Resilience and Frailty in Old Age: What Drives it?
Molecular/Genetic Level
Organ System Level

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The Frailty CORE

- Slow Walking
- Unreliable
- Poor strength
- Lonely
- Wrinkled
- Poor Vision
- Belly fat
- Breaks easily
- Needs Help
- Unstable
- Annoying
- Slow movement
- Tired
- Multi-morbidity
- Sad
- Sedentary
- Greying
- Sick
- Forgetful
- Sagging skin
- Wide waist
- Lost appetite
- Poor earing
- Poor sleep
- Rigid
- Stooped
- Unreliable
- Appendage
- Breaks easily
- Slow movement
Clinical Frailty Scale*

1 Very Fit – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.

2 Well – People who have no active disease symptoms but are less fit than category 1. Often, they exercise or are very active occasionally, e.g. seasonally.

3 Managing Well – People whose medical problems are well controlled, but are not regularly active beyond routine walking.

4 Vulnerable – While not dependent on others for daily help, often symptoms limit activities. A common complaint is being “slowed up”, and/or being tired during the day.

5 Mildly Frail – These people often have more evident slowing, and need help in high order IADLs (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.

6 Moderately Frail – People need help with all outside activities and with keeping house. Inside, they often have problems with stairs and need help with bathing and might need minimal assistance (cuing, standby) with dressing.

7 Severely Frail – Completely dependent for personal care, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).

8 Very Severely Frail – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.

9 Terminally Ill - Approaching the end of life. This category applies to people with a life expectancy <6 months, who are not otherwise evidently frail.

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common symptoms in mild dementia include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In moderate dementia, recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In severe dementia, they cannot do personal care without help.


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Rigid
Poor Bearing
Breaks easily
Poor Vision
Greying
Slow movement
Unstable
Unreliable
Annoying
Stooped
Sagging skin
Belly fat
Forgetful
Tired
Lonely
Sick
Sad
Wide waist
Needs Help
Lost appetite
Poor strength
Sedentary
Multi-morbidity
The Frailty BLOB
The Cycle of Frailty (one of the many versions)

- **Lost appetite**
  - Neuroendocrine Dysregulation
  - Anorexia of aging
  - Total Energy Expenditure
  - Activity
- **Tired**
- **Slow movement**
  - Walking Speed
  - Disability
  - Dependency
- **Poor strength**
  - Resting Metabolic Rate
  - Strength & Power
  - VO$_2$max
- **Sedentary**
- Disease
  - Aging: Senescent musculoskeletal changes
  - Negative Energy Balance
  - Negative Nitrogen Balance
  - Loss of muscle mass Sarcopenia

What are the mechanisms by which aging and disease affect aging phenotypes and longevity?

Changes in Body Composition
Energy Imbalance Production/Utilization
Homeostatic Dysregulation
Neurodegeneration

Physical and Cognitive FRAILTY

Disease Susceptibility
Reduced Functional Reserve
Reduced Healing Capacity and Stress Resistance
Unstable Health
Failure to Thrive

AGING and DISEASES

Stem Cells Exhaustion
Altered Intercellular Communication
Genomic Instability
Telomere Attrition
Epigenetic Alterations
Loss of Proteostasis
Deregulated Nutrient Sensing
Mitochondrial Dysfunction
Cellular Senescence

Ferrucci L, Studenski S. Clinical Problems of Aging. In: Harrison’s Principles of Internal Medicine, 18th Ed. – 2011

The Hallmarks of Aging
Carlos Lo´pez-Ol´n, Maria A. Blasco, Linda Partridge, Manuel Serrano, and Guido Kroemer. Cell 3013, 153: 1194
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?
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Altered Intercellular Communication
Aging is associated with up-regulation of immune function genes.

Transcriptome-wide meta-analysis of genes whose expression differs by age in 7,257 individuals of European ancestry. Findings replicated in another 8,009 individuals. 1,497 genes were differentially expressed with age. The major cluster of positively age-correlated genes (GeneNetwork pathway, 77 genes) was related to innate and adaptive immunity, suggesting that dysregulation of the immune leading to a pro-inflammatory state is a hallmark of aging.
Interleukin-6 Serum Levels Predict Incident Disability
A Case Cohort Study Nested in the EPESE

Ferrucci et al. JAGS 1999;47: 639-44
4. IL-6 as risk factor for multimorbidity.

A mild chronic pro-inflammatory state, characterized by high levels of IL-6, is a typical phenotype associated with older age and has been implicated in the pathogenesis of many age-related chronic diseases. In the InCHIANTI study (n=914, over 6-year follow-up) we demonstrated that IL-6 was a strong cross sectional and longitudinal correlate of multimorbidity, and increase in IL-6 over time independently predicted even higher increase in multimorbidity.
Systemic Effects of Localized Inflammation

Harmful stimuli:
- Damaged cells
- Irritant chemicals
- Pathogens

INFLAMMATION attempts to remove damaged cells, irritants or pathogens

Effective
Eliminates the cause of inflammation

“Switch off” inflammation

Healing

Systemic Effects

G.I. System
- Reduces food absorption
- Causes insulin resistance
- Stimulates glycogenolysis
- Down-regulates somatostatin

Muscle
- Inhibits muscle growth
- Down-regulates IGF-1 signaling

Bone
- Stimulates osteoclasts
- Down-regulates Osteocalcin

Bone Marrow
- Inhibits Hematopoiesis
- Down-regulates EPO signaling

Arteries
- Stimulates atherosclerosis
- Inhibit endothelial reactivity

Brain
- Activates microglia
- Inhibits Neurogenesis
- Down-regulates BDNF

INFLAMMATION attempts to remove damaged cells, irritants or pathogens

Healing

Healing

Healing

Healing
Changes in basal gene expression in CD4+ cells

cytapheresis
  ↓
  PBMC
  ↓
CD4+ T cells
  ↓
activation (anti-CD3 0, 2, 4h)

RNA
  ↓
  protein

nuclear
  ↓
microarray

immunoblot
  ↓
mass spec.
Cell-intrinsic activation of NF-κB target genes

Hypothesis: Effects of *in vivo* inflammatory milieu should be reflected in the gene expression pattern in freshly isolated cells compared to cells cultured *ex vivo*.

Elevated expression of putative NF-κB target genes is the consequence of metabolic activity.
Proteomic analyses of age-associated changes in CD4^+ T cells

cytapheresis
  ↓
PBMC
  ↓
CD4^+ T cells
  ↓
activation
  (anti-CD3 0, 2, 4h)
  ↓
RNA
  ↓
protein
  ↓
nuclear
  ↓
microarray
  ↓
immunoblot
  ↓
cytoplasmic
  ↓
mass spec.
  ↓
Protein quantitation by iTRAQ

LC/MS/MS
### Experimental design

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<th>iTRAQ Reagents</th>
<th>iTRAQ 1 Sample #</th>
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<th>Age</th>
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<th>Age</th>
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<td>BL4877</td>
<td>83</td>
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<td>76</td>
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</table>

4 young (20-34y)  
18 old (68-83y)  
ND617 used as control in each assay  
ND620 repeated in 3 assays (tech control)  
2 ‘old’ repeats in independent assays

27 differentially expressed proteins between Y and O (>1.5x, p<0.05)
Pathways identified based on differential protein expression

(DAVID analysis, REACTOME pathway database)

Metabolism of nucleotides
Diabetes pathway
Integration of energy metabolism

% genes 0 10 20 30 40 50

Cardiac muscle contraction
Alzheimer’s disease
Huntington’s disease
Oxidative phosphorylation
Parkinson disease

% genes 0 10 20 30 40 50

(DAVID analysis, KEGG pathway database)
Multiple components of the mitochondrial electron transport chain are up-regulated in CD4^+ T cells from older individuals.
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?
Age-dependent changes in mitochondrial morphology and volume are not predictors of lifespan
Saroj G. Regmi et al.

Age-dependent mitochondrial changes in C. elegans body wall muscle cells

AGING, February 2014, Vol. 6 No.2
Age Changes in Size and Number of Mitochondria of Human Hepatic Cells

Fig. 1. Changes with age in number of mitochondria in a given area.

Fig. 2. Changes with age in size of mitochondria.

Fig. 3. Changes with age in circumference of mitochondria.

Fig. 4. Distribution pattern of circumference of mitochondria according to age.
$P_{31}$ MRS: Rate of Phosphocreatine Replenishment and Aging

\[ \beta = 0.38 \]
\[ P = 0.002 \]

- **Aging**
- **Sarcopenia**: Loss of muscle mass
- **Whole body aerobic capacity** (VO$_2$peak)
- **Muscle mitochondrial capacity and efficiency**
- **Oxygen** (O$_2$ consumption – State 3 respiration)
- **ATP** (ATP production – ATP$_{max}$)
- **Efficiency** - ATP$_{max}$/State 3 respiration
- **Capacity** - ATP$_{max}$
  - State 3 respiration*Quadriceps volume
- **Energetic cost of walking**
- **Walking speed**
- **Mobility**
- **Disability**
- **Hospitalization**
- **Mortality**
Skeletal Muscle Mitochondrial Energetics Are Associated With Maximal Aerobic Capacity and Walking Speed in Older Adults
High Basal Metabolic Rate Is a Risk Factor for Mortality: The Baltimore Longitudinal Study of Aging

Multi-morbidity and Resting Metabolic Rate (RMR): longitudinal association

*adjusted for baseline age, sex, baseline total body lean mass and fat mass

BLSA (unpublished data)
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?

Epigenetic Alterations
DNAm Age Correlates with Chronologic Age

cor = 0.85, p < 1e-200
Ticking rate of the epigenetic clock
= rate of change of the average DNA meth levels
= rate of change of the red curve
Chronologic vs DNAm Age (test sets by tissue)
DNA methylation age of human tissues and cell types
Steve Horvath at http://genomebiology.com//14/10/R115

B Training data cor=0.92, p<1e-200
C Test data cor=0.92, p<1e-200

InCHIANTI Baseline (n=499)  InCHIANTI 9-year Followup (n=499)  BLSA (n=1105)
DNAm Age Tracks Chronologic Age Over a 9-year Period
DNA methylation age of blood predicts all-cause mortality in later life


Riccardo E Marioni, Sonia Shah, Allan F McRae, Brian H Chen et al.
Is the Biology of Aging at the Core Of Frailty?
Can This Hypothesis be Tested?

Telomere Attrition
Telomere length declines with aging. Attrition of telomere length in peripheral blood mononuclear cells with age is documented from cross-sectional studies (left). Average decline in telomere length was recently confirmed in the Baltimore Longitudinal Study of Aging (below).
Telomere length vs. DNAm Age

DNAm Age

Telomere Length
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?

Cellular Senescence
In 1961, L. Hayflick proposed that the limited lifespan of cells in culture represented the phenomenon of aging at the cellular level.
In 1998, it was conclusively demonstrated that replicative cell senescence is caused by telomere shortening.

In these experiments, the authors introduced telomerase, and that was sufficient to abrogate cell senescence (as shown by lack of SA-bGal staining in the upper panels).

Telomere shortening is only one of many paths to cell senescence

Differentiated Cell Fates Under Stress

- Proliferation
- Senescence
- Apoptosis

p53

Cancer
Degeneration
Do they exist in vivo?

Monitoring Tumorigenesis and Senescence In Vivo with a $p16^{INK4a}$-Luciferase Model

Burd et al. – Cell 152:340 (2013)
Chemotherapy-induced senescence *in vivo* (mice)

**HT1080 fibrosarcoma xenograft**

*Roninson et al., Drug Res. Updat. 4, 303, 2001*
Senescent cells secrete a large number (and large amounts) of biologically active factors with the potential of affecting cellular physiology / responses in neighboring, non-senescent cells.
Purging Cells in Mice Is Found to Combat Aging Ills

Two 9-month-old mice from the study. The one on the right received the drug to eliminate senescent cells.

By NICHOLAS WADE
Published: November 2, 2011

In a potentially fundamental advance, researchers have opened up a novel approach to combating the effects of aging with the discovery that a special category of cells, known as senescent cells, are bad actors that promote the aging of the tissues. Cleansing the body of the
Clearance of p16\textsuperscript{Ink4a}–positive senescent cells delays ageing–associated disorders

Darren J. Baker\textsuperscript{1,2,3}, Tobias Wijshake\textsuperscript{1,4}, Tamar Tchkonia\textsuperscript{3}, Nathan K. LeBrasseur\textsuperscript{3,5}, Bennett G. Childs\textsuperscript{1}, Bart van de Sluis\textsuperscript{4}, James L. Kirkland\textsuperscript{3} & Jan M. van Deursen\textsuperscript{1,2,3}
Expression of p16INK4a in peripheral blood T-cells is a biomarker of human aging


(B) $R^2 = 0.40$
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?
SATELLITE CELLS AND MYONUCLEI IN YOUNG AND ELDERLY WOMEN AND MEN


Table 2. Muscle fibers, satellite cells, and myonuclei in the tibialis anterior muscle of young and elderly women and men.

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (n = 16)</td>
<td>Men (n = 15)</td>
</tr>
<tr>
<td>No. of muscle fibers (f)</td>
<td>218 ± 12 (210–243)</td>
<td>212 ± 8 (192–223)</td>
</tr>
<tr>
<td>No. of satellite cells (s)</td>
<td>37 ± 10 (19–63)</td>
<td>40 ± 11 (24–58)</td>
</tr>
<tr>
<td>No. of myonuclei (m)</td>
<td>493 ± 72 (391–655)</td>
<td>545 ± 54 (461–627)</td>
</tr>
<tr>
<td>Total number of nuclei (s+m)</td>
<td>530 ± 73 (430–700)</td>
<td>585 ± 56 (505–683)</td>
</tr>
<tr>
<td>No. of satellite cells/muscle fiber (s/f)</td>
<td>0.17 ± 0.04 (0.09–0.28)</td>
<td>0.19 ± 0.05 (0.11–0.29)</td>
</tr>
<tr>
<td>No. of myonuclei/muscle fiber (m/f)</td>
<td>2.25 ± 0.27 (1.89–2.76)</td>
<td>2.57 ± 0.23 (2.28–2.97)</td>
</tr>
<tr>
<td>Relative number of satellite cells [s/(m + s) × 100] (%)</td>
<td>7.1 ± 1.9 (3.4–11.2)</td>
<td>6.9 ± 1.8 (4.4–11.2)</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD and range.
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?
CONSEQUENCES OF DNA DAMAGE

DEFECTS in repair of DNA damage associate with aging and age-related disease

STRESS
- Endogenous
- Environmental

DNA Lesion

DNA REPAIR SYSTEMS

TRANSCRIPTION:
- Blockage or Error

REPLICATION:
- Blockage or Error
  - Cellular Dysfunction
  - Genetic Instability
  - Cell Death
  - CANCER
  - NEURODEGENERATION
  - AGING PROCESS
DNA lesions are introduced into fluorescent reporter plasmids in vitro. Numbers labeling the plasmids represent the dose (in joules per square meter) of UV radiation. Following treatment, plasmids were combined and cotransfected into cells. After 18 or 40 h incubation, cells were assayed for fluorescence by flow cytometry. Comparison of fluorescence signals with those from cells transfected with undamaged plasmids yields a dose–response curve.
The Green Ear by Gianni Rodari

One day as I took the train direct to Capranica-Viterbo a man got on with an ear as green as an unripe tomato. He wasn’t exactly young at all, but rather somewhat older. Except for his bright green ear, he was totally, totally in order.

I quickly moved and changed my seat to study this phenomenon from head to feet. “Sir,” I said to him, “I see you’ve reached a certain age, so why a green ear at this late stage?”

“Just say,” he answered with courtesy, “that I’ve become quite old. This ear is now the only thing left from my youth—if truth be told. This ear, a child’s ear, is used to help me grasp what I can—those voices adults don’t ever hear and will never understand.

I listen to what the birds say, to the words of all the trees. I listen to the clouds that pass as well as the rocks and streams. I understand the children when they say some things I hear, those things that seem so strange to every grown-up’s ear.”

That’s what he said. There with an ear as green as an unripe tomato on the day that I took the train direct to Capranica-Viterbo.
Is the Biology of Aging at the Core Of Frailty? Can This Hypothesis be Tested?

Deregulated Nutrient Sensing
Is the Biology of Aging at the Core Of Frailty?
Can This Hypothesis be Tested?

Loss of Proteostasis