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Muscle protein turnover in the elderly and its potential contribution to the development of sarcopenia

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The underlying aetiology of sarcopenia appears multifaceted and not yet fully defined, but ultimately involves the gradual loss of muscle protein content over time. The present evidence suggests that the loss of lean tissue in the elderly is exacerbated by low dietary protein intake. Moreover, acute stable-isotope-based methodologies have demonstrated that the muscle anabolic response to a given amount of protein may decline with age, a phenomenon that has been termed anabolic resistance. Although the mechanism responsible for the inability of muscle to mount a satisfactory anabolic response to protein provision with increasing age is presently unknown, it does not appear due to impaired digestion or absorption of dietary protein. Rather, the issue could reside with any combination of: a diminished delivery of amino acids to peripheral tissues, impaired uptake of amino acids into muscle cells, or an inability of amino acids to elicit intracellular events pivotal for anabolism to occur. Despite the presence of anabolic resistance to dietary protein, present evidence suggests that protein supplementation may be able to overcome these issues, particularly when combined with resistance exercise programmes. As such, protein supplementation may prove to be an effective approach to delay the loss of muscle mass with age and has led to calls for the recommended daily intake of protein to be increased for the elderly population.

Muscle protein: Amino acids: Sarcopenia: Exercise

Muscle mass and contractile function are intricately associated with perceived quality of life, and outcome following subsequent illness, disease or surgery[1–4]. As such, when a significant loss of muscle mass occurs, individuals are at a heightened risk of fall-related fractures and their ability to live an independent life is severely compromised. Of concern, the loss of muscle mass and strength are natural hallmarks of the ageing process, being termed sarcopenia and dynapenia, respectively. As early as age 40 years, leg muscle mass and strength start to decline, with the rate of loss accelerating from 70 years onwards[5,6]. In the USA, where figures are readily available, it is estimated that sarcopenia is indirectly responsible for US$18·5 billion in annual health care expenditure[7]. With the projected increase in size of the global elderly population anticipated in subsequent decades, healthcare resources required for the prevention and treatment of sarcopenia are only set to grow. It is therefore imperative that an understanding of the underlying scientific basis for the loss of muscle mass with age is established so that effective and acceptable pharmaceutical, nutraceutical and lifestyle modifications can be developed and instigated in at risk populations.

There are numerous factors that could contribute to the development of sarcopenia, including but not limited to: reduced habitual food intake, increase in sedentary

Abbreviations: mTORc1, mammalian target of rapamycin complex 1; LAT1, L-type amino acid transporter 1; NO, nitric oxide; SNAT, sodium-coupled neutral amino acid transporter.

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behaviour, poor vitamin D status, loss of motorneurones, development of chronic low-grade inflammation and increased oxidative stress. In reality, it is likely that the aetiology of sarcopenia is multifactorial and moreover, the underlying causative factors could differ between sarcopenic individuals. Regardless of the mechanism(s) involved, the insipid loss of muscle mass observed during sarcopenia will necessitate the net loss of muscle myofibrillar contractile protein, thereby representing a key target for therapeutic intervention. Thus, strategies that reverse or limit the age related decline in muscle protein content are likely to be advantageous. This review will focus on the mechanisms responsible for regulating muscle mass in human subjects and discuss how they are perturbed as part of the ageing process. Furthermore, the use of protein and amino acids as strategies to enhance muscle anabolism will be considered and possible challenges in their adoption highlighted.

The role of protein turnover in the regulation of muscle mass

Muscle mass, being typically composed of 88 % protein by dry weight[8], is largely dependent upon the intricate balance between the rate at which muscle proteins are synthesised and degraded. Where an imbalance in the magnitude of these two opposing processes occurs, a net change in muscle protein content ensues and when sustained over chronic periods, is reflected by a reciprocal change in muscle mass. During sarcopenia, and in other conditions characterised by cachexia (e.g. cancer, sepsis, acquired immune deficiency syndrome and chronic obstructive pulmonary disease), rates of muscle proteolysis exceeds those of muscle protein synthesis, often leading to the debilitating loss of muscle mass over time.

Crucially, the processes of muscle protein synthesis and muscle protein breakdown are dynamic, changing in response to nutritional, hormonal and contractile cues. Importantly, following protein consumption, muscle protein synthesis is enhanced[9]; an effect that can be recreated solely through the administration of the essential amino acids[10]. In particular, the branched chain amino acid leucine appears particularly effective at stimulating muscle protein synthesis (see[11]). Viewed in conjunction with observations that hyperinsulinaemia stimulates muscle protein synthesis in human subjects under conditions of increased amino acid availability[12], it highlights the important roles played by both insulin and leucine in the induction of muscle protein anabolism post-meal consumption. The manner in which insulin and amino acids collectively elicit these effects within the muscle cell appears through stimulation of the mammalian target of rapamycin complex 1 (mTORc1) signalling cascade. Recent evidence has provided the notion that mTORc1 acts as a nutrient sensor, integrating signals on amino acid availability, energy status via feedback from 5′ adenosine monophosphate-activated protein kinase, and status of the insulin-signalling pathway through AKT-mediated phosphorylation of mTORc1 residue Ser^{2448} (Fig. 1; see [13]). As such, mTORc1 co-ordinates the promotion of

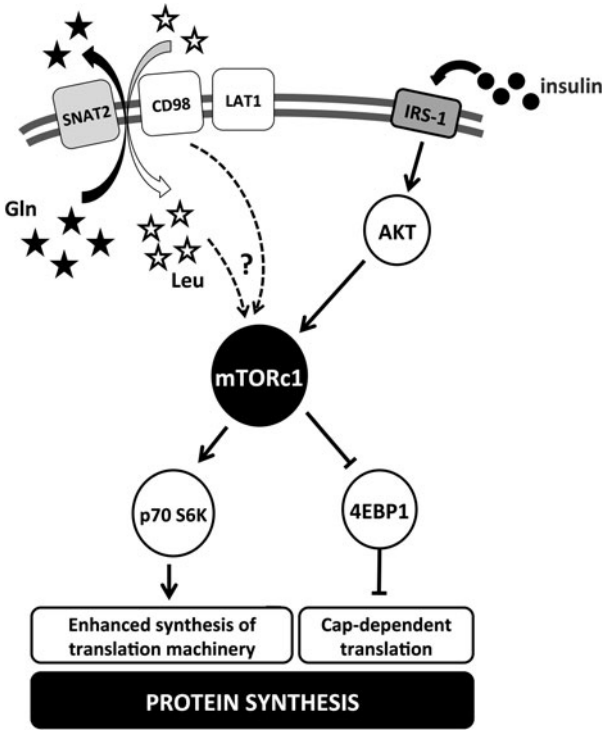

Fig. 1. A simplified diagram representing the role of mammalian target of rapamycin complex 1 (mTORc1) signalling in regulating muscle protein synthesis in response to extracellular cues. SNAT, sodium-coupled neutral amino acid transporter; LAT1, L-type amino acid transporter 1; IRS-1, insulin receptor substrate-1; Gln, glutamine; Leu, leucine; 4EBP1, eukaryotic initiation factor 4E binding protein 1.

translation initiation in the postprandial period when substrates (amino acids) are readily available and ATP generation is sufficient to support the high-energy demands of protein synthesis. This is achieved by mTORc1 eliciting two main effects. Firstly, when active, mTORc1 phosphorylates residue Thr^{389} of p70 S6 K, promoting the increased translation of mRNA containing a 5′ oligopyrimidine tract, typically represented in mRNA encoding ribsosomal proteins and translation elongation factors. Secondly, mTORc1 phosphorylates and thereby inactivates eukaryotic initiation factor 4E binding protein 1 (also known as PHAS-1), a protein that when hypophosphorylated is known to repress translation by preventing the binding of the 7-methylguanylate cap, a structural feature of most mRNA transcripts, to the forming ribosome complex (see[14]). The demonstration that administration of the potent mTORc1 inhibitor rapamycin to healthy human volunteers blunts the ability of amino acid feeding to stimulate muscle protein synthesis[15], highlights the essential requirement of this kinase in eliciting feeding gains in muscle protein content.

While the postprandial stimulation of muscle protein synthesis has historically received the most attention when events responsible for the net loss of muscle protein content in human subjects have been investigated, this is not to suggest that muscle protein breakdown also plays an essential, if yet to be fully defined, role. Indeed, in

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rodents enhanced muscle proteolysis has been repeatedly demonstrated to represent a major contributor to the loss of muscle mass in a number of atrophy-inducing conditions[16], including sarcopenia[17]. The paucity of data in human subjects is more a reflection of the experimental challenges and technical requirements faced when trying to obtain accurate surrogate measures of muscle protein breakdown. However, in reports where attempts have been made to obtain an index of muscle protein breakdown by examining arteriovenous dilution of isotopically labelled amino acids across the leg, it has been consistently observed that during conditions of hyperinsulinaemia, as observed in the postprandial period, muscle protein breakdown is suppressed[18]. Collectively therefore, the consumption of food plays an essential role in dictating the diurnal pattern of muscle protein synthesis and muscle protein breakdown.

As mentioned earlier, the maintenance of protein intake and its effectiveness at stimulating muscle protein synthesis are central to the maintenance of muscle mass in the elderly population. However, several aspects from the process of meal consumption to the eventual stimulation of muscle protein synthesis by amino acids have been proposed as negatively impacted as part of the ageing process. These include decreased food intake, impaired digestion and absorption of nutrients, altered splanchnic uptake of amino acids, insufficient delivery of nutrients through the microvascular circulation, blunted uptake of amino acids into the muscle cell and perturbed cellular response to increased amino acid availability. Each of these potential contributory factors will be discussed in turn.

Changes in protein intake requirements with age

It has been suggested that decreased protein intake with age, set in the wider context of reduced meal consumption, plays a major role in the aetiology of sarcopenia[19]. Certainly, for a subset of individuals dental issues, blunted olfactory and taste perception, combined with social and economic factors contribute to reduced meal consumption or removal of high-quality animal protein from the diet[20], and likely compound the loss of muscle experienced by these individuals. However, for the majority of the elderly population the issue appears to be far greater than just insufficient protein intake alone. Observational studies performed in Western populations have demonstrated that a significant proportion (60–85 %) of older men and women meet the present adult recommendations for protein intake of 0·8 g/kg per d (see[19]). However, of concern, a study by Campbell *et al.* in older adults reported that a 14-week carefully controlled and monitored eucaloric diet containing 0·8 g protein/kg per d was insufficient to stave off the 1·5 % decline in the thigh muscle cross-sectional area seen during the experimental period[21]. Similarly, in a preceding smaller study by the same researchers, they demonstrated that older healthy adults consuming the protein RDA for 10 d resulted in a negative nitrogen balance[22]. A 3-year longitudinal study conducted in 2066 North American septuagenarians provided further evidence that weight-losing and weight-stable individuals meeting or exceeding current protein recommendations still experienced the loss of appendicular lean mass[23]. Collectively, these important observations have led to the suggestion that the current RDA for adults aged over 18 years is insufficient to maintain muscle mass in the elderly, with the higher recommendations of 1–1·5 g/kg per d suggested as an appropriate intake to reduce risk of sarcopenia[19]. Moreover, it identifies that the ability to elicit muscle anabolism per unit dietary protein is reduced in the elderly[19], potential reasons for which are discussed later.

Protein absorption and digestion

Independent of any purported change in food intake in the elderly, the maintenance of adequate protein digestion and absorption is essential. An inability to retain comparable digestion and absorption kinetics to young adults could underpin the failure of dietary protein to maintain muscle mass in the elderly. Given the highly complex nature of the gastrointestinal tract, involving the coordinated action of a wide variety of specialised cells, the exact impact of ageing *per se* on gastrointestinal function remains unclear (see[24]). However, delayed gastric emptying, increased colonic transit time and increased incidence of chronic constipation have all been reported[24]. Likewise, clinical disorders of the gastrointestinal tract, including susceptibility to gut infections, gastroesophageal reflux disease, irritable bowel syndrome, peptic ulcer formation and diverticulosis, all increase in incidence with age. Therefore, the impaired digestion and absorption of food constituents in the elderly is a genuine concern.

Typically, the direct study of protein digestion and absorption kinetics in human subjects is fraught with technical challenges, and as such rarely attempted. However, in a recent series of detailed experiments, the absorption and digestion characteristics of animal-derived protein in older adults were investigated. By intravenously administering [1–^{13}C]phenylalanine to a cow over a 48 h period followed by milking and slaughter of the animal, Pennings *et al.* were able to generate intrinsically labelled milk protein and meat[25]. Utilising the casein fraction of the intrinsically labelled milk, they were able to demonstrate through oral administration of a 35 g bolus to young and elderly individuals that the rate of appearance of the labelled phenylalanine in the circulation was equivalent between the age groups studied[26]. This demonstrated that in healthy elderly men the digestion and absorption of casein protein is not impaired. Moreover, despite previous suggestions that splanchnic uptake of absorbed amino acids may increase with age[27,28], the authors were able to demonstrate that splanchnic uptake was largely equivalent between the two age groups studied, at about 72 % each. This latter observation is noteworthy, if splanchnic uptake was found to be enhanced with age; this could impair the availability of dietary-

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consumed amino acids for utilisation in muscle protein synthesis.

It could be argued that the administration of a large casein bolus in beverage form is not representative of the types of foods ingested by elderly adults or the manner in which they typically consume meals. As such, the relevance of these findings to the real-world situation could be questioned. In a subsequent report, the same authors demonstrate that the manner in which food is processed can impact significantly on protein digestion and absorption kinetics. By comparing 135 g cooked minced beef with beef steak, both intrinsically labelled with [1–^{13}C]phenylalanine and consumed by elderly male volunteers, they reported that minced beef is more rapidly digested and absorbed than its steak counterpart[29]. Likewise, small-animal-based studies have demonstrated that protein digestibility is considerably lower in older animals compared with their young counterparts when protein is consumed in the presence of anti-nutritional factors such as phytases[30]; an issue of particular concern in developing countries where there is a large reliance on crop-based protein sources high in concentrations of such anti-nutritional factors (see[31]). Collectively, this suggests that a more holistic approach reflecting the standard eating practices in the elderly would be beneficial when considering questions relating to digestion and absorption.

Given that the work of Koopman *et al.* tentatively suggests that the digestion and absorption of protein is not impaired in the elderly[26], it would thereby propose that the basis for the inability of dietary protein to maintain muscle mass in the elderly must reside elsewhere. In support of this notion, the intravenous administration of mixed amino acids to young and elderly volunteers under hyperinsulinaemic euglycaemic conditions, a strategy that thereby circumvents any potential issues associated with altered digestion, absorption or splanchnic uptake of orally administered protein, elicits greater increases in muscle protein synthesis in young compared with elderly individuals[32]. The blunting of the stimulatory effect of amino acids on muscle protein synthesis in elderly individuals has since been reported by others, following either intravenous or oral feeding[33] of amino acids, with the phenomenon subsequently termed anabolic resistance[33].

Impact of age on postprandial hyperaemia

The enhanced delivery of circulating amino acids to muscle tissue is an essential requisite for postprandial elevations in muscle protein synthesis to occur, with delivery dependent on both arterial amino acid concentration and arterial blood flow to the muscle. The administration of insulin to produce physiological hyperinsulinaemia has been repeatedly shown to stimulate enhanced blood flow to muscle tissue[12,34,35] and therefore, the incretin effect of food consumption acts to prime the system to assist in the delivery of glucose and amino acids to peripheral tissues. As such, linear relationships have been described in healthy young subjects between the stimulation of muscle protein synthesis during graded localised insulin administration and the change seen in femoral arterial blood flow and amino acid delivery to the leg[12]. Given the progressive development of insulin resistance in the elderly population[36], it would appear to follow that an impairment of insulin-mediated increases in amino acid delivery under postprandial conditions may contribute to anabolic resistance.

In support of this notion, leg blood flow under hyperinsulinaemic euglycaemic clamp conditions, appears impaired in old volunteers compared with their young healthy counterparts[37]. The same finding has also been observed when performed in conjunction with exogenously administered amino acids[38] or in response to intermittent feeding of an oral mixed-feed over a 2 h period[39]. Thus, with the stated requirement to enhance amino acid delivery to sufficiently increase muscle protein synthesis, it would be judicious to employ strategies that safely stimulate blood flow or improve the vasodilatory effects of insulin. In this regard, the principal mechanism by which insulin appears to impart is vasodilatory effects via stimulating the synthesis and release of endothelin-derived nitric oxide (NO)[40], thereby promoting a potent relaxation of arterial smooth muscle. Therefore, pharmacological stimulation of increased NO synthesis may represent one such strategy. In support, Dillon *et al.* were able to demonstrate that sodium nitroprusside, a known NO donor with potent vasodilatory effects, was able to enhance leg blood flow and stimulate muscle protein synthesis to similar degrees in young and elderly subjects when administered in combination with amino acids[41]. However, their decision not to include a control group who did not receive the nitroprusside treatment means caution has to be applied as to the significance of these findings.

In addition to pharmacological approaches, the use of nutrition to promote increased NO formation in the elderly is an area of current investigation. Although the primary focus in the majority of the studies performed to date has been to improve vascular function so as to reduce the incidence of CVD, an unintended benefit may be an improvement in the hyperaemic response to food consumption. Common strategies employed have included increasing dietary nitrate intake through the consumption of high-nitrate containing foods or by high-nitrate containing oral supplements, often in the form of beetroot concentrate. Although the number of studies conducted to date is limited, the acute (3-d) consumption of beetroot supplements by elderly subjects has been shown to increase plasma nitrite concentrations 4-fold and result in a 3 mm Hg decrease in mean arterial blood pressure with no change in resting heart rate[42]; changes consistent with increased NO-mediated smooth muscle relaxation. While the ability of a nitrate supplement to enhance a postprandial rise in blood flow has yet to be demonstrated in the elderly, reports of concentrated beetroot juice successfully enhancing brachial artery endothelial function in overweight and obese men 2 h post-meal consumption is promising[43]. Separately, while the administration of the NO precursor, L-arginine, has been proposed as an alternative

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mechanism by which to enhance NO species generation[44], the inability of large bolus doses of L-arginine to increase plasma nitrate and nitrite concentrations in healthy young individuals[45] suggests that this may not represent a viable strategy. Likewise, administration of citrulline, the endogenous precursor for the synthesis of arginine, does not appear to increase the responsiveness of muscle blood flow or muscle protein synthesis to protein feeding in elderly men[46]. Therefore, on the basis of current evidence, it would appear that nitrate supplements might represent the most viable strategy to reverse age-related impairments in hyperaemia following feeding. However, a crucial requirement before its adoption would be the need to understand the potential side effect of chronic high-nitrate intake in this population.

A second important modulator of muscle blood flow is exercise. While elderly men display a blunted hyperaemic response to both resistance-based and aerobic exercise across a spectrum of workloads[47,48], exercise appears to retain its ability to improve the hyperaemic response to feeding. Specifically, it has been demonstrated in elderly men that a 20-week whole-body resistance exercise training programme is able to restore the hyperaemic response to both feeding and acute bouts of resistance exercise[39]. This capacity of resistance exercise, combined with its widely known function to act as a potent stimulator of muscle growth, reinforces the need to consider resistance exercise as a central treatment to prevent and/or limit sarcopenia. While concerns may exist as to the suitability of this form of exercise in frail populations, work by Evans and co-workers has shown high-intensity resistance exercise to be both feasible in frail very elderly populations (age 72–98 years), and moreover, able to increase muscle mass and strength[49].

Alterations in the muscle cell response to hyperaminoacidaemia with age

Regardless of purported impairments in the hyperaemic response to food consumption in the elderly, it is also feasible that the uptake of, or responsiveness of the muscle cell to, amino acids is no longer satisfactory to stimulate mTORc1 activity and initiate translation initiation. Certainly, it has been demonstrated that even after the consumption of large doses (≥40 g) of essential amino acids, phosphorylation of mTORc1 is blunted in elderly muscle *v.* the young[33]. Moreover, the blunted response of muscle protein synthesis to amino acid provision in the elderly remains even when intramuscular concentrations of leucine are increased to a greater extent than that observed in the young[33], suggesting that impediments at the muscle cell level exist.

Leucine, the predominant amino acid that results in increased mTORc1 phosphorylation[50], and stimulates muscle protein synthesis[11], is unable to freely traverse the muscle cell membrane. Instead, in common with the other branched-chain amino acids, uptake into the muscle cell is regulated via System L transport through a protein complex consisting of a heterodimer between amino acid transporters L-type amino acid transporter 1 (LAT1) and CD98, with leucine import requiring the bidirectional transport of glutamine out of the muscle cell (Fig. 1; see[13]). Therefore, the maintenance of a glutamine concentration gradient is essential to retain the cell's ability to import leucine, achieved through the concerted action of the System A sodium-coupled neutral amino acid transporter (SNAT2) and System N amino acid transporters (SNAT3). As a result, intracellular concentrations of essential amino acids can be maintained under conditions of hypoaminoacidaemia[51]. Likewise, except when concentrations of plasma amino acids are in excess of the amount required to maximally stimulate muscle protein synthesis[52], intracellular concentrations of essential amino acids are maintained at relatively constant concentrations[51,53]. Proton-assisted amino acid transporter 1, an intracellular transporter primarily located on the late endosome and lysosomes within cells has also been implicated in the activation of mTORc1 in response to amino acid availability[54]; deletion in *Drosophila* flies is known to impair the ability of amino acids to activate mTORc1 and as a consequence their overall growth is retarded[55]. As such, it collectively suggests that amino acid transporters may play a role in regulating mTORc1 activity and by virtue, muscle protein metabolism. Although the simplest scenario would be for the amino acid transporters to directly regulate intracellular leucine concentrations ultimately leading to the activation of mTORc1, this does not appear to be the case. By examining the dose–response relationship between muscle protein synthesis and extracellular and intracellular amino acid concentrations, Bohé *et al.* were able to demonstrate that the anabolic response to amino acids is not responsive to intracellular amino acid availability[52]. Subsequent cell culture experiments using methylaminoisobutyric acid, a non-metabolisable System A amino acid analogue, have demonstrated that SNAT2 appears able to induce p70 S6 K phosphorylation in a rapacmycin-sensitive manner, in spite of reduced intracellular amino acid concentrations[56]. This potential ability of amino acid transporters to elicit intracellular signalling events has led to them being termed transceptors (transporter + receptor) and provides credence to their study in the relation to sarcopenia. In particular, an inability of the amino acid transporters to allow sufficient essential amino acids import or initialise signalling events leading to the activation of mTORc1 appear plausible areas of interest.

Although the study of the amino acid transporters in human skeletal muscle is limited, the evidence suggests that LAT1, SNAT2, CD98 and proton-assisted amino acid transporter 1 are transcriptionally up-regulated rapidly (1 h) following essential amino acids ingestion, and that this is paralleled by increases in intracellular leucine concentrations and muscle protein synthesis[57]. However, in the same study, increases in muscle intracellular leucine concentrations and muscle protein synthesis were found to precede detectable increases in LAT1 and SNAT2 protein levels, suggesting that increased transporter protein expression is not involved in the chain of events responsible for increased leucine uptake into the muscle cell or in enhanced muscle protein anabolism post-amino acid consumption. Indeed, the clearance of blood amino acids has been shown to be complete 3 h following meal

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consumption, suggesting that the changes in transporter protein expression would need to be rapid[58]. Subsequent work in murine muscle cells has demonstrated SNAT2, proton-assisted amino acid transporter 1 and LAT1 to be transcriptionally up-regulated in response to insulin administration, with LAT1 increased in an apparent mTORc1-dependent manner[59], providing further evidence that transporter expression may lag behind the stimulation of muscle protein synthesis. Therefore, the changes observed in transporter expression in human muscle following essential amino acids ingestion may merely be a reflection of the known incretin effect of amino acids rather than any direct stimulatory effect on transporter expression to drive anabolism, although this remains to be determined. However, given that intracellular amino acid concentrations are thought to be regulated though the integration of protein synthesis, breakdown, movement into and out of the extracellular pool and where applicable, oxidation[51,60], a role for changes in amino acid transporter expression post-stimulation of muscle protein synthesis may prove important for the maintenance of intracellular amino acid concentrations.

Notably, while the constitutive activation of the insulin-signalling kinase AKT, results in substantial muscle growth in rodents[61], the effects of insulin and amino acids on human muscle protein synthesis appear distinct. The provision of a high-dose mixed-amino acid infusion concomitant to an insulin clamp designed to maintain serum insulin concentrations at post-absorptive levels has been shown to significantly stimulate muscle protein synthesis[62]. Moreover, no further enhancement in muscle protein synthesis rates are observed with stepwise increases in serum insulin concentrations despite elevated AKT phosphorylation. Therefore, the ability of amino acids to stimulate muscle protein synthesis appears independent of insulin action. Given the reported role of insulin in stimulating amino acid transporter expression, this would provide further evidence that transcriptional up-regulation of the amino acid transporters may not be a key process in the anabolic response to amino acid feeding. However, caution has to be applied to current findings; transporters by virtue of their function are localised to cellular membranes and in the case of specific transport systems (e.g. system L), have to form heterodimers before they are able to perform transport functions. Measures restricted to 'readouts' of mRNA and protein levels for the amino acid transporters are unable to take these key variables into account.

Although the role of altered amino acid transporter expression as a manner in which the cell regulates the stimulation of muscle anabolism remains unclear, impaired amino acid transporter expression may remain a contributor to sarcopenia and/or muscle disuse atrophy. Seven day bed rest in elderly individuals has been shown to prevent the stimulatory effect of essential amino acids on SNAT2 and LAT1 protein expression, concomitant to a partial blunting of mTORc1 phosphorylation and failure to enhance muscle protein synthesis rates over the period examined[63]. Further work is required to place these findings into the context of sarcopenia; however, studies are currently limited by the availability of experimental tools for the study of amino acid transporter function in human subjects. Until such challenges are overcome the contribution of such findings to the aetiology of sarcopenia will remain unclear.

Impact of inactivity on muscle protein turnover

Like sarcopenia itself, the presence of anabolic resistance has not been universally observed in all elderly individuals. Indeed, some researchers have failed to observe anabolic resistance in their recruited elderly volunteers[26,64], leading some to question the applicability of acute stable-isotope-based methods to detect such changes[65]. The crux of their argument centres on two main ideas. Firstly, that the insipid loss of muscle mass with age, predicted to be 1–2 % per year[66], is unlikely to be of sufficient magnitude to be detectable by commonly employed stable-isotope approaches. Secondly, they argue that the short timeframes often examined with stable-isotope approaches ($\leq$3 h) fail to take account for the possibility of a delayed rather than reduced anabolic response to amino acid provision. Certainly, a delay in the stimulation of muscle protein synthesis to amino acids has been reported in the elderly, with the cumulative protein synthetic response found to be equivalent between young and elderly subjects when long (5 h) postprandial periods are considered[67] (a process that by its own virtue will diminish the sensitivity required to detect differences). While renewed vigour in the use of deuterium-labelled water to allow the chronic assessment of muscle protein synthesis[68,69] should overcome both of these limitations and permit the habitual examination of elderly individuals, an alternative explanation for this discord in the literature may exist. Notably, the vast majority of studies interested in the impact of ageing on muscle protein turnover tend to recruit healthy, non-sarcopenic and active elderly individuals. As such, the anabolic resistance observed in such studies could be primarily a reflection of the activity status of the recruited individuals.

Muscle disuse, induced by bed-rest or limb immobilisation, is widely reported to result in a decline in insulin sensitivity and an impaired anabolic response of muscle to feeding in human subjects[70,71]. In stark contrast to small-animal models where protein turnover rates are significantly greater than human subjects[72], current evidence would suggest that the overall contribution of muscle protein breakdown is likely small and transient, probably confined to the first few days post-immobilisation[73]; albeit this remains an area of significant controversy[74,75]. While such gross declines in activity would not be evident in a healthy recruited population, recent evidence has demonstrated that a decline and not necessarily cessation of daily activity is sufficient at inducing significant anabolic resistance in the elderly[76]. Reducing daily step-count by 76 % for 14 d, from a habitual level of about 5900 to about 1400 steps daily, was shown to reduce leg fat-free mass by 3·9 % and attenuate the rise in postprandial muscle protein synthesis rates by 26 %, independent of

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mTORc1 signalling. This highlights the need to carefully consider and monitor habitual physical activity levels in recruited subjects, especially as periods of reduced ambulatory activity are common in the elderly. It also highlights the utility of physical activity to maintain muscle mass and provides further credence to its use in this population.

Possible non-pharmacological treatment strategies

While the underlying aetiology for sarcopenia remains to be resolved, the current research has indicated several non-pharmacological approaches that may be applicable to stave off the age-dependent loss of muscle. A major area of focus has been on the use of protein and/or amino acid supplements to promote increased muscle anabolism. While there has been comprehensive discussion of the effectiveness of such approaches to impact on muscle mass, human metabolic studies have revealed several aspects that warrant consideration when developing effective supplementation approaches.

First and foremost, while protein supplementation may be beneficial for individuals not meeting protein intake requirements through habitual means, simply providing large single doses of protein with the premise that you can 'game the system' and stimulate anabolism above and beyond the response to standard protein intake is unrealistic. In response to prolonged intravenous infusions of amino acids, muscle protein synthesis is only elevated for the first 2 h of the infusion in young individuals[77]. Somewhat remarkably, rates of muscle protein synthesis subsequently return to post-absorptive values even in the face of persistently elevated blood amino acid concentrations[77]. This has led to the concept of the muscle full hypothesis, a ceiling upon which delivered amino acids are no longer incorporated into muscle protein but rather diverted towards oxidation[78]. Of concern, the ceiling at which this effect takes place appears equivalent in healthy young and older individuals, with 10 g essential amino acids able to maximally stimulate muscle protein synthesis in both groups, with lower absolute rates of myofibrillar protein synthesis seen in the elderly volunteers[33]. This highlights the potential futility of administering protein in large single doses to elderly individuals. The known properties of protein to impact on satiety are also of concern and it is perhaps not surprising that the use of nutritional supplements in the elderly has been associated with compromising subsequent food intake[79]. Therefore, the dose at which a protein supplement is administered requires consideration of the balance between providing sufficient substrate to avoid negative nitrogen balance while avoiding the potential to impact on food intake or promote the oxidation of administered amino acids.

At the same time, a certain level of hyperaminoacidaemia is required to adequately stimulate muscle protein synthesis. Pennings *et al.*, by orally administering to elderly men 20 g of either casein, casein hydrosylate, or whey protein on three separate occasions, with the known differences in digestion kinetics between the three proteins utilised, were able to demonstrate a

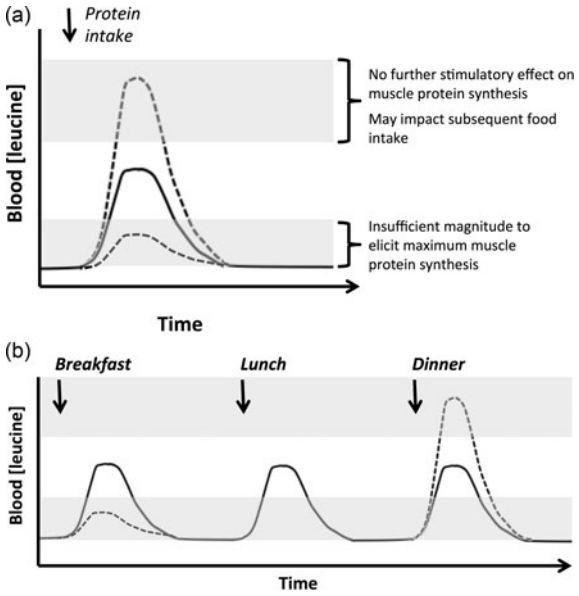

Fig. 2. (a) An illustrative example of the benefit of maintaining postprandial hyperleucinaemia within a set threshold. (b) The anticipated consequences of a skewed diurnal pattern of protein intake (- - -) *v.* a balanced approach to protein intake across meals (___) on blood leucine concentrations.

correlation between the peak hyperacidaemia elicited by the test drink and the relative increase in muscle protein synthesis observed[9]. Collectively, this suggests that an optimal window for the quantity of protein consumed in a single sitting exists, within which muscle protein synthesis is meaningfully stimulated while avoiding issues associated with overconsumption (Fig. 2).

Such a scenario also has ramifications for the effectiveness of the diurnal pattern in which protein is consumed in the Western world. With standard food habits, protein intake is typically orientated towards the evening meal with examination of the North American diet revealing breakfast containing approximately one-third of the amount of protein contained in the evening meal (13 *v.* 38 g protein, respectively[80]). The skewed manner in which protein is typically consumed may not be optimal, and as a consequence, impinge on the ability of elderly individuals to maintain sufficient anabolic potential to maintain muscle mass. When recreated experimentally over a 7-d period, protein intake spread equally throughout the day in three sets of about 30 g doses results in significantly greater 24 h muscle protein synthesis than 90 g split into 11, 16 and 63 g for the three daily meals[80]. This has potential implications for the timing of any protein supplementation strategy, suggesting that they are best consumed around low protein intake meals, most likely breakfast. Utilising such an approach would allow postprandial increase in blood amino acid concentrations to be within the proposed optimum operating window for muscle protein synthesis.

While the utility of resistance exercise to increase muscle mass in the elderly has already been introduced, one aspect where resistance exercise may prove particularly useful is in sensitising the muscle to subsequent amino

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acid ingestion. The incorporation of resistance exercise during periods of excess amino acid supply has been shown in healthy young individuals to result in greater net muscle protein balance than either resistance exercise or amino acid ingestion alone[81]. Moreover, a recent meta-analysis of the literature has demonstrated that protein supplementation is able to enhance both muscle mass and strength in response to chronic (>6 weeks) resistance exercise training[82]. Therefore, resistance exercise would appear to represent a useful strategy to raise the threshold before which circulating amino acids are no longer incorporated into muscle protein as described in the muscle full hypothesis. In support, Yang *et al.* by instructing elderly men to perform an acute bout of one-legged resistance exercise prior to the consumption of whey protein, demonstrated that muscle protein synthesis was greater in the exercised leg *v.* the non-exercised contralateral limb across a range of protein doses[83]. Moreover, when frail elderly subjects were supplemented with 15 g protein consumed twice daily during a 24-week progressive resistance exercise programme, significant improvements were observed in lean body mass, strength and physical performance[84]. Most notably, elderly adults administered with a placebo for the 24-week period in place of the protein supplement saw no detectable gains in muscle mass, albeit similar improvements in strength and physical performance to the protein group were recorded[84].

Reduced mobility and joint pain is a common complaint among the elderly, particularly in frail individuals. As a result, the muscle contractile forces that they can comfortably generate could be potentially compromised and not operating near the typically high-intensity (>70 % of 1-repitition maximum) contractions employed in resistance exercise programmes for the young. Of relevance to affected individuals, recent evidence suggests that high-volume low-intensity resistance exercise is just as effective at eliciting the same degree of muscle protein synthesis as low-volume high-intensity exercise. In particular, young subjects performing leg extension exercise at 30 % of 1-repitition maximum until failure were able to acutely stimulate muscle protein synthesis to the same degree as that observed when exercise was performed at the much higher intensity of 90 % 1-repitition maximum [85]. Likewise, comparable observations have been made in older individuals[86], thereby suggesting that increasing volume rather than intensity is an effective process by which to stimulate muscle protein synthesis in the elderly and would likely assist in encouraging compliance in this population.

Conclusions

The inability of habitual protein intake to maintain muscle mass in the elderly is potentially the culmination of a number of events impairing the muscle protein synthetic response to food. Current evidence suggests that protein supplementation may be able to overcome these issues, particularly when combined with resistance exercise programmes. The role that inactivity plays in the aetiology of sarcopenia remains unclear, but could transpire to be a major contributor and should be the focus of future work.

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Conflicts of Interest

None.

Authorship

A. J. M. was responsible for all aspects related to the authoring of this paper.

References

1. Rantanen T, Harris T, Leveille SG *et al.* (2000) Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. *J Gerontol A Biol Sci Med Sci* **55**, M168–M173.
2. Streja E, Molnar MZ, Kovesdy CP *et al.* (2011) Associations of pretransplant weight and muscle mass with mortality in renal transplant recipients. *Clin J Am Soc Nephrol* **6**, 1463–1473.
3. Han SS, Kim KW, Kim KI *et al.* (2010) Lean mass index: a better predictor of mortality than body mass index in elderly Asians. *J Am Geriatr Soc* **58**, 312–317.
4. Lee CG, Boyko EJ, Nielson CM *et al.* (2011) Mortality risk in older men associated with changes in weight, lean mass, and fat mass. *J Am Geriatr Soc* **59**, 233–240.
5. Kitamura I, Koda M, Otsuka R *et al.* (2014) Six-year longitudinal changes in body composition of middle-aged and elderly Japanese: age and sex differences in appendicular skeletal muscle mass. *Geriatr Gerontol Int* **14**, 354–361.
6. Lindle RS, Metter EJ, Lynch NA *et al.* (1997) Age and gender comparisons of muscle strength in 654 women and men aged 20–93 yr. *J Appl Physiol* **83**, 1581–1587.
7. Sayer AA (2010) Sarcopenia. *BMJ* **341**, c4097.
8. Heymsfield SB, Stevens V, Noel R *et al.* (1982) Biochemical composition of muscle in normal and semistarved human subjects: relevance to anthropometric measurements. *Am J Clin Nutr* **36**, 131–142.
9. Pennings B, Boirie Y, Senden JM *et al.* (2011) Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *Am J Clin Nutr* **93**, 997–1005.
10. Volpi E, Kobayashi H, Sheffield-Moore M *et al.* (2003) Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* **78**, 250–258.
11. Garlick PJ (2005) The role of leucine in the regulation of protein metabolism. *J Nutr* **135**, 1553S–1556S.
12. Fujita S, Rasmussen BB, Cadenas JG *et al.* (2006) Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. *Am J Physiol Endocrinol Metab* **291**, E745–E754.
13. Frost RA, Lang CH (2011) mTor signaling in skeletal muscle during sepsis and inflammation: where does it all go wrong? *Physiology* **26**, 83–96.

https://doi.org/10.1017/S0029665115000130 Published online by Cambridge University Press

14. Nader GA (2005) Molecular determinants of skeletal muscle mass: getting the "AKT" together. *Int J Biochem Cell Biol* **37**, 1985–1996.
15. Dickinson JM, Fry CS, Drummond MJ *et al.* (2011) Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. *J Nutr* **141**, 856–862.
16. Lecker SH, Jagoe RT, Gilbert A *et al.* (2004) Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J* **18**, 39–51.
17. Altun M, Besche HC, Overkleeft HS *et al.* (2010) Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. *J Biol Chem* **285**, 39597–39608.
18. Wilkes EA, Selby AL, Atherton PJ *et al.* (2009) Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *Am J Clin Nutr* **90**, 1343–1350.
19. Morley JE, Argiles JM, Evans WJ *et al.* (2010) Nutritional recommendations for the management of sarcopenia. *J Am Med Dir Assoc* **11**, 391–396.
20. Boyce JM, Shone GR (2006) Effects of ageing on smell and taste. *Postgrad Med J* **82**, 239–241.
21. Campbell WW, Trappe TA, Wolfe RR *et al.* (2001) The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci* **56**, M373–M380.
22. Campbell WW, Crim MC, Dallal GE *et al.* (1994) Increased protein requirements in elderly people: new data and retrospective reassessments. *Am J Clin Nutr* **60**, 501–509.
23. Houston DK, Nicklas BJ, Ding J *et al.* (2008) Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the health, aging, and body composition (Health ABC) study. *Am J Clin Nutr* **87**, 150–155.
24. Saffrey MJ (2014) Aging of the mammalian gastrointestinal tract: a complex organ system. *Age* **36**, 9603.
25. Pennings B, Pellikaan WF, Senden JM *et al.* (2011) The production of intrinsically labeled milk and meat protein is feasible and provides functional tools for human nutrition research. *J Dairy Sci* **94**, 4366–4373.
26. Koopman R, Walrand S, Beelen M *et al.* (2009) Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein synthetic response do not differ between young and elderly men. *J Nutr* **139**, 1707–1713.
27. Volpi E, Mittendorfer B, Wolf SE *et al.* (1999) Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* **277**, E513–E520.
28. Boirie Y, Gachon P, Beaufrere B (1997) Splanchnic and whole-body leucine kinetics in young and elderly men. *Am J Clin Nutr* **65**, 489–495.
29. Pennings B, Groen BB, van Dijk JW *et al.* (2013) Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. *Am J Clin Nutr* **98**, 121–128.
30. Gilani GS, Sepehr E (2003) Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. *J Nutr* **133**, 220–225.
31. Gilani GS, Cockell KA, Sepehr E (2005) Effects of antinutritional factors on protein digestibility and amino acid availability in foods. *J AOAC Int* **88**, 967–987.
32. Guillet C, Prod'homme M, Balage M *et al.* (2004) Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J* **18**, 1586–1587.
33. Cuthbertson D, Smith K, Babraj J *et al.* (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* **19**, 422–424.
34. Vollenweider P, Tappy L, Randin D *et al.* (1993) Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J Clin Invest* **92**, 147–154.
35. Laakso M, Edelman SV, Brechtel G *et al.* (1990) Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest* **85**, 1844–1852.
36. Karakelides H, Irving BA, Short KR *et al.* (2010) Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. *Diabetes* **59**, 89–97.
37. Meneilly GS, Elliot T, Bryer-Ash M *et al.* (1995) Insulin-mediated increase in blood flow is impaired in the elderly. *J Clin Endocrinol Metab* **80**, 1899–1903.
38. Volpi E, Mittendorfer B, Rasmussen BB *et al.* (2000) The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab* **85**, 4481–4490.
39. Phillips B, Williams J, Atherton P *et al.* (2012) Resistance exercise training improves age-related declines in leg vascular conductance and rejuvenates acute leg blood flow responses to feeding and exercise. *J Appl Physiol* **112**, 347–353.
40. Steinberg HO, Brechtel G, Johnson A *et al.* (1994) Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J Clin Invest* **94**, 1172–1179.
41. Dillon EL, Casperson SL, Durham WJ *et al.* (2011) Muscle protein metabolism responds similarly to exogenous amino acids in healthy younger and older adults during NO-induced hyperemia. *Am J Physiol Regul Integr Comp Physiol* **301**, R1408–R1417.
42. Kelly J, Fulford J, Vanhatalo A *et al.* (2013) Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults. *Am J Physiol Regul Integr Comp Physiol* **304**, R73–R83.
43. Joris PJ, Mensink RP (2013) Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal. *Atherosclerosis* **231**, 78–83.
44. Paddon-Jones D, Borsheim E, Wolfe RR (2004) Potential ergogenic effects of arginine and creatine supplementation. *J Nutr* **134**, 2888S–2894S; discussion 95S.
45. Tang JE, Lysecki PJ, Manolakos JJ *et al.* (2011) Bolus arginine supplementation affects neither muscle blood flow nor muscle protein synthesis in young men at rest or after resistance exercise. *J Nutr* **141**, 195–200.
46. Churchward-Venne TA, Cotie LM, MacDonald MJ *et al.* (2014) Citrulline does not enhance blood flow, microvascular circulation, or myofibrillar protein synthesis in elderly men at rest or following exercise. *Am J Physiol Endocrinol Metab* **307**, E71–E83.
47. Donato AJ, Uberoi A, Wray DW *et al.* (2006) Differential effects of aging on limb blood flow in humans. *Am J Physiol Heart Circ Physiol* **290**, H272–H278.
48. Poole JG, Lawrenson L, Kim J *et al.* (2003) Vascular and metabolic response to cycle exercise in sedentary humans: effect of age. *Am J Physiol Heart Circ Physiol* **284**, H1251–H1259.
49. Fiatarone MA, O'Neill EF, Ryan ND *et al.* (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* **330**, 1769–1775.

https://doi.org/10.1017/S0029665115000130 Published online by Cambridge University Press

50. Atherton PJ, Smith K, Etheridge T *et al.* (2010) Distinct anabolic signalling responses to amino acids in C2C12 skeletal muscle cells. *Amino Acids* **38**, 1533–1539.
51. Borsheim E, Kobayashi H, Traber DL *et al.* (2006) Compartmental distribution of amino acids during hemodialysis-induced hypoaminoacidemia. *Am J Physiol Endocrinol Metab* **290**, E643–E652.
52. Bohe J, Low A, Wolfe RR *et al.* (2003) Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose–response study. *J Physiol* **552**, 315–324.
53. Tipton KD, Ferrando AA, Phillips SM *et al.* (1999) Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* **276**, E628–E634.
54. Ogmundsdottir MH, Heublein S, Kazi S *et al.* (2012) Proton-assisted amino acid transporter PAT1 complexes with Rag GTPases and activates TORC1 on late endosomal and lysosomal membranes. *PLoS ONE* **7**, e36616.
55. Heublein S, Kazi S, Ogmundsdottir MH *et al.* (2010) Proton-assisted amino-acid transporters are conserved regulators of proliferation and amino-acid-dependent mTORC1 activation. *Oncogene* **29**, 4068–4079.
56. Pinilla J, Aledo JC, Cwiklinski E *et al.* (2011) SNAT2 transceptor signalling via mTOR: a role in cell growth and proliferation? *Front Biosci* **3**, 1289–1299.
57. Drummond MJ, Glynn EL, Fry CS *et al.* (2010) An increase in essential amino acid availability upregulates amino acid transporter expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* **298**, E1011–E1018.
58. Bergstrom J, Furst P, Vinnars E (1990) Effect of a test meal, without and with protein, on muscle and plasma free amino acids. *Clin Sci (Lond)* **79**, 331–337.
59. Walker DK, Drummond MJ, Dickinson JM *et al.* (2014) Insulin increases mRNA abundance of the amino acid transporter SLC7A5/LAT1 via an mTORC1-dependent mechanism in skeletal muscle cells. *Physiol Rep* **2**, e00238.
60. Wolfe RR, Miller SL (1999) Amino acid availability controls muscle protein metabolism. *Diab Nutr Metab* **12**, 322–328.
61. Bodine SC, Stitt TN, Gonzalez M *et al.* (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* **3**, 1014–1019.
62. Greenhaff PL, Karagounis L, Peirce N *et al.* (2008) Disassociation between the effects of amino acids and insulin on signalling, ubiquitin-ligases and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* **295**, E595–E604.
63. Drummond MJ, Dickinson JM, Fry CS *et al.* (2012) Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults. *Am J Physiol Endocrinol Metab* **302**, E1113–E1122.
64. Symons TB, Schutzler SE, Cocke TL *et al.* (2007) Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* **86**, 451–456.
65. Burd NA, Wall BT, van Loon LJ (2012) The curious case of anabolic resistance: old wives' tales or new fables? *J Appl Physiol* **112**, 1233–1235.
66. Frontera WR, Hughes VA, Fielding RA *et al.* (2000) Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol* **88**, 1321–1326.
67. Drummond MJ, Dreyer HC, Pennings B *et al.* (2008) Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. *J Appl Physiol* **104**, 1452–1461.
68. Robinson MM, Turner SM, Hellerstein MK *et al.* (2011) Long-term synthesis rates of skeletal muscle DNA and protein are higher during aerobic training in older humans than in sedentary young subjects but are not altered by protein supplementation. *FASEB J* **25**, 3240–3249.
69. Wilkinson DJ, Franchi MV, Brook MS *et al.* (2014) A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *Am J Physiol Endocrinol Metab* **306**, E571–E579.
70. Glover EI, Phillips SM, Oates BR *et al.* (2008) Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. *J Physiol* **586**, 6049–6061.
71. Wall BT, Snijders T, Senden JM *et al.* (2013) Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men. *J Clin Endocrinol Metab* **98**, 4872–4881.
72. Rennie MJ, Selby A, Atherton P *et al.* (2010) Facts, noise and wishful thinking: muscle protein turnover in aging and human disuse atrophy. *Scand J Med Sci Sports* **20**, 5–9.
73. Glover EI, Yasuda N, Tarnopolsky MA *et al.* (2010) Little change in markers of protein breakdown and oxidative stress in humans in immobilization-induced skeletal muscle atrophy. *Appl Physiol Nutr Metab* **35**, 125–133.
74. Phillips SM, McGlory C (2014) Rebuttal from Stuart M. Phillips and Chris McGlory. *J Physiol* **592**, 5349.
75. Reid MB, Judge AR, Bodine SC (2014) CrossTalk opposing view: the dominant mechanism causing disuse muscle atrophy is proteolysis. *J Physiol* **592**, 5345–5347.
76. Breen L, Stokes KA, Churchward-Venne TA *et al.* (2013) Two weeks of reduced activity decreases leg lean mass and induces 'anabolic resistance' of myofibrillar protein synthesis in healthy elderly. *J Clin Endocrinol Metab* **98**, 2604–2612.
77. Bohe J, Low JF, Wolfe RR *et al.* (2001) Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* **532**, 575–579.
78. Millward DJ, Pacy PJ (1995) Postprandial protein utilization and protein quality assessment in man. *Clin Sci (Lond)* **88**, 597–606.
79. Fiatarone Singh MA, Bernstein MA, Ryan AD *et al.* (2000) The effect of oral nutritional supplements on habitual dietary quality and quantity in frail elders. *J Nutr Health Aging* **4**, 5–12.
80. Mamerow MM, Mettler JA, English KL *et al.* (2014) Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. *J Nutr* **144**, 876–880.
81. Wolfe RR (2006) Skeletal muscle protein metabolism and resistance exercise. *J Nutr* **136**, 525S–528S.
82. Cermak NM, Res PT, de Groot LC *et al.* (2012) Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr* **96**, 1454–1464.
83. Yang Y, Breen L, Burd NA *et al.* (2012) Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* **108**, 1780–1788.
84. Tieland M, Dirks ML, van der Zwaluw N *et al.* (2012) Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* **13**, 713–719.
85. Burd NA, West DW, Staples AW *et al.* (2010) Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE* **5**, e12033.
86. Kumar V, Selby A, Rankin D *et al.* (2009) Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol* **587**, 211–217.

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