

1 Review

## 3 Protein considerations for optimising skeletal muscle 4 mass in healthy young and older adults.

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12 **Abstract:** Skeletal muscle is critical for human health. Protein feeding, alongside resistance exercise,  
13 is a potent stimulus for **muscle protein synthesis (MPS)** and is a key factor that regulates skeletal  
14 muscle mass (SMM). The main purpose of this narrative review was to evaluate the latest evidence  
15 for optimising the amino acid or protein source, dose, timing, pattern and macronutrient coingestion  
16 for increasing or preserving SMM in healthy young and healthy older adults. We used a systematic  
17 search strategy of PubMed and Web of Science to retrieve all articles related to this review objective.  
18 In summary, our findings support the notion that protein guidelines for increasing or preserving  
19 SMM are more complex than simply recommending a total daily amount of protein. Instead,  
20 multifactorial interactions between protein source, dose, timing, pattern and macronutrient  
21 coingestion, alongside exercise, influence the **stimulation of MPS**, and thus should be considered in  
22 the context of protein recommendations for regulating SMM. To conclude, on the basis of currently  
23 available scientific literature, protein recommendations for optimising SMM should be tailored to  
24 the population or context of interest, with consideration given to age and resting/post **resistance**  
25 exercise conditions.

26 **Keywords:** Muscle protein synthesis, muscle hypertrophy, amino acid availability, **protein source,**  
27 **protein dose, protein timing, protein pattern, macronutrient coingestion.**

### 28 1. Introduction

29 Skeletal muscle is crucial for metabolic health and sport performance. Beyond the positive  
30 relationship between skeletal muscle mass (SMM), strength and athletic performance, skeletal muscle  
31 also plays an important, and often underappreciated, role in reducing risk of diseases such as obesity,  
32 cardiovascular disease, insulin resistance, diabetes and osteoporosis [1]. Therefore, strategies to  
33 maintain or increase SMM are vitally important for both clinical and athletic populations.

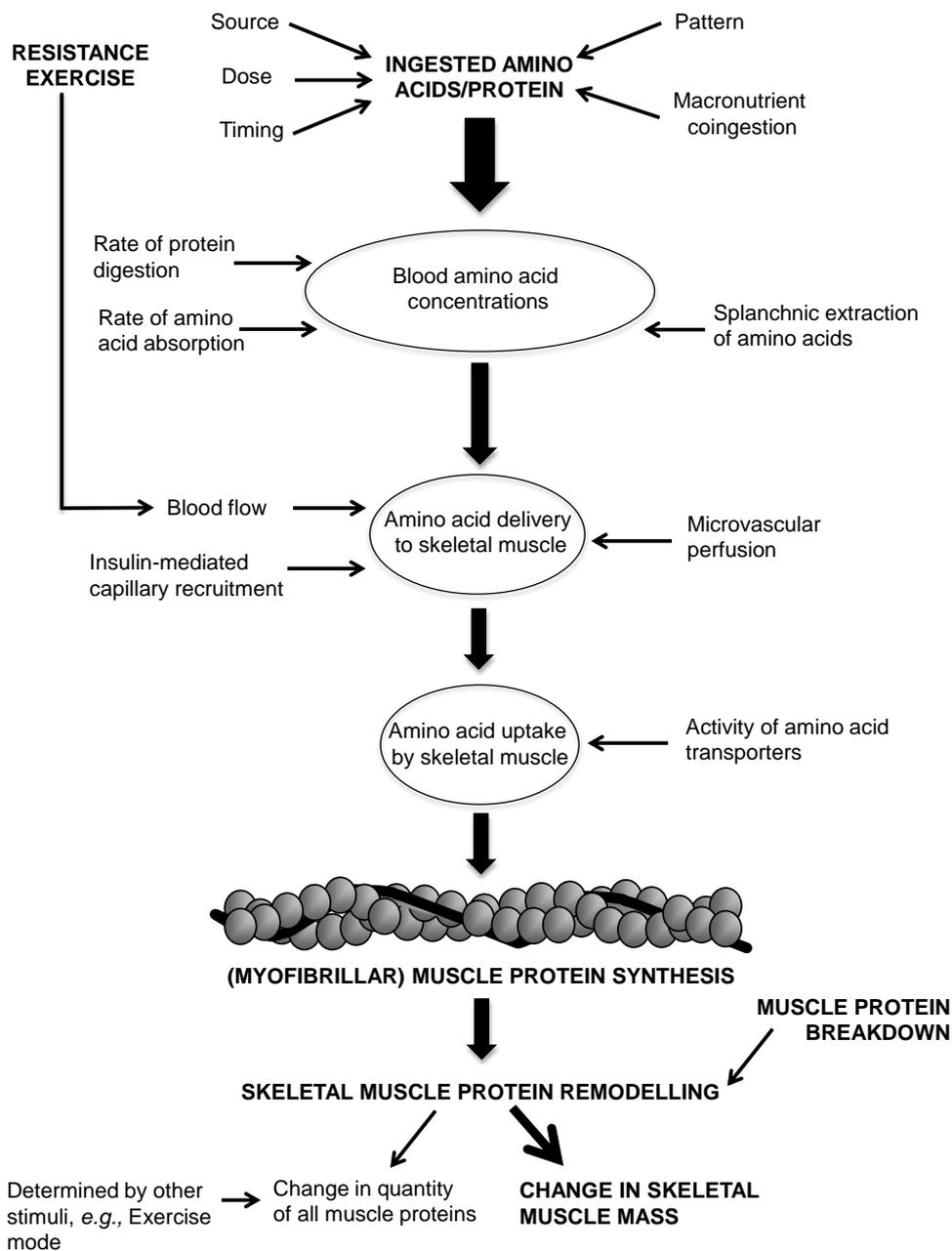
34 Skeletal muscle tissue displays remarkable plasticity. This plasticity allows for adaptation, including  
35 an increase in SMM. Skeletal muscle proteins are continuously being remodelled through the  
36 simultaneous processes of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). In  
37 turn, **skeletal muscle protein remodeling** is a prerequisite for increasing SMM [2]. Exercise and  
38 nutrition influence SMM through changes in MPS more than MPB [3]. Thus, MPS is accepted to be  
39 the dominant process of **muscle remodelling** responsible for regulating SMM in healthy adult  
40 humans. Whilst a high degree of **muscle remodelling** also is associated with other phenotypic  
41 adaptations, including the repair of old and/or damaged muscle proteins and modifications to the  
42 type and functionality of muscle proteins, the present review refers to skeletal muscle protein  
43 remodelling in the context of optimising muscle mass.

44 Protein or **amino acid** feeding stimulates MPS at rest [4] and during exercise recovery [5]. Thus, it  
45 follows that protein ingestion is a key stimulus for preserving SMM under resting conditions and  
46 increasing SMM under exercise training conditions. The stimulation of MPS is fundamentally  
47 regulated by extracellular and intracellular **amino acid** availability [6]. **Figure 1** depicts the role of  
48 **amino acid** availability in regulating MPS in response to **amino acid**/protein ingestion and exercise.  
49 **Amino acid** availability is modulated by several dietary factors, including the **amino acid**/protein  
50 source, amount ingested (as a single dose), timing, pattern and macronutrient coingestion. These  
51 factors independently and synergistically impact rates of protein digestion and **amino acid**  
52 absorption, splanchnic extraction of **amino acid**, microvascular perfusion (capillary recruitment and  
53 dilation), the delivery of **amino acid** to skeletal muscle and the uptake of **amino acid** by skeletal  
54 muscle, and thus regulate postprandial rates of MPS. In addition, exercise enhances the ability of  
55 skeletal muscle to respond to **amino acid** provision [7,8]. The most likely contributing mechanism is  
56 an exercise-induced increase in blood flow to the muscle [5] that increases the delivery of **amino acid**  
57 to the muscle, thus increasing the provision of substrate for MPS [9]. Crucially, the responsiveness of  
58 MPS to **amino acid** ingestion deteriorates with advancing age [10-12]. This phenomenon is referred  
59 to as ‘anabolic resistance’ and is thought to be mediated by impairments in each of the dietary factors  
60 **introduced** above.

61 To our knowledge, no previous authors have conducted a narrative review, using a systematic search  
62 strategy, to evaluate scientific evidence used to inform the latest protein recommendations for  
63 **optimising MPS** and SMM in healthy adult humans. Therefore, the primary objective of this review  
64 was to examine the impact of five key factors related to protein nutrition that regulate **MPS**, defined  
65 herein as follows:

- 66 i. **Amino acid**/protein *source* refers to the origin source of ingested protein, *e.g.*, isolated intact whey,  
67 casein or soy; animal or plant. **Amino acid**/protein *form* refers to the matrix form of ingested  
68 protein, *e.g.*, liquid or solid.
- 69 ii. **Amino acid**/protein *dose* refers to the quantity of **Amino acid**/protein contained in a single  
70 serving.
- 71 iii. **Amino acid**/protein *timing* refers to the timed intake of **Amino acid**/protein in relation to exercise  
72 (before and after) or to ingestion of other nutrients.
- 73 iv. **Amino acid**/protein *pattern* refers to the distribution pattern of ingested **Amino acid**/protein over  
74 a given period of time, accounting for the dose, timing and frequency of **Amino acid**/protein  
75 ingestion.
- 76 v. *Macronutrient co-ingestion* refers to the concurrent ingestion of carbohydrate (CHO) and/or fat  
77 alongside an **Amino acid**/protein source.

78 For clarity, this review has been structured to address each factor of protein nutrition independently.  
 79 However, an important point of discussion concerns the interaction of these factors for **modulating**  
 80 **MPS** in healthy young and older adults. An understanding of recommended protein nutrition  
 81 practice for **optimising** MPS and SMM could lead to the provision of improved advice to aid the  
 82 muscle health of young and older adults.



**Figure 1:** Simplified diagram detailing role of amino acid availability in regulating muscle protein synthesis with protein ingestion and exercise. Whilst resistance exercise preferentially stimulates the synthesis of contractile myofibrillar proteins (e.g. actin, myosin, troponin), resistance exercise also stimulates the synthesis of non-contractile proteins (e.g. mitochondrial and sarcoplasmic) in skeletal muscle.

## 84 2. Methods

85 A systematic search strategy was employed to identify citations for this narrative review. We  
86 searched the National Library of Medicine database (PubMed) and Web of Science from their  
87 inception through to December 2015. The terms “muscle anabolism” OR “muscle protein synthesis”  
88 OR “muscle hypertrophy” OR “skeletal muscle protein remodelling” AND “protein feeding” OR  
89 “protein ingestion” OR “protein supplementation” OR “AA ingestion” AND “humans” were entered  
90 into both databases and filters including “articles” and “humans” were used to refine the search.  
91 After initial screening of title and abstracts, selected papers were examined, including the reference  
92 lists of the retrieved articles.

93 Studied participants met the eligibility criteria if classified as healthy with no medical  
94 contraindications. Participants were young (mean age of studied cohort  $\leq 35$  y) and older (mean age  
95 of studied cohort  $\geq 65$  y) adult men and women, resistance-trained ( $\geq 2$  exercise sessions/wk) or  
96 untrained volunteers, who were studied under resting or post resistance exercise conditions in the  
97 fed or fasted state. Several exclusion criteria were applied. We excluded intervention studies where  
98 the control condition was not considered appropriate to answer the question. For example, in the  
99 context of macronutrient coingestion, several studies included an iso-energetic CHO only [13] or a  
100 non-energetic placebo [14] rather than an amino acid/protein- only control condition. Also excluded  
101 were case studies and descriptive studies whereby no control group was used. Studies were excluded  
102 if they had a specific purpose of weight loss, if the method of protein intake was not oral (e.g.,  
103 nasogastric/enteral intake of protein or the infusion of amino acids) and the exercise mode was not  
104 resistance-based. Finally, we excluded studies where participants were classified as patient groups  
105 (i.e., not healthy, including overweight) and any non-human studies. Screening of studies resulted in  
106 the assessment of 64 citations for this narrative review. Of these, 24 citations were focused on amino  
107 acid /protein source, 8 dose, 11 timing, 6 pattern and 15 macronutrient coingestion.

## 108 3. Synthesis of findings

### 109 3.1 Amino acid/protein source

110 Amino acid composition and digestive properties can vary between different protein comparisons of  
111 different isolated types of intact proteins, protein blends vs. isolated intact proteins and different  
112 forms of the same protein source. The Digestible Indispensable Amino Acid Score (DIAAS) is the  
113 latest and preferred index for differentiating between protein sources. The DIAAS score reflects the  
114 essential amino acids (EAA) content protein and digestion properties of any given protein source.

#### 115 3.1.1 Isolated types of intact protein

116 The most common comparison of intact proteins is between rapidly digested whey protein that is  
117 high in leucine content (~12.5% of total protein) and slowly digested casein protein that exhibits a  
118 relatively lower (~8.5% of total protein) leucine content. Studies in young [15] and older [16,17] adults  
119 have consistently demonstrated a greater resting postprandial stimulation of mixed-MPS with  
120 ingestion of whey compared with casein protein. However, studies that compared the response of  
121 MPS or net muscle protein balance (NBAL; difference between MPS and MPB and thus indicative of  
122 the aggregate muscle protein anabolic response) to the post-exercise ingestion of whey and casein  
123 protein report equivocal results in both young [15,18,19] and older [16,20] adults. In young adults,  
124 studies report both a greater post-exercise response of mixed-MPS to ingestion of whey protein  
125 compared with micellar casein protein [15] and also no differences in the post-exercise response of  
126 NBAL (measured over 5 h) [19] and myofibrillar-MPS (measured over a 6 h period) [18] between  
127 whey and casein conditions. Additionally, a recent study in young adults reported no difference in  
128 the chronic resistance training-induced increase in lean body mass (LBM) between whey and casein  
129 protein conditions [21]. Similarly, studies in older adults have reported both a greater post-exercise

130 stimulation of myofibrillar-MPS (measured over a 4 h period) following ingestion of whey protein  
131 isolate compared to micellar casein [16] and also no difference in the post-exercise response of mixed-  
132 MPS (measured over a 6 h period) [20] between whey and casein protein conditions. No longitudinal  
133 endpoint study in older adults has compared intact whey and casein protein sources on any outcome  
134 measure of SMM.

135 The discrepant findings between studies that fed whey and casein protein after exercise, at least in  
136 terms of acute measurements of MPS and NBAL, may be reconciled by general differences in study  
137 design. These differences include the form of intact protein ingested post-exercise (whey hydrolysate,  
138 whey isolate, micellar casein or calcium caseinate), the chosen endpoint measurement of muscle  
139 anabolism (e.g. mixed-MPS, myofibrillar-MPS or NBAL) and/or the time period over which MPS or  
140 NBAL was measured after protein ingestion. Micellar casein is insoluble and therefore is often treated  
141 with alkaline compounds such as calcium hydroxide to produce calcium caseinate. This treatment  
142 alters the digestion kinetics of casein, such that the rate of blood amino acid appearance with  
143 caseinate ingestion more closely mimics whey protein compared with micellar casein protein.  
144 Interestingly, acute studies that reported a differential post-exercise response of MPS between whey  
145 and casein protein ingestion administered micellar casein [15,16]. Conversely, those studies that  
146 reported a similar post-exercise response of MPS or NBAL between whey and casein protein  
147 conditions administered calcium caseinate protein [24-26]. Taken together, these data suggest that  
148 ingesting the more rapidly absorbed caseinate elicits a greater anabolic stimulus compared with  
149 ingesting micellar casein. This insight expands other reviews [22] and the common perception that  
150 whey protein, due to amino acid composition (high EAA, BCAA and leucine content) and rapid  
151 digestibility properties, is the highest-quality intact protein source popularised in protein  
152 supplements. In summary, these data consistently demonstrate that ingestion of whey protein  
153 stimulates a greater resting postprandial response of MPS compared to casein protein in young and  
154 older adults. Similarly, a direct comparison between “fast” whey protein and “slow” micellar casein  
155 protein reveals a superior post-exercise response of MPS to whey protein ingestion in young and  
156 older adults.

157 Variation in the time periods over which MPS or NBAL was measured also may explain the  
158 discrepant findings. An interesting observation is that studies reporting a greater response of MPS to  
159 whey compared with casein protein conducted measurements of MPS over a 4 h period or less after  
160 protein ingestion [15,16], whereas studies reporting no differences between whey and casein  
161 conditions obtained measurements of MPS or NBAL over 5 h or more [18,19]. It is conceivable that  
162 “rapidly” digested whey protein stimulates a greater response of MPS in the early postprandial  
163 period ( $\leq 4$  h), however this advantageous ‘muscle protein anabolic response’ is cancelled out in the  
164 late ( $\geq 4$  h) postprandial period by the more “slowly” digested casein. Whereas this notion is  
165 supported by currently available data, more studies are necessary to substantiate this speculation.  
166 Moreover, given the disparate digestive properties and subsequent differences in pattern of blood  
167 amino acid appearance between whey and micellar casein protein, physiological rationale underpins  
168 the notion that casein should be ingested pre-exercise, whereas whey protein should be ingested post-  
169 exercise. However, despite promising rationale [23] surprisingly no study has directly compared the  
170 post-exercise response of MPS to ingestion of casein protein before exercise vs. whey protein after  
171 exercise. Future confirmatory work in young and older adults is necessary to strengthen the quality  
172 of this evidence.

173 Three other direct comparisons of isolated types of intact protein have been studied in young adults:  
174 whey vs. soy protein which is relatively low in leucine (~7.5% of total protein) content, whey vs. rice  
175 protein which is slowly digested and relatively low in leucine (~8% of total protein) and casein vs.  
176 soy protein. A similar resting postprandial response of mixed-MPS to ingestion of whey and soy  
177 protein has been reported [15]. However, acute metabolic data that demonstrate a greater post-  
178 exercise response of mixed-MPS with whey compared with soy protein ingestion [15] are consistent  
179 with a tightly controlled longitudinal endpoint study of ~20 participants [24] that measured greater

180 gains in (LBM) during a 9-month resistance training period with whey compared to soy protein  
181 supplementation. A smaller-scale (n=12 per condition) well-controlled (administration of meal plans)  
182 study that compared whey and rice protein isolate supplementation observed similar gains in LBM  
183 between conditions during an 8-wk training period [25]. Finally, greater rested and post-exercise  
184 responses of MPS were reported with soy compared with casein protein ingestion [15]. In summary,  
185 given the sparse body of evidence for each comparison (1 or 2 studies), there remains ample scope  
186 for future work that compares the response of MPS and SMM to ingestion of various isolated types  
187 of intact protein, both from animal (*e.g.*, egg, fish, *etc.*) and plant (*e.g.*, lentil, quinoa, maize, hemp,  
188 *etc.*) sources in young and older adults [26].

### 189 3.1.2. Protein blends

190 A protein blend combines two or more intact proteins. The scientific rationale for ingesting a protein  
191 blend is that combining more than one type of protein will capitalise on the unique digestive  
192 properties of each type of protein, allowing for an optimal blood availability of amino acid to increase  
193 the amplitude and duration of MPS stimulation. The efficacy of a protein blend for the stimulation of  
194 MPS was first evaluated by two studies in young adults that compared the ingestion of skimmed  
195 milk (casein + whey protein) with isolated soy protein [27,28]. The finding of a greater acute post-  
196 exercise response of mixed-MPS and NBAL with milk compared to soy protein ingestion [27] was  
197 extended by a longitudinal study that measured a greater increase in LBM after 12 wk of resistance  
198 training in the milk compared to soy protein condition [28]. However, a recent study demonstrated  
199 milk ingestion elicits a similar post-exercise response of MPS compared with beef ingestion in young  
200 adults [29]. Two other studies compared the post-exercise response of MPS to ingestion of a protein  
201 blend (soy + casein + whey protein) with an isolated whey protein control in young adult men [30,31].  
202 The protein blend composition was 25% whey protein, 50% casein and 25% soy protein. Conditions  
203 were matched for total EAA (~8.8 g) and leucine (~1.9 g) content, however, the blend condition  
204 comprised a greater total protein content compared with the whey protein condition (~19.3 *vs.* ~17.7  
205 g). As anticipated, in both studies [30,31] the amplitude of rise in amino acid concentrations during  
206 the early postprandial period was greater in the whey protein compared with protein blend  
207 condition. However, with the exception of valine, and to a lesser extent phenylalanine, ingestion of  
208 the protein blend failed to sustain elevated plasma amino acid (leucine, isoleucine, total BCAA)  
209 concentrations during the late (2-4 h) postprandial period compared with whey protein ingestion.  
210 Since the casein source included in the blend was sodium caseinate, which exhibits similar transient  
211 amino acid kinetics to whey protein [17,18], it was not surprising that no difference in the duration  
212 of increased amino acid availability was observed between protein blend and whey protein  
213 conditions. In both studies, the response of mixed [30] and myofibrillar [31] MPS followed the same  
214 pattern. At 0-2 and 0-4 h post protein ingestion, a similar increase in the response of MPS above basal  
215 values was observed between conditions. These data suggest that whey protein ingestion is similarly  
216 effective compared to a dose-matched (for leucine content) protein blend for the stimulation of MPS.  
217 Interestingly, despite a similar amino acid profile during late recovery, over the 2-4 h postprandial  
218 period, the response of MPS was increased above basal rates in the protein blend condition only.  
219 Although these data imply that the duration of MPS stimulation may be extended with a protein  
220 blend compared with an isolated type of intact whey protein, this observation also may be an artifact  
221 of the additional total protein content of the blend condition compared with the whey protein control.  
222 Moreover, the physiological significance of stimulating a greater response of MPS during the late (2-  
223 4 h) acute recovery period, without augmenting the aggregate (0-4 h) acute response of MPS, is not  
224 obviously apparent. Future work also is warranted to evaluate the response of MPS and SMM to  
225 other protein blend combinations, including egg, rice and hemp protein. The implications of these  
226 data are of particular relevance to the protein industry that is interested in producing cheaper and  
227 more sustainable protein-based products.

228 An important line of research worthy of future investigation is comparing the response of MPS to  
229 animal and plant-derived protein sources, or blends of plant-derived proteins [26]. In particular,

230 combinations of plant-derived protein sources with divergent amino acid profiles that when  
231 combined allow for a 'complete' EAA profile (e.g., relative to animal-derived proteins, wheat is low  
232 in lysine yet high in methionine, whereas lentil is high in lysine, yet low in methionine). A recent  
233 study reported a similar increase in SMM with the post-exercise ingestion of pea protein compared  
234 with whey protein [32]. However, the limited information available in humans implies that animal-  
235 derived protein sources stimulate a greater response of MPS compared with plant-derived protein  
236 sources [15,28]. However, the overall completeness, applicability and quality of evidence are weak.  
237 To date, a limited number of controlled laboratory studies in humans has directly compared the acute  
238 response of MPS to ingesting an animal-derived compared to a plant-derived protein source. No  
239 acute metabolic studies in humans have compared other animal-derived protein-rich foods, such as  
240 eggs, yoghurts, meat and fish with other plant-derived protein-rich foods, such as lentil, maize, pea,  
241 rice and wheat. The implications of these data are particularly relevant to the protein industry for  
242 aiding the production of more economically and environmentally sustainable protein-based products  
243 [33].

### 244 3.1.3 Manipulating amino acid composition

245 Several studies have investigated the impact of manipulating the composition of an amino  
246 acid/protein source for stimulating an increased response of MPS to amino acid/protein ingestion [34-  
247 37]. In terms of amino acid profile, the leucine content of a protein source is of particular importance  
248 for stimulating a postprandial response of MPS. Leucine not only provides substrate for the synthesis  
249 of new muscle protein, but also serves as a key anabolic signal for skeletal muscle by activating  
250 enzymes within the mammalian target of rapamycin (mTOR) signalling pathway [38]. Indeed, the  
251 leucine threshold hypothesis [39] has been proposed to explain the observation that young muscle  
252 appears relatively sensitive to the anabolic action of small (~1 g) quantities of ingested leucine,  
253 whereas older muscle requires  $\geq 2$  g of leucine (typically contained in ~20 g of high-quality protein)  
254 to increase MPS above resting rates [40]. Accordingly, studies have manipulated amino acid  
255 composition in two ways: by adding leucine to an amino acid source or modifying the leucine profile  
256 of an AA source. In addition, longitudinal studies have investigated the impact of chronic leucine  
257 supplementation on long-term changes in SMM.

258 Based on available evidence, the efficacy of adding leucine to an amino acid source or modifying the  
259 leucine profile of an amino acid source for increasing the stimulation of MPS depends on the  
260 interaction of two factors. These factors include the leucine content of the original amino acid source  
261 and whether the amino acid source was ingested at rest or after exercise. Two studies in older adults  
262 demonstrated the addition of leucine (3.5/2.5 g) to a casein protein (30/20 g) source increased the  
263 resting postprandial stimulation of mixed-MPS [39,41]. Conversely, studies in young [42] and older  
264 [43] adults reported a similar post-exercise response of mixed-MPS to coingesting leucine (3.4 g) with  
265 a whey protein (16.6 g) plus CHO mixture compared to whey protein alone. With regards to  
266 modifying leucine profile, studies in young [34] and older [44,45] adults matched the dose of ingested  
267 EAA (6.7/10/10 g) between conditions, but manipulated the leucine content (2.8/3.5/3.5 g) of the  
268 ingested EAA source. Study outcomes were dependent on the dose of ingested EAA. Leucine-  
269 enriched EAA ingestion increased the resting postprandial [34] and post-exercise [44] response of  
270 MPS to a suboptimal (for maximal stimulation of MPS— see Amino Acid/Protein dose) dose of EAA,  
271 but not to an optimal (for maximal stimulation of MPS) dose of EAA in young [34,45] and [44] older  
272 adults. In summary, on the basis of available evidence, leucine coingestion and leucine enrichment  
273 effectively stimulates an increased resting postprandial response of MPS to an amino acid source,  
274 such as casein protein, that contains a relatively low leucine content (vs. whey). In contrast, adding  
275 leucine to an amino acid source such as whey protein that already contains sufficient leucine to  
276 stimulate a pronounced rise in blood leucine concentration, and thus surpass the leucine threshold  
277 for stimulation of MPS, is surplus to increasing post-exercise rates of MPS.

278 Other studies have manipulated the leucine content of a protein source. A recent study in young  
279 adults measured the resting postprandial and post-exercise response of myofibrillar-MPS to ingestion  
280 of 25 g whey protein (optimal dose) compared to 6.25 g of whey protein (suboptimal dose) in young  
281 adults [46]. Whereas the protein dose was not matched between conditions, leucine intake was  
282 equated by adding 2.25 g of leucine (to match the leucine content of the 25 g whey protein dose) to  
283 the lower protein dose, thus introducing a leucine-enriched suboptimal dose of whey protein. The  
284 impact of leucine-enriching a lower dose of whey protein on the stimulation of MPS differed between  
285 resting and post-exercise conditions. In rested muscle, ingestion of a leucine-enriched 6.25 g dose of  
286 whey protein resulted in rates of MPS similar to those stimulated with ingestion of a 25 g dose of  
287 whey protein. Likewise, ingestion of an EAA-enriched (with the exception of leucine) suboptimal  
288 dose of whey protein stimulated a similar MPS response compared with the ingestion of 25 g whey  
289 protein. However, notwithstanding the equivalent amount of leucine ingested, an inferior post-  
290 exercise response of MPS was observed with ingestion of 6.25 g of leucine-enriched whey protein  
291 compared to 25 g of whey protein. This differential response between rested and exercised states may  
292 be reconciled by the enhanced ability of muscle to utilise ingested amino acid for the stimulation of  
293 MPS following exercise [47]. Hence, it may be speculated that in this study [46], EAA availability was  
294 rate limiting for potentiating the post-exercise response of MPS to a suboptimal dose of whey protein.  
295 These results support the notion that, rather than blood leucine availability *per se*, the availability of  
296 a full complement of EAA is the critical factor for stimulating a maximal response of MPS during  
297 exercise recovery.

298 A follow-up study in young adults by the same authors [35] demonstrated a greater post-exercise  
299 response of MPS to ingestion of 25 g of whey protein compared with ingestion of a low dose (6.25 g)  
300 of whey protein plus additional leucine (totally 3 g of leucine) when ingested as part of a mixed  
301 macronutrient beverage. However, ingestion of a higher dose of leucine added to 6.25 g of whey  
302 protein (totalling 5 g of leucine) resulted in a similar post-exercise response of myofibrillar-MPS to  
303 ingestion of 25 g of whey protein. Collectively, these data [38,47] suggest that enriching a suboptimal  
304 dose of whey protein with leucine may potentiate the post-exercise response of MPS to a suboptimal  
305 protein dose, but only when the suboptimal protein dose is consumed alongside other  
306 macronutrients and is leucine-enriched above a certain undetermined threshold.

307 Based on the rationale that older adults often experience low levels of appetite [48] and routinely  
308 consume suboptimal doses of protein, a similar study [49] has recently been conducted in older  
309 adults. The ingestion of a leucine-enriched (1.2 g) suboptimal dose of EAA (3 g) stimulated a similar  
310 resting postprandial and post-exercise response of myofibrillar-MPS compared to a 20 g whey protein  
311 bolus containing 9.6 g of EAA and 2 g of leucine. These data suggest that a less satiating (low energy)  
312 leucine-enriched suboptimal dose of EAA stimulates a similar resting and post-exercise response of  
313 myofibrillar-MPS compared with ingestion of a larger bolus dose of whey protein in older adults.  
314 Hence, fortifying a suboptimal quantity of protein with leucine may be a viable strategy for  
315 promoting MPS and increasing SMM in older adults. Given that the optimal dose of whey protein to  
316 stimulate a maximal post-exercise response of MPS has been shown to exceed 20 g in older adults  
317 (see Amino acid/Protein dose), it remains unknown if a leucine enriched protein source rescues a  
318 maximal response of MPS in older adults. Future studies should be designed to provide a similar  
319 comparison between a leucine-enriched suboptimal protein dose (*i.e.* 20 g of whey protein) and an  
320 optimal protein dose (~40 g of whey protein) in older adults during exercise recovery.

321 Finally, two studies in older adults have evaluated the impact of chronic leucine supplementation on  
322 outcome measures of SMM and reported equivocal findings [50,51]. Whereas 2 wk of leucine  
323 supplementation increased the resting postabsorptive and postprandial response of MPS to a  
324 suboptimal dose of EAA plus CHO in one study [50], Verhoeven et al. [51] reported no change in  
325 LBM after 12 wk of leucine supplementation. Based on these contrasting findings, the efficacy of a  
326 prolonged period of leucine supplementation on outcome measures of SMM remains unclear in older  
327 adults and warrants investigation in young adults.

## 328 3.1.4 Protein form

329 Three studies in older adults have manipulated the form of an amino acid/protein source and  
330 measured resting postprandial rates of MPS [17,52-54]. Koopman et al. [52] compared liquid  
331 supplements of intact casein and casein hydrolysate and reported a greater blood amino acid  
332 availability, and a trend for a greater response of MPS, to ingestion of casein hydrolysate. The same  
333 research group recently reported that ingestion of casein in its naturally occurring milk matrix form  
334 resulted in a reduced blood amino acid availability (possibly due to delayed amino acid  
335 digestion/absorption kinetics), but did not modulate postprandial rates of MPS compared with  
336 ingestion of isolated intact micellar casein [53]. A similar result was reported by Pennings et al. [54]  
337 whereby the ingestion of minced beef, that is easily masticated and digested, stimulated a more rapid  
338 increase in arterialised blood EAA availability compared with an equivalent amount of intact steak,  
339 however no difference in the 6 h postprandial response of MPS was observed between conditions.  
340 These findings [17,53] suggest that, at least in the early resting postprandial period, the rate of blood  
341 amino acid availability does not translate into an increased stimulation of MPS. However, it must be  
342 recognised that these findings are in the context of a single feeding period under resting conditions.  
343 Whether a more rapid blood amino acid availability stimulates a greater response of MPS in the  
344 context of repeated feeding and/or during exercise recovery deserves consideration.

## 345 3.2 Amino acid/protein dose

346 Several acute metabolic dose-response studies have been designed to characterise the optimum dose  
347 of amino acid/protein contained in a single serving for the maximal stimulation of MPS [10,47,55-58].  
348 These studies examined a range of protein sources, including free crystalline amino acid, intact  
349 proteins and complete foods in young and older adults at rest and during exercise recovery.

## 350 3.2.1 Young adults

351 The optimal dose of ingested amino acid/protein for stimulating a maximal resting postprandial  
352 response of MPS is well established in young adults. In the context of a realistic meal-like setting,  
353 ingesting a standard portion of lean beef (containing ~30 g protein) was shown to stimulate a similar  
354 response of MPS compared with an over-sized portion of lean beef (containing ~90 g protein) [59].  
355 Although a study design that compares only two conditions does not allow for a true dose-response  
356 relationship to be characterised, these data suggest a saturable protein dose exists regarding the  
357 feeding-induced stimulation of MPS. Consistent with the notion of a saturable dose of protein, we  
358 [47] and others [10] observed a plateau in the resting postprandial response of MPS to ingesting 10 g  
359 of EAA ( $2.5 < 5 < 10 = 20$  g) [10] or 20 g of intact whey protein ( $10 < 20 = 40$  g) [47]. The ingestion of 20  
360 g EAA [10] or 40 g intact protein [47] failed to elicit an additional resting postprandial stimulation of  
361 MPS. Instead, we [47] reported a pronounced stimulation of irreversible amino acid oxidation and  
362 ureagenesis, implicating a shift toward fates of ingested amino acid other than MPS. Taken together,  
363 these data [10,47] often are interpreted to suggest that, when expressed as an absolute intake, 10 g of  
364 EAA (equivalent to ~20 g of protein) is the optimal dose for stimulating a maximal response of MPS  
365 in young adults at rest. Expanding these data, a retrospective analysis of previous studies revealed  
366 that, expressed relative to body mass, the optimal protein dose for maximal stimulation of MPS in  
367 young adults at rest is 0.24 g/kg body mass/serving [60].

368 In young adults, the optimum dose of protein to ingest during exercise recovery is less well defined.  
369 We [47] and others [61] reported no statistical difference in the post-exercise response of MPS to  
370 ingestion of 20 compared to 40 g of protein. However, it was intriguing that both studies [47,61]  
371 reported an ~10% increase in mean values for the post-exercise stimulation of MPS when the protein  
372 dose was increased from 20 to 40 g. Given that increasing the dose of ingested protein from 10 to 20  
373 g stimulated a ~20% greater post-exercise response of MPS without a marked increase in amino acid  
374 oxidation of urea production, a diminishing return in terms of stimulating MPS, at the very least, was

375 achieved with ingestion of >20 g of protein [47,61]. The physiological relevance, in terms of long-term  
376 changes in SMM, of a 10% increase in the response of MPS during exercise recovery is unknown and  
377 warrants further investigation.

### 378 3.2.2 Older adults

379 In older adults, the optimal dose of ingested protein at rest and during exercise recovery **is not well**  
380 **established**. Consistent with young adults, Symons et al. [59] reported a similar resting postprandial  
381 response of MPS to ingesting 113 g (~30 g protein) compared with 340 g (~90 g protein) of lean beef.  
382 **Moreover, the seminal EAA dose-MPS response study by Cuthbertson and colleagues [10] reported**  
383 **a similar resting stimulation of myofibrillar-MPS with the ingestion of 20 (≈40 g protein) or 40 g (≈80**  
384 **g protein) of EAA in older adults**. Hence, in the context of stimulating a postprandial response of  
385 MPS, a saturable dose of ingested protein also exists in older adults. However, several recent dose-  
386 response studies of intact protein sources [55,57,58] and protein-rich foods [56] in middle-aged (~60  
387 y) [56] and older adults [55,57,58] failed to observe a saturated response of MPS to graded protein  
388 intakes. These studies reported a dose-dependent, graded increase in the response of MPS to  
389 increasing doses (0-40 g) of intact whey protein [55,58], soy protein [57] and minced beef [56]. Since  
390 no previous study has observed a plateau in the response of MPS to increasing doses of ingested  
391 protein [55-58], the optimal single bolus dose of ingested protein for stimulating a maximal response  
392 of MPS in older adults cannot be firmly established.

393 Despite being inconclusive, two lines of evidence provide an informed estimate of the optimal protein  
394 dose for stimulating a maximal response of MPS in older adults. First, previous work has  
395 demonstrated that ingesting >36 g of beef protein [56] or 35-40 g of whey protein [55,58] stimulated a  
396 pronounced increase in the rate of irreversible **amino acid** oxidation. These data [55,58] imply the rate  
397 of MPS was approaching, or had indeed reached, an upper limit with ingestion of 35-40 g of protein.  
398 Second, the maximal effective protein dose at rest is higher in older compared with young adults. A  
399 retrospective analysis of previous studies [60] estimated that, when expressed relative to body mass,  
400 the dose of protein required to stimulate a maximal response of MPS at rest was ~68 % greater in  
401 older (~0.40 g/kg body mass) *vs.* young (0.24 g/kg body mass) adults. Moving forward, to refine the  
402 optimal protein dose for the maximal stimulation of MPS in middle-aged or older adults, future  
403 studies should measure the postprandial response of myofibrillar-MPS to 0, 20-40 and 50-60 g doses  
404 of ingested protein.

405 In addition to age, several other nutritional, physiological and/or methodological factors could  
406 impact the optimal dose of protein for the maximal postprandial stimulation of MPS in young and  
407 older adults. Protein source has been shown to affect the dose-response relationship in older adults.  
408 A greater dose of soy protein (≥ 40 g) [57] was required to stimulate a comparable postprandial MPS  
409 response to whey (≥ 20 g) protein [58]. As such, a rightwards shift in the dose-response relationship  
410 was observed with soy protein compared with whey protein. Intuitively, these findings suggest that  
411 protein source alters the optimal protein dose for the maximal stimulation of MPS in older adults.

412 Physiological factors, including body composition and sex-differences, also may impact the dose-  
413 response relationship. It is intuitive that individual differences in SMM will affect the optimal protein  
414 dose for maximal stimulation of MPS. However, no study has compared the dose-response  
415 relationship between individuals with higher vs. lower amounts of SMM. Hence, a protein dose  
416 exceeding 20 g may be optimal in young adults with high amounts of SMM, particularly during  
417 exercise recovery when muscle is sensitised to protein ingestion [8]. Whereas a sex-specific difference  
418 in the response of MPS to exercise and nutrition has not been consistently shown in young adults  
419 [62-64], sexually dimorphic postprandial responses of MPS have been shown in older adults [65].  
420 Thus, although not directly evaluated, these data suggest that sex-specific differences are more likely  
421 to affect the optimal single bolus dose of protein in older compared with young adults. Future studies  
422 are warranted to test this thesis.

## 423 3.3 Amino acid/protein timing

424 The majority of studies have focused on the timing of amino acid/protein ingestion after exercise.  
425 Whereas resistance exercise stimulates MPS for at least 48 h during recovery, the magnitude of the  
426 post-exercise response of MPS diminishes over time (i.e., 3 > 24 > 48 h) [66]. This time resolution could  
427 be explained by the notion that, as time elapses, muscle progressively loses anabolic sensitivity to  
428 protein ingestion. An extreme interpretation of this concept is the belief that the anabolic  
429 responsiveness of skeletal muscle will be impaired– or even abolished– if an amino acid/protein  
430 source is not ingested within as little as 45-60 min following exercise [67]. This time period has been  
431 coined the “anabolic window of opportunity.”

432 The timing of amino acid/protein ingestion before and during exercise also should be considered in  
433 the context of stimulating MPS. In theory, amino acid/protein ingestion before and/or during exercise  
434 increases blood amino acid concentrations at a time when blood flow also is increased by exercise.  
435 During exercise, a net loss of muscle protein is apparent because MPS is either decreased [68] or  
436 unchanged [69], whereas MPB is (generally) increased [66]. Moreover, the stimulation of MPS by  
437 protein ingestion is refractory, with a latent period of ~1 h [70]. Intuitively, ingestion of an amino acid  
438 /protein source before or during exercise, will increase amino acid delivery to skeletal muscle during  
439 and immediately post-exercise and counteract the net loss of muscle protein during exercise and in  
440 the initial post-exercise recovery period by providing additional substrate for the stimulation of MPS.

441 Scientific rationale exists also to support the notion that post-exercise amino acid/protein ingestion  
442 should be timed in relation to CHO intake. The post-exercise response of NBAL to CHO ingestion is  
443 delayed until ~1 h after CHO ingestion [71]. Given that the post-exercise response of NBAL to  
444 ingested amino acid is rapid [72], one may speculate that delaying protein ingestion for 1 h after CHO  
445 ingestion may superimpose these muscle protein anabolic responses. Thus, it could be argued that  
446 amino acid/protein timing should consider the timing of other ingested nutrients, as well as  
447 proximity to exercise.

## 448 3.3.1 Time-focused vs. time-divided amino acid/protein timing

449 Surprisingly few studies have compared the impact of time-focused (amino acid/protein ingestion in  
450 close temporal proximity to exercise) and time-divided (amino acid/protein ingestion at times other  
451 than close to exercise) amino acid/protein ingestion on MPS or SMM. Acute metabolic studies do not  
452 support the notion that timing amino acid/protein ingestion immediately post-exercise is critical for  
453 optimizing the muscle anabolic response. These data reveal a similar response of MPS and NBAL to  
454 EAA ingestion 1, 2 or 3 h following resistance exercise in untrained young men [73-75]. Hence, it has  
455 been argued that the purported “anabolic window of opportunity” may extend beyond the first hour  
456 or less following exercise [76]. In addition, a recent study demonstrated protein ingestion 24 h  
457 following resistance exercise resulted in a greater response of MPS than protein ingested with no  
458 exercise [77]. A direct comparison of the response of MPS to ingestion of protein immediately and 24  
459 h following exercise has yet to be made and thus the stimulation of MPS could, in fact, be slightly  
460 greater with protein ingestion immediately following, rather than 24 h after exercise. Nonetheless, it  
461 is clear, at least in young adults, that skeletal muscle is still responsive to protein ingestion for at least  
462 24 h following exercise [77]. Thus, according to results from acute metabolic studies, the importance  
463 of immediate post-exercise amino acid/protein ingestion does not seem as critical as has often been  
464 championed [67,78].

465 Longitudinal endpoint studies that investigated the efficacy of timing amino acid/protein ingestion  
466 in close temporal proximity to exercise for increasing SMM, report inconsistent and, in some cases,  
467 puzzling results. A study by Cribb and Hayes [79] reported the ingestion of protein immediately  
468 before and after each training session (time-focussed protein supplementation regimen) over a 10 wk  
469 training period resulted in greater improvements in LBM, cross-sectional area of type II muscle fibres

470 and strength compared with ingestion of protein before breakfast and prior to bedtime (time-divided  
471 protein supplementation regimen). Similarly, Esmarck et al. [80] reported SMM gains after 12 wk of  
472 resistance training in a group of older adults that consumed a protein supplement (within a mixed  
473 macronutrient beverage) immediately after a training session, whereas no change in SMM and  
474 negligible strength gains were achieved in the group that consumed protein 2 h after exercise.  
475 However, it is easy to be sceptical about these data [80]. The magnitude of muscle hypertrophy  
476 measured with immediate post-exercise ingestion of the protein supplement was similar to that  
477 reported in other resistance training studies with older adult volunteers that included no particular  
478 feeding intervention [81,82]. Hence, on closer inspection, the results of this study [80] suggest that  
479 immediate post-exercise ingestion of protein does not confer any advantage over resistance training  
480 with unsupervised nutrition, at least in older adults. Moreover, it should be noted that waiting 2 h to  
481 ingest the protein actually inhibited the 'normal' anabolic response to resistance training, making  
482 these results [80] puzzling and difficult to interpret. In contrast, other longitudinal studies in young  
483 adults fail to support the notion that protein ingestion in close temporal proximity to resistance  
484 exercise is critical for maximising SMM. Accordingly, studies by Burk et al. [83] and Hoffman et al.  
485 [84] reported time-focused protein supplementation resulted in a similar [84] or inferior [83] change  
486 in LBM after training compared to time-divided protein supplementation. Given that resistance  
487 training is an established anabolic stimulus for increasing SMM, it may be considered surprising that  
488 no improvement in LBM was observed following the training period with the time-focused  
489 supplementation regimen.

#### 490 3.3.2 Pre- vs. post-exercise timing of protein ingestion

491 Other timing considerations may hold similar importance as post-exercise protein timing for  
492 optimising the response of MPS. Indeed, ingestion of an EAA plus CHO mixture immediately pre-  
493 exercise stimulated a greater response of MPS during 2 h of exercise recovery compared with  
494 ingesting an identical EAA-CHO mixture immediately post-exercise [74]. However, an acute study  
495 of similar design in young adults, but this time ingesting intact whey protein, reported no difference  
496 in NBAL during exercise recovery between pre and post-exercise whey protein conditions [85].  
497 Moreover, the exercise-induced stimulation of MPS was similar when a protein-containing meal was  
498 ingested 2 h prior to exercise [86] compared with when an amino acid source was provided after  
499 exercise [9,87]. Accordingly, a longitudinal endpoint study reported similar increases in LBM after  
500 12 wk of resistance training between groups of older adults that consumed a protein blend  
501 supplement either before or after each exercise session [88]. Taken together, these data [9,86-88]  
502 suggest that skeletal muscle is, at the very least, comparatively responsive to amino acid/protein  
503 ingested pre or post-exercise.

#### 504 3.3.3. Timing of amino acid/protein ingestion in relation to other nutrients

505 Only one study has tested the hypothesis that separating, rather than combining, the post-exercise  
506 ingestion of amino acid and CHO increases the muscle anabolic response during exercise recovery  
507 [75]. However, despite the separate ingestion of EAA and CHO stimulating a transient physiological  
508 increase in NBAL in the first 2 h of recovery, no difference in NBAL was demonstrated between  
509 combined or separate ingestion of EAA and CHO over an extended 6 h recovery period [75]. Thus,  
510 from a practical perspective, separating ingestion of EAA and CHO should be considered unlikely to  
511 be an important component of protein recommendations for maximising the muscle protein anabolic  
512 response during exercise recovery. Instead, a more simple approach of ingesting CHO and EAA  
513 together is sufficient to engender increased muscle anabolism.

#### 514 3.3.4 Bedtime protein feeding

515 The timed ingestion of amino acid/protein in relation to overnight recovery is a topic of recent  
516 investigation [89,90]. It has been proposed that ingesting a protein source that releases amino acids

517 slowly into the blood immediately prior to sleep promotes a more positive NBAL during overnight  
518 recovery [89,91]. By maintaining increased blood **amino acid** availability throughout the night, it may  
519 be possible to stimulate MPS and/or attenuate MPB, thereby improving NBAL during overnight  
520 recovery from exercise—a period often associated with an extended phase of negative NBAL. Indeed,  
521 the timed ingestion of protein before bedtime has been shown to increase the nighttime stimulation  
522 of MPS in young and older adults [89,91], and thus may be an effective strategy to increase **muscle**  
523 **anabolism** during overnight recovery. However, in previous studies [89,90], no time control  
524 condition was included, *e.g.*, protein ingestion at a time point other than before bedtime. Hence, the  
525 impact of protein timing *per se* cannot be distinguished from the increased protein intake over the  
526 day.

### 527 3.4 Amino acid/protein pattern

528 **Amino acid**/protein pattern accounts for the dose, timing and frequency of ingestion. A balanced  
529 pattern is characterised by the equal spread of total daily protein intake between servings, whereas,  
530 an unbalanced pattern—shown to be the norm for young [92] and older [93] adults—is characterised  
531 by consuming **a large proportion** of total daily protein intake in a single serving, usually in the  
532 evening meal. The aggregate daytime response of **MPS** is a direct function of the cumulative **MPS**  
533 response to each individual protein serving during the course of a day. In theory, the divergent  
534 profiles of blood **amino acid** concentrations associated with manipulating the timing and frequency  
535 of **amino acid**/protein intake during the course of a day will explain differences in the cumulative  
536 response to **MPS** to balanced and unbalanced protein meal patterns. Accordingly, acute metabolic  
537 studies have investigated the influence of **amino acid** /protein feeding pattern on the aggregate  
538 daytime stimulation of MPS while longitudinal endpoint studies have investigated the influence of  
539 protein meal pattern on chronic changes in SMM and strength.

#### 540 3.4.1 Young adults

541 Four studies in young adults have investigated the influence of protein pattern on **the daytime**  
542 **stimulation of MPS** or chronic changes in SMM [94–97]. **Acute metabolic studies are not comparable**  
543 **given the discrepancies in research design including exercise state (rest vs. post-exercise), and protein**  
544 **feeding regimen (intact protein vs. mixed macronutrient meals). Moreover, the unbalanced pattern**  
545 **implemented in these study designs may be considered somewhat extreme and not reflective of real-**  
546 **world practice. These studies provide ~70% of total daily protein intake in the evening meal [96]**  
547 **which is more than typically consumed during dinner under free-living conditions.** Areta et al. [94]  
548 demonstrated a greater 12 h post-exercise response of myofibrillar-MPS to distributing 80 g of whey  
549 protein as 4 × 20 g servings compared with 2 × 40 g servings 6 h apart, or 8 × 10 g servings 1.5 h apart.  
550 In a more practical study design, Mamerow et al. [96] demonstrated a greater 24 h resting  
551 postprandial response of MPS to a balanced meal pattern that distributed 90 g of protein evenly  
552 between three meals (3 × 30 g), spaced 3.5 - 4 h apart *vs.* a conventional [92,93] unbalanced protein  
553 meal pattern that biased 70% of daily protein intake towards the evening meal. Hence, despite an  
554 equal total daily protein intake (90 g) between conditions, the aggregate daytime stimulation of MPS  
555 was greater with a balanced compared to unbalanced protein feeding pattern. A theoretical  
556 explanation for the improved aggregate daytime stimulation of MPS with a balanced protein meal  
557 pattern may be attributed to the muscle full effect [98] and thus repeatedly reaching the leucine  
558 threshold for the maximal acute stimulation of MPS. However, these data are not supported by a  
559 recent short-term acute metabolic study [97] that demonstrated no difference in the 3 h resting  
560 response of MPS to ingestion of 15 g of EAA either as a single bolus or distributed between four small  
561 boluses. Moreover, the only published chronic study by Arnal and colleagues [95] reported no  
562 changes in LBM following 14 days of either a balanced or unbalanced protein meal pattern. However,  
563 a drawback of this study [95] was that 2/4 meals contained 13–15 g of protein, rather than the optimal  
564 20 g dose [47,61]. At this juncture, acute [96,97] and chronic studies [95] in young adults investigating  
565 the influence of protein pattern on **MPS** and SMM provide inconsistent results. Future studies in

566 young adults should be designed to compare a balanced vs. unbalanced distribution pattern of daily  
567 protein intake on **the daytime stimulation of MPS (under reasting and post-exercise conditions)** and  
568 training-induced changes in SMM, whilst taking into consideration the established optimal dose of  
569 protein contained in a single serving for young adults.

### 570 3.4.2 Older adults

571 Two studies have investigated the influence of protein meal pattern on the **response of MPS** and SMM  
572 in older adults [99,100]. In contrast to studies in young adults, no study has reported that protein  
573 meal pattern affects the aggregate response of MPS to total daily protein intake. Kim and colleagues  
574 [100] reported no difference in the 22 h response of MPS to an unbalanced pattern that biased 65 %  
575 of daily protein intake towards the evening meal compared with a balanced pattern that spread total  
576 daily protein intake evenly between meals. In this study [100], the balanced pattern consisted of three  
577 meals that each contained a protein dose (~37 g) that was likely sufficient for stimulating a maximal  
578 resting postprandial response of MPS in older adults [55,58,100]. However, the statistical power of  
579 this dataset [100] may be considered to be insufficient given that the sample size of the unbalanced  
580 group was only four participants. The only published chronic study by Arnal and colleagues [99]  
581 reported no changes in LBM following 14 d of either a balanced or unbalanced protein meal pattern.  
582 Thus, on the basis of statistical analysis, results are consistent between acute [100]and chronic [99]  
583 studies that investigate the influence of protein pattern on **MPS** and SMM. To date, no study has  
584 investigated the influence of protein feeding pattern on the aggregate post-exercise response of MPS  
585 to daily protein intake in older adults.

### 586 3.5 Macronutrient coingestion

587 Irrespective of whether protein is consumed in food (mixed-macronutrient meal) or supplement  
588 (liquid beverage or solid bar) form, it is often coingested with CHO and/or fat. Hence, it is important  
589 to understand the impact of macronutrient coingestion on **MPS** and SMM.

#### 590 3.5.1 Carbohydrate coingestion

591 Macronutrient coingestion alters physiological factors known to regulate the stimulation of MPS.  
592 **CHO** coingestion increases plasma insulin concentrations compared to CHO [101] or protein  
593 [102]alone and the anabolic action of insulin on muscle protein metabolism is two-fold. First, under  
594 conditions of sufficient **amino acid** availability [103,104], insulin increases **amino acid** delivery to  
595 skeletal muscle (a rate limiting step in the stimulation of MPS) by increasing capillary recruitment  
596 and microvascular perfusion [105]. Second, insulin initiates a suppression of MPB via the ubiquitous  
597 proteasome pathway [106]. Therefore, CHO coingestion theoretically has the potential to facilitate  
598 the stimulation of MPS and suppress the stimulation of MPB.

599 A systematic series of hypothesis-driven studies has investigated the influence of CHO coingestion  
600 on the response of **muscle anabolic response** to an **amino acid**/protein source. Based on available  
601 evidence, the efficacy of CHO coingestion to increase the **muscle anabolic response** and SMM in  
602 response to **amino acid**/protein ingestion is dependent, at least in young adults, on the dose of  
603 ingested **amino acids**/protein. Two acute metabolic studies indicate that coingesting CHO with ~6 g  
604 of **amino acid** increased the muscle protein anabolic response in young adults, compared with the  
605 independent ingestion of AA [107,108]. These findings of a 60 % greater utilisation of ingested **amino**  
606 **acid** [108] and suppression of urinary 3-MH excretion [107]– a crude marker of MPB– in response to  
607 exercise with CHO-**amino acid** coingestion indicate a greater acute stimulation of MPS and inhibition  
608 of myofibrillar-MPB, respectively. Accordingly, the findings of Bird et al. [107] were extended to a  
609 longitudinal training study [109] whereby young adults achieved greater gains in type II muscle fibre  
610 cross-sectional area after 12 wk resistance training when consuming a CHO plus **amino acid**-  
611 containing supplement during each exercise session compared with an **amino acid**-only supplement.

612 As detailed previously, in the absence of sufficient blood amino acid availability [9], the anabolic  
613 action of a CHO-mediated increase in blood insulin concentration is likely to target a suppression of  
614 MPB, rather than stimulation of MPS [3]. Prior work demonstrated the insulin-mediated suppression  
615 of MPB to be linearly graded up to an insulin concentration of ~30 uU/mL [106]. Taken together, these  
616 data in young adults suggest the increased muscle anabolic response to coingesting CHO with small  
617 ( $\leq 6$  g) doses of EAA is mediated by a suppressed response of MPB [106,107,109]. To date, no study  
618 has investigated the impact of coingesting CHO with a suboptimal dose of protein (rather than amino  
619 acids) on MPS in young or older adults.

620 A handful of acute metabolic studies in young [3,102,110,111] and older [110,112] adults report that  
621 coingesting CHO with a moderate/large dose of amino acid/protein elicits no change in rested  
622 [102,110,112] or post-exercise rates of MPS [3,102,111] or MPB [102]. Consistent with these data  
623 [3,102,110-112], similar improvements in LBM, fibre-specific muscle hypertrophy and strength were  
624 reported when resistance-trained young males consumed either a protein or mixed protein-CHO  
625 supplement immediately after each exercise bout of a 10 wk resistance-training period [79]. This  
626 absence of an additive effect of protein and CHO was evident despite CHO coingestion stimulating  
627 a robust increase in circulating insulin concentrations [102,111]. Given that basal insulin  
628 concentrations are known to be sufficient for stimulating MPS in the presence of amino acid [106],  
629 the insulin response to moderate or large protein doses could be considered sufficient to saturate  
630 mTORC1 signalling, thus rendering the CHO-mediated increase in insulin concentration permissive  
631 for increasing the stimulation of MPS.

### 632 3.5.2 Fat coingestion

633 Preliminary, albeit inconsistent, evidence also suggests that fat coingestion increases the muscle  
634 anabolic response [113-115]. Mechanistic studies have demonstrated that increasing free fatty acid  
635 concentrations in blood had no impact on the responsiveness of NBAL to amino acid ingestion  
636 [114,115]. Moreover, results from a recent study demonstrated that coingesting milk fat with casein  
637 protein failed to increase the postprandial stimulation of MPS in older adults [53]. In contrast, a study  
638 of greater physiological relevance by Elliot et al. [113] demonstrated that ingestion of whole-fat milk  
639 stimulated a superior post-exercise utilisation of ingested amino acid compared with ingestion of  
640 skimmed-fat milk matched for volume (239 g) and similar in protein content (8.0 vs. 8.8 g,  
641 respectively). To date, no study has directly assessed the response of MPS to coingesting fat with an  
642 amino acid/protein source under rested or exercised conditions in young or older adults.

643 A topic of recent interest is the role for fish oil derived long chain n-3 polyunsaturated fatty acids (LC  
644 n-3PUFA) for increasing MPS and SMM [116-119]. Studies in young and middle-aged [119] or older  
645 [118] adults have demonstrated that 8 wk of LC n-3PUFA supplementation increased MPS rates, and  
646 the phosphorylation status of signalling proteins (mTORC1-p70S6k1 signalling) known to regulate  
647 MPS, in response to the intravenous infusion of combined amino acids and insulin. Irrespective of  
648 age, no change in basal MPS was observed with LC n-3PUFA supplementation [118,119]. These data  
649 [118,119] suggest that, rather than exerting a direct anabolic effect on muscle protein, LC n-3 PUFA  
650 sensitise skeletal muscle to potent anabolic stimuli, such as amino acids and insulin. Moreover, a  
651 prolonged period of supplementation with LC n-3PUFA was shown to enhance muscle mass and  
652 function at rest [117] and resistance training-induced improvements in muscle strength and  
653 functional capacity in older adults [116]. However, in this study [116], no measurements of SMM  
654 were collected and therefore the impact of LC n-3PUFA supplementation, in combination with  
655 exercise training, on chronic changes in SMM remains unknown.

656 Two causal mechanisms are proposed to underpin the anabolic action of LC n-3PUFA. First, LC n-  
657 3PUFA may exhibit intrinsic muscle protein anabolic properties by modifying the lipid profile of the  
658 muscle phospholipid membrane [118,119]. These structural changes in membrane properties may  
659 activate membrane-bound anabolic signalling proteins, such as focal adhesion kinase (FAK) and the

660 downstream anabolic target proteins, protein kinase B (PKB) and mechanistic target of rapamycin  
661 (mTORC1) [120]. Secondly, the potential anabolic action of LC n-3PUFA supplementation also may  
662 be related to a modulated inflammatory response [121]. The next logical step for this **new** research  
663 topic is to investigate the role of LC n-3PUFA supplementation in sensitising skeletal muscle to more  
664 physiologically relevant anabolic stimuli, such as resistance exercise and protein feeding in young  
665 and older adults.

#### 666 4. Conclusions and future perspectives

667 Protein guidelines for increasing or preserving SMM are more complex than simply recommending  
668 a total daily amount of protein. We have identified several factors involved in protein nutrition,  
669 including the source, dose, timing, pattern and coingestion of other nutrients that independently,  
670 concurrently and additively influence **MPS** under resting and post-exercise conditions.  
671 Consequently, understanding the interaction between these aforementioned factors of protein  
672 nutrition and **MPS** is critical for contextualising protein recommendations for increasing or  
673 preserving SMM in healthy young and older adults.

##### 674 4.1 Implications for practice

675 On the basis of published literature collated in this review, we propose the following evidence-based  
676 implications for practice.

- 677 i. Protein guidelines should be customised to the population (young or older adults) and situation  
678 (resting or post-exercise condition) of interest. For example, (a) the optimal dose of protein for  
679 maximal stimulation of MPS during exercise recovery is greater for older compared to young  
680 adults and (b) whey protein has been shown to stimulate a greater response of MPS compared  
681 with soy protein during exercise recovery, but not at rest.
- 682 ii. Chronic periods of leucine supplementation will not necessarily facilitate long-term  
683 improvements in SMM, given that a full complement of EAA is critical for stimulating a  
684 maximal and sustained response of **MPS**.
- 685 iii. Manipulating the leucine content of a protein source that lacks quality (*i.e.*, the protein source  
686 constitutes a low leucine composition) and/or quantity (*i.e.*, an insufficient protein dose for the  
687 maximal stimulation of MPS) effectively rescues a submaximal resting postprandial stimulation  
688 of **MPS**. This phenomenon has particular implications for older adults or other populations that  
689 often experience difficulties in consuming a sufficiently large dose of protein in each meal  
690 serving to stimulate a maximal response of MPS.
- 691 iv. Timing protein intake in close temporal proximity to exercise is recommended, although not  
692 critical, for stimulating **a maximal response of MPS**.
- 693 v. Coingesting CHO with a suboptimal dose of **amino acid**/protein may be an effective strategy  
694 for 'rescuing' a submaximal response of **MPS** associated with a suboptimal dose of **amino acid**  
695 protein. However, no additional benefit is gained from adding CHO to a dose of **amino acid**  
696 /protein known to saturate **the response of MPS**.
- 697 vi. Any beneficial impact of fat coingestion on **MPS** is likely mediated by the anabolic action of the  
698 LC *n*-3 PUFA.

##### 699 4.2 Implications for research

700 **Table 1** extracts from the main body of text a multitude of future academic research directions in the  
701 field of protein nutrition. As a general point, current protein recommendations are primarily  
702 informed by research designs whereby protein beverages are administered commonly as an isolated  
703 protein source. By characterising the response of **MPS** to the single and multiple bolus ingestion of  
704 mixed-macronutrient meals or supplements, it will be possible to tailor more practical and  
705 personalised nutrition advice regarding what foods/supplements should be consumed, how much of

706 a food/supplement should be consumed and when food/supplements should be consumed on both  
707 rest and exercise training days.

708 In terms of future perspectives, from a methodological standpoint we are entering an exciting period  
709 to study the role of protein nutrition in modulating muscle protein metabolism [122]. Specifically, a  
710 recently validated oral deuterium oxide isotope tracer protocol allows for the relatively non-invasive  
711 measurement of free-living, integrated rates of MPS over an intermediate time period (e.g., 1-14 days)  
712 [123,124] that, in the future, should be extended to longer time periods [125]. Hence, quantifying  
713 fraction-specific rates of MPS to represent skeletal muscle protein remodelling in response to  
714 perturbations such as resistance exercise and amino acid ingestion is possible over acute,  
715 intermediate and potentially chronic time periods. Such tools will inevitably expand our existing  
716 knowledge regarding protein considerations for optimizing SMM in both healthy young and older  
717 adults.

718 As a closing remark, there are a distinct lack of data in females and middle-aged (40-55 year old)  
719 adults. Since sex-differences in the response of MPS to feeding have been reported [63,65], future  
720 studies should investigate the impact of protein feeding on MPS and SMM in cohorts of female  
721 volunteers.

722

723 **Table 1:** Proposed future research directions to promote understanding of how several factors of  
724 protein nutrition interact to impact the stimulation of muscle protein synthesis (MPS) at rest and  
725 during exercise recovery in young and older adults.

	Source	Dose	Timing	Pattern	Coingestion
Source	<p>Can plant protein sources stimulate a similar response of MPS compared to animal protein sources in young and older adults?</p> <p>Do liquid-based forms of protein stimulate a greater response of MPS compared to solid-based forms of protein foods?</p>		<p>What impact does protein source have on the optimal timing of protein ingestion in young adults?</p>	<p>What impact does protein source have on the optimal protein meal pattern for the daytime stimulation of MPS in young and older adults?</p>	
Dose	<p>What impact does protein source have on the optimal protein dose for stimulation of MPS in young adults?</p>	<p>What is the maximal effective dose of protein for the stimulation of MPS in older adults?</p> <p>What influence does individual LBM have on the optimal protein dose for stimulation of MPS?</p>			<p>What impact does macronutrient coingestion have on the optimal protein dose for stimulation of MPS in young adults?</p>
Timing	<p>How does the response of MPS during exercise recovery compare between the pre-exercise ingestion of</p>		<p>Does the overnight stimulation of MPS with bedtime protein feeding translate into long-term gains in SMM?</p>		<p>What impact does macronutrient coingestion have on the optimal protein timing for stimulation of</p>

	<b>casein vs. the post-exercise ingestion of whey protein?</b>				<b>MPS in young and older adults?</b>
<b>Pattern</b>		<b>What impact does protein dose have on the optimal pattern of protein feeding for the daytime stimulation of MPS?</b>		<b>What is the impact of protein feeding pattern, combined with exercise, on the aggregate daytime stimulation of MPS in older adults?</b>	
<b>Coingestion</b>		<b>What impact does coingesting CHO with a suboptimal dose of protein have on MPS in young and older adults?</b>		<b>Does the ingestion of protein within mixed macronutrient meals impact the optimal protein meal pattern for the daytime stimulation of MPS?</b>	<b>What is the impact of LC <i>n</i>-3PUFA supplementation on the response of MPS to exercise and protein feeding in young and older adults?</b>

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The grid has been designed to illustrate the independent or interactive effects of the several factors of protein nutrition on the stimulation of muscle protein synthesis. The placement of each question is dependent on the factor of protein nutrition address by the question. For example, the question ‘Can plant-based protein sources stimulate a similar response of MPS compared with animal-based protein sources?’ relates to the independent impact of *protein source* on MPS and thus fits in the protein source-protein source box. The question, ‘What impact does coingesting CHO with a suboptimal dose of protein have on the stimulation of MPS in young and older adults?’ relates to the interactive effect of *protein dose* and *macronutrient coingestion* on MPS and thus fits in the protein dose-macronutrient coingestion box. SMM, skeletal muscle mass. MPS, muscle protein synthesis. LC *n*-3PUFA, long chain *n*-3 polyunsaturated fatty acids.

735

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740 manuscript. OCW, SLW, LSM, **AH and KDT** contributed to subsequent drafts and approved the manuscript for  
741 submission.

742 **Conflicts of Interest:** No conflict of interest Abbreviations

743

744 The following abbreviations are used in this manuscript:

745 BCAA: branched chain amino acids

746 EAA: Essential amino acids

747 LBM: lean body mass

748 MPS: muscle protein synthesis

749 MPB: muscle protein breakdown

750 NBAL: net muscle protein balance

751 SMM: skeletal muscle mass

752

753 **References:**

754

755 [1] Wolfe, R.R. The Underappreciated Role of Muscle in Health and Disease. *Am. J. Clin. Nutr.* **2006**, *84*, 475-  
756 482.

757

758 [2] Mitchell, C.J.; Churchward-Venne, T.A.; Cameron-Smith, D.; Phillips, S.M. Last Word on Viewpoint: What  
759 is the Relationship between the Acute Muscle Protein Synthetic Response and Changes in Muscle Mass?  
760 *J. Appl. Physiol.* (1985.) **2015**, *118*, 503.

761

762 [3] Glynn, E.L.; Fry, C.S.; Drummond, M.J.; Dreyer, H.C.; Dhanani, S.; Volpi, E.; Rasmussen, B.B. Muscle  
763 Protein Breakdown has a Minor Role in the Protein Anabolic Response to Essential Amino Acid and  
764 Carbohydrate Intake Following Resistance Exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2010**, *299*,  
765 R533-R540.

766

767 [4] Rennie, M.J.; Edwards, R.H.; Halliday, D.; Matthews, D.E.; Wolman, S.L.; Millward, D.J. Muscle Protein  
768 Synthesis Measured by Stable Isotope Techniques in Man: The Effects of Feeding and Fasting. *Clin. Sci.* **1982**,  
769 *63*, 519-523.

770

771 [5] Biolo, G.; Maggi, S.P.; Williams, B.D.; Tipton, K.D.; Wolfe, R.R. Increased Rates of Muscle Protein  
772 Turnover and Amino Acid Transport After Resistance Exercise in Humans. *Am. J. Physiol.* **1995**, *268*, E514-  
773 E520.

774

775 [6] Kimball, S.R.; Jefferson, L.S. Control of Protein Synthesis by Amino Acid Availability. *Curr. Opin. Clin.*  
776 *Nutr. Metab. Care* **2002**, *5*, 63-67.

- 777 [7] Timmerman, K.L.; Dhanani, S.; Glynn, E.L.; Fry, C.S.; Drummond, M.J.; Jennings, K.; Rasmussen, B.B.;  
778 Volpi, E. A Moderate Acute Increase in Physical Activity Enhances Nutritive Flow and the Muscle Protein  
779 Anabolic Response to Mixed Nutrient Intake in Older Adults. *Am. J. Clin. Nutr.* **2012**, *95*, 1403-1412.  
780
- 781 [8] Pennings, B.; Koopman, R.; Beelen, M.; Senden, J.M.; Saris, W.H.; van Loon, L.J. Exercising before Protein  
782 Intake Allows for Greater use of Dietary Protein-Derived Amino Acids for De Novo Muscle Protein Synthesis  
783 in both Young and Elderly Men. *Am. J. Clin. Nutr.* **2011**, *93*, 322-331.  
784
- 785 [9] Biolo, G.; Tipton, K.D.; Klein, S.; Wolfe, R.R. An Abundant Supply of Amino Acids Enhances the  
786 Metabolic Effect of Exercise on Muscle Protein. *Am. J. Physiol.* **1997**, *273*, E122-E129.  
787
- 788 [10] Cuthbertson, D.; Smith, K.; Babraj, J.; Leese, G.; Waddell, T.; Atherton, P.; Wackerhage, H.; Taylor, P.M.;  
789 Rennie, M.J. Anabolic Signaling Deficits Underlie Amino Acid Resistance of Wasting, Aging Muscle. *FASEB*  
790 *J.* **2005**, *19*, 422-424.  
791
- 792 [11] Guillet, C.; Prod'homme, M.; Balage, M.; Gachon, P.; Giraudet, C.; Morin, L.; Grizard, J.; Boirie, Y.  
793 Impaired Anabolic Response of Muscle Protein Synthesis is Associated with S6K1 Dysregulation in Elderly  
794 Humans. *FASEB J.* **2004**, *18*, 1586-1587.  
795
- 796 [12] Katsanos, C.S.; Kobayashi, H.; Sheffield-Moore, M.; Aarsland, A.; Wolfe, R.R. Aging is Associated with  
797 Diminished Accretion of Muscle Proteins After the Ingestion of a Small Bolus of Essential Amino Acids. *Am.*  
798 *J. Clin. Nutr.* **2005**, *82*, 1065-1073.  
799
- 800 [13] Ferreira, M.P.; Li, R.; Cooke, M.; Kreider, R.B.; Willoughby, D.S. Periexercise Coingestion of Branched-  
801 Chain Amino Acids and Carbohydrate in Men does Not Preferentially Augment Resistance Exercise-Induced  
802 Increases in Phosphatidylinositol 3 Kinase/Protein Kinase B-Mammalian Target of Rapamycin Pathway  
803 Markers Indicative of Muscle Protein Synthesis. *Nutr. Res.* **2014**, *34*, 191-198.  
804
- 805 [14] Vieillevoye, S.; Poortmans, J.R.; Duchateau, J.; Carpentier, A. Effects of a Combined Essential Amino  
806 Acids/Carbohydrate Supplementation on Muscle Mass, Architecture and Maximal Strength Following Heavy-  
807 Load Training. *Eur. J. Appl. Physiol.* **2010**, *110*, 479-488.  
808
- 809 [15] Tang, J.E.; Moore, D.R.; Kujbida, G.W.; Tarnopolsky, M.A.; Phillips, S.M. Ingestion of Whey  
810 Hydrolysate, Casein, Or Soy Protein Isolate: Effects on Mixed Muscle Protein Synthesis at Rest and Following  
811 Resistance Exercise in Young Men. *J. Appl. Physiol.* **2009**, *107*, 987-992.  
812
- 813 [16] Burd, N.A.; Yang, Y.; Moore, D.R.; Tang, J.E.; Tarnopolsky, M.A.; Phillips, S.M. Greater Stimulation of  
814 Myofibrillar Protein Synthesis with Ingestion of Whey Protein Isolate V. Micellar Casein at Rest and After  
815 Resistance Exercise in Elderly Men. *Br. J. Nutr.* **2012**, 1-5.  
816

- 817 [17] Pennings, B.; Boirie, Y.; Senden, J.M.; Gijsen, A.P.; Kuipers, H.; van Loon, L.J. Whey Protein Stimulates  
818 Postprandial Muscle Protein Accretion More Effectively than do Casein and Casein Hydrolysate in Older Men.  
819 Am. J. Clin. Nutr. **2011**, *93*, 997-1005.
- 820 [18] Reitelseder, S.; Agergaard, J.; Doessing, S.; Helmark, I.C.; Lund, P.; Kristensen, N.B.; Frystyk, J.;  
821 Flyvbjerg, A.; Schjerling, P.; van, H.G. *et al.* Whey and Casein Labeled with L-[1-13C]Leucine and Muscle  
822 Protein Synthesis: Effect of Resistance Exercise and Protein Ingestion. Am. J. Physiol. Endocrinol. Metab.  
823 **2011**, *300*, E231-E242.
- 824
- 825 [19] Tipton, K.D.; Elliott, T.A.; Cree, M.G.; Wolf, S.E.; Sanford, A.P.; Wolfe, R.R. Ingestion of Casein and  
826 Whey Proteins Result in Muscle Anabolism After Resistance Exercise. Med. Sci. Sports Exerc. **2004**, *36*, 2073-  
827 2081.
- 828
- 829 [20] Dideriksen, K.J.; Reitelseder, S.; Petersen, S.G.; Hjort, M.; Helmark, I.C.; Kjaer, M.; Holm, L. Stimulation  
830 of Muscle Protein Synthesis by Whey and Caseinate Ingestion After Resistance Exercise in Elderly Individuals.  
831 Scand. J. Med. Sci. Sports **2011**, *21*, e372-e383.
- 832
- 833 [21] Wilborn, C.D.; Taylor, L.W.; Outlaw, J.; Williams, L.; Campbell, B.; Foster, C.A.; Smith-Ryan, A.;  
834 Urbina, S.; Hayward, S. The Effects of Pre- and Post-Exercise Whey Vs. Casein Protein Consumption on Body  
835 Composition and Performance Measures in Collegiate Female Athletes. J.Sports Sci.Med. **2013**, *12*, 74-79.
- 836
- 837 [22] Devries, M.C.; Phillips, S.M. Supplemental Protein in Support of Muscle Mass and Health: Advantage  
838 Whey. J. Food Sci. **2015**, *80 Suppl 1*, A8-A15.
- 839
- 840 [23] Burke, L.M.; Hawley, J.A.; Ross, M.L.; Moore, D.R.; Phillips, S.M.; Slater, G.R.; Stellingwerff, T.; Tipton,  
841 K.D.; Garnham, A.P.; Coffey, V.G. Preexercise Aminoacidemia and Muscle Protein Synthesis After Resistance  
842 Exercise. Med. Sci. Sports Exerc. **2012**, *44*, 1968-1977.
- 843
- 844 [24] Volek, J.S.; Volk, B.M.; Gomez, A.L.; Kunces, L.J.; Kupchak, B.R.; Freidenreich, D.J.; Aristizabal, J.C.;  
845 Saenz, C.; Dunn-Lewis, C.; Ballard, K.D. *et al.* Whey Protein Supplementation during Resistance Training  
846 Augments Lean Body Mass. J. Am. Coll. Nutr. **2013**, *32*, 122-135.
- 847
- 848 [25] Joy, J.M.; Lowery, R.P.; Wilson, J.M.; Purpura, M.; De Souza, E.O.; Wilson, S.M.; Kalman, D.S.; Dudeck,  
849 J.E.; Jager, R. The Effects of 8 Weeks of Whey Or Rice Protein Supplementation on Body Composition and  
850 Exercise Performance. Nutr.J. **2013**, *12*, 86.
- 851
- 852 [26] van Vliet, S.; Burd, N.A.; van Loon, L.J.C. The Skeletal Muscle Anabolic Response to Plant- Versus  
853 Animal-Based Protein Consumption. J. Nutr. **2015**, *Ahead of print*.
- 854
- 855 [27] Wilkinson, S.B.; Tarnopolsky, M.A.; Macdonald, M.J.; MacDonald, J.R.; Armstrong, D.; Phillips, S.M.  
856 Consumption of Fluid Skim Milk Promotes Greater Muscle Protein Accretion After Resistance Exercise than  
857 does Consumption of an Isonitrogenous and Isoenergetic Soy-Protein Beverage. Am. J. Clin. Nutr. **2007**, *85*,  
858 1031-1040.

859

860 [28] Hartman, J.W.; Tang, J.E.; Wilkinson, S.B.; Tarnopolsky, M.A.; Lawrence, R.L.; Fullerton, A.V.; Phillips,  
861 S.M. Consumption of Fat-Free Fluid Milk After Resistance Exercise Promotes Greater Lean Mass Accretion  
862 than does Consumption of Soy Or Carbohydrate in Young, Novice, Male Weightlifters. *Am. J. Clin. Nutr.* **2007**,  
863 *86*, 373-381.

864

865 [29] Burd, N.A.; Gorissen, S.H.; van Vliet, S.; Snijders, T.; van Loon, L.J. Differences in Postprandial Protein  
866 Handling After Beef Compared with Milk Ingestion during Postexercise Recovery: A Randomized Controlled  
867 Trial. *Am. J. Clin. Nutr.* **2015**, *102*, 828-836.

868

869 [30] Reidy, P.T.; Walker, D.K.; Dickinson, J.M.; Gundermann, D.M.; Drummond, M.J.; Timmerman, K.L.;  
870 Fry, C.S.; Borack, M.S.; Cope, M.B.; Mukherjea, R. *et al.* Protein Blend Ingestion Following Resistance  
871 Exercise Promotes Human Muscle Protein Synthesis. *J. Nutr.* **2013**, *143*, 410-416.

872

873 [31] Reidy, P.T.; Walker, D.K.; Dickinson, J.M.; Gundermann, D.M.; Drummond, M.J.; Timmerman, K.L.;  
874 Cope, M.B.; Mukherjea, R.; Jennings, K.; Volpi, E. *et al.* Soy-Dairy Protein Blend and Whey Protein Ingestion  
875 After Resistance Exercise Increases Amino Acid Transport and Transporter Expression in Human Skeletal  
876 Muscle. *J. Appl. Physiol.* (1985.) **2014**, *116*, 1353-1364.

877

878 [32] Babault, N.; Paizis, C.; Deley, G.; Guerin-Deremaux, L.; Saniez, M.H.; Lefranc-Millot, C.; Allaert, F.A.  
879 Pea Proteins Oral Supplementation Promotes Muscle Thickness Gains during Resistance Training: A Double-  
880 Blind, Randomized, Placebo-Controlled Clinical Trial Vs. Whey Protein. *J. Int. Soc. Sports Nutr.* **2015**, *12*, 3-  
881 014-0064-5. eCollection 2015.

882

883 [33] Macdiarmid, J.I.; Kyle, J.; Horgan, G.W.; Loe, J.; Fyfe, C.; Johnstone, A.; McNeill, G. Sustainable Diets  
884 for the Future: Can we Contribute to Reducing Greenhouse Gas Emissions by Eating a Healthy Diet? *Am. J.*  
885 *Clin. Nutr.* **2012**, *96*, 632-639.

886

887 [34] Katsanos, C.S.; Kobayashi, H.; Sheffield-Moore, M.; Aarsland, A.; Wolfe, R.R. A High Proportion of  
888 Leucine is Required for Optimal Stimulation of the Rate of Muscle Protein Synthesis by Essential Amino Acids  
889 in the Elderly. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *291*, E381-E387.

890

891 [35] Churchward-Venne, T.A.; Breen, L.; Di Donato, D.M.; Hector, A.J.; Mitchell, C.J.; Moore, D.R.;  
892 Stellingwerff, T.; Breuille, D.; Offord, E.A.; Baker, S.K. *et al.* Leucine Supplementation of a Low-Protein  
893 Mixed Macronutrient Beverage Enhances Myofibrillar Protein Synthesis in Young Men: A Double-Blind,  
894 Randomized Trial. *Am. J. Clin. Nutr.* **2014**, *99*, 276-286.

895

896 [36] Koopman, R.; Verdijk, L.; Manders, R.J.; Gijsen, A.P.; Gorselink, M.; Pijpers, E.; Wagenmakers, A.J.;  
897 van Loon, L.J. Co-Ingestion of Protein and Leucine Stimulates Muscle Protein Synthesis Rates to the Same  
898 Extent in Young and Elderly Lean Men. *Am. J. Clin. Nutr.* **2006**, *84*, 623-632.

899

- 900 [37] Koopman, R.; Verdijk, L.B.; Beelen, M.; Gorselink, M.; Kruseman, A.N.; Wagenmakers, A.J.; Kuipers,  
901 H.; van Loon, L.J. Co-Ingestion of Leucine with Protein does Not further Augment Post-Exercise Muscle  
902 Protein Synthesis Rates in Elderly Men. *Br. J. Nutr.* **2008**, *99*, 571-580.
- 903 [38] Anthony, J.C.; Anthony, T.G.; Kimball, S.R.; Jefferson, L.S. Signaling Pathways Involved in Translational  
904 Control of Protein Synthesis in Skeletal Muscle by Leucine. *J. Nutr.* **2001**, *131*, 856S-860S.
- 905
- 906 [39] Rieu, I.; Balage, M.; Sornet, C.; Giraudet, C.; Pujos, E.; Grizard, J.; Mosoni, L.; Dardevet, D. Leucine  
907 Supplementation Improves Muscle Protein Synthesis in Elderly Men Independently of Hyperaminoacidaemia.  
908 *J. Physiol. (Lond.)* **2006**, *575*, 305-315.
- 909
- 910 [40] Phillips, S.M. Nutritional Supplements in Support of Resistance Exercise to Counter Age-Related  
911 Sarcopenia. *Adv.Nutr.* **2015**, *6*, 452-460.
- 912
- 913 [41] Wall, B.T.; Hamer, H.M.; de, L.A.; Kiskini, A.; Groen, B.B.; Senden, J.M.; Gijsen, A.P.; Verdijk, L.B.;  
914 van Loon, L.J. Leucine Co-Ingestion Improves Post-Prandial Muscle Protein Accretion in Elderly Men. *Clin.*  
915 *Nutr.* **2013**, *32*, 412-419.
- 916
- 917 [42] Koopman, R.; Wagenmakers, A.J.; Manders, R.J.; Zorenc, A.H.; Senden, J.M.; Gorselink, M.; Keizer,  
918 H.A.; van Loon, L.J. Combined Ingestion of Protein and Free Leucine with Carbohydrate Increases Postexercise  
919 Muscle Protein Synthesis in Vivo in Male Subjects. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *288*, E645-E653.
- 920
- 921 [43] Tipton, K.D.; Elliott, T.A.; Ferrando, A.A.; Aarsland, A.A.; Wolfe, R.R. Stimulation of Muscle Anabolism  
922 by Resistance Exercise and Ingestion of Leucine Plus Protein. *Appl.Physiol Nutr.Metab* **2009**, *34*, 151-161.
- 923
- 924 [44] Dickinson, J.M.; Gundermann, D.M.; Walker, D.K.; Reidy, P.T.; Borack, M.S.; Drummond, M.J.; Arora,  
925 M.; Volpi, E.; Rasmussen, B.B. Leucine-Enriched Amino Acid Ingestion After Resistance Exercise Prolongs  
926 Myofibrillar Protein Synthesis and Amino Acid Transporter Expression in Older Men. *J. Nutr.* **2014**, *144*, 1694-  
927 1702.
- 928
- 929 [45] Glynn, E.L.; Fry, C.S.; Drummond, M.J.; Timmerman, K.L.; Dhanani, S.; Volpi, E.; Rasmussen, B.B.  
930 Excess Leucine Intake Enhances Muscle Anabolic Signaling but Not Net Protein Anabolism in Young Men and  
931 Women. *J. Nutr.* **2010**, *140*, 1970-1976.
- 932
- 933 [46] Churchward-Venne, T.A.; Burd, N.A.; Mitchell, C.J.; West, D.W.; Philp, A.; Marcotte, G.R.; Baker, S.K.;  
934 Baar, K.; Phillips, S.M. Supplementation of a Suboptimal Protein Dose with Leucine Or Essential Amino Acids:  
935 Effects on Myofibrillar Protein Synthesis at Rest and Following Resistance Exercise in Men. *J. Physiol. (Lond.)*  
936 **2012**, *590*, 2751-2765.
- 937
- 938 [47] Witard, O.C.; Jackman, S.R.; Breen, L.; Smith, K.; Selby, A.; Tipton, K.D. Myofibrillar Muscle Protein  
939 Synthesis Rates Subsequent to a Meal in Response to Increasing Doses of Whey Protein at Rest and After  
940 Resistance Exercise. *Am. J. Clin. Nutr.* **2014**, *99*, 86-95.
- 941

- 942 [48] Westenhoefer, J. Age and Gender Dependent Profile of Food Choice. *Forum Nutr.* **2005**, 44-51.  
943
- 944 [49] Bukhari, S.S.; Phillips, B.E.; Wilkinson, D.J.; Limb, M.C.; Rankin, D.; Mitchell, W.K.; Kobayashi, H.;  
945 Greenhaff, P.L.; Smith, K.; Atherton, P.J. Intake of Low-Dose Leucine-Rich Essential Amino Acids Stimulates  
946 Muscle Anabolism Equivalently to Bolus Whey Protein in Older Women, at Rest and After Exercise. *Am. J.*  
947 *Physiol. Endocrinol. Metab.* **2015**, ajpendo.  
948
- 949 [50] Casperson, S.L.; Sheffield-Moore, M.; Hewlings, S.J.; Paddon-Jones, D. Leucine Supplementation  
950 Chronically Improves Muscle Protein Synthesis in Older Adults Consuming the RDA for Protein. *Clin. Nutr.*  
951 **2012**, 31, 512-519.  
952
- 953 [51] Verhoeven, S.; Vanschoonbeek, K.; Verdijk, L.B.; Koopman, R.; Wodzig, W.K.; Dendale, P.; van Loon,  
954 L.J. Long-Term Leucine Supplementation does Not Increase Muscle Mass Or Strength in Healthy Elderly Men.  
955 *Am. J. Clin. Nutr.* **2009**, 89, 1468-1475.  
956
- 957 [52] Koopman, R.; Crombach, N.; Gijsen, A.P.; Walrand, S.; Fauquant, J.; Kies, A.K.; Lemosquet, S.; Saris,  
958 W.H.; Boirie, Y.; van Loon, L.J. Ingestion of a Protein Hydrolysate is Accompanied by an Accelerated in Vivo  
959 Digestion and Absorption Rate when Compared with its Intact Protein. *Am. J. Clin. Nutr.* **2009**, 90, 106-115.  
960
- 961 [53] Churchward-Venne, T.A.; Snijders, T.; Linkens, A.M.; Hamer, H.M.; van Kranenburg, J.; van Loon, L.J.  
962 Ingestion of Casein in a Milk Matrix Modulates Dietary Protein Digestion and Absorption Kinetics but does  
963 Not Modulate Postprandial Muscle Protein Synthesis in Older Men. *J. Nutr.* **2015**, 145, 1438-1445.  
964
- 965 [54] Pennings, B.; Groen, B.B.; van Dijk, J.W.; de, L.A.; Kiskini, A.; Kuklinski, M.; Senden, J.M.; van Loon,  
966 L.J. Minced Beef is More Rapidly Digested and Absorbed than Beef Steak, Resulting in Greater Postprandial  
967 Protein Retention in Older Men. *Am. J. Clin. Nutr.* **2013**, 98, 121-128.  
968
- 969 [55] Pennings, B.; Groen, B.; de, L.A.; Gijsen, A.P.; Zorenc, A.H.; Senden, J.M.; van Loon, L.J. Amino Acid  
970 Absorption and Subsequent Muscle Protein Accretion Following Graded Intakes of Whey Protein in Elderly  
971 Men. *Am. J. Physiol. Endocrinol. Metab.* **2012**, 302, E992-E999.  
972
- 973 [56] Robinson, M.J.; Burd, N.A.; Breen, L.; Rerecich, T.; Yang, Y.; Hector, A.J.; Baker, S.K.; Phillips, S.M.  
974 Dose-Dependent Responses of Myofibrillar Protein Synthesis with Beef Ingestion are Enhanced with  
975 Resistance Exercise in Middle-Aged Men. *Appl. Physiol. Nutr. Metab.* **2013**, 38, 120-125.  
976
- 977 [57] Yang, Y.; Churchward-Venne, T.A.; Burd, N.A.; Breen, L.; Tarnopolsky, M.A.; Phillips, S.M. Myofibrillar  
978 Protein Synthesis Following Ingestion of Soy Protein Isolate at Rest and After Resistance Exercise in Elderly  
979 Men. *Nutr. Metab. (Lond)* **2012**, 9, 57.  
980
- 981 [58] Yang, Y.; Breen, L.; Burd, N.A.; Hector, A.J.; Churchward-Venne, T.A.; Josse, A.R.; Tarnopolsky, M.A.;  
982 Phillips, S.M. Resistance Exercise Enhances Myofibrillar Protein Synthesis with Graded Intakes of Whey  
983 Protein in Older Men. *Br. J. Nutr.* **2012**, 108, 1780-1788.

- 984 [59] Symons, T.B.; Sheffield-Moore, M.; Wolfe, R.R.; Paddon-Jones, D. A Moderate Serving of High-Quality  
985 Protein Maximally Stimulates Skeletal Muscle Protein Synthesis in Young and Elderly Subjects. *J. Am. Diet.*  
986 *Assoc.* **2009**, *109*, 1582-1586.
- 987
- 988 [60] Moore, D.R.; Churchward-Venne, T.A.; Witard, O.; Breen, L.; Burd, N.A.; Tipton, K.D.; Phillips, S.M.  
989 Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in  
990 Healthy Older Versus Younger Men. *J. Gerontol. A Biol. Sci. Med. Sci.* **2014**.
- 991
- 992 [61] Moore, D.R.; Robinson, M.J.; Fry, J.L.; Tang, J.E.; Glover, E.I.; Wilkinson, S.B.; Prior, T.; Tarnopolsky,  
993 M.A.; Phillips, S.M. Ingested Protein Dose Response of Muscle and Albumin Protein Synthesis After  
994 Resistance Exercise in Young Men. *Am. J. Clin. Nutr.* **2009**, *89*, 161-168.
- 995
- 996 [62] Dreyer, H.C.; Fujita, S.; Glynn, E.L.; Drummond, M.J.; Volpi, E.; Rasmussen, B.B. Resistance Exercise  
997 Increases Leg Muscle Protein Synthesis and mTOR Signalling Independent of Sex. *Acta Physiol (Oxf)* **2010**,  
998 *199*, 71-81.
- 999
- 1000 [63] Scalzo, R.L.; Peltonen, G.L.; Binns, S.E.; Shankaran, M.; Giordano, G.R.; Hartley, D.A.; Klochak, A.L.;  
1001 Lonac, M.C.; Paris, H.L.; Szallar, S.E. *et al.* Greater Muscle Protein Synthesis and Mitochondrial Biogenesis  
1002 in Males Compared with Females during Sprint Interval Training. *FASEB J.* **2014**, *28*, 2705-2714.
- 1003
- 1004 [64] Smith, G.I.; Atherton, P.; Reeds, D.N.; Mohammed, B.S.; Jaffery, H.; Rankin, D.; Rennie, M.J.;  
1005 Mittendorfer, B. No Major Sex Differences in Muscle Protein Synthesis Rates in the Postabsorptive State and  
1006 during Hyperinsulinemia-Hyperaminoacidemia in Middle-Aged Adults. *J. Appl. Physiol.* **2009**, *107*, 1308-  
1007 1315.
- 1008
- 1009 [65] Smith, G.I.; Atherton, P.; Villareal, D.T.; Frimel, T.N.; Rankin, D.; Rennie, M.J.; Mittendorfer, B.  
1010 Differences in Muscle Protein Synthesis and Anabolic Signaling in the Postabsorptive State and in Response to  
1011 Food in 65-80 Year Old Men and Women. *PLoS.One.* **2008**, *3*, e1875.
- 1012
- 1013 [66] Phillips, S.M.; Tipton, K.D.; Aarsland, A.; Wolf, S.E.; Wolfe, R.R. Mixed Muscle Protein Synthesis and  
1014 Breakdown After Resistance Exercise in Humans. *Am. J. Physiol.* **1997**, *273*, E99-107.
- 1015
- 1016 [67] Lemon, P.W.; Berardi, J.M.; Noreen, E.E. The Role of Protein and Amino Acid Supplements in the  
1017 Athlete's Diet: Does Type Or Timing of Ingestion Matter? *Curr.Sports Med.Rep.* **2002**, *1*, 214-221.
- 1018
- 1019 [68] Dreyer, H.C.; Fujita, S.; Cadenas, J.G.; Chinkes, D.L.; Volpi, E.; Rasmussen, B.B. Resistance Exercise  
1020 Increases AMPK Activity and Reduces 4E-BP1 Phosphorylation and Protein Synthesis in Human Skeletal  
1021 Muscle. *J. Physiol. (Lond. )* **2006**, *576*, 613-624.
- 1022
- 1023 [69] Durham, W.J.; Miller, S.L.; Yeckel, C.W.; Chinkes, D.L.; Tipton, K.D.; Rasmussen, B.B.; Wolfe, R.R.  
1024 Leg Glucose and Protein Metabolism during an Acute Bout of Resistance Exercise in Humans. *J.Appl.Physiol*  
1025 (1985.) **2004**, *97*, 1379-1386.

- 1026 [70] Bohe, J.; Low, J.F.; Wolfe, R.R.; Rennie, M.J. Latency and Duration of Stimulation of Human Muscle  
1027 Protein Synthesis during Continuous Infusion of Amino Acids. *J. Physiol. (Lond. )* **2001**, *532*, 575-579.  
1028
- 1029 [71] Borsheim, E.; Cree, M.G.; Tipton, K.D.; Elliott, T.A.; Aarsland, A.; Wolfe, R.R. Effect of Carbohydrate  
1030 Intake on Net Muscle Protein Synthesis during Recovery from Resistance Exercise. *J. Appl. Physiol.* **2004**, *96*,  
1031 674-678.  
1032
- 1033 [72] Borsheim, E.; Tipton, K.D.; Wolf, S.E.; Wolfe, R.R. Essential Amino Acids and Muscle Protein Recovery  
1034 from Resistance Exercise. *Am. J. Physiol. Endocrinol. Metab.* **2002**, *283*, E648-E657.  
1035
- 1036 [73] Rasmussen, B.B.; Tipton, K.D.; Miller, S.L.; Wolf, S.E.; Wolfe, R.R. An Oral Essential Amino Acid-  
1037 Carbohydrate Supplement Enhances Muscle Protein Anabolism After Resistance Exercise. *J. Appl. Physiol.*  
1038 **2000**, *88*, 386-392.  
1039
- 1040 [74] Tipton, K.D.; Rasmussen, B.B.; Miller, S.L.; Wolf, S.E.; Owens-Stovall, S.K.; Petrini, B.E.; Wolfe, R.R.  
1041 Timing of Amino Acid-Carbohydrate Ingestion Alters Anabolic Response of Muscle to Resistance Exercise.  
1042 *Am. J. Physiol. Endocrinol. Metab.* **2001**, *281*, E197-E206.  
1043
- 1044 [75] Witard, O.C.; Cocke, T.L.; Ferrando, A.A.; Wolfe, R.R.; Tipton, K.D. Increased Net Muscle Protein  
1045 Balance in Response to Simultaneous and Separate Ingestion of Carbohydrate and Essential Amino Acids  
1046 Following Resistance Exercise. *Appl. Physiol. Nutr. Metab.* **2014**, *39*, 329-339.  
1047
- 1048 [76] Witard, O.C.; KD, T. Defining the Anabolic Window of Opportunity. *Agro FOOD Industry Hi-Tech* **2014**,  
1049 *25*, 10-13.  
1050
- 1051 [77] Burd, N.A.; West, D.W.; Moore, D.R.; Atherton, P.J.; Staples, A.W.; Prior, T.; Tang, J.E.; Rennie, M.J.;  
1052 Baker, S.K.; Phillips, S.M. Enhanced Amino Acid Sensitivity of Myofibrillar Protein Synthesis Persists for Up  
1053 to 24 H After Resistance Exercise in Young Men. *J. Nutr.* **2011**, *141*, 568-573.  
1054
- 1055 [78] Ivy, J.; Portman, R. Nutrient timing. In *The Future of Sports Nutrition: Nutrient Timing*; Carol Rosenberg,  
1056 Ed.; Basic Health Publications Inc: California, USA, 2007, pp. 7-14.  
1057
- 1058 [79] Cribb, P.J.; Hayes, A. Effects of Supplement Timing and Resistance Exercise on Skeletal Muscle  
1059 Hypertrophy. *Med. Sci. Sports Exerc.* **2006**, *38*, 1918-1925.  
1060
- 1061 [80] Esmarck, B.; Andersen, J.L.; Olsen, S.; Richter, E.A.; Mizuno, M.; Kjaer, M. Timing of Postexercise  
1062 Protein Intake is Important for Muscle Hypertrophy with Resistance Training in Elderly Humans. *J. Physiol.*  
1063 *(Lond. )* **2001**, *535*, 301-311.  
1064
- 1065 [81] Fiatarone, M.A.; O'Neill, E.F.; Ryan, N.D.; Clements, K.M.; Solares, G.R.; Nelson, M.E.; Roberts, S.B.;  
1066 Kehayias, J.J.; Lipsitz, L.A.; Evans, W.J. Exercise Training and Nutritional Supplementation for Physical  
1067 Frailty in very Elderly People. *N. Engl. J. Med.* **1994**, *330*, 1769-1775.

- 1068 [82] Frontera, W.R.; Meredith, C.N.; O'Reilly, K.P.; Knuttgen, H.G.; Evans, W.J. Strength Conditioning in  
1069 Older Men: Skeletal Muscle Hypertrophy and Improved Function. *J. Appl. Physiol* (1985.) **1988**, *64*, 1038-1044.  
1070
- 1071 [83] Burk, A.; Timpmann, S.; Medijainen, L.; Vahi, M.; Oopik, V. Time-Divided Ingestion Pattern of Casein-  
1072 Based Protein Supplement Stimulates an Increase in Fat-Free Body Mass during Resistance Training in Young  
1073 Untrained Men. *Nutr. Res.* **2009**, *29*, 405-413.  
1074
- 1075 [84] Hoffman, J.R.; Ratamess, N.A.; Tranchina, C.P.; Rashti, S.L.; Kang, J.; Faigenbaum, A.D. Effect of  
1076 Protein-Supplement Timing on Strength, Power, and Body-Composition Changes in Resistance-Trained Men.  
1077 *Int. J. Sport Nutr. Exerc. Metab.* **2009**, *19*, 172-185.  
1078
- 1079 [85] Tipton, K.D.; Elliott, T.A.; Cree, M.G.; Aarsland, A.A.; Sanford, A.P.; Wolfe, R.R. Stimulation of Net  
1080 Muscle Protein Synthesis by Whey Protein Ingestion before and After Exercise. *Am. J. Physiol. Endocrinol.*  
1081 *Metab.* **2007**, *292*, E71-E76.  
1082
- 1083 [86] Witard, O.C.; Tieland, M.; Beelen, M.; Tipton, K.D.; van Loon, L.J.; Koopman, R. Resistance Exercise  
1084 Increases Postprandial Muscle Protein Synthesis in Humans. *Med. Sci. Sports Exerc.* **2009**, *41*, 144-154.  
1085
- 1086 [87] Phillips, S.M.; Parise, G.; Roy, B.D.; Tipton, K.D.; Wolfe, R.R.; Tamopolsky, M.A. Resistance-Training-  
1087 Induced Adaptations in Skeletal Muscle Protein Turnover in the Fed State. *Can. J. Physiol. Pharmacol.* **2002**,  
1088 *80*, 1045-1053.  
1089
- 1090 [88] Candow, D.G.; Chilibeck, P.D.; Facci, M.; Abeysekara, S.; Zello, G.A. Protein Supplementation before  
1091 and After Resistance Training in Older Men. *Eur. J. Appl. Physiol.* **2006**, *97*, 548-556.  
1092
- 1093 [89] Res, P.T.; Groen, B.; Pennings, B.; Beelen, M.; Wallis, G.A.; Gijsen, A.P.; Senden, J.M.; van Loon, L.J.  
1094 Protein Ingestion before Sleep Improves Postexercise Overnight Recovery. *Med. Sci. Sports Exerc.* **2012**, *44*,  
1095 1560-1569.  
1096
- 1097 [90] Betts, J.A.; Beelen, M.; Stokes, K.A.; Saris, W.H.; van Loon, L.J. Endocrine Responses during Overnight  
1098 Recovery from Exercise: Impact of Nutrition and Relationships with Muscle Protein Synthesis. *Int. J. Sport*  
1099 *Nutr. Exerc. Metab.* **2011**, *21*, 398-409.  
1100
- 1101 [91] Groen, B.B.; Res, P.T.; Pennings, B.; Hertle, E.; Senden, J.M.; Saris, W.H.; van Loon, L.J. Intra-gastric  
1102 Protein Administration Stimulates Overnight Muscle Protein Synthesis in Elderly Men. *Am. J. Physiol.*  
1103 *Endocrinol. Metab.* **2012**, *302*, E52-E60.  
1104
- 1105 [92] Valenzuela, R.E.; Ponce, J.A.; Morales-Figueroa, G.G.; Muro, K.A.; Carreon, V.R.; eman-Mateo, H.  
1106 Insufficient Amounts and Inadequate Distribution of Dietary Protein Intake in Apparently Healthy Older Adults  
1107 in a Developing Country: Implications for Dietary Strategies to Prevent Sarcopenia. *Clin. Interv. Aging* **2013**, *8*,  
1108 1143-1148.

- 1109 [93] Tieland, M.; Borgonjen-Van den; van Loon, L.J.; de Groot, L.C. Dietary Protein Intake in Community-  
1110 Dwelling, Frail, and Institutionalized Elderly People: Scope for Improvement. *Eur. J. Nutr.* **2012**, *51*, 173-179.  
1111
- 1112 [94] Areta, J.L.; Burke, L.M.; Ross, M.L.; Camera, D.M.; West, D.W.; Broad, E.M.; Jeacocke, N.A.; Moore,  
1113 D.R.; Stellingwerff, T.; Phillips, S.M. *et al.* Timing and Distribution of Protein Ingestion during Prolonged  
1114 Recovery from Resistance Exercise Alters Myofibrillar Protein Synthesis. *J. Physiol. (Lond.)* **2013**, *591*, 2319-  
1115 2331.  
1116
- 1117 [95] Arnal, M.A.; Mosoni, L.; Boirie, Y.; Houlier, M.L.; Morin, L.; Verdier, E.; Ritz, P.; Antoine, J.M.;  
1118 Prugnaud, J.; Beaufre, B. *et al.* Protein Feeding Pattern does Not Affect Protein Retention in Young Women.  
1119 *J. Nutr.* **2000**, *130*, 1700-1704.  
1120
- 1121 [96] Mamerow, M.M.; Mettler, J.A.; English, K.L.; Casperson, S.L.; rentson-Lantz, E.; Sheffield-Moore, M.;  
1122 Layman, D.K.; Paddon-Jones, D. Dietary Protein Distribution Positively Influences 24-H Muscle Protein  
1123 Synthesis in Healthy Adults. *J. Nutr.* **2014**, *144*, 876-880.  
1124
- 1125 [97] Mitchell, W.K.; Phillips, B.E.; Williams, J.P.; Rankin, D.; Lund, J.N.; Smith, K.; Atherton, P.J. A Dose-  
1126 rather than Delivery Profile-Dependent Mechanism Regulates the "Muscle-Full" Effect in Response to Oral  
1127 Essential Amino Acid Intake in Young Men. *J. Nutr.* **2015**, *145*, 207-214.  
1128
- 1129 [98] Atherton, P.J.; Etheridge, T.; Watt, P.W.; Wilkinson, D.; Selby, A.; Rankin, D.; Smith, K.; Rennie, M.J.  
1130 Muscle Full Effect After Oral Protein: Time-Dependent Concordance and Discordance between Human Muscle  
1131 Protein Synthesis and mTORC1 Signaling. *Am. J. Clin. Nutr.* **2010**, *92*, 1080-1088.  
1132
- 1133 [99] Arnal, M.A.; Mosoni, L.; Boirie, Y.; Houlier, M.L.; Morin, L.; Verdier, E.; Ritz, P.; Antoine, J.M.;  
1134 Prugnaud, J.; Beaufre, B. *et al.* Protein Pulse Feeding Improves Protein Retention in Elderly Women. *Am. J.*  
1135 *Clin. Nutr.* **1999**, *69*, 1202-1208.  
1136
- 1137 [100] Kim, I.Y.; Schutzler, S.; Schrader, A.; Spencer, H.; Kortebein, P.; Deutz, N.E.; Wolfe, R.R.; Ferrando,  
1138 A.A. Quantity of Dietary Protein Intake, but Not Pattern of Intake, Affects Net Protein Balance Primarily  
1139 through Differences in Protein Synthesis in Older Adults. *Am. J. Physiol. Endocrinol. Metab.* **2014**, *ajpendo*.  
1140
- 1141 [101] Breen, L.; Philp, A.; Witard, O.C.; Jackman, S.R.; Selby, A.; Smith, K.; Baar, K.; Tipton, K.D. The  
1142 Influence of Carbohydrate-Protein Co-Ingestion Following Endurance Exercise on Myofibrillar and  
1143 Mitochondrial Protein Synthesis. *J. Physiol. (Lond.)* **2011**, *589*, 4011-4025.  
1144
- 1145 [102] Staples, A.W.; Burd, N.A.; West, D.W.; Currie, K.D.; Atherton, P.J.; Moore, D.R.; Rennie, M.J.;  
1146 Macdonald, M.J.; Baker, S.K.; Phillips, S.M. Carbohydrate does Not Augment Exercise-Induced Protein  
1147 Accretion Versus Protein Alone. *Med. Sci. Sports Exerc.* **2011**, *43*, 1154-1161.  
1148

- 1149 [103] Fryburg, D.A.; Jahn, L.A.; Hill, S.A.; Oliveras, D.M.; Barrett, E.J. Insulin and Insulin-Like Growth  
1150 Factor-I Enhance Human Skeletal Muscle Protein Anabolism during Hyperaminoacidemia by Different  
1151 Mechanisms. *J. Clin. Invest.* **1995**, *96*, 1722-1729.
- 1152
- 1153 [104] Biolo, G.; Williams, B.D.; Fleming, R.Y.; Wolfe, R.R. Insulin Action on Muscle Protein Kinetics and  
1154 Amino Acid Transport during Recovery After Resistance Exercise. *Diabetes* **1999**, *48*, 949-957.
- 1155
- 1156 [105] Timmerman, K.L.; Lee, J.L.; Dreyer, H.C.; Dhanani, S.; Glynn, E.L.; Fry, C.S.; Drummond, M.J.;  
1157 Sheffield-Moore, M.; Rasmussen, B.B.; Volpi, E. Insulin Stimulates Human Skeletal Muscle Protein Synthesis  
1158 Via an Indirect Mechanism Involving Endothelial-Dependent Vasodilation and Mammalian Target of  
1159 Rapamycin Complex 1 Signaling. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 3848-3857.
- 1160
- 1161 [106] Greenhaff, P.L.; Karagounis, L.G.; Peirce, N.; Simpson, E.J.; Hazell, M.; Layfield, R.; Wackerhage, H.;  
1162 Smith, K.; Atherton, P.; Selby, A. *et al.* Disassociation between the Effects of Amino Acids and Insulin on  
1163 Signaling, Ubiquitin Ligases, and Protein Turnover in Human Muscle. *Am. J. Physiol. Endocrinol. Metab.* **2008**,  
1164 *295*, E595-E604.
- 1165
- 1166 [107] Bird, S.P.; Tarpinning, K.M.; Marino, F.E. Liquid Carbohydrate/Essential Amino Acid Ingestion during  
1167 a Short-Term Bout of Resistance Exercise Suppresses Myofibrillar Protein Degradation. *Metab. Clin. Exp.*  
1168 **2006**, *55*, 570-577.
- 1169
- 1170 [108] Miller, S.L.; Tipton, K.D.; Chinkes, D.L.; Wolf, S.E.; Wolfe, R.R. Independent and Combined Effects of  
1171 Amino Acids and Glucose After Resistance Exercise. *Med. Sci. Sports Exerc.* **2003**, *35*, 449-455.
- 1172
- 1173 [109] Bird, S.P.; Tarpinning, K.M.; Marino, F.E. Independent and Combined Effects of Liquid  
1174 Carbohydrate/Essential Amino Acid Ingestion on Hormonal and Muscular Adaptations Following Resistance  
1175 Training in Untrained Men. *Eur. J. Appl. Physiol.* **2006**, *97*, 225-238.
- 1176
- 1177 [110] Gorissen, S.H.; Burd, N.A.; Hamer, H.M.; Gijzen, A.P.; Groen, B.B.; van Loon, L.J. Carbohydrate  
1178 Coingestion Delays Dietary Protein Digestion and Absorption but does Not Modulate Postprandial Muscle  
1179 Protein Accretion. *J. Clin. Endocrinol. Metab.* **2014**, *99*, 2250-2258.
- 1180
- 1181 [111] Koopman, R.; Beelen, M.; Stellingwerff, T.; Pennings, B.; Saris, W.H.; Kies, A.K.; Kuipers, H.; van  
1182 Loon, L.J. Coingestion of Carbohydrate with Protein does Not further Augment Postexercise Muscle Protein  
1183 Synthesis. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *293*, E833-E842.
- 1184
- 1185 [112] Hamer, H.M.; Wall, B.T.; Kiskini, A.; de, L.A.; Groen, B.B.; Bakker, J.A.; Gijzen, A.P.; Verdijk, L.B.;  
1186 van Loon, L.J. Carbohydrate Co-Ingestion with Protein does Not further Augment Post-Prandial Muscle Protein  
1187 Accretion in Older Men. *Nutr. Metab. (Lond)* **2013**, *10*, 15.
- 1188
- 1189 [113] Elliot, T.A.; Cree, M.G.; Sanford, A.P.; Wolfe, R.R.; Tipton, K.D. Milk Ingestion Stimulates Net Muscle  
1190 Protein Synthesis Following Resistance Exercise. *Med. Sci. Sports Exerc.* **2006**, *38*, 667-674.

- 1191 [114] Katsanos, C.S.; Aarsland, A.; Cree, M.G.; Wolfe, R.R. Muscle Protein Synthesis and Balance  
1192 Responsiveness to Essential Amino Acids Ingestion in the Presence of Elevated Plasma Free Fatty Acid  
1193 Concentrations. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 2984-2990.  
1194
- 1195 [115] Svanberg, E.; Moller-Loswick, A.C.; Matthews, D.E.; Korner, U.; Andersson, M.; Lundholm, K. The  
1196 Role of Glucose, Long-Chain Triglycerides and Amino Acids for Promotion of Amino Acid Balance Across  
1197 Peripheral Tissues in Man. *Clin. Physiol.* **1999**, *19*, 311-320.  
1198
- 1199 [116] Rodacki, C.L.; Rodacki, A.L.; Pereira, G.; Naliwaiko, K.; Coelho, I.; Pequito, D.; Fernandes, L.C. Fish-  
1200 Oil Supplementation Enhances the Effects of Strength Training in Elderly Women. *Am. J. Clin. Nutr.* **2012**, *95*,  
1201 428-436.  
1202
- 1203 [117] Smith, G.I.; Julliand, S.; Reeds, D.N.; Sinacore, D.R.; Klein, S.; Mittendorfer, B. Fish Oil-Derived N-3  
1204 PUFA Therapy Increases Muscle Mass and Function in Healthy Older Adults. *Am. J. Clin. Nutr.* **2015**.  
1205
- 1206 [118] Smith, G.I.; Atherton, P.; Reeds, D.N.; Mohammed, B.S.; Rankin, D.; Rennie, M.J.; Mittendorfer, B.  
1207 Dietary Omega-3 Fatty Acid Supplementation Increases the Rate of Muscle Protein Synthesis in Older Adults:  
1208 A Randomized Controlled Trial. *Am. J. Clin. Nutr.* **2011**, *93*, 402-412.  
1209
- 1210 [119] Smith, G.I.; Atherton, P.; Reeds, D.N.; Mohammed, B.S.; Rankin, D.; Rennie, M.J.; Mittendorfer, B.  
1211 Omega-3 Polyunsaturated Fatty Acids Augment the Muscle Protein Anabolic Response to Hyperinsulinaemia-  
1212 Hyperaminoacidaemia in Healthy Young and Middle-Aged Men and Women. *Clin. Sci.* **2011**, *121*, 267-278.  
1213
- 1214 [120] McGlory, C.; Galloway, S.D.; Hamilton, D.L.; McClintock, C.; Breen, L.; Dick, J.R.; Bell, J.G.; Tipton,  
1215 K.D. Temporal Changes in Human Skeletal Muscle and Blood Lipid Composition with Fish Oil  
1216 Supplementation. *Prostaglandins Leukot. Essent. Fatty Acids* **2014**, *90*, 199-206.  
1217
- 1218 [121] Fetterman, J.W., Jr.; Zdanowicz, M.M. Therapeutic Potential of N-3 Polyunsaturated Fatty Acids in  
1219 Disease. *Am. J. Health. Syst. Pharm.* **2009**, *66*, 1169-1179.  
1220
- 1221 [122] McGlory, C.; Phillips, S.M. Assessing the Regulation of Skeletal Muscle Plasticity in Response to Protein  
1222 Ingestion and Resistance Exercise: Recent Developments. *Curr. Opin. Clin. Nutr. Metab. Care* **2014**, *17*, 412-  
1223 417.  
1224
- 1225 [123] MacDonald, A.J.; Small, A.C.; Greig, C.A.; Husi, H.; Ross, J.A.; Stephens, N.A.; Fearon, K.C.; Preston,  
1226 T. A Novel Oral Tracer Procedure for Measurement of Habitual Myofibrillar Protein Synthesis. *Rapid*  
1227 *Commun. Mass Spectrom.* **2013**, *27*, 1769-1777.  
1228
- 1229 [124] Wilkinson, D.J.; Franchi, M.V.; Brook, M.S.; Narici, M.V.; Williams, J.P.; Mitchell, W.K.; Szewczyk,  
1230 N.J.; Greenhaff, P.L.; Atherton, P.J.; Smith, K. A Validation of the Application of D(2)O Stable Isotope Tracer  
1231 Techniques for Monitoring Day-to-Day Changes in Muscle Protein Subfraction Synthesis in Humans. *Am. J.*  
1232 *Physiol. Endocrinol. Metab.* **2014**, *306*, E571-E579.

1233 [125] Miller, B.F.; Wolff, C.A.; Peelor, F.F.,III; Shipman, P.D.; Hamilton, K.L. Modeling the Contribution of  
1234 Individual Proteins to Mixed Skeletal Muscle Protein Synthetic Rates Over Increasing Periods of Label  
1235 Incorporation. J.Appl.Physiol (1985.) **2015**, *118*, 655-661.  
1236



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