Oxidative stress, antioxidant intervention and sarcopenia

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Body composition changes and aging

Age (years)

Body composition

Non-muscle
FFM
Fat
Muscle

Skeletal Muscle—Single Muscle Fiber

- Endomysium
- Striations
- Sarcolemma
- Sarcoplasm
- Filaments
- Nuclei
- Myofibrils
Sarcopenia: Definition, Epidemiology, and Pathophysiology

J Bone Metab 2013;20:1-10

Diagram:
- **Sarcopenia**
  - **Endocrine**
    - Corticosteroids, GH, IGF-1
    - Abnormal thyroid function
    - Insulin resistance
  - **Age-related (Primary)**
    - Sex hormones
    - Apoptosis
    - Mitochondrial dysfunction
  - **Neuro-degenerative diseases**
    - Motor neuron loss
  - **Inadequate nutrition / Malabsorption**
  - **Disuse**
    - Immobility
    - Physical inactivity
    - Zero gravity
  - **Cachexia**
Sarcopenia: Definition, Epidemiology, and Pathophysiology

J Bone Metab 2013;20:1-10

Diagram:
- Skeletal muscle
  - "Myokines"
- Adiposity
  - "Adipokines"
- Cardiovascular & metabolic diseases
### Adipo-Myokines: Two Sides of the Same Coin—Mediators of Inflammation and Mediators of Exercise

#### Myokines
- BDNF [135]
- IL-7 [138, 139]
- IL-15 [46, 138]
- Irisin
- LIF
- Myonectin [86]
- Secretome studies [25–29, 147, 148]

#### Adipo-myokines
- ANGPTL4 [133, 134]
- FGF21 [137]
- Fstl1 [111]
- IL-6 [138]
- IL-8 [140]
- MCP-1 [12]
- Myostatin (Table 3)
- PAI-1 [142]
- PEDF [141]
- VEGF (Table 3)

#### Adipokines
- Adiponectin [143, 144]
- Leptin [146]
- Resistin [145]
- Secretome studies [149–156]
Is there skeletal muscle fiber loss with age in humans and animals?


A partial list of mechanisms/consequences of sarcopenia.

- Cell
  - Declines in neuron number and conduction velocity
  - Muscle fiber changes
  - Reduced excitation-contraction coupling

- Tissues
  - Oxidative damage
  - Reduced satellite cell activation/proliferation

- Organs
  - Reduced expression of contractile protein genes
  - Reduced translation of contractile protein mRNAs

- Whole body
  - Changes in muscle metabolism
  - Increased cytokines
  - Endocrine changes and/or reduced tissue response to hormones

- Behavior
  - Reduced response to nutrients and/or malnutrition
  - Inactivity

Aging

Oxidative stress

Inflammation

Changes in body composition
Two-year changes in fat-free mass (FFM) by IL-6 quartiles in the Framingham Heart Study

Adjusted for sociodemographics, comorbidities, biological markers and medications
Hazard ratios (95% CI)

MOBILITY LIMITATION

- Normal oxLDL/LDL and IL-6
  - N=1,336
- High oxLDL/LDL only
  - N=567
- High IL-6 only
  - N=572
- High oxLDL/LDL and IL-6
  - N=379
Mitochondrial apoptotic signaling is elevated in rat gastrocnemius muscle at advanced age, likely contributing to the age-related muscle loss. Activation of mitochondrial apoptotic signaling may be due to modification of the Bcl-2 proteins pattern of expression, possibly supported by enhanced levels of oxidative and nitrosative stress.
This study shows for the first time that apoptotic signaling is correlated with indices of sarcopenia (i.e., decreased muscle mass and function) in a cohort of relatively healthy, community-dwelling older persons.
Oxidative damage, mtDNA deletions, and ETC abnormalities are colocalized along a single muscle fiber and exhibit atrophy.

Wanagat et al. 2001. Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. Faseb J. 15:322-32

### Associations of specific dietary factors with muscle mass and function

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Evidence</th>
<th>Some open questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Inadequate intake may accelerate the loss of lean mass (43)</td>
<td>Potential attenuation of the loss of lean mass with higher protein intakes?</td>
</tr>
<tr>
<td></td>
<td>Protein anabolism can be stimulated by increased essential amino acid availability (44)</td>
<td>Optimal level and type of proteins?</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Low serum vitamin D has been associated with poor and decreasing muscle strength (45)</td>
<td>Observational studies are inconsistent (47)</td>
</tr>
<tr>
<td></td>
<td>Vitamin D supplementation trial showed a substantial increase in quadriceps strength and functional performance in older persons (46)</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>Low serum magnesium has been associated with reduced muscle strength (48)</td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Low serum carotenoids have been associated with poorer muscle strength in older adults (49)</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>Low plasma selenium has been associated with poorer muscle strength in older adults (50)</td>
<td></td>
</tr>
</tbody>
</table>
Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants

Jeong-Su Kim*, Jacob M. Wilson, Sang-Rok Lee

Department of Nutrition, Food and Exercise Sciences, College of Human Sciences, The Florida State University, Tallahassee, FL 32306-1493, USA
Review Article

Rationale for Antioxidant Supplementation in Sarcopenia

Francesco Cerullo,¹ Giovanni Gambassi,¹ and Matteo Cesari²
Antioxidant Supplementation Restores Defective Leucine Stimulation of Protein Synthesis in Skeletal Muscle from Old Rats

Barbara Marzani, Michèle Balage, Annie Vénien, Thierry Astruc, Isabelle Papet, Dominique Dardevet, and Laurent Mosoni

INRA, Centre de Clermont-Ferrand-Theix, UMR 1019, Unité Nutrition Humaine, Saint-Genès-Champanelle, F-63122 and Univ Clermont 1, UFR Médecine, UMR 1019, Unité Nutrition Humaine, Clermont-Ferrand, F-63001 France and INRA, UR 370 QuaPA, F-63122 Saint-Genès-Champanelle, France

Aging is characterized by a progressive loss of muscle mass that could be partly explained by a defect in the anabolic effect of food intake. We previously reported that this defect resulted from a decrease in the protein synthesis response to leucine in muscles from old rats. Because aging is associated with changes in oxidative status, we hypothesized that reactive oxygen species–induced oxidative damage may be involved in the impairment of the anabolic effect of leucine with age. The present study assessed the effect of antioxidant supplementation on leucine-regulated protein metabolism in muscles from adult and old rats. Four groups of 8- and 20-mo-old male rats were supplemented or not for 7 wk with an antioxidant mixture containing rutin, vitamin E, vitamin A, zinc, and selenium. At the end of supplementation, muscle protein metabolism was examined in vitro using epitrochlearis muscles incubated with increasing leucine concentrations. In old rats, the ability of leucine to stimulate muscle protein synthesis was significantly decreased compared with adults. This defect was reversed when old rats were supplemented with antioxidants. It was unrelated to increased oxidative damage to 70-kDa ribosomal protein S6 kinase that is involved in amino acid signaling. These effects could be mediated through a reduction in the inflammatory state, which decreased with antioxidant supplementation. Antioxidant supplementation could benefit muscle protein metabolism during aging, but further studies are needed to determine the mechanism involved and to establish if it could be a useful nutritional tool to slow down sarcopenia with longer supplementation. J. Nutr. 138: 2205–2211, 2008.
### TABLE 2

Body, skeletal muscle, and organ weights in adult and old rats supplemented (Aox+) or not (Aox−) with antioxidants

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th></th>
<th>Dld</th>
<th></th>
<th>2-Way ANOVA²</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Aox−</td>
<td>Aox+</td>
<td>Aox−</td>
<td>Aox+</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>692 ± 11</td>
<td>704 ± 12</td>
<td>675 ± 24</td>
<td>681 ± 23</td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius, g</td>
<td>5.96 ± 0.15</td>
<td>6.11 ± 0.16</td>
<td>4.95 ± 0.08</td>
<td>5.20 ± 0.11</td>
<td>A</td>
</tr>
<tr>
<td>Tibialis anterior, g</td>
<td>1.98 ± 0.05</td>
<td>2.04 ± 0.06</td>
<td>1.72 ± 0.06</td>
<td>1.81 ± 0.05</td>
<td>A</td>
</tr>
<tr>
<td>EDL, mg</td>
<td>510 ± 14</td>
<td>508 ± 12</td>
<td>439 ± 11</td>
<td>458 ± 10</td>
<td>A</td>
</tr>
<tr>
<td>Soleus, mg</td>
<td>415 ± 11</td>
<td>433 ± 13</td>
<td>360 ± 15</td>
<td>371 ± 18</td>
<td>A</td>
</tr>
<tr>
<td>Heart, g</td>
<td>1.69 ± 0.02</td>
<td>1.60 ± 0.03</td>
<td>1.78 ± 0.09</td>
<td>1.59 ± 0.07</td>
<td>D</td>
</tr>
<tr>
<td>Liver, g</td>
<td>16.9 ± 0.5</td>
<td>16.9 ± 0.4</td>
<td>16.0 ± 0.8</td>
<td>16.1 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Spleen, g</td>
<td>1.18 ± 0.05</td>
<td>1.10 ± 0.03</td>
<td>1.59 ± 0.08</td>
<td>1.42 ± 0.05</td>
<td>A, D</td>
</tr>
<tr>
<td>Kidneys, g</td>
<td>3.42 ± 0.10</td>
<td>3.17 ± 0.07</td>
<td>4.94 ± 0.70</td>
<td>4.05 ± 0.39</td>
<td>A</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM, n = 10–12.
² A: Significant effect of age, *P* < 0.005; D: significant effect of diet, *P* < 0.05.
**FIGURE 2** Dose-response curves for leucine stimulated muscle protein synthesis in adult (A) and old (O) rats supplemented (Aox+) or not (Aox−) with antioxidants for 7 wk. Rate of protein synthesis was measured as in vitro incorporation of $^{14}$C phenylalanine into epitrochlearis muscles in the presence of increasing leucine concentrations.

*Oxidation state of S6K.* The carbonyl content, a marker of oxidative damages, detected by DNPH associated to S6K (expressed as a ratio to total S6K) was similar in adult and old rats and did not change by Aox supplementation (data not shown).
Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse

The main purpose of this study was to investigate whether consumption of diets enriched in antioxidants attenuates the level of oxidative stress in the senescence-accelerated mouse (SAM). In separate and independent studies, two different dietary mixtures, one enriched with vitamin E, vitamin C, L-carnitine, and lipoic acid (Diet I) and another diet including vitamins E and C and 13 additional ingredients containing micronutrients with bioflavonoids, polyphenols, and carotenoids (Diet II), were fed for 8 and 10 months, respectively. The amounts of glutathione (GSH) and glutathione disulfides (GSSG) and GSH:GSSG ratios were determined in plasma, tissue homogenates, and mitochondria isolated from five different tissues of SAM (P8) mice. Both diets had a reductive effect in plasma; however, Diet I had relatively little effect on the glutathione redox status in tissue homogenates or mitochondria. Remarkably, Diet II caused a large increase in the amount of glutathione and a marked reductive shift in glutathione redox state in mitochondria. Overall, the effects of Diet II were tissue and gender specific. Results indicated that the glutathione redox state in mitochondria and tissues can be altered by supplemental intake of a relatively complex mixture of dietary antioxidants that contains substances known to induce phase 2 enzymes, glutathione, and antioxidant defenses. Whether corresponding attenuations occur in age-associated deleterious changes in physiological functions or life span remains unknown.
Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Diet I</th>
<th>Diet II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>170</td>
<td>170</td>
<td>190</td>
</tr>
<tr>
<td>Fat</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>200 ppm$^b$</td>
<td>500 ppm$^b$</td>
<td>500 ppm$^b$</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>&lt;32 ppm</td>
<td>80 ppm$^b$</td>
<td>80 ppm$^b$</td>
</tr>
<tr>
<td>L-Carnitine</td>
<td>10 ppm$^b$</td>
<td>300 ppm$^b$</td>
<td>–</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>–</td>
<td>125 ppm</td>
<td>–</td>
</tr>
<tr>
<td>±Broccoli$^c$</td>
<td>–</td>
<td>–</td>
<td>15</td>
</tr>
<tr>
<td>±Rice bran$^c$</td>
<td>–</td>
<td>–</td>
<td>10</td>
</tr>
<tr>
<td>Marine oil</td>
<td>–</td>
<td>–</td>
<td>8.8</td>
</tr>
<tr>
<td>Glutamine dipeptide$^d$</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Methionine$^d$</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
</tr>
<tr>
<td>±Selenium-yeast$^d$</td>
<td>–</td>
<td>–</td>
<td>0.3</td>
</tr>
<tr>
<td>±Algae$^d$</td>
<td>–</td>
<td>–</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Threonine$^d$</td>
<td>–</td>
<td>–</td>
<td>0.25</td>
</tr>
<tr>
<td>Lutein (5%)</td>
<td>–</td>
<td>–</td>
<td>0.15</td>
</tr>
<tr>
<td>Lycopene (5%)</td>
<td>–</td>
<td>–</td>
<td>0.15</td>
</tr>
<tr>
<td>Astaxanthin (8%)</td>
<td>–</td>
<td>–</td>
<td>0.094</td>
</tr>
<tr>
<td>β-Carotene (10%)</td>
<td>–</td>
<td>–</td>
<td>0.075</td>
</tr>
<tr>
<td>Curcumin</td>
<td>–</td>
<td>–</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse

A  Diet I

B  Diet II

Redox Potential (mV)

Liver  Kidney  Heart  Brain  Skel. muscle
Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats

**Conclusion**

The increased levels of endogenous antioxidant enzymes after Vitamin E and C supplementation appear to be regulated by post-transcriptional modifications that are affected differently by age, exercise, and supplementation. These data suggest that antioxidant supplementation improves indices of oxidative stress associated with repetitive loading exercise and aging and improve the positive work output of muscles in aged rodents.
Antioxidant SkQ1 delays sarcopenia-associated damage of mitochondrial ultrastructure

Statistic analysis of the volume occupied by the skeletal muscle mitochondria

mitochondria-targeted antioxidant SkQ1[10-(6'-plastoquinonyl) decyltriphenylphosphonium]
A fragment of the muscle fiber from a 24-month-old Wistar rat treated with SkQ1. Subsarcolemmal population of mitochondria (1) and interfibrillar stretched mitochondria (2).
Effects of Resveratrol on the Recovery of Muscle Mass Following Disuse in the Plantaris Muscle of Aged Rats

The data show that resveratrol supplementation improved muscle mass during reloading after hindlimb suspension. Although resveratrol did not prevent fiber atrophy during the period of disuse, it increased the fiber cross sectional area of type IIA and IIB fibers in response to reloading.
Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia.

**O2 levels in aging skeletal muscle, by histological DHE examination**

![Bar graph showing DHE fluorescence area by age (months)]

**Enzyme activity and hydrogen peroxide levels in aging skeletal muscle**

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Age (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 (n = 16)</td>
</tr>
<tr>
<td>SOD1 (U/mg protein)</td>
<td>19.4 ± 1.3</td>
</tr>
<tr>
<td>SOD2 (U/mg protein)</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Catalase (U/mg protein)</td>
<td>9.3 ± 0.9</td>
</tr>
<tr>
<td>Hydrogen Peroxide (μM)</td>
<td>1.12 ± 0.07</td>
</tr>
<tr>
<td>Superoxide (AFU)</td>
<td>8.1 ± 1.3</td>
</tr>
</tbody>
</table>

**Gene expression**
- **++ +**
- **++**
- **++**
- **+++**
The impact of mechanical ventilation and antioxidant administration (Trolox) on cross-sectional areas (CSA) of different muscle fibres (type I, IIa and IIb/x)
Figure 4. The impact of 8 (T8) and 35 (T35) days of bed rest on cross-sectional area (CSA) of muscle fibres and on protein oxidation (Oxy/RP) of muscle samples from the vastus lateralis muscle of humans. 

A, mean values of CSA of muscle fibres before bed rest (T0) and at T8 and T35. B, protein oxidation index (Oxy/RP). *Significantly different from T0 (P < 0.05). C, regression analysis of normalized values of muscle protein oxidation (Oxy/RP) plotted against the percentage change of fibre CSA of the same muscles, determined at T8 and T35; the slope of the line was significantly different from zero (P < 0.05), reprinted from Dalla Libera et al 2009 used with permission from The American Physiological Society.
Alterations of redox homeostasis through muscles, species and models

<table>
<thead>
<tr>
<th>muscle</th>
<th>VL</th>
<th>gas</th>
<th>sol</th>
<th>sol</th>
<th>dia</th>
</tr>
</thead>
<tbody>
<tr>
<td>species</td>
<td>humans</td>
<td>rat &amp; mice</td>
<td>rat &amp; mice</td>
<td>rat &amp; mice</td>
<td>rat &amp; mice humans</td>
</tr>
<tr>
<td>model</td>
<td>BR, imm., ULLS</td>
<td>HU</td>
<td>HU</td>
<td>imm.</td>
<td>MV</td>
</tr>
<tr>
<td>rate of atrophy</td>
<td>5-25% in 23 w</td>
<td>11% in 14d</td>
<td>24% in 14d</td>
<td>50% in 8d</td>
<td>In 15-18% 18h</td>
</tr>
</tbody>
</table>

- Slow phenotype
- Rate of oxidative metabolism
- Relative decrease in load & in neuromuscular activity
- Extent of ROS production
- Rate of increased proteolysis due to large scale oxidative effect

Less determinant → More determinant
Sarcopenia With Limited Mobility: An International Consensus

John E. Morley, MB, BCh, Angela Marie Abbatecola, BS, MD, PhD, Josep M. Argiles, PhD, Vickie Baracos, BSc, PhD, Juergen Bauer, MD, PhD, Shalender Bhasin, MD, Tommy Cedernolm, MD, PhD, Andrew J. Stewart Coats, DM, DSc, Steven R. Cumming, PhD, Jack M. Guralnik, PhD, FAAP, FACP, FAAN, Anne B. Newman, PhD, Ronnen Roubenoff, MD, Bruno Vellas, MD, PhD, Roger A. Fielding, PhD, Bahar Zadeh, MD, PhD, MPH, Andrea D. Díaz-Cardelino, MD, Ph D, FESC, Francesco Fabbri, MD, PhD, FESC, Omar Elahi, MD, PhD, D. Anker, MD, PhD, THE

2011; 12: 403–409
Lifestyle and Advanced Glycation End Products (AGEs) Burden: Its Relevance to Healthy Aging

Chandan Prasad1*, Victorine Imrhan1, Francesco Marotta2, Shanil Juma1 and Parakat Vijayagopal1

Aging & Disease, June 2014

Diagram:
- Aging → Oxidants (ROS ↑, RNS ↑, Others ↑ (e.g. AGE)) → Oxidative Damage (DN, RN, Protein, Lipids) → Altered Gene Expression, Compromised DNA Repair, Membrane Alteration, Dysfunctional Mitochondria, Dysfunctional Proteins → Age Related Diseases
- Lifestyle (Diet, Exercise, Smoking, Health Management) → Antioxidants
With Sarcopenic Obesity increases in Intermuscular Fat

and Intramuscular fat: Low Density Lean Tissue by CT
Prevalence of obesity, sarcopenia, and sarcopenic-obesity according to age

Baumgartner RN Ann NY Acad Sci 2000;904:437-448