Healthspan and Frailty: Bedside to Benchtop (and Back?)

Bruce R. Troen, M.D.
Division of Gerontology and Geriatric Medicine
University of Miami Miller School of Medicine
Geriatric Research, Education, and Clinical Center
Miami VA Healthcare System

Normal bone  Osteoporosis

Multiple factors bind the CTSK promoter

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Research / Interests

• Healthspan & Frailty
  – obesity / sarcopenia / inflammaging
• Osteoporosis & related pathologies
  – stem cells / osteoclastogenesis
  – growth factor signaling, nuclear factors & transcription
• Vitamin D, Resveratrol & Sirtuins
  – STACs, Sirt1 transgenic mice
  – animal model of vitamin D insufficiency

Challenges / Care Gap

• Recognize the importance of the frailty syndrome in the development of disability and adverse health outcomes
• Understand the roles of subclinical inflammation, vitamin D insufficiency, and sarcopenic obesity
• Identify potential underlying mechanisms
• Develop potential strategies to reduce frailty and enhance healthspan

Healthspan

• Number of years lived in a healthy, vital, and functionally capable state with a good quality of life
• Lifespan might far exceed healthspan
• Goals: prevent, treat, or even reverse diseases associated with older age
• Goal: prolong the healthspan for a greater portion of life, until a time that is closer to the actual end of life

Life Expectancy and Health Status

• At age 65, people can now expect to live another 15-20 years, on the average
• Health status during this time is of great importance:
  – 30% robust
  – 50% have 2 or more diseases
  – 40% have difficulty walking or doing other essential activities
  – 7-10% are frail

Frailty Syndrome

1- Weight loss: >10 lb unintentionally in prior year
2- Weakness: grip strength lowest 20% (by gender and body mass index)
3- Exhaustion: self report of exhaustion (CED-Depression Scale)
4- Slowness: walking time/15 feet slowest 20% (by gender and height)
5- Low activity: Kcal/week lowest 20% (Minnesota Leisure Time activity questionnaire)

Frailty: ≥ 3 criteria
Prefrailty: 1 or 2 criteria

Frailty and Aging

• 7 % ≥ 65 living alone had at least three criteria for frailty, while 46 % had none
• more likely to affect women, African Americans, ≥75, less educated, and the poor
• Co-existing chronic diseases: including arthritis, hypertension, and diabetes
• Death 6 X more likely in the frail vs. non-frail after 3 years
• Death 3 X more likely after 7 years
• Death after 7 years, 43 % of frail vs. 23 % of intermediate vs. 12 % of non-frail
Frailty Syndrome: Definition

- A physiologic state of increased vulnerability to stressors that results from decreased physiologic reserves, and even dysregulation, of multiple physiologic systems.
- Frailty may lead to adverse health outcomes including: disability, dependency, falls, need for long-term care, and mortality.

Fried et al., Journal of Gerontology 2004

Obesity and Frailty

- 96% of obese are frail
  - sarcopenia, poor muscle quality, decreased QOL
- Obesity ∝ frailty (OR = 3.52)
- Fat mass ∝ decrease walking speed and functional limitations
- Highest quintile of fat mass ∝ 2.6 OR of disability 3 years later
- BMI of > 30 ∝ twice likelihood of functional limitations
- BMI of > 30 had greater rate of nursing home admission


Inflammation markers ∝ frailty

- Frailty status was determined in 110 patients >75 years (~83.9 years) according to:
  - function (dependent, intermediate, independent)
  - Fried Index (≥ 3 items)
  - Frailty Index (a measure of accumulated deficits)
- Increasing frailty defined by function and by Fried:
  - ↑TNF-alpha, ↑IL-6, ↑CRP
  - albumin was lowest in the frailest subjects
- The greatest differences were seen between intermediate and dependent groups and between the pre-frail and frail.
- Frailty Index ∝ log-transformed CRP (r= 0.221, P < 0.05), IL-6 (r= 0.369, P < 0.01), TNF-alpha (r= 0.379, P < 0.01) and 1/albumin (r= -0.545, P < 0.01)

Hubbard JCEM 2009

Weight Loss + Exercise Ameliorate Frailty

- 27 frail obese
- 6 months: weekly behavior training for wt. loss & exercise 3x/week
- Rx – lost 8.4% wt., Control – no wt. loss
  - Decreased fat mass
  - Improved Physical Performance score (including strength walking speed, obstacle course, 1 leg stand), peak $O_2$, functional status score

Villareal et al, 2006

Low 25-OH D levels are associated with frailty

- Taiwan, 215 subjects, ~71.1 yrs
  - < 20 ng/ml: OR 10.74
- USA, 4 centers, 6307 subjects ≥ 69 yrs
  - <15 ng/ml: OR 1.47, ≥ 30ng/ml: OR 1.32
- 444 men, 561 women ≥ 65
  - < 20 ng/ml: OR 4.94 (not in women)
- NHANES III, 5048 subjects, > 60 yrs
  - <15 ng/ml: OR 3.7 whites, OR 4 non-whites, 15-30 ng/ml: OR 2.7 non-whites
- USA, 6 centers, 1606 males ≥ 65 yrs
  - < 20 ng/ml: OR 1.5

Low 25-OH D levels are associated with frailty

- 988 community subjects, 77-100 yrs
  - 30.8% < 20 ng/ml, 66.7% < 30 ng/ml
  - ↓ SPPB, ↓ grip
  - ↓ ADL OR 1.51
  - ↓ future mobility OR 1.56
- 368 community subjects, 70-89 yrs
  - 50% < 20 ng/ml
  - ↓ SPPB, ↓ gait speed
  - ↑ 25-OH D > 20 ng/ml - ↑ SPPB

Low vitamin D levels ∝ inflammation

- Bellia: ↑ CRP, IL-6 and TNF-α
- Hyppönen: ↑ CRP, IL-6 and TNF-α, but accounted for by adiposity
- Vilarrasa: NO ↑ IL-18, soluble TNF receptors 1 and 2, or CRP
- Pittas: D3 500 IU/d ➞ CRP, IL-6 in ICU
- Liu: calcitriol 0.5 µg/d ➞ IL-1, TNF-α
- Schleithoff: D3 2000 IU/d ➞ IL-10 in CHF

Inflammaging, HVD, & Fat: A Multi-Pathway to Frailty? 

VitD & Exercise Promote Healthy Aging: Reversal of Fat and Inflammaging Paths

Resveratrol

RSV enhances healthspan

High cal (60% fat) - obesity, insulin R, CVD

Vitamin D and Healthy Lifestyle Modifications Reduce Inflammaging, Improve Physical and Vascular Function, and Promote Healthier Aging

Florez & Troen JAGS 2008 (adapted)
RSV prevents diet-induced obesity

RSV - 400 mpk (4-8 weeks onward)

RSV increases performance

RSV decreases falls

RSV decreases signs of aging

Resveratrol Delays Age-Related Deterioration and Mimics Transcriptional Aspects of Dietary Restriction without Extending Life Span

- ↓ inflammation, ↓ endothelial apoptosis
- ↓ albuminuria, ↓ cataract
- ↑ aortic elasticity, ↑ motor coordination
- preserved BMD

RSV enhances healthspan!

Small molecule activators of SIRT1

SyRMss
Synthetic Resveratrol Mimetics
SRT1720 extends healthspan in mice fed a HFD

Aging and Bone Cells

Role of resveratrol and sirtuins?

RSV ↑ BMD & bone strength

Can RSV treat osteoporosis?

SRT2183 & SRT1720 inhibit OC formation, but RSV does not

SRT2183/1720 inhibit resorption, but RSV does not
RSV and SRT2183 stimulate phosphorylation of AMPK

RSV in vivo inhibits OC-genesis, but stimulates OB-genesis

RSV treatment (6 wks) enhances bone quality in old mice

Sirtuin 1 Knockout Mice

Sirt1 knockout mice exhibit diminished bone quality

Suppression of Sirt1 gene expression (in both OB and OC specific knockouts) results in diminished bone volume and trabecular thickness.

Edwards 2010

SRT1720 action depends upon Sirt1

Minor 2011
SRT2183/1720 inhibit OC- genesis in OC-specific Sirt1-KO BM cells (RSV does not)

SRT2183/1720 inhibit OC- genesis in OC-specific Sirt1-KO BM cells (RSV does not)

SRT2183/1720 prevent actin ring formation in Sirt1-KO BM cells (RSV does not)

SRT2183/1720 inhibit phosphorylation of c-Src (in KO mice), but RSV does not

Sirt1 regulates bone, but SyRMs also act independent of Sirt1

Sirtuin 1 Knockout Mice
Transgenic Mice

**Inducible Cre-Lox Recombination**

- **Osteoblast-Sirt1-KO**
- **Osteoclast-Sirt1-KO**
- **Inducible-Sirt1-KO**
- **Inducible-Sirt1-KO**
- **Whole Body**

**Inducible Sirt1-KO**

**Whole Body**

**Sirt1**

**UBC Cre-ERT2 Mouse**

**Sirt1 Loxp/UBC Cre-ERT2 Mouse**

Sirt1 exon 4 is excised by the translocated Cre recombinase in response to Tamoxifen treatment.

**Tamoxifen in vivo induces down regulation of Sirt1 expression**

- **Spleen**
- **Lung**
- **Kidney**
- **Brain**
- **BM**

Tm (2mg/d) IP injection for 5d, tail-DNA PCR and Western blot done by 3, 7 and 10 days after IP injection finished.

**Tamoxifen inhibits Sirt1 expression ex vivo**

- **Bone marrow from mouse UBC-Cre Slxp+/+ Cre+/- 4 month old; +20ng/ml MCSF ± 4-OH-Tamoxifen**

**Tamoxifen inhibits Sirt1 immunofluorescence ex vivo**

- **Control**
- **Tam-72h**

**Tamoxifen inhibits Sirt1 expression during OCgenesis**

- **TREATMENT**

- **Harvest**

- **OC's**
Research Questions I

- What is the age-related role of Sirt1 in bone?
  - Does this vary depending upon whole body vs. OB or OC specific expression?
- Do RSV and SRT1720 exert similar benefits in different age mice?
- Do RSV and SRT1720 exert similar benefits in Sirt1 knockout mice?
  - Does this vary depending upon whole body vs. OB or OC specific expression?

Research Questions II

What are the impacts of inducible whole-body Sirt1 knockout in vivo on:
  - Muscle function, Metabolism, Renal function, Brain function, GI function
What are the impacts of inducible tissue/organ-specific Sirt1 knockout in vivo?

Healthspan and Aging in the context of Vitamin D insufficiency

Impact of vitamin D insufficiency and aging on fat infiltration of muscle

Physical Performance in an Animal Model of Vitamin D Insufficiency

Hypothesis: low vitamin D ∆ aging ± obesity

Impact of vitamin D insufficiency and aging on fat infiltration of muscle

Specific Aim 1: To establish a model of vitamin D insufficiency in healthy, obese, and aged mice

Specific Aim 2: To determine if vitamin D insufficiency is associated with reduced physical performance, increased inflammation, and/or a decline in mitochondrial biogenesis

1000 U Vit D₃/kg chow/day yields replete mice regardless of age

- **YOUNG**
  - Age: 6 months
  - Strain: C57/Bl6 Mouse
  - Diet: Standard (~4 IU Vitamin D₃/day)
  - Weight: 32.4 ± 1.5 g
  - 25-OH Vit D: 37.2 ± 4.1 ng/ml

- **OLD**
  - Age: 22 months
  - Strain: C57/Bl6 Mouse
  - Diet: Standard (~4 IU Vitamin D₃/day)
  - Weight: 36.7 ± 2.7 g
  - 25-OH Vit D: 34.2 ± 1.3 ng/ml
**Young and old mice have similar body composition**

Mice were subjected to DEXA, and percentages of lean and fat tissue were assessed (n=7, *p=0.0497). Bone mineral density was also measured: young (0.051 ± 0.003 g/cm³) and old (0.052 ± 0.002 g/cm³) were not statistically different.

**Old Mice have worse rotarod performance than young mice**

Rotarod performance was the average time before falling of 3 trials whereby speeds increase from 4-40 RPM over 5 minutes (n=7, ***p<0.0001).

**25-OH vitamin D levels respond quickly to changes in cholecalciferol supplementation**

C57BL/6 mice (6 mo.) were fed standard diet containing either 125 IU or 1000 IU cholecalciferol / kg chow, or switched from 125 IU → 1000 IU chow after 8 weeks. Serum 25OH vitamin D levels were measured every two weeks using ELISA (n=6 1000IU, 10 125IU, 11 125/1000IU, ± stdev).

**Vitamin D insufficient mice have increased total fat, body weight**

Mice were weighed before and after receiving 125 IU or 1000 IU cholecalciferol / kg of chow for 4 months, after which, body fat percentage was measured using DEXA (n=6 1000IU, n=10 125IU, ± stdev, body weight *p=0.0023, body fat % *p=0.0191).

**Vitamin D insufficiency does not impact rotarod performance in young mice**

Mice were fed a diet containing either 125, 1000 or 125→1000 IU cholecalciferol / kg chow. Data represent the mean of three trials, the rotarod accelerated from 4-40 RPM over 5 minutes (n=6-1000IU, 10-125 IU, 11-RE1000IU, ± stdev).
**Impact of vitamin D insufficiency and aging on fat infiltration of muscle**

**Specific Aims:**

1. Assess the impact of vitamin D insufficiency on fat composition and physical performance in the context of aging and/or obesity.

2. Identify the influence of vitamin D on the cytokine/adipokine expression of adipocytes.

**Vitamin D insufficiency appears to promote higher levels of TNF-α and IL-6 in epididymal fat**

Male mice (age 6 mo.) were fed diets containing either 125 IU or 1000 IU cholecalciferol / kg chow for 4 months and then sacrificed. Protein extracts were derived from epididymal fat pads and assessed for total TNF-α and IL-6 levels using ELISA (n=3, ± stdev).

**Mice on a high fat diet exhibit poorer rotarod performance**

Male mice (age 6 mo.) were fed diets containing either LFD or HFD for 4 months and then performance assessed. N=28 LFD, 24=HFD, **p<.0001**
**Frailty Pathways**

- inactivity, obesity
- TOR, angiotensin
- inflammation
- DNA damage, ROS, catabolism

↑ diseases → ↓ cell function, survival, regeneration → ↓ homeostasis → dependence & frailty

Troen 2007

**Frailty ➔ Healthspan**

- inactivity, obesity
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Troen 2007

**Healthier Aging Pathways**

- resveratrol, sirtuins
- TOR, angiotensin
- inflammation
- DNA damage, ROS, catabolism

↓ diseases → ↑ cell function, survival, regeneration → ↑ homeostasis → independence & quality of life

Troen 2007

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Troen 2007