1 Mechanisms of protein balance in skeletal muscle.

2 Anthony, T.G.

3 *Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ 08901 USA*

4

5 Corresponding Author:

6 Tracy G. Anthony, Ph.D.

7 Associate Professor

8 59 Dudley Rd.

9 New Brunswick, NJ 08901

10 USA

11 Phone: 848-932-6331

12 Email: tracy.anthony@rutgers.edu

13

Abstract

Increased global demand for adequate protein nutrition against a backdrop of climate change and concern for animal agriculture sustainability necessitates new and more efficient approaches to livestock growth and production. Anabolic growth is achieved when rates of new synthesis exceed turnover, producing a positive net protein balance. Conversely, deterioration or atrophy of lean mass is a consequence of a net negative protein balance. During early life and periods of growth, muscle mass is driven by increases in protein synthesis at the level of mRNA translation. Throughout life, muscle mass is further influenced by degradative processes such as autophagy and the ubiquitin proteasome pathway. Multiple signal transduction networks guide and coordinate these processes alongside quality control mechanisms to maintain protein homeostasis (proteostasis). Genetics, hormones and environmental stimuli each influence proteostasis control, altering capacity and/or efficiency of muscle growth. An overview of recent findings and current methods to assess muscle protein balance and proteostasis is presented. Current efforts to identify novel control points have the potential through selective breeding design or development of hormetic strategies to better promote growth and health span during environmental stress.

Key Words: proteostasis, muscle protein synthesis, mRNA translation, muscle protein degradation, proteolysis

1. Introduction

Worldwide population growth has increased global demand for adequate protein nutrition [1]. Novel strategies to increase meat production are needed while minimizing the adverse effects on the environment [2]. Genetic approaches to increase production of animal products through selective breeding are successful but also result in economic, environmental and ethical complications [3,4]. Overall, efforts to meet the world's protein needs against a back drop of environmental stress (i.e., physical, chemical and biological constraints on the productivity of the species [5]) are creating greater pressures on animal agriculture than ever before. For these reasons, a greater understanding of the fundamental control points in determining muscle protein balance is relevant to animal agriculture sustainability.

Over the past two decades, advances in genomics allowed for selective breeding to be more informed and thus targeted. Recent developments in technology have further bolstered if not replaced the genomic age with an age of proteomics and metabolomics. These technologies allow for even more sophisticated questions to be asked, moving the field from monitoring genotype to phenotype [6]. A deeper understanding of the phenotypic mechanisms that regulate muscle mass will in turn provide new insight about how to best address environmental challenges to animal growth and improve overall health of livestock. With the above in mind, the following perspective was crafted to provide a basic overview of recent advances in the study of skeletal muscle protein balance *in vivo*. This information is aimed to inform the fields of domestic and livestock animal production about ways to better monitor or alter capacity and efficiency of growth, with an emphasis on skeletal muscle.

2. Evolution of methodology in assessing protein balance

A table summarizing past, current and emerging technologies to evaluate skeletal muscle protein synthesis and turnover in animals are found in Table 1. Nitrogen balance has

traditionally served as a surrogate marker to assess whole body protein balance and growth [7,8]. Subtracting nitrogen intake from output produces a value which reflects growth (>0), maintenance ($=0$) or atrophy (<0). While this measure remains the fundamental basis for determining dietary protein requirements in mammals, this technique reflects whole body and not skeletal muscle specifically. MPS capacity can be reflected by the RNA to protein ratio, a classic measurement in skeletal muscle [9-11]. While still in use today, most research settings use instead a variety of techniques that measure the rate of skeletal muscle protein synthesis (MPS) and turnover more directly. These methods use an assortment of metabolic compounds and labeling techniques in combination with highly sensitive and specialized instruments to calculate rates of MPS and/or muscle protein breakdown (MPB) or simply visualize the expression of one or more individual proteins as biomarkers of muscle growth. To estimate global rates of MPS or MPB, the incorporation and/or flux of an injected or infused metabolic tracer (i.e., radioactive or stable amino acid isotope mixed with an unlabeled amino acid or tracee) is measured into and/or out of muscle tissues over a relatively short and defined period of time [12-16]. Analyses of precursor and product tracer/tracee enrichment involve sophisticated methods of chromatography and mass spectrometry and in some cases complex tracer kinetic calculations [17]. MPS measurements over longer periods of time (d or wk) can be accomplished by ingestion of deuterium oxide (heavy water) [18,19]. This method allows for calculation of DNA synthesis in addition to MPS and turnover [20]. Metabolic tracer approaches are very useful in generating tissue averages, changes over short periods of time, assessing muscle fiber-type differences and detecting changes within subcellular organelles (e.g., mitochondrial protein synthesis) [21,22]. Synthesis rates of individual proteins can also be assessed [23,24].

Applications that rely on antibody-based detection methods such as immunoblotting, immunofluorescence, and flow cytometry are commonly used to visualize protein expression

and provide a qualitative measure of the proteome. The tagging of newly synthesized proteins with puromycin is a more recent method being used to estimate new protein synthesis in muscle [25,26]. Another approach uses biotinylated puromycin to label newly synthesized proteins in cell-free conditions, followed by proteomic analysis to generate a snapshot of the translome [27,28]. These protein tagging approaches to assess the proteome are faster and easier than two-dimensional gel electrophoresis methods to assess the proteome [10,29].

The use of whole genome sequencing is now emerging as a powerful approach to assess the translome [30]. Genome-wide analyses of mRNA expression by microarray or RNA sequencing is now being applied to monitor *in vivo* mRNA translation. One of these approaches is called ribosomal profiling or Ribo-Seq. This method conducts deep sequencing of ribosome-protected RNA fragments to determine which mRNAs are being actively translated at a specific point in time [31-34]. A related approach to examine gene-specific translation involves whole genome sequencing of mRNA bound to varying numbers of polysomes separated via density gradient centrifugation [35]. The technology and instrumentation behind many of these high-throughput systems-based methods are advancing at a pace that precludes independent validation against other more traditional measures of growth. Furthermore, the value of next-generation sequencing data requires bioinformatics pipelines that are not only fast, accurate and easy to use but also take into account animal to animal variability and complex experimental designs. As such, the usefulness of these findings as a phenotypic measure or accurate reflection of animal physiology is uncertain. Nevertheless, there is great potential for these emerging technologies to improve assessment of protein balance and meat quality in livestock as the cost of conducting genome-wide sequencing becomes more affordable and bioinformatics pipelines become more widely accessible and applicable to animal physiology [33,36,37].

3. The proteostasis network in skeletal muscle

Skeletal muscle mass is regulated by dynamic control of the processes of protein synthesis and degradation in combination with systems in place to manage folding, trafficking and clearance. In this regard, protein balance does not function as a binary operation akin to a balance scale (i.e., synthesis or degradation) but instead is a summation of multiple processes that operate according to highly interconnected signaling networks. These signaling networks coordinate quality control mechanisms to maintain protein homeostasis, or proteostasis [38]. Proteostasis control is complex and dynamic, manifesting phenotypic outcomes over months or years. In this regard, visualizing proteostasis as gears to a clock mechanism is an effective analogy [39]. Each step or process in the making and degrading of a protein impacts the function of the entire mechanism. External pressure on the network can alter the pace of the mechanism (i.e., aging) or result in its collapse (i.e., cellular damage or disease). The proteostasis network is a subject of intense study in humans, aimed toward discovering new approaches to delay aging and prevent disease [40]. Proteostasis control concepts are relevant throughout lifespan of domestic animals, even in rapid growth states, particularly under conditions of environmental stress (e.g., changes in temperature, humidity, nutrition), where growth among breeds or species can differ [41]. A greater understanding of how proteostasis is regulated in the different muscle fiber types in each species will help shape future genetic, hormonal and environmental approaches to maximize muscle growth and animal production. It will also help understand differences in meat quantity between breeds, provide novel targets that have efficient growth potential through selective breeding design or perhaps assist in the development of novel *in vitro* strategies to produce meat [2,42].

4. Muscle protein synthesis is controlled at the level of mRNA translation

Studies in rodents and pigs show that postnatal skeletal muscle growth occurs via increases in MPS that are controlled at the initiation step of mRNA translation [43,44]. Two events in translation initiation are identified as contributing factors in the regulation of muscle

mass: 1) the creation of a ternary initiation complex, which consists of methionyl tRNA, eukaryotic initiation factor 2 (eIF2) and GTP; and 2) formation of the eIF4 ribosomal complex . Both of these cellular events are regulated by phosphorylation of specific eIFs which can be monitored using immunoblotting techniques and then used as biomarkers of MPS in response to environmental factors. Among various environmental factors, nutritional factors are of primary importance. Feeding increases MPS, via enhanced formation of the eIF4 complex [45,46] whereas fasting and low protein reduces MPS in concert with reduced eIF4 formation [47]. Stimulation of MPS by feeding is developmentally regulated, with the capacity of the translational machinery reducing with age [43]. Furthermore, recent evidence points to bolus feeding as more effective in stimulating MPS than continuous feeding in neonates [48]. Catabolic stimuli such as infection, inflammation, disuse and aging blunt or block feeding-induced stimulations in MPS at the level of translation initiation and correspond with reductions in the formation of the ternary initiation complex and/or the eIF4 complex [49-51].

Among nutritional strategies examined in recent years, dietary amino acid supply and composition have been a focus [52]. Some of this work began with the seminal observations that the branched chain amino acids and especially leucine stimulated MPS independent of insulin or energy intake [53,54]. More recent efforts have further identified a potentially important role for the leucine metabolite, beta-hydroxy-beta-methylbutyrate [55-57]. Other related research efforts have studied the impact of dose and timing of amino acid intake on maximizing MPS over time, particularly in relation to physical activity and aging [58-61]. The logic behind these studies is clear: maximize MPS with each meal and over time muscle mass will increase. Many examples are to be found in the literature showing impressive increases in MPS in response to feeding protein or leucine more frequently and/or in greater amounts [58,62]. Nonetheless, long term studies examining the impact of protein or amino acid supplementation on lean mass gains are inconsistent or equivocal [63,64] with significant differences between

breeds and among phases of growth evident [65,66]. Thus, it is difficult to assign a single conclusion about the effect of supplementing dietary protein or specific amino acids on skeletal muscle growth; sometimes a reduction rather than an increase in dietary protein or amino acids improves quality of finished product [67]. The use of dietary protein and amino acids must therefore be utilized according to the biological goal in mind alongside an appreciation for the underlying genetic and hormonal background.

5. Protein degradation pathways regulate muscle mass

Skeletal muscle cell mass is influenced by protein turnover/degradation in all phases of life. Enhanced degradation not only reduces muscle mass but also alters muscle fiber type composition [68]. Fasting, glucocorticoids, sepsis and aging shifts fast (Type II) fiber types to slow (Type I) while inactivity and denervation causes a slow to fast fiber type shift [68]. How the different proteolytic systems regulate the capacity and efficiency of growth in the different muscle fiber types, particularly during environmental stress, are important research questions without clearly defined answers. The difficulty in answering these questions lies in the complexity of proteolysis. The stability or half-life of any single protein is modulated by the activity of assorted, overlapping degradative systems in the body. The main proteolytic processes influencing skeletal muscle mass are the 1) autophagy lysosomal system, 2) ubiquitin proteasome pathway, 3) calcium-dependent calpains and 4) cysteine protease caspase enzyme cascade (for recent review see [69]). The relative contribution of these processes in determining muscle mass fluctuates according to genetics, life stage, hormones and environmental stimuli. Furthermore, these catabolic processes interact through associated quality-control signaling networks and gene expression events that modulate one another [70]. As such, a deeper understanding of the regulatory processes guiding activation of each of these modes of proteolysis is a subject of intense investigation.

The two most influential cellular processes in muscle protein turnover and especially during muscle wasting are the autophagy lysosomal system and ubiquitin proteasome pathway [71]. The autophagy lysosomal system is well-recognized for its important role in conducting bulk degradation and recycling of cellular material and organelles. Recent descriptions of the different types or forms of autophagy relevant to muscle metabolism and mass are published [69,72-77] and include microautophagy, macroautophagy, and chaperone mediated autophagy. Microautophagy describes direct engulfment of small portion of cellular material (e.g., glycogen) into the lysosome; its contribution to normal skeletal muscle metabolism is not well-defined. On the other hand, macroautophagy is recognized as a major regulator of muscle mass and activation of macroautophagy corresponds with muscle atrophy. During macroautophagy, the process of “self-eating” occurs through highly-regulated expansion of a double-membraned vesicle that encapsulates cytoplasmic material. Concomitant maturation and closure of the membrane produces an autophagic vacuole which subsequently fuses with a lysosome. While the cellular material degraded can be nonselective, targeted forms of macroautophagy do exist and are increasingly becoming recognized as important for maintenance of muscle homeostasis. The selectivity of client substrate is also specified during chaperone-mediated autophagy (CMA). During CMA, soluble cytosolic proteins containing a targeting motif (KFERQ) are shuttled to the lysosomal surface without the need for vesicular transfer. A role for CMA in normal muscle maintenance is identified in flies, rodents and humans [72] and certain degenerative muscle disease states indicate involvement of CMA [78].

Targeted mutations in components of the autophagic machinery in the skeletal muscle of mice have revealed that macroautophagy is essential for muscle remodeling and quality-control. In particular, selective forms of macroautophagy such as mitophagy have important roles in mitochondrial function and oxidative stress defense during aging [79]. Macroautophagy is regulated by nutritional state, with fasting stimulating and feeding inhibiting biomarkers of

autophagosome formation [77]. In neonatal pigs, both insulin and amino acids play a role in inhibiting macroautophagy whereas CMA is not altered [80]. It should be noted that functional assessment of autophagy is complex and some controversy surrounding valid biomarkers of autophagosome maturation exists. The reader is directed to a formal position statement describing these considerations [81].

The other important proteolytic system in skeletal muscle is the ubiquitin proteasome pathway [71]. The ubiquitin proteasome pathway accomplishes selective protein degradation via ATP hydrolysis and the tagging of client protein with a polyubiquitin chain. In a series of three cooperative catalytic reactions mediated by the E1 (ubiquitin activating), E2 (ubiquitin conjugating), and E3 (ubiquitin ligase) enzymes, four or more ubiquitin monomers are covalently attached to proteins selected for destruction by the 26S proteasome. Because E3 ligases catalyze the final and rate-limiting step of the ubiquitination cascade, research efforts have been directed toward identifying the determinants of substrate selection. However, less than half of the known E3 ligases harbor enzymatic activity by themselves; most require interaction with the proper E2 in order to correctly target substrates for degradation [82]. These E2-E3 relationships in developing or atrophying skeletal muscle are for the most part unknown.

Many catabolic conditions correspond with an increase the expression or activity of a number of E3 ligases, some which exist broadly in different cell types and others with expression exclusive to skeletal muscle [83-85]. Two muscle-specific E3 ubiquitin ligases, named Muscle RING Finger 1 (MuRF1) and Muscle Atrophy F-box (MAFbx)/Atrogin-1 are well-studied in their expression levels during various states of muscle catabolism and atrophy [84]. Other E3 ligases such as Nedd4-1, Trim32 and TRAF6 play critical roles in different models of atrophy and different stages of muscle development [71]. A full list of E3 ligases relevant to the control of muscle mass has yet to be generated, as does the identity of the E2s that pair with

them. A detailed understanding of this level of control will reveal novel means to improve environmental resilience.

6. Signaling pathways coordinate muscle protein balance

Intracellular signaling events are initiated by a variety of chemical signals that reflect nutrition and hormonal status (e.g., insulin/IGF-I), energy state and activity (e.g., AMP kinase, phosphatidic acid), and other mediators of environmental stress (e.g., glucocorticoids, cytokines) (Figure 1). A key point of integration in muscle growth and development is the protein kinase B/Akt kinase [86]. The insulin/IGF-I- Akt pathway increases MPS via inhibiting glycogen synthase kinase 3 β (an inhibitor of eIF2 ternary complex formation) and activating mechanistic target of rapamycin complex 1 (mTORC1) signaling [87]. Akt also reduces MPB via phosphorylation of the Forkhead box class O (FOXO) transcription factors [70,88].

Assembly of mTORC1, a major hub for sensing nutrients, redox and energy state, consists of the core proteins mTOR, Raptor and mLST8, and can include associated ancillary proteins such as Rag A-D, Deptor, PRAS40 and Rheb-GTP [89]. A second complex called mTORC2 is required for Akt signaling to FOXO but the role of mTORC2 in muscle growth is unclear [87] [90]. The activity of mTORC1 is controlled by its localization and assembly at or near the lysosome [91]. Increased mTORC1 lysosomal assembly and signaling corresponds with increased MPS at the translation initiation step and inhibition of autophagy whereas reduced mTORC1 assembly and signaling lowers MPS and increases autophagy. The ubiquitin proteasome pathway is not directly inhibited by mTORC1 but rather is regulated by PI3K/Akt signaling upstream of mTORC1 [86]. In general, anabolic stimuli (e.g., growth hormone, insulin/IGF-I, amino acids, testosterone, β -agonist) activates the mTORC1 signaling pathway whereas catabolic stimuli (e.g., inflammatory cytokines, glucocorticoids, myostatin, fasting, low protein) represses mTORC1 signaling [73,92,93]. Nutrients, especially the branched chain

amino acids, are potent activators of mTORC1 in muscle independent of insulin/IGF-I-Akt [52,54]. Likewise growth factors can stimulate mTORC1 signaling in skeletal muscle independent of amino acid nutrition [94]. A variety of plant steroid compounds called phytoecdysteroids are found to increase protein synthesis and activate Akt signaling similarly to IGF-I in cultured myocytes [95,96]. Feeding these and other phytoecdysteroids produce an anti-obesity effect in mice [97]. Nonetheless, recent feeding trials are unable to identify an acute impact of 20-hydroxyecdysone on Akt or mTORC1 signaling in skeletal muscle [98] suggesting that phytoecdysteroids may require other factors for activity and/or regulate longer-term transcriptional changes in MPB versus signaling mechanisms that regulate MPS.

The control of gene expression through key transcription factors plays a major role in regulating muscle mass. Many of these proteins such as the FOXO family promotes muscle atrophy through increased expression of both E3 ubiquitin ligases as well as inducing autophagosome membrane components [70]. These discoveries are revealing how the ubiquitin proteasome and autophagy lysosome pathways often work together versus separate from each other. Furthermore, hormones and other growth factors can alter the activities and function of both mTORC1 and transcription factors [73,83]. For example, insulin/IGF-I treatment promotes increases Akt signaling, which promotes mTORC1 complex assembly and MPS but also inhibits MPB via reduced proteolytic gene expression under control of the FOXO transcription factors [86,87]. Myostatin treatment blocks Akt, which reduces MPS via mTORC1 complex assembly and increases MPB by activating the FOXO transcription factors [99] as well as other transcriptional regulators such as SMAD family members [100].

An emerging pathway of muscle mass control involves regulation of the Activating Transcription Factor 4 (ATF4) transcription factor [101]. Early studies in non-myofibrillar tissues characterized ATF4 as part of the integrated stress response, a signaling pathway that controls adaptation to nutrient and oxidative stress by altering gene-specific translation and the

transcriptome [102,103]. Knockout and transgenic studies in rodents find that ATF4 is necessary and sufficient for muscle atrophy during fasting and aging [101,104]. Subsequently, unbiased mRNA expression signatures of muscle atrophy in combination with connectivity maps identified two natural compounds, ursolic acid and tomatidine, as small molecule inhibitors of ATF4 activity which prevent age-related loss of muscle mass [105-107]. Because these compounds are naturally occurring, the potential for their use as dietary additives is feasible. This innovative approach highlights the potential of mRNA expression signatures to discover novel regulators of protein muscle mass for use in domestic animal production [108].

7. Proteostasis meets hormesis

A loss of proteostasis control can speed up the aging process whereas enhancement of the proteostasis network extends lifespan and promotes disease resistance [40]. Current evidence suggests that dietary restriction or reductions in insulin/IGF-I signaling improves proteostasis in association with increased life span [20,109-111]. However, these life span benefits can associate with reduced fertility and/or increased development time [112,113]. The balance between adverse (reduced fecundity and growth) versus beneficial (increased resistance to environmental stress and disease) outcomes can be seen as a function of preconditioning or hormesis in which physiological outcomes are influenced by exposure level or dose [114]. Hormetic responses by definition are multi-phasic, and in certain dose ranges, both adverse and beneficial outcomes can coexist or overlap [115]. Benefits of hormesis can also be 'off-target', so that exposure to one type of stress can impart protection from another, different types of stress. These concepts are relevant to the study of quality versus quantity of muscle mass, not only with respect to an animal's age and sex, but also with respect to future progeny. For example, previous work in cows suggested that during nutrient restriction, muscle protein degradation pathways were activated in pregnant cows but not their fetuses [116]. However, a recent study in rats shows that protein restriction during pregnancy limits muscle

growth capacity of male progeny later in life [117] and a another study in pigs shows that feeding a high protein diet during gestation permanently alters the offspring's genome [118]. These data highlight how adaptive responses to protein nutrition can cross generations in their influence. In addition to nutrition, climate conditions are a major influence on the development, performance and health of livestock [119] yet much remains unknown regarding the regulation of protein balance under these conditions. In an age of increased environmental pressures, these factors are important to consider in the search for novel therapeutic and prophylactic strategies to optimize if not maximize muscle growth and meat production.

Acknowledgments

The author gratefully acknowledges research funding from the National Institutes of Health (grant HD070487), NJ Agricultural Experimental Station and USDA NIFA Multistate (NC1184).

References

- [1] WHO. **Availability and changes in consumption of animal products.** In: Nutrition health topics: Global and regional food consumption patterns and trends, 2015, http://www.who.int/nutrition/topics/3_foodconsumption/en/index4.html.
- [2] Post, M.J. **Cultured meat from stem cells: Challenges and prospects.** *Meat science*. 2012;92:297-301.
- [3] Humane Society of the United States. **An HSUS report: Welfare issues with selective breeding for rapid growth in broiler chickens and turkeys.** pp 1-9, http://www.humanesociety.org/assets/pdfs/farm/welfiss_breeding_chickens_turkeys.pdf.
- [4] Bassett, A. **Welfare and belgian blue cattle.** In: Animal Welfare Approved Technical Advice Fact Sheet, Animal Welfare Approved, 2009, pp 1-8, <http://animalwelfareapproved.org/wp-content/uploads/2013/07/TAFS-1-Welfare-and-Belgian-Blue-Cattle-v1.pdf>.
- [5] Zuo, J., Xu, M., Abdullahi, Y.A., Ma, L., Zhang, Z., Feng, D. **Constant heat stress reduces skeletal muscle protein deposition in broilers.** *J Sci Food Agric*. 2015;95:429-436.
- [6] Boggess, M.V., Lippolis, J.D., Hurkman, W.J., Fagerquist, C.K., Briggs, S.P., Gomes, A.V., Righetti, P.G., Bala, K. **The need for agriculture phenotyping: "Moving from genotype to phenotype".** *J Proteomics*. 2013;93:20-39.
- [7] Tome, D. **Criteria and markers for protein quality assessment - a review.** *Br J Nutr*. 2012;108 Suppl 2:S222-229.
- [8] Kopple, J.D. **Uses and limitations of the balance technique.** *JPEN J Parenter Enteral Nutr*. 1987;11:79S-85S.
- [9] Millward, D.J., Garlick, P.J., James, W.P., Nnanyelugo, D.O., Ryatt, J.S. **Relationship between protein synthesis and RNA content in skeletal muscle.** *Nature*. 1973;241:204-205.

344 [10] Fu, W., Duan, Y., Wang, S., Ni, Y., Grossmann, R., Zhao, R. **Comparative proteomic**
345 **analysis of the breast muscle response to chronic corticosterone administration in**
346 **broiler chickens showing long or short tonic immobility.** *Poult Sci.* 2014;93:784-793.

347 [11] Gautsch, T.A., Kandl, S.M., Donovan, S.M., Layman, D.K. **Response of the IGF-I**
348 **system to prolonged undernutrition and its involvement in somatic and skeletal muscle**
349 **growth retardation in rats.** *Growth Dev Aging.* 1998;62:13-25.

350 [12] Fiorotto, M.L., Sosa, H.A., Jr., Davis, T.A. **In vivo measurement of muscle protein**
351 **synthesis rate using the flooding dose technique.** *Methods Mol Biol.* 2012;798:245-264.

352 [13] Smith, G.I., Patterson, B.W., Klein, S.J., Mittendorfer, B. **Effect of hyperinsulinaemia-**
353 **hyperaminoacidaemia on leg muscle protein synthesis and breakdown: Reassessment of**
354 **the two-pool arterio-venous balance model.** *J Physiol.* 2015;593:4245-4257.

355 [14] Holm, L., Kjaer, M. **Measuring protein breakdown rate in individual proteins in vivo.**
356 *Curr Opin Clin Nutr Metabol Care.* 2010;13:526-531.

357 [15] Holm, L., O'Rourke, B., Ebenstein, D., Toth, M.J., Bechshoeft, R., Holstein-Rathlou,
358 N.H., Kjaer, M., Matthews, D.E. **Determination of steady-state protein breakdown rate in**
359 **vivo by the disappearance of protein-bound tracer-labeled amino acids: A method**
360 **applicable in humans.** *Am J Physiol Endocrinol Metabol.* 2013;304:E895-907.

361 [16] Price, J.C., Ghaemmaghami, S. **Analysis of proteome dynamics in mice by isotopic**
362 **labeling.** *Methods Mol Biol.* 2014;1156:111-131.

363 [17] Tuvdendorj, D., Chinkes, D.L., Herndon, D.N., Zhang, X.J., Wolfe, R.R. **A novel stable**
364 **isotope tracer method to measure muscle protein fractional breakdown rate during a**
365 **physiological non-steady-state condition.** *Am J Physiol Endocrinol Metabol.* 2013;304:E623-
366 630.

367 [18] Busch, R., Kim, Y.K., Neese, R.A., Schade-Serin, V., Collins, M., Awada, M., Gardner,
368 J.L., Beysen, C., Marino, M.E., Misell, L.M., Hellerstein, M.K. **Measurement of protein**

369 **turnover rates by heavy water labeling of nonessential amino acids.** *Biochim Biophys Acta.*
370 2006;1760:730-744.

371 [19] Gasier, H.G., Fluckey, J.D., Previs, S.F. **The application of $^2\text{H}_2\text{O}$ to measure skeletal**
372 **muscle protein synthesis.** *Nutr Metab (Lond).* 2010;7:31.

373 [20] Miller, B.F., Drake, J.C., Naylor, B., Price, J.C., Hamilton, K.L. **The measurement of**
374 **protein synthesis for assessing proteostasis in studies of slowed aging.** *Ageing Res Rev.*
375 2014;18:106-111.

376 [21] Burd, N.A., Tardif, N., Rooyackers, O., van Loon, L.J. **Optimizing the measurement of**
377 **mitochondrial protein synthesis in human skeletal muscle.** *Appl Physiol Nutr Metabol.*
378 2015;40:1-9.

379 [22] Jaleel, A., Short, K.R., Asmann, Y.W., Klaus, K.A., Morse, D.M., Ford, G.C., Nair, K.S. **In**
380 **vivo measurement of synthesis rate of individual skeletal muscle mitochondrial proteins.**
381 *Am J Physiol Endocrinol Metabol.* 2008;295:E1255-1268.

382 [23] Miller, B.F., Wolff, C.A., Peelor, F.F., 3rd, Shipman, P.D., Hamilton, K.L. **Modeling the**
383 **contribution of individual proteins to mixed skeletal muscle protein synthetic rates over**
384 **increasing periods of label incorporation.** *J Appl Physiol (1985).* 2015;118:655-661.

385 [24] Papageorgopoulos, C., Caldwell, K., Schweingrubber, H., Neese, R.A., Shackleton,
386 C.H., Hellerstein, M. **Measuring synthesis rates of muscle creatine kinase and myosin with**
387 **stable isotopes and mass spectrometry.** *Anal Biochem.* 2002;309:1-10.

388 [25] Goodman, C.A., Mabrey, D.M., Frey, J.W., Miu, M.H., Schmidt, E.K., Pierre, P.,
389 Hornberger, T.A. **Novel insights into the regulation of skeletal muscle protein synthesis as**
390 **revealed by a new nonradioactive in vivo technique.** *FASEB J.* 2011;25:1028-1039.

391 [26] Goodman, C.A., Hornberger, T.A. **Measuring protein synthesis with sunset: A valid**
392 **alternative to traditional techniques?** *Exerc Sport Sci Rev.* 2013;41:107-115.

393 [27] Aviner, R., Geiger, T., Elroy-Stein, O. **Novel proteomic approach (PUNCH-P) reveals**
394 **cell cycle-specific fluctuations in mrna translation.** *Genes Dev.* 2013;27:1834-1844.

395 [28] Aviner, R., Geiger, T., Elroy-Stein, O. **Genome-wide identification and quantification**
396 **of protein synthesis in cultured cells and whole tissues by puromycin-associated**
397 **nascent chain proteomics (PUNCH-P).** *Nat Protoc.* 2014;9:751-760.

398 [29] Li, Q. **Advances in protein turnover analysis at the global level and biological**
399 **insights.** *Mass Spectrom Rev.* 2010;29:717-736.

400 [30] Kuersten, S., Radek, A., Vogel, C., Penalva, L.O. **Translation regulation gets its**
401 **'omics' moment.** *Wiley Interdiscip Rev RNA.* 2013;4:617-630.

402 [31] Ingolia, N.T., Brar, G.A., Rouskin, S., McGeachy, A.M., Weissman, J.S. **Genome-wide**
403 **annotation and quantitation of translation by ribosome profiling.** *Curr Protoc Mol Biol.*
404 2013;Chapter 4:Unit 4 18.

405 [32] Gao, X., Wan, J., Liu, B., Ma, M., Shen, B., Qian, S.B. **Quantitative profiling of**
406 **initiating ribosomes in vivo.** *Nat Methods.* 2015;12:147-153.

407 [33] Jackson, R., Standart, N. **The awesome power of ribosome profiling.** *RNA.*
408 2015;21:652-654.

409 [34] Ingolia, N.T., Brar, G.A., Rouskin, S., McGeachy, A.M., Weissman, J.S. **The ribosome**
410 **profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-**
411 **protected mRNA fragments.** *Nat Protoc.* 2012;7:1534-1550.

412 [35] King, H.A., Gerber, A.P. **Translatome profiling: Methods for genome-scale analysis**
413 **of mRNA translation.** *Brief Funct Genomics.* 2014.

414 [36] Jung, W.Y., Kwon, S.G., Son, M., Cho, E.S., Lee, Y., Kim, J.H., Kim, B.W., Park da, H.,
415 Hwang, J.H., Kim, T.W., Park, H.C., Park, B.Y., Choi, J.S., Cho, K.K., Chung, K.H., Song, Y.M.,
416 Kim, I.S., Jin, S.K., Kim, D.H., Lee, S.W., Lee, K.W., Bang, W.Y., Kim, C.W. **RNA-seq**
417 **approach for genetic improvement of meat quality in pig and evolutionary insight into the**
418 **substrate specificity of animal carbonyl reductases.** *PLoS ONE.* 2012;7:e42198.

419 [37] Sodhi, S.S., Song, K.D., Ghosh, M., Sharma, N., Lee, S.J., Kim, J.H., Kim, N., Mongre,
420 R.K., Adhikari, P., Kim, J.Y., Hong, S.P., Oh, S.J., Jeong, D.K. **Comparative transcriptomic**

analysis by RNA-seq to discern differential expression of genes in liver and muscle tissues of adult berkshire and jeju native pig. *Gene*. 2014;546:233-242.

[38] Morimoto, R.I., Cuervo, A.M. **Proteostasis and the aging proteome in health and disease.** *J Gerontol A Biol Sci Med Sci*. 2014;69 Suppl 1:S33-38.

[39] Noack, J., Brambilla Pisoni, G., Molinari, M. **Proteostasis: Bad news and good news from the endoplasmic reticulum.** *Swiss Med Wkly*. 2014;144:w14001.

[40] Labbadia, J., Morimoto, R.I. **Proteostasis and longevity: When does aging really begin?** *F1000prime reports*. 2014;6:7.

[41] Rivera-Ferre, M.G., Aguilera, J.F., Nieto, R. **Muscle fractional protein synthesis is higher in iberian than in landrace growing pigs fed adequate or lysine-deficient diets.** *J Nutr*. 2005;135:469-478.

[42] Xu, Y.J., Jin, M.L., Wang, L.J., Zhang, A.D., Zuo, B., Xu, D.Q., Ren, Z.Q., Lei, M.G., Mo, X.Y., Li, F.E., Zheng, R., Deng, C.Y., Xiong, Y.Z. **Differential proteome analysis of porcine skeletal muscles between meishan and large white.** *J Anim Sci*. 2009;87:2519-2527.

[43] Davis, T.A., Suryawan, A., Orellana, R.A., Nguyen, H.V., Fiorotto, M.L. **Postnatal ontogeny of skeletal muscle protein synthesis in pigs.** *J Anim Sci*. 2008;86:E13-18.

[44] Jefferson, L.S., Kimball, S.R. **Translational control of protein synthesis: Implications for understanding changes in skeletal muscle mass.** *Int J Sport Nutr Exer Metabol*. 2001;11 Suppl:S143-149.

[45] Wilson, F.A., Suryawan, A., Orellana, R.A., Kimball, S.R., Gazzaneo, M.C., Nguyen, H.V., Fiorotto, M.L., Davis, T.A. **Feeding rapidly stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing translation initiation.** *J Nutr*. 2009;139:1873-1880.

[46] Kimball, S.R., Jefferson, L.S., Nguyen, H.V., Suryawan, A., Bush, J.A., Davis, T.A. **Feeding stimulates protein synthesis in muscle and liver of neonatal pigs through an mTOR-dependent process.** *American journal of physiology Endocrinology and metabolism*. 2000;279:E1080-1087.

- [47] Liu, Y., Li, F., Kong, X., Tan, B., Li, Y., Duan, Y., Blachier, F., Hu, C.A., Yin, Y. **Signaling pathways related to protein synthesis and amino acid concentration in pig skeletal muscles depend on the dietary protein level, genotype and developmental stages.** *PLoS ONE*. 2015;10:e0138277.
- [48] Davis, T.A., Fiorotto, M.L., Suryawan, A. **Bolus vs. Continuous feeding to optimize anabolism in neonates.** *Curr Opin Clin Nutr Metabol Care*. 2015;18:102-108.
- [49] Goodman, C.A., Mayhew, D.L., Hornberger, T.A. **Recent progress toward understanding the molecular mechanisms that regulate skeletal muscle mass.** *Cell Signal*. 2011;23:1896-1906.
- [50] Laufenberg, L.J., Pruznak, A.M., Navaratnarajah, M., Lang, C.H. **Sepsis-induced changes in amino acid transporters and leucine signaling via mTOR in skeletal muscle.** *Amino Acids*. 2014;46:2787-2798.
- [51] You, J.S., Anderson, G.B., Dooley, M.S., Hornberger, T.A. **The role of mTOR signaling in the regulation of protein synthesis and muscle mass during immobilization in mice.** *Disease models & mechanisms*. 2015;8:1059-1069.
- [52] Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., Anthony, T.G., Watford, M., Hou, Y., Wu, G., Yin, Y. **The role of leucine and its metabolites in protein and energy metabolism.** *Amino Acids*. 2016;48:41-51.
- [53] Anthony, J.C., Anthony, T.G., Kimball, S.R., Jefferson, L.S. **Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine.** *J Nutr*. 2001;131:856S-860S.
- [54] Columbus, D.A., Fiorotto, M.L., Davis, T.A. **Leucine is a major regulator of muscle protein synthesis in neonates.** *Amino Acids*. 2015;47:259-270.
- [55] Qiao, X., Zhang, H.J., Wu, S.G., Yue, H.Y., Zuo, J.J., Feng, D.Y., Qi, G.H. **Effect of beta-hydroxy-beta-methylbutyrate calcium on growth, blood parameters, and carcass qualities of broiler chickens.** *Poult Sci*. 2013;92:753-759.

473 [56] Wheatley, S.M., El-Kadi, S.W., Suryawan, A., Boutry, C., Orellana, R.A., Nguyen, H.V.,
 474 Davis, S.R., Davis, T.A. **Protein synthesis in skeletal muscle of neonatal pigs is enhanced**
 475 **by administration of beta-hydroxy-beta-methylbutyrate.** *Am J Physiol Endocrinol Metabol.*
 476 2014;306:E91-99.

477 [57] Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., Anthony, T.G., Watford, M., Hou, Y.,
 478 Wu, G., Yin, Y. **The role of leucine and its metabolites in protein and energy metabolism.**
 479 *Amino Acids.* 2015.

480 [58] Gazzaneo, M.C., Suryawan, A., Orellana, R.A., Torrazza, R.M., El-Kadi, S.W., Wilson,
 481 F.A., Kimball, S.R., Srivastava, N., Nguyen, H.V., Fiorotto, M.L., Davis, T.A. **Intermittent bolus**
 482 **feeding has a greater stimulatory effect on protein synthesis in skeletal muscle than**
 483 **continuous feeding in neonatal pigs.** *J Nutr.* 2011;141:2152-2158.

484 [59] Mitchell, W.K., Phillips, B.E., Williams, J.P., Rankin, D., Lund, J.N., Wilkinson, D.J.,
 485 Smith, K., Atherton, P.J. **The impact of delivery profile of essential amino acids upon**
 486 **skeletal muscle protein synthesis in older men: Clinical efficacy of pulse vs. Bolus**
 487 **supply.** *Am J Physiol Endocrinol Metabol.* 2015;309:E450-457.

488 [60] Pasiakos, S.M., McLellan, T.M., Lieberman, H.R. **The effects of protein supplements**
 489 **on muscle mass, strength, and aerobic and anaerobic power in healthy adults: A**
 490 **systematic review.** *Sports Med.* 2015;45:111-131.

491 [61] Layman, D.K., Anthony, T.G., Rasmussen, B.B., Adams, S.H., Lynch, C.J., Brinkworth,
 492 G.D., Davis, T.A. **Defining meal requirements for protein to optimize metabolic roles of**
 493 **amino acids.** *Am J Clin Nutr.* 2015.

494 [62] Arnal, M.A., Mosoni, L., Dardevet, D., Ribeyre, M.C., Bayle, G., Prugnaud, J., Patureau
 495 Mirand, P. **Pulse protein feeding pattern restores stimulation of muscle protein synthesis**
 496 **during the feeding period in old rats.** *J Nutr.* 2002;132:1002-1008.

497 [63] Columbus, D.A., Steinhoff-Wagner, J., Suryawan, A., Nguyen, H.V., Hernandez-Garcia,
 498 A., Fiorotto, M.L., Davis, T.A. **Impact of prolonged leucine supplementation on protein**

499 **synthesis and lean growth in neonatal pigs.** *Am J Physiol Endocrinol Metabol.*
500 2015;309:E601-610.

501 [64] Pasiakos, S.M., McClung, J.P. **Supplemental dietary leucine and the skeletal muscle**
502 **anabolic response to essential amino acids.** *Nutr Rev.* 2011;69:550-557.

503 [65] Goodband, B., Tokach, M., Dritz, S., Derouchey, J., Woodworth, J. **Practical starter pig**
504 **amino acid requirements in relation to immunity, gut health and growth performance.** *J*
505 *Anim Sci Biotechnol.* 2014;5:12.

506 [66] Otten, C., Berk, A., Muller, S., Weber, M., Danicke, S. **Influence of dietary amino acid**
507 **level on chemical body composition and performance of growing-finishing boars of two**
508 **sire lines.** *Arch Anim Nutr.* 2013;67:477-491.

509 [67] Madeira, M.S., Costa, P., Alfaia, C.M., Lopes, P.A., Bessa, R.J., Lemos, J.P., Prates,
510 J.A. **The increased intramuscular fat promoted by dietary lysine restriction in lean but not**
511 **in fatty pig genotypes improves pork sensory attributes.** *J Anim Sci.* 2013;91:3177-3187.

512 [68] Greising, S.M., Gransee, H.M., Mantilla, C.B., Sieck, G.C. **Systems biology of skeletal**
513 **muscle: Fiber type as an organizing principle.** *Wiley Interdiscip Rev Syst Biol Med.*
514 2012;4:457-473.

515 [69] Pasiakos, S.M., Carbone, J.W. **Assessment of skeletal muscle proteolysis and the**
516 **regulatory response to nutrition and exercise.** *IUBMB Life.* 2014;66:478-484.

517 [70] Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J.H., Frasson, L., Seydel, A.,
518 Zhao, J., Abraham, R., Goldberg, A.L., Blaauw, B., DePinho, R.A., Sandri, M. **Regulation of**
519 **autophagy and the ubiquitin-proteasome system by the FOXO transcriptional network**
520 **during muscle atrophy.** *Nat Commun.* 2015;6:6670.

521 [71] Sandri, M. **Protein breakdown in muscle wasting: Role of autophagy-lysosome and**
522 **ubiquitin-proteasome.** *Int J Biochem Cell Biol.* 2013;45:2121-2129.

523 [72] Arndt, V., Dick, N., Tawo, R., Dreiseidler, M., Wenzel, D., Hesse, M., Furst, D.O., Saftig,
524 P., Saint, R., Fleischmann, B.K., Hoch, M., Hohfeld, J. **Chaperone-assisted selective**
525 **autophagy is essential for muscle maintenance.** *Curr Biol.* 2010;20:143-148.

526 [73] Bonaldo, P., Sandri, M. **Cellular and molecular mechanisms of muscle atrophy.** *Dis*
527 *Models Mech.* 2013;6:25-39.

528 [74] Boya, P., Reggiori, F., Codogno, P. **Emerging regulation and functions of**
529 **autophagy.** *Nat Cell Biol.* 2013;15:713-720.

530 [75] Sanchez, A.M., Bernardi, H., Py, G., Candau, R.B. **Autophagy is essential to support**
531 **skeletal muscle plasticity in response to endurance exercise.** *Am J Physiol Regul Integr*
532 *Comp Physiol.* 2014;307:R956-969.

533 [76] Vainshtein, A., Grumati, P., Sandri, M., Bonaldo, P. **Skeletal muscle, autophagy, and**
534 **physical activity: The menage a trois of metabolic regulation in health and disease.** *J Mol*
535 *Med (Berl).* 2014;92:127-137.

536 [77] Fan, J., Kou, X., Jia, S., Yang, X., Yang, Y., Chen, N. **Autophagy as a potential target**
537 **for sarcopenia.** *J Cell Physiol.* 2015.

538 [78] Cacciottolo, M., Nogalska, A., D'Agostino, C., Engel, W.K., Askanas, V. **Chaperone-**
539 **mediated autophagy components are upregulated in sporadic inclusion-body myositis**
540 **muscle fibres.** *Neuropathol Appl Neurobiol.* 2013;39:750-761.

541 [79] Leduc-Gaudet, J.P., Picard, M., St-Jean Pelletier, F., Sgarioto, N., Auger, M.J., Vallee,
542 J., Robitaille, R., St-Pierre, D.H., Gouspillou, G. **Mitochondrial morphology is altered in**
543 **atrophied skeletal muscle of aged mice.** *Oncotarget.* 2015;6:17923-17937.

544 [80] Suryawan, A., Davis, T.A. **Regulation of protein degradation pathways by amino**
545 **acids and insulin in skeletal muscle of neonatal pigs.** *J Anim Sci Biotechnol.* 2014;5:8.

546 [81] Klionsky, D.J., Abdalla, F.C., Abeliovich, H., Abraham, R.T., Acevedo-Arozena, A., Adeli,
547 K., Agholme, L., Agnello, M., Agostinis, P., Aguirre-Ghiso, J.A., Ahn, H.J., Ait-Mohamed, O., Ait-
548 Si-Ali, S., Akematsu, T., Akira, S., Al-Younes, H.M., Al-Zeer, M.A., Albert, M.L., Albin, R.L.,

549 Alegre-Abarrategui, J., Aleo, M.F., Alirezaei, M., Almasan, A., Almonte-Becerril, M., Amano, A.,
 550 Amaravadi, R., Amarnath, S., Amer, A.O., Andrieu-Abadie, N., Anantharam, V., Ann, D.K.,
 551 Anoopkumar-Dukie, S., Aoki, H., Apostolova, N., Arancia, G., Aris, J.P., Asanuma, K., Asare,
 552 N.Y., Ashida, H., Askanas, V., Askew, D.S., Auberger, P., Baba, M., Backues, S.K., Baehrecke,
 553 E.H., Bahr, B.A., Bai, X.Y., Bailly, Y., Baiocchi, R., Baldini, G., Balduini, W., Ballabio, A.,
 554 Bamber, B.A., Bampton, E.T., Banhegyi, G., Bartholomew, C.R., Bassham, D.C., Bast, R.C., Jr.,
 555 Batoko, H., Bay, B.H., Beau, I., Bechet, D.M., Begley, T.J., Behl, C., Behrends, C., Bekri, S.,
 556 Bellaire, B., Bendall, L.J., Benetti, L., Berliocchi, L., Bernardi, H., Bernassola, F., Besteiro, S.,
 557 Bhatia-Kissova, I., Bi, X., Biard-Piechaczyk, M., Blum, J.S., Boise, L.H., Bonaldo, P., Boone,
 558 D.L., Bornhauser, B.C., Bortoluci, K.R., Bossis, I., Bost, F., Bourquin, J.P., Boya, P., Boyer-
 559 Guittaut, M., Bozhkov, P.V., Brady, N.R., Brancolini, C., Brech, A., Brenman, J.E., Brennand, A.,
 560 Bresnick, E.H., Brest, P., Bridges, D., Bristol, M.L., Brookes, P.S., Brown, E.J., Brumell, J.H.,
 561 Brunetti-Pierri, N., Brunk, U.T., Bulman, D.E., Bultman, S.J., Bultynck, G., Burbulla, L.F.,
 562 Bursch, W., Butchar, J.P., Buzgariu, W., Bydlowski, S.P., Cadwell, K., Cahova, M., Cai, D., Cai,
 563 J., Cai, Q., Calabretta, B., Calvo-Garrido, J., Camougrand, N., Campanella, M., Campos-
 564 Salinas, J., Candi, E., Cao, L., Caplan, A.B., Carding, S.R., Cardoso, S.M., Carew, J.S., Carlin,
 565 C.R., Carmignac, V., Carneiro, L.A., Carra, S., Caruso, R.A., Casari, G., Casas, C., Castino, R.,
 566 Cebollero, E., Cecconi, F., Celli, J., Chaachouay, H., Chae, H.J., Chai, C.Y., Chan, D.C., Chan,
 567 E.Y., Chang, R.C., Che, C.M., Chen, C.C., Chen, G.C., Chen, G.Q., Chen, M., Chen, Q., Chen,
 568 S.S., Chen, W., Chen, X., Chen, Y.G., Chen, Y., Chen, Y.J., Chen, Z., Cheng, A., Cheng, C.H.,
 569 Cheng, Y., Cheong, H., Cheong, J.H., Cherry, S., Chess-Williams, R., Cheung, Z.H., Chevet, E.,
 570 Chiang, H.L., Chiarelli, R., Chiba, T., Chin, L.S., Chiou, S.H., Chisari, F.V., Cho, C.H., Cho,
 571 D.H., Choi, A.M., Choi, D., Choi, K.S., Choi, M.E., Chouaib, S., Choubey, D., Choubey, V., Chu,
 572 C.T., Chuang, T.H., Chueh, S.H., Chun, T., Chwae, Y.J., Chye, M.L., Ciarcia, R., Ciriolo, M.R.,
 573 Clague, M.J., Clark, R.S., Clarke, P.G., Clarke, R., Codogno, P., Collier, H.A., Colombo, M.I.,
 574 Comincini, S., Condello, M., Condorelli, F., Cookson, M.R., Coombs, G.H., Coppens, I.,

575 Corbalan, R., Cossart, P., Costelli, P., Costes, S., Coto-Montes, A., Couve, E., Coxon, F.P.,
 576 Cregg, J.M., Crespo, J.L., Cronje, M.J., Cuervo, A.M., Cullen, J.J., Czaja, M.J., D'Amelio, M.,
 577 Darfeuille-Michaud, A., Davids, L.M., Davies, F.E., De Felici, M., de Groot, J.F., de Haan, C.A.,
 578 De Martino, L., De Mito, A., De Tata, V., Debnath, J., Degterev, A., Dehay, B., Delbridge, L.M.,
 579 Demarchi, F., Deng, Y.Z., Dengjel, J., Dent, P., Denton, D., Deretic, V., Desai, S.D., Devenish,
 580 R.J., Di Gioacchino, M., Di Paolo, G., Di Pietro, C., Diaz-Araya, G., Diaz-Laviada, I., Diaz-Meco,
 581 M.T., Diaz-Nido, J., Dikic, I., Dinesh-Kumar, S.P., Ding, W.X., Distelhorst, C.W., Diwan, A.,
 582 Djavaheri-Mergny, M., Dokudovskaya, S., Dong, Z., Dorsey, F.C., Dosenko, V., Dowling, J.J.,
 583 Doxsey, S., Dreux, M., Drew, M.E., Duan, Q., Duchosal, M.A., Duff, K., Dugail, I., Durbeej, M.,
 584 Duszenko, M., Edelstein, C.L., Edinger, A.L., Egea, G., Eichinger, L., Eissa, N.T., Ekmekcioglu,
 585 S., El-Deiry, W.S., Elazar, Z., Elgendy, M., Ellerby, L.M., Eng, K.E., Engelbrecht, A.M.,
 586 Engelender, S., Erenpreisa, J., Escalante, R., Esclatine, A., Eskelinen, E.L., Espert, L., Espina,
 587 V., Fan, H., Fan, J., Fan, Q.W., Fan, Z., Fang, S., Fang, Y., Fanto, M., Fanzani, A., Farkas, T.,
 588 Farre, J.C., Faure, M., Fechheimer, M., Feng, C.G., Feng, J., Feng, Q., Feng, Y., Fesus, L.,
 589 Feuer, R., Figueiredo-Pereira, M.E., Fimia, G.M., Fingar, D.C., Finkbeiner, S., Finkel, T., Finley,
 590 K.D., Fiorito, F., Fisher, E.A., Fisher, P.B., Flajolet, M., Florez-McClure, M.L., Florio, S., Fon,
 591 E.A., Fornai, F., Fortunato, F., Fotedar, R., Fowler, D.H., Fox, H.S., Franco, R., Frankel, L.B.,
 592 Fransen, M., Fuentes, J.M., Fueyo, J., Fujii, J., Fujisaki, K., Fujita, E., Fukuda, M., Furukawa,
 593 R.H., Gaestel, M., Gailly, P., Gajewska, M., Galliot, B., Galy, V., Ganesh, S., Ganetzky, B.,
 594 Ganley, I.G., Gao, F.B., Gao, G.F., Gao, J., Garcia, L., Garcia-Manero, G., Garcia-Marcos, M.,
 595 Garmyn, M., Gartel, A.L., Gatti, E., Gautel, M., Gawriluk, T.R., Gegg, M.E., Geng, J., Germain,
 596 M., Gestwicki, J.E., Gewirtz, D.A., Ghavami, S., Ghosh, P., Giammarioli, A.M., Giatromanolaki,
 597 A.N., Gibson, S.B., Gilkerson, R.W., Ginger, M.L., Ginsberg, H.N., Golab, J., Goligorsky, M.S.,
 598 Golstein, P., Gomez-Manzano, C., Goncu, E., Gongora, C., Gonzalez, C.D., Gonzalez, R.,
 599 Gonzalez-Estevez, C., Gonzalez-Polo, R.A., Gonzalez-Rey, E., Gorbunov, N.V., Gorski, S.,
 600 Goruppi, S., Gottlieb, R.A., Gozuacik, D., Granato, G.E., Grant, G.D., Green, K.N., Gregorc, A.,

601 Gros, F., Grose, C., Grunt, T.W., Gual, P., Guan, J.L., Guan, K.L., Guichard, S.M., Gukovskaya,
 602 A.S., Gukovsky, I., Gunst, J., Gustafsson, A.B., Halayko, A.J., Hale, A.N., Halonen, S.K.,
 603 Hamasaki, M., Han, F., Han, T., Hancock, M.K., Hansen, M., Harada, H., Harada, M., Hardt,
 604 S.E., Harper, J.W., Harris, A.L., Harris, J., Harris, S.D., Hashimoto, M., Haspel, J.A., Hayashi,
 605 S., Hazelhurst, L.A., He, C., He, Y.W., Hebert, M.J., Heidenreich, K.A., Helfrich, M.H.,
 606 Helgason, G.V., Henske, E.P., Herman, B., Herman, P.K., Hetz, C., Hilfiker, S., Hill, J.A.,
 607 Hocking, L.J., Hofman, P., Hofmann, T.G., Hohfeld, J., Holyoake, T.L., Hong, M.H., Hood, D.A.,
 608 Hotamisligil, G.S., Houwerzijl, E.J., Hoyer-Hansen, M., Hu, B., Hu, C.A., Hu, H.M., Hua, Y.,
 609 Huang, C., Huang, J., Huang, S., Huang, W.P., Huber, T.B., Huh, W.K., Hung, T.H., Hupp, T.R.,
 610 Hur, G.M., Hurley, J.B., Hussain, S.N., Hussey, P.J., Hwang, J.J., Hwang, S., Ichihara, A.,
 611 Ilkhanizadeh, S., Inoki, K., Into, T., Iovane, V., Iovanna, J.L., Ip, N.Y., Isaka, Y., Ishida, H.,
 612 Isidoro, C., Isobe, K., Iwasaki, A., Izquierdo, M., Izumi, Y., Jaakkola, P.M., Jaattela, M.,
 613 Jackson, G.R., Jackson, W.T., Janji, B., Jendrach, M., Jeon, J.H., Jeung, E.B., Jiang, H., Jiang,
 614 J.X., Jiang, M., Jiang, Q., Jiang, X., Jimenez, A., Jin, M., Jin, S., Joe, C.O., Johansen, T.,
 615 Johnson, D.E., Johnson, G.V., Jones, N.L., Joseph, B., Joseph, S.K., Joubert, A.M., Juhasz, G.,
 616 Juillerat-Jeanneret, L., Jung, C.H., Jung, Y.K., Kaarniranta, K., Kaasik, A., Kabuta, T.,
 617 Kadowaki, M., Kagedal, K., Kamada, Y., Kaminsky, V.O., Kampinga, H.H., Kanamori, H.,
 618 Kang, C., Kang, K.B., Kang, K.I., Kang, R., Kang, Y.A., Kanki, T., Kanneganti, T.D., Kanno, H.,
 619 Kanthasamy, A.G., Kanthasamy, A., Karantza, V., Kaushal, G.P., Kaushik, S., Kawazoe, Y., Ke,
 620 P.Y., Kehrl, J.H., Kelekar, A., Kerkhoff, C., Kessel, D.H., Khalil, H., Kiel, J.A., Kiger, A.A.,
 621 Kihara, A., Kim, D.R., Kim, D.H., Kim, E.K., Kim, H.R., Kim, J.S., Kim, J.H., Kim, J.C., Kim, J.K.,
 622 Kim, P.K., Kim, S.W., Kim, Y.S., Kim, Y., Kimchi, A., Kimmelman, A.C., King, J.S., Kinsella, T.J.,
 623 Kirkin, V., Kirshenbaum, L.A., Kitamoto, K., Kitazato, K., Klein, L., Klimecki, W.T., Klucken, J.,
 624 Knecht, E., Ko, B.C., Koch, J.C., Koga, H., Koh, J.Y., Koh, Y.H., Koike, M., Komatsu, M.,
 625 Kominami, E., Kong, H.J., Kong, W.J., Korolchuk, V.I., Kotake, Y., Koukourakis, M.I., Kouri
 626 Flores, J.B., Kovacs, A.L., Kraft, C., Krainc, D., Kramer, H., Kretz-Remy, C., Krichevsky, A.M.,

627 Kroemer, G., Kruger, R., Krut, O., Ktistakis, N.T., Kuan, C.Y., Kucharczyk, R., Kumar, A.,
 628 Kumar, R., Kumar, S., Kundu, M., Kung, H.J., Kurz, T., Kwon, H.J., La Spada, A.R., Lafont, F.,
 629 Lamark, T., Landry, J., Lane, J.D., Lapaquette, P., Laporte, J.F., Laszlo, L., Lavandero, S.,
 630 Lavoie, J.N., Layfield, R., Lazo, P.A., Le, W., Le Cam, L., Ledbetter, D.J., Lee, A.J., Lee, B.W.,
 631 Lee, G.M., Lee, J., Lee, J.H., Lee, M., Lee, M.S., Lee, S.H., Leeuwenburgh, C., Legembre, P.,
 632 Legouis, R., Lehmann, M., Lei, H.Y., Lei, Q.Y., Leib, D.A., Leiro, J., Lemasters, J.J., Lemoine,
 633 A., Lesniak, M.S., Lev, D., Levenson, V.V., Levine, B., Levy, E., Li, F., Li, J.L., Li, L., Li, S., Li,
 634 W., Li, X.J., Li, Y.B., Li, Y.P., Liang, C., Liang, Q., Liao, Y.F., Liberski, P.P., Lieberman, A., Lim,
 635 H.J., Lim, K.L., Lim, K., Lin, C.F., Lin, F.C., Lin, J., Lin, J.D., Lin, K., Lin, W.W., Lin, W.C., Lin,
 636 Y.L., Linden, R., Lingor, P., Lippincott-Schwartz, J., Lisanti, M.P., Liton, P.B., Liu, B., Liu, C.F.,
 637 Liu, K., Liu, L., Liu, Q.A., Liu, W., Liu, Y.C., Liu, Y., Lockshin, R.A., Lok, C.N., Lonial, S., Loos,
 638 B., Lopez-Berestein, G., Lopez-Otin, C., Lossi, L., Lotze, M.T., Low, P., Lu, B., Lu, Z., Luciano,
 639 F., Lukacs, N.W., Lund, A.H., Lynch-Day, M.A., Ma, Y., Macian, F., MacKeigan, J.P., Macleod,
 640 K.F., Madeo, F., Maiuri, L., Maiuri, M.C., Malagoli, D., Malicdan, M.C., Malorni, W., Man, N.,
 641 Mandelkow, E.M., Manon, S., Manov, I., Mao, K., Mao, X., Mao, Z., Marambaud, P., Marazziti,
 642 D., Marcel, Y.L., Marchbank, K., Marchetti, P., Marciniak, S.J., Marcondes, M., Mardi, M., Marfe,
 643 G., Marino, G., Markaki, M., Marten, M.R., Martin, S.J., Martinand-Mari, C., Martinet, W.,
 644 Martinez-Vicente, M., Masini, M., Matarrese, P., Matsuo, S., Matteoni, R., Mayer, A., Mazure,
 645 N.M., McConkey, D.J., McConnell, M.J., McDermott, C., McDonald, C., McInerney, G.M.,
 646 McKenna, S.L., McLaughlin, B., McLean, P.J., McMaster, C.R., McQuibban, G.A., Meijer, A.J.,
 647 Meisler, M.H., Melendez, A., Melia, T.J., Melino, G., Mena, M.A., Menendez, J.A., Menna-
 648 Barreto, R.F., Menon, M.B., Menzies, F.M., Mercer, C.A., Merighi, A., Merry, D.E., Meschini, S.,
 649 Meyer, C.G., Meyer, T.F., Miao, C.Y., Miao, J.Y., Michels, P.A., Michiels, C., Mijaljica, D.,
 650 Milojkovic, A., Minucci, S., Miracco, C., Miranti, C.K., Mitroulis, I., Miyazawa, K., Mizushima, N.,
 651 Mograbi, B., Mohseni, S., Molero, X., Mollereau, B., Mollinedo, F., Momoi, T., Monastyrska, I.,
 652 Monick, M.M., Monteiro, M.J., Moore, M.N., Mora, R., Moreau, K., Moreira, P.I., Moriyasu, Y.,

653 Moscat, J., Mostowy, S., Mottram, J.C., Motyl, T., Moussa, C.E., Muller, S., Munger, K., Munz,
 654 C., Murphy, L.O., Murphy, M.E., Musaro, A., Mysorekar, I., Nagata, E., Nagata, K., Nahimana,
 655 A., Nair, U., Nakagawa, T., Nakahira, K., Nakano, H., Nakatogawa, H., Nanjundan, M., Naqvi,
 656 N.I., Narendra, D.P., Narita, M., Navarro, M., Nawrocki, S.T., Nazarko, T.Y., Nemchenko, A.,
 657 Netea, M.G., Neufeld, T.P., Ney, P.A., Nezis, I.P., Nguyen, H.P., Nie, D., Nishino, I., Nislow, C.,
 658 Nixon, R.A., Noda, T., Noegel, A.A., Nogalska, A., Noguchi, S., Notterpek, L., Novak, I., Nozaki,
 659 T., Nukina, N., Nurnberger, T., Nyfeler, B., Obara, K., Oberley, T.D., Oddo, S., Ogawa, M.,
 660 Ohashi, T., Okamoto, K., Oleinick, N.L., Oliver, F.J., Olsen, L.J., Olsson, S., Opota, O.,
 661 Osborne, T.F., Ostrander, G.K., Otsu, K., Ou, J.H., Ouimet, M., Overholtzer, M., Ozpolat, B.,
 662 Paganetti, P., Pagnini, U., Pallet, N., Palmer, G.E., Palumbo, C., Pan, T., Panaretakis, T.,
 663 Pandey, U.B., Papackova, Z., Papassideri, I., Paris, I., Park, J., Park, O.K., Parys, J.B.,
 664 Parzych, K.R., Patschan, S., Patterson, C., Pattingre, S., Pawelek, J.M., Peng, J., Perlmutter,
 665 D.H., Perrotta, I., Perry, G., Pervaiz, S., Peter, M., Peters, G.J., Petersen, M., Petrovski, G.,
 666 Phang, J.M., Piacentini, M., Pierre, P., Pierrefite-Carle, V., Pierron, G., Pinkas-Kramarski, R.,
 667 Piras, A., Piri, N., Platanias, L.C., Poggeler, S., Poirot, M., Poletti, A., Pous, C., Pozuelo-Rubio,
 668 M., Praetorius-Ibba, M., Prasad, A., Prescott, M., Priault, M., Produit-Zengaffinen, N.,
 669 Progulske-Fox, A., Proikas-Cezanne, T., Przedborski, S., Przyklenk, K., Puertollano, R., Puyal,
 670 J., Qian, S.B., Qin, L., Qin, Z.H., Quaggin, S.E., Raben, N., Rabinowich, H., Rabkin, S.W.,
 671 Rahman, I., Rami, A., Ramm, G., Randall, G., Randow, F., Rao, V.A., Rathmell, J.C.,
 672 Ravikumar, B., Ray, S.K., Reed, B.H., Reed, J.C., Reggiori, F., Regnier-Vigouroux, A., Reichert,
 673 A.S., Reiners, J.J., Jr., Reiter, R.J., Ren, J., Revuelta, J.L., Rhodes, C.J., Ritis, K., Rizzo, E.,
 674 Robbins, J., Roberge, M., Roca, H., Roccheri, M.C., Rocchi, S., Rodemann, H.P., Rodriguez de
 675 Cordoba, S., Rohrer, B., Roninson, I.B., Rosen, K., Rost-Roszkowska, M.M., Rouis, M.,
 676 Rouschop, K.M., Rovetta, F., Rubin, B.P., Rubinsztein, D.C., Ruckdeschel, K., Rucker, E.B.,
 677 3rd, Rudich, A., Rudolf, E., Ruiz-Opazo, N., Russo, R., Rusten, T.E., Ryan, K.M., Ryter, S.W.,
 678 Sabatini, D.M., Sadoshima, J., Saha, T., Saitoh, T., Sakagami, H., Sakai, Y., Salekdeh, G.H.,

679 Salomoni, P., Salvaterra, P.M., Salvesen, G., Salvioli, R., Sanchez, A.M., Sanchez-Alcazar,
 680 J.A., Sanchez-Prieto, R., Sandri, M., Sankar, U., Sansanwal, P., Santambrogio, L., Saran, S.,
 681 Sarkar, S., Sarwal, M., Sasakawa, C., Sasnauskiene, A., Sass, M., Sato, K., Sato, M., Schapira,
 682 A.H., Scharl, M., Schatzl, H.M., Scheper, W., Schiaffino, S., Schneider, C., Schneider, M.E.,
 683 Schneider-Stock, R., Schoenlein, P.V., Schorderet, D.F., Schuller, C., Schwartz, G.K.,
 684 Scorrano, L., Sealy, L., Seglen, P.O., Segura-Aguilar, J., Seiliez, I., Seleverstov, O., Sell, C.,
 685 Seo, J.B., Separovic, D., Setaluri, V., Setoguchi, T., Settembre, C., Shacka, J.J., Shanmugam,
 686 M., Shapiro, I.M., Shaulian, E., Shaw, R.J., Shelhamer, J.H., Shen, H.M., Shen, W.C., Sheng,
 687 Z.H., Shi, Y., Shibuya, K., Shidoji, Y., Shieh, J.J., Shih, C.M., Shimada, Y., Shimizu, S.,
 688 Shintani, T., Shirihai, O.S., Shore, G.C., Sibirny, A.A., Sidhu, S.B., Sikorska, B., Silva-Zacarin,
 689 E.C., Simmons, A., Simon, A.K., Simon, H.U., Simone, C., Simonsen, A., Sinclair, D.A., Singh,
 690 R., Sinha, D., Sinicrope, F.A., Sirko, A., Siu, P.M., Sivridis, E., Skop, V., Skulachev, V.P., Slack,
 691 R.S., Smaili, S.S., Smith, D.R., Soengas, M.S., Soldati, T., Song, X., Sood, A.K., Soong, T.W.,
 692 Sotgia, F., Spector, S.A., Spies, C.D., Springer, W., Srinivasula, S.M., Stefanis, L., Steffan, J.S.,
 693 Stendel, R., Stenmark, H., Stephanou, A., Stern, S.T., Sternberg, C., Stork, B., Stralfors, P.,
 694 Subauste, C.S., Sui, X., Sulzer, D., Sun, J., Sun, S.Y., Sun, Z.J., Sung, J.J., Suzuki, K., Suzuki,
 695 T., Swanson, M.S., Swanton, C., Sweeney, S.T., Sy, L.K., Szabadkai, G., Tabas, I.,
 696 Taegtmeyer, H., Tafani, M., Takacs-Vellai, K., Takano, Y., Takegawa, K., Takemura, G.,
 697 Takeshita, F., Talbot, N.J., Tan, K.S., Tanaka, K., Tang, D., Tanida, I., Tannous, B.A.,
 698 Tavernarakis, N., Taylor, G.S., Taylor, G.A., Taylor, J.P., Terada, L.S., Terman, A., Tettamanti,
 699 G., Thevissen, K., Thompson, C.B., Thorburn, A., Thumm, M., Tian, F., Tian, Y., Tocchini-
 700 Valentini, G., Tolkovsky, A.M., Tomino, Y., Tonges, L., Tooze, S.A., Tournier, C., Tower, J.,
 701 Towns, R., Trajkovic, V., Travassos, L.H., Tsai, T.F., Tschan, M.P., Tsubata, T., Tsung, A.,
 702 Turk, B., Turner, L.S., Tyagi, S.C., Uchiyama, Y., Ueno, T., Umekawa, M., Umemiya-Shirafuji,
 703 R., Unni, V.K., Vaccaro, M.I., Valente, E.M., Van den Berghe, G., van der Klei, I.J., van Doorn,
 704 W., van Dyk, L.F., van Egmond, M., van Grunsven, L.A., Vandenabeele, P., Vandenberghe,

705 W.P., Vanhorebeek, I., Vaquero, E.C., Velasco, G., Vellai, T., Vicencio, J.M., Vierstra, R.D.,
 706 Vila, M., Vindis, C., Viola, G., Viscomi, M.T., Voitsekhovskaja, O.V., von Haefen, C., Votruba,
 707 M., Wada, K., Wade-Martins, R., Walker, C.L., Walsh, C.M., Walter, J., Wan, X.B., Wang, A.,
 708 Wang, C., Wang, D., Wang, F., Wang, G., Wang, H., Wang, H.G., Wang, H.D., Wang, J., Wang,
 709 K., Wang, M., Wang, R.C., Wang, X., Wang, Y.J., Wang, Y., Wang, Z., Wang, Z.C., Wansink,
 710 D.G., Ward, D.M., Watada, H., Waters, S.L., Webster, P., Wei, L., Weihl, C.C., Weiss, W.A.,
 711 Welford, S.M., Wen, L.P., Whitehouse, C.A., Whitton, J.L., Whitworth, A.J., Wileman, T., Wiley,
 712 J.W., Wilkinson, S., Willbold, D., Williams, R.L., Williamson, P.R., Wouters, B.G., Wu, C., Wu,
 713 D.C., Wu, W.K., Wyttenbach, A., Xavier, R.J., Xi, Z., Xia, P., Xiao, G., Xie, Z., Xu, D.Z., Xu, J.,
 714 Xu, L., Xu, X., Yamamoto, A., Yamashina, S., Yamashita, M., Yan, X., Yanagida, M., Yang,
 715 D.S., Yang, E., Yang, J.M., Yang, S.Y., Yang, W., Yang, W.Y., Yang, Z., Yao, M.C., Yao, T.P.,
 716 Yeganeh, B., Yen, W.L., Yin, J.J., Yin, X.M., Yoo, O.J., Yoon, G., Yoon, S.Y., Yorimitsu, T.,
 717 Yoshikawa, Y., Yoshimori, T., Yoshimoto, K., You, H.J., Youle, R.J., Younes, A., Yu, L., Yu,
 718 S.W., Yu, W.H., Yuan, Z.M., Yue, Z., Yun, C.H., Yuzaki, M., Zabinnyk, O., Silva-Zacarin, E.,
 719 Zacks, D., Zacksenhaus, E., Zaffaroni, N., Zakeri, Z., Zeh, H.J., 3rd, Zeitlin, S.O., Zhang, H.,
 720 Zhang, H.L., Zhang, J., Zhang, J.P., Zhang, L., Zhang, M.Y., Zhang, X.D., Zhao, M., Zhao, Y.F.,
 721 Zhao, Y., Zhao, Z.J., Zheng, X., Zhivotovsky, B., Zhong, Q., Zhou, C.Z., Zhu, C., Zhu, W.G.,
 722 Zhu, X.F., Zhu, X., Zhu, Y., Zoladek, T., Zong, W.X., Zorzano, A., Zschocke, J., Zuckerbraun, B.
 723 **Guidelines for the use and interpretation of assays for monitoring autophagy.** *Autophagy*.
 724 2012;8:445-544.
 725 [82] Polge, C., Attaix, D., Taillandier, D. **Role of E2-ub-conjugating enzymes during**
 726 **skeletal muscle atrophy.** *Front Physiol.* 2015;6:59.
 727 [83] Schakman, O., Kalista, S., Barbe, C., Loumaye, A., Thissen, J.P. **Glucocorticoid-**
 728 **induced skeletal muscle atrophy.** *Int J Biochem Cell Biol.* 2013;45:2163-2172.
 729 [84] Bodine, S.C., Baehr, L.M. **Skeletal muscle atrophy and the E3 ubiquitin ligases**
 730 **murf1 and mafbx/atrogin-1.** *Am J Physiol Endocrinol Metabol.* 2014;307:E469-484.

731 [85] Brooks, N.E., Myburgh, K.H. **Skeletal muscle wasting with disuse atrophy is multi-**
732 **dimensional: The response and interaction of myonuclei, satellite cells and signaling**
733 **pathways.** *Front Physiol.* 2014;5:99.

734 [86] Glass, D.J. **Pi3 kinase regulation of skeletal muscle hypertrophy and atrophy.** *Curr*
735 *Top Microbiol Immunol.* 2010;346:267-278.

736 [87] Schiaffino, S., Mammucari, C. **Regulation of skeletal muscle growth by the IGF1-**
737 **Akt/PKB pathway: Insights from genetic models.** *Skeletal muscle.* 2011;1:4.

738 [88] Sanchez, A.M., Candau, R.B., Bernardi, H. **Foxo transcription factors: Their roles in**
739 **the maintenance of skeletal muscle homeostasis.** *Cell Mol Life Sci.* 2014;71:1657-1671.

740 [89] Laplante, M., Sabatini, D.M. **mTOR signaling in growth control and disease.** *Cell.*
741 2012;149:274-293.

742 [90] Wang, P., Drackley, J.K., Stamey-Lanier, J.A., Keisler, D., Loor, J.J. **Effects of level of**
743 **nutrient intake and age on mammalian target of rapamycin, insulin, and insulin-like**
744 **growth factor-1 gene network expression in skeletal muscle of young holstein calves.** *J*
745 *Dairy Sci.* 2014;97:383-391.

746 [91] Bar-Peled, L., Sabatini, D.M. **Regulation of mTORC1 by amino acids.** *Trends Cell*
747 *Biol.* 2014;24:400-406.

748 [92] Braun, T.P., Marks, D.L. **The regulation of muscle mass by endogenous**
749 **glucocorticoids.** *Front Physiol.* 2015;6:12.

750 [93] Orellana, R.A., Jeyapalan, A., Escobar, J., Frank, J.W., Nguyen, H.V., Suryawan, A.,
751 Davis, T.A. **Amino acids augment muscle protein synthesis in neonatal pigs during acute**
752 **endotoxemia by stimulating mtor-dependent translation initiation.** *Am J Physiol Endocrinol*
753 *Metabol.* 2007;293:E1416-1425.

754 [94] O'Connor, P.M., Kimball, S.R., Suryawan, A., Bush, J.A., Nguyen, H.V., Jefferson, L.S.,
755 Davis, T.A. **Regulation of translation initiation by insulin and amino acids in skeletal**
756 **muscle of neonatal pigs.** *Am J Physiol Endocrinol Metabol.* 2003;285:E40-53.

757 [95] Gorelick-Feldman, J., Maclean, D., Ilic, N., Poulev, A., Lila, M.A., Cheng, D., Raskin, I.
758 **Phytoecdysteroids increase protein synthesis in skeletal muscle cells.** *J Agric Food*
759 *Chem.* 2008;56:3532-3537.

760 [96] Gorelick-Feldman, J., Cohick, W., Raskin, I. **Ecdysteroids elicit a rapid Ca^{2+} flux**
761 **leading to Akt activation and increased protein synthesis in skeletal muscle cells.**
762 *Steroids.* 2010;75:632-637.

763 [97] Kizelsztejn, P., Govorko, D., Komarnytsky, S., Evans, A., Wang, Z., Cefalu, W.T.,
764 Raskin, I. **20-hydroxyecdysone decreases weight and hyperglycemia in a diet-induced**
765 **obesity mice model.** *Am J Physiol Endocrinol Metab.* 2009;296:E433-439.

766 [98] Anthony, T.G., Mirek, E.T., Bargoud, A.R., Phillipson-Weiner, L., DeOliveira, C.M.,
767 Wetstein, B., Graf, B.L., Kuhn, P.E., Raskin, I. **Evaluating the effect of 20-hydroxyecdysone**
768 **(20HE) on mechanistic target of rapamycin complex 1 (mTORC1) signaling in the skeletal**
769 **muscle and liver of rats.** *Appl Physiol Nutr Metabol.* 2015;40:1324-1328.

770 [99] Rodriguez, J., Vernus, B., Chelh, I., Cassar-Malek, I., Gabillard, J.C., Hadj Sassi, A.,
771 Seilliez, I., Picard, B., Bonnieu, A. **Myostatin and the skeletal muscle atrophy and**
772 **hypertrophy signaling pathways.** *Cell Mol Life Sci.* 2014;71:4361-4371.

773 [100] Han, H.Q., Zhou, X., Mitch, W.E., Goldberg, A.L. **Myostatin/activin pathway**
774 **antagonism: Molecular basis and therapeutic potential.** *Int J Biochem Cell Biol.*
775 2013;45:2333-2347.

776 [101] Ebert, S.M., Monteys, A.M., Fox, D.K., Bongers, K.S., Shields, B.E., Malmberg, S.E.,
777 Davidson, B.L., Suneja, M., Adams, C.M. **The transcription factor ATF4 promotes skeletal**
778 **myofiber atrophy during fasting.** *Mol Endocrinol.* 2010;24:790-799.

779 [102] Harding, H.P., Zhang, Y., Zeng, H., Novoa, I., Lu, P.D., Calfon, M., Sadri, N., Yun, C.,
780 Popko, B., Paules, R., Stojdl, D.F., Bell, J.C., Hettmann, T., Leiden, J.M., Ron, D. **An**
781 **integrated stress response regulates amino acid metabolism and resistance to oxidative**
782 **stress.** *Mol Cell.* 2003;11:619-633.

783 [103] Dey, S., Baird, T.D., Zhou, D., Palam, L.R., Spandau, D.F., Wek, R.C. **Both**
784 **transcriptional regulation and translational control of ATF4 are central to the integrated**
785 **stress response.** *J Biol Chem.* 2010;285:33165-33174.

786 [104] Ebert, S.M., Dyle, M.C., Bullard, S.A., Dierdorff, J.M., Murry, D.J., Fox, D.K., Bongers,
787 K.S., Lira, V.A., Meyerholz, D.K., Talley, J.J., Adams, C.M. **Identification and small molecule**
788 **inhibition of an ATF4-dependent pathway to age-related skeletal muscle weakness and**
789 **atrophy.** *J Biol Chem.* 2015.

790 [105] Dyle, M.C., Ebert, S.M., Cook, D.P., Kunkel, S.D., Fox, D.K., Bongers, K.S., Bullard,
791 S.A., Dierdorff, J.M., Adams, C.M. **Systems-based discovery of tomatidine as a natural**
792 **small molecule inhibitor of skeletal muscle atrophy.** *J Biol Chem.* 2014;289:14913-14924.

793 [106] Kunkel, S.D., Elmore, C.J., Bongers, K.S., Ebert, S.M., Fox, D.K., Dyle, M.C., Bullard,
794 S.A., Adams, C.M. **Ursolic acid increases skeletal muscle and brown fat and decreases**
795 **diet-induced obesity, glucose intolerance and fatty liver disease.** *PloS one.* 2012;7:e39332.

796 [107] Kunkel, S.D., Suneja, M., Ebert, S.M., Bongers, K.S., Fox, D.K., Malmberg, S.E.,
797 Alipour, F., Shields, R.K., Adams, C.M. **mRNA expression signatures of human skeletal**
798 **muscle atrophy identify a natural compound that increases muscle mass.** *Cell Metab.*
799 2011;13:627-638.

800 [108] Adams, C.M., Ebert, S.M., Dyle, M.C. **Use of mRNA expression signatures to**
801 **discover small molecule inhibitors of skeletal muscle atrophy.** *Curr Opin Clin Nutr Metab*
802 *Care.* 2015;18:263-268.

803 [109] Levine, M.E., Suarez, J.A., Brandhorst, S., Balasubramanian, P., Cheng, C.W., Madia,
804 F., Fontana, L., Mirisola, M.G., Guevara-Aguirre, J., Wan, J., Passarino, G., Kennedy, B.K.,
805 Wei, M., Cohen, P., Crimmins, E.M., Longo, V.D. **Low protein intake is associated with a**
806 **major reduction in IGF-I, cancer, and overall mortality in the 65 and younger but not older**
807 **population.** *Cell Metab.* 2014;19:407-417.

808 [110] Mirzaei, H., Suarez, J.A., Longo, V.D. **Protein and amino acid restriction, aging and**
809 **disease: From yeast to humans.** *Trends Endocrinol Metab.* 2014;25:558-566.

810 [111] Drake, J.C., Bruns, D.R., Peelor, F.F., 3rd, Biela, L.M., Miller, R.A., Miller, B.F.,
811 Hamilton, K.L. **Long-lived snell dwarf mice display increased proteostatic mechanisms**
812 **that are not dependent on decreased mTORC1 activity.** *Aging Cell.* 2015;14:474-482.

813 [112] Aguilaniu, H. **The mysterious relationship between reproduction and longevity.**
814 *Worm.* 2015;4:e1020276.

815 [113] Adler, M.I., Cassidy, E.J., Fricke, C., Bonduriansky, R. **The lifespan-reproduction**
816 **trade-off under dietary restriction is sex-specific and context-dependent.** *Exp Gerontol.*
817 2013;48:539-548.

818 [114] Mattson, M.P. **Hormesis defined.** *Ageing Res Rev.* 2008;7:1-7.

819 [115] Hine, C.M., Mitchell, J.R. **Nrf2 and the phase ii response in acute stress resistance**
820 **induced by dietary restriction.** *J Clin Exp Pathol.* 2012;S4.

821 [116] Du, M., Zhu, M.J., Means, W.J., Hess, B.W., Ford, S.P. **Nutrient restriction**
822 **differentially modulates the mammalian target of rapamycin signaling and the ubiquitin-**
823 **proteasome system in skeletal muscle of cows and their fetuses.** *J Anim Sci.* 2005;83:117-
824 123.

825 [117] Wang, H., Wilson, G.J., Zhou, D., Lezmi, S., Chen, X., Layman, D.K., Pan, Y.X.
826 **Induction of autophagy through the activating transcription factor 4 (atf4)-dependent**
827 **amino acid response pathway in maternal skeletal muscle may function as the molecular**
828 **memory in response to gestational protein restriction to alert offspring to maternal**
829 **nutrition.** *Br J Nutr.* 2015:1-14.

830 [118] Oster, M., Murani, E., Metges, C.C., Ponsuksili, S., Wimmers, K. **A gestational high**
831 **protein diet affects the abundance of muscle transcripts related to cell cycle regulation**
832 **throughout development in porcine progeny.** *PLoS ONE.* 2012;7:e34519.

833 [119] Roland, L., Drillich, M., Klein-Jobstl, D., Iwersen, M. **Invited review: Influence of**
834 **climatic conditions on the development, performance, and health of calves.** *J Dairy Sci.*
835 2016.

836

837

838 Table 1. Common and emerging technologies to assess muscle protein balance in domestic animals and livestock.

Method	Applications	Strengths	Limitations	Refs
Nitrogen Balance	Whole body protein balance and growth	Simple, sensitive, noninvasive	Cannot assess muscle-specific effects; difficult to measure losses precisely	[7,8]
RNA/protein ratio	Estimates protein synthesis capacity	Simple, inexpensive	Crude	[9-11]
Amino acid isotope tracer kinetics	Whole body protein synthesis and breakdown, Tissue and fiber-type-specific protein synthesis and breakdown, individual protein synthesis and breakdown	Sensitive, can assess change over short time frames	May not capture proteins with longer half-lives; cannot assess free-living environments	[12-17,21,22,24,29]
Deuterium oxide/ heavy water enrichment	Protein synthesis, protein breakdown and DNA synthesis in whole body, skeletal muscle, specific fiber-types and individual proteins	Can assess free living conditions over extended periods of time	Isotope expense, sophisticated equipment and calculations required	[18-20,23]
Two-dimensional electrophoresis	Differential expression of specific tissue proteins using mass spectrometry	Can visualize modifications affecting protein activity in addition to changes in expression	Protein identification is often targeted versus global	[10]
SUnSET - non-isotopic immunodetection of puromycin	Skeletal muscle protein synthesis (whole tissue and fiber-type)	Simple, inexpensive snapshot of nascent MPS	Qualitative, puromycin dose may inhibit protein synthesis in other tissues; cannot assess breakdown	[25,26]
PUNCH-P - puromycin-associated nascent chain proteomics	Genome-wide identification and quantification of protein synthesis in tissues	<i>Ex vivo</i> , relatively simple, less expensive than Ribo-Seq	Qualitative, sophisticated equipment required; cannot assess breakdown	[27,28]
Ribosomal profiling	Tissue-specific global quantification of mRNA translation	<i>Ex vivo</i> , global snapshot of both translated and untranslated mRNAs	Conducting gene expression across polysome fractions is time consuming and expensive; cannot assess breakdown	[30,35]
Ribo-Seq	Global quantification of the average ribosome density on mRNA	<i>Ex vivo</i> , comprehensive evaluation of nascent mRNA translation	Expensive; technology not yet optimized for use in animal tissues; unable to reveal the proportion of untranslated mRNA relative to polysome bound mRNA; cannot assess breakdown	[31-34]

839 **Figure Legend**

840 Figure 1. Signaling pathways coordinate muscle protein balance. Anabolic and catabolic
841 stimuli are integrated through the PKB/Akt-mTORC1 signaling to regulate mechanisms that
842 control muscle protein synthesis and breakdown.

843

Figure 1