Nutrient Sensing in Hospitalized Older Adults with Immobility

Chris Adams, MD, PhD

Professor of Internal Medicine
Division of Endocrinology & Metabolism
Fraternal Order of Eagles Diabetes Research Chair
The University of Iowa
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Hospitalized Older Adults with Immobility
Immobilization-Induced Skeletal Muscle Atrophy

Acute Illness or Injury

\[\downarrow\]

Immobility

\[\downarrow\]

Muscle Atrophy
Immobilization-Induced Skeletal Muscle Atrophy

Acute Illness or Injury

Immobility

Muscle Atrophy

Independent Contributions from Co-Existing Conditions
(Advanced Age, Malnutrition, Chronic Disease)
Immobilization-Induced Skeletal Muscle Atrophy

Acute Illness or Injury

Independent Contributions from Co-Existing Conditions (Advanced Age, Malnutrition, Chronic Disease)

Immobility

Muscle Atrophy

Delayed Recovery

Increased Economic Costs

Decreased Quality of Life

Complications of Prolonged Hospitalization

Vicious Cycle

Vicious Cycle
ATF4
(Activating Transcription Factor 4)

- Evolutionarily ancient mediator of nutrient sensing (starvation) responses
- Stress-inducible subunit of several different bZIP transcription factors
- Had an unknown role in skeletal muscle but ATF4 expression in muscle correlated with muscle atrophy

ATF4
C/EBPβ

ATF4 bZIP Domain Structure
Podust et al. JBC (2001)
Muscle-Specific ATF4 Knockout Mice (ATF4 mKO Mice)

- Lifelong absence of ATF4 in skeletal muscle fibers
- Develop normally and exhibit no basal phenotype into middle-age

| Control Littermate (ATF4^{f/f}) | ATF4 mKO (ATF4^{f/f} ; MCK-Cre) |
Is ATF4 Required for Immobilization-Induced Skeletal Muscle Atrophy?

Young Adult Control Littermates

Young Adult ATF4 mKO

Unilateral Hindlimb Immobilization
Targeted Reduction in ATF4 Reduces Immobilization-Induced Muscle Atrophy

Young Adult Control Littermates

Young Adult ATF4 mKO

Muscle Fiber Diameter (Immobile: Mobile)

Days of Immobilization

Ctrl.

ATF4 mKO
Targeted Reduction in ATF4 Reduces Age-Related Muscle Atrophy

- **Mean Fiber Diameter (μm)**: The graph shows a decrease in mean fiber diameter from 6 months to 22 months. The ATF4 mKO group has a significantly lower mean fiber diameter compared to the control group.

  - **Control**: The mean fiber diameter decreases from approximately 45 μm at 6 months to 35 μm at 22 months.
  - **ATF4 mKO**: The mean fiber diameter decreases from approximately 40 μm at 6 months to 30 μm at 22 months.

- **% Fibers vs. Fiber Diameter (μm)**: The distribution of fiber diameters is shown for 6 and 22 months.
  - **6 Mo.**
    - Control: A normal distribution with a peak around 30 μm
    - ATF4 mKO: A narrower distribution with a peak around 25 μm
  - **22 Mo.**
    - Control: A normal distribution with a peak around 20 μm
    - ATF4 mKO: A narrower distribution with a peak around 15 μm
ATF4 Is Required for Loss of Strength & Muscle Quality During Aging

**Grip Strength (g)**

- ATF4 mKO
- Control

<table>
<thead>
<tr>
<th>Age (Mo.)</th>
<th>6</th>
<th>22</th>
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<tbody>
<tr>
<td>Grip</td>
<td>160</td>
<td>130</td>
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**Specific Force (kN/m²)**

- ATF4 mKO
- Control

<table>
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<th>Age (Mo.)</th>
<th>6</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific</td>
<td>140</td>
<td>110</td>
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**Statistical Significance**

- Control vs. ATF4 mKO:
  - **P < 0.01** (***)
  - P < 0.05 (*)

Healthy Skeletal Muscle

Immobilization
Fasting
Aging

ATF4-Dependent Gene Expression in Muscle Fibers

Muscle Weakness and Atrophy
Immobilization, Fasting, Aging

ATF4 Heterodimer

Gadd45a Gene

Gadd45a Protein

Gadd45a mRNA

MEKK4

M KK3 MKK4 MKK6 MKK7

10 Other Protein Kinases

53 Other Proteins

3 Tyrosine Phosphatases

Unidentified Protein Target(s)

Skeletal Muscle Atrophy

ATF4 Heterodimer

p21 Gene

p21 Protein

p21 mRNA

Unidentified Protein Target(s)

Smox Expression

Other Mechanisms

Systems-Based Discovery Strategy

Muscle Atrophy Stimuli (Fasting & Spinal Cord Injury)

Human & Mouse Skeletal Muscle

Muscle Atrophy mRNA Expression Signatures

Connectivity Map
Small Molecule Library

Human Cell Lines

Small Molecule mRNA Expression Signatures

Identify Negative Correlations

Small Molecule Inhibitor of Muscle Atrophy?
Systems-Based Discovery Strategy

Muscle Atrophy Stimuli (Fasting & Spinal Cord Injury)

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Ursolic Acid & Tomatidine

Connectivity Map Small Molecule Library

Human Cell Lines

Small Molecule mRNA Expression Signatures
Ursolic Acid

Tomatidine

Inhibition of ATF4 & Other Atrophy Pathways

Decreased Muscle Atrophy & Weakness During Immobilization, Aging & Other Stress Conditions

Natural Dietary Compounds (Ursolic Acid & Tomatidine)

Dietary Supplements & Functional Foods to Improve Nutrition During Aging, Chronic Illness, Hospitalization and Rehabilitation
Natural Dietary Compounds (Ursolic Acid & Tomatididine)

Nutrition Products for Muscle Health & Wellness

Search for Novel Analogs with Improved Pharmacologic Properties
Example of a Novel Tomatididine Analog with Increased In Vivo Potency & Efficacy

3-month-old C57BL/6 mice

Mobile

Immobile

Muscle Weight (Immobile / Mobile)

Vehicle EMMY1-19

P < 0.0001
• In hospitalized older adults with immobility, ATF4-dependent gene expression in muscle fibers may be a cause of muscle atrophy & weakness.

• Ursolic acid, tomatidine & related small molecules could potentially benefit hospitalized older adults with immobility.
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Targeted Reduction in ATF4 Decreases Fasting-Induced Muscle Atrophy

Young Adult Littermate Controls

Young Adult ATF4 mKo Mice

Muscle Fiber Diameter (μm)

Fed
Fasted 48 Hrs

Control
ATF4 mKo
Control
ATF4 mKo
ATF4 Is Required for Loss of Endurance Exercise Capacity During Aging

Distance Run on an Accelerating Treadmill (m)

ATF4 mKO
Control

Age (Mo.)
6 22

**
Is ATF4 Expression Sufficient to Induce Skeletal Muscle Atrophy?

2-Month-Old Mice

Control Plasmid

Plasmid Containing ATF4 cDNA

Mice Return to Normal Activities for 1 Week

Electroporate Plasmid DNA into Skeletal Muscle Fibers