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Making Sense of Muscle Protein Synthesis: A Focus on Muscle Growth During Resistance Training

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The acute response of muscle protein synthesis (MPS) to resistance exercise and nutrition is often used to inform recommendations for exercise programming and dietary interventions, particularly protein nutrition, to support and enhance muscle growth with training. Those recommendations are worthwhile only if there is a predictive relationship between the acute response of MPS and subsequent muscle hypertrophy during resistance exercise training. The metabolic basis for muscle hypertrophy is the dynamic balance between the synthesis and degradation of myofibrillar proteins in muscle. There is ample evidence that the process of MPS is much more responsive to exercise and nutrition interventions than muscle protein breakdown. Thus, it is intuitively satisfying to translate the acute changes in MPS to muscle hypertrophy with training over a longer time frame. Our aim is to examine and critically evaluate the strength and nature of this relationship. Moreover, we examine the methodological and physiological factors related to measurement of MPS and changes in muscle hypertrophy that contribute to uncertainty regarding this relationship. Finally, we attempt to offer recommendations for practical and contextually relevant application of the information available from studies of the acute response of MPS to optimize muscle hypertrophy with training.

Keywords: myofibrillar protein synthesis, muscle hypertrophy, muscle remodeling, translational efficiency, ribosomal biogenesis, stable isotopes

Muscle protein synthesis (MPS) is the metabolic process that describes the incorporation of amino acids into bound skeletal muscle proteins. Muscle proteins can be crudely classified into the contractile myofibrillar proteins (i.e., myosin, actin, tropomyosin, troponin) and the energy producing mitochondrial proteins. The synthesis of myofibrillar proteins is primarily responsible for changes in skeletal muscle mass following resistance training; whereas, mitochondrial proteins are primarily synthesized in response to endurance type training (Wilkinson et al., 2008). The MPS is most commonly expressed as a rate of amino acid incorporation into bound muscle protein over a given time period, typically a single hour or a single day. Conversely, the metabolic process of muscle protein breakdown describes the degradation of bound muscle proteins into their amino acid precursors that occurs continuously and concurrently with MPS. As such, the aggregate difference in rates of MPS and muscle protein breakdown determines whether muscle protein is gained (MPS exceeds muscle protein breakdown) or muscle protein is lost (muscle protein breakdown exceeds MPS). Of the two metabolic processes, MPS is more responsive to exercise and nutritional stimuli (Tipton et al., 2018), at least in healthy individuals, and thus has garnered most scientific attention in the context of muscle adaptations to exercise training.

The assessment of the acute response of MPS to combined exercise and nutrition interventions is commonly used as the scientific basis to inform sport and exercise nutrition, in particular protein nutrition, recommendations for the training and performance of athletes and other exercisers. This longstanding assumption is predicated on a direct relationship existing between the acute

response of MPS to a single bout of resistance exercise combined with a nutritional intervention (REx) and chronic phenotypic adaptations (i.e., muscle hypertrophy) to resistance exercise training and repeated dietary manipulation (RET). An early report by Balagopal et al. (1997) supported the idea that acute measurements of MPS are predictive of chronic muscle adaptation, correlating basal rates of MPS with measurements of muscle mass, strength, and muscle mass per unit muscle mass (indirect marker of muscle quality), albeit in untrained individuals across a mix of young, middle-aged, and older adult cohorts (Balagopal et al., 1997). In addition, more recent studies have demonstrated that the acute response of MPS to REx (Tang et al., 2009; Wilkinson et al., 2007) may predict longer term muscle growth with RET (Hartman et al., 2007; Volek et al., 2013). However, this relationship has been challenged (Tipton & Wolfe, 2001) with experimental evidence that a disconnect exists between acute measurements of MPS in response to REx and chronic changes in skeletal muscle mass following RET in previously untrained young men (Mayhew et al., 2009; Mitchell et al., 2014). As such, these data have cast into doubt the predictive value of acute measurements of MPS to inform evidence-based nutrition interventions, with clear implications for practitioners across various disciplines related to sport and exercise nutrition.

Viewed through the lens of the applied sport and exercise nutrition practitioner, it is crucial to understand the real-world significance of the stated and/or perceived superiority of one nutritional strategy over another. Clearly, there are many potential dietary, exercise training, and performance interventions available. An accurate translation of scientific evidence for these interventions to applied practice is going to be most relevant to the exerciser, and especially for competitive athletes. Indeed, this translation applies to whether the focus is on, for instance, the

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total intake, type, or timing of a nutritional intervention. Nutritional strategies to enhance muscle hypertrophy are commonly determined on the basis of controlled laboratory studies that report MPS as the primary outcome measurement. Thus, it is crucial that the real-world significance of acute MPS measurements that are used to determine the superiority of nutritional interventions for muscle hypertrophy is understood within the context and limitations of these methods.

Therefore, the main purpose of this narrative review is to critically evaluate the relationship between acute measurements of MPS and chronic measurements of muscle adaptation, with specific reference to RET and muscle hypertrophy. We comprehensively discuss a range of physiological and methodological variables that, in our view and others (Mitchell et al., 2015a), underpin the complex relationship between the acute response of MPS to exercise and nutrition and chronic changes in muscle mass. Physiological variables relate to inherent variability in the response of MPS to exercise and nutrition, the modulation of muscle protein metabolism with changing training status, the influence of a multitude of training paradigms, and genetics. Methodological variables relate to subtle, yet important, technical considerations with regard to measurements of MPS and muscle hypertrophy. As such, our aim is to "make sense of muscle protein synthesis" by providing a balanced and contextually relevant interpretation of the relationship between the acute response of MPS and chronic changes in muscle mass through a lens of translating the science of MPS into real-world practice for the end user practitioner (physiologist or nutritionist), coach, athlete, and/or researcher.

Metabolic Basis of Muscle Hypertrophy

Muscle hypertrophy represents the primary phenotypic adaptation to RET (Goldberg et al., 1975; McGlory et al., 2017) owing, in large part, to the plasticity of skeletal muscle tissue in response to REx and nutrition. The precise definition of skeletal muscle hypertrophy is a topic of current debate among the scientific community (Damas et al., 2015; Figueiredo, 2019; Haun et al., 2019; Joanisse et al., 2020; Roberts et al., 2020). Traditionally, muscle hypertrophy is defined as an increase in skeletal muscle mass and cross-sectional area (CSA) at the whole tissue and cellular levels (Haun et al., 2019; Russell et al., 2000). This definition is underpinned by the notion that an accretion of contractile (i.e., myofibrillar) proteins occurs due to an increased abundance of sarcomeres within the preexisting myofibrils of muscle fibers, and leads to an increase in muscle fiber CSA (Russell et al., 2000).

The plasticity of skeletal muscle is mediated, at least in part, by the constant turnover or remodeling of muscle proteins. In this regard, two metabolic processes, MPS and muscle protein breakdown, act concurrently in response to various stimuli to repair, replace, and generate new muscle proteins leading to phenotypic adaptations. There is evidence that the fold change in MPS in response to REx and/or protein feeding is greater (as much as 2.5-fold) than muscle protein breakdown (Biolo et al., 1995, 1997), suggesting that MPS is the primary metabolic driver of RET-induced muscle hypertrophy (Tipton & Wolfe, 1998). Accordingly, it has been proposed that muscle hypertrophy following RET stems from a cumulative accretion of muscle proteins resulting from the repeated increase in response of myofibrillar–MPS to successive bouts of REx (Hawley et al., 2006). Hence, according to this traditional definition of muscle hypertrophy, it may seem intuitively satisfying that assessment of the acute response of MPS

to REx provides an informative tool when devising RET and nutritional interventions to maximize muscle hypertrophy in athletes and other exercisers.

We acknowledge that an alternative definition of muscle hypertrophy relates to an increase in skeletal muscle size accompanied by an increase in mineral, protein, or substrate abundance (e.g., glycogen and intramuscular triglyceride) (Haun et al., 2019). This contemporary, and arguably more comprehensive, model of muscle hypertrophy also accounts for the growth of nonmyofibril components. Accordingly, three different types of muscle hypertrophy have been proposed, namely myofibrillar hypertrophy, sarcoplasmic hypertrophy, and connective tissue hypertrophy, each with their own biological definition (Haun et al., 2019). Myofibrillar hypertrophy is defined as an increase in the size and/or number of myofibrils accompanied by an increase in sarcomere number or sarcomere protein abundance directly related to the structure or contractile force generation of the muscle, that is, directly related to the more traditional definition of hypertrophy described above. Sarcoplasmic hypertrophy relates to a chronic increase in volume of the sarcolemma and/or sarcoplasm accompanied by an increase in the volume of mitochondria, sarcoplasmic reticulum, t-tubules, and/or sarcoplasmic enzyme or substrate content. Finally, connective tissue hypertrophy is defined as an increase in volume of the extracellular matrix of skeletal muscle accompanied by an increase in mineral or protein content. A critical evaluation of skeletal muscle hypertrophy as a biological construct is beyond the scope of this text, and the reader is referred to several recent reviews on this topic (Damas et al., 2018; Haun et al., 2019; Roberts et al., 2020). Nonetheless, we suggest that all three types of hypertrophy likely contribute to measured changes in muscle mass with RET, almost certainly to varying degrees depending on the type of training, as well as the type and timing of the measurement. Moreover, these factors potentially add to variability in the measurement of muscle hypertrophy with RET, leading to potential confusion for informing practice.

Muscle Protein Synthesis

The acute measurement of in vivo human rates of MPS in response to REx dates back to the 1990s (Tipton & Wolfe, 1998). A seminal study by Chesley et al. (1992) demonstrated that REx, performed in the fasted state, stimulated MPS (Chesley et al., 1992). Subsequently, it was shown that this response persisted for up to 48 hr postexercise (Phillips et al., 1997). Biolo et al. (1997) first demonstrated that hyperaminoacidemia (elevated arterial amino acid concentrations) following exercise further stimulated MPS (Biolo et al., 1997). Next, the first studies were published that demonstrated ingestion of essential amino acids immediately following REx increased the postexercise stimulation of MPS, resulting in a net accretion of muscle protein (Rasmussen et al., 2000; Tipton et al., 1999, 2001). Collectively, these data provided a platform for studies over the next 20 years (~2000 to present) to systematically investigate the interaction of exercise and nutrition for stimulation of MPS, with direct application to sport and exercise nutrition and exercise science.

Several methods have been used to measure the acute response of MPS to exercise and nutrition in humans. A comprehensive discussion of the methods used to measure MPS is beyond the scope of this review, but the interested reader is directed to a number of excellent recent reviews (Brook & Wilkinson, 2020; Millward & Smith, 2019; Wilkinson et al., 2017). The most common approach is the precursor–product method that allows

for the determination of muscle protein fractional synthesis rate (FSR). In practice, this method utilizes stable isotope labeled amino acids (i.e., $^{13}C_6$ phenylalanine, $1-^{13}C$ leucine), usually administered by intravenous infusion under controlled laboratory conditions, to directly trace the incorporation of free amino acids into newly synthesized bound muscle proteins, typically over an acute 3–12 hr time period following a single exercise and/or nutrition stimulus. Traditionally, FSR was calculated for mixed muscle proteins, that is, all muscle protein fractions combined. Methodological advances during the 1990s allowed for the separation of muscle protein fractions (Hasten et al., 1998; Rooyackers et al., 1996) and thus acute measurements of muscle myofibrillar FSR or muscle mitochondrial FSR were possible in an exercise science setting, dependent on the mode (resistance or endurance-based) of exercise stimulus (Wilkinson et al., 2008). Another recent advancement in the field is centered around the re-emergence of the orally administered deuterium oxide (D_2O) tracer method to measure free-living integrative rates of MPS. Rather than providing a single snapshot of the acute MPS response in just a few hours under tightly controlled laboratory conditions, the D_2O technique quantifies multiple acute MPS responses to exercise and/or nutritional stimuli integrated over hours, days (Wilkinson et al., 2014), weeks, or even months (Brook et al., 2015) providing greater real-world application to the athlete. Today, separation techniques have evolved further to measure FSR at the individual muscle protein level using D_2O. The focus of the review is on studies that directly determined MPS using the measurement of FSR.

The Controversy

The controversy surrounding the value of acute (i.e., 3–6 hr) measurements of MPS for predicting chronic (i.e., 10–16 weeks) changes in muscle mass with RET has been evident essentially since the measurement of MPS has been used to assess the metabolic response of muscle to REx (Tipton & Wolfe, 2001). More recently, an elegant study by Mitchell et al. (2014) cast doubt on the relationship. This study was novel in examining the within-participant (i.e., muscle mass of the same participants was measured pre and post RET) relationship between the acute response of myofibrillar–MPS to REx and protein feeding (administered as a single 30 g milk protein bolus), and the muscle hypertrophic response to 16 weeks of progressive RET in previously untrained young men. No measurement of muscle protein breakdown was conducted in this study. As such, this study design offered insight into whether any heterogeneity in the muscle hypertrophic response to RET could be explained by differences in the acute response of MPS to REx between the 23 participants that conducted the study. The muscle hypertrophic response was determined by measurement of pre–post RET changes in quadriceps volume and lean body mass using magnetic resonance imaging and dual-energy X-ray absorptiometry, respectively. The acute response of MPS was measured over a 6-hr recovery period following the first (of 64) bout of REx.

Perhaps surprisingly to many at the time, and refuting their original hypothesis, the study by Mitchell et al. (2014) revealed no association between the rate of myofibrillar–MPS measured over 6 hr following the initial bout of REx and protein ingestion and the change in muscle volume or lean body mass following 16 weeks of RET. Moreover, no correlation of the change in MPS from rest with the change in muscle volume was reported (Mitchell et al., 2014). Indeed, this observation is consistent with the results of a comparable 16 weeks RET study by Mayhew et al. (2009) that was conducted in previously untrained young and older adult men (Mayhew et al., 2009). In this study, no relationship was observed between the acute response of mixed MPS measured in the fasted state 24 hr after the initial bout of REx and muscle hypertrophy as determined by measurement of muscle fiber cross-sectional area. Taken together, these data suggest that acute measurements of MPS offer limited quantitative value for predicting individualized chronic changes in muscle mass following progressive RET, at least when the acute response of MPS is measured following the initial exercise session of the RET period. These studies have contributed to some confusion—particularly for practitioners, students, and others without specialist knowledge of the strengths and limitation of stable isotope methodology—and controversy over the interpretation of data from the measurement of MPS in response to exercise and nutrition (Mitchell et al., 2015b).

In contrast, multiple lines of evidence support the notion that the acute response of MPS to REx, with or without nutritional intervention, is predictive of chronic changes in muscle mass with RET when repeatedly exposed to a comparable exercise or nutritional intervention, at least when studied on an averaged, group basis. First, ingesting an 18 g bolus of milk protein immediately after REx stimulated a greater acute response of MPS than a dose-matched soy protein beverage in young men (Wilkinson et al., 2007). This finding was consistent with a longitudinal training study that reported a greater change in muscle hypertrophy when a milk protein beverage was consumed immediately after each REx session of a 12-week RET program versus a soy protein beverage in young men (Hartman et al., 2007). Similarly, the greater acute response of MPS to ingesting 20 g of whey protein versus casein immediately post REx (Tang et al., 2009) translated to greater muscle hypertrophy following 10 weeks of RET (Volek et al., 2013). Second, the acute response of MPS to REx when manipulating exercise workload (low vs. high) (Burd, West, et al., 2010) and exercise volume (i.e., 1 vs. 3 sets of REx) (Burd, Holwerda, et al., 2010) corresponded with the muscle hypertrophic response to RET protocols that manipulated these same training variables (Mitchell et al., 2012). Finally, REx-induced increases in putative anabolic hormones were not shown to increase the acute response of MPS (West et al., 2009) or enhance RET-induced muscle hypertrophy (West et al., 2010) in young men. When combined with data generated by Mitchell et al. (2014), these data highlight the complexity of the relationship between the acute response of MPS to REx and nutrition and subsequent changes in muscle mass with RET. In our view, and that of others (Damas et al., 2018; Mitchell et al., 2015), a series of physiological and methodological factors mediate this complex relationship between acute measurements of MPS and chronic changes in muscle hypertrophy, as detailed below.

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Physiological Factors

Several physiological factors, related to both the acute response of MPS to REx and the muscle hypertrophic response to RET, appear to contribute to the observed discrepancy between measured rates of MPS and muscle hypertrophy. Muscle hypertrophy is a complex physiological process that is altered as training progresses. For the initial response of MPS to predict subsequent muscle hypertrophy during RET, it must be assumed that the measured response of MPS to REx is uniform throughout the training period. However, it is clear that the response of MPS is modified from the initiation of RET and as training progresses (Kim et al., 2005; Phillips et al., 2002; Tang et al., 2008). This modification takes place on a number

of levels that include the timecourse (amplitude and duration) and nature (directed to anabolic or nonanabolic processes) of the MPS response, as discussed below.

There is considerable evidence from both cross-sectional (Phillips et al., 1999) and longitudinal (Kim et al., 2005; Phillips et al., 2002; Tang et al., 2008) studies that the training status of an individual modifies the amplitude and duration of the acute response of MPS to REx. In the untrained state, the acute response of MPS has been shown to peak later, but remain elevated for longer, after REx compared with the trained state (Phillips et al., 2002; Tang et al., 2008). Conversely, in the trained state, the acute response of MPS to REx is more rapid but shorter lived than the untrained state (Phillips et al., 1999). As a result, the overall acute stimulation of MPS after REx is generally considered to be greater in untrained versus trained individuals, at least when the absolute workload of REx is matched between training states (Damas et al., 2015). Given that training status clearly modulates the acute response of MPS to REx, it follows that the relationship between the acute MPS response to REx and chronic muscle growth response to RET may be altered over the time course of the training process.

To date, the most comprehensive study to examine the influence of training status on the relationship between the acute MPS response to REx and the muscle growth response to RET was conducted by Damas et al. (2016). In this study, 10 untrained young (~27 years) men performed 10 weeks of RET consisting of two sessions of REx per week. The RET program was divided into three phases, namely the initial (i.e., at baseline), early (after 3 weeks of RET), and late (after 10 weeks of RET) phase of RET. Measurements of the acute MPS response to REx and muscle mass were obtained at each phase of RET. This elegant study design offered unique insight into the temporal relationship between acute measurements of MPS in response to REx, assessed in both the trained and untrained state, and the subsequent muscle growth response during RET.

The study by Damas, Phillips, Libardi, et al. (2016) presents data that reveals a time course-dependent relationship between the acute response of MPS to REx and chronic changes in muscle mass during RET. In this regard, no relationship was observed between the acute response of myofibrillar–MPS to the initial REx bout of the RET period and the change in muscle mass following 10 weeks of RET. As detailed above, this observation is consistent with previous studies that reported no association between the acute response of MPS to the initial REx bout and the change in muscle volume (Mitchell et al., 2014) and fiber cross-sectional area (Mayhew et al., 2009) following 16 weeks of RET in previously untrained men. In contrast, the acute response of MPS to REx measured at Weeks 3 and 10 were associated with chronic changes in muscle mass over the 10-week RET period (Damas, Phillips, Libardi, et al., 2016). These data are consistent with recent studies that reported associations between acute measurements of MPS and muscle hypertrophy over 3 (Brook et al., 2015) and 12 weeks (Reidy et al., 2017) of RET. Taken together, these data indicate the relationship between acute measurements of MPS and chronic changes in the muscle growth response becomes apparent as the training status of the individual progresses (Table 1). The predictive value of the acute response of MPS to nutrition and exercise interventions seems to be greater in trained than untrained individuals, who are not accustomed to muscle loading during REx (Damas et al., 2018). Thus, the researcher or practitioner may wish to consider the relative value of acute measurements of MPS for predicting chronic changes in muscle growth when formulating

training and nutrition recommendations, at least for trained individuals.

One physiological mechanism proposed to explain the temporal relationship between acute measurements of MPS in response to REx and chronic changes in the muscle growth response to RET relates to the nature of the response of MPS to REx (Damas et al., 2018). Damas et al. (2016) reported a greater acute response of MPS to the initial REx bout compared with the MPS response to REx performed during the early (Week 3) and later (Week 10) phase of RET. This trend aligned with the acute (48 hr) muscle damage response to REx that was highest after the initial unaccustomed REx bout, but was attenuated by the early (Week 3) phase of RET. The authors reasoned that during the early phase of a training program, the increased response of MPS to REx and protein ingestion is related more to the repair and remodeling of existing older, perhaps damaged, proteins (Damas, Phillips, Lixandrao, et al., 2016) than to hypertrophy. These early, more global, metabolic responses not only lead to the repair and remodeling of proteins, but also set the stage for future deposition of muscle proteins and muscle growth (Brook, Wilkinson, Smith, & Atherton, 2016; Burd & De Lisio, 2017; Joanisse et al., 2020). Consistent with this notion, the greater muscle damage response to unaccustomed eccentric-based exercise versus a work-matched bout of concentric exercise has been shown to correspond with a greater acute response of MPS to eccentric REx (Moore et al., 2005; Pavis et al., 2021). As RET progresses, the responses of MPS to REx and nutrition become more refined toward muscle hypertrophy. This notion is supported by data showing that both mitochondrial and myofibrillar–MPS are increased following a REx in the untrained state (Wilkinson et al., 2008). However, following 10 weeks of RET, only myofibrillar FSR is increased. Taken together, these data suggest that with the progression of RET, and as the degree of exercise-induced muscle damage starts to diminish, the acute stimulation of MPS is directed almost exclusively to the accretion of new muscle proteins, thus explaining the correlation between acute rates of MPS and the muscle growth response during the later phase of RET (Trommelen et al., 2019).

The inherent variability in the response of MPS to REx and nutrition, as well as the response of muscle hypertrophy to RET, also contributes to our inability to utilize acute metabolic data to predict an individual response to RET (Figure 1). Individual responses to REx and nutrition may vary by as much as 100%, even within groups subjected to identical nutrition and exercise conditions. This variability in response to exercise and nutrition is reported consistently (Jackman et al., 2017; Macnaughton et al., 2016; McGlory et al., 2016) and is considered to represent normal physiological variability (Smith et al., 2011). While the source of this individual variability is not fully understood at this time, genetic variability must be a contributing factor (Clarkson et al., 2005; Pescatello et al., 2006; Riechman et al., 2004). Attempts to control prestudy activity and diet are common in these studies, yet the variability is evident. Moreover, in many studies, the population from which participants are selected is kept fairly tight. Yet, even when the range of muscle mass is restricted, there is considerable variation in the response of MPS (Macnaughton et al., 2016). The methodological conditions under which MPS is determined that may influence the measured response will be discussed below. However, in the examples illustrated in Figure 1, the method used to determine MPS, as well as the conditions under which it was measured, in each individual were identical within studies. Hence, methodological issues alone do not account for all the observed

Table 1 Relationship Between Acute Measurements of MPS and Chronic Changes in Muscle Mass in Response to RET

Reference	Participants	Study design	Measurement of MPS	Measurement of muscle mass	Relationship
Balagopal et al. (1997)	24 healthy UT males and females Three age groups: Young (23 ± 1 years) Middle aged (52 ± 1 years) Older (77 ± 2 years)	Cross-sectional No exercise training All measurements collected in basal state	Mixed muscle protein FSR MHC FSR Sarcoplasmic protein FSR L-[1–^{13}C] leucine infusion 3-hr tracer incorporation period in laboratory setting	Estimated from daily urinary creatinine	Mixed muscle protein FSR correlated with muscle mass ($r = .30$, $p = .220$) MHC FSR correlated with muscle mass ($r = .48$, $p = .020$) No correlation between sarcoplasmic protein FSR and muscle mass
Mayhew et al. (2009)	36 healthy UT males and females Two age groups: Young (28 ± 1 years) Older (64 ± 1 years)	Longitudinal 16 weeks of progressive RET (3 days/week) MPS measured following first REx session of RET period Muscle mass measured immediately pre and post RET	Mixed muscle protein FSR L-[$ring$–^{2}H$_5$] phenylalanine infusion 3-hr tracer incorporation period in laboratory setting	fCSA by immunofluorescence microscopy Thigh lean mass by DXA Total lean (fat and bone free) mass by DXA	No correlation between mixed muscle FSR and changes in muscle mass (all measurements) following RET
Mitchell et al. (2014)	23 UT young (24 ± 1 year) males	Longitudinal 16 weeks of progressive RET (4 day/week) m·s measured following 1st REx session of RET period Muscle mass measured immediately pre and post RET	Muscle myofibrillar protein FSR L-[$ring$–^{13}C$_6$] phenylalanine infusion 6-hr tracer incorporation period in laboratory setting	Quadriceps volume by MRI Total lean (fat and bone free) mass by DXA	No correlation between muscle myofibrillar protein FSR and changes in quadriceps volume following RET ($r = .10$, $p > .050$) No correlation between muscle myofibrillar protein FSR and changes in total lean mass following RET ($r = .13$, $p > .050$)
Damas, Phillips, Libardi, et al. (2016)	10 UT young (27 ± 1 years) males	Longitudinal 10 weeks of progressive RET (2 days/week) MPS measured during Weeks 1, 3, and 10 of RET period Muscle mass measured at pre, Weeks 3 and 10 of RET	Integrated muscle myofibrillar protein FSR Oral deuterium oxide tracer 48-hr tracer incorporation period under free-living conditions	fCSA by microscopy vCSA	No correlation between integrated muscle myofibrillar protein FSR at Week 1 and changes in fCSA following RET Integrated muscle myofibrillar protein FSR at Week 3 correlated with changes in vCSA following RET ($r = .9$, $p = .002$) Integrated muscle myofibrillar protein FSR at Week 10 correlated with changes in fCSA ($r = .9$, $p = .032$) and vCSA following RET ($r = .9$, $p = .000$)

(continued)

Table 1 *(continued)*

Reference	Participants	Study design	Measurement of MPS	Measurement of muscle mass	Relationship
Brook et al. (2015)	10 UT young (23 ± 1 years) males	Longitudinal 6 weeks of progressive unilateral lower limb RET (3 days/week) MPS measured over 3 and 6 weeks of RET period Muscle mass measured at pre, Weeks 3 and 6 of RET	Integrated muscle myofibrillar protein FSR Oral deuterium oxide tracer Tracer incorporation over 6 weeks under free-living conditions	Thigh muscle thickness by ultrasound Thigh muscle mass by DXA	Integrated muscle myofibrillar protein FSR at Week 3 correlated with changes in thigh muscle thickness ($r_2 = .52$, $p = .010$)
Reidy et al. (2017)	31 UT young (25 ± 2 years) males	Longitudinal 12 weeks of progressive whole-body RET (3 days/ week) MPS measured pre and post RET	Mixed muscle protein FSR Muscle myofibrillar protein FSR L-[$ring$—$^{13}C_6$] phenylalanine infusion 6-hr tracer incorporation period in laboratory setting	Total lean (fat and bone free) mass by DXA *Vastus lateralis* muscle thickness by ultrasound fCSA by immunohistochemistry	The pre–post RET change in mixed muscle FSR was correlated with the change in *vastus lateralis* muscle thickness ($r = .22$, $p = .003$)

Note. DXA = dual-energy X-ray absorptiometry; fCSA = muscle fiber cross-sectional area; FSR = fractional synthesis rates; MHC = myosin heavy chain; MRI = magnetic resonance spectroscopy; RET = resistance exercise training; REx = resistance exercise; vCSA = *vastus lateralis* cross-sectional area; MPS = muscle protein synthesis.
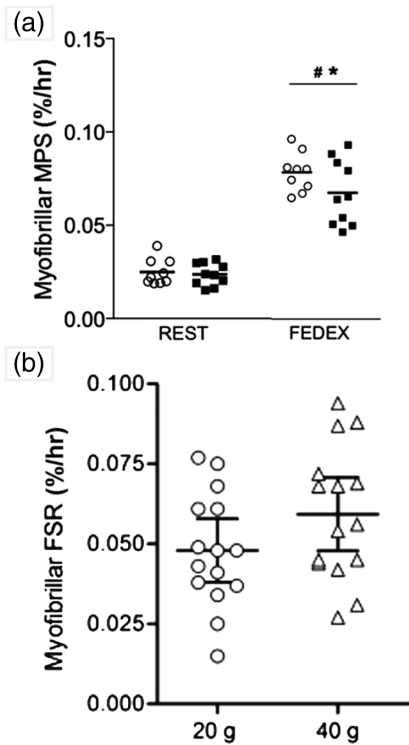
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Figure 1 — Individual FSR responses to a combination of REx followed by protein ingestion in two previous studies. (a) Individual fasted FSR at rest (REST) and with ingestion of 30 g protein following resistance exercise (FEDEX) in two groups of trained young weightlifters (adapted from McGlory et al., 2016) and (b) individual FSR in response to ingestion of 20 and 40 g whey protein following REx in trained young weightlifters (adapted from Macnaughton et al., 2016). MPS = muscle protein synthesis; REx = resistance exercise; FSR = fractional synthetic rate.

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variability. Inherent variability in the metabolic response to REx and nutrition contributes to uncertainty in predicting muscle growth based on measured rates of MPS in individuals.

One potential contributing factor to the variability of the response of MPS to identical REx and protein feeding conditions (Figure 1) might be differences in translational capacity, that is, the total number of ribosomes capable of producing peptide chains (Wen et al., 2016). The MPS is the metabolic process from which functional proteins are produced from polypeptide chains created by ribosomes. The measurement of FSR essentially represents translational efficiency, that is, the rate of translation for a given number of ribosomes. It is clear that ribosome number, that is, translational capacity, does not change acutely following REx (Brook, Wilkinson, Mitchell, et al., 2016; Chesley et al., 1992). However, differences in translational capacity between individuals would result in differences in FSR in response to a given REx and/ or nutrition stimulus. Thus, translational capacity may help explain the individual variability in response of MPS to anabolic stimuli.

Methodological Factors

The lack of ability to predict long-term muscle hypertrophic responses to RET with the acute measurement of MPS does not necessarily reflect the overall worth, or lack thereof, of information obtained from acute metabolic studies. Contributing factors to the uncertain relationship between the acute MPS response to REx and

nutrition, and the muscle hypertrophic response to RET, include a lack of consistency in methods utilized, as well inherent variability resulting from the methods used (Mitchell et al., 2015). There also is heterogeneity in the response of muscle mass to RET that contributes to this disconnect. Accordingly, there are numerous reasons to suggest that the study design and methods chosen to determine hypertrophy in RET studies contributes to this quite heterogeneous response. A full evaluation of these methods is beyond the scope of this review, so interested readers are referred to an excellent presentation of the methodology by Haun et al. (2019).

Several factors related to study design and methods used to assess MPS must be considered when interpreting the relationship between the acute response of MPS- and RET-induced changes in muscle mass. Over the past 25–30 years, the vast majority of studies investigating the response of MPS have utilized the precursor–product method with direct incorporation of the stable isotopically labeled amino acids into muscle protein to determine FSR. Accurate prediction of muscle hypertrophy during RET by determining FSR in response to REx and nutrition requires certain assumptions to be made and met. First, we must assume that the initial measurement of FSR is representative of every subsequent stimulation of MPS for the remainder of the RET period, that is, the responses remain unchanged throughout RET (see discussion above). Next, the measured FSR captures the true response of MPS to REx and protein ingestion. Thus, methodological choices will be critical for determining the true response of MPS.

Methodological considerations influence the ability to capture the true response of MPS with measurement of the FSR in response to exercise and nutrition. Until recently, the majority of studies measuring FSR included an infusion of a labeled amino acid and multiple muscle biopsy samples. The FSR is reported as an hourly rate of synthesis in the time between the muscle samples. An important issue for any infusion study to determine FSR is the limited time period for incorporation of the labeled amino acid. One critical assumption is that the time between biopsies captures the true period of stimulation of MPS. Thus, regardless of the maximal magnitude of the response, if the second muscle sample is taken before the response of MPS returns to baseline, a portion of the true response of MPS may be missed and the determined FSR would be an underestimation (Figure 2). Of course, the converse would be true if the biopsy is taken too late to capture the true response. We must assume that the duration of the true MPS response is captured in the time between muscle biopsies and that this duration is not different between trials assessing the response to different nutrition and/or exercise interventions.

Another factor that contributes to a mismatch between the true response of MPS to REx is the prolonged enhancement of the utilization of amino acids from protein ingestion for MPS following a REx bout (Figure 3). The REx sensitizes the muscle to the anabolic stimulation of elevated amino acid levels from protein feeding (Biolo et al., 1997; Witard et al., 2014). It is clear that the sensitivity of muscle to amino acids remains enhanced for at least 24 hr following the exercise (Burd et al., 2011). Thus, any protein containing meal consumed within this 24-hr time period will result in a MPS response that is greater than that in response to a meal not preceded by REx. An acute measurement of MPS based on an infusion of labeled amino acids and biopsies for only a few hours after exercise would not be capable of capturing the contribution to muscle hypertrophy resulting from all of these enhanced postprandial elevations of MPS (Figure 3a). Thus, an acute measurement limited to only a few hours after REx would not reflect the entire influence of the exercise on MPS and subsequent muscle hypertrophy further contributing to the observed mismatch
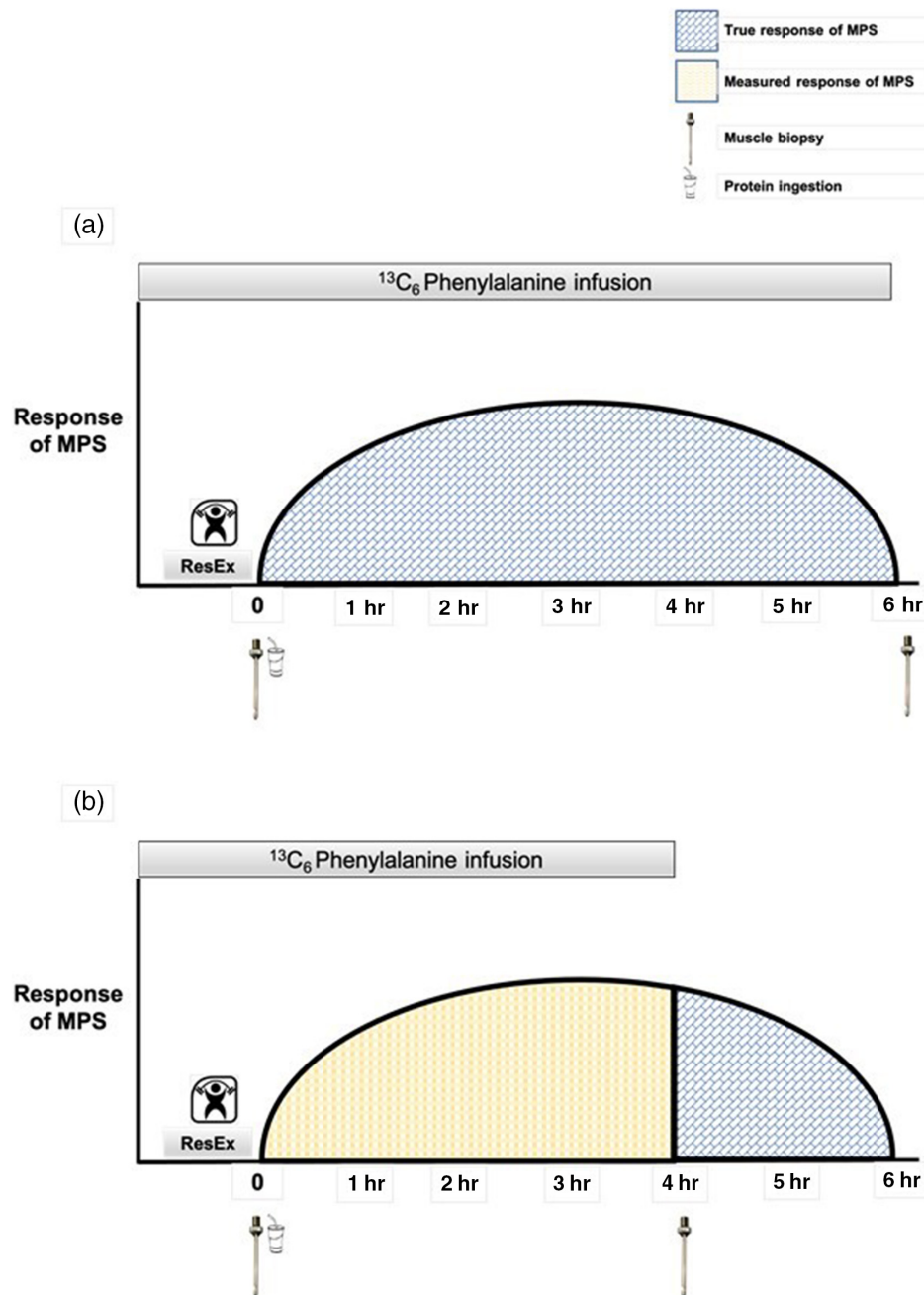
Figure 2 — The response of MPS to a bout of REx and protein ingestion. (a) Infusion of $[^{13}C_6]$ phenylalanine and muscle samples taken at timepoints that capture the entire true response of MPS and (b) infusion ends and muscle samples are taken at 0 and 4 hr, but the true response of MPS remains elevated above baseline for 6 hr, so the response is underestimated. MPS = muscle protein synthesis; REx = resistance exercise.

between measurement of MPS and changes in muscle mass with training.

Over the past 15 years, another method has been revisited to determine an integrated FSR in free-living participants over a time period that is not limited by an infusion, that is, the D_2O method (Figure 3). Thus, MPS in various situations and in response to various exercise and nutrition interventions can be determined over the time course of days to weeks. The determined rate of MPS integrates the response to all physical activity and nutrient consumption during that time, including the prolonged response of MPS to subsequent meals following REx (Figure 3b). Thus, the D_2O method could be argued to provide a more holistic assessment of MPS without the limitations inherent with the requirement for infusion of stable isotopes for measurement of MPS. It is perhaps not particularly surprising that integrated rates of MPS over longer time periods than are possible with isotope infusion studies, as well as inclusion of habitual physical activity and enhanced periods of postprandial MPS in response to exercise hours to days earlier, are better correlated with subsequent muscle hypertrophy. Several studies utilizing the D_2O measurement of FSR have reported correlations of MPS with subsequent muscle hypertrophy (Brook et al., 2015; Damas, Phillips, Libardi, et al., 2016; Franchi et al., 2015). Therefore, this method for assessing MPS seems to be more suitable for predicting muscle hypertrophy with RET.

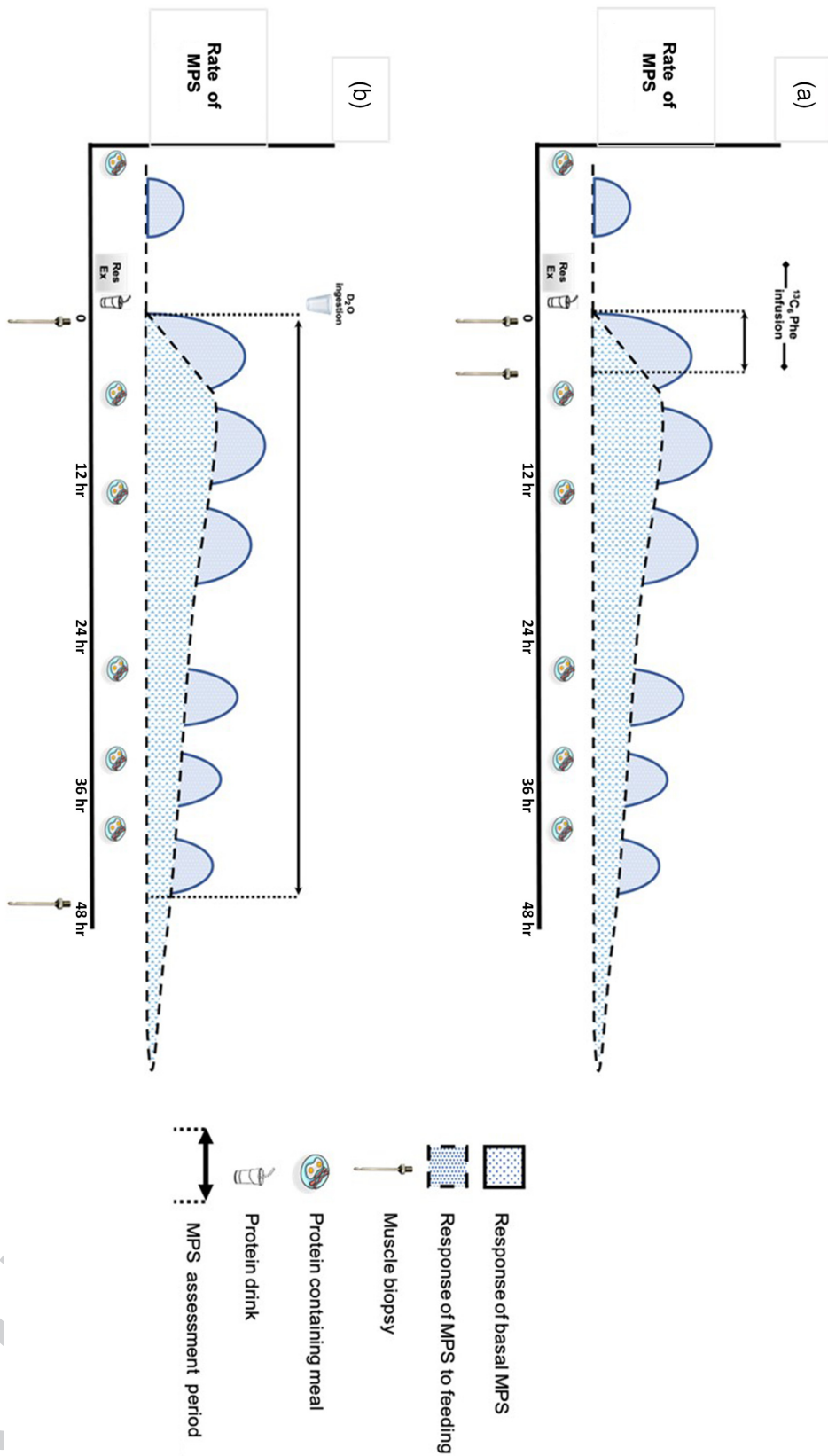**Figure 3** — Comparison of measurement of MPS with (a) an infusion of labeled amino acids ($^{13}C_6$ Phe) or (b) ingestion of deuterated water (D_2O). The response of MPS is enhanced following REx and this is captured by D_2O measurement of MPS. MPS = muscle protein synthesis; REx = resistance exercise.

The disconnect between the initial measurement of MPS and subsequent muscle hypertrophy during RET may be due to methodological choices made for measurement of changes in muscle mass in addition to MPS. Differences in study design and methods chosen to determine changes in muscle mass, in addition to inherent individual variability in the response of muscle to training (Mobley et al., 2018), contribute to variable results among RET studies. Factors including training duration, sleep quality, nontraining physical activity, nutrition, and other lifestyle variables may impact the training response (Haun et al., 2019; Mitchell et al., 2014). Proper control of many of these factors is virtually impossible in most RET study situations. This variability is further complicated by the various permutations possible with various combinations of these factors (Haun et al., 2019).

Perhaps a more prosaic factor contributing to the disconnect between the acute response of MPS and subsequent muscle hypertrophy with RET relates to the inherent limitations of methods used to measure changes in muscle mass in humans. Reported changes in muscle mass with RET are heavily dependent on the method chosen to assess those changes. Hence, the critical reader should consider the limitations of these methods when evaluating any particular training study. Changes in muscle mass may be measured on one or more of several levels, that is, biochemical, ultrastructural, histological, and gross anatomical levels. When multiple methods from these levels of hypertrophy are used, the agreement between methods is often poor (Haun et al., 2019). Moreover, as detailed above, there are different types of hypertrophy that must be considered in combination with the method chosen to assess changes in muscle mass. Strict control of methodological conditions, both at the time of measurement and/or laboratory conditions, is necessary (Haun et al., 2019). Three types of hypertrophy have been proposed: connective tissue, sarcoplasmic, and myofibrillar. Contributions of each type of hypertrophy to measured hypertrophy may vary with training status and/or the method used to assess hypertrophy. For example, there is evidence that hypertrophy measured at the early stage of a RET program may result from edema-induced, that is, muscle swelling and sarcoplasmic hypertrophy (Damas, Phillips, Libardi, et al., 2016). This means that if muscle hypertrophy is based on dual-energy X-ray absorptiometry or other methods without consideration of changes in intramuscular fluid, overestimations of true hypertrophy will be made. Clearly, changes in muscle mass with fluid infiltration are not related to MPS. These methodological factors should be considered when assessing the relationship between the acute response of MPS to changes in muscle mass with RET.

Practical Implications

Translating the science behind this complex relationship between the acute response of MPS to exercise and/or nutrition into clear, contextually relevant and practical messages is a priority for practitioners, coaches, athletes, and researchers. Based on our critical evaluation of existing evidence, we can make three practical implications.

(a) The acute response of MPS to REx plus protein ingestion will translate to chronic adaptations in muscle mass only in trained individuals. The predictive value of acute measurements of MPS for chronic adaptations in muscle mass in individuals at the beginning of a period of RET is limited due to the multiple regenerative roles of MPS beyond the accumulation of new muscle protein during the early stages of the training process. Nevertheless, greater rates of MPS in untrained individuals still may be considered beneficial since they are indicative of greater rates of protein turnover and muscle remodeling following exercise.

(b) The predictive value of the acute response of MPS in distinguishing between the anabolic capacity of an exercise training or nutritional intervention warrants consideration when offering practical recommendations at a group level. In this regard, the practitioner may use this information as a general starting point to trial the effectiveness of an exercise or nutritional stimulus. However, the practitioner should remain open minded that a "one-size-fits-all" approach almost certainly does not apply, and there will likely be some athletes that do not respond to the intervention.

(c) Finally, any recommendations made based on information, such as is described in (b), should not be based on quantitative differences between interventions that stimulate MPS. Despite one intervention being X% better than another according to acute metabolic data, this will not translate directly, at least quantitatively, to the magnitude of change for the parameter (i.e., muscle hypertrophy) of interest. Hence, the practitioner should manage expectations when explaining the potential gains afforded to the intervention of interest.

Conclusions

In this review, we have attempted to provide an evidence-based critical evaluation for the use of results from acute metabolic studies to predict changes in muscle mass with RET. It is clear that the measured acute response of MPS to an exercise/nutrition intervention is not predictive of muscle hypertrophy for any individual participating in a RET and nutrition program based on that particular combination of exercise parameters and nutrition. This lack of predictive power is especially true if the individual is beginning an unaccustomed exercise program. Nevertheless, this discrepancy should not be used to determine the value of studies measuring MPS in response to REx and protein nutrition. There are multiple examples of studies in which the acute response of MPS does predict the average hypertrophy on a group level (Hartman et al., 2007; Tang et al., 2009; Volek et al., 2013; West et al., 2009, 2010; Wilkinson et al., 2007). Moreover, measurement of the acute response of MPS to REx and nutrition interventions can provide valuable information. Regardless of training status, the acute response of MPS is indicative of protein turnover and muscle remodeling critical for recovery from exercise and adaptation to training.

The measurement of integrated MPS that includes the enhanced postprandial response of MPS to protein ingestion in free-living individuals certainly may provide predictive information about subsequent muscle growth, albeit not in individuals undergoing unaccustomed exercise. Moreover, the acute measurement of MPS also provides more sensitivity than chronic training studies over a much shorter time frame and can thus be viewed as a good starting point for determining nutritional recommendations. Given the nature of measurement of FSR, if a difference is detected in an acute study, for example, between different protein sources, then we can conclude with high confidence that the measured difference is physiologically relevant, at least qualitatively. In this regard, the protein source that engenders the greater FSR may be considered the higher quality protein source irrespective of whether chronic studies are able to detect differences in muscle hypertrophy

under comparable conditions of protein source manipulation. Thus, we can use that information to inform subsequent RET studies.

Finally, the acute measurement of MPS in response to exercise and nutrition offers valuable mechanistic information. In fact, delineation of mechanisms of muscle protein metabolism was the aim of many of the seminal studies that are now used to contribute to the development of recommendations (Biolo et al., 1997; Phillips et al., 1997; Tipton et al., 1999, 2001). Thus, whereas practitioners should be aware of the potential pitfalls with reliance on acute metabolic studies for making nutritional recommendations for athletes and exercisers, with proper interpretation a great deal of valuable information may be gleaned from these studies. Acute measurement of MPS in response to various nutrition and exercise interventions should be viewed as yet another tool in the toolbox for use by practitioners and others.

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