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REVIEW ARTICLE

Dietary proteins and amino acids in the control of the muscle mass during immobilization and aging: role of the MPS response

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Abstract Dietary proteins/essential amino acids (EAAs) are nutrients with anabolic properties that may increase muscle mass or attenuate muscle loss during immobilization and aging via the stimulation of muscle protein synthesis (MPS). An EAA's anabolic threshold, capable to maximize the stimulation of MPS has been hypothesized, but during certain conditions associated with muscle loss, this anabolic threshold seems to increase which reduces the efficacy of dietary EAAs to stimulate MPS. Preliminary studies have demonstrated that acute ingestion of dietary proteins/EAA (with a sufficient amount of leucine) was capable to restore the postprandial MPS during bed rest, immobilization or aging; however, whether these improvements translate into chronic increases (or attenuates loss) of muscle mass is equivocal. For example, although free leucine supplementation acutely increases MPS and muscle mass in some chronic studies, other studies have reported no increases in muscle mass following chronic leucine supplementation. In contrast, chronically increasing leucine intake via the consumption of an overall increase in dietary protein appears to be the most effective dietary intervention toward increasing or attenuating lean mass during aging; however, more research investigating the optimal dose and timing of protein ingestion is necessary. Several studies have demonstrated that decreases in postprandial MPS as a result of increased circulating oxidative and inflammatory are more responsible than muscle protein breakdown for the decreases in muscle mass during disuse and health aging. Therefore, nutritional interventions that reduce oxidation or inflammation in conjunction with higher protein intakes that overcome the anabolic resistance may enhance the MPS response to feeding and either increase muscle mass or attenuate loss. In preliminary studies, antioxidant vitamins and amino acids with antioxidant or anti-inflammatory properties show potential to restore the anabolic response associated with protein ingestion. More research, however, is required to investigate if these nutrients translate to increases in MPS and, ultimately, increased lean mass in aging humans. The purpose of the present review is to discuss the role of protein/EAA intake to enhance postprandial MPS during conditions associated with muscle loss, and bring new perspectives and challenges associated nutritional interventions aimed to optimize the anabolic effects of dietary protein/EAAs ingestion.

Keywords Proteins · Betaine · Amino acids · Essential amino acids · Leucine · Glycine · Postprandial muscle protein synthesis · Atrophy · Hypertrophy

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Introduction

Proteins and essential amino acids (EAAs) are of the most popularly supplemented nutrients worldwide, especially

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among individuals who seek to increase muscle mass. Although United States National Academy of Medicine's recommendation for protein intake as part of a balanced diet expected to meet the needs of 97% of the population is 0.83 g/kg/day, dietary proteins consumed in amounts threefold higher than the recommended dietary allowance have been shown to be safe (no changes in blood lipids or markers of hepatic or renal function) and effective at increasing lean mass (Antonio et al. 2015). The main mechanism associated with increases in muscle protein accretion as a result of postprandial protein ingestion is an alteration in the cellular balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB), such that MPS is favoured. Both the quantity of protein consumed and the composition/quality of the protein source, such as EAA and leucine content, will affect the postprandial MPS (for review see: Guimarães-Ferreira et al. 2014). Varying physio-pathological conditions will also alter the MPS response to protein consumption. For example, it has been repeatedly demonstrated that postprandial MPS after a resistance training session is enhanced via EAA ingestion (Atherton and Smith 2012). The opposite seems to occur during certain muscle disuse conditions in which the acute consumption of dietary proteins fails to maximally stimulate postprandial MPS (Phillips et al. 2009). Since muscle disuse (over different clinical manifestations such as bed rest, immobilization and/or sarcopenia) leads to reductions in basal metabolic rate, glucose disposal, muscular strength, and increases in morbidity and mortality (Kim and Choi 2013), new strategies aiming to enhance both the acute response and chronic adaptations to protein ingestion are of interest to basic scientists and practitioners. This review discusses protein metabolism in the regulation of muscle mass during catabolic conditions with a special emphasis on how dietary protein and amino acid intake impacts postprandial MPS and muscle protein accretion during periods of bed rest, immobilization or aging. Also, we will present how amino acid and antioxidant interventions may improve the preservation of muscle mass during catabolic states.

Muscle disuse as a general descriptor of different clinical manifestations associated with muscle atrophy and decreased MPS

Muscle disuse is part of a central paradigm explaining losses in muscle mass during several common conditions such as cast immobilization, extended bed rest or reduced ambulation. In the literature, this is termed "simple atrophy" as the absence of a disease-associated state limits the muscle atrophy process to the affected limb, rather than systemically (Atherton et al. 2016; Bell et al. 2016). Thus, disuse is a process intrinsic to the muscles that are exposed to it (Atherton et al. 2016). Although episodic periods of disuse such as limb immobilization or bed rest clearly accelerate the loss of muscle mass and strength in young and older adults (Kim and Choi 2013), it is difficult to identify what components are intrinsic from muscle atrophy and what components are derived from disease-related atrophy aspects. As an example, in humans, immobilization results in the development of whole body and muscle insulin resistance (MIR) within 3–5 days (Hamburg et al. 2007; Sonne et al. 2011; Stuart et al. 1988). Also, certain models of partial physical inactivity or "moderate muscle disuse", such as reduced ambulatory activity, induce MIR after just 3–14 days (Hamburg et al. 2007; Krogh-Madsen et al. 2010; Olsen et al. 2008). The impact that insulin resistance has on muscle protein metabolism following different catabolic conditions is not clear, especially in humans. In obese subjects for example, a marked blunting in the leg glucose disposal (under insulin clamp) is seen but this event was not linked to lower lean tissue mass compared with their age-matched counterparts (Atherton et al. 2016). Corroborating the absence of direct link between muscle mass and insulin resistance, during a period of reduced ambulation, insulin resistance develops rapidly in younger men and precedes any measurable decreases in lean mass or increases in adipose tissue mass (Knudsen et al. 2012). In rodents, a more direct relationship seems to occur. In this regard, Wang et al. (2006) studied the impact of obesity and MIR on skeletal muscle proteolysis. In muscle of insulin-resistant db/db mice (a routinely used model of type 2 diabetes in rodents), protein degradation and activities of the major proteolytic systems were increased. Interestingly, treatment with the hypoglycemic agent rosiglitazone improved insulin resistance and decreased activities of major proteolytic systems (namely caspase-3 and the proteasome) in muscle, leading to a normalization of proteolysis. However, there was only partial recovery of the cross-sectional area and muscle mass. The authors suggested that the recovery may not have been complete because rosiglitazone did not stimulate protein synthesis. Collectively, such information suggests that insulin resistance may increase muscle proteolysis and impair muscle protein synthesis but this has not been observed in human models of disuse. The degree of insulin resistance may be predictive of muscle losses associated with muscle atrophy, but this hypothesis requires further examination.

Another important condition associated with muscle atrophy and reductions in physical activity is the aging process. Aging is accompanied by a slow and inevitable age-related decline in skeletal muscle mass referred to as sarcopenia. Sarcopenia, in turn, contributes to increased risk of falls and fractures in older individuals, as well to the incidence of metabolic disorders like type 2 diabetes mellitus. As with muscle disuse, investigators have tried

to categorize muscle sarcopenia to better understand this phenomenon. For example, it has been suggested that the term "primary sarcopenia" (or age-related) should be used to define sarcopenia that is caused by aging itself. On the other hand, the term "secondary sarcopenia" has been used to describe sarcopenia that is caused by additional disuse, disease, and/or inadequate nutrition and malabsorption (Janssen 2011; Cruz-Jentoft et al. 2010). In our view, it can be very difficult to separate primary sarcopenia from secondary sarcopenia in a given older person. During conditions of primary sarcopenia, co-morbidities often develop that lead to reduced physical activity and periods of bed rest (i.e.: secondary sarcopenia), which may further the development of an increased production of catabolic hormones such as cortisol, proinflammatory molecules such as cytokines, and increased ROS (reactive oxygen species) formation, which may result in additional decreases in MPS or increases in MPB (Santilli et al. 2014; Brocca et al. 2012). Since not all aged persons are sarcopenic, the term "healthy" elderly individuals or non-sarcopenic older adults are also widely used in the literature to describe subjects without significant muscle loss or disuse-associated diseases. Therefore, this paper will only classify between non-sarcopenic and sarcopenic older adults, referred to as "healthy" and "sarcopenic", respectively.

Basal protein turnover in the regulation of muscle mass

The term "proteostasis" (the fusion of the words protein and homeostasis), was first coined to designate the balance between synthetic (biogenesis, folding, trafficking and export) and proteolytic (degradation of proteins present within and outside the cell) pathways integrating the protein metabolism in a cell (Powers et al. 2009). At the macroscopic level, the term proteostasis strictly relates to protein metabolism and can be interpreted as the balance between the rates of MPS and MPB, the main determinants of skeletal muscle turnover. Although imbalances in the basal (postabsorptive) rates of protein metabolism in favour of increased MPB are responsible for decreases in muscle mass during several disuse-induced atrophies (sepsis, cancer, heart failure, glucocorticoid treatment), it is becoming clear that during conditions such as immobilization, extended bed-rest or sarcopenia, a basal increase in MPB may not be observed (Dardevet et al. 2000; Phillips et al. 2009).

In this regard, markers of basal MPS do not seem to be affected by 24 days of bed rest (Brocca et al. 2012) or 14 days of step-reduction (Breen et al. 2013). On the other hand, a more prolonged immobilization period seems to reduce basal MPS in the immobilized leg, compared to the control leg (Gibson et al. 1987; Glover et al. 2009). This suggests that basal MPS may or may not be reduced during disuse inducing muscle atrophy, likely depending on if muscle disuse is induced for a short or long term. Another recurrent finding from disuse studies is a reduction in the fed-state gains in muscle protein synthesis in response to amino acid infusion (Glover et al. 2009) or following the ingestion of protein (Wall et al. 2013), a condition termed "anabolic resistance". Considering that the two main determinants of MPS (postabsorptive and postprandial), are reduced under different muscle disuse conditions, is correct to consider that a chronic daily reduction in MPS is the major determinant of skeletal muscle atrophy during disuse. In this regard, it has been estimated (based on acute measurements) that reductions to the magnitude of 40–50% are expected to occur both in early (10 days or less) and later (beyond 10 days) disuse (Crossland et al. 2010). In addition, it seems that MPB is slightly elevated in the early disuse, but in late periods, MPB seems to be adaptively reduced, which explains why muscle atrophy is less in chronic observations compared to acute models of disuse (Baehr et al. 2016). Thus, considering that MPB is not robustly affected during periods of muscle disuse, the loss of lean tissue due to reduced activity appears to be primarily driven by both a reduced basal MPS and a blunting of MPS response to the ingestion of dietary protein. Corroborating such information, in a recent study, Wall et al. (2013) have reported that during reduced ambulation, fed-state gains in muscle protein are reduced and that fasted losses remain the same (or are slightly increased transiently), with a large part of the muscle atrophy being due to a reduction in the fed-state MPS response. A similar phenomenon seems to occur during healthy aging. Compared to healthy young adults, decrements in the postabsorptive MPS are barely detectable in the healthy elderly (Breen et al. 2012). In contrast, MPS becomes partially insensitive to the stimulating effect of a normal protein meal, thus partially explaining why the muscle mass is eroded over time during the aging process (Dardevet et al. 2012).

Role of decreased postprandial MPS in the regulation of the muscle mass during aging and muscle disuse

The switch from a fasted to a fed state results in changes in MPS that are 10–20-fold greater than any measured change in MPB (Tang and Phillips 2009). In catabolic conditions where the postabsorptive rates of MPB are not increased, this specific but robust regulation carries the potential to be a major target for the metabolic control of muscle mass. Since the human organism is under the influence of food intake for 40% of the day, it is predictable that the quantitative and qualitative characteristics of the food intake will strongly condition the maintenance of muscle mass. In healthy conditions, a standard dose of proteins (20–25 g composed by 8–9 g of EAA with 2.5–3.5 g of leucine)

is sufficient to maximally increase MPS in young men (Moore et al. 2015. In states of muscle atrophy, however, this stereotypic response has been shown to be altered. With a quadriceps immobilization, for example, a "resistance" of MPS to amino acids has been demonstrated, which appears to explain one half of the muscle loss (Glover et al. 2008).

The intrinsic reasons why the skeletal muscle presents anabolic resistance in some physio-pathological conditions are still unclear. In nature, not all animals exposed to a prolonged physical inactivity present increased muscle atrophy associated to muscle disuse. In bears, for example, hibernating periods are linked to a diminishment in the adipose tissue *reservoir* with no major muscle loss, even under extreme starvation (Harlow et al. 2001; Fuster et al. 2007). In rodents, on the other hand, in models mimicking human-based immobilization, a marked increase in MPB is seen (together with decreased MPS rates) (Krawiec et al. 2005). Thus, across species, a general mechanism explaining how muscle disuse induces muscle atrophy does not seem to exist. In humans, however, as previously described, reductions in MPS seem to contribute to the majority of muscle loss during disuse.

The dampened MPS response to feeding occurs during aging in humans, although the causative mechanisms are still unknown. Two possible factors linked to anabolic resistance in the aging muscle are a gradual decline in physical activity or an age-related increase in inflammation, especially low-grade inflammation. In this regard, Balage et al. (2010) demonstrated in old rats that the presence of low-grade inflammation did not change postabsorptive MPS but robustly blunted the postprandial MPS. In humans, Toth et al. (2005) demonstrated, in elderly subjects, increased circulating concentrations of several markers of immune activation which were related to reduced MPS rates. The reason why decreased MPS, and not increased MPB, is the major determinant of muscle mass loss during disuse and the aging process requires further investigation. One possible explanation is that there are significantly less circulating stimulants of muscle proteolysis (hypercortisolemia, inflammation, ROS) in healthy older adults compared to what has been observed during diseases like cancer cachexia, sepsis, infections, chronic heart failure, or uncontrolled diabetes (Fuster et al. 2007; Crossland et al. 2008; Crossland et al. 2010).

Role of dietary proteins and leucine on postprandial MPS

Considering that the stimulation of MPS is dependent on both the dose and quality of dietary proteins/EAAs and the muscle sensitivity to amino acid changes, it is interesting to consider how to deal with such a variety of factors to maximize postprandial MPS. In the case of both muscle disuse and aging, it has been postulated that increasing the amount of dietary proteins (or specific amino acids) in each meal would be enough to overcome the anabolic resistance in skeletal muscle. In this respect, it has been observed that the difference in the MPS response to food intake between adult and old rats was explained by the dose–response curves of leucine action in incubated epitrochlearis muscles (Dardevet et al. 2000). Specifically, MPS in vitro still responded to leucine in old rats but at leucine concentrations two- to threefold greater than in healthy adult rats. In elderly subjects, a similar phenomenon has been observed with an EAA supplementation mimicking a whey protein dosage of 15 g, which increased postprandial MPS but to a lesser extent when compared to young adults (Katsanos et al. 2006). During bed-rest immobilization in elderly subjects, Ferrando et al. (2010) reported the maintenance of the 24-h FSR (fractional synthetic rate, an index of MPS) with a supplementation of EAAs (15 g, 3× per day), whereas a significant decrease in FSR occurred in the non-supplemented control group (Ferrando et al. 2010). This evidence demonstrates that, at least acutely, protein doses must be doubled during the aging process to achieve a substantial MPS response.

The beneficial effect of such protein/EAA supplementation on muscle mass in the long term is less clear since there is a lack of consistent evidence in humans. In aged animals, an increased leucine intake for several days under free leucine supplementation or whey proteins (Rieu et al. 2003, 2007) was capable to restore MPS chronically; however, there were no changes in muscle mass (Rieu et al. 2007). Similar observations have been made by Verhoeven et al. (2009) with long-term leucine supplementation which did not increase muscle mass or strength in healthy elderly men. During bed-rest conditions, a leucine-supplemented diet (0.06 g/kg/meal) has also been shown to protect against muscle loss after 7 days, but not after 14 days in middle-aged subjects (English et al. 2016). However, several reports related that muscle atrophy during short-term (28 days) and long-term bed rest (60 days) (Trappe et al. 2007a, b, 2008) failed to be impacted by daily AA supplementation or by a daily leucine-enriched whey protein supplement, respectively. On the other hand, several recent investigations have demonstrated positive effects of protein/AA supplementation not only on MPS but also on lean mass tissue. In a recent investigation, Murphy et al. (2016) observed in healthy old subjects that leucine supplementation increased the integrated MPS response. However, this effect was observed only in the group who consumed low amounts of dietary proteins. In a recent study, the relationship between dietary protein and leucine consumption and lean mass was assessed over 6 years among younger and older adult Danes (aged 35–65 years). In this study, adults over 65 years of age consumed less protein and leucine

than those of 35–55 years of age. Moreover, protein and leucine intake was associated with positive LBM change in those older than 65 years, with no effect seen in those younger than 55 years. Older participants in the highest leucine intake (7 × 1 g/day) experienced LBM maintenance, whereas lower intakes were associated with LBM loss over 6 years (McDonald et al. 2016).

It is not clear why chronic leucine supplementation, despite its powerful effects on acute MPS, sometimes translates and sometimes does not translate into increased muscle mass when chronically evaluated. Several possible mechanisms have been postulated, including a "desynchronization effect" of leucine (Dardevet et al. 2012). It seems that leucine excess can stimulate key enzymes in BCAA catabolism (BCAA aminotransferase and BCKA dehydrogenase), thus increasing the oxidation of serum leucine. Excess leucine intake can also decrease the plasma concentration of the other EAAs such as valine and isoleucine, an effect called antagonism of BCAAs (May et al. 1991). More research is currently needed to confirm the "desynchronization effect" hypothesis. Collectively, however, these results in addition to the results of Murphy et al. (2016) discussed above suggest that the efficacy of leucine (in the isolated form) to increase muscle mass mainly occurs when a low-protein diet is present, and consuming more leucine through additional dietary protein is likely the most effective dietary intervention to increase lean mass.

Indeed, a recent consensus statement suggests that a protein intake above the RDA may be of benefit to the preservation of lean mass in healthy older adults (Bauer et al. 2013). However, the ideal strategy of optimal distribution of protein in meals and total daily protein consumption are poorly defined. To address these questions, Norton et al. (2016) provided a high-quality protein supplement (1.6 g/kg/d in the supplemented group versus 1.2 g/kg/day in the control group) for 24 weeks to healthy, independent-living older adults. Supplemental protein, equivalent to 0.33 g protein/kg per day, was consumed in two equal parts with the lower protein-containing meals of the day (i.e., breakfast and lunch). As a result of the intervention, protein intakes in the protein (PRO) group increased to ~0.4 g/kg per meal. Leucine intakes at breakfast and midday and evening meals increased to 1.8, 1.9, and 1.5 g, respectively, and were closer to the 3 g required to maximally stimulate MPS in older adults (Paddon-Jones and Rasmussen 2009). Total lean mass and appendicular lean mass increased by 0.45 and 0.28 kg, respectively, in the protein-supplemented group but not in the control group. These results suggest that an optimized dose and balanced distribution (in the levels herein tested) of protein intake between meals could be beneficial in the preservation of lean mass in the aging population.

As previously discussed, an important mechanism explaining losses in the muscle mass during aging or immobilization seems to be a reduced MPS response to protein feeding. Thus, identifying factors that lead to a reduced efficacy of dietary amino acids to stimulate MPS is of importance. One potential mediator of reduced MPS and subsequent smaller increases in lean mass in response to protein ingestion is through the large neutral amino acid transporter 1 (LAT1) which preferentially transports leucine and the other branched chain amino acids into the cell, and, together with the system A amino acid transporter (SNAT2/SLC38A2), has been shown to activate mTORC1 (Evans et al. 2007). LAT1 expression and activity have been shown to decrease in the postprandial but not postabsorptive state following a 7-day bed-rest period in older adults (Drummond et al. 2012). Although changes in the postabsorptive expression of the LAT1 and SNAT2 have not been observed between young adult (30 ± 2 years) and older (70 ± 2 years) subjects, SNAT2 expression and activity were reduced in response to post-exercise protein ingestion in older compared to younger subjects (Dickinson et al. 2013). Thus, a dampening of LAT1 and SNAT2 and, therefore, a reduced transport of amino acids into the muscle cell, in response to protein ingestion could partially contribute to lower postprandial MPS and reduced increases in lean mass as a result of aging. However, further research examining age-related differences in amino acid transporter responses to protein ingestion in the absence of exercise is required to substantiate this hypothesis.

Adding stimulatory co-factors to enhance postprandial MPS

Besides leucine, BCAA, EAA supplementation or increased quantity of dietary protein, another possibility to increase the postprandial MPS is to re-sensitize the protein synthetic pathways with co-factors bearing antioxidant properties. The main rationale of such strategy is that many muscle-wasting conditions, including immobilization, aging, cancer cachexia, and sepsis are associated with a proinflammatory and/or pro-oxidative environment. Although the causes are specific for each catabolic state, the overproduction of proinflammatory cytokines and ROS is believed to play a central role in impaired muscle protein turnover (Balage et al. 2010; Ham et al. 2014; Cuthbertson et al. 2005) as proteins involved in the antioxidant defence system (superoxide dismutase, carbonic anhydrase III, peroxiredoxin 3, α,β-crystallin, heat shock protein B6, heat shock protein B1 and heat shock protein 70) were all downregulated following 8 and 35 days of bed rest in the vastus lateralis (Brocca et al. 2012). Specifically, inflammation (NF-kappaB) and ROS inhibit the activity of important enzymes involved in protein translation initiation, namely

mTOR and p70 S6 kinase 1 (S6K1) (Frost and Lang 2011) and enhance protein degradation through the ubiquitin proteasome system (Li et al. 2003). However, since makers of MPB and autophagy were not upregulated following bed rest (Brocca et al. 2012), reductions in MPS due appear to have the greatest influence on muscle atrophy during disuse. Therefore, consuming or supplementing with nutrients that reduce oxidative stress and/or inflammation combined with increased protein intake is a promising nutritional intervention to overcome anabolic resistance and maintain muscle mass during disuse and aging.

Since oxygen-derived free radicals are involved in several catabolic states, a rational strategy to decrease ROS production and increase postprandial MPS could be antioxidant supplementation. To test this hypothesis, Marzani et al. (2008) supplemented old rats for 7 weeks with a diet containing antioxidants (a mixture containing rutin, vitamin E, vitamin A, zinc, and selenium) and compared the MPS response to increasing leucine concentrations. As expected, in old rats, the ability of leucine to stimulate muscle protein synthesis was significantly decreased compared to young adults. However, this defect was reversed when old rats were supplemented with antioxidants. Following 8 days of immobilization in rats, muscle mass recovery during the next 40 days was accelerated by the antioxidant diet plus leucine supplementation due to higher MPS rates in both the postabsorptive and postprandial states (Savary-Auzeloux et al. 2013). Summing up, antioxidant supplementation seems to benefit postprandial MPS stimulation during both immobilization and aging conditions, but more research is required to test if this strategy will translate to humans.

Another co-factor with antioxidant properties that may enhance the MPS response to feeding is *N*-acetylcysteine (NAC). NAC comprises a cysteine with an acetyl group attached to the nitrogen atom and is used to treat a variety of conditions marked by increased oxidative stress (Tse and Tseng 2014). NAC appears to protect against oxidative stress by scavenging ROS and providing cysteine for glutathione synthesis (Zafarullah et al. 2003). NAC has been shown to protect the sarcoplasmic reticulum and myofibrils in *ryr* zebra fish against oxidative stress-induced swelling and myofibril disruption, respectively (Dowling et al. 2012). In an acute model of muscle disuse, adult mice were placed on a mechanical ventilator for 24 h resulting in a state of diaphragmatic oxidative stress and proteolysis, and treatment with 150 mg/kg NAC prevented oxidative stress and markers of proteolysis in diaphragm muscle fibres. In contrast, adult mice were exposed to 11 days of hind-limb suspension and treated with a 1% NAC-enriched diet. NAC prevented an increase in NF-kappaB but did not attenuate muscle atrophy or functional decline (Farid et al. 2005). The effects of NAC on MPS following feeding have yet to be studied, and, thus, warrants further research.

In addition to antioxidants, amino acids (without anabolic properties) have also been studied to improve the postprandial MPS response. For example, the nonessential amino acid glycine (see Wang et al. 2013 for review) is often considered biologically neutral and sometimes used as an isonitrogenous control in supplementation studies (Ham et al. 2016). Evidence that glycine has profound inhibitory effects on inflammatory cell activation has accumulated (Zhong et al. 2003), and has been hypothesized to enhance MPS in inflammatory conditions. In rats treated with LPS (lipopolysaccharide), glycine supplementation was capable to restore the postprandial MPS response to leucine feeding. The improvement in protein metabolism was associated with a reduction in skeletal muscle ROS (mainly superoxide anions) but did not alter skeletal muscle inflammatory signalling in vivo or in vitro (Ham et al. 2016). While glycine may be a promising nutrient in the treatment of attenuation disuse atrophy, further investigations should focus on whether these increases in MPS translate to improvements in muscle mass.

A final nutrient that merits further research as an agent that may enhance the MPS response is trimethylated glycine (betaine). Betaine is a naturally occurring compound found in dark green leafy vegetables, beets, and whole wheat. In humans, higher consumptions and greater concentrations of plasma betaine are associated with lower markers of inflammation (Detopoulou et al. 2008) and betaine treatment has been shown to reduce NF-kappaB expression in mice (Lee et al. 2013). In young adults, betaine supplementation has been shown to increase lean mass in conjunction with resistance training (Cholewa et al. 2013) possibly due to enhanced Akt/PKB signalling (Apicella et al. 2012). In particular, elevated plasma homocysteine and its cyclized derivative, homocysteine thiolactone, directly inhibit insulin-mediated Akt/PKB and p70 S6K phosphorylation (Najib and Sánchez-Margalet 2005) and diminish myogenic satellite cell regenerative capabilities by increasing oxidative stress and p38-MAPK (Veeranki et al. 2015). Increasing plasma homocysteine has been associated with the development of sarcopenia (Park and Georgiades 2013) and betaine supplementation has been shown to reduce homocysteine and homocysteine thiolactone in young and older adults (Cholewa et al. 2014). Therefore, betaine supplementation may protect against homocysteine-induced inhibitions in protein synthesis and possibly attenuate sarcopenia; however, further research in both animal models of aging and translational research in humans is needed to test this hypothesis.

Given the partial failure of dietary amino acid administration as a strategy to overcome anabolic resistance and attenuate muscle wasting, glycine or an antioxidant-rich diet plus leucine/protein consumption are promising strategies to combat muscle wasting in states where anabolic resistance

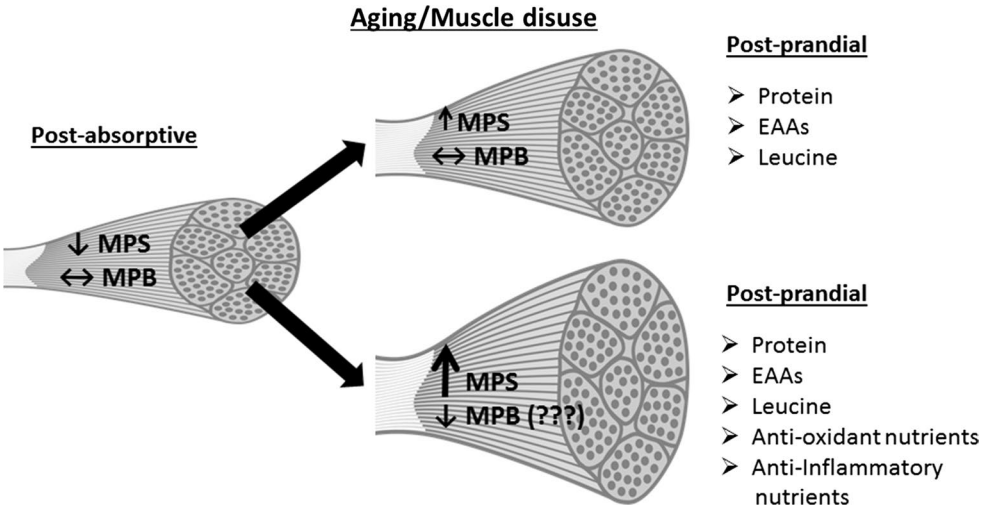

Fig. 1 Postprandial MPS in young/adult subjects and during aging/muscle disuse conditions in response to protein/AA feeding alone or combined with antioxidants, including the AA glycine

to amino acids is prominent. Figure 1 summarizes the current state of the research in young adult subjects and during muscle disuse and aging conditions in regards to the postprandial MPS response to protein/AA feeding alone or combined with antioxidants, including the AA glycine.

Conclusion and perspectives

Postprandial MPS can be stimulated by dietary proteins/EAAs intake such that if a threshold quantity of high-quality protein is consumed, maximal MPS values may be reached. However, during non-pathological conditions associated with muscle loss such as muscle disuse (immobilization) and aging, the anabolic threshold for amino acids to stimulate MPS is increased and the anabolic potential of a stereotypical protein intake to enhance muscle anabolism is reduced. Several years ago, pioneering studies demonstrated that increasing protein consumption or increasing the consumption of isolated amino acids with anabolic properties (i.e., leucine) was capable to restore the postprandial MPS response. However, this increase in postprandial MPS did not always translate into muscle tissue sparing during catabolic conditions. Several recent investigations have demonstrated positive effects of protein/AA supplementation not only on MPS but also on lean mass tissue, which suggests that an optimized and balanced distribution of protein intake between meals could be beneficial in the preservation of lean mass in the aging population. However, why certain studies result in a lack of positive effects (i.e., induces a maladaptation response of muscle protein metabolism to increased amounts of dietary proteins) during conditions associated with muscle loss, still remains unclear and requires further investigation. However, from a practical point of view, neutralizing the secondary factors linked to anabolic resistance such as increased ROS or inflammation markers may be a promising strategy. Although antioxidant excess can potentially lead to increased ROS production or be innocuous (Abdali et al. 2015), antioxidant-rich diets and, more recently, glycine supplementation, has demonstrated a strong potential to restore postprandial MPS during immobilization, aging and even sepsis. More chronic studies, however, are needed to evaluate if this renewed increase in postprandial MPS, indeed, leads to muscle restoration, and under which specific catabolic states that it may be most beneficial. If, on the one hand, the main dietary concern in treating atrophy is to increase the anabolic stimuli (aminoacidemia), on the other hand, decreasing factors related to the anabolic resistance is mandatory (ROS, inflammation and others) and warrants further investigation.

Compliance with ethical standards

Conflict of interest We have no conflict of interest to declare.

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