Pathways to Longevity

intracellular biochemical associated with aging and long

Robert Rountree, MD
Boulder, Colorado
“The most fundamental defining characteristic of living organisms compared to machines is that living organisms have the ability to repair themselves”

Why We Age
Steven Austad
She may be the world's oldest person, if she cares to claim it

SACABA, Bolivia -- Julia Flores Colque still sings with joy in her indigenous Quechua tongue and strums the five strings of a tiny Andean guitar known as the charango, despite a recorded age of almost 118 years. In her long life, she has witnessed two world wars, revolutions in her native Bolivia and the transformation of her rural town of Sacaba from 3,000 people to a bustling city of more than 175,000 in five decades.

Her national identity card says Flores Colque was born on Oct. 26, 1900, in a mining camp in the Bolivian mountains. At 117 and just over 10 months, she would be the oldest woman in the Andean nation and perhaps the oldest living person in the world.
Maximum Human Life Span: 122 years

Average Life Expectancy at Birth in US

• Circa 100 AD: 30 years
• Circa 1900 AD: 45 years
• Circa 2015 AD: 78 years
• Circa 2040 AD: 95 years?
United States of America / Life expectancy

78.74 years (2015)

- Canada: 82.14 years
- United Kingdom: 81.60 years
- United States of America: 78.74 years
By the year 2050....

Source: United Nation’s Population Division

- The median age will increase from 26 to 36.
- The number of people older than 60 will triple.
- These two billion people will constitute one third of the world’s population.
Aging populations: the challenges ahead

• If the pace of increase in life expectancy in developed countries over the past two centuries continues through the 21st century, in France, Germany, Italy, the UK, the USA, Canada, Japan, and other countries with long life expectancies, most babies born since 2000 will celebrate their 100th birthdays.

• A key question is: are increases in life expectancy accompanied by a concurrent postponement of functional limitations and disability?

Will these people be living well or just living longer?
A Pessimist’s View

(Westendorp)
What is healthy aging in the 21st century?

Rudi GJ Westendorp

*Am J Clin Nutr* 2006;83(suppl):404S-9S.

“The increase in average life span observed in all developed countries is accompanied by an incremental burden of age-associated diseases”
Aging and Morbidity

• Physical dysfunction and incapacity to respond to stresses become increasingly prevalent toward the end of life, with up to 45% of people over the age of 85 being frail.

• This physical dysfunction is associated with considerable morbidity, including decreased mobility and increased burden of age-related chronic diseases, loss of independence, nursing home and hospital admissions, and mortality.

_Nat Med._ 2018 Aug;24(8):1246-1256
**Golden Years?**

Though Americans live longer than they did 20 years ago, the amount of time they spend with chronic disability is also on the rise.

### U.S. life expectancy at birth

<table>
<thead>
<tr>
<th></th>
<th>Healthy Life Expectancy</th>
<th>Years with Disability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>68.1 years</td>
<td>10.1</td>
<td>78.2</td>
</tr>
<tr>
<td>1990</td>
<td>65.8</td>
<td>9.4</td>
<td>75.2</td>
</tr>
</tbody>
</table>

### Rank of OECD* nations by healthy life expectancy

<table>
<thead>
<tr>
<th>Year</th>
<th>Nation</th>
<th>Healthy Life Expectancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>U.S. (13)</td>
<td>65.8</td>
</tr>
<tr>
<td></td>
<td>Turkey (34)</td>
<td>57.7</td>
</tr>
<tr>
<td>2010</td>
<td>Japan (1)</td>
<td>73 years</td>
</tr>
<tr>
<td></td>
<td>U.S. (26)</td>
<td>68.1</td>
</tr>
<tr>
<td></td>
<td>Turkey (34)</td>
<td>64</td>
</tr>
</tbody>
</table>

*Organization for Economic Cooperation and Development

Source: Institute for Health Metrics and Evaluation, Seattle

The Wall Street Journal
Despite significant increases in life expectancy, “morbidity and chronic disability now account for nearly half of the US health burden, and improvements in population health in the United States have not kept pace with advances in population health in other wealthy nations.”
An Optimist’s View
(Fries)
“Aging, natural death and the compression of morbidity”
Fries, JF, NEJM, 1980, Vol 303: 130-135

• Decreases in health risks from chronic diseases lead to increases in average age at death.
• The physiologic and psychology markers of aging are modifiable: primary prevention measures such as stopping smoking, reducing BMI, and regular exercise can reduce cumulative lifetime disability.
“Aging, natural death and the compression of morbidity”
Fries, JF, NEJM, 1980, Vol 303: 130-135

• By instituting these measures, age at the time of initial infirmity will increase more than the gain in longevity (morbidity will be compressed later in life).

• The advantage of delaying the inevitable: we don’t need a cure if we can postpone the onset
Aging and Morbidity

A major question in biology is whether interventions can be devised that enhance healthspan in tandem with increasing remaining lifespan so that the period of morbidity near the end of life is not increased.

Nat Med. 2018 Aug;24(8):1246-1256
Lessons on Aging Well, From a 105-Year-Old Cyclist

Robert Marchand, age 105, in Paris on Jan. 5, 2017, a day after setting a new one-hour cycling record.
Joel Saget/Agence France-Presse — Getty Images
Lessons On Aging Well

The New York Times

February 13, 2017

A diminutive 5 feet in height and weighing about 115 pounds, Mr Marchand said he had not exercised regularly during most of his working life as a truck driver, gardener, firefighter and lumberjack.

But since his retirement, he had begun cycling most days of the week, either on an indoor trainer or the roads near his home in suburban Paris.
Case Studies in Physiology: Maximal oxygen consumption and performance in a centenarian cyclist

This study shows, for the first time, that maximal oxygen consumption (+13%) and performance (+11%) can still be increased between 101 and 103 yr old with 2 yr of training and that a centenarian is able, at 103 yr old, to cover 26.9 km/h in 1 h.

Opening the door to treating ageing as a disease

• Death in old age was historically attributed to just that—ie, old age and natural causes.

• However, with increasing life expectancy and the desire to maintain good health in older age for as long as possible (so-called healthy ageing), calls to recognise ageing as a disease that can be treated are increasingly relevant and debated, despite the lack of a universally accepted set of ageing biomarkers and uncertainty about the time of transition to disease.

Risk factors for exposures that contribute to chronic-disease mortality. Chart compiled from WHO estimates of exposures affecting 50 million global deaths in 2010

Environ Health Perspect 2014; 122(8): 769–774
A Central Question of Biology

Is aging simply the consequence of declining organ reserve, resulting from cumulative exogenous and endogenous insults?

Or is cellular senescence a physiologic “program” that is potentially reversible?
Key Physiologic Pathways Associated with Senescence
Pathways that Accelerate Senescence

- Telomere attrition
- Oxidative stress
- NFkB – “Inflammaging”
- mTOR
What We Lose With Age

As cells divide over time...

Telomeres: end caps that protect the chromosome...

telomeres shorten, and eventually cell division stops

http://2014hs.igem.org/Team:TAS_Taipei/project/abstract
Telemore Attrition: Risk Factors

• Chronic severe stress, anxiety, or depression.
• Chronic insomnia or sleep deprivation
• Lack of social support from friends and family.
• Sedentary lifestyle
• Processed foods & sweetened beverages
• Oxidative stress: cigarette smoke, air pollution
• Chronic toxic exposure: pesticides and other chemical toxins

Factors Associated with Longer Telomeres

- Regular exercise
- Good social support
- Meditation
- Low stress (empowerment)
- Good quality sleep
- Omega-3 fatty acids (EPA – DHA)
- Multivitamins
- Vitamin D
- Green tea

Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease.

*JAMA*, 2010;303:250-257.

- Researchers measured telomere length in leukocytes of 608 people with stable heart disease at the beginning of the study and again five years later.
- People who had the lowest blood levels of two key omega-3 fats, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), experienced the most rapid rate of telomere shortening during the study.
- In contrast, those with the highest blood levels of EPA and DHA had the slowest rate of telomere shortening.
Multivitamin use and telomere length in women

- Compared with nonusers, the relative telomere length of leukocyte DNA was on average 5.1% longer among daily multivitamin users.
- Higher intakes of vitamins C and E from foods were each associated with longer telomeres, even after adjustment for multivitamin use.
- Intakes of both nutrients were associated with telomere length among women who did not take multivitamins.
Higher serum vitamin D concentrations are associated with longer leukocyte telomeres in women


• Measured serum 25-OH-vit D in 2,160 women, aged 18-79 years
• Higher serum vit D levels significantly associated with longer telomeres
• Difference in telomere length between highest & lowest levels of D equivalent to five years of aging
Chinese tea consumption is associated with longer telomere length in elderly Chinese men

- Analysis of food group intake & telomere length in 2006 Chinese men & women >64 years old
- Men who drank average of 3 cups of Chinese tea per day had telomeres 4.6 kilobases longer than those of people who drank an average of 1/4 cup daily
TL;DR

Space made Scott Kelly taller and younger

By Loren Grush on March 3, 2016 03:15 pm .twitter.com/lorengrush
Telomere Length increased!

- ** (p=0.0073)
- * (p=0.0480)
Free Radicals and Aging

A missing electron creates a “Free Radical”, highly reactive

Orbitals

Electrons

Nucleus
Denham Harman, MD, PhD

- **1956**: proposed idea that “free radicals” damage macromolecules: proteins, lipids, nuclear and mitochondrial DNA
- This free radical damage plays a major role in aging
- **1972**: revised hypothesis: mitochondrial health determines lifespan
- **1998**: died at age 98 (after short illness)

*J Gerontol 1956;11:298-300.*
*Age, 1983, Vol 6: 86-94*
Healthy Mitochondrion in Young Cell:
- Relatively few free radicals attack cell.

Damaged Mitochondrion in Distressed Old Cell:
- Free-radical damage increases.
- ATP supply shrinks.

Abundant ATP powers cellular activities.

Nutrients and Oxygen flow into the cell through the mitochondrial membrane.

Energy-producing machinery operates within the mitochondrial matrix.

Time progresses, leading to mitochondrial damage and reduced ATP production.
Mitochondrial function → Generation of ROS → Oxidative damage mtDNA mutations → Mitochondrial dysfunction → Ageing

«The vicious cycle»
Oxidative Stress
Positive Correlation with Chronic Disease and Aging

Are free radicals are always harmful?
How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis).

In contrast with Denham Harman’s free radical theory of aging, increased formation of ROS within the mitochondria cause an adaptive response (mitohormesis) that culminates in increased stress resistance and a long-term reduction in oxidative stress.

*Exp Gerontol.* 2010 Jun;45(6):410-8
Antioxidants prevent health-promoting effects of physical exercise in humans

• Exercise-induced oxidative stress ameliorates insulin resistance and causes an adaptive response promoting endogenous antioxidant defense capacity.

• Supplementation with antioxidants may preclude these health-promoting effects of exercise in humans

Michael Ristow et al. PNAS 2009;106:21:8665-8670
Mitohormesis links physical exercise and subsequent formation of reactive oxygen species to insulin sensitivity and antioxidant defense.

Physical exercise

\[ \rightarrow \text{Antioxidants} \]

Transient increase of oxidative stress (ROS)

Target gene induction (\textit{PGC1}α/β, \textit{PPAR}γ, \textit{SOD}1/2, \textit{GPx}1)

Induction of insulin sensitivity & endogenous ROS defense (\textit{Mitohormesis})

Reduced disease risk

Michael Ristow et al. PNAS 2009;106:21:8665-8670
Figure 2. Differential responses to rising oxidative stress.
Inflammaging
Inflammation, But Not Telomere Length, Predicts Successful Ageing at Extreme Old Age: A Longitudinal Study of Semi-supercentenarians

- Analysis of data from multiple Japanese studies of centenarians and supercentenarians
- Biomarkers of inflammation (CRP/IL6) were best predictor of all-cause mortality
- “We conclude that inflammation is an important malleable driver of extreme old age in humans.”
Inflammatory Signal Transduction

MODIFIED FROM MAX PLANCK INSTITUTE

Signal (TNFα, AGE, PAMP, DAMP)

Receptor (TLR, TNFR, RAGE)

Cell

Kinases

Transcription Factors (NFκB, AP-1)

Nucleus

Gene Expression

DNA

Biological Answer (Pro-inflammatory cytokines)
“The motif most strongly associated with aging was that of the transcription factor NF-κB. Inducible genetic blockade of NF-κB for 2 wk in the epidermis of chronologically aged mice reverted the tissue characteristics and global gene expression programs to those of young mice.”

Inflammatory Foods!!
Increase in intranuclear nuclear factor kB and decrease in inhibitor kB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect

• “…a moderate-sized mixed meal results in a significant increase in the proinflammatory transcription factor NF-kB in the nucleus

• It is possible that chronic overeating may result in permanent increases in NF-KB binding activity and in total expression as a protein in the cell.

Nuclear Factor Kappa B Activators

- oxidative stress (e.g. superoxide, H$_2$O$_2$, oxidized LDL), ionizing radiation
- pathogenic microbes / endotoxin (LPS)
- tissue damage (damage-associated patterns)
- advanced glycation end products
- trans fats
- synthetic toxins; heavy metals
- inflammatory cytokines (IFN-γ, TNF-α)
NF-κB Activation: Inhibitors and Modulators

- Glucocorticoids
- Calorie restriction / fasting
- Omega-3 fatty acids (EPA, DHA)
- Alpha lipoic Acid
- N-Acetyl Cysteine (NAC)
- Antioxidants: vitamin C & E

NF-κB Activation: Plant-derived Inhibitors and Modulators

- Flavonoids & related compounds: quercetin, grape seed polyphenols, soy isoflavones, resveratrol, curcuminoids, green tea catechins, ellagitannins (pomegranate)


Matthew J. Killeen, Mark Linder, Paolo Pontoniere, Roberto Crea, NF-κB signaling and chronic inflammatory diseases: exploring the potential of natural products to drive new therapeutic opportunities, Drug Discovery Today, Volume 19, Issue 4, 2014, Pages 373-378
Curcuma Longa

Curcumin calms inflammation and stimulates circulation.
Curcumin: Anti-inflammatory Mechanism of Action
Senolytics improve physical function and increase lifespan in old age

- We demonstrate that transplanting relatively small numbers of senescent cells into young mice is sufficient to cause persistent physical dysfunction, as well as to spread cellular senescence to host tissues.

- Transplanting even fewer senescent cells had the same effect in older recipients and was accompanied by reduced survival, indicating the potency of senescent cells in shortening health and lifespan.

*Nat Med.* 2018 Aug;24(8):1246-1256
Senolytics improve physical function and increase lifespan in old age

- intermittent oral administration of the senolytics quercetin plus dasatinib (tyrosine kinase inhibitor) to both senescent cell–transplanted young mice and naturally aged mice alleviated physical dysfunction and increased post-treatment survival by 36% while reducing mortality hazard to 65%

*Nat Med.* 2018 Aug;24(8):1246-1256
Senolytics improve physical function and increase lifespan in old age

• Our study shows that health and lifespan are curtailed by increased senescent cell abundance and, conversely, are enhanced by reducing proinflammatory... senescent cell burden in mice, even late in life

Nat Med. 2018 Aug;24(8):1246-1256
The bacterium *Streptomyces hygroscopicus* is found only on Easter Island.

Photographer: Tomas Munita/NY Times/Redux
Rapamycin (Sirolimus)

• Potent antifungal made by soil-based bacterium (*Streptomyces hygroscopicus*) found on Rapa Nui

• Initially developed as immunomodulator for transplant recipients ($100s of millions in sales); and cytostatic anti-cancer agent

• Subject of >1200 studies (PubMed)

• Improves biomarkers of longevity in yeasts, nematodes, flies, old mice, & dogs

Bloomberg.com/news, 2/12/2015: Does a Real Anti-Aging Pill Already Exist?
Rapamycin Effects (in Animals)

- Increases lifespan
- Reduces age-related bone loss
- Decreases tendon stiffening
- Reverses cardiac aging
- Reduces chronic inflammation
- Reverses cognitive decline
- Inhibits growth of certain cancers

Rapamycin’s Exclusive Mechanism of Action: Direct Inhibition of mTOR
mTOR: Mammalian/Mechanistic Target of Rapamycin

- Major nutrient sensor that regulates cellular growth, development, proliferation, metabolism (while generating cellular debris)
- Multi-organ command and control center: the body’s “general contractor” (David Sabatini)
- Protein kinase - core component of two multiple protein complexes

PNAS, 2017 114 (45) 11818-11825 (Sabatini)
mTOR: Mammalian/Mechanistic Target of Rapamycin

• mTOR C1: anabolic growth and proliferation
  persistent activation inhibits autophagy leading to accumulation of inflammatory misfolded proteins

• mTOR C2: insulin signaling pathways

PNAS, 2017 114 (45) 11818-11825 (Sabatini)
Hypothesis: aging and age-related disease are the result of chronic mTOR upregulation, which signals for growth and development (hyperfunction) at a time when they aren’t needed.

Chronic mTOR Upregulation and Disease

- Cancer
- Obesity
- T2DM
- Depression
- Neurodegenerative disease
- Autoimmune disease

Cell, 2012, 149(2): 274-293
Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition

- Cell senescence is associated with activation of the TOR (target of rapamycin) nutrient- and mitogen-sensing pathway, which promotes cell growth, even though the cell cycle is blocked.
- As an anti-aging drug, rapamycin will prevent diseases rather than cure complications of diseases.

Blagosklonny MV, Cell Cycle. 2006 Sep;5(18):2087-102
One small catch: long-term daily use of rapamycin could increase risk of insulin resistance, glucose intolerance, and hyperlipidemia.

Transplant Direct. 2016 Feb; 2(2): e65
Br J Pharmacol. 2012 Apr; 165(7): 2325–2340
mTOR: Mammalian/Mechanistic Target of Rapamycin

- Rapamycin inhibits mTOR
- Daily use strongly inhibits mTORC1, weakly inhibits mTORC2
- mTORC1 inhibition: extends lifespan, autophagy, enhances immunity
- mTORC2 inhibition: alters insulin signaling cascade = glucose dysregulation

mTOR inhibition improves immune function in the elderly

- The mTOR inhibitor RAD001 (everolimus) enhanced the response to the influenza vaccine by about 20% at doses that were relatively well tolerated.
- RAD001 also reduced the percentage of CD4 and CD8 T lymphocytes expressing the programmed death-1 (PD-1) receptor, which inhibits T cell signaling and is more highly expressed with age.
- These results raise the possibility that mTOR inhibition may have beneficial effects on immunosenescence in the elderly.

mTOR Inhibitors

• Sirolimus/Rapamycin
• Everolimus (mTORC1 selective)
• Metformin
• Calorie restriction:
  – Chronic
  – Intermittent (overnight fasting)
• Amino acid (leucine) restriction?
• Ketogenic diets (?)

Cell Metab. 2017; 25(2): 463–471
Anticancer Agents Med Chem. 2010; 10(7): 571–581
Epilepsia. 2011; 52(3): e7–e11.
Metformin inhibits hepatic mTORC1 signaling via dose-dependent mechanisms involving AMPK and the TSC complex

• we show that metformin robustly inhibits mTORC1 in mouse liver tissue and primary hepatocytes.
• Using mouse genetics, we find that at the lowest concentrations of metformin that inhibit hepatic mTORC1 signaling, this inhibition is dependent on AMPK and the tuberous sclerosis complex (TSC) protein complex (TSC complex).
• Finally, we show that metformin profoundly inhibits hepatocyte protein synthesis in a manner that is largely dependent on its ability to suppress mTORC1 signaling.

Cell Metab. 2017 February 07; 25(2): 463–471
Food Energy Restriction

• **Caloric Restriction:**
  – 20-30% less BMR
  – or 600 cal/day

• **Intermittent Fasting:**
  – 24 hr fast monthly
  – 16-18 hr fast weekly
  – 12+ hour overnight fast
  – Every other day fast
“natural” mTOR inhibitors (in vitro or animal studies)

- Berberine
- Resveratrol
- N-acetylcysteine
- Curcumin
- Quercetin
- Lipoic acid
- Green tea (EGCG)
- Ellagic acid (pomegranate)
- Di-indolylmethane
- Olive oil polyphenols

Counteracting Senescence
FOXO3A genotype is strongly associated with human longevity

• Assessed a large, homogeneous, long-lived population of men well-characterized for aging phenotypes

• Analysis of five candidate genes within the human insulin/IGF-1 signaling pathway and/or oxidative stress response system demonstrated that one gene clearly stood out from the others in terms of a potential human longevity gene—FOXO3A

Proc Natl Acad Sci U S A. 2008 Sep 16; 105(37): 13987–13992
FOXO3A genotype is strongly associated with human longevity

• Long-lived men also presented several additional phenotypes linked to healthy aging, including lower prevalence of cancer and cardiovascular disease, better self-reported health, and high physical and cognitive function, despite significantly older ages than controls.

• Several of these aging phenotypes were associated with FOXO3A genotype

*Proc Natl Acad Sci U S A*. 2008 Sep 16; 105(37): 13987–13992
FOXO3A genotype is strongly associated with human longevity

- FOXO may provide a potential forkhead or bridge between insulin signaling, free radicals, and human aging/longevity.

Proc Natl Acad Sci U S A. 2008 Sep 16; 105(37): 13987–13992
Pathways That Actively Counteract Senescence

• Mitochondrial energy/biogenesis pathway
  – AMKP
  – NAD+ -- Sirtuin1/Sirtuin3
  – PGC1-alpha
  – FOX01 / FOXO3

• Cytoprotective pathway
  – Nrf2/Keap1
  – Antioxidant response element (ARE)
Healthy cristae (left) vs damaged (right)
“A progressive decline in the expression of mitochondrial genes is a central feature of normal human aging.”

Mitochondrial QC & Aging

• Healthy mitochondrial function is maintained by elaborate mechanisms of homeostatic “quality control”

• QC results from balance between
  – Mitophagy: elimination of damaged DNA, membranes and proteins
  – Biogenesis: regeneration of healthy organelles
Mitochondrial Dynamics

- Biogenesis (synthesis)
- Fusion: increase in mitochondrial mass
- Mitochondrial fusion and fission cycle
- Fission: increase in mitochondrial numbers
- Mitophagy (degradation)
- Fused mitochondria
- Endoplasmic reticulum and mitochondria interact to form microenvironments for the assembly of DRP1, MFF, and Bax.

Beginner’s HIIT Protocol

http://www.acefitness.org/acefit/fitness-fact-article/3317/high-intensity-interval-training/

- Protocols vary
- No one size fits all
- Experiment with shorter and longer speed and recovery periods
- Work up to 8 speed intervals
- 1-2 times a week
- 6 weeks in a row to enhance regular training

<table>
<thead>
<tr>
<th>Time</th>
<th>Interval</th>
<th>Exertion Level (0-10)</th>
</tr>
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<tbody>
<tr>
<td>5 min.</td>
<td>Warm-up</td>
<td>3–4</td>
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<tr>
<td>1 min.</td>
<td>Speed</td>
<td>7–9</td>
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<tr>
<td>2 min.</td>
<td>Recovery</td>
<td>5–6</td>
</tr>
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<tr>
<td>1 min.</td>
<td>Speed</td>
<td>7–9</td>
</tr>
<tr>
<td>5 min</td>
<td>Cool-down</td>
<td>3–4</td>
</tr>
</tbody>
</table>

22 min. Total Time
(4 min. total speed)
12 Weeks Exercise Training in Younger and Older People

High Intensity Training

Resistance Training

Increased

Increased

DNA → mRNA → tRNA + Ribosome → Protein → Mitochondria → Hypertrophy

Increased

↑ Oxidation capacity

↑ Muscle mass and strength

Skeletal Muscle Adaptation to Exercise Training

Cell Metabolism 2017 25, 581-592
EXERCISE TRAINING

- BIODGENERESIS
- FUSION
- FISSION
- MITOPHAGY

ADDITION OF HEALTHY MITOCHONDRIA

REMOVAL OF DAMAGED MITOCHONDRIA

ENHANCED METABOLIC FUNCTION/PERFORMANCE

Source: Exerc Sport Sci Rev © 2012 American College of Sports Medicine
How does HIIT training lead to more mitochondria? ATP depletion activates stress pathways
ATP depletion:
Calorie restriction, Exercise, Cold Temperatures

AMPK  
SIRT1

PGC-1alpha  
(muscle, brain, liver, brown adipose tissue)

Gene regulation

Mitochondrial biogenesis:
↑ATP, ↑Fatty acid oxidation, ↑Energy, Endurance, Weight loss, Longevity
AMPK-Sirtuin pathway

- AMPK/Sirtuins (1 & 3)
- PGC1-alpha--FOXO (1 & 3)
- Core regulator of cellular energy metabolism
- "Upstream" of mTOR

Int. J. Mol. Sci. 2017, 18, 288
5‘AMP-Activated Kinase (AMPK): A Metabolic Master Switch

- Major intracellular metabolic sensing enzyme, involved in cellular energy homeostasis
- Active in liver, skeletal muscle, brain
- Dysregulated in
  - Obesity
  - T2DM
  - Fatty liver disease
  - Cardiovascular disease
  - Cancer

Int. J. Mol. Sci. 2017, 18, 288
AMPK Activators

- Low ATP: high AMP
- Exercise (muscle contraction)
- Hypoxia/ischemia
- Leptin, adiponectin
- Thyroxine (increases production of AMPK subunits)
- Metformin
- Berberine
- Trans-resveratrol
- Quercitin
- Curcumin
- Green tea polyphenols
- Alpha lipoic acid

Int. J. Mol. Sci. 2017, 18, 288
Am J Physiol Endocrinol Metab. 2010; 298(4): E751–E760
Hypoxia!!!!
Could we forget the high altitude hikes, and take metformin instead?
Huang Lian
*Coptis Chinensis*
rhizome: Source of berberine powder
Could berberine substitute for metformin?
Rhizoma Coptidis and Berberine as a Natural Drug to Combat Aging and Aging-Related Diseases via Anti-Oxidation & AMPK Activation

• Two different mechanisms are thought to be primary causes of aging. One is cumulative DNA damage induced by a perpetuating cycle of oxidative stress;

• the other is nutrient-sensing adenosine monophosphate-activated protein kinase (AMPK) and rapamycin (mTOR)/ ribosomal protein S6 (rpS6) pathways.

Aging and Disease, 2017, Vol 8(6): 760-777
Rhizoma Coptidis and Berberine as a Natural Drug to Combat Aging and Aging-Related Diseases via Anti-Oxidation & AMPK Activation

• Berberine is a promising anti-aging natural product, and has pharmaceutical potential in combating aging-related diseases via anti-oxidation and AMPK cellular kinase activation.

Aging and Disease, 2017, Vol 8(6): 760-777
Sirtuins
Silent Information Regulators of Transcription

- Family of 7 NAD-dependent deacetylases
- Found in most living organisms
- Activation linked to longevity, insulin sensitivity & protection against diet-induced obesity

Annu Rev Pathol. 2010; 5: 253–295
Sirtuin 1

- Found in cytoplasm and nucleus
- Coded by SIRT1 gene
- Supports cell survival during stress, especially from lack of food
- Improves insulin sensitivity; downregulated with insulin resistance
- Induced by caloric restriction, exercise

Proc Natl Acad Sci 2008;105:9793-9798
Sirtuin 1

- Protect cells from metabolic damage by glucose (glucotoxicity) & fat (lipotoxicity)
- Enhances autophagy
- Activates PGC1-alpha (via deacetylation)
- Inhibits NFKB
- Activated by polyphenols/resveratrol

Proc Natl Acad Sci 2008;105:9793-9798
PGC1α (PPARγ co-activator 1α)

- **Master regulator of mitochondrial biogenesis**: most genes coding for mitochondrial proteins have binding sites for PCG1α on their regulatory regions.

- **Activated by**
  - AMPK: in response to low energy stores
  - Sirtuins (deacetylases)
  - Nitric oxide
  - Calcium-dependent enzymes (CaMK; calcineurin)
PGC-1α  (PPARγ co-activator 1α)

• Predominantly expressed in mitochondrial-rich tissues such as heart, skeletal muscle, brown adipose tissue, and to some extent liver
• Increases metabolic switch toward β-oxidation, OXPHOS, antioxidant protection, and uncoupling proteins (exercise, cold)
• Increased PGC1α activity =
  – improved athletic performance
  – Enhanced lipolysis & insulin sensitivity

Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha

- “RSV treatment protected mice against diet-induced-obesity and insulin resistance.”
- “These pharmacological effects of RSV combined with the association of three Sirt1 SNPs and energy homeostasis in Finnish subjects implicates SIRT1 as a key regulator of energy and metabolic homeostasis.”

Cell 2006; Vol 127(6): 1109-22
If resveratrol is so powerful for activating sirtuins, why has it failed in clinical trials?

Answer: sirtuins require NAD+ as a cofactor
NAD+ Regulation of Sirtuins

NAD+

SIRT1

Brain/CNS
- Neurodegeneration

Heart
- Inflammation
- Cardioprotection

Liver
- Fatty acid oxidation
- Gluconeogenesis

WAT
- Lipogenesis

Pancreas
- Insulin secretion

Skeletal Muscle
- Insulin sensitivity
- Fatty acid oxidation

FOXO NF-κB
LXR PGC-1α PPARα
PPARY
FOXO UCP2
PGC-1α FOXO
NAD+ (Nicotinamide Adenine Dinucleotide)

- Enzymatic cofactor for metabolic activators, cell signaling, DNA repair
- Activates mitohormesis
- Metabolic regulator of ROS/ATP
  - Modulates free radical production
  - Controls rate of oxidative phosphorylation & ATP production

NAD+ Depletion

• NAD+ is consumed & depleted by
  – Poly-ADP ribose polymerases: DNA repair
  – sirtuins (deacetylases): metabolic activators
  – cADP-ribose synthasases: intracellular signaling

• As result, NAD+ levels decline with aging and stress – a hallmark of senescence

NAD+ Repletion

• Calorie restriction/\textit{fasting}
• Oral (dietary) NAD+ precursors:
  – Tryptophan (inefficient source)
  – Niacin/nicotinic acid
  – Niacinamide/nicotinamide (sirtuin inhibitor)
  – \textit{Nicotinamide riboside} (via NR kinases – highly efficient)
• Synergistic with
  – Resveratrol
  – Quercetin
**NAD⁺ production**
- e.g. Trp, NA, NAM, NR

**NAD⁺ consumption**
- e.g. PARP-1, CD38

**STACs**

**Resveratrol**

**Exercise**

**Calorie restriction**

**Fasting**

**Aging**

**Sirtuins**
- e.g. SIRT1, SIRT3

**Mitochondrial diseases, Obesity, diabetes, neurodegeneration**

- Deacetylation of transcriptional regulators (e.g. PGC-1α, FOXO1)

- Metabolic adaptation

- Mitochondrial biogenesis and function

- Protection against mitochondrial and age-related disorders

**Mitochondrial unfolded protein response (UPR⁰mt)**

- Mitohormetic response

- Mitochondrial biogenesis and function

- Longevity
Nicotinamide Riboside (NR)

- Vitamin B3 analog, produced endogenously and found in food (beer, cow’s milk)
- Extensively researched at Dartmouth Medical School, Cornell, & Univ of Iowa
- Efficient dietary precursor to cellular NAD+ (Brenner, 2004)
- Activates sirtuins 1 & 3 resulting in
  - mitochondrial biogenesis
  - improved energy metabolism
The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet induced obesity

“our work shows that nicotinamide riboside is a powerful supplement to boost NAD+ levels, activate sirtuin signaling and improve mitochondrial function, suggesting that this vitamin could be used to prevent and treat the mitochondrial decline that is a hallmark of many diseases associated with aging”
The Mitochondrial Biogenesis Triumvirate

PGC1α

Nitric Oxide

AMPK

Sirt 1

Metformin
Berberine
Curcumin
Quercetin
Lipoic acid

Curcumin
Resveratrol
Nicotinamide riboside

Green tea
Beets
Arugula

Increased strength in Men.
Increased lean body mass in Women.

Influences same genes as calorie restriction.

Adjuncts to Exercise and CR for Disease Prevention, Healthy Aging
The hierarchical oxidative stress model

A. Nel et al., Science 311, 622-627 (2006)
Adaptive Stress Response

- Activated when mammals consume low doses of certain phytochemicals
- A complex but coordinated molecular & cellular response pattern
- Achieved by turning on specific group of genes, resulting in increased expression of cytoprotective and longevity enhancing enzymes
HORMETIC FACTORS
- Lifestyle
- exercise
- dietary energy restriction
- dietary phytochemicals
- cognitive stimulation
- Environmental Exposures
- toxins
- radiation
- temperature
- water
- Intrinsic Factors
- ischemia
- endocrine status
- neurotransmitters

CELL STRESS/SIGNALING
- Free radicals
- Ion fluxes
- Energy depletion
- Receptors
- Kinases and phosphatases
- Deacetylases
- Transcription factors
  - Nrf-2
  - FOXOs
  - CREB
  - NF-κB

HORMETIC EFFECTORS
- Antioxidants
- superoxide dismutases
- catalase
- glutathione peroxidase
- glutathione
- Protein chaperones
- HSP-70
- GRP-78
- Growth factors
- BDNF
- VEGF
- bFGF
- Others
- mitochondrial proteins
- calcium-regulating proteins
Hormetic Phytochemicals = Indirect Antioxidants: Superior to Chemical Antioxidants for Enhancing Health & Longevity
Nrf2

- Intracellular sensor that regulates redox balance & stress response—activates Antioxidant Response Element (ARE) on DNA
- Master switch for cellular response against oxidative stress
Antioxidant Response Element (aka Electrophile Response Element)

- DNA binding site that primarily activates antioxidant, phase II, & cytoprotective enzymes
- ARE genes are activated by Nrf2
- ARE gene activation enhances detoxification, decreases inflammation & inhibits cancer growth
- This mechanism may explain many observed beneficial effects of detoxifying phytochemicals
Nrf2 as a Master Redox Switch in Turning on the Cellular Signaling Involved in the Induction of Cytoprotective Genes by Some Chemopreventive Phytochemicals


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Abstract

A wide array of dietary phytochemicals have been reported to induce the expression of enzymes involved in both cellular antioxidant defenses and elimination/inactivation of electrophilic carcinogens. Induction of such cytoprotective enzymes by edible phytochemicals largely accounts for their cancer chemopreventive and chemoprotective activities. Nuclear factor-erythroid-2-related factor 2 (Nrf2) plays a crucial role in the coordinated induction of those genes encoding many stress-responsive and cytoprotective enzymes and related proteins. These include NAD(P)H:quinone oxidoreductase-1, heme oxygenase-1, glutathione S-transferase, glutathione peroxidase, thioredoxin, etc. In resting cells, Nrf2 is sequestered in the cytoplasm as an inactive complex with the repressor Kelch-like ECH-associated protein 1 (Keap1). The release of Nrf2 from its repressor is most likely to be achieved by alterations in the structure of Keap1. Keap1 contains several reactive cysteine residues that function as sensors of cellular redox changes. Oxidation or covalent modification of some of these critical cysteine thiols would stabilize Nrf2, thereby facilitating nuclear accumulation of Nrf2. After translocation into nucleus, Nrf2 forms a heterodimer with other transcription factors, such as small Maf, which in turn binds to the 5'-upstream cis-acting regulatory sequence, termed antioxidant response elements (ARE) or electrophile response elements (EpRE), located in the promoter region of genes encoding various antioxidant and phase 2 detoxifying enzymes. Certain dietary chemopreventive agents target Keap1 by oxidizing or chemically modifying one or more of its specific cysteine thiols, thereby stabilizing Nrf2. In addition, phosphorylation of specific serine or threonine residues present in Nrf2 by upstream kinases may also facilitate the nuclear localization of Nrf2. Multiple mechanisms of Nrf2 activation by signals mediated by one or more of the upstream kinases, such as mitogen-activated protein kinases, phosphatidylinositol-3-kinase/Akt, protein kinase C, and casein kinase-2 have recently been proposed. This review highlights the cytoprotective gene expression induced by some representative dietary chemopreventive phytochemicals with the Nrf2-Keap1 system as a prime molecular target.
Chemopreventive phytochemicals

activation of upstream kinases

Nrf2-Keap1

oxidation/modification of Keap1 cysteine thiol

Nrf2

Induction of anti-oxidant/detoxifying enzymes and other cytoprotective proteins

Oxidative and electrophilic stress, inflammatory injury, etc.

DNA damage/mutation or epigenetic changes for malignant transformation

ATP depletion:
- Calorie restriction
- Exercise
- Cold temperatures
- Alpha Lipoic Acid
- Resveratrol

AMPK

SIRT1

PGC-1alpha
(muscle, brain, liver, brown adipose tissue)

Mitochondrial biogenesis
- ↑ATP
- ↑Fatty acid oxidation, ↑Energy, Endurance, Weight loss, Longevity

Nrf2

Sulforaphane
- Curcumin
- Green tea polyphenols
- Resveratrol

Gene regulation (numerous)

Gene regulation (Antioxidant Response Element)

Antioxidant enzymes
- Phase II detoxification enzymes
- Neuroprotective proteins
- Longevity
Plants & Constituents That Induce ARE via Nrf2

- Sulforaphane (broccoli), PEITC (watercress)
- Curcumin
- Trans-resveratrol
- Green tea polyphenols (EGCG)
- Quercetin & related polyphenols
- Ginkgo biloba
- Garlic (allyl sulfide)
- Carnosol (rosemary)
- Milk thistle
- Alpha lipoic acid
- Coffee (kahweol)
Life Extension: Potential Strategies

• Daily meditation
• Active social network
• Regular exercise, HIIT plus resistance
• Dietary strategies
  – Caloric restriction
  – Time-restricted feeding
  – Intermittent fasting / fasting mimicking
• Hormone replacement
Categories of Longevity Enhancing Agents

– Telomerase activators
– Anti-inflammatory (NFKB downregulators)
– mTOR inhibitors
– AMPK-SIRT-PGC1-alpha activators
– Nrf2 activators (indirect antioxidants)
Research-Worthy Longevity Agents

- Rapamycin (intermittent?)
- Metformin
- Berberine
- N-acetylcysteine (NAC); alpha lipoic acid
- Quercetin; curcumin; green tea
- Trans-resveratrol + nicotinamide riboside
- Sulforaphane
- Omega-3 fatty acids