Overview

- Silver tsunami
- Skeletal muscle
  - Protein synthesis—blunted anabolic response
  - Heightened inflammation
- Challenges faced
  - Poorly defined therapies to attenuate muscle deterioration
  - Poor understanding of etiology
- A new therapy?

The “Graying” of society: Global

Life expectancy at birth (2013) 78.8 years

- Chronic disease
  - Impaired Life 12.7 y
- Acute illness
- Healthy Life 66.1 y
“...the prevalence of health problems increases with age while people become increasingly dependent on healthcare and community support to survive.”

**Skeletal muscle**
- Skeletal muscle is the largest organ (system)
  - ~45% of body weight (BW) in young men
  - ~35% of BW in young women
  - 25-30% of BW by 70 y (**sarcopenia**)
- Functions
  - Strength, power, endurance
  - Skeleton support
  - Metabolic processes
  - Endocrine functions

**Age-related change in thigh muscle and fat masses in BMI and sex matched adults**

**Etiology**

Skeletal muscle deterioration
Pathological remodeling
Metabolic disease
Loss of independence
**Blunted anabolic response in older human skeletal muscle**

- Anabolic response in older adult skeletal muscle can be stimulated with:
  - Large bolus of dietary protein
  - Greater amounts of essential amino acids
  - Greater amounts of leucine

**Skeletal muscle gene expression profile reflects an accommodative response to dietary protein in old males**

<table>
<thead>
<tr>
<th>Dietary Protein Intake</th>
<th>Mean transcript level (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPro = 0.50, MPro = 0.75, HPro=1.00 g•kg⁻¹•d⁻¹</td>
<td>Young vs Old</td>
</tr>
</tbody>
</table>

**Older adult skeletal muscle has heightened inflammation and stress**

- Differentially expressed genes 24-h post RL:
  - Old: 128 ↓ and 223 ↑
  - Young: 36 ↓ and 55 ↑

- Among old, inflammation and stress at the center of primary damage responses

- Old have heightened inflammatory protein signaling before injury

**Heightened inflammation and stress response—impaired regenerative capacity**
Challenges faced—Therapies

- Appropriate therapies to attenuate sarcopenia and functional decline are not clearly defined for all adults.
- Current therapies
  - Resistance exercise training
  - Dietary protein
  - Leucine, an essential amino acid

Not everyone has the same hypertrophic response to RT

16 weeks resistance exercise training
- Extreme Responders
  \[ Xtr = +2,475 \pm 140 \, \mu m^2 \]
- Moderate Responders
  \[ Mod = +1,111 \pm 46 \, \mu m^2 \]
- Non Responders
  \[ Non = -16 \pm 99 \, \mu m^2 \]

*Responses independent of age and sex

Whey protein stimulates muscle protein synthesis more effectively in older men—Acute response

- 20 g of protein, No exercise stimulus

*WHEY significantly different from CAS, \( P < 0.01 \).
#WHEY significantly different from CASH, \( P < 0.05 \).
Milk and milk proteins result in greater lean mass gains with RE training in young and old adults

HOWEVER...
- Large inter-individual variation in muscle gains
- Gains in muscle can be marginal

![Graph showing lean mass gains with different protein supplements.](image)

Higher dairy intake is associated with greater muscle mass in older age women

![Bar chart showing differences in lean mass by milk intake.](image)

Challenges faced
- **Therapies:** Appropriate therapies to attenuate sarcopenia and functional decline are **not clearly defined for all adults**

  - **Etiology:** Underlying etiology not well-understood
    - What causes chronic inflammation?
    - What causes anabolic resistance to stimuli?

  **Can we maximize gains or maintenance of skeletal muscle by understanding the etiology?**

Serum metabolite profile of skeletal muscle mass in older adults

- **Purpose:** To identify serum metabolites and metabolic pathways associated with skeletal muscle mass in older adults

  - Develop hypotheses about the metabolic changes that underlie sarcopenia—**potential for new therapies**
**Study design**

- **Participants:** 19 older adults
  - 60-75 years
  - 13 female, 6 male
- **DXA:** measured appendicular lean mass
- **Skeletal Muscle Index (SMI):** appendicular lean mass (kg) / height² (m²)
- **Blood draw:** collected serum after an overnight fast
- **Metabolomics:** Measured metabolite concentrations for ~340 polar metabolites
- **MetaboAnalyst:** Determined metabolic pathways associated with SMI based on the serum metabolites

**Arginine and proline metabolism**—most strongly associated with SMI in older adults

Arginine and proline metabolism—most strongly associated with SMI in older adults

**Arginine and proline metabolism**

- Low concentrations in older adults with low SMI
- High concentrations in older adults with low SMI

**Amino acids**

- Basic building blocks of protein
  - **Amino acids**
    - Non-essential
    - Essential
    - Conditionally Essential
  - **Arginine** = a conditionally essential amino acid
    - Growth
    - Trauma and stress
Supplements containing arginine increase skeletal muscle mass

- Change in Lean Body Mass (kg)
  - Supplement: Arginine (14 g)
  - Hydroxymethylbutyrate (3 g)
  - Glutamate (14 g)
  - Control: Maltodextrin (isocaloric)

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Week</th>
<th>Change</th>
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<tbody>
<tr>
<td>Arginine</td>
<td>Baseline</td>
<td>-0.5</td>
</tr>
<tr>
<td></td>
<td>Week 4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Week 24</td>
<td>2</td>
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<tr>
<td>Control</td>
<td>Baseline</td>
<td>0</td>
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<tr>
<td></td>
<td>Week 4</td>
<td>2</td>
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<td></td>
<td>Week 24</td>
<td>3</td>
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- Whole-body protein synthesis
  - Supplement: Arginine (5 g)
  - Hydroxymethylbutyrate (2 g)
  - Lysine (1.5 g)
  - Control: Maltodextrin (isocaloric)


How could arginine affect muscle mass?

- **Attenuating inflammatory susceptibility**
  - Arginine attenuates hindlimb-suspension-induced increases in the E3 ubiquitin ligases MURF-1 and atrogin-1 in rats (Lemonsinos, Biochem [Mosi], 2011)
  - Arginine is important for attenuating NF-kB activity (inflammation induced signaling) (Magemane et al., J Mol Med 2007)

- **Anabolic response to stimuli**
  - Arginine stimulates mTORC1, mediated by the lysosomal protein SLC38A9 (Wang, Science, 2015)

- **Alternative mechanism**
  - A metabolite of arginine could have an undefined effect on muscle mass

Cationic Amino Acid Transporters (CAT)

- Bidirectional, sodium-independent transport
- Transport arginine, ornithine, and lysine
- CAT-1 and CAT-2 isoforms are found in skeletal muscle
- CAT-2 gene expression is stimulated by inflammatory mediators, insulin, and glucocorticoids in human endothelial cells (Kisiel, Biochimie et Biophysica Acta – Biomembranes, 2006)

Supplements are effective in populations with elevated inflammation—is there an interaction between arginine metabolism and inflammation?
Purpose
• To investigate the effect of age on inflammatory signaling and arginine transport in human skeletal muscle.

Hypotheses:
— Older adults will have elevated inflammatory signaling in skeletal muscle
— Older adults will have increased expression of the arginine transporters, CAT-1 and CAT-2, in skeletal muscle

Study design
• Participants were healthy adults
  — Young: 11 adults (21-39 years)
  — Old: 10 adults (68-80 years)
• Skeletal muscle biopsies after an overnight fast
• qPCR to measure gene expression in muscle samples
• Statistics – ANOVA to assess the effects of age (and sex) on expression of arginine transport and inflammation genes

Inflammatory biomarkers and arginine transporters do not differ with age(?)

<table>
<thead>
<tr>
<th>Inflammatory biomarkers</th>
<th>IL-6</th>
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<tbody>
<tr>
<td>Young Male</td>
<td></td>
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<tr>
<td>Young Female</td>
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<tr>
<td>Old Male</td>
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<tr>
<td>Old Female</td>
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<table>
<thead>
<tr>
<th>Arginine transporters</th>
<th>CAT-1</th>
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<tbody>
<tr>
<td>Young Male</td>
<td></td>
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<tr>
<td>Young Female</td>
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<td>Old Male</td>
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<td>Old Female</td>
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Purpose
• To investigate the effects of exogenous TNFα on skeletal muscle arginine transporters in human primary skeletal muscle cells

Hypotheses:
— TNFα will elevate inflammatory signaling
— TNFα will increase gene expression of the arginine transporters, CAT-1 and CAT-2
Study design
• Subjects – 11 young adults
• Myoblast cell culture
  – Growth media for 7 days
  – Differentiation media for 3 days
  – TNFα incubation (10 ng/mL) for 2 days
• Gene expression in harvested RNA
  – qPCR
  – PCR array—arginine metabolism enzymes
• Statistics
  – Student’s t-test (TNFα vs control cultures)
  – False discovery rate correction

Conclusions
• Arginine and proline metabolism is the pathway most closely associated with SMI in older adults
• In an inflammatory state, gene expression of CAT-2 in skeletal muscle cells increases, which may be a signal of increased demand for arginine
• Inflammation decreases the expression of genes regulating arginine metabolism suggesting preservation of arginine in the inflamed cells
**Working hypothesis**

*One of the biggest challenges faced is sarcopenia—appropriate therapies to offset muscle loss are needed.*

*Therapies to attenuate inflammation and improve anabolic responses are necessary.*

*Therapies to manipulate arginine availability could improve skeletal muscle health in populations with elevated muscle inflammation, including many older adults.*

**Summary:**

- One of the biggest challenges faced is sarcopenia—appropriate therapies to offset muscle loss are needed.
- Therapies to attenuate inflammation and improve anabolic responses are necessary.
- Therapies to manipulate arginine availability could improve skeletal muscle health in populations with elevated muscle inflammation, including many older adults.

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