Domestic Animal Endocrinology 56 (2016) S23–S32

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Mechanisms of protein balance in skeletal muscle

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ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 23 February 2016
Accepted 29 February 2016

Keywords:
Proteostasis
Muscle protein synthesis
mRNA translation
Muscle protein degradation
Proteolysis

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.

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 backdrop of environmental stress (ie, 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 2 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

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http://dx.doi.org/10.1016/j.domaniend.2016.02.012

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). Although this measure remains the fundamental basis for determining dietary protein requirements in mammals, this technique reflects whole body and not skeletal muscle specifically. Muscle protein synthesis (MPS) capacity can be reflected by the RNA-to-protein ratio, a classic measurement in skeletal muscle [9–11]. Although still in use today, most research settings use instead a variety of techniques that measure the rate of skeletal 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 (ie, 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]. Muscle protein synthesis measurements over longer periods of time (d or wk) can be accomplished by ingestion of deuterium oxide (heavy water) [22,23]. This method allows for calculation of DNA synthesis in addition to MPS and turnover [24]. 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 (eg, mitochondrial protein synthesis) [18,19]. Synthesis rates of individual proteins can also be assessed [20,25].

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 [26,27]. Another approach uses biotinylated puromycin to label newly synthesized proteins in cell-free conditions, followed by proteomic analysis to generate a snapshot of the translatome [28,29]. These protein tagging approaches to assess the proteome are faster and easier than 2-dimensional gel electrophoresis methods to assess the proteome [10,21].

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	[12–21]
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	[22–25]
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	[26,27]
PUNCH-P - puromycin-associated nascent chain proteomics	Genome-wide identification and quantification of protein synthesis in tissues	*Ex vivo*, relatively simple, less expensive than Ribo-Seq	Qualitative, sophisticated equipment required; cannot assess breakdown	[28,29]
Ribosomal profiling	Tissue-specific global quantification of mRNA translation	*Ex vivo*, global snapshot of both translated and untranslated mRNAs	Conducting gene expression across polysome fractions is time consuming and expensive; cannot assess breakdown	[30,31]
Ribo-Seq	Global quantification of the average ribosome density on mRNA	*Ex vivo*, 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	[32–35]

Abbreviation: MPS, muscle protein synthesis.