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Dietary protein, exercise, ageing and physical inactivity: interactive influences on skeletal muscle proteostasis

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Dietary protein is a pre-requisite for the maintenance of skeletal muscle mass; stimulating increases in muscle protein synthesis (MPS), via essential amino acids (EAA), and attenuating muscle protein breakdown, via insulin. Muscles are receptive to the anabolic effects of dietary protein, and in particular the EAA leucine, for only a short period (i.e. about 2–3 h) in the rested state. Thereafter, MPS exhibits tachyphylaxis despite continued EAA availability and sustained mechanistic target of rapamycin complex 1 signalling. Other notable characteristics of this 'muscle full' phenomenon include: (i) it cannot be overcome by proximal intake of additional nutrient signals/substrates regulating MPS; meaning a refractory period exists before a next stimulation is possible, (ii) it is refractory to pharmacological/nutraceutical enhancement of muscle blood flow and thus is not induced by muscle hypo-perfusion, (iii) it manifests independently of whether protein intake occurs in a bolus or intermittent feeding pattern, and (iv) it does not appear to be dependent on protein dose *per se*. Instead, the main factor associated with altering muscle full is physical activity. For instance, when coupled to protein intake, resistance exercise delays the muscle full set-point to permit additional use of available EAA for MPS to promote muscle remodelling/growth. In contrast, ageing is associated with blunted MPS responses to protein/exercise (anabolic resistance), while physical inactivity (e.g. immobilisation) induces a premature muscle full, promoting muscle atrophy. It is crucial that in catabolic scenarios, anabolic strategies are sought to mitigate muscle decline. This review highlights regulatory protein turnover interactions by dietary protein, exercise, ageing and physical inactivity.

Dietary protein: Exercise: Ageing: Physical inactivity: Proteostasis

A prominent feature of many communicable infectious, non-communicable and non-disease-related conditions, such as ageing[1], cancer[2], diabetes[3], rheumatoid arthritis[4], physical inactivity[5] and bed-rest/immobilisation[6], is the undesirable loss of skeletal muscle mass (atrophy). As the largest organ in the body occupying about 45–55 % of body mass, skeletal muscle plays pivotal roles in locomotion, structural support and metabolic health, serving as the largest reservoir of amino acids (AA)[7] and acting as a key storage site for glucose and intramuscular lipids for

Abbreviations: AA, amino acids; BCAA, branched-chain amino acids; EAA, essential amino acids; eIF, eukaryotic initiation factor; HMB, β-hydroxy-β-methylbutyrate; KIC, α-ketoisocaproate; MPB, muscle protein breakdown; MPS, muscle protein synthesis; mTORC1, mechanistic target of rapamycin complex 1; NEAA, non-essential amino acids; RE, resistance exercise; RET, resistance exercise training; 1-RM, 1-repetition maximum.

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energy production[8,9]. As such, maintenance of a healthy muscle mass is critical to whole-body health and well-being across the lifespan[10]. It therefore follows that the consequences of muscle atrophy are not trivial; demonstrable by robust associations with numerous negative health-related outcomes[11,12], such as an increased risk of frailty-related falls[13], morbidity[14] and even mortality[15].

The intake of dietary protein is a pre-requisite for the day-to-day maintenance of skeletal muscle mass, whereby muscle protein breakdown (MPB) exceeds muscle protein synthesis (MPS) in the fasted state and MPS exceeds MPB in the fed state (and so MPS and MPB are equal across diurnal fasted-fed cycles[16]). Indeed, the quantity and composition of dietary protein are factors known to dictate the anabolic response[16,17]; namely the magnitude and duration of MPS and MPB. In certain (patho)physiological conditions, the anabolic response to dietary protein/exercise can be heightened or impaired. For example, in response to exercise, both MPS and MPB are transiently increased, where protein provision means MPS is enhanced while MPB is put under restraint, contributing to a positive net protein balance, therein driving skeletal muscle growth (hypertrophy)[16]. On the contrary, during ageing and/or physical inactivity, dietary protein can fail to robustly stimulate MPS, contributing to negative net protein balance and driving muscle atrophy. As such, interactions between dietary protein and muscle metabolism remain an area of intense research, driven by a clinical need to identify countermeasures against atrophy, but also in light of the emerging need for sustainable protein sources of high-biological value[18].

Previous reviews have discussed the regulation of human skeletal muscle protein metabolism by dietary protein, yet such reviews are often more singularly focused e.g. on protein quantity (e.g.[19]), protein quality (e.g.[17]), the exercise stimulus (e.g. resistance exercise (RE)[20,21]) or population (e.g. ageing[22]) in isolation or myriad aspects of dietary protein modulation (i.e. dose, quantity) in human subjects *per se* (i.e. not population specific (e.g.[16]). This review focuses upon the anabolic effects of dietary protein, including quantity and quality/composition, in the context of: (i) exercise, (ii) ageing, (iii) physical inactivity and (iv) physical inactivity during ageing, as conditions which can significantly influence the anabolic response to protein feeding. As such, this review will mainly be of interest to scientists, nutritionists, dietitians and clinicians interested in frameworks about protein feeding to potentiate muscle health in situations of exercise, and offset muscle decline in situations of ageing and inactivity (e.g. hospitalisation). Out of the scope of this review is discussion regarding methodologies used to quantify human skeletal muscle protein metabolism (e.g. stable isotope tracers) and so we direct readers to the following in-depth focused publications[23–28].

Dietary protein induces transient muscle anabolism until 'muscle full'

The anabolic effects of feeding, which are driven by the transfer and incorporation of AA obtained from dietary sources into skeletal muscle proteins[16], were realised >30 years ago when the provision of a mixed-macronutrient meal (containing protein, carbohydrate and fat) was shown to stimulate acute MPS in human subjects[29]. It later transpired this robust MPS stimulation is highly dependent on the AA content of the meal[30], which was uniquely attributed to the essential amino acids (EAA)[31,32]. This was eloquently demonstrated where bolus feeding of EAA (e.g. leucine, phenylalanine, threonine) stimulated MPS, but non-essential amino acids (NEAA; e.g. arginine, glycine, serine) did not[31,32]. This AA-induced stimulation of MPS is purportedly dose-dependent with maximal anabolic stimulation being achieved with about 10–20 g EAA[33] or about 20–40 g high-quality protein[34–38]. However, of all EAA, the branched-chain amino acid (BCAA), leucine, can robustly stimulate MPS in isolation[39], and so it is likely that the dose–response of MPS to protein/EAA is not driven by AA quantity *per se* but instead by the leucine content[40]. Reflecting this, it was shown that 3 g EAA containing 40 % leucine elicited comparable MPS to that of 20 g whey in older women[40]. An important implication of this is that low doses of leucine-enriched EAA may provide less satiating yet anabolically robust nutritional protein sources (which is particularly relevant for older adults where the satiety of a feed is a key consideration[40,41]). The digestion rate of the protein source is also purported to determine the subsequent anabolic impact, whereby 'fast' (e.g. whey) proteins result in rapid aminoacidemia and greater protein accretion compared to 'slow' (e.g. casein) proteins. Whilst there is data to show that 20 g whey elicits a greater MPS response *v.* a matched dose of casein, it is important to note that these protein sources differed in AA constituency, particularly in leucine content[42,43]. Consequently, to address the importance of the EAA delivery profile upon muscle anabolism, Mitchell *et al.* provided older males with EAA consumed as either a single 15 g bolus (eliciting rapid aminoacidemia) or in four smaller pulse doses of 3·75 g received every 45 min (eliciting slower aminoacidemia), and demonstrated no differences in MPS[44]. As such, it is plausible to suggest that the content of leucine is a major determinant of the ensuing anabolic response. However, it should be noted that although leucine stimulates MPS in the absence of other AA via depletion of endogenous intra-myocellular pools, MPS would eventually become limited by the availability of other EAA[20] i.e. as an extreme example, if one were to replace protein in meals to leucine alone. In addition to acting as a signal and substrate for MPS, as a BCAA, and along with other BCAA (isoleucine and valine)[45], leucine may also be metabolised within skeletal muscle. This occurs via BCAA deamination and decarboxylation with ensuing metabolic steps leading to the formation of derivative molecules which feed into myriad metabolic pathways, such as the tricarboxylic acid cycle. On this basis, it has been suggested that BCAA metabolites may possess anabolic properties beyond the intact BCAA[46]. Indeed, α-ketoisocaproate (KIC), which is the resultant keto-acid from leucine transamination, stimulates comparable MPS rates to leucine, albeit not in human muscle[47].

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However, plasma leucine concentrations increase with KIC infusion and the reversible transamination of KIC back to leucine may mean that leucine was accountable for the stimulation of MPS[46]. Whilst the KIC-branched-chain α-keto acid dehydrogenase pathway is the fate of most leucine metabolism, a minority (5 %) is converted to the metabolite, β-hydroxy-β-methylbutyrate (HMB)[48] via KIC dioxygenase. Although primarily involved in cholesterol synthesis, HMB possesses robust anabolic and anti-catabolic properties[39,46,49], demonstrated by the provision of 3 g free acid-HMB stimulating MPS, similar to that of leucine, while also suppressing MPB in young human subjects[39,49]. Importantly, despite proposed differences in bioavailability, the pro-anabolic and anti-catabolic properties of HMB also extend to the calcium salt form of HMB[49].

Notably, skeletal muscles are receptive to the anabolic effects of AA for only a short period in the rested state; thus exhibiting temporal regulation. Following feeding, there is an initial lag of about 45–90 min post AA intake due to the time taken for digestion, absorption, arrival at the target tissue (i.e. muscle capillary perfusion and crossing the interstitium) and activation of intracellular anabolic signalling pathways[16]. Subsequently, MPS increases about 2–3-fold, peaking between about 1·5–2 h, prior to returning to baseline a total of about 2–3 h later[16]. Thereafter, MPS displays tachyphylaxis despite continued EAA availability and elevated intracellular anabolic signalling[50], a phenomenon termed muscle full[51], whereby muscle cells intrinsically sense excess EAA and divert them away from incorporation into muscle proteins, instead towards oxidation[23,37]. The notion that MPS may not be continually stimulated by dietary protein intake is reflected by the fact that dietary protein alone, even in excess, cannot induce muscle hypertrophy[46]. Despite this muscle full phenomenon, the mechanisms regulating MPS-related tachyphylaxis despite adequate EAA provision and appropriate intracellular signalling remain elusive, although may reflect attenuated translation elongation[52,53] or endoplasmic reticulum stress[46]. Another notable characteristic of the muscle full state is the fact that it cannot be overcome by proximal intake of additional nutrient signals/substrates regulating MPS (i.e. leucine)[54]. Therefore, a refractory period must exist before a further stimulation of MPS is possible, although the precise kinetics (i.e. duration, etc.) of this period remain ill-defined. Finally, muscle full is also refractory to pharmacological and nutraceutical enhancement of nutritive muscle blood flow, demonstrated by vasodilatory methacholine[55] and cocoa flavanols[56] having no added effects on MPS despite enhancing leg blood flow and microvascular blood volume; as such muscle full is not a state induced by muscle hypo-perfusion. Finally, muscle full manifests independently of whether protein intake occurs in a bolus or intermittent feeding pattern[44], thus suggesting differing rates of aminoacidemia (and dose-dependence) do not dictate muscle full.

Whilst being substrates for MPS, certain EAA also act as key signalling molecules regulating MPS responses[46]. Following *trans*-sarcolemmal EAA transport, leucine (and perhaps other EAA) activates the mechanistic target of rapamycin complex-1 (mTORC1), independent of proximal insulin (e.g. PI3 K/AKT) signalling[16]. mTORC1 activation induces phosphorylation of 4E-binding protein and ribosomal protein S6 kinase, stimulating the binding of the eukaryotic initiation factor (eIF) 4A and eIF4E to eIF4G, forming the eIF4F complex[57]. Thereafter, the eIF4F complex mediates mRNA binding to the 43S preinitiation complex, ultimately resulting in the formation of the 48S preinitiation complex[57]. This relay system triggered by intracellular EAA accumulation results in an upregulation in rates of mRNA translation and thus, MPS. Whilst the EAA-induced upstream regulation of mTORC1 is incompletely defined, recent evidence suggests leucine binding to Sestrin-2 (an intracellular leucine sensor of the mTOR pathway) and subsequent dissociation of Sestrin-2 and the GTPase-activating protein, GATOR2, leads to mTORC1 activation[58]. For more in-depth discussions on nutrient-led signalling to MPS, readers are encouraged towards the following reviews[57,59,60].

In addition to stimulating MPS, protein/EAA intake (with or without carbohydrate) provides a second route for anabolism via suppression of MPB. This is a facet of nutrient-mediated protein accretion which is entirely attributable to insulin action[16]. Illustrating this, insulin at 3× postabsorptive concentrations (i.e. about 15 μU/ml) was sufficient to inhibit MPB by about 50 %[61]; an observation which could not be recapitulated by the provision of EAA infusions during postabsorptive insulin clamps (i.e. at 5 μU/ml)[62]. Thus, since the provision of protein/AA only suppresses MPB when insulin is allowed to rise above post-absorptive levels[62], it is insulin and not EAA that is responsible for the anti-catabolic effects of feeding[61]. Supporting this, a recent systematic review and meta-analysis of >40 human studies concluded insulin has a permissive role in MPS but does have a prominent anti-catabolic role in attenuating MPB[63]. Nonetheless, changes in MPS are likely to be greater than those of MPB (at least in healthy muscle), and so MPS remains the key driver of protein feeding-induced anabolism[16].

Dietary protein and exercise interactions delay the onset of muscle full

In addition to being a prerequisite for skeletal muscle maintenance, dietary protein is critical for the ensuing muscle adaptation in response to RE. This is evidenced by the fact that exercise-induced increases in MPS in the absence of protein/EAA result in a prolonged elevation in MPB, therein contributing to an overall negative muscle protein balance[64]. As such, exercise must be coupled with sufficient protein/EAA in order to achieve positive net protein balance, yielding small amounts of muscle protein accrual after each individual bout of RE, that ultimately culminate over a period of resistance exercise training (RET) resulting in muscle hypertrophy[20].

A prominent feature of exercise is that it increases the anabolic sensitivity of the muscle therein delaying the

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onset of muscle full. Specifically, increasing the availability of dietary EAA post-exercise enhances the magnitude and duration of the MPS response[65], with no further anabolic effects (i.e. increase in MPS and/or decrease in MPB) evidenced with the addition of carbohydrates[66]. To further evidence this, older women exhibited comparable increases in MPS for 2 h following whey or low-dose leucine-enriched EAA feeding in the absence of exercise, whereas exercise (i.e. RE) combined with protein feeding (regardless of composition) prolonged this response, stimulating MPS for 4 h[40]. In some cases, these exercise-enhanced MPS responses to protein intake can last for 24 h[67]. The maximisation of the post-exercise MPS response also relates to studies investigating the optimal timing of protein intake in and around exercise. However, considering an acute bout of RE delays muscle full considerably (i.e. up to 24 h[67]), nutrient consumption pre-, during or post-exercise is perhaps not as critical as presumed; and rather, protein sufficiency (that being quantity and quality) is the most fundamental consideration[16]. Despite exercise sensitising the muscle to protein feeding, limits still exist whereby muscle full is still reached. Specifically, in response to unilateral leg-based RE, MPS displays a dose–response relationship to dietary protein ingestion until maximal stimulation with 20 g intact protein, where doses above which (i.e. 40 g) lead to irreversible oxidation and thus excess protein is catabolised[16,35]. However, in situations of whole-body RE the protein requirements tend to be greater where 40 g whey protein stimulated MPS to a greater extent than 20 g, although the dose needed to reach muscle full was not determined[38]. Taken together, protein-intake coupled with RE delays, but does not fully overcome an eventual muscle full, to permit additional use of available EAA for MPS to promote appropriate muscle remodelling and/or growth.

Older skeletal muscle displays 'anabolic resistance' to dietary protein intake and exercise (i.e. resistance exercise)

For more than a decade, work developing the concept of 'anabolic resistance' has greatly aided explanation of age-related declines in skeletal muscle mass[68]. Although some early, small cohort studies reported depressed basal (post-absorptive, rested) MPS rates in older compared to younger age[69,70], it is now largely accepted that post-absorptive rates of MPS and MPB are unchanged in healthy ageing[22,34,71,72]. Anabolic resistance, therefore, centres upon the blunting of increases in MPS and/or suppression of MPB to key anabolic drivers, namely exercise and nutrition[34,71–73]. Reflecting this, a plethora of accumulating evidence (i.e.[34,71–75]) suggests that anabolic resistance is likely a major driver of age-related muscle atrophy (sarcopenia); though that is not to say mixed results engender this contentious[36,76]. Seminal work by Cuthbertson *et al.* demonstrated a maximal MPS response in young adults to 10 g EAA, with older adults having suppressed rates of MPS above 5 g EAA[33]. Furthermore, a 40 g bolus of EAA failed to elicit a similar MPS response in older adults to that seen in young adults at 10 g EAA[33]. Two recent meta-analyses further support the existence of anabolic resistance in aged muscle, with Wall *et al.* reporting depressed post-prandial MPS rates (−16 %) in older adults[72] and Moore *et al.* reporting a 'rightward shift' whereby myofibrillar fractional synthesis rate (i.e. MPS) plateaus with respect to relative protein intake in older (about 0·4 g/kg) compared to young (about 0·24 g/kg) men[34].

Fortifying protein with leucine may provide another nutraceutical avenue for combating anabolic resistance in ageing muscle. Older adults typically exhibit increased satiety after consuming a meal[33], which likely contributes to the inadequate daily consumption of protein (RDA: 1·0–1·2 g/kg BW/d[77]) commonly seen in the older population[33,73]. As such, specific EAA, such as leucine, may be considered as an adjuvant nutritional strategy to maximise MPS that can also alleviate satiating effects[73]. Through the consumption of a submaximal protein drink (10 g) enriched with 4·5 g leucine, older adults exhibited elevated MPS (0·14 (SEM 0·01) %/4 h), compared to enrichment with 4·5 g alanine (0·11 (SEM 0·01) %/4 h), with fractional synthesis rate remaining elevated above baseline beyond the measured 4 h[78]. Moreover, anabolic signalling was robustly triggered when administering about 6 g BCAA (about 2·6 g leucine), hence leading to an increase in acute MPS response in both young muscle post-RE[79] and in older adults[80]. Conversely, it was reported that ingestion of leucine alone failed to stimulate MPS in the absence of a full EAA profile in postmenopausal (aged 50–65 years) women[81]. Bell *et al.* reported that with 12 weeks of combined RET (about 80 % 1-repetition maximum (1-RM)) and high-intensity interval training (about 90 % maximal heart-rate), alongside consumption of a multi-ingredient supplement (consisting of 30 g whey protein, 2·5 g creatine, 500 IU vitamin D, 400 mg calcium and 1500 mg *n*-3 PUFA), myofibrillar fractional synthesis rate post-training was elevated by about 30 % and 7 % at 0–24 and 24–48 h, respectively; however, the control group demonstrated about 20 % increase in MPS compared to resting levels 24–48 h post-training[82]. Therefore, it is inconclusive whether standalone or adjuvant supplementation of leucine is effective to sufficiently stimulate MPS across populations, and it remains to be investigated whether submaximal doses of complete protein enriched with leucine (at least 3 g[39]) or multi-ingredient supplementation may lead to enhanced muscle anabolism in older adults[73].

Similar to protein feeding, there is evidence of anabolic resistance in response to exercise in older age. Kumar *et al.* demonstrated at 1–2 h post-RET (20–90 % 1-RM), that MPS responses were blunted in older compared to younger adults[83], and with Fry *et al.* highlighting impaired skeletal muscle mTORC1 signalling (a possible mechanism underlying age-related blunting of MPS) and MPS up to 24 h post-exercise[84]. Brook *et al.* also highlighted the presence of anabolic resistance following 6 weeks of unilateral leg RET (75 % 1-RM), with MPS becoming elevated after 3 weeks in young (1·6 (SEM 0·01) % daily) but not older (1·49 (SEM 0·08) % daily) adults, which was coupled with an absence of

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thigh fat-free mass gains in older muscle[74]. Similarly, Durham *et al.* reported a reduced sensitivity to AA following endurance type (i.e. walking) exercise at about 40 % VO_{2peak} in older compared to younger adults[85]. In contrast, Symons *et al.* reported a 50 % increase in MPS in young and older muscle when exercise was coupled to a 30 g protein meal[36], suggesting aged muscle simply exhibits delays in the anabolic response to exercise coupled to nutrition[86]. In terms of exercise intensity, it has been suggested that low-intensity high-volume RET may have comparative effects to high-intensity low-volume RET, a modality which would be of particular practicable benefit to older individuals[22]. Indeed, results from Fry *et al.* highlight, in older compared to young adults, low-intensity RET (20 % 1-RM) coupled with blood flow occlusion heightened mTORC1 signalling and MPS by 56 % above basal levels 3 h post-exercise[84]. However, in the absence of blood flow occlusion, MPS remained unchanged post-exercise[84]. Interestingly, although in younger adults, it was demonstrated that unilateral leg extension exercise at 30 % *v.* 90 % 1-RM to failure was more effective at increasing and maintaining elevated MPS (199 %) 24 h post-exercise[87]. If RET volume rather than intensity is key for stimulating MPS, a greater portion of older adults may be able to perform RET due to associated sarcopenic comorbidities (e.g. arthritis) which may limit the performance of high-intensity exercise[22]; thus offering a potential effective intervention to offset the impact of anabolic resistance and age-related muscle functional declines, although this requires further investigation.

Physical inactivity induces a premature muscle full state in response to dietary protein

Physical inactivity, such as sedentarism or immobilisation due to bed-rest, induces a premature muscle full state characterised by blunted fasted and fed-state MPS (i.e. anabolic resistance)[88–93], prompting insulin resistance[94] and muscle atrophy[6], which over time may play a key role in the aetiology of sarcopenia[95]. In certain cases, inactivity-induced atrophy is not fully recovered despite resumption of normal ambulation[96] or even following RET[97]. As such, protein intervention, as a means to stimulate inactivity-induced declines in MPS, is a primary research avenue for offsetting inactivity-induced atrophy, particularly since modulating the type[98] and amount[99] of dietary protein can somewhat overcome anabolic resistance in ageing[98,99].

To date, results surrounding the anabolic efficacy of protein feeding during inactivity have been mixed. For example, provision of 20 g protein daily (on top of a partially controlled diet 1 g/kg/d) during 14 d of unilateral leg immobilisation did not attenuate muscle loss in middle-aged adults[100]. More recently, a high protein diet (1·6 g protein/kg/d) provided at evenly spaced intervals throughout the day (strategically done so in order to avoid the refractory period), was shown to have no positive influence on MPS or muscle volume observed with 3 d of unilateral limb immobilisation in young males[101]. This led the authors to suggest that dietary protein consumption of ≤1·6 g/kg/d during inactivity does not modulate muscle loss[101], perhaps calling for future studies to investigate whether larger protein doses can overcome the anabolic resistance that manifests during inactivity. On the contrary, higher dose EAA/leucine supplementation has shown more promising results. For example, high-dose EAA combined with carbohydrate during 28 d of bed-rest preserved MPS rates and ameliorated the loss of lean muscle mass[102,103]. Further, middle-aged adults consuming leucine supplementation (0·06 g/kg/meal) during 14 d of bed rest illustrated attenuated reductions in post-absorptive MPS and reduced whole-body lean mass loss after 7 d (compared to an alanine control group)[104]. However, not all studies are in agreement; 7·5 g/d of free leucine did not attenuate muscle mass declines in young adults undergoing 7 d of unilateral limb immobilisation[105]. Whilst the precise cause(s) of discrepancies between study findings are unknown, the model of inactivity used (i.e. bed rest, unilateral limb immobilisation), volunteer characteristics, dietary control within studies, and importantly, the differing amount and quality of protein provided are likely factors. In line with the general consensus of the literature thus far, it is plausible that higher dose EAA/leucine, as opposed to high protein diets, may be a more effective intervention to overcome the premature onset of muscle full during inactivity. If so, it has been suggested that it is not the availability of AA *per se* that is limiting muscle anabolism during inactivity, but rather inactivity induces a significant increase in the threshold required for EAA/leucine to stimulate anabolism (e.g. intracellular signalling pathways)[101]. To test this, future research should thus focus on protein strategies that maximise intracellular transport and delivery of AA, therein maximising anabolic signals during inactivity[101]. In the search for alternative/effective protein therapeutics, NEAA (which seemingly play a trivial role in healthy muscle[106]), may be important in ameliorating inactivity-induced muscle decline[107]. For example, glutamine is purported to harbour anti-catabolic properties[108] while arginine is implicated in the maintenance of muscle perfusion[109] contributing to substrate delivery or MPS stimulation[107,110]. As such, dietary formulations combining EAA and NEAA may confer unique mechanistic advantages over either AA profile in isolation due to the simultaneous targeting and therapizing of multiple inactivity-activated molecular processes contributing to muscle decline. Indeed, a novel AA formulation (AXA2678) containing select anabolic EAA (e.g. leucine) and NEAA (glutamine, arginine and n-acetylcysteine) consumed thrice daily, preserved muscle volume and cross-sectional area during 7 d of immobilisation in young men[107]. However, a confounder within this study was the use of a carbohydrate placebo, meaning the treatment group (AXA2678) received an additional about 66·8 g AA daily, maybe explaining the positive findings[107]. Future studies are warranted that compare supplements such as AXA2678 to appropriate controls, and to test their efficacy in situations of anabolic resistance (i.e. ageing).

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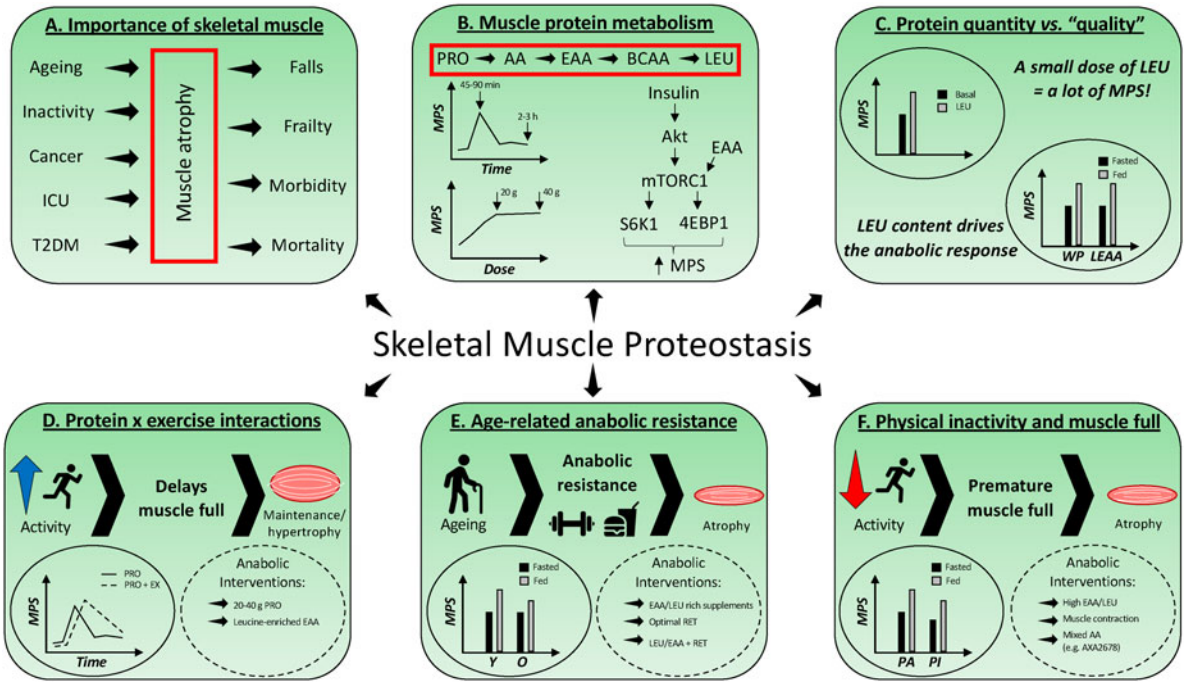

Fig. 1. Summary of skeletal muscle proteostasis in relation to dietary protein, exercise, ageing and physical inactivity. (A) Skeletal muscle atrophy is a prominent feature of many pathological conditions, associating with a multitude of negative outcomes, (B) the essential amino acid (EAA) and branched-chain amino acid (BCAA), leucine (LEU), is the most anabolic constituent of protein feeding, where amino acids (AA) act as signals and substrates for the transient and purportedly dose-dependent regulation of muscle protein synthesis (MPS), (C) small (about 3 g) quantities of LEU elicit robust MPS and so, it is likely that the MPS response to protein/EAA is not driven by AA quantity *per se* but instead by the LEU content, (D) protein/LEU feeding in combination with exercise (resistance exercise (RE)) delays the onset of muscle full, (E) anabolic resistance contributes to age-related muscle atrophy, which may be (partially) overcome by EEA/LEU supplementation and RE and (F) physical inactivity (e.g. bed rest, immobilisation) induces a premature onset of muscle full, which may be circumvented by high EAA/LEU supplementation, muscle contraction (e.g. neuromuscular electrical stimulation) and/or non-essential amino acids (NEAA). Akt, protein kinase B; eIF4E, eukaryotic initiation factor 4E; EX, exercise; ICU, intensive care unit; LEAA, leucine-rich essential amino acids; mTORC1, mechanistic target of rapamycin complex 1; O, older adults; PA, physical activity; PI, physical inactivity; PRO, protein; S6K1, ribosomal protein S6 kinase; T2DM, type II diabetes mellitus; WP, whey protein; Y, young adults; 4EBP1, 4E-binding protein.

A parallel anabolic intervention to protein feeding in order to negate the premature onset of muscle full during inactivity is the re-introduction of some form of muscle contraction during the period of inactivity[111,112]. For example, RE performed every other day during 14 d bed rest or immobilisation in young adults prevented declines in type I and II myofibres[111] and muscle cross-sectional area[112], respectively. However, the ability to perform exercise during inactivity, both in the context of physical capacity and having access to an appropriate environment (e.g. equipment and expertise) are key considerations for this type of intervention, particularly in a clinical setting. In light of this, some researchers have applied neuromuscular electrical stimulation which, from a practical standpoint, has many benefits such as its relatively low cost, ease for hospital staff to apply, tolerance of volunteers, and no reported contraindications or side effects, and importantly, it is feasible and applicable during situations of inactivity or incapacity[113]. Indeed, neuromuscular electrical stimulation prevented declines in quadricep cross-sectional area in young healthy males following 5 d unilateral leg immobilisation, with corresponding attenuations in the atrophy-associated markers, myostatin and muscle atrophy F-box[114]. However, the interactions of neuromuscular electrical stimulation and protein feeding in the context of inactivity remain to be investigated, and across populations. Thus, whilst inactivity–activity-protein interactions show tremendous potential for mitigating inactivity-associated muscle decline, studies are still very much in the infancy stage, even more so in the context of a young clinical setting (e.g. hospitalisation).

Dietary protein to mitigate the premature muscle full state during physical inactivity in ageing

From a clinical perspective, older adults are more likely to experience bouts of inactivity (e.g. hospitalisation/physical debilitation with ensuing bed rest)[97,115], with most hospitalised patients over 65 years old and having longer lengths of hospital stays[116]. This may induce acute sarcopenia. Indeed, the associated loss of muscle mass is significant and rapid, with older adults losing

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detectable muscle mass (about 1·5 %) within as few as 5 d unilateral limb immobilisation[117] and losing up to about 6 % (about 1 kg) of lean mass during 10 d bed rest[118]; this is purportedly greater than the loss of muscle seen in younger subjects following 14 or 28 d bed rest[103,119]. In older individuals, the premature onset of muscle full associated with inactivity may be further confounded by age-related anabolic resistance to nutrition. As such, ageing populations subjected to periods of physical inactivity stand to benefit considerably from optimised dietary protein–exercise intervention.

As with younger adults, the ability of protein feeding in older adults to offset the premature muscle full state associated with inactivity has produced mixed results. For example, twice daily supplementation of protein (20·7 g), fat (3·0 g) and carbohydrate (9·3 g) on top of an ample protein diet (1·1 g/kg/d) during 5 d unilateral leg immobilisation did not attenuate muscle mass declines in older males[117]. This led the authors to speculate that maintaining dietary protein intake is necessary to prevent muscle loss during inactivity, but hyperprotein consumption would not further circumvent muscle loss[117], although more recent data from the same group obtained in younger adults no longer supports this hypothesis[101]. Further studies show that high-dose EAA supplementation (15 g thrice daily) on top of a diet containing 0·8 g/kg/d protein attenuated the decline in 24 h fractional synthesis rate during 10 d bed rest in older adults[120]. Similarly, healthy older men and women consuming daily meals supplemented with leucine (3–4 g/meal) during 7 d bed rest reduced the loss of leg lean mass (compared to alanine control)[115]. This demonstrates the anabolic potential of small amounts of supplemental leucine and highlights practical advantages such as the cost and ease of incorporating leucine into meal plans[115]. Although muscle loss is largely attributed to marked declines in MPS, potential undetectable changes in MPB cannot be disregarded; particularly in cohorts where the MPB response to inactivity is not well characterised (i.e. older adults), most likely due to difficulties surrounding MPB-related methodologies. As such, HMB has been tested as a potential therapeutic in inactive older adults due to its pro-anabolic and anti-catabolic properties[39] and its efficacy for preserving older muscle health in the context of habitual living[121]. In these trials, older men and women consuming 3 g/d HMB during bed rest confinement for 10 d maintained MPS rates pre- to post-bed rest and preserved muscle mass (control volunteers lost about 2 kg lean mass)[122]. As such, data thus far in older adults echo that of the young, where EAA/leucine, and possibly HMB, may demonstrate greater anabolic effects during inactivity, compared to high protein diets. As a collective, these studies demonstrate there is potential for refined nutritional interventions to offset inactivity-induced atrophy in ageing.

Considering protein feeding in isolation may not fully counteract the premature onset of muscle full during inactivity in older adults; adjunct interventions, such as clinically relevant, feasible (i.e. financially) and effective exercise interventions, are needed. Importantly, hospitalised older adults experience functional decline and are often limited to about 4 min walking every 3 h, which is even less so in women who typically engage in about 2 min walking every 3 h[123]. As such, neuromuscular electrical stimulation is a logical adjunct intervention that is (as previously discussed) clinically feasible even for those who are totally incapable of weight bearing. Investigations thus far have shown that following 1 d best rest, older adults subjected to unilateral neuromuscular electrical stimulation followed by 40 g casein ingestion prior to sleep led to an about 18 % greater increase in MPS overnight (compared to the unstimulated leg)[113]. In the context of longer periods of inactivity, 5 d bed rest combined with neuromuscular electrical stimulation (40 min, thrice daily) and supplemental protein (17 g/d) maintained thigh lean mass and attenuated increases in catabolic biomarkers, myostatin and muscle atrophy F-box, perhaps (at least partly) underlying the preservation of lean mass observed in this trial[124].

Table 1. Key research gaps surrounding dietary protein, exercise, ageing and physical inactivity in relation to skeletal muscle proteostasis

Protein
Identification of the precise AA sensor(s) coupling intracellular AA to mTORC1 signalling
Uncover the mechanisms underlying the 'switch' from stimulated MPS back to fasting MPS in the face of continued AA availability and mTORC1 activity (i.e. mechanisms regulating muscle full)
Discover how long is required after maximal MPS stimulation, before the next stimulation of MPS is possible (i.e. refractory period kinetics)
Exercise
What minimum threshold of combined exercise (i.e. type, duration, intensity) and protein (i.e. type, amount) is required to delay muscle full?
What combination of exercise (i.e. type, duration, intensity) and protein (i.e. type, amount) can minimise the refractory period?
Ageing
Investigate the additive anabolic effects of optimal RET volume/intensity and optimal protein nutrition to overcome anabolic resistance
Physical inactivity
Establish the optimal protein quantity to maximally stimulate MPS during inactivity, across age
Impact of novel protein supplements (e.g. AXA2678) on MPS, in the context of physical inactivity *and* ageing
Better understand the impacts of physical inactivity in clinical populations (i.e. hospitalised patients as opposed to healthy volunteers, ±inflammation)
Investigate protein-exercise interactions in young healthy and clinical populations undergoing periods of inactivity (i.e. ±inflammation)
Devise protein-exercise interactions that specifically target older female clinical populations undergoing periods of inactivity

AA, amino acids; MPS, muscle protein synthesis; mTORC1, mechanistic target of rapamycin complex 1; RET, resistance exercise training.

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Protein–exercise interventional approaches such as this may have translational ramifications for preserving muscle anabolic responses in older adults subjected to periods of inactivity in a clinical setting (i.e. hospitalisation). Future studies should aim to optimise such 'EX-NUT' interventions, particularly in women, considering apparent sexual dimorphism in ambulation in hospital settings.

It should be noted that whilst inactivity studies (i.e. bed rest, immobilisation, reduced physical activity) in healthy young and older volunteer populations allow for the metabolic and molecular investigations of inactivity-induced muscle decline, whilst controlling for lifestyle factors that can drastically influence metabolism (e.g. diet, activity, etc.), clinical translation must be treated with caution. For example, the onset of illness or occurrence of injury often results in hospital admittance where a hyper-metabolism (or other catabolic stressors such as inflammation) may result in excessive or accelerated muscle loss, particularly in those with comorbidities, which may have specific dietary challenges and requirements[125] and, importantly, may not be recapitulated in healthy research volunteers[73]. As such, investigations into the effects of protein nutrition in both young and older clinical populations undergoing inactivity are imminently needed in order to optimise appropriate anabolic interventions. Furthermore, in an attempt to effectively and fully translate clinical trials into the clinical setting, it is imperative that day-to-day concerns, such as the quantity, taste and palatability of protein feeding[125] and tolerability and compliance to exercise interventions are thoroughly trialled in appropriate clinical populations.

Conclusion

In summary (Fig. 1), dietary protein is a pre-requisite for the day-to-day maintenance of skeletal muscle mass, supported through the stimulation of MPS and attenuation of MPB. The anabolic effects of dietary protein are driven mainly by EAA, particularly leucine, and are transient in nature (lasting about 2–3 h). This muscle full state cannot be overcome by further feeding meaning a refractory period must exist before the next MPS stimulation is possible. However, muscle full can be delayed by exercise via increased sensitivity of muscle to EAA extending the duration of MPS, whereas ageing/inactivity are associated with a blunted synthetic response to exercise/protein feeding (anabolic resistance) resulting in a premature onset of muscle full. In an attempt to promote the translation of the currently available evidence, we provide the following frameworks that we believe may help delay muscle full or negate the premature onset in each given scenario; (i) in conjunction with a bout of RE, 20 g protein/low-dose leucine-enriched EAA (3 g, 40 % leucine) should be consumed, although these protein recommendations may need to be increased in the context of whole body RE, (ii) in older adults, greater quantities of protein (about 40 g)/fortifying protein with leucine (about 3 g) should be consumed each meal in conjunction with performing optimal RE, and (iii) in situations of physical inactivity (and physical inactivity during ageing), heightened EAA/leucine (as opposed to high protein diets) supplementation, potentially in combination with NEAA cocktails, should be consumed alongside some form of muscle contraction (e.g. neuromuscular electrical stimulation), if physically/logistically possible to do so. Despite the substantial body of research to date, it is clear that a number of gaps remain in our understanding of how dietary protein, exercise, ageing and physical inactivity influence skeletal muscle proteostasis, and so we direct and encourage our target audience to refer to Table 1, which highlights (what we regard as) important research questions worthy of future robust clinical trial investigation/s.

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Conflict of Interest

P. J. A. has received research funding from Ajinomoto, Abbott Nutrition and Fresenius-Kabi.

Authorship

All authors wrote and approved the manuscript.

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