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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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Running title: Muscle anabolic resistance in older age

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Abstract

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 synthesised 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: i) exercise alone; ii) amino acid-based nutrition alone; or iii) 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'* whilst in response to amino acid-based nutrition alone, 8 of the 21 study arms provided sufficient *'evidence of age-related 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 optimisation of exercise and amino acid-based nutrition is sufficient to induce a comparable MPS response between young and older individuals. However, the exercise volume completed and/or the amino acid/protein dose and leucine content must exceed a certain threshold to stimulate equivalent MPS rates in young and older adults, below which age-related muscle anabolic resistance may become apparent.

Keywords: Skeletal muscle, anabolic resistance, sarcopenia, resistance exercise

Introduction

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 2 billion in 2050, whilst 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 co-morbidities (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 fibre size (34, 74, 79, 102). In young healthy individuals, mechanical loading (i.e. exercise contraction) in the fasted, post-absorptive 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 supress 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 hyperinsulemic 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 signalling (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, whilst 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, i) the time frame of MPS assessment, ii) analysis of specific muscle protein sub-fractions and iii) 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 between 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 23rd May 2016) databases was performed with the final literature search completed on 23rd May 2016. These databases were chosen due to the extensive cover 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 utilised. 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: Randomised controlled trials, non-randomised clinical trials or comparative studies that directly compared young and older participants within the same study were eligible for inclusion. Non-randomised 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 randomisation 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 yrs of age (inclusive). The mean age of the older group was required to be ≥55 yrs of age. These

criteria were chosen as age-related sarcopenia tends to manifest in the 4-5th decade in humans (23, 50), and thus we reasoned that an age range of 18-35 yrs would provide a fair reflection of younger individuals that had not yet reached the threshold for development of sarcopenia. Similarly, we posited that ≥55 yrs of age for older individuals would ensure that the threshold for development of age-related sarcopenia had been reached. Accordingly, any studies that utilised young or older groups with a mean age between 36 and 54 yrs (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 characterised 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, non-diseased populations (25).

Types of Interventions: This systematic review was limited to studies utilising 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 (i.e. carbohydrates and fats) were provided in addition to amino acid/protein provision were deemed eligible for inclusion as co-ingestion of carbohydrate and/or fat does not appear to significantly modulate the postprandial MPS response to protein ingestion (44, 45, 60). Interventions that co-administered pharmaceutical drugs that were not designed to incur hyper and/or hypo aminoacidemia, insulinemia, or glycemia were excluded, as these 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 programme were also excluded as this 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 post-stimulus 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 the 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 sub-fractions (i.e. myofibrillar, mitochondrial and sarcoplasmic). Therefore, any studies that used the 2-pool or 3-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 sub-fractions 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 (BJ Shad and JL Thompson). All titles

and abstracts deemed ineligible were excluded, whilst 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 the two independent reviewers (BJ 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, version X7). Duplicate records were removed using the 'find duplicates' function in Endnote.

Data Extraction and Management: Two reviewers (BJ Shad and L Breen) independently extracted all data (i.e. study characteristics and outcome data) from all included studies using a customised 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 utilised within the same study (e.g. provision of different essential amino acid (EAA) doses). Categories of data extracted included: a) participant characteristics (e.g. age, number, gender and body mass), b) type of intervention (e.g. exercise mode, exercise intensity and amino acid dose), c) details of the method of MPS assessment (e.g. measurement period, muscle sub-fraction used and precursor pool used) and d) 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 synthesise the data from the included studies as the heterogeneous experimental methodology employed when assessing MPS (e.g. amino acid stable isotope tracer, muscle protein sub-fraction, duration of tracer incorporation, and precursor pool) can result in varying rates of MPS between studies (86), meaning

quantitative analysis across studies was not feasible. As part of the data extraction process, both reviewers were required to qualitatively synthesise 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; i) a significantly ($P < 0.05$) greater MPS response in young compared with older participants in response to an anabolic stimulus, or ii) 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 only reported 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 only reported 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 and colleagues (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 utilised exercise as the only form of anabolic stimulus were included to examine age-related differences in the MPS response to an isolated contractile bout. In Model 2, study arms that utilised 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 utilised 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 5 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 (BJ Shad and JL 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 (BJ Shad and L Breen) independently. Of these 32 full-text articles, 8 were excluded for reasons including; use of the 3-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 utilised (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 in the results text below, 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, whilst 1 study failed to declare any such assessment (5). A total of 15 of the included studies recruited males only, 1 study included females only, 7 studies included both males and females, and 1 study did not report the gender of participants (46). The age range of the young participant groups was between 20 and 35 yrs, whereas the age range of the older participant groups was between 64 and 76 yrs. Body mass of the young participant groups ranged from 62 kg to 88.9 kg, whilst body mass in the older participant group ranged from 60.8 kg to 88 kg.

Anabolic stimulus

Of the 24 studies, 12 included some form of acute exercise stimulus. Resistance exercise was utilised in 10 of the 12 studies, and endurance exercise in 2 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, whilst 3 studies administered amino acids through intravenous (IV) infusion. A total of 6 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 h to ~24 h. MPS in a mixed muscle fraction was assessed in 19 studies, whilst 5 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, whilst 8 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 utilised 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 (Figure 2). Of these 48 study arms, 18 were considered to provide sufficient evidence of age-related muscle anabolic resistance (5, 27, 35, 37, 44, 46, 53, 58, 61, 62, 65, 84, 85, 104), whereas 30 were considered to provide insufficient evidence of age-related muscle anabolic resistance (2, 24, 27, 35, 36, 44, 53, 57, 59, 61, 62, 76, 78, 84, 85, 90, 91, 105) (Figure 2). In order to further examine age-related differences in MPS in response to various anabolic stimuli, we constructed three models that included study arms based on the anabolic stimulus provided (outlined above in 'methods').

In Model 1, study arms were included if they utilised exercise as the only form of anabolic stimulus. As a result, 17 study arms were included in Model 1, with 8 providing sufficient evidence (37, 61, 62, 65, 84, 85) and 9 providing insufficient evidence of age-related muscle anabolic resistance (61, 62, 84, 85). Fourteen of the 17 study arms assessed age-related differences in MPS following resistance exercise, with 7 providing sufficient and 7 providing insufficient evidence of age-related muscle anabolic resistance (Table 1). Two of the three study arms that applied endurance exercise as the contractile stimulus provided insufficient evidence of age-related muscle anabolic resistance.

In Model 2, study arms were included if they utilised amino acid/protein administration/feeding as the only anabolic stimulus. As a result, 21 study arms were included in Model 2, with 8 providing sufficient evidence (5, 27, 44, 46, 53, 104) and 13 providing insufficient evidence of age-related muscle anabolic resistance (24, 27, 44, 53, 57, 59, 76, 78, 91, 105). Ten of the 21 study arms provided oral free amino acids, with 5 providing sufficient and 5 providing insufficient evidence of age-related muscle anabolic resistance. Casein protein was orally administered in 7 of the 21 study arms, with 2 providing sufficient and 5 providing insufficient evidence of age-related muscle anabolic resistance. The 2 study arms, which administered lean ground beef as the protein source, provided insufficient evidence of age-related muscle anabolic resistance. Two of the 21 study arms administered amino acids intravenously, with 1 providing sufficient and 1 providing insufficient evidence of age-related muscle anabolic resistance (Table 2).

Finally, in Model 3, study arms that utilised 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 2 study arms providing sufficient evidence (35, 58) and 8 study arms providing insufficient evidence of age-related muscle anabolic resistance (2, 35, 36, 78, 90). Nine of the 10 study arms utilised resistance exercise as the contractile stimulus, with 2 providing sufficient evidence and 7 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 artefact of lifestyle modifications (15, 107), or a combination of these two factors. Whilst 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 (Figure 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) using exercise or amino acid/protein administration/feeding alone or in combination; and 4) differences in experimental methodology and design.

359 ***Exercise Volume and Intensity***

360 It has been documented that both endurance and resistance exercise robustly stimulate
361 mitochondrial and myofibrillar MPS, respectively, in young and older individuals (29, 33, 61,
362 111). However, it is not yet fully known how the MPS response to exercise differs between
363 young and older individuals. To this end, we constructed a model which included only those
364 study arms that assessed the MPS response to exercise alone in the postabsorptive state
365 (Figure 2, Model 1 and Table 1). Interestingly, whilst 8 study arms provided sufficient
366 evidence of age-related muscle anabolic resistance (37, 61, 62, 65, 84, 85), 9 study arms did
367 not (61, 62, 84, 85). One potential explanation for the lack of congruency may be the
368 difference in exercise volume between studies. For example, in a well-controlled study from
369 Kumar and colleagues (61), MPS post-exercise was significantly lower in the older group
370 compared with the young when a relatively low volume of work was completed (3 sets of
371 knee extension exercise at 40% one repetition maximal strength (1RM)). However, the
372 authors noted that when the volume of work completed was doubled, the MPS response was
373 comparable between young and older groups (61). These data infer the possibility of an age-
374 related exercise volume 'threshold', whereby older individuals are required to complete
375 greater exercise volumes to elicit a comparable MPS response to the young. Alternatively, the
376 relative loading intensity of resistance exercise may also explain differences in the MPS
377 response to exercise observed between studies. Specifically, whilst 3 sets of knee extensions
378 at 40% of 1RM induced greater rates of MPS in the young compared with the older group, 3
379 sets at 75% of 1RM, with volume-matched to that completed at 40% 1RM (i.e. fewer
380 repetitions), overcame the age-related blunting of MPS (61). The position that a greater
381 volume and/or heavier load exercise can overcome age-related differences in MPS may
382 explain why Sheffield-Moore et al. failed to detect any age-related deficit in MPS following 6

sets of knee extensions at 80% 1RM (84). However, this fails to explain the occurrence of age-related muscle anabolic resistance following 8 sets of knee extensions at 70% of 1RM by Fry and colleagues over numerous post-exercise time points and using a larger sample size (37). Exactly why the findings of Kumar et al. (61) and Fry and colleagues (37) differ is difficult to reconcile but may relate to the habitual physical activity levels of the young and older participants, which were not objectively measured in either study (discussed in further detail below). The lack of a within-subject comparison group in the study by Fry et al. (37) 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 utilising larger sample sizes, multiple post-exercise 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 essential amino acids (EAA's) (96, 103). Accordingly, we constructed a second model in an attempt to examine

whether age-related differences in MPS exist following the provision of amino acids/protein alone (Figure 2, Model 2 and Table 2). Of the 21 study arms included in this model, 8 provided sufficient evidence of age-related muscle anabolic resistance (5, 27, 44, 46, 53, 104) whilst 13 did not (24, 27, 44, 53, 57, 59, 76, 78, 91, 105). However, although the MPS response between young and older adults was only significantly different in 8 of 21 study arms in Model 2, when study arms were pooled together we observed that the general pattern of the magnitude of the MPS response appeared to be lower in older individuals compared with the young (Figure 3). Further, we believe there are a number of factors that may explain the lack of agreement as to the presence or absence of muscle anabolic resistance in older adults in response to orally ingested amino acid-based nutrition. Firstly, the dose of amino acids/protein ingested varied considerably between studies. For example, whilst one of the study arms provided just 2.5g of crystalline EAA's (27), equivalent to that contained in ~5g of high-quality supplemental protein, a number of other study arms provided as much as 35-40g of amino acids/protein (27, 59, 104, 105) and one study provided 90g of protein in the form of 340g 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 and colleagues provided strong evidence that the relative amount of protein required to maximally stimulate MPS is considerably greater in older adults (~0.4g/kg) compared with the young (~0.24g/kg) (69). Put into context, for an average 75-80kg older individual, this equates to ≥30g of high-quality protein to maximally stimulate MPS. In support of these data, others have demonstrated that the MPS response to 20g of casein protein ingestion is ~16% lower in older vs. young individuals (106). Based on these data, it could be expected that the study arms in this systematic review

that provided ≥0.4g/kg of high quality protein would fail to provide evidence of age-related muscle anabolic resistance. To this end, we analysed study arms that provided either, i) ≥0.4g/kg of amino acids/protein or ii) an amount of EAA's equivalent to that contained in a dose of high-quality protein corresponding to ≥0.4g/kg (49), finding that 4 of 5 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 Model 2 might also be explained by the source of amino acids/protein administered. Specifically, the digestion/absorption properties and leucine content of ingested protein are thought to play a key role in the acute MPS response (77). Of the 17 study arms that provided amino acids/protein orally and in liquid form, 10 study arms provided crystalline amino acids whilst 7 provided casein (Table 2). Crystalline free-form amino acids are more rapidly digested and absorbed than amino acid constituents of protein-rich supplemental and whole-food sources (27, 53). On the other hand, casein protein is predominantly acid insoluble and thus coagulates within the acidic environment of the stomach, which increases gastric transit time, resulting in a 'slow' digestion/absorption profile (12). The 'slow' digestion/absorption kinetics of casein protein, coupled with the relatively low leucine content, results in inferior acute postprandial MPS stimulation compared to an equivalent amount of rapidly digested, leucine-rich whey protein in both young and older men at rest (11, 77, 92, 108). With this in mind, it may be expected that the study arms utilising casein, particularly in low doses (containing very little leucine) would be more likely to observe evidence of age-related

muscle anabolic resistance than those administering free amino acids or whey protein. However, 5 of the 7 study arms (44, 57, 59, 78) in which casein protein was provided observed no age-related differences in postprandial MPS (Table 2). This observation is perhaps surprising given that the postprandial MPS response to 20g of casein in a relatively large cohort is significantly lower (~16%) in older vs. young individuals (106), but may be explained by the relatively long time-frame over which MPS was assessed (discussed in further detail below).

An important question that must also be posed is which of the amino acid/protein sources provided in the study arms included in Model 2 most accurately reflect the habitual food choices of free-living young and older individuals? As previously mentioned, 10 of the 21 study arms provided oral free amino acids, with 7 providing protein in the form of casein, 2 providing protein in the form of lean ground beef, and 2 providing free amino acids intravenously. It is clear that intravenous and oral provision of free amino acids do not accurately reflect the typical route or form in which amino acids/protein are consumed. Thus, findings from these studies could be suggested to hold less significance than those which provided protein in the form of casein (the main protein constituent of milk) and lean ground beef, which are likely to be more reflective of the typical food sources consumed on a day-to-day basis in free-living scenarios. However, the importance of utilising 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 utilise 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), and thus this highlights the importance of tailoring the study design towards 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 explaining the apparent presence or absence of age-related differences in postprandial MPS between studies, 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 which 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 6.7g EAA's containing ~1.8g leucine (26% of the total content, equivalent to that contained in ~15g whey protein). However, when the leucine content was enriched to ~3g (41% of the total content, equivalent to that contained in ~25g of whey protein), an equivalent stimulation of MPS was observed between young and older individuals. Furthermore, others demonstrate a strong positive association between peak plasma leucine concentrations and postprandial MPS in older individuals (77). In support of these findings, of the 9 study arms included in Model 2 that reported the leucine content of the amino acid/protein source administered, 6 provided no evidence of age-related muscle anabolic resistance (44, 53, 76, 91). Interestingly, 4 of these study arms provided a leucine dose of ~2g or more. In contrast, the 3 study arms that failed to provide evidence of age-

related muscle anabolic resistance all provided amino acid/protein sources containing a 'sub-optimal' 1.4-1.7g 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 (Figure 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 accordance with this assumption, 7 of the 9 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-moderate intensities (40-75% of 1RM). However, given that Durham et al. (36) observed no age-related impairment in MPS following 45 minutes 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 8 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 ~3h post-ingestion in young and older adults (1, 67), whereas the maximal MPS response to resistance exercise in the absence of post-exercise 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

550 selecting appropriate muscle biopsy sampling time-points to enable sufficient temporal
551 resolution. This point is well highlighted by Gorissen et al. (44), who demonstrated that whilst
552 the MPS response to casein ingestion was greater over 0-2 h postprandial period in the young
553 compared with older individuals, the response over 0-5 h postprandial period showed no age-
554 related difference. Thus, it is perhaps not surprising that the 6 study arms (44, 57, 59, 78) that
555 assessed MPS in response to casein alone (Model 2) or coupled with exercise (Model 3) over
556 a 5-6 h incorporation period, reported no evidence of age-related muscle anabolic resistance.
557 Indeed, when we analysed study arms from Model 2 that assessed MPS over a postprandial
558 period of ≤3 h, 6 out of 10 study arms reported evidence of age-related muscle anabolic
559 resistance, whereas when MPS was assessed over a postprandial period of >3 h, only 2 out of
560 11 study arms demonstrated evidence of age-related muscle anabolic resistance (Table 2).
561 This would suggest that age-related muscle anabolic resistance predominates in the early
562 postprandial period as opposed to the later postprandial period where a more sustained and
563 comparable MPS response is observed in young and older individuals (44). Given that the
564 MPS response to bolus protein ingestion returns to baseline by ~3h post-ingestion (1, 67), we
565 postulate that the occurrence of age-related muscle anabolic resistance may have been masked
566 in studies assessing postprandial MPS over a prolonged measurement period (e.g. 6 hours),
567 over which the peak stimulation may be somewhat diluted by the lower MPS response in the
568 later postprandial phase (e.g. 3-6 hours). Although MPS rates are comparable over a relatively
569 longer postprandial period between young and older individuals, the physiological relevance
570 of muscle anabolic resistance over the early postprandial period requires further investigation.
571
572 The choice of muscle sub-fraction used in the calculation of MPS differed between studies
573 and could explain some of the conflicting findings. Whilst 34 of the study arms calculated

574 mixed MPS (i.e. an aggregate of all muscle protein sub-fractions), 14 study arms chose to
575 calculate MPS in isolated myofibrillar proteins (Tables 1, 2 and 3). Myofibrillar proteins
576 comprise the contractile apparatus within skeletal muscle (i.e. myosin, actin, titin), the
577 synthesis of which can increase by 2-to-3-fold above basal, postabsorptive values following a
578 single bout of high intensity/volume resistance exercise in young and older individuals (62,
579 71, 111). On the other hand, proteins that comprise a mixed fraction include sarcoplasmic and
580 mitochondrial proteins, and may display lower acute responsiveness than myofibrillar
581 proteins to resistance exercise alone or combined with amino acid-based nutrition (71, 111).
582 For example, in well-trained individuals an acute bout of resistance exercise stimulates rates
583 of myofibrillar, but not mixed MPS (55). Herein, we were unable to detect any age-related
584 differences in the MPS response in myofibrillar vs. mixed fractions due to the highly variable
585 experimental methods between studies (i.e. specifics of the anabolic stimulus, tracer
586 incorporation time, etc.). Thus, we cannot rule out the possibility that, under certain
587 experimental conditions, the choice of muscle protein sub-fraction used for the calculation of
588 MPS may be important in detecting difference in MPS between young and older individuals.
589
590 Finally, and perhaps most importantly, whilst a number of studies provided instructions to
591 participants regarding physical activity in the days leading up to the trials, only one study
592 objectively measured habitual physical activity (via accelerometry) in the days immediately
593 prior to the experimental trials (24). The importance of controlling for prior physical activity
594 when assessing MPS cannot be overstated, as recent work demonstrated that just 2 weeks of
595 reduced ambulation (~75% daily step reduction) resulted in muscle atrophy and anabolic
596 resistance in older individuals (15). Given emerging evidence that the proposed post-exercise
597 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. Whilst 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 sub-fraction, 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 focussed) 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 remodelling 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 utilising relatively low intensity/volume protocols or low dose protein/amino acid provision (sub-optimal leucine). However, we cannot dismiss the fact that some study arms failed to observe age-related muscle anabolic resistance in response to sub-optimal anabolic stimuli and that others observed age-related muscle anabolic resistance following robust anabolic stimuli. We postulate that this inconsistency between studies can largely be attributed 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 ~12g 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 non-sedentary behaviour 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 weight 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 sub-optimal 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 ~0.4g/kg body weight per meal, distributed evenly across the day (8, 28). Based on 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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Figure Captions

Figure 1: Study identification process flowchart.

Figure 2: Diagrammatic illustration of the different models constructed for reporting evidence or no evidence of age-related muscle anabolic resistance.

Figure 3: Study arms in Model 2 comparing the magnitude of the MPS response to provision of a source of amino acids (AA)/protein in young vs. older adults (expressed as the % change from basal postabsorptive values). NB: the dose, protein source, leucine content, FSR incorporation period and route of administration differ between, but not within studies (see Table 2). FSR values for study arms were obtained directly from published manuscripts or, when not available, through requesting the information directly from the authors. In 5 of the 21 study arms, precise FSR values were unavailable and therefore estimated visually from the manuscript figure. Three of the 21 study arms were excluded from the comparison as they failed to assess MPS in the basal, postabsorptive state.

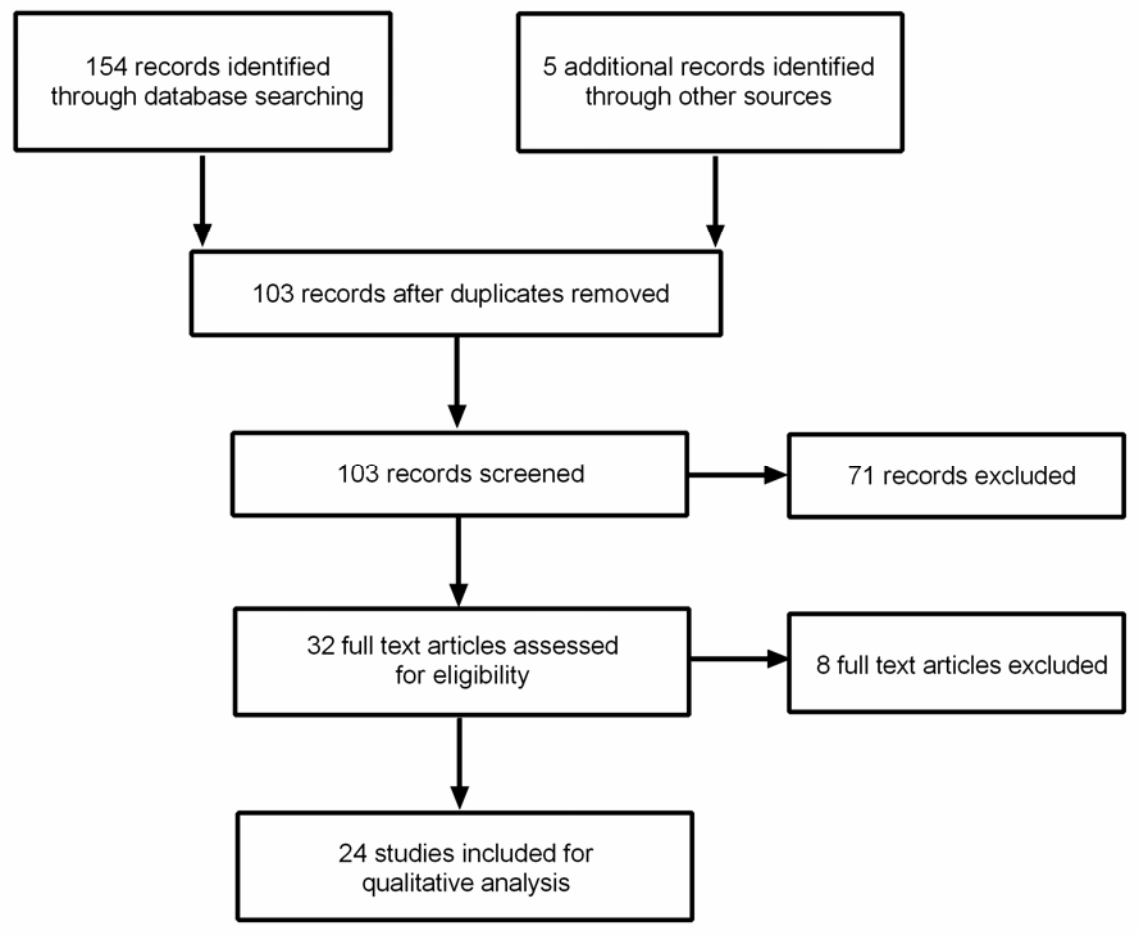

154 records identified through database searching
5 additional records identified through other sources
103 records after duplicates removed
103 records screened
71 records excluded
32 full text articles assessed for eligibility
8 full text articles excluded
24 studies included for qualitative analysis

Figure 1

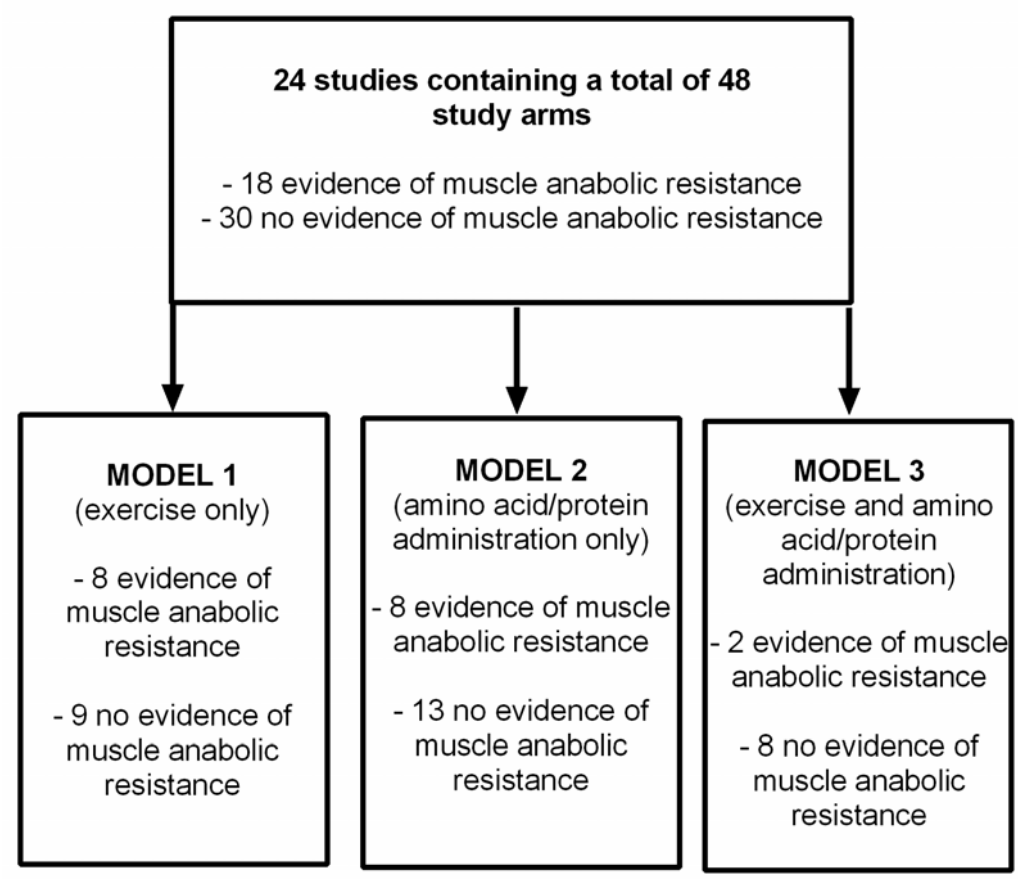
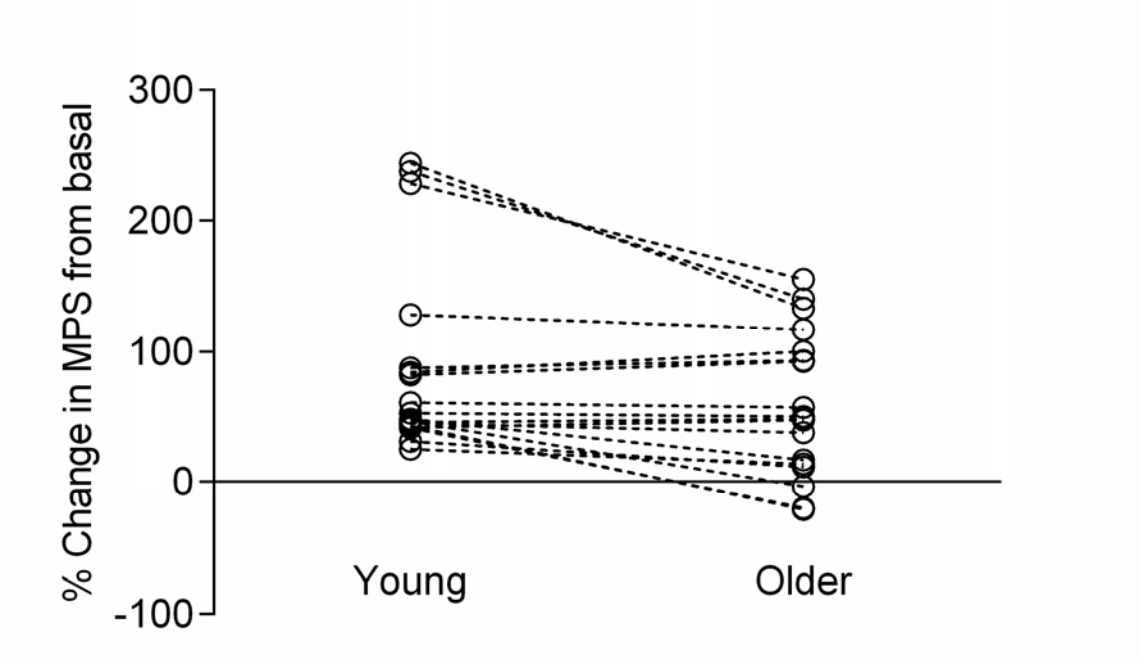

24 studies containing a total of 48 study arms
- 18 evidence of muscle anabolic resistance
- 30 no evidence of muscle anabolic resistance
MODEL 1
(exercise only)
- 8 evidence of muscle anabolic resistance
- 9 no evidence of muscle anabolic resistance
MODEL 2
(amino acid/protein administration only)
- 8 evidence of muscle anabolic resistance
- 13 no evidence of muscle anabolic resistance
MODEL 3
(exercise and amino acid/protein administration)
- 2 evidence of muscle anabolic resistance
- 8 no evidence of muscle anabolic resistance

Figure 2



1133

1134 Figure 3

Table 1. Summary of studies included in Model 1

Reference	Group, age (years)	Sex (n)	Body mass (kg)	Exercise protocol	Incorporation period	Muscle sub-fraction	Precursor pool	Evidence of age-related muscle anabolic resistance	Main findings
Fry et al. (2011) **(37)**	Young 27 ± 2	M/F n = 16	70.2 ± 3.1	8x10 sets of KE at 70% 1RM	0-3h	Mixed	IC	Yes	MPS was increased in both Y and O and was greater in Y at all time points.
					3-6h			Yes	
	Older 70 ± 2	M/F n = 16	66.9 ± 3.0		22-24h			Yes	
Kumar et al. (2009) **(62)**	Young 24 ± 6	M n = 25	-	Unilateral KE at intensities from 20-90% 1RM (volume matched)	0-1h	Myo	IC	No	The overall MPS response (AUC) across all intensities was 30% higher in Y compared with O at 1-2h. MPS was not different between Y and O at 0-1h or 2-4h.
					1-2h			Yes	
	Older 70 ± 5	M n = 25	-		2-4h			No	
Kumar et al. (2012) **(61)**	Young 24 ± 6	M n = 12	72 ± 11	1. 3 sets of KE at 40% 1RM	0-4h	Myo	IC	Yes	At 40% 1RM (3 sets), AUC for MPS over entire 0-4h post-exercise was higher in Y than O. At 40% (6 sets) and 75% (3 and 6 sets) 1RM, AUC for MPS was not different between Y and O.
	Older 70 ± 5	M n = 12	72 ± 16	2. 6 sets of KE at 40% 1RM				No	
				3. 3 sets of KE at 75% 1RM				No	

				4. 6 sets of KE at 75% 1RM				No	
Mayhew et al. (2009) **(65)**	Young 27 ± 1	M n = 8	75.4 ± 3.0	3x8-12 RM of squat, LP and KE	21-24h	Mixed	IC	Yes	MPS was increased above baseline at 21-24h post-exercise in Y only.
	Older 64 ± 1	M n = 6	76.8 ± 3.9						
Sheffield-Moore et al. (2004) **(85)**	Young 29 ± 2	M n = 6	80 ± 4	Treadmill exercise (walking) for 45 min at ~ 40% Vo_2 peak	0-10min	Mixed	IC	No	MPS at 0-10min and 0-3h was not different between Y and O but MPS was increased only in Y at 0-1h.
					0-1h			Yes	
	Older 69 ± 1	M n = 6	88 ± 7		0-3h			No	
Sheffield-Moore et al. (2005) **(84)**	Young 29 ± 2	M n = 6	78 ± 3	6x8 sets of KE at 80% 1RM	0-10min	Mixed	IC	No	MPS was increased at 0-10min in O only. MPS was not elevated in Y or O at 0-1h. MPS was increased at 0-3h in Y only.
					0-1h			No	
	Older 69 ± 1	M n = 6	86 ± 2		0-3h			Yes	

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

Reference	Group, age (years)	Sex (n)	Body mass (kg)	Amino acid/protein protocol	Incorporation period	Muscle sub-fraction	Precursor pool	Evidence of age-related muscle anabolic resistance	Main findings
Babraj et al. (2005) **(5)**	Young 28 ± 6	M n = 4	-	20g of EAA orally consumed	0-3h	Myo	Plasma	Yes	Y and O increased MPS, but increase was lower in O.
	Older 70 ± 6	M n = 4	-						
Chevalier et al. (2011) **(24)**	Young 24 ± 1	F n = 8	62.0 ± 3.6	Hyperinsulinemic, hyperglycemic, and hyperaminoacidemic clamp (IV)	0-2h	Mixed	IC	No	Both Y and O increased MPS with no difference between groups.
	Older 73 ± 3	F n = 8	60.8 ± 3.5						
Cuthbertson et al. (2005) **(27)**	Young 28 ± 6	M n = 16	75 ± 10	1. 2.5g EAA orally	0-3h	Myo	IC	No	No difference in MPS between Y and O at 2.5g and 5g EAA. MPS in Y was greater than O at 10g and 20g EAA.
				2. 5g EAA orally				No	
	Older 70 ± 6	M n = 16	79 ± 13	3. 10g EAA orally				Yes	
				4. 20g EAA orally				Yes	
Gorissen et al. (2014) **(44)**	1.Young 20 ± 1	M n = 12	76.1 ± 2.8	1. 20g of casein orally consumed with 60g carbohydrate	0-2h	Mixed	Plasma	Yes	MPS was increased only in Y at 0-2h, but MPS over entire 0-5h was not different between Y and O for either
	1.Older 76 ± 1	M n = 13	79.6 ± 2.7		0-5h			No	

	2.Young 21 ± 1	M n = 12	70.9 ± 3.2	2. 20g of casein orally consumed without 60g carbohydrate	0-2h			Yes	intervention.
	2.Older 74 ± 1	M n = 12	75.0 ± 4.2		0-5h			No	
Guillet et al. (2004) **(46)**	Young 25 ± 1	- n = 6	78.7 ± 3.3	Hyperinsulinemic, hyperaminoacidemic clamp (IV)	0-4h	Mixed	IC	Yes	MPS was increased in both Y and O and was greater in Y.
	Older 72 ± 2	- n = 8	75.4 ± 3.3						
Katsanos et al. (2006) **(53)**	1.Young 31 ± 2	M/F n = 8	70.1 ± 4.7	1. 6.7g of EAA orally consumed with 26% leucine	0-3.5h	Mixed	Plasma	Yes	MPS was increased equally after EAA with 41% leucine but MPS was only increased in Y after EAA with 26% leucine.
	1.Older 67 ± 2	M/F n = 10	81.7 ± 3.6						
	2.Young 29 ± 3	M/F n = 8	76.6 ± 7.7	2. 6.7g of EAA orally consumed with 41% leucine				No	
	2.Older 67 ± 2	M/F n = 10	74.5 ± 4.7						
Kiskini et al. (2013) **(57)**	Young 21 ± 1	M n = 12	74.4 ± 2.2	20g of casein orally consumed with 40g carbohydrate	0-6h	Mixed	Plasma	No	MPS over entire 0-6h did not differ between Y and O.
	Older 75 ± 1	M n = 12	78.4 ± 2.1						
Koopman et al. (2009) **(59)**	Young 23 ± 1	M n = 10	76.8 ± 2.0	35g of casein orally consumed	0-6h	Mixed	Plasma	No	MPS over entire 0-6h did not differ between Y and O.

	Older 64 ± 1	M n = 10	78.8 ± 3.1						
Paddon-Jones et al. (2004) **(76)**	Young 34 ± 4	M/F n = 6	63 ± 3	15g of EAA orally consumed	0-3.5/4h	Mixed	IC	No	MPS was increased similarly in both Y and O.
	Older 67 ± 2	M/F n = 7	71 ± 5						
Pennings et al. (2011) **(78)**	Young 21 ± 1	M n = 12	76.2 ± 3.6	20g of casein orally consumed	0-6h	Mixed	Plasma	No	MPS over entire 0-6h did not differ between Y and O.
	Older 75 ± 1	M n = 12	74.4 ± 2.3						
Symons et al. (2009) **(91)**	Young 35 ± 3	M/F n = 17	79.2 ± 7.0	1. 113g (30g protein) of lean ground beef	0-5h	Mixed	IC	No	MPS was increased similarly in both Y and O with 113g and 340g lean ground beef.
	Older 68 ± 2	M/F n = 17	77.5 ± 8.0	2. 340g (90g protein) of lean ground beef				No	
Volpi et al. (1999) **(105)**	Young 30 ± 2	M/F n = 7	72 ± 3	40g of amino acids orally consumed in boluses every 10mins	0-3h	Mixed	IC	No	MPS was increased similarly in both Y and O.
	Older 71 ± 2	M/F n = 8	74 ± 4						
Volpi et al. (2000) **(104)**	Young 30 ± 3	M/F n = 5	-	40g amino acids with 40g carbohydrate orally consumed in boluses every 10mins	0-3h	Mixed	IC	Yes	MPS was increased only in Y.
	Older 72 ± 1	M/F n = 5	-						

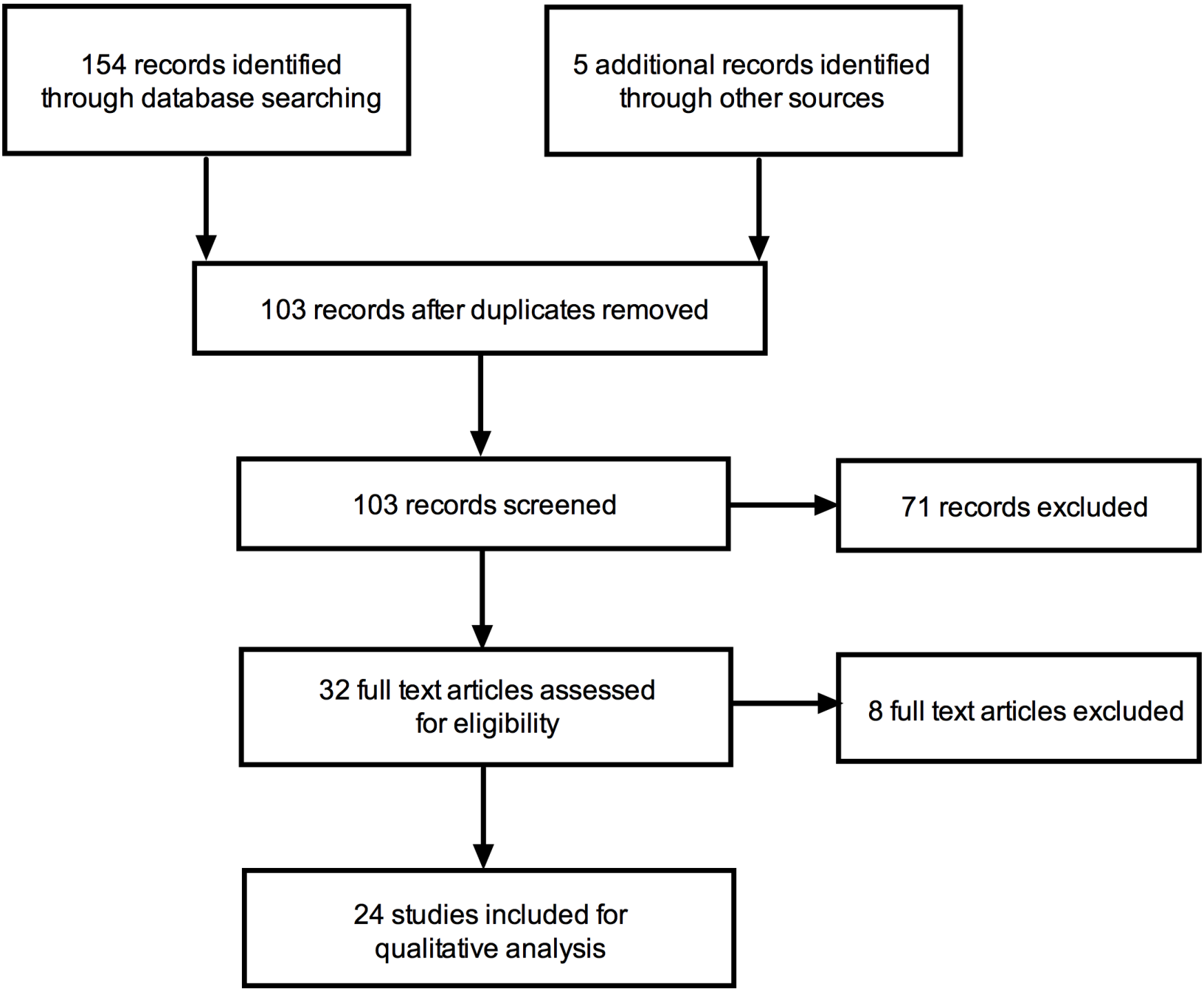
Y = young; O = older; M = male; F = female; IV = intravenous; EAA = essential amino acids; MPS = muscle protein synthesis; AUC = area under curve; Myo = myofibrillar; IC = intracellular.

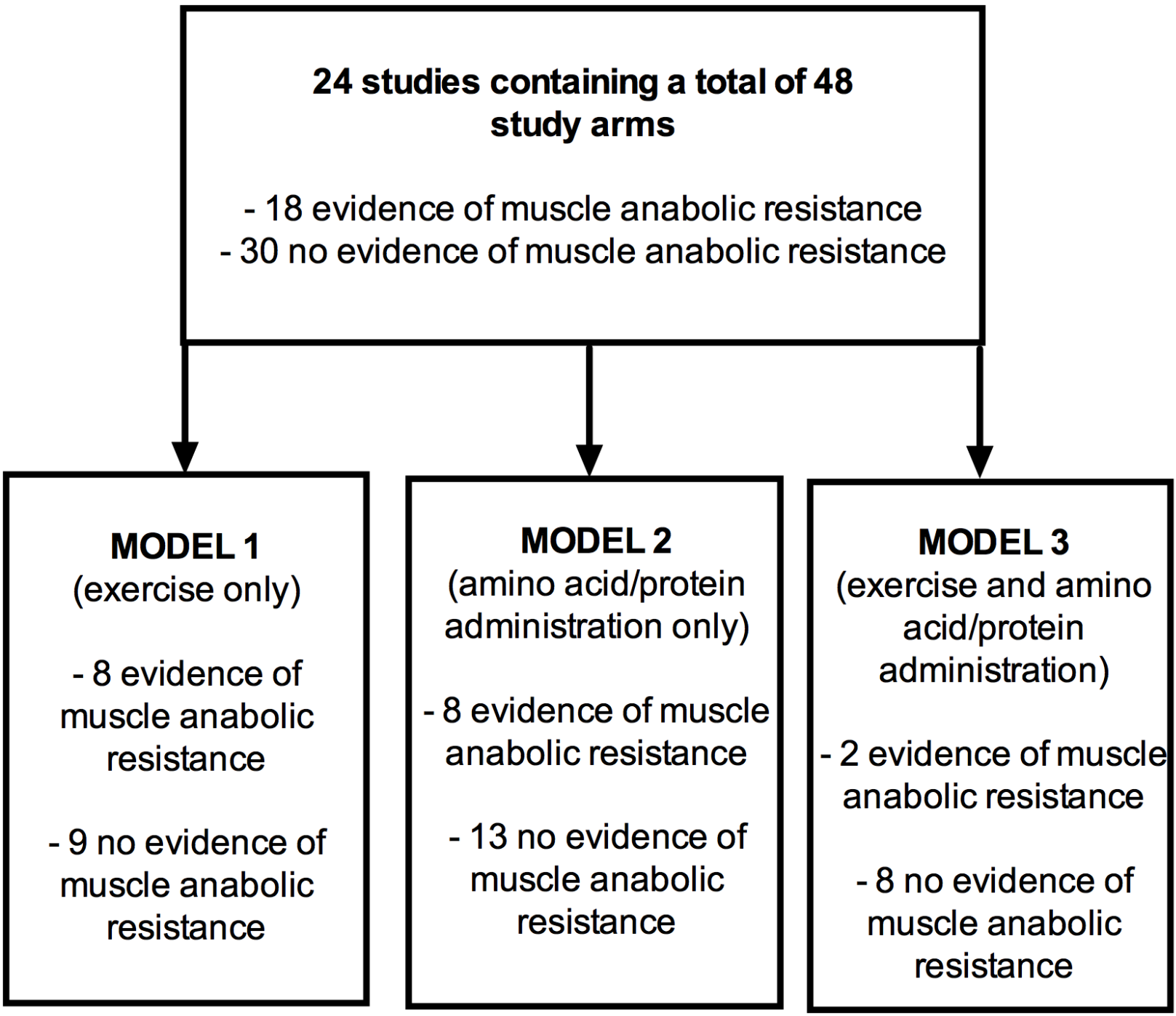
Table 3. Summary of studies included in Model 3

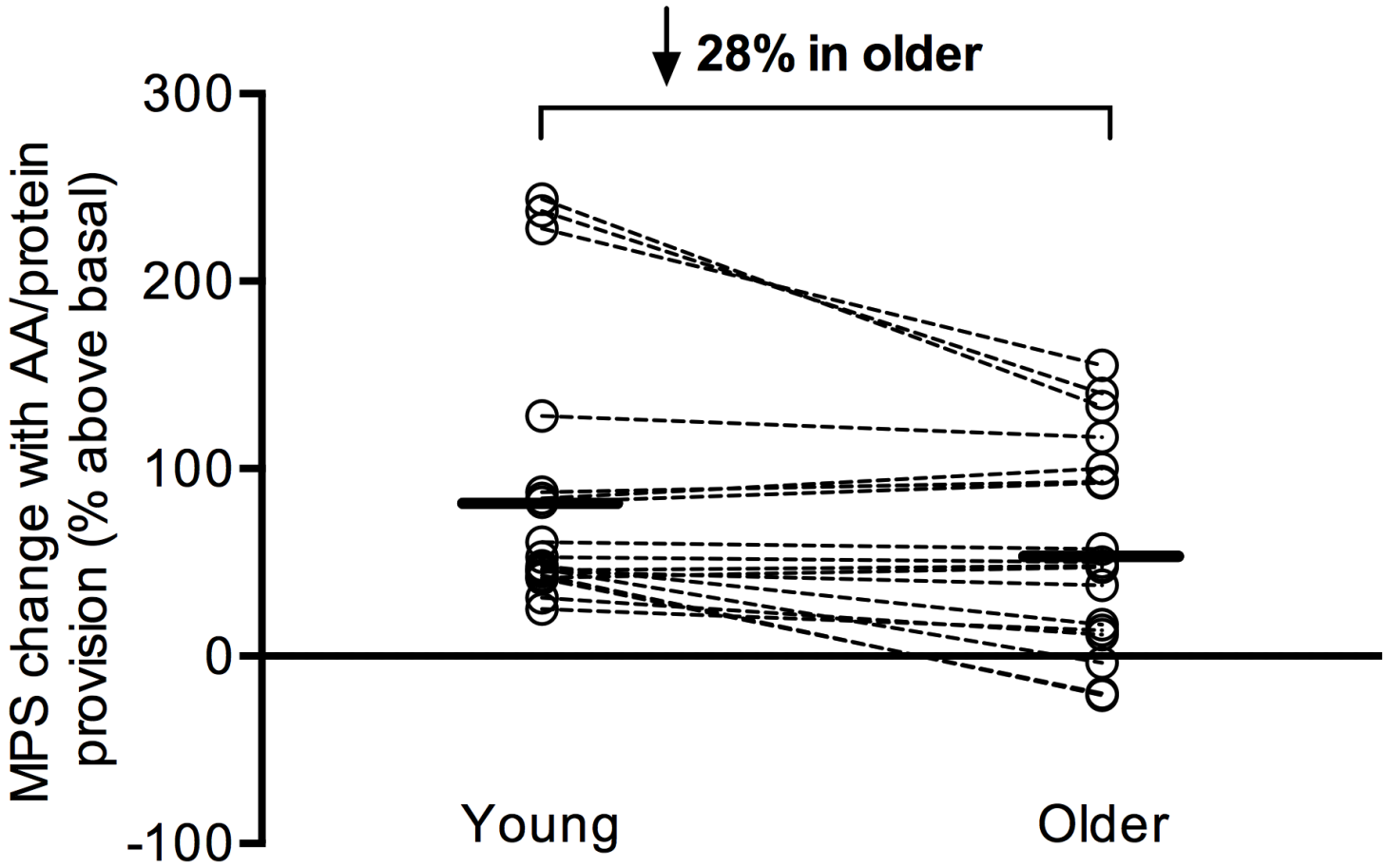
Reference	Group, age (years)	Sex (n)	Body mass (kg)	Exercise and Amino acid/protein protocol	Incorporation period	Muscle sub-fraction	Precursor pool	Evidence of age-related muscle anabolic resistance	Main findings
Atherton et al. (2016) **(2)**	Young 24 ± 6	M n = 18	75 ± 10	1. 6x8 sets of KE at 75% 1RM followed by 10g of protein (8g casein, 2g whey), 24g carbohydrate and 4.2g leucine	0-4h	Myo	Plasma	No	AUC for 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.
	Older 70 ± 5	M n = 18	76 ± 10						
				2. 6x8 sets of KE at 75% 1RM followed by 10g of protein (8g casein, 2g whey), 24g carbohydrate and 4.2g alanine				No	
Drummond et al. (2008) **(35)**	Young 30 ± 2	M n = 7	88.9 ± 5.4	8x10 sets of KE at 70% 1RM followed by 20g oral EAA 1h post-exercise	0-1h	Mixed	IC	No	MPS was higher in Y than O at 1-3h, but MPS over 0-1h, 3-6h and entire 1-6h was not different.
					1-3h			Yes	
	Older 70 ± 2	M n = 6	81.3 ± 5.2		3-6h			No	
					1-6h			No	
Durham et al. (2010) **(36)**	Young 30 ± 2	M n = 9	78 ± 2	Treadmill exercise (walking) for 45 min at ~ 40% Vo_2 peak with amino acids infused throughout recovery	10min-3h	Mixed	IC	No	MPS was increased in both Y and O with no differences between groups.
	Older 67 ± 2	M n = 8	84 ± 4						

Koopman et al. (2006) **(58)**	Young 20 ± 1	M n = 8	73.7 ± 3.2	6x10 sets of LP and 6x10 sets of KE at 40-75% 1RM followed by small repeated boluses of ~60g whey with ~184g carbohydrate	0-6h	Mixed	Plasma	Yes	MPS over entire 0-6h was lower in the O compared to the Y.
	Older 75 ± 1	M n = 8	75.5 ± 2.1						
Pennings et al. (2011) **(78)**	Young 21 ± 1	M n = 12	76.1 ± 2.8	6x10 sets of LP and 6x10 sets of KE at 40-75% 1RM followed by 20g of casein orally consumed	0-6h	Mixed	Plasma	No	MPS over entire 0-6h did not differ between Y and O.
	Older 73 ± 1	M n = 12	79.6 ± 2.7						
Symons et al. (2011) **(90)**	Young 29 ± 3	M/F n = 7	79 ± 10	340g (90g protein) of lean ground beef followed 60mins later by 6x8 sets of KE at 80% 1RM	0-5h	Mixed	IC	No	MPS was increased similarly in both Y and O.
	Older 67 ± 2	M/F n = 7	76 ± 5						

Y = young; O = older; M = male; F = female; EAA = essential amino acids; KE = knee extension; LP = leg press; 1RM = One repetition maximum; MPS = muscle protein synthesis; AUC = area under curve; IC = intracellular.


154 records identified through database searching
5 additional records identified through other sources
103 records after duplicates removed
103 records screened
71 records excluded
32 full text articles assessed for eligibility
8 full text articles excluded
24 studies included for qualitative analysis


24 studies containing a total of 48 study arms
- 18 evidence of muscle anabolic resistance
- 30 no evidence of muscle anabolic resistance
MODEL 1
(exercise only)
- 8 evidence of muscle anabolic resistance
- 9 no evidence of muscle anabolic resistance
MODEL 2
(amino acid/protein administration only)
- 8 evidence of muscle anabolic resistance
- 13 no evidence of muscle anabolic resistance
MODEL 3
(exercise and amino acid/protein administration)
- 2 evidence of muscle anabolic resistance
- 8 no evidence of muscle anabolic resistance


28% in older
300
200
100
0
-100
MPS change with AA/protein provision (% above basal)
Young
Older