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

3

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5

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10 Running title: Muscle anabolic resistance in older age

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25 **Abstract**

26 The precise role of age-related muscle anabolic resistance in the progression of sarcopenia
27 and functional decline in older individuals is unclear. The present aim was to assess whether
28 the muscle protein synthesis (MPS) response to acute exercise (endurance or resistance)
29 and/or amino acid-based nutrition is attenuated in older compared with young individuals. A
30 systematic review was conducted on studies that directly examined the influence of age on the
31 MPS response to exercise and/or amino acid-based nutrition. Each study arm was synthesised
32 and reported as providing sufficient or insufficient '*evidence of age-related muscle anabolic*
33 *resistance*'. Subsequently, three models were established to compare age-related differences
34 in the MPS response to: i) exercise alone; ii) amino acid-based nutrition alone; or iii) the
35 combination of exercise and amino acid-based nutrition. Following exercise alone, 8 of the 17
36 study arms provided sufficient '*evidence of age-related muscle anabolic resistance*' whilst in
37 response to amino acid-based nutrition alone, 8 of the 21 study arms provided sufficient
38 '*evidence of age-related muscle anabolic resistance*'. When exercise and amino acid-based
39 nutrition were combined, only 2 of the 10 study arms provided sufficient '*evidence of age-*
40 *related muscle anabolic resistance*'. Our results highlight that optimisation of exercise and
41 amino acid-based nutrition is sufficient to induce a comparable MPS response between young
42 and older individuals. However, the exercise volume completed and/or the amino acid/protein
43 dose and leucine content must exceed a certain threshold to stimulate equivalent MPS rates in
44 young and older adults, below which age-related muscle anabolic resistance may become
45 apparent.

46

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

48

49 **Introduction**

50 It is well documented that we are in the midst of a global shift towards an expanding aging
51 demographic. Recent estimates predict that the number of people aged 60 years and over is
52 expected to more than double from 901 million in 2015 to over 2 billion in 2050, whilst the
53 number of people aged 80 years and over (the ‘oldest old’) is expected to more than triple
54 (100). Advancing age is closely associated with a number of debilitating health consequences,
55 including the loss of skeletal muscle mass and strength (termed sarcopenia), which is strongly
56 associated with an increased incidence of falls (63), loss of independence (9), increased risk
57 of age-related co-morbidities (4, 32) and, in severe cases, premature mortality (16, 88). As
58 such, sarcopenia and associated comorbidities place a considerable burden on healthcare
59 resources (51). Therefore, clear understanding of the metabolic and molecular mechanisms
60 that underpin sarcopenia is of paramount importance in order to develop targeted therapeutic
61 strategies to prevent and/or treat this age-related phenomenon.

62

63 The underlying pathology of sarcopenia is highly complex and remains to be fully elucidated.
64 Sarcopenia may result from factors including inactivity/disuse, inadequate dietary protein
65 intake, chronic low-grade inflammation and hormonal dysregulation, summarized succinctly
66 by others (73). Regardless of the precise contribution of each of these factors, sarcopenia is
67 due to muscle protein loss resulting from an imbalance between muscle protein synthesis
68 (MPS) and breakdown (MPB), which manifests primarily as a reduction in type II muscle
69 fibre size (34, 74, 79, 102). In young healthy individuals, mechanical loading (i.e. exercise
70 contraction) in the fasted, post-absorptive state increases MPS and, to a lesser extent MPB,
71 resulting in an improved, yet negative net protein balance (NBAL) (10, 80). In contrast,
72 amino acid-based nutrition serves primarily to increase MPS, with the impact on MPB less

73 clear due to the methodological difficulties encountered when assessing MPB under non-
74 steady state conditions. In general, most studies appear to demonstrate a small suppression of
75 MPB in response to amino acid-based nutrition, which in conjunction with the postprandial
76 rise in MPS results in a positive NBAL in both young and older individuals (43, 71, 103,
77 105). Combined, mechanical loading and amino acid-based nutrition act synergistically to
78 enhance MPS and suppress MPB and thus promote net muscle protein accretion (22, 42, 71,
79 78). Most (27, 35, 64, 76, 105), but not all (7, 48, 117) studies to date have observed no
80 evidence of age-related differences in postabsorptive, basal rates of MPS. Likewise, although
81 methodologically challenging to measure, rates of MPB are comparable between healthy
82 younger and older individuals in the postabsorptive, basal state and following resistance
83 exercise (38, 110). Evidence of an age-related impairment in the suppression of MPB under
84 hyperaminoacidemic and/or hyperinsulemic conditions has been limited and relatively
85 inconsistent to date (81, 104, 110). The absence of age-related differences in postabsorptive,
86 basal state rates of MPS and MPB, coupled with inconsistent findings on age-related
87 differences in postprandial rates of MPB, has led to the hypothesis that dysregulation of the
88 MPS response to normally robust anabolic stimuli (i.e. exercise and/or amino acid-based
89 nutrition), termed ‘anabolic resistance’ (83), may underpin the progression of sarcopenia.
90

91 Age-related muscle anabolic resistance may be related to diminished mRNA translational
92 signalling (27, 37, 46, 62), impaired transport of amino acids into muscle (30, 31), lipid-
93 induced muscle insulin resistance (89), attenuated protein digestion and absorption (13) and
94 dysregulation of nutritive blood flow to skeletal muscle (39, 66, 81). However, these defects
95 may be a consequence of declining habitual activity levels (15), protracted disuse events (41,
96 107), obesity (72) and chronic inflammation (6, 97) superimposed on the natural biological

97 ageing process. Interestingly, whilst some studies support the development of age-related
98 muscle anabolic resistance (27, 46, 53), other studies have failed to observe any difference in
99 the MPS response to anabolic stimuli between young and older adults (59, 76, 90). This lack
100 of agreement between studies on whether or not differences in MPS exist between young and
101 older individuals may be due to differences in the experimental methodology used to assess
102 MPS (18). For example, i) the time frame of MPS assessment, ii) analysis of specific muscle
103 protein sub-fractions and iii) volume of exercise and dose/source of amino acid-based
104 nutrition can profoundly influence the observed MPS response in young and older adults.
105 Furthermore, participant habitual physical activity levels and metabolic health status may also
106 explain the incongruous findings of previous studies (15, 17). With this in mind, it is
107 imperative that we explore the possible cause of discrepancies between studies and delineate
108 whether age-related differences in MPS between young and older individuals do exist. This
109 approach will allow us to identify whether (or not) strategies to restore muscle anabolic
110 sensitivity in older individuals have the capacity to prevent or slow sarcopenic progression.

111

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

119

120 **Methods**

121 ***Search Strategy***

122 A systematic literature search of the Ovid MEDLINE (1946 to May 2016) and EMBASE
123 (1974 to 23rd May 2016) databases was performed with the final literature search completed
124 on 23rd May 2016. These databases were chosen due to the extensive cover of journal articles
125 in the area of health and clinical sciences. Search terms used were: protein synth*, muscle
126 protein synth*, MPS, fractional synth*, FSR, myofibrillar, muscle protein accru*, protein
127 balance, phenylalanine, exercise*, contraction*, resistance exercise*, amino acid*, EAA*,
128 essential amino*, dietary protein, protein-rich, beef, leucine, young*, old* and elder*. The
129 medical subject headings (MeSH) “muscle proteins” and “humans” were also utilised.
130 Boolean operators “and” and “or” were used to combine search terms. Additional studies
131 were identified through the reference lists of articles (e.g. reviews) from relevant fields of
132 study.

133

134 ***Eligibility Criteria***

135 *Types of Studies:* Randomised controlled trials, non-randomised clinical trials or comparative
136 studies that directly compared young and older participants within the same study were
137 eligible for inclusion. Non-randomised studies were eligible as the majority of studies that
138 explore age-related differences in MPS in response to an anabolic stimulus intentionally
139 group subjects based on their age (i.e. young vs. older) and thus randomisation is not always
140 possible. Studies were restricted to those written in the English language and no publication
141 date restrictions were applied.

142 *Types of Participants:* Healthy young and older humans, both male and female, were
143 included. The mean age of the young group was required to be in the range of 18 and 35 yrs
144 of age (inclusive). The mean age of the older group was required to be ≥ 55 yrs of age. These

145 criteria were chosen as age-related sarcopenia tends to manifest in the 4-5th decade in humans
146 (23, 50), and thus we reasoned that an age range of 18-35 yrs would provide a fair reflection
147 of younger individuals that had not yet reached the threshold for development of sarcopenia.
148 Similarly, we posited that ≥ 55 yrs of age for older individuals would ensure that the threshold
149 for development of age-related sarcopenia had been reached. Accordingly, any studies that
150 utilised young or older groups with a mean age between 36 and 54 yrs (inclusive) were
151 excluded. To ensure that we addressed the influence of age on the MPS response to anabolic
152 stimuli *per se*, participants with any form of diabetes or chronic disease condition
153 characterised by rapid inflammation-induced muscle atrophy (e.g. chronic obstructive
154 pulmonary disease, cancer cachexia, arthritis or congestive heart failure), were excluded, as
155 such conditions are known to dramatically alter postabsorptive and postprandial muscle
156 protein turnover beyond that expected in healthy, non-diseased populations (25).

157 *Types of Interventions:* This systematic review was limited to studies utilising a single, acute
158 bout of resistance exercise (e.g. free-weight, guided range-of-motion machines, dynamometry
159 or body weight exercises) and/or endurance exercise (e.g. walking, cycling or running) and/or
160 amino acid/protein administration. Amino acids/protein could be provided either orally (e.g.
161 supplemental protein beverages or protein-rich solid foods) or intravenously (e.g.
162 hyperaminoacidemic clamp). Studies in which additional macronutrients (i.e. carbohydrates
163 and fats) were provided in addition to amino acid/protein provision were deemed eligible for
164 inclusion as co-ingestion of carbohydrate and/or fat does not appear to significantly modulate
165 the postprandial MPS response to protein ingestion (44, 45, 60). Interventions that co-
166 administered pharmaceutical drugs that were not designed to incur hyper and/or hypo
167 aminoacidemia, insulinemia, or glycemia were excluded, as these drugs could confound some
168 of the age-related differences in the MPS response to anabolic stimuli between young and

169 older individuals. Interventions that assessed acute MPS rates following a chronic resistance
170 training programme were also excluded as this could abrogate potential age-related
171 differences in MPS (48).

172 *Types of Outcome Measures:* The primary outcome measure from eligible studies was a
173 qualitative appraisal of muscle anabolic resistance, i.e. sufficient evidence of age-related
174 differences in MPS rates, or insufficient evidence of age-related differences in MPS rates in
175 response to a given anabolic stimulus. Assessment of MPS was required to be completed
176 within 24 h of the given stimulus, as it has previously been demonstrated that the increase in
177 MPS rates is most pronounced in the immediate hours following an anabolic stimulus,
178 gradually subsiding by 24 h post-stimulus in young individuals (19, 80). All studies included
179 were required to assess MPS via calculation of the muscle fractional synthetic rate (FSR)
180 using the precursor-product model. The precursor-product model measures the rate at which
181 the tracer is incorporated into bound muscle protein between sequential muscle biopsies over
182 a specified period of time, and is considered the gold-standard for assessing *in vivo* MPS in
183 humans (14, 54, 114). Furthermore, this approach allows the assessment of MPS within
184 specific protein sub-fractions (i.e. myofibrillar, mitochondrial and sarcoplasmic). Therefore,
185 any studies that used the 2-pool or 3-pool arteriovenous balance method (indirect estimate of
186 MPS) were excluded. Included studies were required to assess at least one of the following:
187 mixed-muscle, myofibrillar or myosin heavy chain muscle protein synthesis, as these protein
188 sub-fractions comprise the contractile apparatus of skeletal muscle.

189

190 ***Data Collection and Analysis***

191 *Selection of Studies:* Eligibility appraisal of the titles and abstracts generated by the literature
192 search was conducted independently by two reviewers (BJ Shad and JL Thompson). All titles

193 and abstracts deemed ineligible were excluded, whilst those determined to be potentially
194 eligible for inclusion in the systematic review were reserved and the full-text articles
195 obtained. Full-text articles were subsequently screened by the two independent reviewers (BJ
196 Shad and L Breen) for relevance using the eligibility criteria described above. Any
197 disagreements between the two reviewers were resolved by consensus. All records generated
198 by the literature search of Ovid MEDLINE and EMBASE databases were managed using the
199 reference management software package EndNote (Thomson Reuters, version X7). Duplicate
200 records were removed using the ‘find duplicates’ function in Endnote.

201 *Data Extraction and Management:* Two reviewers (BJ Shad and L Breen) independently
202 extracted all data (i.e. study characteristics and outcome data) from all included studies using
203 a customised data extraction form. Any disagreements were resolved by consensus between
204 the two reviewers. Data were extracted on a study arm level. This ensured that all relevant
205 data were extracted in circumstances where multiple interventions were utilised within the
206 same study (e.g. provision of different essential amino acid (EAA) doses). Categories of data
207 extracted included: a) participant characteristics (e.g. age, number, gender and body mass), b)
208 type of intervention (e.g. exercise mode, exercise intensity and amino acid dose), c) details of
209 the method of MPS assessment (e.g. measurement period, muscle sub-fraction used and
210 precursor pool used) and d) data outcome details (i.e. qualitative appraisal of age-related
211 differences in the MPS response and whether the data provided sufficient ‘*evidence of age-*
212 *related muscle anabolic resistance*’ or not (see ‘Method of Data Synthesis’ section below).

213 *Method of Data Synthesis:* We chose to qualitatively synthesise the data from the included
214 studies as the heterogeneous experimental methodology employed when assessing MPS (e.g.
215 amino acid stable isotope tracer, muscle protein sub-fraction, duration of tracer incorporation,
216 and precursor pool) can result in varying rates of MPS between studies (86), meaning

217 quantitative analysis across studies was not feasible. As part of the data extraction process,
218 both reviewers were required to qualitatively synthesise the data of each study by
219 independently determining whether there was sufficient '*evidence of age-related muscle*
220 *anabolic resistance*' or not. If it was deemed that the results of a study provided sufficient
221 '*evidence of age-related muscle anabolic resistance*', the study was given a 'Yes' whereas if it
222 was deemed that the results of a study did not provide sufficient '*evidence of age-related*
223 *muscle anabolic resistance*', the study was given a 'No'. Examples of sufficient '*evidence of*
224 *age-related muscle anabolic resistance*' included data demonstrating; i) a significantly ($P <$
225 0.05) greater MPS response in young compared with older participants in response to an
226 anabolic stimulus, or ii) that only young participants experienced a significant ($P < 0.05$)
227 increase in MPS in response to anabolic stimuli. In the event that a study assessed MPS at
228 multiple time points, but only reported age-related differences in MPS at some, but not all of
229 these time points, data were extracted from the reported time points only. Similarly, in the
230 event that a study assessed the MPS response to multiple exercise stimuli (e.g. a range of
231 exercise intensities) and/or nutritional interventions (e.g. varying amino acid doses) but only
232 reported age-related differences in MPS for some of these interventions, data were extracted
233 from the reported interventions only. Upon completion of data extraction, using a similar
234 analysis approach to Trommelen and colleagues (99), several different models were
235 constructed to compare age-related differences in MPS in response to different anabolic
236 stimuli. In Model 1, study arms that utilised exercise as the only form of anabolic stimulus
237 were included to examine age-related differences in the MPS response to an isolated
238 contractile bout. In Model 2, study arms that utilised amino acid/protein
239 administration/feeding as the only form of anabolic stimuli were included to examine age-
240 related differences in the MPS response to a nutrient stimulus. Finally, Model 3 included

241 study arms that utilised exercise alongside amino acid/protein administration/feeding to
242 examine age-related differences in the MPS response to the combined anabolic stimulus of
243 contraction and amino acid-based nutrition.

244

245 **Results**

246 *Literature Search*

247 The literature search produced 154 records potentially eligible for inclusion. A further 5
248 records were identified through a hand search of reference lists of reviews in the field of
249 study, resulting in a total of 159 records. Following the removal of duplicate records, 103
250 records remained. From the remaining records, titles and abstracts were independently
251 screened by two reviewers (BJ Shad and JL Thompson) to assess eligibility. The screening
252 process resulted in 71 studies being excluded, leaving 32 full-text articles to be assessed for
253 eligibility by two reviewers (BJ Shad and L Breen) independently. Of these 32 full-text
254 articles, 8 were excluded for reasons including; use of the 3-pool arteriovenous balance
255 method to estimate age-related differences in MPS (52), assessment of MPS in the
256 postabsorptive state only (109) and mean age of the young participants falling outside the
257 inclusion range (87). Accordingly, a total of 24 studies met the eligibility criteria and thus
258 were included in the systematic review for qualitative analysis. Figure 1 depicts a flow
259 diagram of the study identification process.

260

261 *Included Studies*

262 Across the 24 studies included, there was a large amount of heterogeneity pertaining to the
263 participant characteristics, the anabolic stimuli utilised (e.g. different exercise regimens and/or
264 route, source and dose of amino acid/protein provision) and the experimental methodology

265 used to determine MPS. A brief overview of between study differences is provided in the
266 results text below, and more comprehensively in Tables 1, 2 and 3.

267

268 ***Participants***

269 All of the included studies reported participants as '*healthy*,' and included a comparison
270 between young and older groups. A total of 23 of the included studies specifically assessed
271 participant health status, whilst 1 study failed to declare any such assessment (5). A total of 15
272 of the included studies recruited males only, 1 study included females only, 7 studies included
273 both males and females, and 1 study did not report the gender of participants (46). The age
274 range of the young participant groups was between 20 and 35 yrs, whereas the age range of
275 the older participant groups was between 64 and 76 yrs. Body mass of the young participant
276 groups ranged from 62 kg to 88.9 kg, whilst body mass in the older participant group ranged
277 from 60.8 kg to 88 kg.

278

279 ***Anabolic stimulus***

280 Of the 24 studies, 12 included some form of acute exercise stimulus. Resistance exercise was
281 utilised in 10 of the 12 studies, and endurance exercise in 2 studies. Eighteen of the included
282 studies involved a form of amino acid/protein administration/feeding. Oral ingestion of amino
283 acids/protein was evident in 15 of the 18 studies, whilst 3 studies administered amino acids
284 through intravenous (IV) infusion. A total of 6 of the 24 studies combined exercise with oral
285 or IV administration of amino acids/protein.

286

287 ***Experimental methodology***

288 Experimental methodology between studies was highly variable. The time point over which
289 the post-stimulus MPS measurement was assessed ranged from 2 h to ~24 h. MPS in a mixed
290 muscle fraction was assessed in 19 studies, whilst 5 studies assessed MPS in the myofibrillar
291 fraction. Sixteen studies used the intracellular free-pool isotopic tracer enrichment as the
292 precursor in the calculation of FSR, whilst 8 studies used the plasma isotopic tracer
293 enrichment as the precursor. All of the included studies measured MPS from muscle biopsy
294 tissue collected from the quadriceps *vastus lateralis* muscle.

295 ***Data Synthesis***

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

303

304 A total of 48 study arms were identified from the 24 included studies (Figure 2). Of these 48
305 study arms, 18 were considered to provide sufficient evidence of age-related muscle anabolic
306 resistance (5, 27, 35, 37, 44, 46, 53, 58, 61, 62, 65, 84, 85, 104), whereas 30 were considered
307 to provide insufficient evidence of age-related muscle anabolic resistance (2, 24, 27, 35, 36,
308 44, 53, 57, 59, 61, 62, 76, 78, 84, 85, 90, 91, 105) (Figure 2). In order to further examine age-
309 related differences in MPS in response to various anabolic stimuli, we constructed three
310 models that included study arms based on the anabolic stimulus provided (outlined above in
311 'methods').

312

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

321

322 In Model 2, study arms were included if they utilised amino acid/protein
323 administration/feeding as the only anabolic stimulus. As a result, 21 study arms were included
324 in Model 2, with 8 providing sufficient evidence (5, 27, 44, 46, 53, 104) and 13 providing
325 insufficient evidence of age-related muscle anabolic resistance (24, 27, 44, 53, 57, 59, 76, 78,
326 91, 105). Ten of the 21 study arms provided oral free amino acids, with 5 providing sufficient
327 and 5 providing insufficient evidence of age-related muscle anabolic resistance. Casein
328 protein was orally administered in 7 of the 21 study arms, with 2 providing sufficient and 5
329 providing insufficient evidence of age-related muscle anabolic resistance. The 2 study arms,
330 which administered lean ground beef as the protein source, provided insufficient evidence of
331 age-related muscle anabolic resistance. Two of the 21 study arms administered amino acids
332 intravenously, with 1 providing sufficient and 1 providing insufficient evidence of age-related
333 muscle anabolic resistance (Table 2).

334

335 Finally, in Model 3, study arms that utilised a combination of both exercise and amino
336 acid/protein administration/feeding were included. As a result, 10 study arms were included in
337 Model 3, with 2 study arms providing sufficient evidence (35, 58) and 8 study arms providing
338 insufficient evidence of age-related muscle anabolic resistance (2, 35, 36, 78, 90). Nine of the
339 10 study arms utilised resistance exercise as the contractile stimulus, with 2 providing
340 sufficient evidence and 7 providing insufficient evidence of age-related muscle anabolic
341 resistance (Table 3). The single study arm that applied endurance exercise as the contractile
342 stimulus provided insufficient evidence of age-related muscle anabolic resistance.

343

344 **Discussion**

345 The aim of this systematic review was to examine the literature on age-related differences in
346 the muscle protein synthetic response to anabolic stimuli (resistance exercise, endurance
347 exercise and/or amino acid/protein administration) between young and older individuals.

348 There has been much debate as to whether muscle anabolic resistance is indeed an inevitable
349 characteristic of the aging process (17, 18), an artefact of lifestyle modifications (15, 107), or
350 a combination of these two factors. Whilst 18 study arms provided findings to support the
351 presence of muscle anabolic resistance in older individuals, 30 study arms provided
352 insufficient evidence of the development of age-related muscle anabolic resistance (Figure 2).

353 As will be discussed in this section, the primary factors that appear to contribute to the
354 discrepancies between study arms include: 1) differences in exercise volume and intensity; 2)
355 the dose, source, and leucine content of amino acids/protein provided; 3) using exercise or
356 amino acid/protein administration/feeding alone or in combination; and 4) differences in
357 experimental methodology and design.

358

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

383 sets of knee extensions at 80% 1RM (84). However, this fails to explain the occurrence of
384 age-related muscle anabolic resistance following 8 sets of knee extensions at 70% of 1RM by
385 Fry and colleagues over numerous post-exercise time points and using a larger sample size
386 (37). Exactly why the findings of Kumar et al. (61) and Fry and colleagues (37) differ is
387 difficult to reconcile but may relate to the habitual physical activity levels of the young and
388 older participants, which were not objectively measured in either study (discussed in further
389 detail below). The lack of a within-subject comparison group in the study by Fry et al. (37)
390 precludes interrogation of the dose-response of MPS to differing volume and intensity of
391 resistance exercise in this group of participants. Taken together, it is clear that future acute
392 dose-response exercise studies utilising larger sample sizes, multiple post-exercise time
393 points, with control/monitoring of habitual physical activity levels are needed to improve our
394 understanding of the importance of exercise volume and intensity in overcoming potential
395 age-related muscle anabolic resistance. In addition, chronic resistance training studies are
396 required to delineate the appropriate exercise training volume and/or intensity to maintain or
397 augment skeletal muscle mass in older individuals. Nonetheless, the findings presented
398 suggest that age-related muscle anabolic resistance may be apparent following low
399 volume/intensity resistance exercise, and that the prescription of higher volume and/or
400 intensity resistance exercise may be a feasible strategy to overcome this impairment and thus
401 maintain skeletal muscle mass.

402

403 *Dose of Amino Acids/Protein*

404 The provision of amino acid-based nutrition is a potent stimulus for MPS in young and older
405 individuals (27, 82, 116), primarily through the action of constituent essential amino acids
406 (EAA's) (96, 103). Accordingly, we constructed a second model in an attempt to examine

407 whether age-related differences in MPS exist following the provision of amino acids/protein
408 alone (Figure 2, Model 2 and Table 2). Of the 21 study arms included in this model, 8
409 provided sufficient evidence of age-related muscle anabolic resistance (5, 27, 44, 46, 53, 104)
410 whilst 13 did not (24, 27, 44, 53, 57, 59, 76, 78, 91, 105). However, although the MPS
411 response between young and older adults was only significantly different in 8 of 21 study
412 arms in Model 2, when study arms were pooled together we observed that the general pattern
413 of the magnitude of the MPS response appeared to be lower in older individuals compared
414 with the young (Figure 3). Further, we believe there are a number of factors that may explain
415 the lack of agreement as to the presence or absence of muscle anabolic resistance in older
416 adults in response to orally ingested amino acid-based nutrition. Firstly, the dose of amino
417 acids/protein ingested varied considerably between studies. For example, whilst one of the
418 study arms provided just 2.5g of crystalline EAA's (27), equivalent to that contained in ~5g of
419 high-quality supplemental protein, a number of other study arms provided as much as 35-40g
420 of amino acids/protein (27, 59, 104, 105) and one study provided 90g of protein in the form of
421 340g of lean ground beef (91). The amount of protein provided is important to consider, as it
422 has been documented that there is a dose-dependent MPS response to protein provision that
423 ultimately plateaus at a given dose, beyond which additional protein is oxidized rather than
424 incorporated into muscle (70, 113). Recently, Moore and colleagues provided strong evidence
425 that the relative amount of protein required to maximally stimulate MPS is considerably
426 greater in older adults (~0.4g/kg) compared with the young (~0.24g/kg) (69). Put into context,
427 for an average 75-80kg older individual, this equates to ≥ 30 g of high-quality protein to
428 maximally stimulate MPS. In support of these data, others have demonstrated that the MPS
429 response to 20g of casein protein ingestion is ~16% lower in older vs. young individuals
430 (106). Based on these data, it could be expected that the study arms in this systematic review

431 that provided $\geq 0.4\text{g/kg}$ of high quality protein would fail to provide evidence of age-related
432 muscle anabolic resistance. To this end, we analysed study arms that provided either, i)
433 $\geq 0.4\text{g/kg}$ of amino acids/protein or ii) an amount of EAA's equivalent to that contained in a
434 dose of high-quality protein corresponding to $\geq 0.4\text{g/kg}$ (49), finding that 4 of 5 study arms
435 demonstrated insufficient evidence of age-related muscle anabolic resistance (59, 76, 91,
436 105). Taken together, these findings suggest the absence of an age-related deficit in the MPS
437 response when a sufficient (i.e. high) dose of high quality amino acids/protein is provided.

438

439 *Source of Amino Acids/Protein*

440 In addition to the amino acid/protein dose, inconsistent findings between studies in Model 2
441 might also be explained by the source of amino acids/protein administered. Specifically, the
442 digestion/absorption properties and leucine content of ingested protein are thought to play a
443 key role in the acute MPS response (77). Of the 17 study arms that provided amino
444 acids/protein orally and in liquid form, 10 study arms provided crystalline amino acids whilst
445 7 provided casein (Table 2). Crystalline free-form amino acids are more rapidly digested and
446 absorbed than amino acid constituents of protein-rich supplemental and whole-food sources
447 (27, 53). On the other hand, casein protein is predominantly acid insoluble and thus
448 coagulates within the acidic environment of the stomach, which increases gastric transit time,
449 resulting in a 'slow' digestion/absorption profile (12). The 'slow' digestion/absorption
450 kinetics of casein protein, coupled with the relatively low leucine content, results in inferior
451 acute postprandial MPS stimulation compared to an equivalent amount of rapidly digested,
452 leucine-rich whey protein in both young and older men at rest (11, 77, 92, 108). With this in
453 mind, it may be expected that the study arms utilising casein, particularly in low doses
454 (containing very little leucine) would be more likely to observe evidence of age-related

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

462

463 An important question that must also be posed is which of the amino acid/protein sources
464 provided in the study arms included in Model 2 most accurately reflect the habitual food
465 choices of free-living young and older individuals? As previously mentioned, 10 of the 21
466 study arms provided oral free amino acids, with 7 providing protein in the form of casein, 2
467 providing protein in the form of lean ground beef, and 2 providing free amino acids
468 intravenously. It is clear that intravenous and oral provision of free amino acids do not
469 accurately reflect the typical route or form in which amino acids/protein are consumed. Thus,
470 findings from these studies could be suggested to hold less significance than those which
471 provided protein in the form of casein (the main protein constituent of milk) and lean ground
472 beef, which are likely to be more reflective of the typical food sources consumed on a day-to-
473 day basis in free-living scenarios. However, the importance of utilising free amino acids
474 orally or intravenously to investigate age-related differences in skeletal muscle protein
475 metabolism should not be discounted. For example, intravenous provision of free amino acids
476 can be a valuable experimental approach to utilise when the research question is focused on
477 controlling for other potential confounding factors (e.g. differences in protein/amino acid

478 digestion and absorption between individuals), and thus this highlights the importance of
479 tailoring the study design towards the experimental hypothesis being investigated.

480

481 ***Leucine Content of Amino Acids/Protein***

482 Although the source of amino acids/protein appears to be of secondary importance to the
483 amount of protein, when explaining the apparent presence or absence of age-related
484 differences in postprandial MPS between studies, the leucine content of the administered
485 amino acid/protein source may offer further insight. The branched-chain amino acid leucine
486 appears to play a key role in the stimulation of MPS (3, 56). Leucine is unique in that it serves
487 not only as a substrate for the synthesis of new muscle proteins, but also as a potent molecular
488 anabolic signal which robustly stimulates MPS (26, 56). Interestingly, two of the included
489 study arms in this review provide strong evidence that the leucine content of a protein source
490 is an important determinant of postprandial MPS, particularly in older individuals. Katsanos
491 et al. (53) demonstrated that postprandial MPS was stimulated in young, but not older
492 individuals following the provision of 6.7g EAA's containing ~1.8g leucine (26% of the total
493 content, equivalent to that contained in ~15g whey protein). However, when the leucine
494 content was enriched to ~3g (41% of the total content, equivalent to that contained in ~25g of
495 whey protein), an equivalent stimulation of MPS was observed between young and older
496 individuals. Furthermore, others demonstrate a strong positive association between peak
497 plasma leucine concentrations and postprandial MPS in older individuals (77). In support of
498 these findings, of the 9 study arms included in Model 2 that reported the leucine content of the
499 amino acid/protein source administered, 6 provided no evidence of age-related muscle
500 anabolic resistance (44, 53, 76, 91). Interestingly, 4 of these study arms provided a leucine
501 dose of ~2g or more. In contrast, the 3 study arms that failed to provide evidence of age-

502 related muscle anabolic resistance all provided amino acid/protein sources containing a ‘sub-
503 optimal’ 1.4-1.7g dose of leucine (44, 53). Taken together, it appears that sources of amino
504 acids/protein that achieve a rapid, high amplitude peak aminoacidemia and leucinemia,
505 maximally stimulate postprandial MPS and thus should be recommended for older individuals
506 to alleviate muscle anabolic resistance.

507

508 ***Exercise and Amino Acid/Protein Provision***

509 The final model constructed (Figure 2, Model 3 and Table 3) included 10 study arms (2, 35,
510 36, 58, 78, 90) that measured the MPS response to the combined stimulus of exercise with
511 amino acid/protein provision. Acutely, combined resistance exercise and protein provision act
512 to synergistically enhance and maximize the stimulation of MPS above rates observed in
513 response to protein provision alone in young and older individuals (20, 78, 115). Chronically,
514 protein supplementation enhances resistance training-induced muscle hypertrophy and
515 strength increases in young and older individuals (22, 94, 112). With this in mind, it could be
516 expected that age-related differences in MPS would be less apparent in studies utilising the
517 combined anabolic stimulus of resistance exercise and amino acid/protein provision. In
518 accordance with this assumption, 7 of the 9 study arms that combined resistance exercise with
519 amino acid/protein provision found no evidence of age-related muscle anabolic resistance.
520 Although Drummond et al. (35) did observe age-related muscle anabolic resistance at 1-3 h
521 following resistance exercise and EAA ingestion, the aggregate MPS response over 1-6 h was
522 not different, suggesting that the MPS response to exercise and amino acid/protein provision
523 may be delayed (rather than attenuated) with advancing age. Precisely why Koopman et al.
524 observed age-related differences in MPS is unclear, but could relate to the exercise intensity
525 chosen, which may have been insufficient to overcome the blunted MPS response in the older

526 group, even in the presence of adequate protein provision (58). Specifically, the authors chose
527 to simulate activities of daily living in older individuals through implementation of resistance
528 exercise at low-to-moderate intensities (40-75% of 1RM). However, given that Durham et al.
529 (36) observed no age-related impairment in MPS following 45 minutes of treadmill walking
530 (at a relatively low exercise intensity) combined with amino acid infusion, the notion that
531 exercise intensity may explain the findings of Koopman et al. (58) requires further
532 clarification. Nonetheless, that 8 of the 10 study arms in Model 3 found no age-related
533 differences in MPS strongly suggests that the combination of exercise and amino acid/protein
534 provision is an effective strategy to restore ‘youthful’ muscle protein synthetic responsiveness
535 in older individuals.

536

537 *Differences in Experimental Methodology*

538 Differences in experimental methodology used to assess MPS between studies may explain
539 the inconsistent findings reported herein. For example, the tracer incorporation period over
540 which MPS was investigated (i.e. timing between sequential muscle biopsy samples) varied
541 widely from 0-2 h (24) to 0-6 h (58, 59, 78). The timing of muscle biopsy sampling is an
542 important consideration when capturing the peak MPS response to a given exercise and/or
543 nutritional stimulus (71). For example, it has been demonstrated that the MPS response to
544 bolus protein ingestion is relatively transient, peaking over ~3h post-ingestion in young and
545 older adults (1, 67), whereas the maximal MPS response to resistance exercise in the absence
546 of post-exercise amino acid/protein provision is thought to occur ~1-2 h after exercise
547 cessation in both young and older individuals (62). Interestingly, the suggestion that the MPS
548 response to combined resistance exercise and amino acid/protein provision may simply be
549 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 ~ 3 h 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

598 ingestion extends beyond the immediate hours of recovery in young individuals (19),
599 excessive physical activity or inactivity in the days prior to experimental trials may confound
600 the assessment of MPS. This is further supported by evidence in older individuals
601 demonstrating that the MPS response to EAA intake can be enhanced by prior low-intensity
602 aerobic exercise in the form of brisk walking (95). As such, it has been hypothesized that
603 physical inactivity may be at the root of muscle anabolic resistance and exacerbate the
604 progression of sarcopenia in the older population (17, 18, 68). With this in mind, it could be
605 speculated that muscle anabolic resistance would be more easily detected in studies involving
606 sedentary older, but not highly functioning, physically active older individuals. Although the
607 evidence to support this position is sparse, the single study arm in which habitual physical
608 activity was reported to be similar between the young and older groups demonstrated an
609 equivalent MPS response to amino acid administration (24). Accordingly, it is imperative that
610 future studies investigating MPS in young and older populations objectively assess habitual
611 physical activity levels.

612

613 ***Conclusions and Future Implications***

614 In this systematic review, 18 study arms provided sufficient evidence of age-related muscle
615 anabolic resistance, whereas 30 study arms did not. Whilst a quantitative appraisal of the
616 presence of age-related differences in the MPS response to anabolic stimuli (i.e. directly
617 contrasting absolute FSR values between young and older individuals) would have been
618 preferable, the variability in experimental methodology used to assess MPS (e.g. amino acid
619 stable isotope tracer, muscle protein sub-fraction, precursor pool and FSR incorporation
620 period) made this approach largely unviable. However, we believe that the variability in
621 experimental methodology is an important factor underlying the inconsistent findings as to the

622 presence or absence of an impaired muscle anabolic response in older age. Although beyond
623 the scope of this systematic review, it is important to acknowledge that MPS (on which we
624 have focussed) is an acute, dynamic assessment that represents only one side of the overall net
625 protein balance (NBAL) equation. Ultimately, overall NBAL dictates long-term skeletal
626 muscle remodelling which is the end-point in the diagnosis of sarcopenia and, as such, the
627 findings of this systematic review should be considered within this broader context. Although
628 our findings suggest that age-related muscle anabolic resistance is infrequently observed in
629 response to a robust muscle anabolic stimuli (i.e. a high-dose of protein and/or a high
630 volume/intensity of exercise), this phenomenon appears to be more frequently observed in
631 response to anabolic stimuli that could be considered as insufficient to maximally stimulate
632 MPS in older muscles, for example, in studies utilising relatively low intensity/volume
633 protocols or low dose protein/amino acid provision (sub-optimal leucine). However, we
634 cannot dismiss the fact that some study arms failed to observe age-related muscle anabolic
635 resistance in response to sub-optimal anabolic stimuli and that others observed age-related
636 muscle anabolic resistance following robust anabolic stimuli. We postulate that this
637 inconsistency between studies can largely be attributed to differences in study population (e.g.
638 habitual physical activity) and experimental methodology (e.g. tracer incorporation period) as
639 outlined in this discussion.

640

641 It has become increasingly evident that older individuals, especially those who are frail or
642 institutionalized, consume less protein than younger individuals (40), particularly at breakfast,
643 where the average protein intake is ~12g and comes largely from low-leucine, non-animal
644 based sources, such as bread and cereals (75, 93, 101). It is also clear that sedentary time
645 increases with advancing age (21, 47, 98) and non-sedentary behaviour is often of a relatively

646 low-intensity (e.g. gentle walking). Thus, the experimental conditions under which age-
647 related muscle anabolic resistance has often been reported (i.e. low-volume exercise and/or
648 low dose protein/amino acid provision) are highly representative of the lifestyle and dietary
649 habits of the average older individual. Accordingly, it is imperative that the mechanisms
650 underpinning age-related muscle anabolic resistance are elucidated, to aid the development of
651 targeted therapeutic strategies to slow the progression of sarcopenia.

652

653 Clinical recommendations for the prevention of sarcopenia are currently lacking. However, in
654 line with the current findings, recent position stands recommend that an average daily protein
655 intake of at least 1-1.2 g/kg body weight in conjunction with regular resistance and/or
656 endurance exercise is the most effective means of maintaining muscle mass/strength for older
657 individuals (8, 28). In agreement with the conclusions of this systematic review (i.e. that age-
658 related muscle anabolic resistance is most frequently observed in response to sub-optimal
659 amino acid/protein feeding), and other recent analyses (69, 106), these recommendations
660 specifically advise that older adults ingest rapidly digested, leucine-rich proteins in doses of
661 ~0.4g/kg body weight per meal, distributed evenly across the day (8, 28). Based on the current
662 findings, we recommend that future position stands should focus on defining optimal training
663 volume/intensity requirements to deliver the greatest benefit for musculoskeletal health in
664 older age.

665

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1081 **Figure Captions**

1082 **Figure 1:** Study identification process flowchart.

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1084 **Figure 2:** Diagrammatic illustration of the different models constructed for reporting
1085 evidence or no evidence of age-related muscle anabolic resistance.

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1087 **Figure 3:** Study arms in Model 2 comparing the magnitude of the MPS response to provision
1088 of a source of amino acids (AA)/protein in young vs. older adults (expressed as the % change
1089 from basal postabsorptive values). NB: the dose, protein source, leucine content, FSR
1090 incorporation period and route of administration differ between, but not within studies (see
1091 Table 2). FSR values for study arms were obtained directly from published manuscripts or,
1092 when not available, through requesting the information directly from the authors. In 5 of the
1093 21 study arms, precise FSR values were unavailable and therefore estimated visually from the
1094 manuscript figure. Three of the 21 study arms were excluded from the comparison as they
1095 failed to assess MPS in the basal, postabsorptive state.

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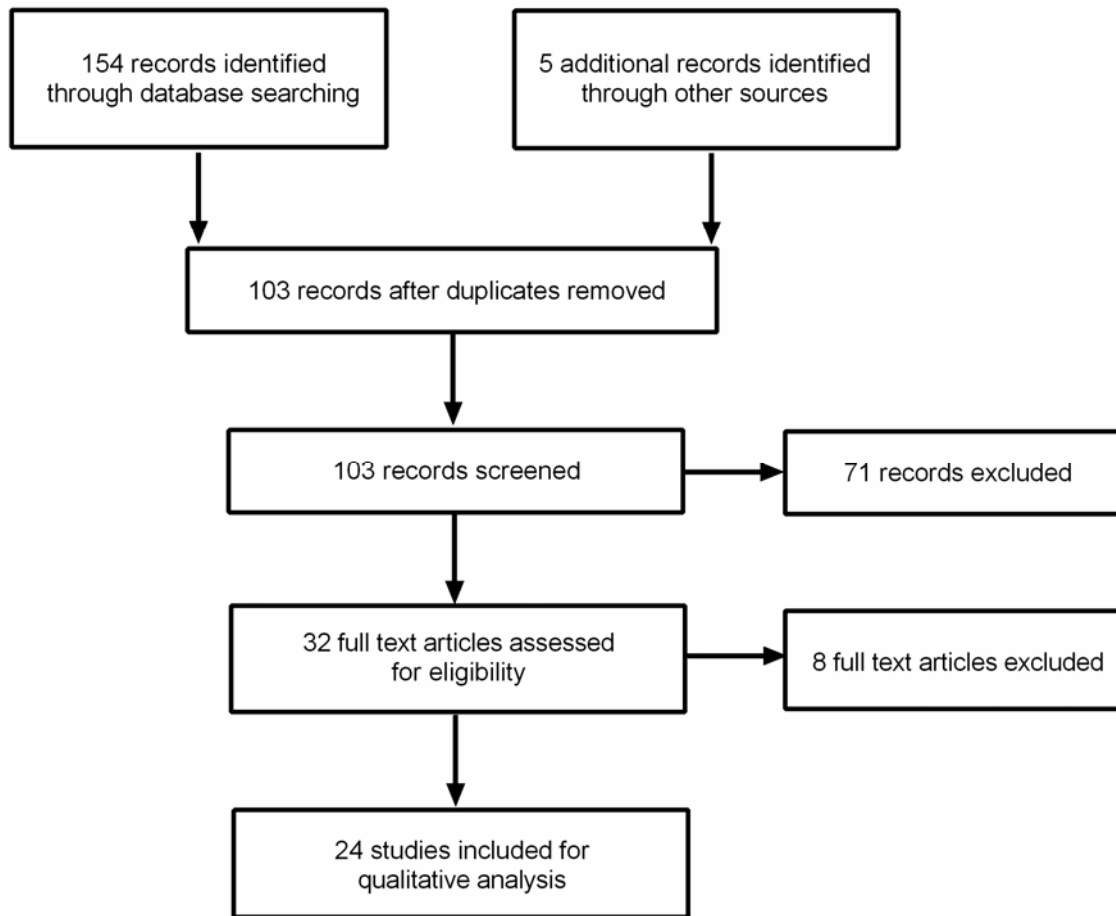
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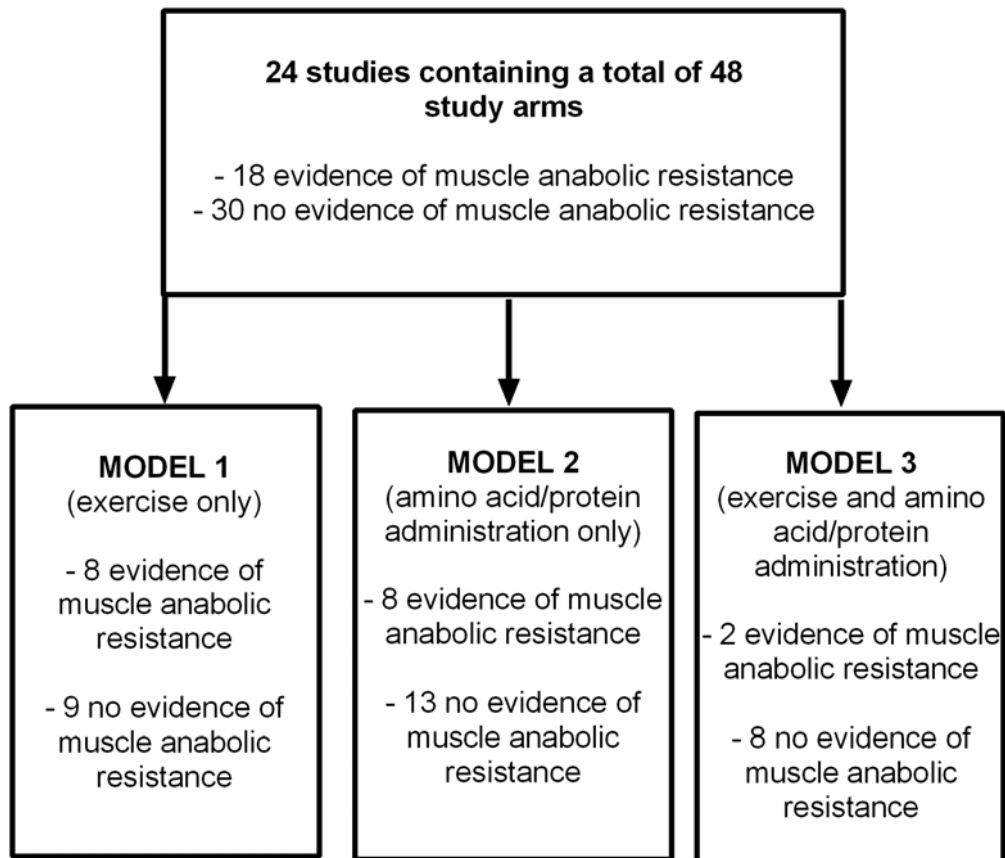
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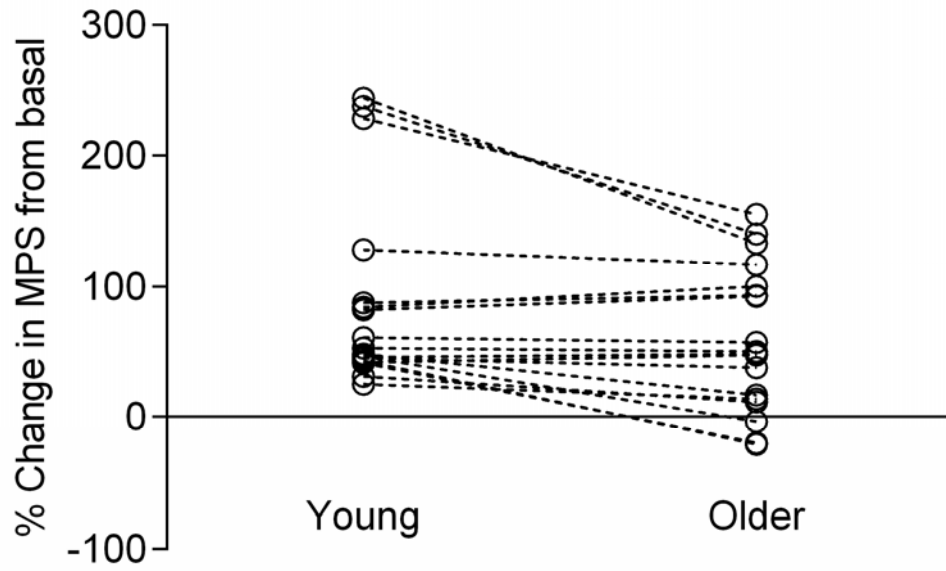
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1134 Figure 3

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	M/F	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.
	27 ± 2	n = 16			3-6h			Yes	
	Older	M/F	66.9 ± 3.0	22-24h	Yes				
Kumar et al. (2009) (62)	Young	M	-	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.
	24 ± 6	n = 25	1-2h		Yes				
	Older	M	-		2-4h			No	
Kumar et al. (2012) (61)	Young	M	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.
	24 ± 6	n = 12	2. 6 sets of KE at 40% 1RM	No					
	Older	M	72 ± 16	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% V _O ₂ peak	0-10min 0-1h 0-3h	Mixed	IC	No Yes 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.
Sheffield-Moore et al. (2005) (84)	Young 29 ± 2	M n = 6	78 ± 3	6x8 sets of KE at 80% 1RM	0-10min 0-1h 0-3h	Mixed	IC	No No Yes	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.

1135 Y = young; O = older; M = male; F = female; KE = knee extension; LP = leg press; 1RM = One repetition maximum; RM =
1136 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	

	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	-							

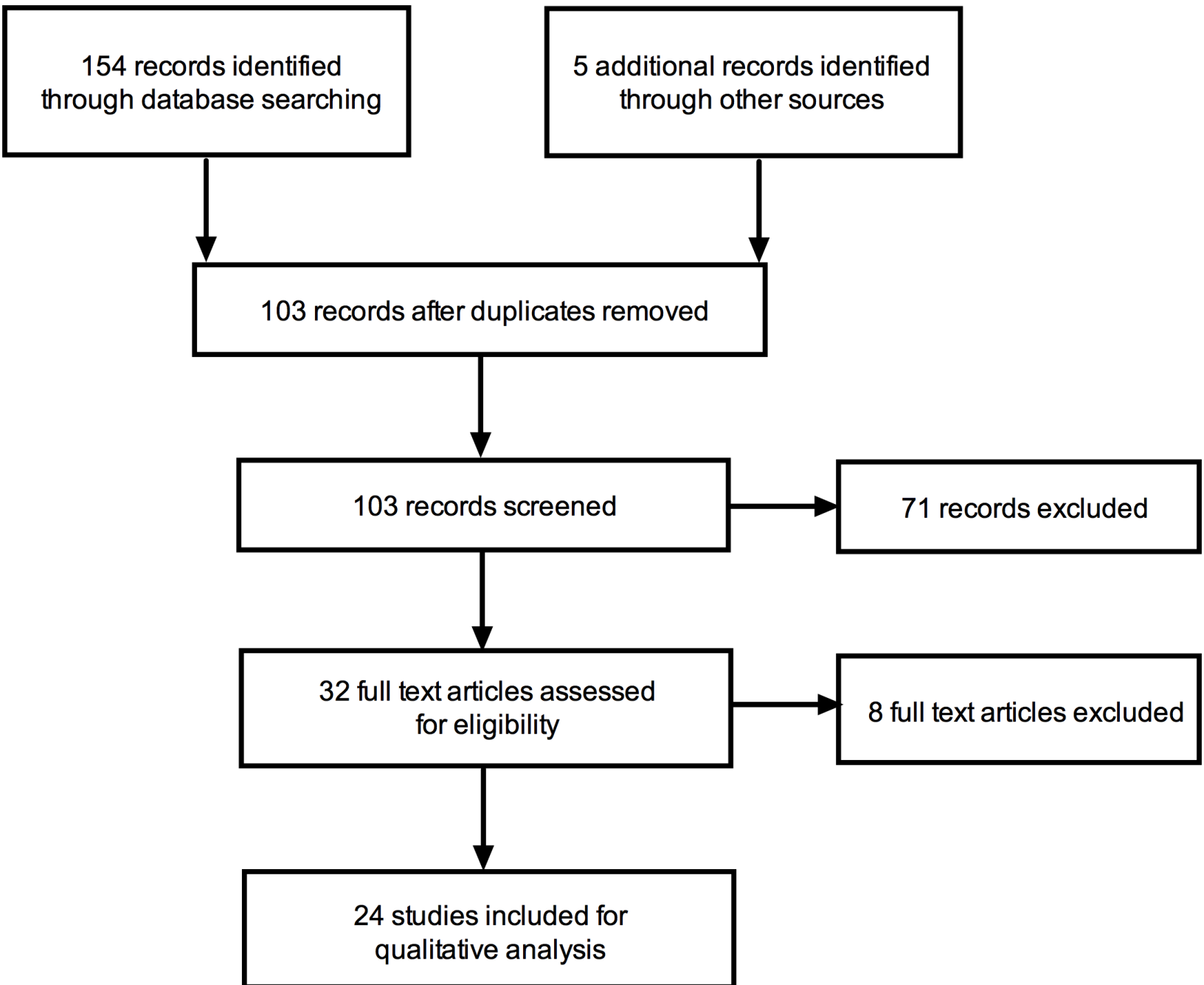
1137 Y = young; O = older; M = male; F = female; IV = intravenous; EAA = essential amino acids; MPS = muscle protein synthesis; AUC = area
1138 under curve; Myo = myofibrillar; IC = intracellular.

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 2. 6x8 sets of KE at 75% 1RM followed by 10g of protein (8g casein, 2g whey), 24g carbohydrate and 4.2g alanine	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					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	
	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% V _O 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	M	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.
	20 ± 1	n = 8							
Pennings et al. (2011) (78)	Young	M	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.
	21 ± 1	n = 12							
Symons et al. (2011) (90)	Young	M/F	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.
	29 ± 3	n = 7							

1139 Y = young; O = older; M = male; F = female; EAA = essential amino acids; KE = knee extension; LP = leg press; 1RM = One repetition maximum;
1140 MPS = muscle protein synthesis; AUC = area under curve; IC = intracellular.



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

