Symposium 14

Understanding Aging Skeletal Muscle and Dynamics

Chairpersons

Kijin Kim
Keimyung University, Korea

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KAIST, Korea

Speakers

William Evans
University of California, Berkeley, USA

Marc Hellerstein
University of California, Berkeley, USA

Il-Young Kim
Gachon University, Korea

Panel Discussion

Seung-Hwan Lee
The Catholic University of Korea, Korea

Justin Y. Jeon
Yonsei University, Korea

William Evans

University of California, Berkeley, USA

• Education

Period	Affiliation	Position
– **1980**	Human BioEnergetics, Ball State University, Human Performance Laboratory	Ph.D.
– **1976**	Biology, Ball State University, Human Performance Laboratory	M.S.
– **1972**	Zoology, University of North Carolina at Chapel Hill	B.A

• Affiliations / Experience

Period	Affiliation	Position
– **2017-Present**	Department of Nutritional Sciences & Toxicology, University of California, Berkeley	Adjunct Professor of Human Nutrition
– **2010-Present**	Division of Geriatrics, Duke University Medical Center	Adjunct Professor of Medicine
– **2014-2016**	Muscle & Health Division, KineMed, Inc	President/ Director
– **2009-2014**	Muscle Metabolism Discovery Unit, GlaxoSmithKline, Research Triangle Park, NC	Vice President
– **1997-2009**	Donald W. Reynolds Institute on Aging at the University of Arkansas for Medical Sciences	Jane and Ed Warmack Chair/ Director

• Committee Memberships

- American Federation for Aging Research
- Skeletal muscle, and exercise physiology study section, Clinical and Integrative Diabetes and Obesity Study Section, and Multicenter AIDS Cohort Study (MACS)- NIH, Small Business Innovative Research grant, Pepper Center for Independent Living grants
- Society on Cachexia and Wasting Disorders" (SCWD)
- UAMS Institutional Review Board
- Neurological, Aging, and Musculoskeletal Epidemiology Study

• Publications

- WJ Evans, M Hellerstein, RJ Butterfield, E Smith, M Guglieri, N Katz, B Nave, L Branigan, S Thera BS3, KL Vordos, L Behar, M Schiava, M James, T Field, H Mohammed, and M Shankaran, Reductions in functional muscle mass measured using D3Creatine dilution and ability to ambulate in Duchenne muscular dystrophy from ages 4 – 24 years, (in review)
- M Hetherington-Rauth , CE McCulloch, SR Cummings, WJ Evans, M Hellerstein, JA Cauley, K Ensrud, L Langsetmo , ES Orwoll, and PM Cawthon Change in D3Cr muscle mass in oldest old men and its association with changes in grip strength and walking speed (in review)
- HR Banack, J Wactawski-Wende, HM Ochs-Balcom, EM Cespedes Feliciano, B Caan, C Lee, G Anderson, M Shankaran, WJ Evans A protocol for remote collection of skeletal muscle mass via D3-creatine dilution in community-dwelling postmenopausal women from the Women's Health Initiative, PLOS One, 19: e0300140, DOI: 10.1371/journal.pone.0300140
- PM Cawthon, Blackwell TL, Kritchevsky SB, Newman AB, Hepple RT, Coen PM, Goodpaster BH, Duchowny K, Hetherington-Rauth M, Mau T, Shankaran M, Hellerstein M, Evans WJ, Cummings SR. Associations between D3Cr muscle mass and MR thigh muscle volume with strength, power, physical performance, fitness, and limitations in older adults in the SOMMA study. J Gerontol A Biol Sci Med Sci. Accepted
- E Cheng, BJ Caan, PM Cawthon, WJ Evans, MK Hellerstein, M Shankaran, KL Campbell, AM Binder, B Sternfeld, JA Meyerhardt, KH Schmitz, EM Cespedes Feliciano, D3-creatine dilution, computed tomography and dual-energy X-ray absorptiometry for assessing myopenia and physical function in colon cancer: A cross-sectional study, J Cachexia Sarcopenia Muscle, 10.1002/jcsm.13353

Symposium 14

Sarcopenia: New Insights for a Unified Definition

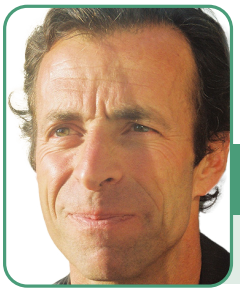
William Evans (University of California, Berkeley, USA)

The decline of muscle mass and muscle strength is one of the phenotypic characteristics of aging. Although this process can be attenuated by moderate-to-intense physical activity, even individuals that faithfully exercise every day see their muscles shrink and their strength decline. When this process is overt and crosses a critical threshold, it leads to a condition called sarcopenia. Over the last three decades, there has been a titanic effort to define standard criteria for the screening and clinical assessment of sarcopenia. Such efforts were driven by the notion that sarcopenia is a major cause of mobility disability in the elderly, and that screening for and "treating" sarcopenia shall result into mobility loss prevention. Yet, despite dozens of dedicated meetings, workshops, scientific articles, statements from professional societies and even the establishment of an ICD-10 code, no definition of sarcopenia that is widely acknowledged by the scientific community is currently available [1]. Lean body mass (LBM) was assumed to be an appropriate surrogate assessment of muscle mass and has been so used in several large cohort studies. To date, cross-sectional and longitudinal aging cohort studies have reported little or no relationship between low LBM and increased risk of health-related outcomes, including functional capacity, disability, and mortality [2]. A meta-analysis3 of longitudinal observational studies in older people (> 65 y) conducted between 1976 and 2012 examined reported data of body composition (BIA, DXA, CT) and physical functional capacity. Although LBM was measured, the authors incorrectly used the term "muscle mass" and concluded that "low muscle mass was not significantly associated with functional decline."This lack of associated of LBM with functional capacity or health related outcomes has led to a type 2 error in the existing literature and as a result there is no consensus on the role of muscle in defining sarcopenia. This lack of consensus has also resulted in several (> 15) 'consensus' definitions with no consensus at all [1]. The use of surrogate measures of muscle mass older people fail to distinguish between contractile proteins and other proteins that accumulate in skeletal muscle with aging, such as collagen and amyloid that thicken the matrix between myofibers [4]. Because this phenomenon is widely heterogeneous between individuals, the use of LBM is noisy, not very useful and the lack of relationship between LBM, strength and other health related outcomes more recent 'consensus' definitions of sarcopenia use no body composition assessment and rely on function and strength.

The D_3Creatine dilution method now allows a non-invasive measurement of total body skeletal muscle mass [5]. Because creatine is actively transported into the sarcomere against a large concentration gradient, almost 98% of the body creatine pool is found in muscle. In addition, creatine and phosphocreatine is co-located with contractile components, potentially providing a measure of the 'functional' components of skeletal muscle. Studies in the (MrOS) population in which 1425 older, community dwelling men were measured demonstrated that muscle mass assessed with D_3Cr muscle was strongly and independently associated with strength, functional capacity[6], risk of disability (including IADL), hip fracture[7], and mortality[8]. In addition, a threshold of approximately 25% muscle mass was described for a high risk of a mobility disability defined by chair stand time[9]. For the first time, we now have data demonstrating that longitudinal loss of muscle mass measured by D_3Cr dilution is significantly associated will decreased strength and walking speed [10].

I propose a simplified definition of sarcopenia as the term implies (lack of flesh) as low muscle mass. The definitions so far attempted are based on three basic variables in various combinations: muscle mass (typically measured by DEXA of computerized tomography), muscle strength (either grip or knee extension strength) and lower extremity performance (typically walking speed). All three dimensions present complex assessment and interpretation problems. A simplified definition will have greater clinical utility. At the present time, most physicians have no idea how to diagnose or treat sarcopenia[11], perhaps because of the lack of consensus of what it is or that it is not recognized indication by the US Food and Drug Administration for development of sarcopenia drugs. Most health care professionals (HCP) do not routinely measure strength or functional capacity in their older patients. A definition of sarcopenia as low % muscle mass will also allow HCPs to determine who may be at risk for several age-associated syndromes that have been associated with low muscle mass and develop a therapy that targets maintenance or improvement skeletal muscle amount. Preservation of muscle mass to combat sarcopenia may prove to be the most effective strategy to preserve independence and face advancing age with dignity. A simplified definition available to all HCPs will go a long way to meet this goal.

1. Evans WJ, Guralnik J, Cawthon P, et al. Sarcopenia: no consensus, no diagnostic criteria, and no approved indication-How did we get here? *Geroscience*. 2023.
2. Studenski SA, Peters KW, Alley DE, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. *J Gerontol A Biol Sci Med Sci*. 2014;69(5):547-558.
3. Schaap LA, Koster A, Visser M. Adiposity, muscle mass, and muscle strength in relation to functional decline in older persons. *Epidemiol Rev*. 2013;35:51-65.
4. McGregor RA, Cameron-Smith D, Poppitt SD. It is not just muscle mass: a review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. *Longev Healthspan*. 2014;3(1):9.
5. Evans WJ, Cawthon PM. D(3)Creatine Dilution as a Direct, Non-invasive and Accurate Measurement of Muscle Mass for Aging Research. *Calcif Tissue Int*. 2023.
6. Cawthon PM, Orwoll ES, Peters KE, et al. Strong Relation Between Muscle Mass Determined by D3-creatine Dilution, Physical Performance, and Incidence of Falls and Mobility Limitations in a Prospective Cohort of Older Men. *J Gerontol A Biol Sci Med Sci*. 2019;74(6):844-852.
7. Cawthon PM, Peters KE, Cummings SR, et al. Association Between Muscle Mass Determined by D3 -Creatine Dilution and Incident Fractures in a Prospective Cohort Study of Older Men. *J Bone Miner Res*. 2022.
8. Cawthon PM, Blackwell T, Cummings SR, et al. Muscle Mass Assessed by the D3-Creatine Dilution Method and Incident Self-reported Disability and Mortality in a Prospective Observational Study of Community-Dwelling Older Men. *J Gerontol A Biol Sci Med Sci*. 2021;76(1):123-130.
9. Zanker J, Patel S, Blackwell T, et al. Walking Speed and Muscle Mass Estimated by the D3-Creatine Dilution Method Are Important Components of Sarcopenia Associated With Incident Mobility Disability in Older Men: A Classification and Regression Tree Analysis. *J Am Med Dir Assoc*. 2020.
10. Duchowny KA, Peters KE, Cummings SR, et al. Association of change in muscle mass assessed by D3 -creatine dilution with changes in grip strength and walking speed. *J Cachexia Sarcopenia Muscle*. 2020;11(1):55-61.
11. Guralnik JM, Cawthon PM, Bhasin S, et al. Limited physician knowledge of sarcopenia: A survey. *J Am Geriatr Soc*. 2023;71(5):1595-1602.



Marc Hellerstein

University of California, Berkeley, USA

• Education

Period	Affiliation	Position
– **1986**	Mass Institute of Technology, Cambridge, MA	Ph.D.
– **1979**	Yale Univ. School of Medicine, New Haven, CT	M.D.
– **1975**	Brandeis University, Waltham, Massachusetts	B.A.

• Affiliations / Experience

Period	Affiliation	Position
– **2023-Present**	Myo Corps, Inc., Chapel Hill, NC	Co-founder, President, and Chairman of the Board
– **2017-Present**	9 Muses-Hellerstein Foundation (a 501(c)3 public charity), Berkeley, CA	Founder and President
– **2002-2017**	KineMed., Inc., Emeryville CA	Co-founder and Chief of SAB; President
– **1999-Present**	University of California at Berkeley, CA and, University of California, San Francisco, CA	Professor of Nutritional Sciences and Professor of Medicine
– **1993-1999**	University of California at Berkeley, CA and, University of California, San Francisco, CA	Associate Professor of Nutritional Sciences and Associate Professor of Medicine

• Committee Memberships

- EXCOM (Faculty Governance), UC Berkeley College of Natural Resources
- Graduate Admissions Committee, DNST, UC Berkeley
- NASA Scientific Advisory Bd, Nutritional and Metabolic Counter-Measures

• Publications

- Smith GI, Shankaran M,..Hellerstein M, Klein S (Co-senior authors). Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. J Clin Invest. 130(3):1453-1460
- Shankaran M, King CL, Angel TE, Hellerstein MK. Circulating protein synthesis rates reveal skeletal muscle proteome dynamics; J Clin Invest. 126(1):288-302. PMID: 26657858
- Holmes WE, Angel TE, Li KW, Hellerstein MK. Dynamic Proteomics: In Vivo Proteome-Wide Measurement of Protein Kinetics Using Metabolic Labeling. Methods Enzymol. 561:219-276
- Clark RV, Walker AC, O'Connor-Semmes RL, Leonard MS, Miller RR, Stimpson SA, Turner SM, Ravussin E, Cefalu WT, Hellerstein MK, Evans WJ. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans.J Appl Physiol. 116(12):1605-13. PMID: 24764133
- Busch R, Neese RA, Awada M, Hayes GM, Hellerstein MK. Measurement of cell proliferation by heavy water labeling. Nat Protoc 2:3045-57, PMID: 18079703

Symposium 14

Understanding Skeletal Muscle Protein Dynamics, Regulation and Function Using New Tracer Techniques

Marc Hellerstein (University of California, Berkeley, USA)

I will review data from the many applications of a new technology that we have developed to measure proteome-wide muscle protein synthesis rates *in vivo*. Metabolic labeling with heavy water is combined with tandem mass spectrometric (LC/MS-MS) analysis of isotope ratios by Mass Isotopomer Distribution Analysis (MIDA). Proteins across the proteome or targeted proteins can be measured. This methodology has allowed several important areas of muscle biology to be explored.

1. *Central role of muscle protein synthesis in both catabolic and anabolic conditions*. Somewhat surprisingly, almost every intervention that either increases or decreases muscle mass has been shown by this labeling method to act by altering muscle protein synthesis (fractional synthesis rate, FSR). On the anabolic side, this includes androgen therapy or resistance exercise in humans, and clenbuterol or weight-bearing exercise in rodents; on the catabolic side, energy restriction, bed rest, immobilization, weight loss, long-term calorie restriction, Duchenne's Muscular Dystrophy or glucocorticoid treatment (the latter widely believed to increase muscle protein breakdown). Of particular interest is the powerful effect of energy deficiency – which prevents concurrent testosterone therapy from increasing muscle FSR in humans, for example. These findings have important implications for prevention and treatment of muscle loss.
2. *Predictive value as an early marker*. In rodent models, increases in muscle protein FSR after several days of androgen therapy predict later gains in muscle mass. In human studies, muscle FSRs can increase within days of starting an intervention.
3. *Discovery*. We have shown that localized experimental muscle injury with cardiotoxin results not only in sequential changes in proteome turnover in the injured muscle but alters turnover in the contralateral muscle – suggesting circulating myokines or neurologic signals in response to local injury. The physiologic effect of an intervention can also be dissected by differential protein responses- e.g., using FSRs of mitochondrial vs. structural and glycolytic proteins to learn whether sprint exercise training increases aerobic, power or both types of muscle response in humans.
4. *Testing drug candidates in animal models and in Ph1 / 2 trials in humans*. We showed in a 15-day study that a candidate drug was effective at reversing the lower protein synthesis response to bed rest in muscle. We established the pharmacodynamics on muscle protein FSRs of a drug candidate in rodents and compared to a known muscle anabolic agent. The effect of a nutritional supplement on muscle FSR in humans was also documented through this highly sensitive method.
5. *Non-invasive measurements*. FSRs of plasma proteins derived from skeletal muscle can be used as a "liquid biopsy" or "virtual biopsy" of muscle protein dynamics and correlate well with muscle protein turnover. This can avoid the need for a muscle biopsy.
6. *Next generation applications*: turning molecular-cell biology into physiology. It is now possible to measure synthesis and breakdown rates of low abundance intracellular proteins that are important in metabolic control. We explored the interactive turnover of the LDL receptor and PCSK-9 in rodent liver in response to dietary cholesterol loading, for example, uncovering a surprising new dimension to the canonical cholesterol homeostasis model. Other intracellular proteins previously characterized mostly at the level of structure and content are now being studied for their kinetic response to interventions, revealing a rich physiologic life of these target molecules.

Many questions can be explored using these powerful tools. The effects of GLP-1-induced weight loss on muscle protein kinetics, mass and function is a topic of central public health importance today. Identifying effective treatments for sarcopenia and cachexia and for genetic disorders or muscle are long-unmet medical needs.

In summary, the ability to easily measure *in vivo* turnover rates of proteins globally across the proteome or for individual proteins in humans and animal models opens a new world of possibilities. Muscle physiology, pathophysiology, molecular control mechanisms and therapeutics

Il-Young Kim

Gachon University, Korea

• Education

Period	Affiliation	Position
– **2007-2011**	University of Texas, Austin	Ph.D.
– **2004-2007**	University of Texas, Austin	M.A.
– **2003-2004**	University of California, Berkeley	Graduate Program in Dept. of Integrative Biology

• Affiliations / Experience

Period	Affiliation	Position
– **2021-Present**	Gachon University College of Medicine	Associate Professor
– **2018-2020**	Gachon University College of Medicine	Assistant Professor
– **2015-2017**	Univ. of Arkansas for Medical Sciences	Assistant Professor
– **2012-2015**	Univ. of Arkansas for Medical Sciences	Post-Doc/ Faculty

• Committee Memberships

- The Korean Society for the Study of Obesity
- The Korean Society of Sports Medicine
- The Korean Society of Sarcopenia

• Publications

- Jang JW et al., Free Essential Amino Acid Feeding Improves Endurance During Resistance Training via DRP1-Dependent Mitochondrial Remodeling, Journal of Cachexia, Sarcopenia, & Muscle (co-corresponding author)
- Choi S et al., Hippo-YAP/TAZ signaling coordinates adipose plasticity and energy balance by uncoupling leptin expression from fat mass, Nature Metabolism
- Jang JW et al., Balanced Free Essential Amino Acids and Resistance Exercise Training Synergistically Improve Dexamethasone-Induced Impairments in Muscle Strength, Endurance, and Insulin Sensitivity in Mice, Int. J. Mol. Sci. (corresponding author)
- Kim I-Y et al., Tracing Metabolic Flux In Vivo: Basic Model Structures of Tracer Methodology, Exp Mol Med (corresponding author)
- Song BS et al., Mitoribosomal defects aggravate liver cancer via aberrant glycolytic flux and T cell exhaustion, Journal for ImmunoTherapy of Cancer (co-corresponding author)

Symposium 14

Overcoming Anabolic Resistance to Exercise in Sarcopenia: Role of Free Essential Amino Acids

Il-Young Kim (Gachon University, Korea)

Sarcopenia is the age-associated progressive loss of muscle mass and function, accompanying with declines in independency and quality of life in older adults. The etiology of sarcopenia is multifactorial; however, a major characteristic phenomenon is the blunted muscle protein synthetic response to anabolic stimuli such as resistance exercise and nutrition (e.g., protein/amino acids), called anabolic resistance. Despite the existence of anabolic resistance of aging muscle in varying degrees, exercise, particularly, resistance exercise, is still the most powerful means to counteract the progression of sarcopenia. We hypothesized that consumption of balanced 9 free essential amino acids will enhance anabolic response to resistance exercise training as 9 essential amino acids as a team serve not only as building blocks for synthesis of new muscle proteins but as potent anabolic stimuli for protein synthesis. In my lab, we have recently demonstrated that consumption of balanced 9 essential amino acids enhances both endurance capacity and muscle mass and strength during resistance exercise training in young mice. To test if this hold in aging, we evaluated these beneficial effects in aging sarcopenic mice. First, we found that resistance exercise training over 8 weeks enhanced both muscle protein synthesis rate and muscle mass and strength in young. However, in old mice, the same resistance exercise training in old mice resulted in blunted muscle strength gains without changes in net muscle protein synthesis and muscle mass. This confirms anabolic resistance in gains in muscle mass and strength in response to resistance exercise training in older mice. Remarkably, consumption of balanced 9 essential amino acids over 8 weeks attenuated the magnitude of anabolic resistance in old mice. To explore these beneficial effects at physiological, metabolic, and molecular aspects in young and old mice, we employed (or will employ as the study is still in progress) both metabolic kinetics or "dynamics" approach using various stable isotope tracing techniques to access cumulative synthesis rates of myofibrillar and mitochondrial protein, turnover fluxes of various metabolites including glucose, amino acids, and fatty acids, TCA cycle fluxes as well as whole-body and muscle insulin sensitivity using hyperinsulinemic-euglycemic clamp in addition to traditional "statomics" (static, snapshot) techniques including RNA-seq, molecular singling, and genetic modifications. In this talk, I will discuss the potential role of balanced essential amino acids in enhancing the efficacy of resistance exercise training in gaining of muscle mass and strength as well as endurance capacity, both of which are the best predictor of all-cause mortality and both healthspan and lifespan.