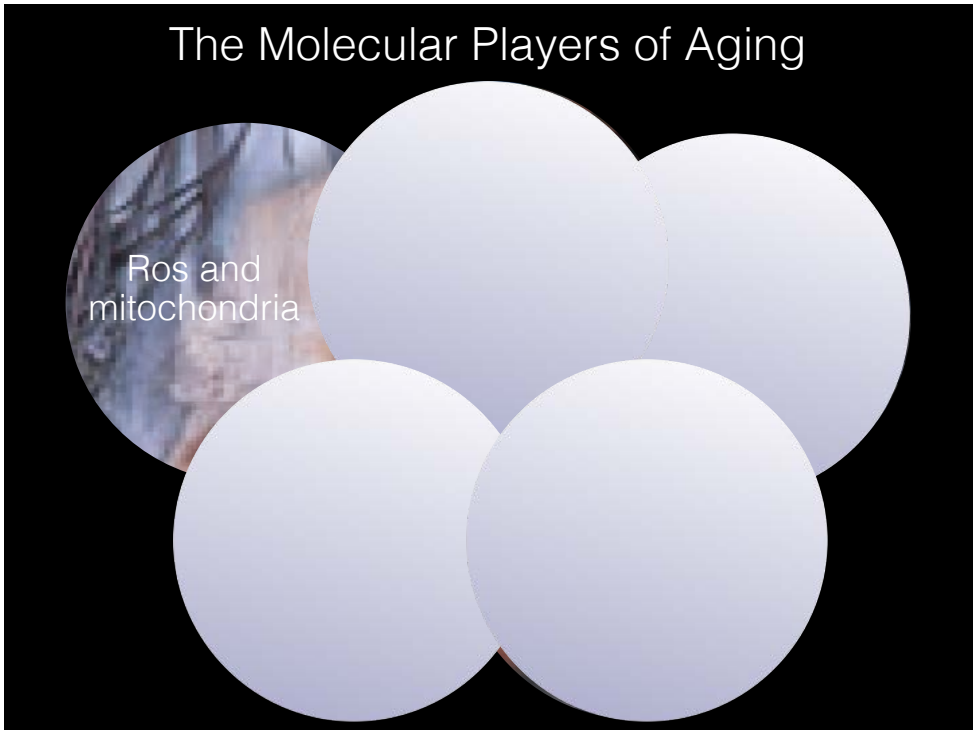
Werner syndrome

14 y

48 y

Werner syndrome is genetic recessive disorder. It is a type of progeria disease that occurs in adults ages twenty to thirty. People who are affected start to age rapidly beginning in their twenties and thirties and look as though they are twenty or more years older that what they actually are. Along with looking older patients develop other types of diseases and disorders that occur with normal aging. Werner's strikes about three in every 1 million people worldwide, although it is slightly more common in Japan.

The Molecular Players of Aging



Cellular senescence

- Normal human cells have a limited ability to proliferate in vitro (Hayflick, 1965)
- Growth potential of a primary cell declines 0.2 population doublings per year of life
- Correlation between the number of senescent cells in vivo and age of donor
- Cells from progeria syndrome patients have limited doubling potential
- Association with several molecular changes
- Overexpression of telomerase overcomes senescence; overexpression of ras induces senescence

TABLE 1. Selected Alterations in Cell Phenotype with the Onset of Senescence

Phenotypic alteration in senescence*	Cell type	Ref.
Permanent growth arrest	All	13, 19, 64, 65
Repression of c-*fos*	Fibroblasts, T lymphocytes	66, 67
Repression of cyclins A and B	Fibroblasts	68
G_2 arrest on restimulation without division	Fibroblasts, T lymphocytes	69, 37
Elevated collagenase	Fibroblasts	42
Elevated TIMP-2	Fibroblasts, endothelial cells	44, 70
Elevated PAI-1	Fibroblasts, endothelial cells	44
Elevated ceramide	Fibroblasts	71
Transcriptional repression of IGF-1	Fibroblasts	72
Induction of Ws3.10 inhibitor of Ca^{2+}-dependent membrane currents	Fibroblasts	73
Elevated IL-1α expression	Fibroblasts	56
Decreased IL-6 expression	Fibroblasts	74
Senescence-associated β-galactosidase	Fibroblasts, keratinocytes, mammary epithelial cells, endothelial cells, neonatal melanocytes	23
Induction of SAG gene	Fibroblasts	75
Repression of 17α-hydroxylase	Adrenocortical cells	11
Elevation of cytochrome *b* and NADH 4/4L subunit	Fibroblasts	76
Elevated *hic-5* expression	Fibroblasts	77

*TIMP-2, tissue inhibitor of metalloprotein 2; PAI-1, plasminogen activator inhibitor 1; IGF-1, insulin-like growth factor 1; IL, interleukin.

Senescent cells accumulate with age and contribute to age-related disease

Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment

Ok Hee Jeon[1,8], Chaekyu Kim[1,2,8], Remi-Martin Laberge[3,4], Marco Demaria[3,5], Sona Rathod[1], Alain P Vasserot[4], Jae Wook Chung[1], Do Hun Kim[1], Yan Poon[4], Nathaniel David[4], Darren J Baker[6], Jan M van Deursen[6], Judith Campisi[3,7] & Jennifer H Elisseeff[1]

Senescent cells (SnCs) accumulate in many vertebrate tissues with age and contribute to age-related pathologies[1–3], presumably through their secretion of factors contributing to the senescence-associated secretory phenotype (SASP)[4–6]. Removal of SnCs delays several pathologies[7–9] and increases healthy lifespan[8]. Aging and trauma are risk factors for the development of osteoarthritis (OA)[10], a chronic disease characterized by degeneration of articular cartilage leading to pain and physical disability. Senescent chondrocytes are found in cartilage tissue isolated from patients undergoing joint replacement surgery[11–14], yet their role in disease pathogenesis is unknown. To test the idea that SnCs might play a causative role in OA, we used the p16-3MR transgenic mouse, which harbors a $p16^{INK4a}$ (*Cdkn2a*) promoter driving the expression of a fusion protein containing synthetic *Renilla* luciferase and monomeric red fluorescent protein domains, as well as a truncated form of herpes simplex virus 1 thymidine kinase (HSV-TK)[15,16]. This mouse strain allowed us to selectively follow and remove SnCs after anterior cruciate ligament transection (ACLT). We found that SnCs accumulated in the articular cartilage and synovium after ACLT, and selective elimination of these cells attenuated the development of post-traumatic OA, reduced pain and increased cartilage development. Intra-articular injection of a senolytic molecule that selectively killed SnCs validated these results in transgenic, non-transgenic and aged mice. Selective removal of the SnCs from *in vitro* cultures of chondrocytes isolated from patients with OA undergoing total knee replacement decreased expression of senescent and inflammatory markers while also increasing expression of cartilage tissue extracellular matrix proteins. Collectively, these findings support the use of SnCs as a therapeutic target for treating degenerative joint disease.

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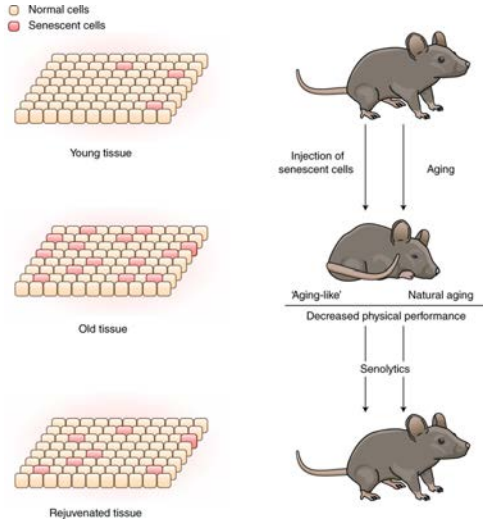
Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline

Tyler J. Bussian[1,3], Asef Aziz[2,3], Charlton F. Meyer[2], Barbara L. Swenson[2], Jan M. van Deursen[1,2] & Darren J. Baker[1,2]*

Cellular senescence, which is characterized by an irreversible cell-cycle arrest[1] accompanied by a distinctive secretory phenotype[2], can be induced through various intracellular and extracellular factors. Senescent cells that express the cell cycle inhibitory protein $p16^{INK4A}$ have been found to actively drive naturally occurring age-related tissue deterioration[3,4] and contribute to several diseases associated with ageing, including atherosclerosis[5] and osteoarthritis[6]. Various markers of senescence have been observed in patients with neurodegenerative diseases[7–9], however, a role for senescent cells in the aetiology of these pathologies is unknown. Here we show a causal link between the accumulation of senescent cells and cognition-associated neuronal loss. We found that the *MAPT*^{P301S}*PS19* mouse model of tau-dependent neurodegenerative disease[10] accumulates $p16^{INK4A}$-positive senescent astrocytes and microglia. Clearance of these cells as they arise using *INK-ATTAC* transgenic mice prevents gliosis, hyperphosphorylation of both soluble and insoluble tau leading to neurofibrillary tangle deposition, and degeneration of cortical and hippocampal neurons, thus preserving cognitive function. Pharmacological intervention with a first-generation senolytic modulates tau aggregation. Collectively, these results show that senescent cells have a role in the initiation and progression of tau-mediated disease, and suggest that targeting senescent cells may provide a therapeutic avenue for the treatment of these pathologies.

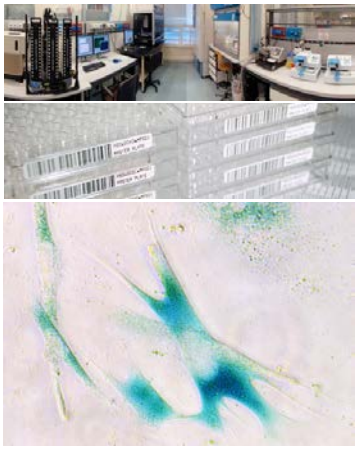
Senolytic drugs contrast aging phenotypes

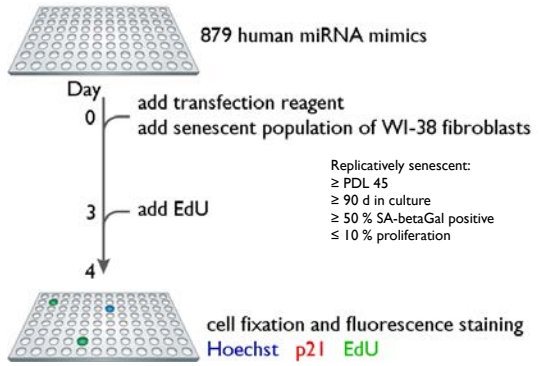
- **Senolytic drugs**: drugs that preferentially kill senescent cells
- In old mice, or mice 'aged' with senescent cells, as senolytic drugs (**dasatinib** and **quercetin**) increase remaining lifespan by 36%, enhance healthspan, reduce frailty and delay age-related diseases



Senolytics improve physical function and increase lifespan in old age, Nature Medicine, August 2018

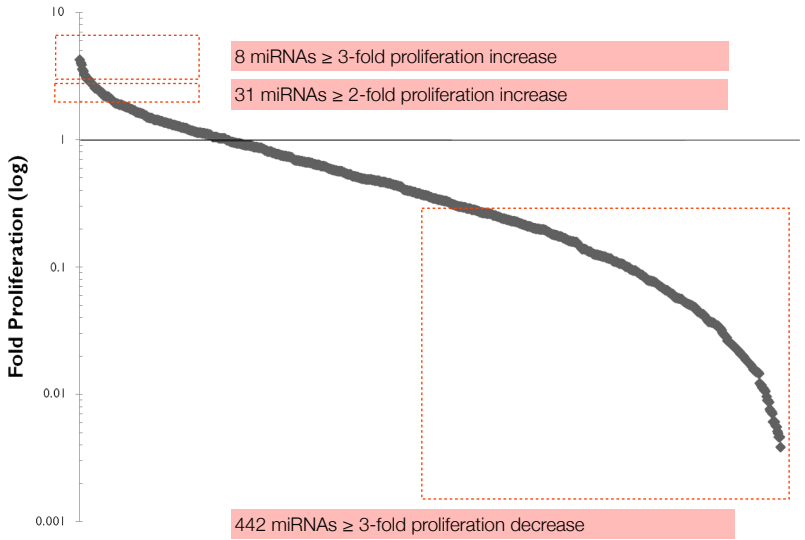
High throughput screening to identify microRNAs bypassing cellular senescence



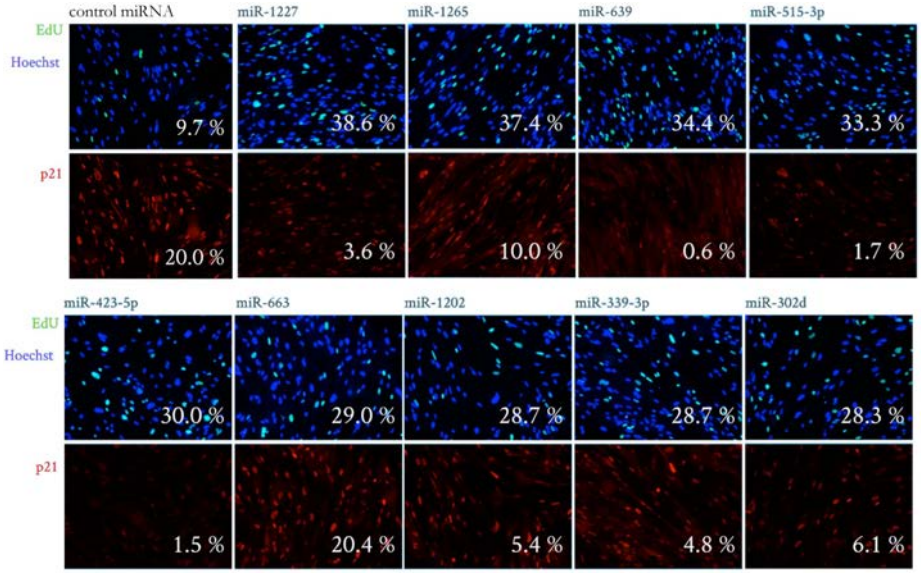


Nadja Ring

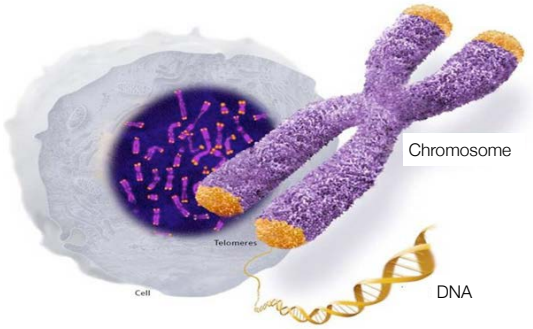
High throughput screening identifies microRNAs bypassing cellular senescence



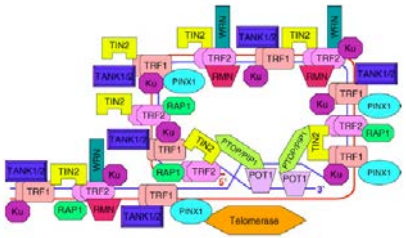
Screening results: top hits



Telomeres are shortened during cellular senescence



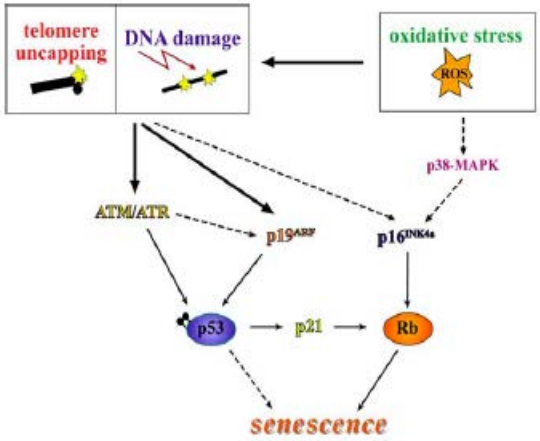
Vertebrate telomeres are long stretches (1-50 kb) of dsDNA containing the repetitive sequence **TTAGGG**, which terminate in 100-200 bases of ss TTAGGG at the 3' end.
This 3' overhang circles back end embed in the duplex DNA



The extended telomeric cap helps maintain the stability of the genome

Telomeres uncapping causes a DNA damage response

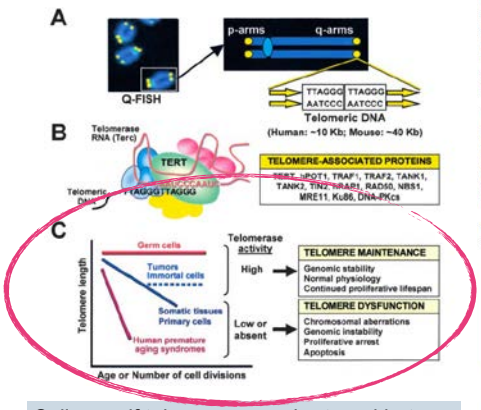
Senescent telomeres lose some of their single-stranded portion - the telomeric overhang - which is crucial for the maintenance of the T-loop and the subsequent formation of the cap



Telomere uncapping (disruption of the proper structure of the protective cap) seems to be recognised as a dsDNA break, activating the DNA damage machinery.

Telomeres

In normal human cells, at every replication cycle the telomere looses its terminal part and gets shortened



Cells age if telomeres are shortened but senescence is delayed if the telomerase is produced and telomeres can be restored

Three Scientists Win Nobel Prize in Medicine

Their work involved the health of cells and the aging process. *Transcript of radio broadcast:*
06 October 2009

This is the VOA Special English Health Report.

Three scientists based in the United States have won the two thousand nine Nobel Prize for Physiology or Medicine. They are being honored for their work in the nineteen eighties about the health of cells and the aging process.

The winners are Elizabeth Blackburn from the University of California, San Francisco; Jack Szostak from Harvard Medical School in Massachusetts and Carol Greider from Johns Hopkins University in Maryland. They will share the one million four hundred thousand dollar prize.

Elizabeth Blackburn, left, and Carol Greider after receiving a science prize in Frankfurt, Germany, earlier this year

The scientists' work begins with telomeres. These are like protective coverings on the ends of chromosomes. Elizabeth Blackburn compares them to the plastic tips on the ends of shoelaces. She says without telomeres the chromosome and the genes it holds would come apart.

Telomeres are necessary for a cell to divide. They also are involved in directing the number of divisions.

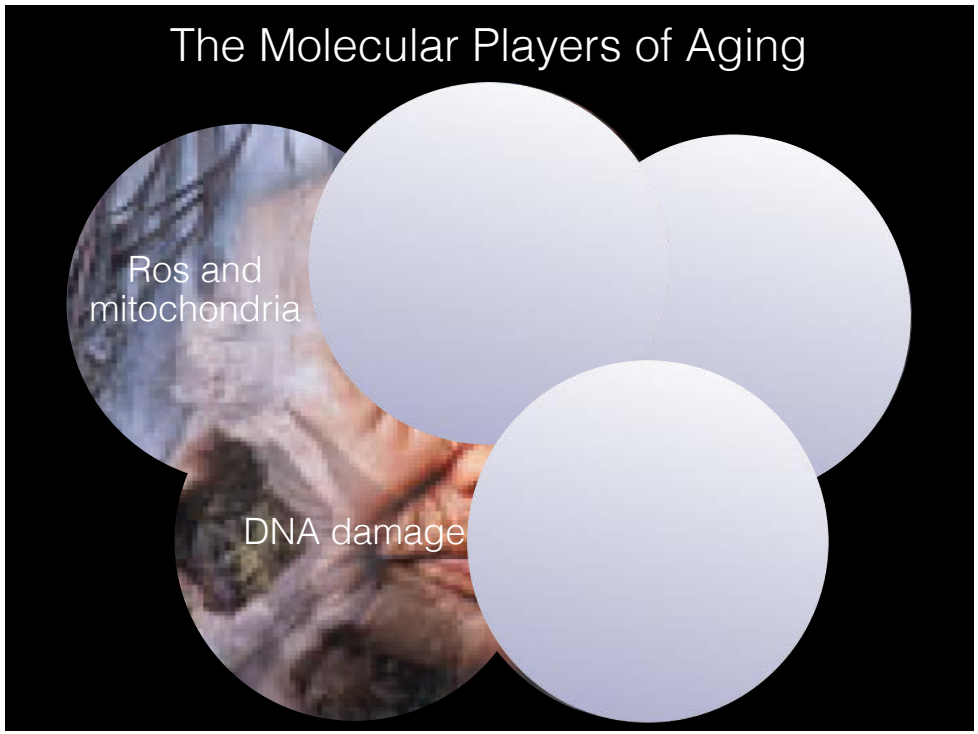
Jack Szostak

Mizz Blackburn and Mister Szostak discovered the special system of genetic information in the telomeres that protects the chromosomes from ruin. Later, Mizz Blackburn and Mizz Greider discovered the substance in the body that builds telomeres. The scientists named the enzyme telomerase.

Their research showed that cells age if telomeres are shortened. But, cell death is delayed if a lot of the enzyme telomerase is produced.

Rune Toftgard is a Nobel Committee member from Sweden's Karolinska Institute. He says the work of telomeres is important to the understanding of how genetic material is copied and saved.

Short telomeres are associated to senescence but there is no proof that telomere shortening is causative in aging



Is longevity controlled by a genetic (biologic) program?

- C. elegans: at 20°C it lives, on average, 17 days, with a maximum of 25 days; in conditions of high density and food, it transforms into a larval form (dauer), which that does not reproduce and lives 60 days

- Social insects: queens and workers are born from the same eggs fertilized by the same drone; workers live a few weeks in summer and a few months in winter; queens live several years

- Some animals (turtles, deep water fishes, american lobster) age very slowly; these animals show no limit to body mass increase

Epigenetic control of longevity and reproductive status

Fertile queens and sterile workers are alternative forms of the adult female honeybee that develop from genetically identical larvae following differential feeding with royal jelly.

Nutritional Control of Reproductive Status in Honeybees via DNA Methylation

R. Kucharski*, J. Maleszka*, S. Foret and R. Maleszka†

Science
www.sciencemag.org
Published Online March 13 2008
Science 28 March 2008:
Vol. 319 no. 5871 pp. 1827–1830
DOI: 10.1126/science.1153069

The "genes of aging"

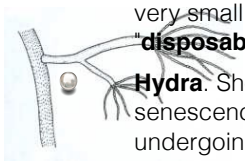
Mutation in single genes decrease life span

Table 1. Features of Selected Models of Premature Aging in the Mouse

Mutant	Cellular Process Affected	Tissues Affected	Increased Rate of Cancer?	Accelerated Fibroblast Senescence?	Citations
Atm	DSB signaling/repair	Cerebellum, Gonad, Hematopoietic organs, Thymus	Yes	Yes	(Ito et al., 2004; Shiloh and Kastan, 2001)
Bub1b$^{H/H}$	Spindle assembly checkpoint	Bone, Lens, Skin, Gonad	No	Yes	(Baker et al., 2004)
BRCA1$^{\Delta 11/\Delta 11}$; p53$^{+/-}$	DSB repair, other	Bone, Eye, Heart, Intestine, Liver, Lymphocytic hyperplasia, Testes +others	Yes	Yes	(Cao et al., 2003b)
DNA-PKcs	NHEJ, other	Bone, Intestine	Yes	No	(Espejel et al., 2004b)
Ercc1, XPF	Nucleotide excision repair, crosslink repair, other	Liver (Ercc1, XPF) + Brain, Kidney, Skin, Spleen—Ercc1	No	Yes (Ercc1)	(McWhir et al., 1993; Tian et al., 2004; Weeda et al., 1997)
Ku80	NHEJ, other	Bone, Liver, Skin	No	Yes	(Vogel et al., 1999)
Dysfunctional p53 (p53^{R172H})	DNA damage response	Bone, Hair, Lymphoid tissue, Skin	No	ND	(Tyner et al., 2002)
Dysfunctional p53 (p44 Tg)	DNA damage response	Bone, Testes	No	Yes	(Maier et al., 2004)
PASG/Lsh	DNA methylation	Bone, Hair, Kidney, Skin, Thymus	No	Yes	(Sun et al., 2004)
PolgA	Mitochondrial DNA polymerase	Bone, Hair, Heart, Hematopoietic organs, Skin, Testes	No	ND	(Trifunovic et al., 2004)
Rad50$^{S/S}$	DSB repair	Hematopoietic organs, Testes	Yes	No	(Bender et al., 2002)
Terc	Telomere maintenance	Hematopoietic organs, Hair, Heart, Intestine, Myometrium, Skin, Testes	Conflicting results	Yes	(Espejel et al., 2004a; Lee et al., 1998)
Terc/Atm	Telomere maintenance/ DSB signaling/repair	Bone, Brain, Hair, Hematopoietic organs, Intestine	No	Yes	(Wong et al., 2003)
Terc/DNA-PKcs	Telomere maintenance/ NHEJ, other	Intestine, Various	No	ND	(Espejel et al., 2004a)
Terc/Parp-1	Telomere maintenance/ DNA repair	Various	No	ND	(Espejel et al., 2004a)
Terc/Ku80	Telomere maintenance/ NHEJ, other	Intestine, Various	No	ND	(Espejel et al., 2004a)
Terc/Wrn	Telomere maintenance/ DNA repair	Bone, Endocrine, Gonad, Hair, Intestine, Lens, Skin, Spleen	Yes	Yes	(Chang et al., 2004)
Terc/Wrn/Blm	Telomere maintenance/ DNA repair	Bone, Endocrine, Gonad, Hair, Intestine	Yes	Yes	(Du et al., 2004)
Top1IIbeta	Topoisomerase	Kidney, Lymphocytic infiltrates, Pancreatic islets, Skin, Testes	No	ND	(Kwan et al., 2003)
Wrn	DNA repair	None	No	Yes	(Lebel and Leder, 1998; Lombard et al., 2000)
XPA/CSB	NER, transcription	Cerebellum	No	ND	(Murai et al., 2001)
Xpd^{TTD}	NER, transcription	Bone, Hair, Ovary, Skin	Yes	ND	(de Boer et al., 2002)
Xpd^{TTD}/XPA	NER, transcription	Bone, Hair, Skin	No	ND	(de Boer et al., 2002)

Other interesting organisms

Semelparous organisms (once-only reproducing species; e.g. Pacific salmon, marsupial male rat; also called "**Big Bang animals**"). Die immediately after mating. Most probably, the mechanism is not active, and due to the fact that natural selection has evolved that a massive effort is made to mobilize all available resources to maximize reproductive success, even if this leaves the adult so severely depleted or damaged that death ensues. This is most likely to occur where ecological circumstances decree that the chance of surviving to breed again are very small (an extreme example of the "**disposable soma theory**").



Hydra. Show slow or negligible rate of senescence. These organisms are capable of undergoing complete regeneration from almost any part of their structure, implying that germ cells permeate the body that there is no true distinction between germline and somatic tissue.

Mutants in the IIS pathway with extended lifespan in the mouse

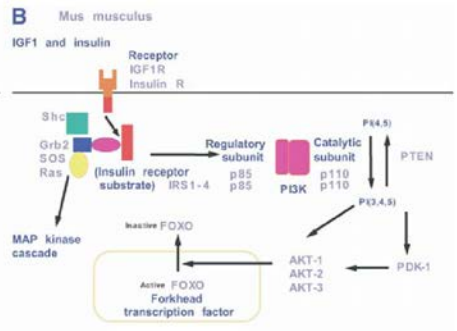
Ames and **Snell Dwarf mice**: miss the growth hormone-IGF-1 axis and other pituitary hormones due to mutations in the pit-1 gene

Little mice: mutations in the GH-releasing hormone receptor

KO mice for ligands (insulin, IGF1, IGF2)

KO mice for receptors (IR, IFG1R, GHR)

KO mice for immediate downstream signaling molecules (IRS proteins and other adaptor molecules including p66Shc)



Cell growth and proliferation

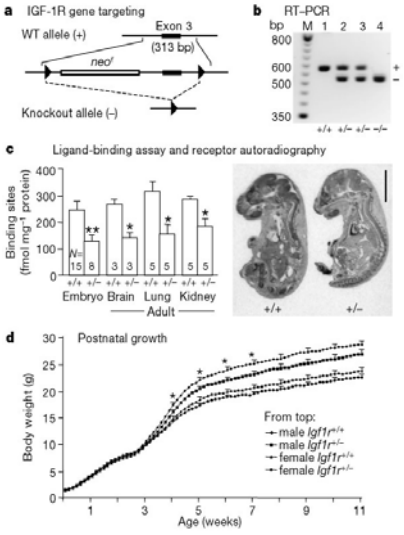
Glucose uptake and metabolic activity

Developmental Biology 229, 141–162 (2001)
doi:10.1006/dbio.2000.9975, available online at http://www.idealibrary.com on IDEAL®

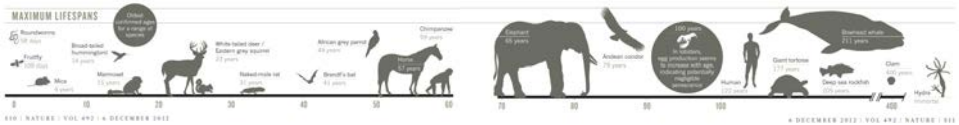
Roles of Growth Hormone and Insulin-like Growth Factor 1 in Mouse Postnatal Growth

Floria Lupu,* Joseph D. Terwilliger,† Kaechoong Lee,‡
Gino V. Segre,‡ and Argiris Efstratiadis*,1

Dwarf mice with mutations that delete the IGF-1 receptor or the GH receptor, which reduces functioning of the insulin/IGF-1 signaling pathway, live longer than normal mice.



The ‘rate of living’ theory of aging



There is a complex relationship between size and longevity in mammals:

Larger species live longer, whereas the opposite is true within a species

Gerontology. 2012;58(4):337-43. doi: 10.1159/000335166. Epub 2012 Jan 18.

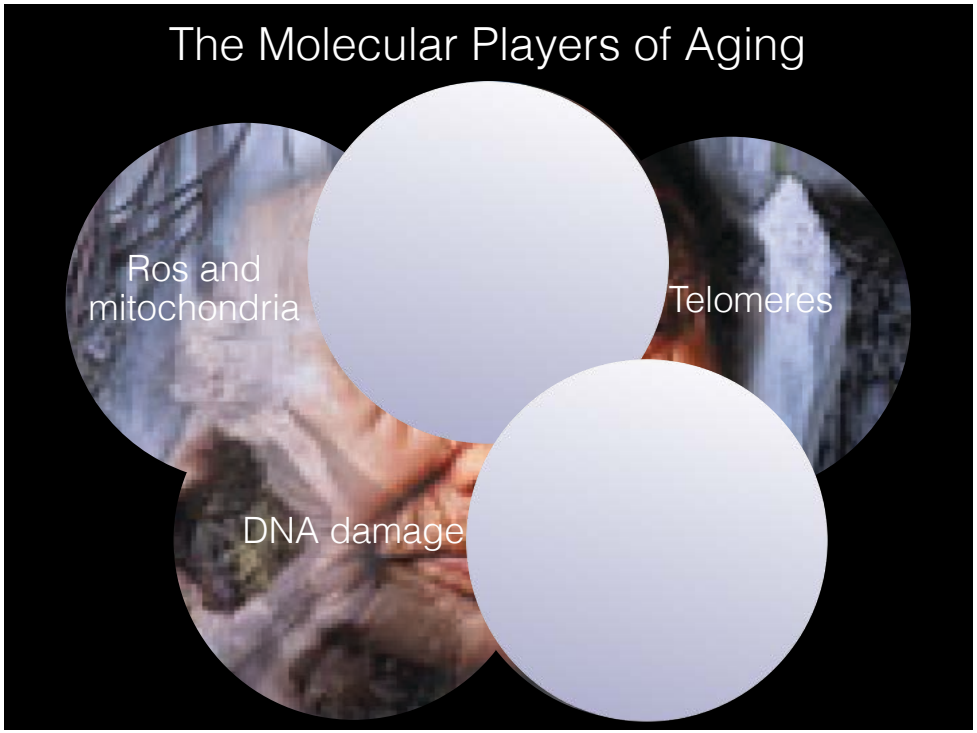
Healthy aging: is smaller better? - a mini-review.

Bartke A.

Department of Internal Medicine, Southern Illinois University School of Medicine, Springfield, 62794-9628, USA.
abartke@siumed.edu

Abstract

A recent report of virtually complete protection from diabetes and cancer in a population of people with hereditary dwarfism revived interest in elucidating the relationships between growth, adult body size, age-related disease and longevity. In many species, smaller individuals outlive those that are larger and a similar relationship was shown in studies of various human populations. Adult body size is strongly dependent on the actions of growth hormone (GH) and the absence of GH or GH receptor in mice leads to a remarkable extension of longevity. Many mechanisms that may account for, or contribute to, this association have been identified. It is suggested that modest modifications of the diet at different ages may extend human healthspan and lifespan by reducing levels of hormones that stimulate growth.



Sir2 genes and aging

Genetic studies indicate that **the major genetic determinant of replicative life span in yeast is SIR2** (loss-of-function mutations shorten life span, increased gene dosage extend it).

The SIR2 ortholog in *C. elegans* is a key determinant of life span in this animal.

The fact that yeast and *C. elegans* diverged from a common ancestor about one billion years ago suggests that all the descendants of that ancestor (including mammals) will possess SIR2-related genes involved in regulating their life span.

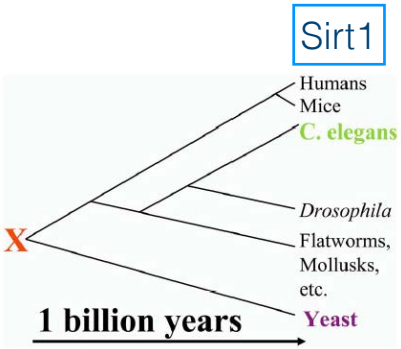


Table 1 | **Sirtuin localization and function**

Sirtuin	Class	Localization	Activity	Targets	Refs
SIRT1	I	Nucleus, cytosol	Deacetylation	PGC1α, FOXO1, FOXO3, p53, Notch, NF-κB, HIF1α, LXR, FXR, SREBP1c and more	5,30,32, 33,39, 41,42
SIRT2	I	Cytosol	Deacetylation	Tubulin, PEPCK, FOXO1, PAR3	58–61
SIRT3	I	Mitochondria	Deacetylation	LCAD, HMGCS2, GDH, OXPHOS complexes, SOD2, IDH2 and more	46–49, 51–57
SIRT4	II	Mitochondria	ADP-ribosylation	GDH	17
SIRT5	III	Mitochondria	Deacetylation, demalonylation, desuccinylation	CPS1	21–23
SIRT6	IV	Nucleus	Deacetylation, ADP-ribosylation	H3K9, H3K56	14,18–20, 63
SIRT7	IV	Nucleolus	Unknown	Unknown	15,64

CPS1, carbamoyl phosphate synthetase 1; FOXO, forkhead box O; FXR, farnesoid X receptor; GDH, glutamate dehydrogenase; HIF1α, hypoxia-inducible factor 1α; HMGCS2, 3-hydroxy-3-methylglutaryl CoA synthase 2; IDH2, isocitrate dehydrogenase 2; LCAD, long-chain acyl CoA dehydrogenase; LXR, liver X receptor; NF-κB, nuclear factor-κB; OXPHOS, oxidative phosphorylation; PAR3, partitioning defective 3 homologue; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, peroxisome proliferator-activated receptor-γ co-activator 1α; SIRT, sirtuin; SOD2, superoxide dismutase 2; SREBP1c, sterol-response element-binding protein 1c.

Differences in Coronary Mortality Can Be Explained by Differences in Cholesterol and Saturated Fat Intakes in 40 Countries but Not in France and Finland

A Paradox

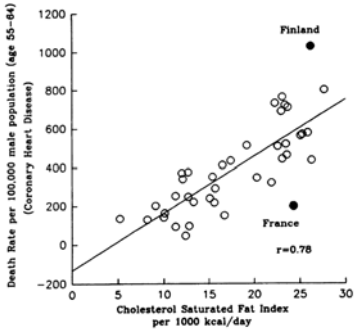
Sabine M. Artaud-Wild, BS, RD; Sonja L. Connor, MS, RD; Gary Sexton, PhD; William E. Connor, MD

Background. For decades, the coronary heart disease (CHD) mortality rate has been four or more times higher in Finland than in France despite comparable intakes of dietary cholesterol and saturated fat. A potential answer to this paradox is provided by this study of 40 countries and the analyses of other nutrients in the diets besides cholesterol and saturated fat.

Methods and Results. CHD death rates for men aged 55 to 64 years were derived from the World Health Organization annual vital statistics. Dietary intakes were gathered from the Food and Agriculture Organization of the United Nations database. Forty countries at various levels of economic development and 40 dietary variables were investigated, including a lipid score that combined the intakes of cholesterol and saturated fat (Cholesterol–Saturated Fat Index [CSI]). The CSI was significantly and positively related to CHD mortality in the 40 countries. The countries with low CSIs had low CHD death rates. Countries with high CSIs had a wide range of CHD death rates. France, Finland, and other Western industrialized countries had similar CSIs. After adjusting for cholesterol and saturated fat, milk and many components of milk (butterfat, milk protein, calcium from milk, and riboflavin) and total calcium remained positively related to CHD mortality for all 40 countries. There were differences in the consumption of these foods and nutrients in France and Finland. Milk and butterfat (fat from milk, cream, cheese, and butter) consumption was higher in Finland than in France. The consumption of plant foods, recently shown to be protective against CHD (vegetables and vegetable oils containing monounsaturated and polyunsaturated fatty acids), was greater in France than in Finland.

Conclusions. Over the years, France and Finland, with similar intakes of cholesterol and saturated fat, consistently have had very different CHD mortality rates. This paradox may be explained as follows. Given a high intake of cholesterol and saturated fat, the country in which people also consume more plant foods, including small amounts of liquid vegetable oils, and more vegetables (more antioxidants) had lower rates of CHD mortality. On the other hand, milk and butterfat were associated with increased CHD mortality, possibly through their effects on thrombosis as well as on atherosclerosis. (*Circulation.* 1993;88:2771-2779.)

KEY WORDS • thrombosis • diet • cholesterol • heart disease • mortality



Circulation *Vol 88, No 6 December 1993*

BMJ helping doctors make better decisions

Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits?

Eric B Rimm, *assistant professor of epidemiology and nutrition*,[a] **Arthur**

BMJ 1996;312:731-736 (23 March)

ORIGINAL ARTICLE

Roles of Drinking Pattern and Type of Alcohol Consumed in Coronary Heart Disease in Men

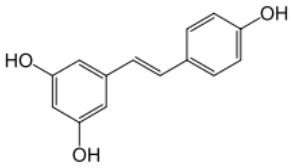
Kenneth J. Mukamal, M.D., M.P.H., Katherine M. Conigrave, M.B., B.S., Ph.D., Murray A. Mittleman, M.D., Dr.P.H., Carlos A. Camargo, Jr., M.D., Dr.P.H., Meir J. Stampfer, M.D., Dr.P.H., Walter C. Willett, M.D., Dr.P.H., and Eric B. Rimm, Sc.D.

Annals of Internal Medicine
Established in 1927 by the American College of Physicians

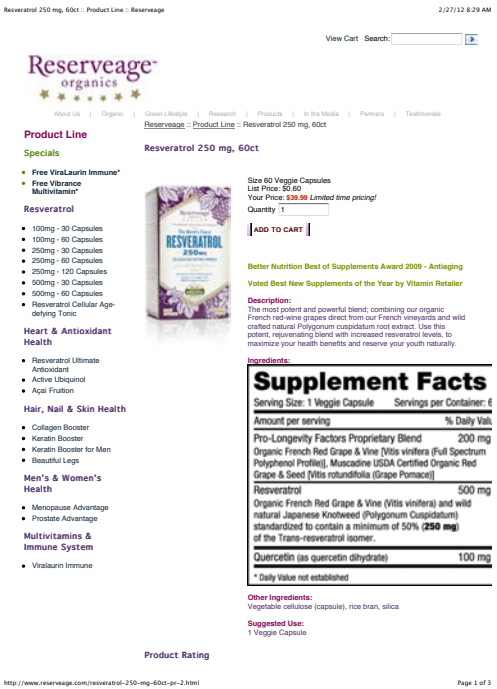
Type of Alcohol Consumed and Mortality from All Causes, Coronary Heart Disease, and Cancer

Morten Grønbæk, MD, DrMedSci; Ulrik Becker, MD, DrMedSci; Ditte Johansen, MSc; Adam Gottschau, MSc, PhD; Peter Schnohr, MD; Hans Ole Hein, MD; Gorm Jensen, MD, DrMedSci; and Thorkild I.A. Sørensen, MD, DrMedSci

Resveratrol



- 3,4',5-trihydrody-trans-stilbene
- a phytoalexin produced naturally by several plants, including berries and grape, especially when under attack by pathogens such as bacteria or fungi
- Activates SIR2 in yeast and Sirt1 in mammals
- Extends life span in yeast, worm and flies (Baur & Sinclair, 2006)


Resveratrol 250 mg, 60ct :: Product Line :: Reserveage 2/27/12 8:29 AM

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Product Line

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- Free Vibrance Multivitamin*

Resveratrol

- 100mg - 30 Capsules
- 100mg - 60 Capsules
- 250mg - 30 Capsules
- 250mg - 60 Capsules
- 250mg - 120 Capsules
- 500mg - 30 Capsules
- 500mg - 60 Capsules
- Resveratrol Cellular Age-defying Tonic

Heart & Antioxidant Health

- Resveratrol Ultimate Antioxidant
- Active Ubiquinol
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Ingredients:

Supplement Facts

Serving Size: 1 Veggie Capsule	Servings per Container: 6
Amount per serving	% Daily Value
Pro-Longevity Factors Proprietary Blend Organic French Red Grape & Vine [Vitis vinifera (Full Spectrum Polyphenol Profile)], Muscadine USDA Certified Organic Red Grape & Seed [Vitis rotundifolia (Grape Pomace)]	200 mg
Resveratrol Organic French Red Grape & Vine (Vitis vinifera) and wild natural Japanese Knotweed (Polygonum Cuspidatum) standardized to contain a minimum of 50% (**250 mg**) of the Trans-resveratrol isomer.	500 mg
Quercetin (as quercetin dihydrate)	100 mg

* Daily Value not established

Other Ingredients:
Vegetable cellulose (capsule), rice bran, silica

Suggested Use:
1 Veggie Capsule

Product Rating

http://www.reserveage.com/resveratrol-250-mg-60ct-pr-2.html Page 1 of 3


NEWS FEATURE

Much ado about ageing

Questions about a laboratory assay are making Sirtris, a high-profile biotechnology company, the talking point of the ageing field. **Heidi Ledford** investigates.

Konrad Howitz wasn't looking for a fountain of youth. As director of biochemistry at BIOMOL International in Plymouth Meeting, Pennsylvania, Howitz wanted to add new molecular assays to the company's catalogue. A protein called a sirtuin had recently been shown to lengthen lifespan in yeast[1], and Howitz was developing methods to measure the activity of one of its mammalian forms, called SIRT1.

But when Howitz and his team stumbled on the discovery that a compound called resveratrol seemed to activate SIRT1, they quickly realized the implications. Resveratrol was rumoured to be the ingredient in red wine that kept the French healthy. In 2003, Howitz shared his findings with David Sinclair, a molecular biologist at Harvard Medical School in Boston, Massachusetts, who had studied sirtuins extensively. Sinclair immediately saw commercial potential for resveratrol as a human anti-ageing drug. Fearing corporate espionage, Sinclair code-named the compound 'R'.

A year later, 'R' became the centrepiece of Sirtris, a company Sinclair co-founded in Cambridge, Massachusetts. Four years after that, Sirtris became a biotechnology success story when a London-based pharmaceutical giant, GlaxoSmithKline (GSK), purchased it for $387.20 million. Yet behind the scenes, rumblers have been voicing concerns about some of the [illegible] that forms the basis for the company. Most recently, questions have resurfaced over whether the interpretation of Howitz's original SIRT1 assay[2] was flawed.

It is a technical debate with big implications, not just for Sirtris but also for the rapidly expanding research community that is now studying sirtuins. Some researchers are questioning whether resveratrol, and other compounds like it that Sirtris is now testing in clinical trials, really does activate sirtuins. Until the mechanism is clear, they are cautious about pursuing drugs that might have unanticipated biological targets and effects. "It is an exciting time in the ageing field," says Brian Kennedy, a molecular biologist at the University of Washington in Seattle. "But this issue has had a polarizing effect. It needs to be resolved."

Helped in no small part by Sirtris's public-relations efforts, sirtuins have been trumpeted in the press as the key to boosting human lifespan. The compounds have a compelling biological narrative: Sinclair and others have proposed that resveratrol and other SIRT1 activators imitate the effects of 'caloric restriction', a drastic reduction in calories that lengthens lifespan in some animals. Compounds that activate sirtuins might therefore have all the benefits of caloric restriction without the starvation.

Biologist David Sinclair co-founded Sirtris to explore sirtuins, enzymes linked to lifespan and ageing.

Seeing the light

Those were the exciting implications when, in 2003, Howitz, Sinclair and their colleagues showed that sirtuin activators could extend yeast lifespan by 70%. Their paper, published in *Nature*[1], also showed results from Howitz's assay. The sirtuins are members of a class of enzyme called deacetylases, which strip acetyl groups from proteins. To measure this activity, Howitz designed an acetylated protein fragment bearing a chemical tag. When activated, SIRT1 deacetylated this peptide substrate and the tag started to fluoresce. The greater the activity of SIRT1, the greater the fluorescence — and in the paper, resveratrol and other chemicals thought to activate SIRT1 generated a mighty glow.

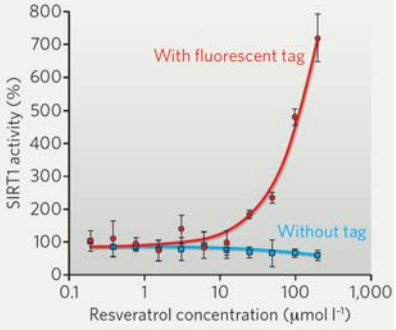
Doubts about the assay first surfaced publicly two years later. Two papers[2,3] showed that resveratrol boosted the activity of SIRT1 only when its peptide substrate contained the fluorescent tag; no tag, no activity (see graph). The papers caused a stir in the field, but failed to register among investors or the public. "The sirtuin stuff has just sort of been a runaway train," says Kennedy, who was lead author on one of the papers[3]. "We might have caused the train to wobble a little, but it kept barrelling down the tracks."

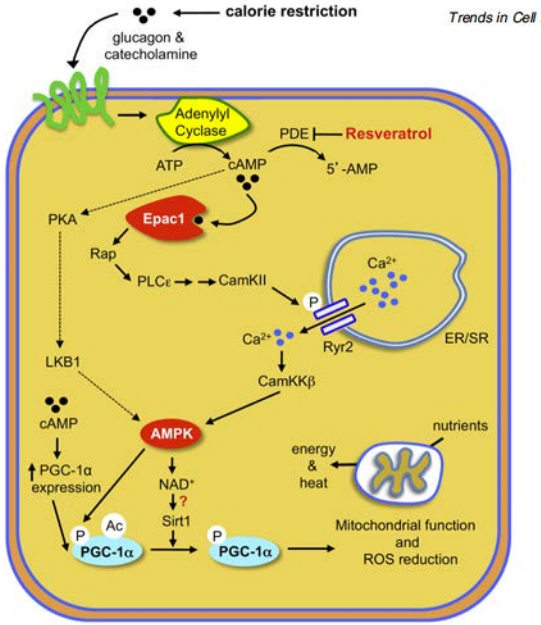
The assay controversy wasn't the only stone on the tracks. Some labs have been unable to consistently reproduce Sinclair's life-extending results in model organisms such as fruitflies and nematodes[4], and debate has simmered over whether SIRT1 activation truly mimics caloric restriction. For Sirtris, however, the arguments have little practical bearing — the company hopes to market its drugs as a way to stave off diabetes and other diseases associated with ageing, rather than a way to extend lives. And by 2006, Sinclair and his colleagues had shown that resveratrol improved the health of mice fed extremely high-fat diets[5]. Then, in a high-profile *Nature* paper published at the end of 2007, Sirtris unveiled a set of compounds that its researchers said were 1,000 times more potent than resveratrol[6]. In April the following year, GSK announced that it planned to

480

SIRTUINS UNDER SCRUTINY

Some researchers claim that an assay designed to measure activation of SIRT1 by resveratrol works only in the presence of a fluorescent tag — as suggested by these data from M. Kaeberlein *et al. J. Biol. Chem.* **280,** 17038–17045 (2005).





Trends in Cell Biology October 2012, Vol. 22, No. 10

Figure 7. Proposed Model of How Resveratrol Mimics CR
Resveratrol inhibits PDE activity and induces cAMP signaling via Epac1, which activates PLCε, resulting in Ca^{2+} release via the Ryr2 Ca^{2+} channel and, ultimately, the activation of the CamKKβ-AMPK pathway. CR increases cAMP levels by increasing glucagon and catecholamine levels, which activate AC activity and cAMP production. AMPK increases mitochondrial biogenesis and function by increasing PGC-1α expression, NAD^+ levels, and Sirt1 activity. An additional pathway that may contribute to resveratrol action is indicated with dotted lines.

New Target for Aspirin

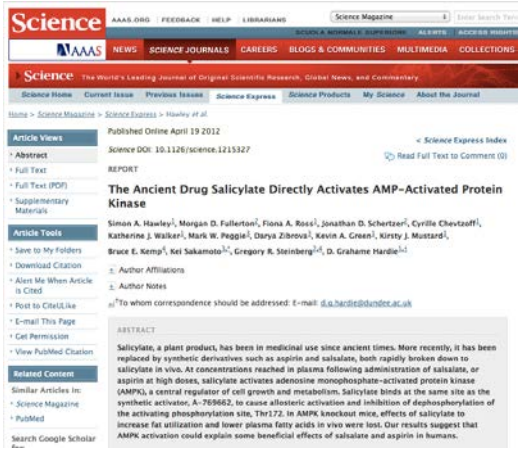
New work on salicylate, a natural component of aspirin, suggests that activation of the energy-sensing AMP kinase may underlie some of aspirin's health benefits.

By Sabrina Richards | April 20, 2012

Wikimedia Commons, Chaval Brasil

Salicylate, the natural compound on which aspirin is based, directly activates a pathway linked to tumor suppression and glucose regulation, which may explain some of aspirin's long-term health benefits, such as its role in cancer prevention. Published this week (April 19) in *Science*, the research shows that salicylate directly activates AMP kinase, which regulates a cell's ATP levels, which in turn promotes fat burning in mice.

"It's a very important paper" that sheds light on the



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Published Online April 19 2012

Science DOI: 10.1126/science.1215327

REPORT

The Ancient Drug Salicylate Directly Activates AMP-Activated Protein Kinase

Simon A. Hawley, Morgan D. Fullerton, Fiona A. Ross, Jonathan D. Schertzer, Cyrille Chevtzoff, Katherine J. Walker, Mark W. Peggie, Darya Zibrova, Kevin A. Green, Kirsty J. Mustard, Bruce E. Kemp, Kei Sakamoto, Gregory R. Steinberg, D. Grahame Hardie

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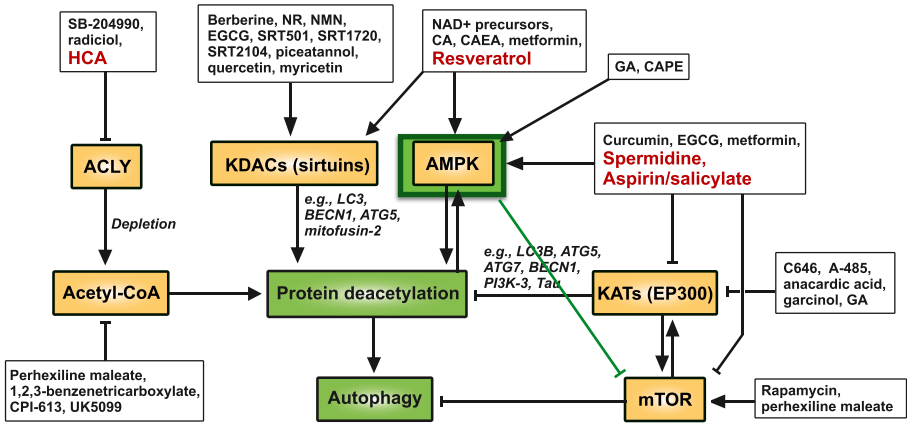
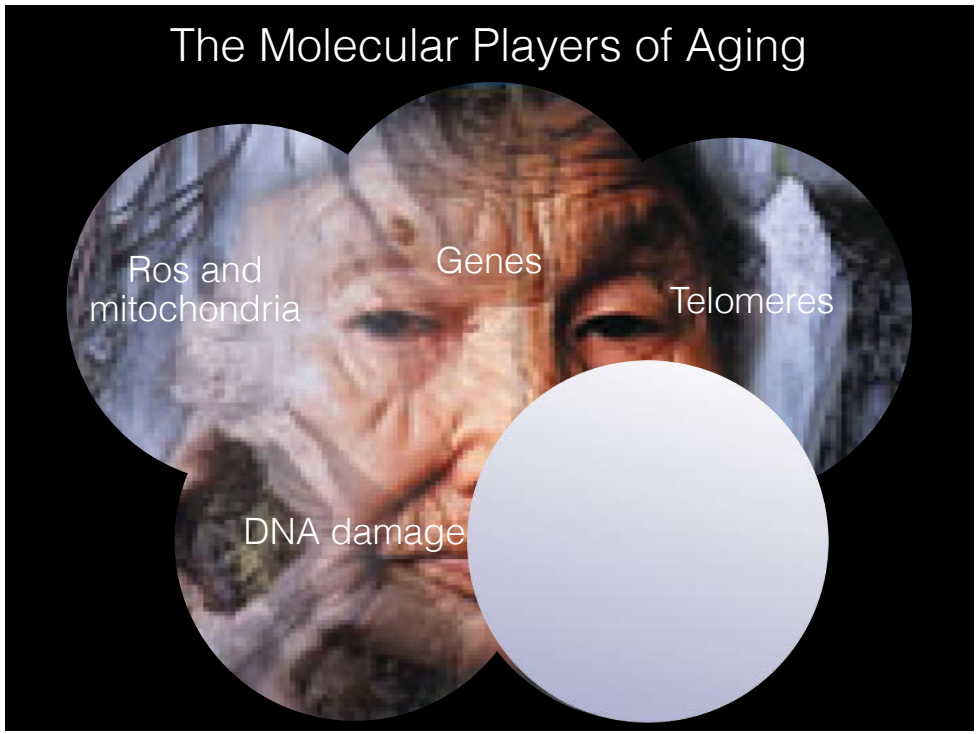
ABSTRACT

Salicylate, a plant product, has been in medicinal use since ancient times. More recently, it has been replaced by synthetic derivatives such as aspirin and salsalate, both rapidly broken down to salicylate in vivo. At concentrations reached in plasma following administration of salsalate, or aspirin at high doses, salicylate activates adenosine monophosphate–activated protein kinase (AMPK), a central regulator of cell growth and metabolism. Salicylate binds at the same site as the synthetic activator, A-769662, to cause allosteric activation and inhibition of dephosphorylation of the activating phosphorylation site, Thr172. In AMPK knockout mice, effects of salicylate to increase fat utilization and lower plasma fatty acids in vivo were lost. Our results suggest that AMPK activation could explain some beneficial effects of salsalate and aspirin in humans.

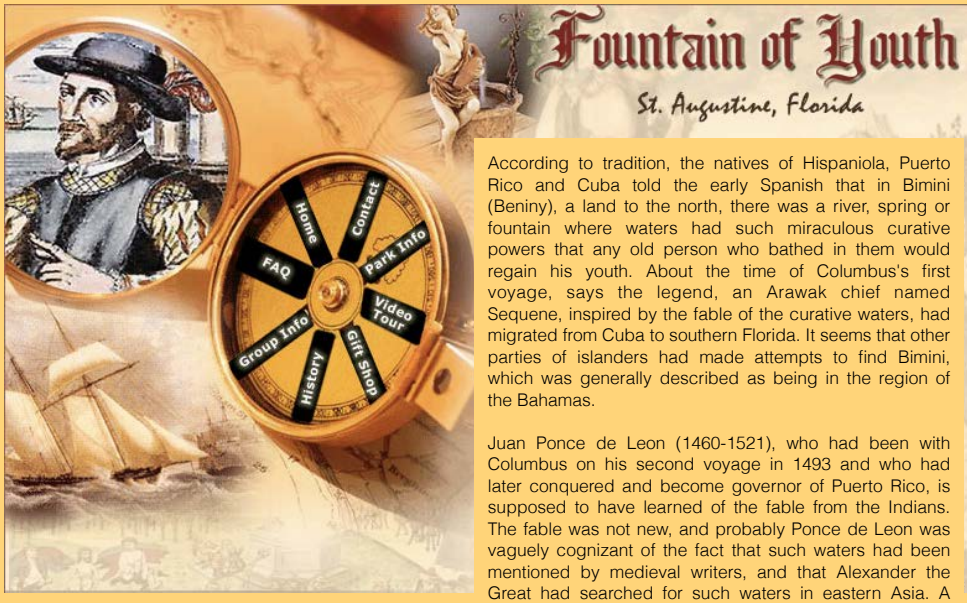
AMPK activation promotes authophagy



Cell Metabolism *29*, March 5, 2019



And so, what can we do?



According to tradition, the natives of Hispaniola, Puerto Rico and Cuba told the early Spanish that in Bimini (Beniny), a land to the north, there was a river, spring or fountain where waters had such miraculous curative powers that any old person who bathed in them would regain his youth. About the time of Columbus's first voyage, says the legend, an Arawak chief named Sequene, inspired by the fable of the curative waters, had migrated from Cuba to southern Florida. It seems that other parties of islanders had made attempts to find Bimini, which was generally described as being in the region of the Bahamas.

Juan Ponce de Leon (1460-1521), who had been with Columbus on his second voyage in 1493 and who had later conquered and become governor of Puerto Rico, is supposed to have learned of the fable from the Indians. The fable was not new, and probably Ponce de Leon was vaguely cognizant of the fact that such waters had been mentioned by medieval writers, and that Alexander the Great had searched for such waters in eastern Asia. A similar legend was known to the Polynesians, whose tradition located the fountain of perpetual youth in Hawaii.

Antiaging therapies

(i) In mice, modest effects with **some antioxidants** and no effects with α-lipoic acid (antioxidant) or coenzyme Q10 (which increases electron transport and has antioxidant activities)

(ii) **Resveratrol** (sirtuin activator)

(iii) **AMPK activators** (aspirin?)

(iv) **Antinflammatory agents** (aspirin and nitroflurbiprofen)

(v) 4-hydrody-phenyl-N-ter-butyl nitrone (free radical scavenger)

(vi) Nordihydroguarietic acid (lipoxygenase inhibitor with structural similarity to resveratrol)

(vii) **Drugs that regulate cholesterol metabolism** (genetically, a relationship exists between exceptional longevity and variants of several genes affecting lipoprotein metabolism)

(viii) **Rapamycin** and other mTOR inhibitors

(ix) Ongoing genetic screens in *C. elegans*, *Drosophila* and mammalian cells aimed at the identification of drugs, **siRNAs** and **microRNAs** that increase life span

Caloric restriction (CR) is the most effective environmental method to increase lifespan (and to prevent late-onset diseases!)

Dietary restriction extends lifespan in S. cerevisiae, C. elegans, D. melanogaster, rodents and primates.

CR = 60-70% of what an animal would eat at libitum

In rodents CR results in as much as a 50% increase in rodent longevity

Physiological effects of CR: acute phase followed by an adaptive period of several weeks to reach a stable, altered physiological state characterized by lower body temperature (possible marker for metabolic rate), lower blood glucose and insulin levels and reduced fat and weight.

The CR animals are more resistant to external stressors, including heat and oxidative stress; organs are typically smaller (except for the brain)

CR may represent an adaptation to scarcity in a boom and bust cycle; any organism that could slow aging and reproduction in times of scarcity and remain able to reproduce when food reappeared would enjoy a competitive advantage. Extremes examples are the formation of spored in microbes and dauer larvae in C. elegans

CR animals are resistant to disease, including **cancer** and **infections**

Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys

Ricki J. Colman,[1]* Rozalyn M. Anderson,[1] Sterling C. Johnson,[1,2,3] Erik K. Kastman,[2,3] Kristopher J. Kosmatka,[2,3] T. Mark Beasley,[4] David B. Allison,[4] Christina Cruzen,[1] Heather A. Simmons,[1] Joseph W. Kemnitz,[1,2,5] Richard Weindruch[1,2,3]*

Caloric restriction (CR), without malnutrition, delays aging and extends life span in diverse species; however, its effect on resistance to illness and mortality in primates has not been clearly established. We report findings of a 20-year longitudinal adult-onset CR study in rhesus monkeys aimed at filling this critical gap in aging research. In a population of rhesus macaques maintained at the Wisconsin National Primate Research Center, moderate CR lowered the incidence of aging-related deaths. At the time point reported, 50% of control fed animals survived as compared with 80% of the CR animals. Furthermore, CR delayed the onset of age-associated pathologies. Specifically, CR reduced the incidence of diabetes, cancer, cardiovascular disease, and brain atrophy. These data demonstrate that CR slows aging in a primate species.

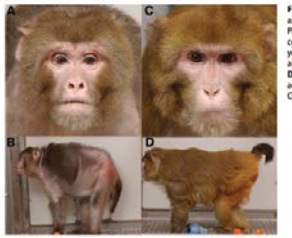
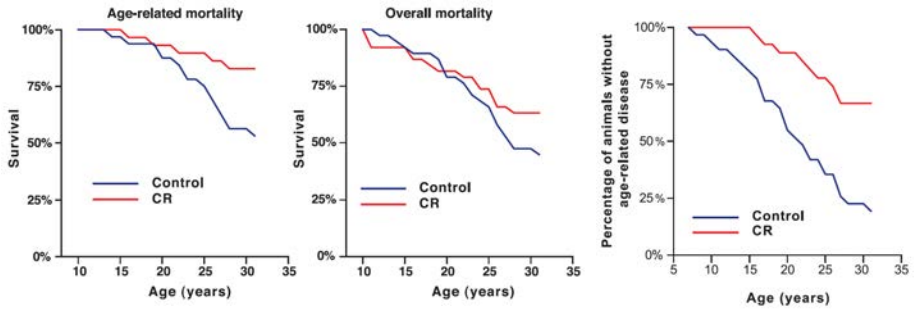


Fig. 1. Animal appearance in old age. (A and B) Photographs of a typical control animal at 27.6 years of age (about the average life span). (C and D) Photographs of an age-matched animal on CR.



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Severe Diet Doesn't Prolong Life, at Least in Monkeys

By GINA KOLATA
Published: August 29, 2012 | 236 Comments

For 25 years, the rhesus monkeys were kept semi-starved, lean and hungry. The males' weights were so low they were the equivalent of a 6-foot-tall man who tipped the scales at just 120 to 133 pounds. The hope was that if the monkeys lived longer, healthier lives by eating a lot less, then maybe people, their evolutionary cousins, would, too. Some scientists, anticipating such benefits, began severely restricting their own diets.

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National Institute on Aging/NIH

A 23-year study comparing calorie restricted rhesus monkeys, left, to normally-fed monkeys, has shown that calorie restriction may not increase one's lifespan.

The results of this major, long-awaited study, which began in 1987, are finally in. But it did not bring the vindication calorie restriction enthusiasts had anticipated. It turns out the skinny monkeys did not live any longer than those kept at more normal weights. Some lab test results improved, but only in monkeys put on the diet when they were old. The causes of death — cancer, heart disease — were the same in both the underfed and the normally fed monkeys.

Caloric restriction (undernutrition without malnutrition)

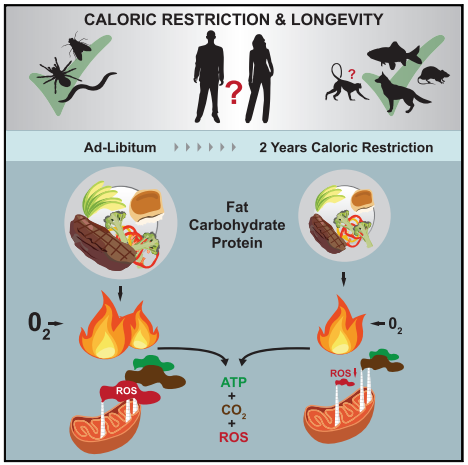
Slows multiple age-related changes, delays the onset of cancer and multiple other age-related pathologies, and extends life span

Periodic food deprivation (every-other-day intermittent feeding) may induce similar physiologic effects even when average caloric intake is not different from ad libitum intake

CALERIE (Comprehensive Assessment of Long-term Effects of Restricted Intake of Energy Intake) trial: has tested effects of 2-3 years of CR (20-30% reduction) in young and middle-aged nonobese persons

Cell Metabolism
Clinical and Translational Report

Metabolic Slowing and Reduced Oxidative Damage with Sustained Caloric Restriction Support the Rate of Living and Oxidative Damage Theories of Aging

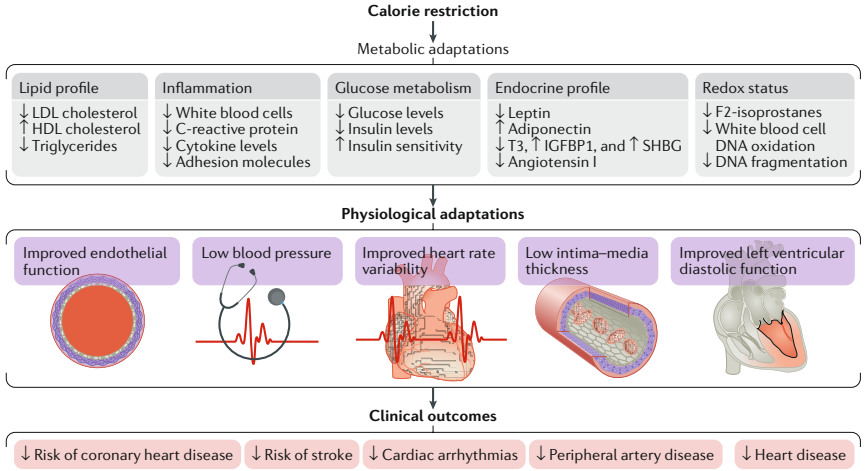


Highlights

- Calorie restriction (CR) extends maximum lifespan in most species
- Young, healthy individuals achieved 15% CR and 8 kg weight loss over 2 years
- Energy expenditure (24 hr and sleep) was reduced beyond weight loss
- Oxidative stress was also reduced, supporting two long-standing theories of aging

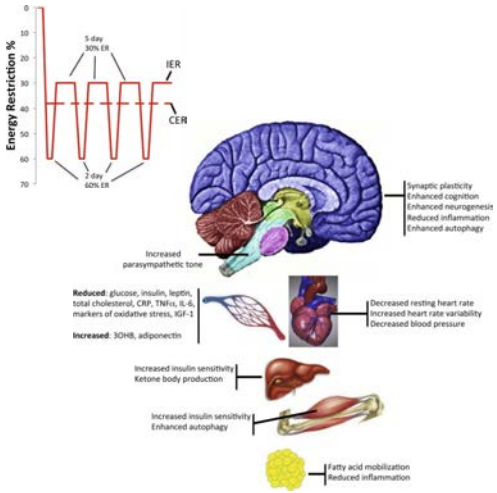
Redman et al., 2018, Cell Metabolism 27, 805–815
April 3, 2018 © 2018 Elsevier Inc.
https://doi.org/10.1016/j.cmet.2018.02.019

Caloric restriction in humans results in sustained beneficial effect on most CVD risk factors



Intermittent fasting and meal time

Both intermittent fasting and time-restricted feeding extend lifespan up to 30% in mice

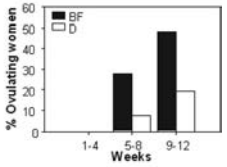


Eating at breakfast and lunch results in better **metabolic adaptation** (weight loss, glucose tolerance and insulin sensitivity) compare to a later meal pattern

Effects of caloric intake timing on insulin resistance and hyperandrogenism in lean women with polycystic ovary syndrome

Daniela JAKUBOWICZ*, Maayan BARNEA†, Julio WAINSTEIN* and Oren FROY†

*Diabetes Unit E. Wolfson Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Holon 58100, Israel
†Institute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel



BF: breakfast diet

Caloric restriction or dietary restriction?

The Ratio of Macronutrients, Not Caloric Intake, Dictates Cardiometabolic Health, Aging, and Longevity in Ad Libitum-Fed Mice

Samantha M. Solon-Biet,[1,2,3,4,13] Aisling C. McMahon,[1,2,3,13] J. William O. Ballard,[5] Kari Ruohonen,[6] Lindsay E. Wu,[7] Victoria C. Cogger,[1,2,3] Alessandra Warren,[1,2,3] Xin Huang,[1,2,3] Nicolas Pichaud,[5] Richard G. Melvin,[8] Rahul Gokarn,[2,3] Mamdouh Khalil,[1] Nigel Turner,[1] Gregory J. Cooney,[9] David A. Sinclair,[7,10] David Raubenheimer,[1,4,11,12] David G. Le Couteur,[1,2,3,4] and Stephen J. Simpson[1,4,*]
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[5]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney NSW 2052, Australia
[6]EWOS Innovation, Dirdal 4335, Norway
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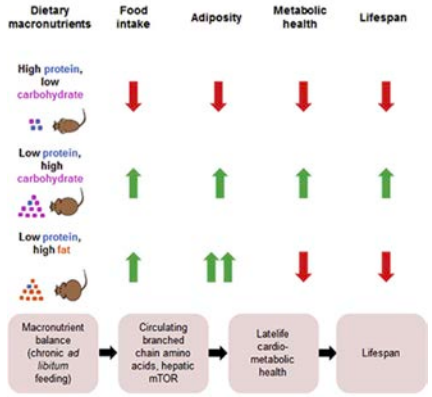
[9]Garvan Institute of Medical Research, University of New South Wales, Darlinghurst NSW 2010, Australia
[10]The Paul F. Glenn Laboratories for the Biological Mechanisms of Aging, Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
[11]Institute of Natural Sciences, Massey University, Auckland 0632, New Zealand
[12]Faculty of Veterinary Science, The University of Sydney, Sydney NSW 2006, Australia
[13]These authors contributed equally to this work
*Correspondence: david.lecouteur@sydney.edu.au (D.G.L.C.), stephen.simpson@sydney.edu.au (S.J.S.)
http://dx.doi.org/10.1016/j.cmet.2014.02.009

Highlights

- Food intake is regulated primarily by dietary protein and carbohydrate
- Low-protein, high-carbohydrate diets are associated with the longest lifespans
- Energy reduction from high-protein diets or dietary dilution does not extend life
- Diet influences hepatic mTOR via branched-chain amino acids and glucose



Reduced physical activity is a strong and independent predictor of CVD mortality although exercise do not eliminate the higher risk of death associated to visceral adiposity

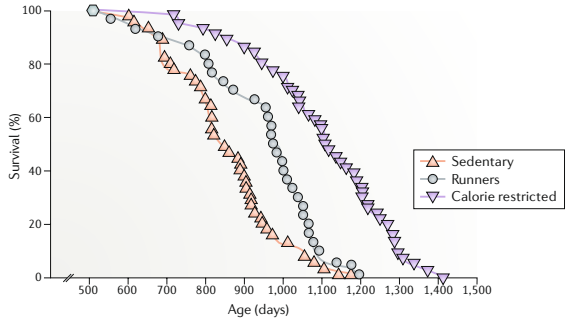
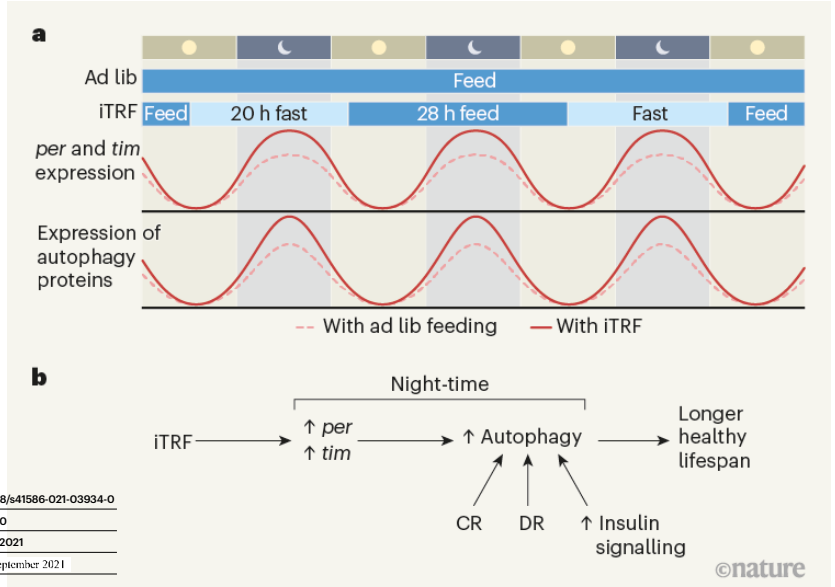


Fig. 4 | **Calorie restriction, but not endurance exercise, increases maximal lifespan in rats.** The survival curve for sedentary control rats is significantly different from that of runners ($P < 0.02$) and calorie-restricted sedentary rats ($P < 0.0001$). The survival curve for runners is also significantly different from that of calorie-restricted sedentary rats ($P < 0.01$). Figure is adapted with permission from REF.[67], American Physiological Society.

Circadian autophagy drives iTRF-mediated longevity



https://doi.org/10.1038/s41586-021-03934-0
Received: 9 June 2020
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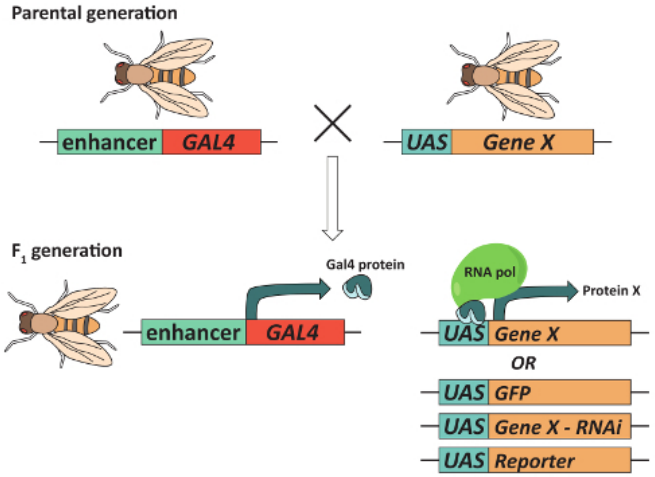
Published online: 29 September 2021

Capillary feeder (CaFe) assay

The Gal4-UAS system in flies



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Moderatori: Antonio Musarò; Alexandra Battaglia Mayer, Carmine Settembre
29 Ottobre 2021 ore 16:00-18:30
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Università Vita-Salute S.Raffaele, Milano:
Sperimentazione animale: comunicazione e bias cognitivi
16:40 - 17:20 **Roberto Caminiti**
Sapienza Università di Roma:
Primati non umani, aspetti evolutivi e ontogenetici
17:20 - 18:00 **Alberto Auricchio**
Telethon Institute of Genetics and Medicine (TIGEM):
Terapia genica in vivo: dal laboratorio alla clinica
18:00 - 18:30 Discussione generale
Per informazioni su questo evento: antonio.musaro@uniroma1.it

01
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Responsabili Proff. Micaela Morelli e Daniela Puzzo
Ricerca sui Primati non umani: quando, come e perché
18 marzo 2021

02
Coordinato da Università Roma Tre e Università di Cagliari
Responsabili proff. Viviana Trezza e Miriam Melis
Perché è necessario studiare le sostanze che provocano abuso
9 Giugno 2021

03
Coordinato da Università di Firenze e Pisa
Responsabili Proff. Nicoletta Berardi, Elisabetta Cerbai, Marco Onorati
Il cervello tra *Homo sapiens* e primati non-umani: sviluppo evoluzione e potenzialità
21 Giugno 2021

04
Coordinato da Università di Ferrara e Università di Camerino
Responsabili Proff. Michele Simonato, Luciano Fadiga, Paolo Pinton, Roberto Ciccocioppo
Sperimentazione animale: aspetti storici, etici, giuridici
23 settembre 2021