Nutrition for Skeletal Muscle Health …in Aging

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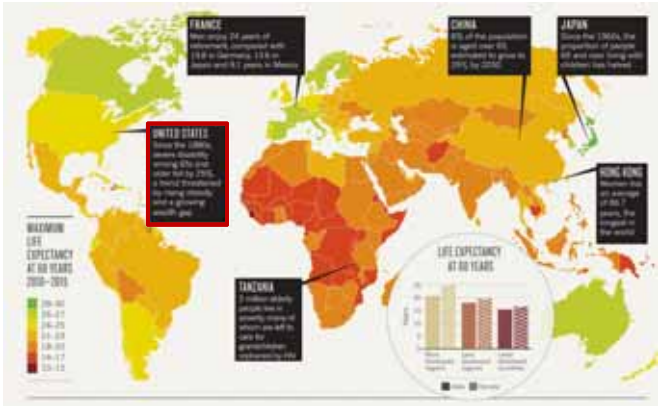
October 19, 2016

Overview

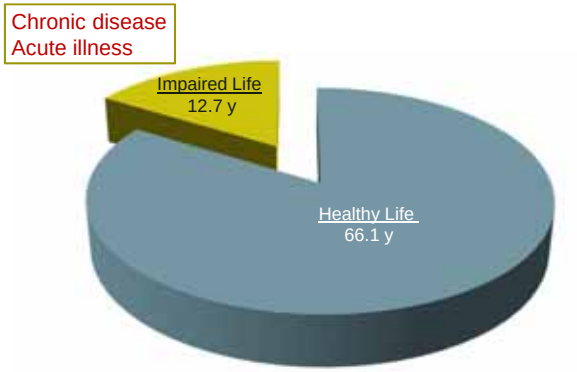
- Silver tsunami
- Skeletal muscle
  - Protein synthesis—blunted anabolic response
  - Heightened inflammation
- Challenges faced
  - Poorly defined therapies to attenuate muscle deterioration
  - Poor understanding of etiology
- A new therapy?

The "Graying" of society: Global

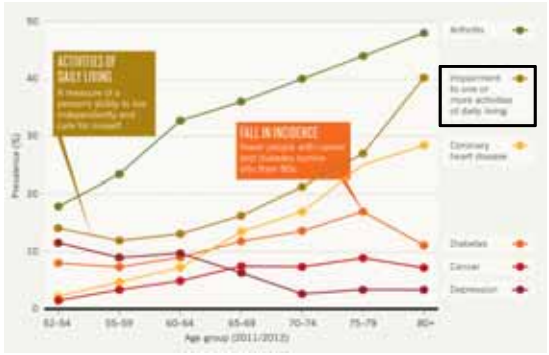


Nature, 2012

Life expectancy at birth (2013) 78.8 years cdc.gov

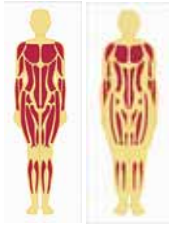


"…the prevalence of health problems increases with age while people become increasingly dependent on healthcare and community support to survive."

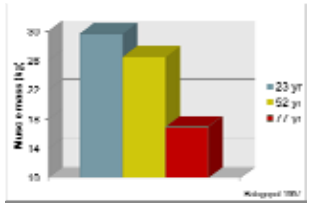


Nature, 2012

Skeletal muscle

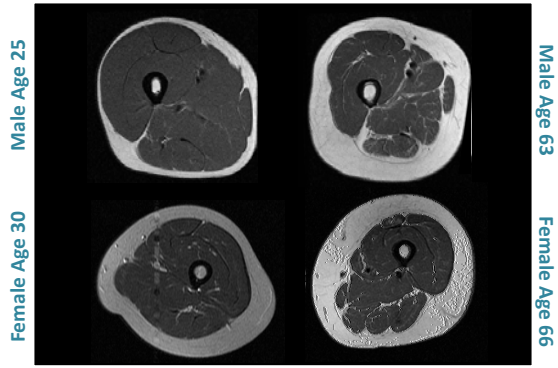


- Skeletal muscle is the largest organ (system)
  - ~45% of body weight (BW) in young men
  - ~35% of BW in young women
  - ⬇ 25-30% of BW by 70 y (**sarcopenia**)



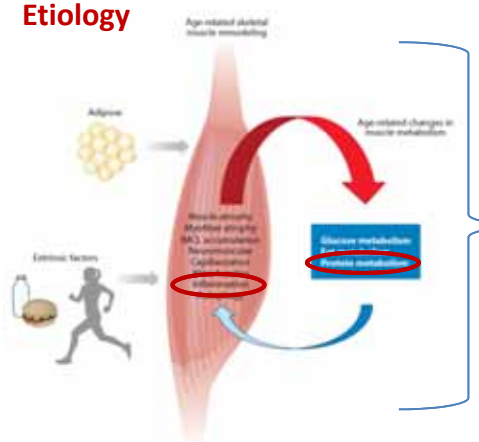
- Functions
  - Strength, power, endurance
  - Skeleton support
  - Metabolic processes
  - Endocrine functions

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



UAB Core Muscle Research Laboratory

Etiology

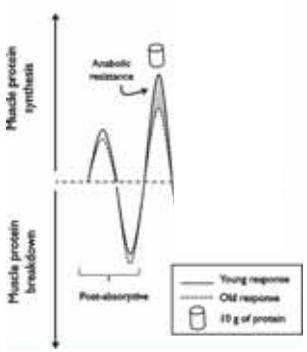


Skeletal muscle deterioration
Pathological remodeling
Metabolic disease

Loss of independence

Gheller et al. Annals Nutrition 2016

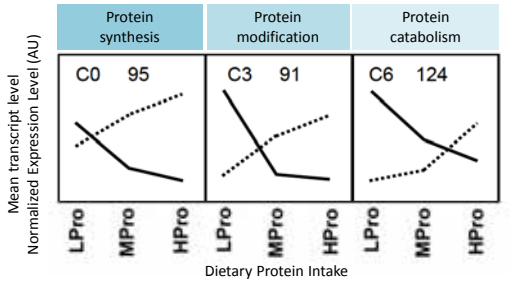
Blunted anabolic response in older human skeletal muscle



- 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

Burd et al, *Medscape* 2013

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



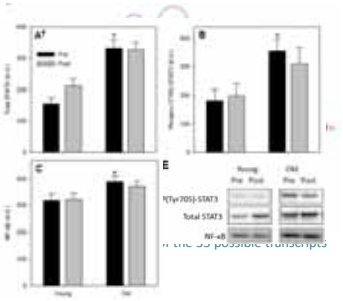
LPro = 0.50, **MPro** = 0.75, **HPro**=1.00 g•kg^{-1} •d^{-1}

Young ---Old ——

Thalacker-Mercer, *JNB* 2010

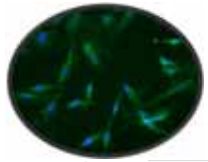
Older adult skeletal muscle has heightened inflammation and stress

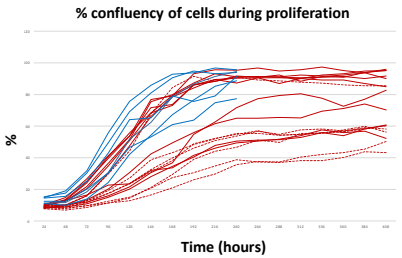
- Differentially expressed genes 24-h post RL:
  - Old: 128 ↓ and 223 ↑
  - Young: 36 ↓ and 55 ↑
- Among old, inflammation and stress at the center of primary damage responses
- Old have heightened inflammatory protein signaling **before injury**

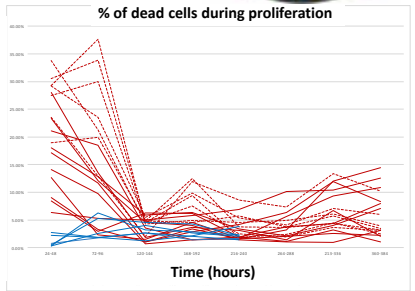


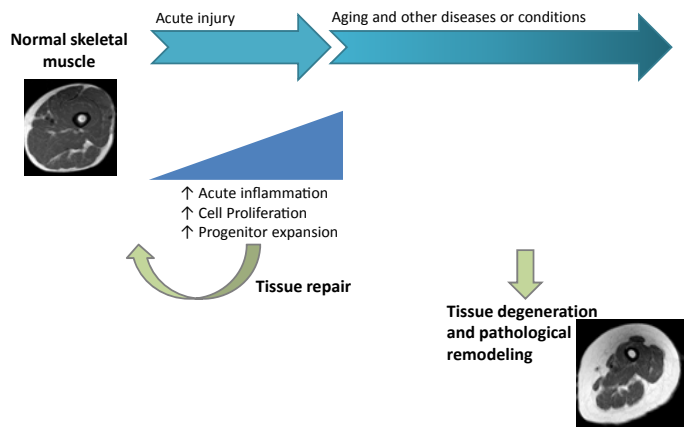
Thalacker-Mercer et al. *Physiol Genomics* 2010

Heightened inflammation and stress response— impaired regenerative capacity









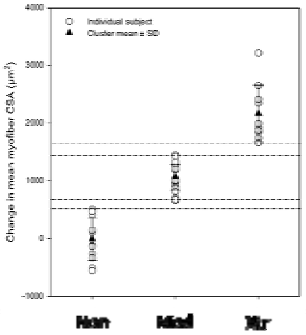
Adopted from Girgenrath et. al 2006

Challenges faced—Therapies

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

"What fits your busy schedule better, exercising one hour a day or being dead 24 hours a day?"

Not everyone has the same hypertrophic response to RT



16 weeks resistance exercise training

- Extreme Responders

  Xtr =+2,475 ± 140 μm²
- Moderate Responders

  Mod = +1,111 ± 46 μm²
- Non Responders

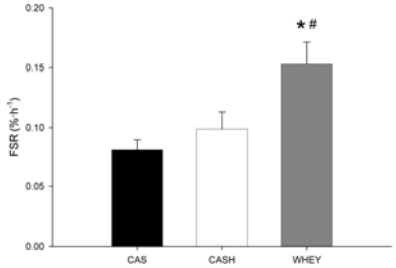
  Non = −16 ± 99 μm²

*Responses independent of age and sex

Bamman et al. 2007

Whey protein stimulates muscle protein synthesis more effectively in <u>older</u> men—*Acute response*

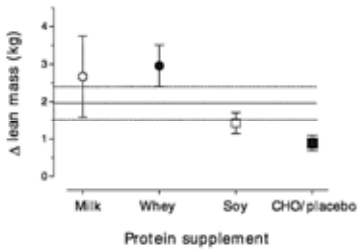
- 20 g of protein, No exercise stimulus



*WHEY significantly different from CAS, $P < 0.01$.
#WHEY significantly different from CASH, $P < 0.05$.

Pennings, *AJCN* 2011

Milk and milk proteins result in greater lean mass gains with RE training in young and old adults

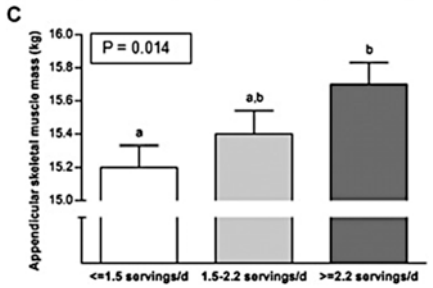


HOWEVER…

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

Phillips et al., *J Am Coll Nutr*, 2009

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



Differences in lean mass are marginal

Milk (serving/d)	**0.8** (0.4,0.8)	**1.5** (0.8,1.5)	**1.5** (1.5,2.5)

*Median (IQR)

Radavelli-Bagatini et al., *J Bone Miner Res*, 2014

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

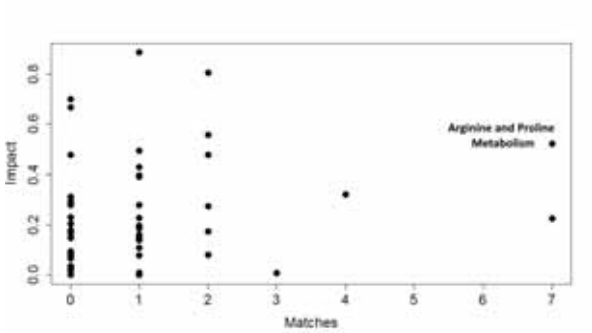
- **Purpose:** To identify serum metabolites and metabolic pathways associated with skeletal muscle mass in older adults

- 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) / height^2 (m^2)
- **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



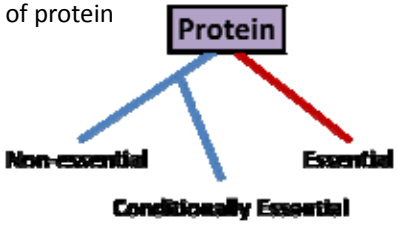
Arginine and proline metabolism

Low concentrations in older adults with low SMI

High concentrations in older adults with low SMI

Amino acids

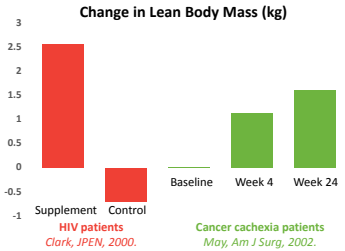
- Basic building blocks of protein



- Amino acids

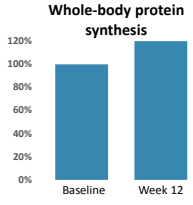
- **Arginine** = a conditionally essential amino acid
  - Growth
  - Trauma and stress

Supplements containing arginine increase skeletal muscle mass



HIV patients
Clark, JPEN, 2000.

Cancer cachexia patients
May, Am J Surg, 2002.



Elderly women
Flakoll, Nutrition, 2004.

Supplement
Arginine (14 g)
Hydroxymethylbutyrate (3 g)
Glutamate (14 g)

Control
Maltodextrin (isocaloric)

Supplement
Arginine (5 g)
Hydroxymethylbutyrate (2 g)
Lysine (1.5 g)

- Arginine supplements are ineffective in young athletes *(Bescós, IJSNEM, 2009., Liu, J Nutr Biochem, 2009., Sunderland, JSCR, 2011., Tsai, Chin J Physiol, 2009.)*

Supplements are effective in populations with elevated *inflammation*—is there an interaction between arginine metabolism and inflammation?

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 *(Lomonosova, Biochem (Mosc), 2011)*
  - Arginine is important for attenuating NF-kB activity (inflammation induced signaling) *(Kagemann 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

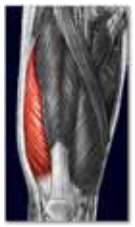
Cationic Amino Acid Transporters (CAT)

- 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 *(Visigalli, Biochimica et Biophysica Acta – Biomembranes, 2004)*

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

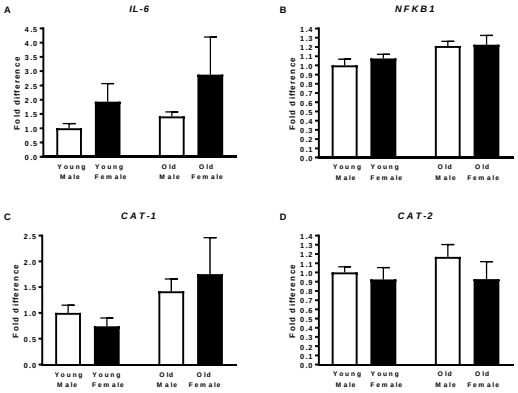


- Participants were healthy adults
  - Young: 11 adults (21-39 years)
  - Old: 10 adults (68-80 years)
- Skeletal muscle biopsies after an overnight fast
- qPCR to measure gene expression in muscle samples
- Statistics – ANOVA to assess the effects of age (and sex) on expression of arginine transport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

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

Inflammatory biomarkers

Arginine transporters

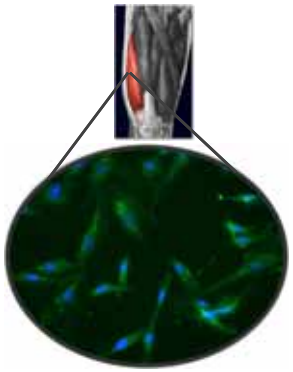


Purpose

- 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*

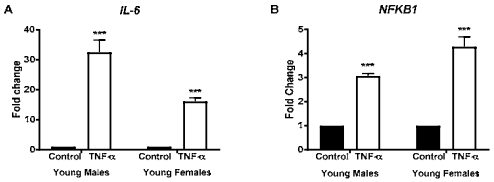
Study design

- 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

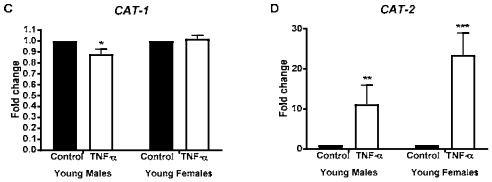


TNFα increased expression of inflammatory biomarkers and arginine transporter

Inflammatory biomarkers



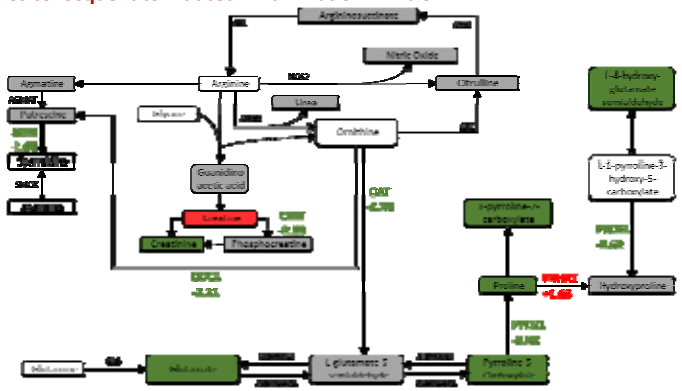
Arginine transporters



Means ± SEM. ***significantly different from control, $p < 0.001$

TNFα decreased expression of arginine metabolism genes

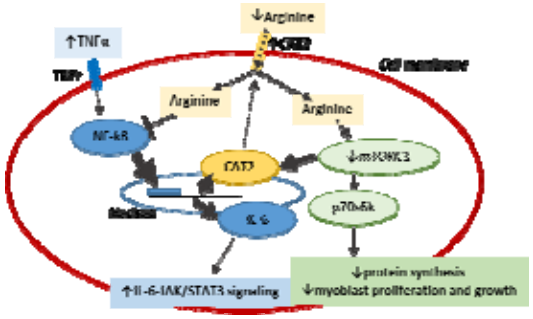
Lower levels of the metabolic products for arginine metabolism, observed among older adults in vivo, are consistent with reduced expression of related metabolic enzymes consequent to induced inflammation in vitro.



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



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.

Acknowledgements

Cornell University
Heather Roman, MS
Diwakar Gupta, MS
Emily Riddle, MS, RD
Brandon Gheller, MS, RD
Jamie Blum
Lynn Johnson, PhD
Erica Bender, CNM, NP
Melinda Lem

U. of Alabama at Birmingham
Marcas Bamman, PhD

Duke University
Jason Locasale, PhD

Purdue University
Wayne Campbell, PhD
James Fleet, PhD

Support:
Cornell-start-up, NIA, NIDDK, UAB-Center for Exercise Medicine, UAB-Center for Aging