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Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review

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Shad BJ, Thompson JL, Breen L. Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am J Physiol Endocrinol Metab* 311: E803–E817, 2016. First published August 23, 2016; doi:10.1152/ajpendo.00213.2016. —The precise role of age-related muscle anabolic resistance in the progression of sarcopenia and functional decline in older individuals is unclear. The present aim was to assess whether the muscle protein synthesis (MPS) response to acute exercise (endurance or resistance) and/or amino acid-based nutrition is attenuated in older compared with young individuals. A systematic review was conducted on studies that directly examined the influence of age on the MPS response to exercise and/or amino acid-based nutrition. Each study arm was synthesized and reported as providing sufficient or insufficient “evidence of age-related muscle anabolic resistance”. Subsequently, three models were established to compare age-related differences in the MPS response to 1) exercise alone, 2) amino acid-based nutrition alone, or 3) the combination of exercise and amino acid-based nutrition. Following exercise alone, 8 of the 17 study arms provided sufficient evidence of age-related muscle anabolic resistance, while in response to amino acid-based nutrition alone, 8 of the 21 study arms provided sufficient evidence of muscle anabolic resistance. When exercise and amino acid-based nutrition were combined, only 2 of the 10 study arms provided sufficient evidence of age-related muscle anabolic resistance. Our results highlight that optimization of exercise and amino acid-based nutrition is sufficient to induce a comparable MPS response between young and older adults, below which age-related muscle anabolic resistance may become apparent.

skeletal muscle; anabolic resistance; sarcopenia; resistance exercise

IT IS WELL DOCUMENTED THAT we are in the midst of a global shift towards an expanding aging demographic. Recent estimates predict that the number of people aged 60 years and over is expected to more than double from 901 million in 2015 to over two billion in 2050, while the number of people aged 80 years and over (the “oldest old”) is expected to more than triple (100). Advancing age is closely associated with a number of debilitating health consequences, including the loss of skeletal muscle mass and strength (termed sarcopenia), which is strongly associated with an increased incidence of falls (63), loss of independence (9), increased risk of age-related comorbidities (4, 32), and, in severe cases, premature mortality (16, 88). As such, sarcopenia and associated comorbidities place a considerable burden on healthcare resources (51). Therefore, clear understanding of the metabolic and molecular mechanisms that underpin sarcopenia is of paramount importance in order to develop targeted therapeutic strategies to prevent and/or treat this age-related phenomenon.

The underlying pathology of sarcopenia is highly complex and remains to be fully elucidated. Sarcopenia may result from factors including inactivity/disuse, inadequate dietary protein intake, chronic low-grade inflammation, and hormonal dysregulation, summarized succinctly by others (73). Regardless of the precise contribution of each of these factors, sarcopenia is due to muscle protein loss resulting from an imbalance between muscle protein synthesis (MPS) and breakdown (MPB), which manifests primarily as a reduction in type II...
muscle fiber size (34, 74, 79, 102). In healthy young individuals, mechanical loading (i.e., exercise contraction) in the fasted postabsorptive state increases MPS and, to a lesser extent MPB, resulting in an improved yet negative net protein balance (NBAL) (10, 80). In contrast, amino acid-based nutrition serves primarily to increase MPS, with the impact on MPB less clear due to the methodological difficulties encountered when assessing MPB under non-steady-state conditions. In general, most studies appear to demonstrate a small suppression of MPB in response to amino acid-based nutrition, which in conjunction with the postprandial rise in MPS results in a positive NBAL in both young and older individuals (43, 71, 103, 105). Combined, mechanical loading and amino acid-based nutrition act synergistically to enhance MPS and suppress MPB and thus promote net muscle protein accretion (22, 42, 71, 78). Most (27, 35, 64, 76, 105), but not all (7, 48, 117) studies to date have observed no evidence of age-related differences in postabsorptive basal rates of MPS. Likewise, although methodologically challenging to measure, rates of MPB are comparable between healthy younger and older individuals in the postabsorptive basal state and following resistance exercise (38, 110). Evidence of an age-related impairment in the suppression of MPB under hyperaminoacidemic and/or hyperinsulinemic conditions has been limited and relatively inconsistent to date (81, 104, 110). The absence of age-related differences in postabsorptive basal state rates of MPS and MPB, coupled with inconsistent findings on age-related differences in postprandial rates of MPB, has led to the hypothesis that dysregulation of the MPS response to normally robust anabolic stimuli (i.e., exercise and/or amino acid-based nutrition), termed “anabolic resistance” (83), may underpin the progression of sarcopenia.

Age-related muscle anabolic resistance may be related to diminished mRNA translational signaling (27, 37, 46, 62), impaired transport of amino acids into muscle (30, 31), lipid-induced muscle insulin resistance (89), attenuated protein digestion and absorption (13), and dysregulation of nutritive blood flow to skeletal muscle (39, 66, 81). However, these defects may be a consequence of declining habitual activity levels (15), protracted disuse events (41, 107), obesity (72), and chronic inflammation (6, 97) superimposed on the natural biological ageing process. Interestingly, while some studies support the development of age-related muscle anabolic resistance (27, 46, 53), other studies have failed to observe any difference in the MPS response to anabolic stimuli between young and older adults (59, 76, 90). This lack of agreement between studies on whether or not differences in MPS exist between young and older individuals may be due to differences in the experimental methodology used to assess MPS (18). For example, 1) the time frame of MPS assessment, 2) analysis of specific muscle protein subfractions, and 3) volume of exercise and dose/source of amino acid-based nutrition can profoundly influence the observed MPS response in young and older adults. Furthermore, participant habitual physical activity levels and metabolic health status may also explain the incongruous findings of previous studies (15, 17). With this in mind, it is imperative that we explore the possible cause of discrepancies between studies and delineate whether age-related differences in MPS between young and older individuals do exist. This approach will allow us to identify whether (or not) strategies to restore muscle anabolic sensitivity in older individuals have the capacity to prevent or slow sarcopenic progression.

Accordingly, the primary aim of this qualitative systematic review was to explore whether the MPS response to exercise (endurance and/or resistance) and/or amino acid/protein administration is attenuated in older compared with young individuals. Given the suggestion that aspects of experimental design and methodology may influence the observed MPS response between young and older individuals (17, 18), a secondary aim of this analysis was to contrast experimental parameters among the included studies to delineate whether design/methodological variables may account for any incongruence observed.

METHODS

Search Strategy

A systematic literature search of the Ovid MEDLINE (1946 to May 2016) and EMBASE (1974 to 23 May 2016) databases was performed with the final literature search completed on 23 May 2016. These databases were chosen due to the extensive coverage of journal articles in the area of health and clinical sciences. Search terms used were: protein synth*, muscle protein synth*, MPS, fractional synth*, FSR, myofibrillar, muscle protein accru*, protein balance, phenylalanine, exercise*, contraction*, resistance exercise*, amino acid*, EAA*, essential amino*, dietary protein, protein-rich, beef, leucine, young*, old* and elder*. The medical subject headings (MeSH) “muscle proteins” and “humans” were also utilized. Boolean operators “and” and “or” were used to combine search terms. Additional studies were identified through the reference lists of articles (e.g., reviews) from relevant fields of study.

Eligibility Criteria

Types of studies. Randomized controlled trials, nonrandomized clinical trials, or comparative studies that directly compared young and older participants within the same study were eligible for inclusion. Nonrandomized studies were eligible, as the majority of studies that explore age-related differences in MPS in response to an anabolic stimulus intentionally group subjects based on their age (i.e., young vs. older), and thus randomization is not always possible. Studies were restricted to those written in the English language, and no publication date restrictions were applied.

Types of participants. Healthy young and older humans, both male and female, were included. The mean age of the young group was required to be in the range of 18 and 35 yr (inclusive). The mean age of the older group was required to be ≥55 yr. These criteria were chosen because age-related sarcopenia tends to manifest in the fourth to fifth decade in humans (23, 50); thus, we reasoned that an age range of 18–35 yr would provide a fair reflection of younger individuals that had not yet reached the threshold for development of sarcopenia. Similarly, we posited that 55 yr of age and older individuals would ensure that the threshold for development of age-related sarcopenia had been reached. Accordingly, any studies that utilized young or older groups with a mean age between 36 and 54 yr (inclusive) were excluded. To ensure that we addressed the influence of age on the MPS response to anabolic stimuli per se, participants with any form of diabetes or chronic disease condition characterized by rapid inflammation-induced muscle atrophy (e.g., chronic obstructive pulmonary disease, cancer cachexia, arthritis, or congestive heart failure) were excluded, as such conditions are known to dramatically alter postabsorptive and postprandial muscle protein turnover beyond that expected in healthy, nondiseased populations (25).

Types of interventions. This systematic review was limited to studies utilizing a single acute bout of resistance exercise (e.g., free-weight, guided range-of-motion machines, dynamometry, or...
body weight exercises) and/or endurance exercise (e.g., walking, cycling, or running) and/or amino acid/protein administration. Amino acids/protein could be provided either orally (e.g., supplemental protein beverages or protein-rich solid foods) or intravenously (e.g., hyperaminoacidemic clamp). Studies in which additional macronutrients (carbohydrates and fats) were provided in addition to amino acid/protein provision were deemed eligible for inclusion, as coingestion of carbohydrate and/or fat does not appear to significantly modulate the postprandial MPS response to protein ingestion (44, 45, 60). Interventions that coadministered pharmaceutical drugs that were not designed to incur hyper- and/or hypoaminoacidemia, insulinemia, or glycemia were excluded, as those drugs could confound some of the age-related differences in the MPS response to anabolic stimuli between young and older individuals. Interventions that assessed acute MPS rates following a chronic resistance training program were also excluded, as that could abrogate potential age-related differences in MPS (48).

Types of outcome measures. The primary outcome measure from eligible studies was a qualitative appraisal of muscle anabolic resistance, i.e., sufficient evidence of age-related differences in MPS rates, or insufficient evidence of age-related differences in MPS rates in response to a given anabolic stimulus. Assessment of MPS was required to be completed within 24 h of the given stimulus, as it has previously been demonstrated that the increase in MPS rates is most pronounced in the immediate hours following an anabolic stimulus, gradually subsiding by 24 h poststimulus in young individuals (19, 80). All studies included were required to assess MPS via calculation of the muscle fractional synthetic rate (FSR) using the precursor-product model. The precursor-product model measures the rate at which a tracer is incorporated into bound muscle protein between sequential muscle biopsies over a specified period of time and is considered the gold standard for assessing in vivo MPS in humans (14, 54, 114). Furthermore, this approach allows the assessment of MPS within specific protein subfractions (i.e., myofibrillar, mitochondrial, and sarcoplasmic). Therefore, any studies that used the two-pool or three-pool arteriovenous balance method (indirect estimate of MPS) were excluded. Included studies were required to assess at least one of the following: mixed-muscle, myofibrillar, or myosin heavy chain muscle protein synthesis, as these protein subfractions comprise the contractile apparatus of skeletal muscle.

Data Collection and Analysis

Selection of studies. Eligibility appraisal of the titles and abstracts generated by the literature search was conducted independently by two reviewers (B.J. Shad and J.L. Thompson). All titles and abstracts deemed ineligible were excluded, while those determined to be potentially eligible for inclusion in the systematic review were reserved and the full-text articles obtained. Full-text articles were subsequently screened by two independent reviewers (B.J. Shad and L. Breen) for relevance using the eligibility criteria described above. Any disagreements between the two reviewers were resolved by consensus. All records generated by the literature search of Ovid MEDLINE and EMBASE databases were managed using the reference management software package EndNote (Thomson Reuters, v. X7). Duplicate records were removed using the “find duplicates” function in Endnote.

Data extraction and management. Two reviewers (B.J. Shad and L. Breen) independently extracted all data (i.e., study characteristics and outcome data) from all included studies using a customized data extraction form. Any disagreements were resolved by consensus between the two reviewers. Data were extracted on a study arm level. This ensured that all relevant data were extracted in circumstances where multiple interventions were utilized within the same study [e.g., provision of different essential amino acid (EAA) doses]. Categories of data extracted included 1) participant characteristics (e.g., age, number, sex, and body mass), 2) type of intervention (e.g., exercise mode, exercise intensity, and amino acid dose), 3) details of the method of MPS assessment (e.g., measurement period, muscle subtraction used, and precursor pool used), and 4) data outcome details (i.e., qualitative appraisal of age-related differences in the MPS response and whether the data provided sufficient “evidence of age-related muscle anabolic resistance” or not; see Method of data synthesis section below).

Method of data synthesis. We chose to qualitatively synthesize the data from the included studies, as the heterogeneous experimental methodology employed when assessing MPS (e.g., amino acid stable isotope tracer, muscle protein subtraction, duration of tracer incorporation, and precursor pool) can result in varying rates of MPS between studies (86), meaning that quantitative analysis across studies was not feasible. As part of the data extraction process, both reviewers were required to qualitatively synthesize the data of each study by independently determining whether there was sufficient evidence of age-related muscle anabolic resistance or not. If it was deemed that the results of a study provided sufficient evidence of age-related muscle anabolic resistance, the study was given a “Yes”, whereas if it was deemed that the results of a study did not provide sufficient evidence of age-related muscle anabolic resistance, the study was given a “No”. Examples of sufficient evidence of age-related muscle anabolic resistance included data demonstrating 1) a significantly (P < 0.05) greater MPS response in young compared with older participants in response to an anabolic stimulus or 2) that only young participants experienced a significant (P < 0.05) increase in MPS in response to anabolic stimuli. In the event that a study assessed MPS at multiple time points but reported only age-related differences in MPS at some but not all of these time points, data were extracted from the reported time points only. Similarly, in the event that a study assessed the MPS response to multiple exercise stimuli (e.g., a range of exercise intensities) and/or nutritional interventions (e.g., varying amino acid doses) but reported only age-related differences in MPS for some of these interventions, data were extracted from the reported interventions only. Upon completion of data extraction, using a similar analysis approach to Trommelen et al. (99), several different models were constructed to compare age-related differences in MPS in response to different anabolic stimuli. In Model 1, study arms that utilized exercise as the only form of anabolic stimuli were included to examine age-related differences in the MPS response to an isolated contractile bout. In Model 2, study arms that utilized amino acid/protein administration/feeding as the only form of anabolic stimuli were included to examine age-related differences in the MPS response to a nutrient stimulus. Finally, Model 3 included study arms that utilized exercise alongside amino acid/protein administration/feeding to examine age-related differences in the MPS response to the combined anabolic stimulus of contraction and amino acid-based nutrition.

RESULTS

Literature Search

The literature search produced 154 records potentially eligible for inclusion. A further five records were identified through a hand search of reference lists of reviews in the field of study, resulting in a total of 159 records. Following the removal of duplicate records, 103 records remained. From the remaining records, titles and abstracts were independently screened by two reviewers (B.J. Shad and J.L. Thompson) to assess eligibility. The screening process resulted in 71 studies being excluded, leaving 32 full-text articles to be assessed for eligibility by two reviewers (B.J. Shad and L. Breen) independently. Of these 32 full-text articles, eight were excluded for reasons including use of the three-pool arteriovenous balance method to estimate age-related differences in MPS (52), assessment of MPS in the postabsorptive state only (109), and...
mean age of the young participants falling outside the inclusion range (87). Accordingly, a total of 24 studies met the eligibility criteria and thus were included in the systematic review for qualitative analysis. Figure 1 depicts a flow diagram of the study identification process.

Included Studies

Across the 24 studies included, there was a large amount of heterogeneity pertaining to the participant characteristics, the anabolic stimuli utilized (e.g., different exercise regimens and/or route, source and dose of amino acid/protein provision) and the experimental methodology used to determine MPS. A brief overview of between-study differences is provided below in RESULTS and more comprehensively in Tables 1, 2, and 3.

Participants

All of the included studies reported participants as “healthy,” and included a comparison between young and older groups. A total of 23 of the included studies specifically assessed participant health status, whereas one study failed to declare any such assessment (5). A total of 15 of the included studies recruited males only, one study included females only, seven studies included both males and females, and one study did not report the sex of participants (46). The age range of the young participant groups was between 20 and 35 yr, while the age range of the older participant groups was between 64 and 76 yr. Body mass of the young participant groups ranged from 62 to 88.9 kg, and body mass in the older participant group ranged from 60.8 to 88 kg.

Anabolic stimulus

Of the 24 studies, 12 included some form of acute exercise stimulus. Resistance exercise was utilized in 10 of the 12 studies, and endurance exercise in two studies. Eighteen of the included studies involved a form of amino acid/protein administration/feeding. Oral ingestion of amino acids/protein was evident in 15 of the 18 studies, while three studies administered amino acids through intravenous (iv) infusion. A total of six of the 24 studies combined exercise with oral or iv administration of amino acids/protein.

Experimental Methodology

Experimental methodology between studies was highly variable. The time point over which the post-stimulus MPS measurement was assessed ranged from 2 to ~24 h. MPS in a mixed muscle fraction was assessed in 19 studies, whereas five studies assessed MPS in the myofibrillar fraction. Sixteen studies used the intracellular free-pool isotopic tracer enrichment as the precursor in the calculation of FSR, while eight studies used the plasma isotopic tracer enrichment as the precursor. All of the included studies measured MPS from muscle biopsy tissue collected from the quadriceps vastus lateralis muscle.

Data Synthesis

Details of the 24 studies identified for inclusion are included in Tables 1 (Model 1), 2 (Model 2), and 3 (Model 3). Several of the included studies utilized experimental designs (e.g., EAA and/or exercise dose-response interventions) that allowed the assessment of multiple anabolic stimuli over several post-intervention time points within the same study. The divergence in experimental designs made it difficult to draw firm conclusions as to whether there was sufficient evidence of age-related muscle anabolic resistance on a study level. Thus, we decided to perform data synthesis on a study arm level.

A total of 48 study arms were identified from the 24 included studies (Fig. 2). Of these 48 study arms, 18 were considered to provide sufficient evidence of age-related muscle anabolic resistance.

Fig. 1. Study identification process flowchart. Y, young; O, older; M, male; F, female; KE, knee extension; LP, leg press; 1RM, one-repetition maximum; RM, repetition maximum; MPS, muscle protein synthesis; AUC, area under curve; Myo, myofibrillar; IC, intracellular.
Finally, in Model 3, study arms that utilized a combination of both exercise and amino acid/protein administration/feeding were included. As a result, 10 study arms were included in Model 3, with two study arms providing sufficient evidence (35, 58) and eight study arms providing insufficient evidence of age-related muscle anabolic resistance (2, 35, 36, 78, 90). Nine of the 10 study arms utilized resistance exercise as the contractile stimulus, with two providing sufficient evidence and seven providing insufficient evidence of age-related muscle anabolic resistance (Table 3). The single study arm that applied endurance exercise as the contractile stimulus provided insufficient evidence of age-related muscle anabolic resistance.

**DISCUSSION**

The aim of this systematic review was to examine the literature on age-related differences in the muscle protein synthetic response to anabolic stimuli (resistance exercise, endurance exercise, and/or amino acid/protein administration) between young and older individuals. There has been much debate as to whether muscle anabolic resistance is indeed an inevitable characteristic of the aging process (17, 18), an artifact of lifestyle modifications (15, 107), or a combination of these two factors. While 18 study arms provided findings to support the presence of muscle anabolic resistance in older individuals, 30 study arms provided insufficient evidence of the development of age-related muscle anabolic resistance (Fig. 2). As will be discussed in this section, the primary factors that appear to contribute to the discrepancies between study arms include 1) differences in exercise volume and intensity, 2) the dose, source, and leucine content of amino acids/protein provided, 3) use of exercise or amino acid/protein administration/feeding alone or in combination, and 4) differences in experimental methodology and design.

**Exercise Volume and Intensity**

It has been documented that both endurance and resistance exercise robustly stimulate mitochondrial and myofibrillar MPS, respectively, in young and older individuals (29, 33, 61, 111). However, it is not yet fully known how the MPS response to exercise differs between young and older individuals. To this end, we constructed a model that included only those study arms that assessed the MPS response to exercise alone in the postabsorptive state (Fig. 2, Model 1, and Table 1). Interestingly, whereas eight study arms provided sufficient evidence of age-related muscle anabolic resistance (37, 61, 62, 65, 84, 85), nine study arms did not (61, 62, 84, 85). One potential explanation for the lack of congruence may be the difference in exercise volume between studies. For example, in a well-controlled study from Kumar et al. (61), MPS postexercise was significantly lower in the older group than in the young when a relatively low volume of work was completed [3 sets of knee extension exercise at 40% one-repetition maximal strength (1RM)]. However, the authors noted that when the volume of work completed was doubled the MPS response was comparable between young and older groups (61). These data imply the possibility of an age-related exercise volume “threshold”, whereby older individuals are required to complete greater exercise volumes to elicit a comparable MPS response to that of the young. Alternatively, the relative loading intensity of resistance exercise may also explain differences in the MPS response.
Habitual physical activity levels are needed to improve our response to exercise studies utilizing larger sample sizes and participants. Taken together, it is clear that future acute dose-ranging volume and intensity of resistance exercise in this group of within-subject comparison group in the study by Fry et al. and older participants, which were not measured objectively in may relate to the habitual physical activity levels of the young et al. (61) and Fry et al. (37) differ is difficult to reconcile but a larger sample size (37). Exactly why the findings of Kumar failed to detect any age-related deficit in MPS following six differences in MPS may explain why Sheffield-Moore et al. volume and/or heavier load exercise can overcome age-related blunting of MPS (61). The position that a greater rate of MPS in the young compared with the older completed at 40% 1RM (i.e., fewer repetitions), overcame the group, three sets at 75% of 1RM, with volume-matched to that greater in Y vs. O at 1–2 h. MPS was not different between Y and O while three sets of knee extensions at 40% of 1RM induced response to exercise observed between studies. Specifically, while three sets of knee extensions at 40% of 1RM induced greater rates of MPS in the young compared with the older group, three sets at 75% of 1RM, with volume-matched to that completed at 40% 1RM (i.e., fewer repetitions), overcame the age-related blunting of MPS (61). The position that a greater volume and/or heavier load exercise can overcome age-related differences in MPS may explain why Sheffield-Moore et al. failed to detect any age-related deficit in MPS following six sets of knee extensions at 80% 1RM (84). However, this fails to explain the occurrence of age-related muscle anabolic resistance following eight sets of knee extensions at 70% of 1RM by Fry et al. over numerous postexercise time points and using a larger sample size (37). Exactly why the findings of Kumar et al. (61) and Fry et al. (37) differ is difficult to reconcile but may relate to the habitual physical activity levels of the young and older participants, which were not measured objectively in either study (discussed in further detail below). The lack of a within-subject comparison group in the study by Fry et al. precludes interrogation of the dose response of MPS to differing volume and intensity of resistance exercise in this group of participants. Taken together, it is clear that future acute dose-response exercise studies utilizing larger sample sizes and multiple postexercise time points, with control/monitoring of habitual physical activity levels are needed to improve our understanding of the importance of exercise volume and intensity in overcoming potential age-related muscle anabolic resistance. In addition, chronic resistance training studies are required to delineate the appropriate exercise training volume and/or intensity to maintain or augment skeletal muscle mass in older individuals. Nonetheless, the findings presented suggest that age-related muscle anabolic resistance may be apparent following low-volume/intensity resistance exercise and that the prescription of higher volume and/or intensity resistance exercise may be a feasible strategy to overcome this impairment and thus maintain skeletal muscle mass.

### Dose of Amino Acids/Protein

The provision of amino acid-based nutrition is a potent stimulus for MPS in young and older individuals (27, 82, 116), primarily through the action of constituent EAAs (96, 103). According to the findings of the studies included in Model 1, amino acids are essential for muscle protein synthesis.

### Summary of studies included in Model 1

<table>
<thead>
<tr>
<th>Reference</th>
<th>Group, Age (yr)</th>
<th>Sex (n)</th>
<th>Body Mass (kg)</th>
<th>Exercise Protocol</th>
<th>Incorporation Period</th>
<th>Muscle Subfraction</th>
<th>Precursor Pool</th>
<th>Evidence of Age-Related Muscle Anabolic Resistance</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fry et al. (2011) (37)</td>
<td>Y 27 ± 2, M 56 ± 3</td>
<td>MF n = 16</td>
<td>70.2 ± 3.1</td>
<td>8x10 sets of KE at 75% 1RM</td>
<td>0–3 h</td>
<td>Mixed</td>
<td>IC</td>
<td>Yes</td>
<td>MPS was increased in both Y and O and was greater in Y at all time points.</td>
</tr>
<tr>
<td>Kumar et al. (2009) (62)</td>
<td>Y 24 ± 6, M 70 ± 25</td>
<td>M n = 25</td>
<td>66.9 ± 3.0</td>
<td>Unilateral KE at intensities from 20–90% 1RM (volume matched)</td>
<td>0–1 h</td>
<td>Myo</td>
<td>IC</td>
<td>No</td>
<td>Overall MPS response (AUC) across all intensities was 30% higher in Y vs. O at 1–2 h. MPS was not different between Y and O.</td>
</tr>
<tr>
<td>Kumar et al. (2012) (61)</td>
<td>Y 24 ± 6, M 70 ± 25</td>
<td>M n = 25</td>
<td>72 ± 12</td>
<td>1. 3 sets of KE at 40% 1RM 2. 6 sets of KE at 40% 1RM 3. 3 sets of KE at 75% 1RM 4. 6 sets of KE at 75% 1RM</td>
<td>0–4 h</td>
<td>Myo</td>
<td>IC</td>
<td>Yes</td>
<td>At 40% 1RM (3 sets), AUC for MPS over entire 0–4 h postexercise was higher in Y vs. O. At 40% (6 sets) and 75% (3 and 6 sets) 1RM, AUC for MPS was not different between Y and O.</td>
</tr>
<tr>
<td>Mayhew et al. (2009) (65)</td>
<td>Y 27 ± 1, O 24 ± 4</td>
<td>M n = 8, M n = 6</td>
<td>75.4 ± 3.0, 76.8 ± 3.0</td>
<td>3x8–12 RM of squat, LP and KE</td>
<td>21–24 h</td>
<td>Mixed</td>
<td>IC</td>
<td>Yes</td>
<td>MPS was increased above baseline at 21–24 h postexercise in Y only.</td>
</tr>
<tr>
<td>Sheffield-Moore et al. (2004) (85)</td>
<td>Y 29 ± 2, M 64 ± 2</td>
<td>M n = 6</td>
<td>75.8 ± 3.0, 76.8 ± 3.0</td>
<td>Treadmill exercise (walking) for 45 min at ~40% VO2 peak</td>
<td>0–10 min</td>
<td>Mixed</td>
<td>IC</td>
<td>No</td>
<td>MPS at 0–10 min and 0–3 h was not different between Y and O but MPS was increased only in Y at 0–1 h.</td>
</tr>
<tr>
<td>Sheffield-Moore et al. (2005) (84)</td>
<td>Y 29 ± 2, O 69 ± 2</td>
<td>M n = 6, M n = 6</td>
<td>78 ± 3, 86 ± 2</td>
<td>6x6 sets of KE at 80% 1RM</td>
<td>0–10 min</td>
<td>Mixed</td>
<td>IC</td>
<td>No</td>
<td>MPS was increased at 0–10 min in O only. MPS was not elevated in Y or O at 0–1 h. MPS was increased at 0–3 h in Y only.</td>
</tr>
</tbody>
</table>

Values are means ± SE. Y, young; O, older; M, male; F, female; KE, knee extension; LP, leg press; 1RM, One repetition maximum; RM, repetition maximum; MPS, muscle protein synthesis; AUC, area under curve; Myo, myofibrillar; IC, intracellular.
Table 2. Summary of studies included in Model 2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Group, Age (yr)</th>
<th>Sex (n)</th>
<th>Body Mass (kg)</th>
<th>Amino Acid/Protein Protocol</th>
<th>Incorporation Period</th>
<th>Muscle Subfraction</th>
<th>Precursor Pool</th>
<th>Evidence of Age-Related Muscle Anabolic Resistance</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babraj et al. (2005) (5)</td>
<td>Y 28 ± 6</td>
<td>M n = 4</td>
<td>20 g EAA orally consumed</td>
<td>0-3 h Myo Plasma</td>
<td>Yes</td>
<td>Y and O increased MPS, but increase was lower in O.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chevalier et al. (2011) (24)</td>
<td>Y 24 ± 1</td>
<td>F n = 8</td>
<td>62.0 ± 3.6</td>
<td>Hyperinsulinemic, hyperglycemic, and hyperaminoacidemic clamp (iv)</td>
<td>0-2 h Mixed IC</td>
<td>No</td>
<td>Both Y and O increased MPS with no difference between groups.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuthbertson et al. (2005) (27)</td>
<td>Y 28 ± 6</td>
<td>M n = 16</td>
<td>75 ± 10</td>
<td>1. 2.5 g EAA orally</td>
<td>0-3 h Myo IC</td>
<td>No</td>
<td>No MPS between Y and O at 2.5 and 5 g EAA. MPS in Y was greater than O at 10 and 20 g EAA.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O 28 ± 6</td>
<td>M n = 16</td>
<td>79 ± 13</td>
<td>2. 5 g EAA orally</td>
<td></td>
<td>No</td>
<td>No MPS between Y and O for either intervention.</td>
<td></td>
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<tr>
<td></td>
<td>O 70 ± 6</td>
<td>M n = 16</td>
<td>20 g EAA orally</td>
<td></td>
<td></td>
<td>Yes</td>
<td>MPS was increased in both Y and O and was greater in Y.</td>
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<tr>
<td>Gorissen et al. (2014) (44)</td>
<td>Y 20 ± 1</td>
<td>M n = 12</td>
<td>79.6 ± 2.7</td>
<td>2. 20 g casein orally consumed without 60 g carbohydrate</td>
<td>0-5 h</td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td>O 76 ± 1</td>
<td>M n = 12</td>
<td>70.9 ± 3.2</td>
<td></td>
<td>0-2 h</td>
<td>Yes</td>
<td></td>
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<tr>
<td></td>
<td>Y 21 ± 1</td>
<td>F n = 8</td>
<td>75.0 ± 4.2</td>
<td></td>
<td>0-5 h</td>
<td>No</td>
<td></td>
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<td></td>
<td>O 74 ± 1</td>
<td>M n = 12</td>
<td>75.4 ± 3.3</td>
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<tr>
<td>Guillet et al. (2004) (46)</td>
<td>Y 25 ± 1</td>
<td>M n = 6</td>
<td>78.7 ± 3.3</td>
<td>Hyperinsulinemic, hyperaminoacidemic clamp (iv)</td>
<td>0-4 h Mixed IC</td>
<td>Yes</td>
<td>MPS was increased in both Y and O and was greater in Y.</td>
<td></td>
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<tr>
<td></td>
<td>O 72 ± 2</td>
<td>F n = 8</td>
<td>75.4 ± 3.3</td>
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<tr>
<td>Katsanos et al. (2006) (53)</td>
<td>Y 1.0 ± 1</td>
<td>M/F n = 8</td>
<td>70.1 ± 4.7</td>
<td>1. 6.7 g EAA orally consumed with 26% leucine</td>
<td>0-3.5 h Mixed Plasma</td>
<td>Yes</td>
<td>MPS was increased equally after EAA with 41% leucine, but MPS was only increased in Y after EAA with 26% leucine.</td>
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<td>No</td>
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<tr>
<td>Kiskini et al. (2013) (57)</td>
<td>1. O 67 ± 2</td>
<td>M/F n = 12</td>
<td>81.7 ± 3.6</td>
<td>20 g casein orally consumed with 40 g carbohydrate</td>
<td>0-6 h Mixed Plasma</td>
<td>No</td>
<td>MPS over entire 0-6 h did not differ between Y and O.</td>
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<td></td>
<td>2. Y 29 ± 3</td>
<td>M/F n = 8</td>
<td>76.6 ± 7.7</td>
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<td></td>
<td>2. O 67 ± 2</td>
<td>M/F n = 10</td>
<td>74.5 ± 4.7</td>
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<tr>
<td>Koopman et al. (2009) (59)</td>
<td>Y 75 ± 1</td>
<td>M n = 12</td>
<td>74.4 ± 2.2</td>
<td>20 g casein orally consumed with 40 g carbohydrate</td>
<td>0-6 h Mixed Plasma</td>
<td>No</td>
<td>MPS over entire 0-6 h did not differ between Y and O.</td>
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<tr>
<td></td>
<td>Y 23 ± 1</td>
<td>M n = 10</td>
<td>76.8 ± 2.0</td>
<td></td>
<td>0-6 h</td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td>O 64 ± 1</td>
<td>M n = 10</td>
<td>78.8 ± 3.1</td>
<td></td>
<td>0-6 h</td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td>Y 34 ± 4</td>
<td>M/F n = 6</td>
<td>63 ± 3</td>
<td>15 g EAA orally consumed</td>
<td>0-3.5/4 h Mixed IC</td>
<td>No</td>
<td>MPS was increased similarly in both Y and O.</td>
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<td></td>
<td></td>
<td></td>
<td>71 ± 5</td>
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<tr>
<td>Paddon-Jones et al. (2004) (76)</td>
<td>O 67 ± 2</td>
<td>M/F n = 7</td>
<td>74.4 ± 3.3</td>
<td>20 g casein orally consumed</td>
<td>0-6 h Mixed Plasma</td>
<td>No</td>
<td>MPS over entire 0-6 h did not differ between Y and O.</td>
<td></td>
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<tr>
<td>Pagwens et al. (2011) (78)</td>
<td>Y 21 ± 1</td>
<td>M n = 12</td>
<td>76.2 ± 3.6</td>
<td>20 g casein orally consumed</td>
<td>0-6 h Mixed Plasma</td>
<td>No</td>
<td>MPS over entire 0-6 h did not differ between Y and O.</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Y 75 ± 1</td>
<td>M n = 12</td>
<td>74.4 ± 2.3</td>
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<tr>
<td></td>
<td>Y 35 ± 3</td>
<td>M/F n = 17</td>
<td>79.2 ± 7.0</td>
<td>1. 113 g (30 g protein) of lean ground beef</td>
<td>0-5 h Mixed IC</td>
<td>No</td>
<td>MPS was increased similarly in both Y and O with 113 g and 340 g lean ground beef.</td>
<td></td>
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<tr>
<td></td>
<td>O 68 ± 2</td>
<td>M/F n = 17</td>
<td>77.5 ± 8.0</td>
<td>2. 340 g (90 g protein) of lean ground beef</td>
<td></td>
<td>No</td>
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</tr>
</tbody>
</table>

Continued
the source of amino acids/protein administered. Specifically, findings between studies in the MPS response when a sufficient (i.e., high) dose of amino acids/protein ingested varied considerably between studies. For example, whereas one of the study arms provided just 2.5 g of crystalline EAAs (27), equivalent to that contained in ~5 g of high-quality supplemental protein, a number of other study arms provided as much as 35–40 g of amino acids/protein (27, 59, 104, 105), and one study provided 90 g of protein in the form of 340 g of lean ground beef (91). The amount of protein provided is important to consider, as it has been documented that there is a dose-dependent MPS response to protein provision that ultimately plateaus at a given dose, beyond which additional protein is oxidized rather than incorporated into muscle (70, 113). Recently, Moore et al. (69) provided strong evidence that the relative amount of protein required to maximally stimulate MPS is considerably greater in older adults (~0.4 g/kg) than in the young (~0.24 g/kg). Put into context, for an average 75- to 80-kg older individual, this equates to ~30 g of high-quality protein to maximally stimulate MPS. In support of these data, others have demonstrated that the MPS response to 20 g of casein protein ingestion is ~16% lower in older than in young individuals (106). Based on these data, it could be expected that the study arms in this systematic review that provided ~0.4 g/kg of high-quality protein would fail to provide evidence of age-related muscle anabolic resistance. To this end, we analyzed study arms that provided either ~0.4 g/kg of amino acids/protein or an amount of EAAAs equivalent to that contained in a dose of high-quality protein corresponding to ~0.4 g/kg (49), finding that four of five study arms demonstrated insufficient evidence of age-related muscle anabolic resistance (59, 76, 91, 105). Taken together, these findings suggest the absence of an age-related deficit in the MPS response when a sufficient (i.e., high) dose of high-quality amino acids/protein is provided.

Source of Amino Acids/Protein

In addition to the amino acid/protein dose, inconsistent findings between studies in the MPS response to orally ingested amino acid-based nutrition. First, the dose of amino acids/protein ingested varied considerably between studies. For example, whereas one of the study arms provided just 2.5 g of crystalline EAAs (27), equivalent to that contained in ~5 g of high-quality supplemental protein, a number of other study arms provided as much as 35–40 g of amino acids/protein (27, 59, 104, 105), and one study provided 90 g of protein in the form of 340 g of lean ground beef (91). The amount of protein provided is important to consider, as it has been documented that there is a dose-dependent MPS response to protein provision that ultimately plateaus at a given dose, beyond which additional protein is oxidized rather than incorporated into muscle (70, 113). Recently, Moore et al. (69) provided strong evidence that the relative amount of protein required to maximally stimulate MPS is considerably greater in older adults (~0.4 g/kg) than in the young (~0.24 g/kg). Put into context, for an average 75- to 80-kg older individual, this equates to ~30 g of high-quality protein to maximally stimulate MPS. In support of these data, others have demonstrated that the MPS response to 20 g of casein protein ingestion is ~16% lower in older than in young individuals (106). Based on these data, it could be expected that the study arms in this systematic review that provided ~0.4 g/kg of high-quality protein would fail to provide evidence of age-related muscle anabolic resistance. To this end, we analyzed study arms that provided either ~0.4 g/kg of amino acids/protein or an amount of EAAAs equivalent to that contained in a dose of high-quality protein corresponding to ~0.4 g/kg (49), finding that four of five study arms demonstrated insufficient evidence of age-related muscle anabolic resistance (59, 76, 91, 105). Taken together, these findings suggest the absence of an age-related deficit in the MPS response when a sufficient (i.e., high) dose of high-quality amino acids/protein is provided.

Table 2.—Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Group, Age (yr)</th>
<th>Sex (n)</th>
<th>Body Mass (kg)</th>
<th>Amino Acid/Protein Protocol</th>
<th>Incorporation Period</th>
<th>Muscle Subfraction</th>
<th>Precursor Pool</th>
<th>Evidence of Age-Related Muscle Anabolic Resistance</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volpi et al. (1999) (105)</td>
<td>Y</td>
<td>M/F</td>
<td>72 ± 3</td>
<td>40 g amino acids orally consumed in boluses every 0–3 h</td>
<td>Mixed IC</td>
<td>No</td>
<td>MPS was increased similarly in both Y and O.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 ± 2</td>
<td>n = 7</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>O</td>
<td>M/F</td>
<td>74 ± 4</td>
<td>10 mins</td>
<td>0–3 h</td>
<td>Mixed IC</td>
<td>Yes</td>
<td>MPS was increased only in Y.</td>
<td></td>
</tr>
<tr>
<td>Volpi et al. (2000) (104)</td>
<td>Y</td>
<td>M/F</td>
<td>72 ± 1</td>
<td>40 g amino acids with 40 g carbohydrate orally consumed in boluses every 10 min</td>
<td>0–3 h</td>
<td>Mixed IC</td>
<td>No</td>
<td>MPS was increased similarly in both Y and O.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 ± 3</td>
<td>n = 5</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>O</td>
<td>M/F</td>
<td>72 ± 1</td>
<td>n = 5</td>
<td>40 g amino acids orally consumed in boluses every 10 min</td>
<td>0–3 h</td>
<td>Mixed IC</td>
<td>Yes</td>
<td>MPS was increased only in Y.</td>
</tr>
</tbody>
</table>

Values are means ± SE. EAA, essential amino acids.
Summary of studies included in Model 3

<table>
<thead>
<tr>
<th>Reference</th>
<th>Group, Age (yr)</th>
<th>Sex (n)</th>
<th>Body Mass (kg)</th>
<th>Exercise and Amino Acid/Protein Protocol</th>
<th>Incorporation Period</th>
<th>Muscle Subtraction</th>
<th>Precursor Pool</th>
<th>Evidence of Age-Related Muscle Anabolic Resistance</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherton et al. (2016)</td>
<td>Y 24 ± 6 M n = 18</td>
<td>M 75 ± 10</td>
<td>0–4 h</td>
<td>6x8 sets of KE at 75% 1RM followed by 10 g protein (8 g casein, 2 g whey), 24 g carbohydrate and 4.2 g leucine</td>
<td>Yes</td>
<td>MPS was greater with added leucine compared to alanine in both Y and O. AUC for MPS not different between Y and O in either condition.</td>
<td></td>
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<tr>
<td></td>
<td>O 70 ± 5 M n = 18</td>
<td>M 76 ± 10</td>
<td></td>
<td>2. 6x8 sets of KE at 75% 1RM followed by 10 g protein (8 g casein, 2 g whey), 24 g carbohydrate and 4.2 g leucine</td>
<td>No</td>
<td></td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Drummond et al. (2008)</td>
<td>Y 30 ± 2 M n = 7</td>
<td>M 88.9 ± 5.4</td>
<td>0–1 h</td>
<td>8x10 sets of KE at 70% 1RM followed by 20 g oral EAA 1 h postexercise</td>
<td>Yes</td>
<td>MPS was higher in Y than O at 1–3 h, but MPS over 0–1 h, 3–6 h and entire 1–6 h was not different.</td>
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<tr>
<td></td>
<td>O 70 ± 2 M n = 6</td>
<td>M 81.3 ± 5.2</td>
<td>3–6 h</td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td>No</td>
<td></td>
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<tr>
<td>Durham et al. (2010)</td>
<td>Y 30 ± 2 M n = 9</td>
<td>M 78 ± 2</td>
<td>10 min-3 h</td>
<td>Treadmill exercise (walking) for 45 min at ~40% VO2 peak with amino acids infused throughout recovery</td>
<td>No</td>
<td>MPS was increased in both Y and O with no differences between groups.</td>
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<tr>
<td></td>
<td>O 67 ± 2 M n = 8</td>
<td>M 84 ± 4</td>
<td>1–3 h</td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td>No</td>
<td></td>
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<tr>
<td>Koopman et al. (2006)</td>
<td>Y 20 ± 1 M n = 8</td>
<td>M 73.7 ± 3.2</td>
<td>0–6 h</td>
<td>6x10 sets of LP and 6x10 sets of KE at 40–75% 1RM followed by small repeated boluses of ~60 g whey with ~184 g carbohydrate and 4.2 g leucine</td>
<td>Yes</td>
<td>MPS over entire 0–6 h was lower in the O vs. Y.</td>
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<tr>
<td></td>
<td>O 75 ± 1 M n = 8</td>
<td>M 75.5 ± 2.1</td>
<td></td>
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<td></td>
<td>No</td>
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<tr>
<td>Pennings et al. (2011)</td>
<td>Y 21 ± 1 M n = 12</td>
<td>M 76.1 ± 2.8</td>
<td>0–6 h</td>
<td>6x10 sets of LP and 6x10 sets of KE at 40–75% 1RM followed by 20 g casein orally consumed</td>
<td>No</td>
<td>MPS over entire 0–6 h did not differ between Y and O.</td>
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<tr>
<td></td>
<td>O 73 ± 1 M n = 12</td>
<td>M 79.6 ± 2.7</td>
<td></td>
<td></td>
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<td></td>
<td>No</td>
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<tr>
<td>Symons et al. (2011)</td>
<td>Y 29 ± 3 M/F n = 7</td>
<td>M/F 79 ± 10</td>
<td>0–5 h</td>
<td>340 g (90 g protein) of lean ground beef followed by 60 mins later by 6x8 sets of KE at 80% 1RM</td>
<td>No</td>
<td>MPS was increased similarly in both Y and O.</td>
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<tr>
<td></td>
<td>O 67 ± 2 M/F n = 7</td>
<td>M/F 76 ± 5</td>
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<td></td>
<td>No</td>
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</table>

Values are means ± SE.

The importance of utilizing free amino acids orally or intravenously to investigate age-related differences in skeletal muscle protein metabolism should not be discounted. For example, intravenous provision of free amino acids can be a valuable experimental approach to utilize when the research question is focused on controlling for other potential confounding factors (e.g., differences in protein/amino acid digestion and absorption between individuals); thus, this highlights the importance of tailoring the study design toward the experimental hypothesis being investigated.

**Leucine Content of Amino Acids/Protein**

Although the source of amino acids/protein appears to be of secondary importance to the amount of protein, when the apparent presence or absence of age-related differences in postprandial MPS between studies is explained, the leucine content of the administered amino acid/protein source may offer further insight. The branched-chain amino acid leucine appears to play a key role in the stimulation of MPS (3, 56). Leucine is unique in that it serves not only as a substrate for the synthesis of new muscle proteins but also as a potent molecular anabolic signal that robustly stimulates MPS (26, 56). Interestingly, two of the included study arms in this review provide strong evidence that the leucine content of a protein source is an important determinant of postprandial MPS, particularly in older individuals. Katsanos et al. (53) demonstrated that postprandial MPS was stimulated in young but not older individuals following the provision of 6.7 g of EAAs containing ~1.8 g leucine (26% of the total content, equivalent to that contained in ~15 g whey protein). However, when the leucine content...
was enriched to ~3 g (41% of the total content, equivalent to that contained in ~25 g of whey protein), an equivalent stimulation of MPS was observed between young and older individuals. Furthermore, others have demonstrated a strong positive association between peak plasma leucine concentrations and postprandial MPS in older individuals (77). In support of these findings, of the nine study arms included in Model 2 that reported the leucine content of the amino acid/protein source administered, six provided no evidence of age-related muscle anabolic resistance (44, 53, 76, 91). Interestingly, four of these study arms provided a leucine dose of ~2 g or more. In contrast, the three study arms that failed to provide evidence of age-related muscle anabolic resistance all provided amino acid/protein sources containing a “suboptimal” 1.4- to 1.7-g dose of leucine (44, 53). Taken together, it appears that sources of amino acids/protein that achieve a rapid, high-amplitude peak aminoacidemia and leucinemia maximally stimulate postprandial MPS and thus should be recommended for older individuals to alleviate muscle anabolic resistance.

Exercise and Amino Acid/Protein Provision

The final model constructed (Fig. 2, Model 3, and Table 3) included 10 study arms (2, 35, 36, 58, 78, 90) that measured the MPS response to the combined stimulus of exercise with amino acid/protein provision. Acutely, combined resistance exercise and protein provision act to synergistically enhance and maximize the stimulation of MPS above rates observed in response to protein provision alone in young and older individuals (20, 78, 115). Chronically, protein supplementation enhances resistance training-induced muscle hypertrophy and strength increases in young and older individuals (22, 94, 112). With this in mind, it could be expected that age-related differences in MPS would be less apparent in studies utilising the combined anabolic stimulus of resistance exercise and amino acid/protein provision. In accord with this assumption, seven of the nine study arms that combined resistance exercise with amino acid/protein provision found no evidence of age-related muscle anabolic resistance. Although Drummond et al. (35) did observe age-related muscle anabolic resistance at 1–3 h following resistance exercise and EAA ingestion, the aggregate MPS response over 1–6 h was not different, suggesting that the MPS response to exercise and amino acid/protein provision may be delayed (rather than attenuated) with advancing age. Precisely why Koopman et al. observed age-related differences in MPS is unclear but could relate to the exercise intensity chosen, which may have been insufficient to overcome the blunted MPS response in the older group even in the presence of adequate protein provision (58). Specifically, the authors chose to simulate activities of daily living in older individuals through implementation of resistance exercise at low-to-modest intensities (40–75% of 1RM). However, given that Durham et al. (36) observed no age-related impairment in MPS following 45 min of treadmill walking (at a relatively low exercise intensity) combined with amino acid infusion, the notion that exercise intensity may explain the findings of Koopman et al. (58) requires further clarification. Nonetheless, that eight of the 10 study arms in Model 3 found no age-related differences in MPS strongly suggests that the combination of exercise and amino acid/protein provision is an effective strategy to restore “youthful” muscle protein synthetic responsiveness in older individuals.

Differences in Experimental Methodology

Differences in experimental methodology used to assess MPS between studies may explain the inconsistent findings reported herein. For example, the tracer incorporation period over which MPS was investigated (i.e., timing between sequential muscle biopsy samples) varied widely from 0–2 h (24) to 0–6 h (58, 59, 78). The timing of muscle biopsy sampling is an important consideration when capturing the peak MPS response to a given exercise and/or nutritional stimulus (71). For example, it has been demonstrated that the MPS response to bolus protein ingestion is relatively transient, peaking over ~3 h postigestion in young and older adults (1, 67), whereas the maximal MPS response to resistance exercise in the absence of postexercise amino acid/protein provision is thought to occur ~1–2 h after exercise cessation in both young and older individuals (62). Interestingly, the suggestion that the MPS response to combined resistance exercise and amino acid/protein provision may simply be delayed (rather than attenuated) with advancing age (35) underlines the importance of selecting appropriate muscle biopsy sampling time points to enable sufficient temporal resolution. This point is well highlighted by Gorissen et al. (44), who demonstrated that whereas the MPS response to casein ingestion was greater over 0–2 h postprandial period in the young compared with older individuals, the response over 0–5 h postprandial period showed no age-related difference. Thus, it is perhaps not surprising that the six study arms (44, 57, 59, 78) that assessed MPS in response to casein alone (Model 2) or coupled with exercise (Model 3) over a 5- to 6-h incorporation period reported no evidence of age-related muscle anabolic resistance. Indeed, when we analyzed study arms from Model 2 that assessed MPS over a postprandial period of ~3 h, six of 10 study arms reported evidence of age-related muscle anabolic resistance, whereas when MPS was assessed over a postprandial period of...
>3 h, only two of 11 study arms demonstrated evidence of age-related muscle anabolic resistance (Table 2). This would suggest that age-related muscle anabolic resistance predominates in the early postprandial period as opposed to the later postprandial period where a more sustained and comparable MPS response is observed in young and older individuals (44). Given that the MPS response to bolus protein ingestion returns to baseline by ~3 h postingestion (1, 67), we postulate that the occurrence of age-related muscle anabolic resistance may have been masked in studies assessing postprandial MPS over a prolonged measurement period (e.g., 6 h), over which the peak stimulation may be somewhat diluted by the lower MPS response in the later postprandial phase (e.g., 3–6 h). Although MPS rates are comparable over a relatively longer postprandial period between young and older individuals, the physiological relevance of muscle anabolic resistance over the early postprandial period requires further investigation.

The choice of muscle subfraction used in the calculation of MPS differed between studies and could explain some of the conflicting findings. Whereas 34 of the study arms calculated mixed MPS (i.e., an aggregate of all muscle protein subfractions), 14 study arms chose to calculate MPS in isolated myofibrillar proteins (Tables 1, 2, and 3). Myofibrillar proteins comprise the contractile apparatus within skeletal muscle (i.e., myosin, actin, titin), the synthesis of which can increase two- to threefold above basal, postabsorptive values following a single bout of high-intensity/volume resistance exercise in young and older individuals (62, 71, 111). On the other hand, proteins that comprise a mixed fraction include sarcoplasmic and mitochondrial proteins and may display lower acute responsiveness than myofibrillar proteins to resistance exercise alone or combined with amino acid-based nutrition (71, 111). For example, in well-trained individuals, an acute bout of resistance exercise stimulates rates of myofibrillar but not mixed MPS (55). Herein, we were unable to detect any age-related differences in the MPS response in myofibrillar vs. mixed fractions due to the highly variable experimental methods between studies (i.e., specifics of the anabolic stimulus, tracer incorporation time, etc.). Thus, we cannot rule out the possibility that, under certain experimental conditions, the choice of muscle protein subfraction used for the calculation of MPS may be important in detecting difference in MPS between young and older individuals.

Finally, and perhaps most importantly, while a number of studies provided instructions to participants regarding physical activity in the days leading up to the trials, only one study objectively measured habitual physical activity (via accelerometry) in the days immediately prior to the experimental trials (24). The importance of controlling for prior physical activity when assessing MPS cannot be overstated, as recent work demonstrated that just 2 wk of reduced ambulation (~75% daily step reduction) resulted in muscle atrophy and anabolic resistance in older individuals (15). Given emerging evidence that the proposed postexercise anabolic “window of opportunity” for the synergistic enhancement of MPS through protein ingestion extends beyond the immediate hours of recovery in young individuals (19), excessive physical activity or inactivity in the days prior to experimental trials may confound the assessment of MPS. This is further supported by evidence in older individuals demonstrating that the MPS response to EAA intake can be enhanced by prior low-intensity aerobic exercise in the form of brisk walking (95). As such, it has been hypothesized that physical inactivity may be at the root of muscle anabolic resistance and exacerbate the progression of sarcopenia in the older population (17, 18, 68). With this in mind, it could be speculated that muscle anabolic resistance would be more easily detected in studies involving sedentary older, but not highly functioning, physically active older individuals. Although the evidence to support this position is sparse, the single study arm in which habitual physical activity was reported to be similar between the young and older groups demonstrated an equivalent MPS response to amino acid administration (24). Accordingly, it is imperative that future studies investigating MPS in young and older populations objectively assess habitual physical activity levels.

Conclusions and Future Implications

In this systematic review, 18 study arms provided sufficient evidence of age-related muscle anabolic resistance, whereas 30 study arms did not. Although a quantitative appraisal of the presence of age-related differences in the MPS response to anabolic stimuli (i.e., directly contrasting absolute FSR values between young and older individuals) would have been preferable, the variability in experimental methodology used to assess MPS (e.g., amino acid stable isotope tracer, muscle protein subfraction, precursor pool, and FSR incorporation period) made this approach largely unviable. However, we believe that the variability in experimental methodology is an important factor underlying the inconsistent findings as to the presence or absence of an impaired muscle anabolic response in older age. Although beyond the scope of this systematic review, it is important to acknowledge that MPS (on which we have focused) is an acute, dynamic assessment that represents only one side of the overall net protein balance (NBAL) equation. Ultimately, overall NBAL dictates long-term skeletal muscle remodeling, which is the end point in the diagnosis of sarcopenia, and as such, the findings of this systematic review should be considered within this broader context. Although our findings suggest that age-related muscle anabolic resistance is infrequently observed in response to a robust muscle anabolic stimuli (i.e., a high dose of protein and/or a high volume/intensity of exercise), this phenomenon appears to be more frequently observed in response to anabolic stimuli that could be considered as insufficient to maximally stimulate MPS in older muscles, for example, in studies utilizing relatively low-intensity/volume protocols or low-dose protein/amino acid provision (suboptimal leucine). However, we cannot dismiss the fact that some study arms failed to observe age-related muscle anabolic resistance in response to suboptimal anabolic stimuli and that others observed age-related muscle anabolic resistance following robust anabolic stimuli. We postulate that this inconsistency between studies can be attributed largely to differences in study population (e.g., habitual physical activity) and experimental methodology (e.g., tracer incorporation period) as outlined in this discussion.

It has become increasingly evident that older individuals, especially those who are frail or institutionalized, consume less protein than younger individuals (40), particularly at breakfast, where the average protein intake is ~12 g and comes largely from low-leucine, non-animal-based sources such as bread and cereals (75, 93, 101). It is also clear that sedentary time...
increases with advancing age (21, 47, 98), and nonsedentary behavior is often of a relatively low intensity (e.g., gentle walking). Thus, the experimental conditions under which age-related muscle anabolic resistance has often been reported (i.e., low-volume exercise and/or low-dose protein/amino acid provision) are highly representative of the lifestyle and dietary habits of the average older individual. Accordingly, it is imperative that the mechanisms underpinning age-related muscle anabolic resistance are elucidated, to aid the development of targeted therapeutic strategies to slow the progression of sarcopenia.

Clinical recommendations for the prevention of sarcopenia are currently lacking. However, in line with the current findings, recent position stands recommend that an average daily protein intake of at least 1–1.2 g/kg body wt in conjunction with regular resistance and/or endurance exercise is the most effective means of maintaining muscle mass/strength for older individuals (8, 28). In agreement with the conclusions of this systematic review (i.e., that age-related muscle anabolic resistance is most frequently observed in response to suboptimal amino acid/protein feeding), and other recent analyses (69, 106), these recommendations specifically advise that older adults ingest rapidly digested, leucine-rich proteins in doses of ~0.4 g/kg body wt per meal, distributed evenly across the day (8, 28). On the basis of the current findings, we recommend that future position stands should focus on defining optimal training volume/intensity requirements to deliver the greatest benefit for musculoskeletal health in older age.

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AUTHOR CONTRIBUTIONS


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Muscle anabolic resistance in older age


