Regulation of apoptotic signaling by lifelong caloric restriction

Emanuele Marzetti, MD, PhD

Postdoctoral Associate
Biology of Aging Laboratory
Department of Aging and Geriatric Research
Institute on Aging
University of Florida
Gainesville (FL)
Sarcopenia and frailty in older adults
Time course of muscle loss

Prevalence of sarcopenia in older adults

Sarcopenia and frailty in older adults

Sarcopenia and frailty in older adults

- Muscular strength decline is a powerful predictor of disability and all-cause mortality even in healthy elderly.
- The estimated healthcare cost attributable to sarcopenia in the US in 2000 was US$ 18.5 billions (1.5% of total healthcare expenditure).
Impact of sarcopenia in older adults

Prevalence of functional limitations 25 years after grip strength was tested in a cohort of initially healthy men.

Rantanen T, et al. JAMA 1999
Impact of sarcopenia in older adults

All-cause mortality according to grip-strength tertiles and BMI over a 30 years follow-up in initially healthy men.

Mechanisms underlying sarcopenia

Mechanisms underlying sarcopenia

- Progressive decrease in muscle fiber size and number, with a preferential loss of type II (fast twitch) fibers.
- At a cellular level, apoptosis may represent the key mechanism driving the onset and progression of muscle wasting.

Siu PM et al. Am J Physiol Regul Integr Comp Physiol 2005
Song W, Kwak HB, Lawler JM. Antioxid Redox Signal 2006
Apoptosis in skeletal muscle
Simplified signaling pathways of apoptosis
Caloric restriction (CR) is the only non-genetic intervention that has consistently shown to slow the intrinsic rate of aging in mammals.

CR attenuates the rate of functional decline and the loss of muscle fibers that occur with age.

Preservation of skeletal muscle mass and function likely results from the effects of CR on the apoptotic potential.
Effects of caloric restriction on aged skeletal muscle

- CR effects on skeletal muscle homeostasis vary depending on fiber type composition.
- Type II fibers are far more benefited from CR than type I, slow-twitch fibers.
- CR is able to counteract the majority of muscle apoptotic pathways.
Effects of age and CR on muscle mass

<table>
<thead>
<tr>
<th></th>
<th>6 AL (n=8)</th>
<th>26 AL (n=8)</th>
<th>26 CR (n=8)</th>
<th>% change 26 AL vs. 6 AL</th>
<th>% change 26 CR vs. 26 AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>363.4 ± 4.0</td>
<td>413.6 ± 5.4*</td>
<td>307.1 ± 2.3</td>
<td>↑13%</td>
<td>↓35%</td>
</tr>
<tr>
<td>Soleus weight (g)</td>
<td>0.147 ± 0.004</td>
<td>0.146 ± 0.005**</td>
<td>0.109 ± 0.003</td>
<td>no change</td>
<td>↓33%</td>
</tr>
<tr>
<td>SW/BW</td>
<td>0.399 ± 0.006</td>
<td>0.345 ± 0.011***</td>
<td>0.355 ± 0.011</td>
<td>↓15%</td>
<td>no change</td>
</tr>
<tr>
<td>SVL weight (g)</td>
<td>0.419 ± 0.003</td>
<td>0.364 ± 0.005*</td>
<td>0.332 ± 0.006</td>
<td>↓15%</td>
<td>↓9%</td>
</tr>
<tr>
<td>SVLW/BW</td>
<td>1.126 ± 0.021</td>
<td>0.850 ± 0.013#</td>
<td>1.089 ± 0.022</td>
<td>↓32%</td>
<td>↑28%</td>
</tr>
</tbody>
</table>

Animals are 6-month and 26-month-old ad libitum fed (AL) and 26-month-old calorie-restricted (CR) male Fischer 344 rats.

*P < 0.001, 26AL vs. 6AL and 26CR; **P < 0.001, 26AL vs. 26CR; ***P = 0.0205, 26AL vs. 6AL; #P < 0.0001, 26AL vs. 6AL and 26CR.

Phillips T, Leeuwenburgh C. FASEB J 2005
Fiber number in soleus and SVL

*P<0.05 26 AL vs. 6 AL; §P<0.05 26 AL vs. 6 AL and 26 CR.

Phillips T, Leeuwenburgh C. FASEB J 2005
Fiber CSA in soleus and SVL

*P<0.05 26 AL vs. 6 AL, **P<0.05 26 AL vs. 6 AL and 26 CR.

Phillips T, Leeuwenburgh C. FASEB J 2005
DNA fragmentation in soleus and SVL

DNA fragmentation in SVL (A) and soleus muscle (B) assessed by PCR.

Phillips T, Leeuwenburgh C. FASEB J 2005
Simplified scheme of receptor-mediated apoptosis

TNF-α

TNFR1

TRADD

FADD

Caspase-8

Caspase-3

APOPTOSIS
Effects of age and CR on TNF-α signaling

*P < 0.0001 26 AL vs. 6 AL; **P < 0.0001 26 CR vs. 26 AL.

Phillips T, Leeuwenburgh C. FASEB J 2005
## Effects of age and CR on TNF-α signaling

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
<th></th>
<th>SVL</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>CR</td>
<td>Age</td>
<td>CR</td>
</tr>
<tr>
<td>TNF-α content</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>TNF-R1 content (cytosol)</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>IKKy content</td>
<td>↑</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>IkB-α</td>
<td>↑</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>p65 content</td>
<td>↑</td>
<td>↓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NF-kB binding activity *</td>
<td>↔</td>
<td>↔</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>FADD content</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Caspase-8 content</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

Phillips T, Leeuwenburgh C. FASEB J 2005
TNF-α is released and binds to TNFR1. This activates a series of events leading to apoptosis.

**Type I fiber**

1. TNF-α binds to TNFR1.
2. TRADD and TRAF2 are recruited.
3. RIP1 is activated.
4. NF-κB is activated, leading to transcription of anti-apoptotic proteins (i.e., cFLIP, IAP).
5. Caspase-8 is activated.
6. Apoptosis.

**Type II fiber**

1. TNF-α binds to TNFR1.
2. TRADD and TRAF2 are recruited.
3. FADD is recruited.
4. Caspase-8 is activated.
5. Apoptosis.
6. Caspase-8 activates CR, leading to apoptosis.

Note: CR likely represents a caspase inhibitor or regulator.
Protein content of procaspase-3, cleaved caspase-3, X-linked inhibitor of apoptosis (XIAP), and enzymatic activity of caspase-3 in the gastrocnemius muscle of 12-mo-old ad libitum fed (12AL, n = 11), 26-mo-old ad libitum fed (26AL, n = 8), and 26-mo-old calorie restricted (26CR, n = 9) male Fischer 344 rats. * P<0.005 26 AL vs. 26 CR.

Mechanisms of mitochondrial-mediated cell death

1. Initiation
   - BAD
   - BIM
   - BAX
   - BAK
   - BCL-XL
   - BCL-2
   - VDAC
   - PT
   - ARC
   - Apoptosome
   - ATP
   - Apaf-1
   - Caspase-9
   - Pro-Caspase-3
   - Caspase-3
   - INHIBITORS OF APOPTOSIS
   - IAPs, i.e. XIAP
   - APOPTOSIS
   - Nuclear Fragmentation

2. Amplification
   - EndoG
   - AIF
   - Nuclear Fragmentation
CR effects on mitochondrial-mediated apoptosis in gastrocnemius muscle

Cytochrome c: *p = 0.03, 12AL vs. 26AL; non significant 26AL vs. 26CR. Apaf-1: *p = 0.03, 12AL vs. 26AL, and **p = 0.001, 26AL vs. 26CR. Procaspase-9 and cleaved caspase-9: non significantly different across groups.

ARC inhibits PTP opening and cytochrome c release
CR modulates subcellular ARC distribution

*P = 0.008, 12AL vs. 26AL; **P < 0.05, 26AL vs. 26CR.

ARC inhibits PTP opening and cytochrome c release
Mechanisms of mitochondrial-mediated cell death
CR counteracts mitochondrial-mediated apoptosis in plantaris muscle

Total and nuclear apoptosis-inducing factor (AIF) levels in the plantaris muscle of 12AL, 26AL, and 26CR male Fischer 344 rats. Total AIF: *p = 0.002, 12AL vs. 26AL, and **p = 0.015, 26AL vs. 26CR. Nuclear AIF: non significantly different across groups.

Modulation of mitochondrial mediated apoptosis by CR

- Aged muscle fibers often display regions harboring ETC abnormalities (i.e., COX\(^{-}\)/SDH\(^{+}\)) that are more likely to undergo apoptosis.
- CR has been shown to reduce the incidence of these abnormalities, thus attenuating the apoptotic potential.
Atrophy is concomitant with ETS abnormality in a rectus femoris fiber from a 38-month-old rat.

Wanagat J et al. FASEB J 2001
CR reduces muscle ETC abnormalities

COX: $P = 0.004$, CR35 vs. CR50; SDH: $P = 0.001$, CR35 vs. CR50; both: $P = 0.012$ CR35 vs. CR50.

Aspnes LE et al. FASEB J 1997
mtDNA deletions with age and CR

Aspnes LE et al. FASEB J 1997
CR reduces mtDNA oxidative damage

8-oxo-7,8-dihydro-2'-deoxyguanosine levels in gastrocnemius muscle mtDNA. P < 0.01 26 CR vs. 26 AL.

Modulation of ER-mediated apoptosis by CR

- Aged skeletal muscle often displays ER stress, which may be due to membrane proteins oxidative damage (Ca$^{2+}$ ATPase).
- Calcium imbalance may also result from mitochondrial inability to properly sequester cytosolic calcium.
- ER stress is able to trigger apoptosis through activation of caspase-12, which initiates the caspase cascade.
Signaling pathway of ER-mediated apoptosis

ENDOPLASMIC RETICULUM STRESS

$[\uparrow \text{Ca}^{2+}]$ m-calpain

Caspase-12

Caspase-3

APOPTOSIS
CR counteracts ER-mediated apoptosis in gastrocnemius muscle

Procaspase-12: *p = 0.0002, 12AL vs. 26AL and **p = 0.006, 26AL vs. 26CR. Cleaved caspase-12: non significant 12AL vs. 26AL, and **p = 0.04, 26AL vs. 26CR.

Functional implications of muscle apoptosis modulation by CR

- CR delays and reduces the age-related muscle loss.
- Conservation of adequate muscle mass at old age assures preservation of physical performance and prevents the onset of physical disability.
CR attenuates age-related loss of muscle strength in EDL muscle

Young AL (n = 14, 12 mo), old AL (n = 10, 26–28 mo), and old CR (n = 10, 27–28 mo) male Fischer 344 rats. Aging decreased force-to-body mass ratio and muscle specific force in the EDL muscle (*P < 0.05, Old AL vs. Young AL), whereas CR increased both (P < 0.05, Old-CR vs. Old-AL).

To what extent is CR applicable to humans?

- 30-40% CR implies a dramatic decrease in food intake that is difficult to hypothesize for humans, especially at old age.
- However, even a slight CR might be effective in counteracting age-related muscle loss.
Slight CR and voluntary exercise counteract muscle loss via apoptosis

Cytosolic mono- and oligo-nucleosomes in plantaris and soleus muscles of young ad libitum fed (6 AL), old ad libitum fed (24 AL), old 8% calorie restricted (24 CR), and old 8% calorie restricted exercised Fisher 344 male rats (n = 6-9/group). p < 0.05 (*); p < 0.01 (**); p < 0.001 (***) versus 24 AL.

Seo AY. Unpublished data
Sarcopenia, frailty and disability in older adults. What is missing?

• Research on animal models supports the key role of deregulated apoptosis in muscle mass and strength loss with age.
• ... but evidence in humans is still desolately lacking.
• Hence, there is a clear need to move a step forward, in order to identify potential strategies (slight CR? physical exercise? others?) to counteract the overwhelming and ever-growing prevalence of physical disability in older populations.
Pilot study on apoptosis, sarcopenia and frailty

Study objectives

1. Changes of skeletal muscle apoptosis potential with age.
2. Extent of skeletal muscle apoptosis in non-frail and frail elderly subjects.
Simplified signaling pathways of apoptosis

- Death Domain Receptor Signaling (TNF-α with TNFR1 and TNFR2)
- Withdrawal of Growth and Survival Factor
- Endoplasmic Reticulum Signaling Pathway
  - ER Stress
  - [Ca^{2+}]
  - Caspase-7
  - Caspase-12
  - Caspase-4
- Caspase-8, -10
- Caspase-9
- Inhibitors of Apoptosis (cIAP1, cIAP2, XIAP)
- NF-κB
- Mitochondrial Signaling Pathways
  - Bcl-XL
  - Bcl-2
  - Cyto C
  - Apaf1
  - Caspase-9
  - Apoptosis
  - Nuclear Fragmentation
  - Nuclear Cell Death

APOPTOSIS
Pilot study on apoptosis, sarcopenia and frailty

Schematic study design

Young
- Muscle strength tests
- Muscle biopsy (apoptosis extent)
- MRI

Old, non-frail
- Muscle strength tests
- Muscle biopsy (apoptosis extent, apoptosis pathways)
- MRI

Old, frail
- SPPB
- Muscle strength tests
- Muscle biopsy (apoptosis extent, apoptosis pathways)
- MRI
Conclusive remarks

• Sarcopenia of aging is associated with considerable disability and mortality, and is a major modifiable risk factors for frailty.
• Accelerated apoptosis of muscle fibers might represent a key mechanism underlying sarcopenia.
• CR is effective in counteracting age-related muscle loss in animal models.
• A greater effort is needed to identify key cellular mechanisms leading to sarcopenia in humans.
ACKNOWLEDGEMENTS

Department of Aging and Geriatric Research and the UF Institute on Aging

Division of Biology of Aging
Biochemistry of Aging Lab
Arnold Seo (S. Korea-USA)
Asimina Hiona (Greece)
Rizwan Kalani (USA)
Shashank Upadhyay (USA)
Stephane Servais (France)
Stephanie Wohlgemuth (Germany)
Tim Hofer (Sweden)
Alberto Sanz (Spain)
Amie Dirks (USA)
Barry Drew (USA)
Colin Selman (Scotland)
Evelyn Kouwenhoven (Netherlands)
Rajani Shelke (India)
Ricardo Gredilla (Spain)
Sharon Judge (USA)
Tracey Philips (Scotland)
Young Mok Jang (S. Korea)

Collaborators
Tomas Prolla (USA)
David Julian (USA)
Gustavo Barja (Spain)
Esther Dupont-Versteegden (USA)
Marco Pahor (USA)
Christy Carter (USA)
Matteo Cesari (Italy)
Irene Mangani (Italy)
Cinzia Maraldi (Italy)
Angela Lezza (Italy)
Nicola Maria Gadaletta (Italy)
Tilman Grune (Germany)
Bill Dunn (USA)
John Aris (USA)
John Speakman (UK)
Tory Hagen (USA)
Shuji Oh-ishi (Japan)

Funding Provided by:
National Institute on Aging
National Institute of Health
American Heart Association
Society of Geriatric Cardiology

The End