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REVIEW ARTICLE

Nutritionally essential amino acids and metabolic signaling in aging

E. Lichar Dillon

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Abstract Aging is associated with a gradual decline in skeletal muscle mass and strength leading to increased risk for functional impairments. Although basal rates of protein synthesis and degradation are largely unaffected with age, the sensitivity of older muscle cells to the anabolic actions of essential amino acids appears to decline. The major pathway through which essential amino acids induce anabolic responses involves the mammalian target of rapamycin (mTOR) Complex 1, a signaling pathway that is especially sensitive to regulation by the branched chain amino acid leucine. Recent evidence suggests that muscle of older individuals require increasing concentrations of leucine to maintain robust anabolic responses through the mTOR pathway. While the exact mechanisms for the age-related alterations in nutritional signaling through the mTOR pathway remain elusive, there is increasing evidence that decreased sensitivity to insulin action, reductions in endothelial function, and increased oxidative stress may be underlying factors in this decrease in anabolic sensitivity. Ensuring adequate nutrition, including sources of high quality protein, and promoting regular physical activity will remain among the frontline defenses against the onset of sarcopenia in older individuals.

Keywords Skeletal muscle · Protein synthesis · Anabolic resistance

Abbreviations

AA	Amino acid
Akt/PKB	Protein kinase B
BCAA	Branched chain AA
EAA	Essential AA
4E-BP1	eIF4E Binding protein
eEf	Eukaryotic elongation factor
eIF	Eukaryotic initiation factor
FSR	Fractional synthetic rate
GβL	G-protein-β-subunit-like protein
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
hVps34	Human vacuolar protein sorting 34
IGF-1	Insulin-like growth factor 1
LRS	Leucyl-tRNA synthetase
mTOR	Mammalian target of rapamycin
NEAA	Nonessential AA
NO	Nitric oxide
PI3K	Phosphatidylinositol 3-kinase
Rag	Ras-related GTPase
Raptor	Regulatory-associated protein of mTOR
Rheb	Ras homologue enhanced in brain
S6K1	p70 ribosomal protein S6 kinase 1
SNP	Sodium nitroprusside
TSC	Tuberous sclerosis complex

E. L. Dillon (✉)
Division of Endocrinology and Metabolism, Department of Internal Medicine, The University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555, USA
e-mail: eldillon@utmb.edu

Introduction

The world population is steadily aging with the proportion of adults aged 65 and older expected to exceed the number of children aged 5 years or younger within the decade (WHO 2011). As life expectancy continuous to increase, so does the need to better understand the aging process in order to promote long-term health, physical independence, and quality of life in our aging population. Not only is adequate strength required to perform activities of daily

living, skeletal muscle is a key metabolic tissue in the regulation of energy homeostasis, and functions as the largest pool of amino acids for utilization by peripheral tissues (Wolfe 2006). Maintaining adequate skeletal muscle mass and strength is essential for life, and aging is associated with a gradual decline in skeletal muscle mass and strength. This decline is generally referred to as sarcopenia, although there is increasing consensus that this term should be reserved for measurable losses that result in lean muscle mass of at least 2 standard deviations below that of healthy young individuals matched for gender and ethnicity (Cruz-Jentoft et al. 2010; Morley 2008; Thomas et al. 2000).

Sarcopenia is evident in approximately 5 % of people at age 65 and nears 50 % of people aged 80 and older (Baumgartner et al. 1998; Morley 2008, 2012). While there are many possible causes for the age-related decline in skeletal muscle mass, at a fundamental level, it is thought that age-related changes in the regulation of skeletal muscle protein metabolism lead to a condition of negative protein balance, where the average rate of protein anabolism no longer keeps up with that of protein catabolism, resulting in a gradual net loss of skeletal muscle protein (Katsanos et al. 2005; Morley 2012). Protein balance is regulated by many factors that are each susceptible to change during the aging process, including hormone status (i.e., insulin, growth hormone, testosterone, and IGF-1), mechanical forces (i.e., physical activity and exercise), and nutrition (i.e., AA intake and metabolism) (Abbatecola et al. 2011; Dillon et al. 2010; Horstman et al. 2012; Kimball et al. 2002; Walker et al. 2011; Wall et al. 2010).

Besides serving as substrates in energy metabolism, AAs are important among the macronutrients as being both the building blocks of proteins as well as potent signaling molecules in the regulation of protein metabolism (Kimball and Jefferson 2006a; Wu 2009). AAs stimulate muscle protein synthesis (Crozier et al. 2005; Liu and Barrett 2002; Paddon-Jones et al. 2004; Watt et al. 1992) and the anabolic effects of AAs on protein synthesis are beyond that which can be explained by the simple increase in their presence as substrate alone (Rennie et al. 2006). Oral AAs acutely induce a stimulatory response in muscle protein anabolism of older individuals similar to that observed in young (Paddon-Jones et al. 2004) and increases in muscle mass (Borsheim et al. 2008; Dillon et al. 2009) or function (Tieland et al. 2012) have been demonstrated in older individuals following chronic AA supplementation. Among the 21 AA necessary for protein synthesis in mammals, 9 are considered nutritionally EAA as they cannot be synthesized in adult humans. In addition, some NEAA can be regarded as conditionally essential in circumstances where rates of de novo synthesis are unable to keep up with physiological demands (Wu 2009). The majority of the anabolic effects during AA feeding can be attributed to the presence of EAAs (Freudenberg et al. 2012; Tipton et al. 1999; Volpi et al. 2003). Among the EAA, the BCAA leucine is a key regulator of protein synthesis (Atherton et al. 2009; Buse and Reid 1975; Kimball and Jefferson 2006b).

Because of the complexity of anabolic signals involved in protein metabolism, much of the research into the regulation of skeletal muscle anabolism is therefore understandably focused on not only the individual contributions of the major regulators (i.e., hormonal (Sattler et al. 2009; Sheffield-Moore et al. 2011) vs. nutritional (Paddon-Jones et al. 2004) vs. mechanical (Fujita et al. 2007; Sheffield-Moore et al. 2004)), but also on the interactions between combinations of these stimuli (i.e., hormones + AA, (Dennis et al. 2011; Sheffield-Moore 2000; Volpi et al. 2000); AA + physical activity, (Dreyer et al. 2008; Drummond et al. 2008; Durham et al. 2010; Ferrando et al. 2009); physical activity + insulin, (Biolo et al. 1999; Fujita et al. 2007)). Elucidating the age-related differences in the mechanisms of muscle protein synthesis is of great clinical importance. Halting or slowing the gradual loss of lean body mass that occurs during sarcopenia likely involves long-term combinatorial measures aimed at improving both postabsorptive and postprandial rates of muscle protein synthesis in elderly. While the main focus of this review will be on the contributions of AA nutrition in the regulation of protein metabolism during aging, it is necessary to appreciate the complexity of the many other factors that are necessary to maximize the anabolic effect of these nutrients and signaling regulators.

Postabsorptive protein synthesis in aging

In the absence of anabolic stimuli, such as at rest in the postabsorptive (fasted) state, the rate of protein synthesis is lower than that of protein degradation resulting in a net loss of skeletal muscle protein during these periods (Fig. 1). Fasting rates of protein degradation are similar between old and young adults but there has been a fair amount of discourse regarding whether basal rates of skeletal muscle protein synthesis are further reduced with aging. Some studies have reported lower rates of protein synthesis of older individuals when compared with young (Guillet et al. 2004; Rooyackers et al. 1996; Welle et al. 1993; Yarasheski et al. 1993), while others have not (Cuthbertson et al. 2005; Dillon et al. 2011; Katsanos et al. 2005, 2006; Paddon-Jones et al. 2004; Symons et al. 2009; Volpi et al. 1999, 2000, 2001).

Chronic dietary supplementation with EAAs has been shown to increase fasting rates of muscle protein synthesis in older individuals by some investigators (Casperson et al.

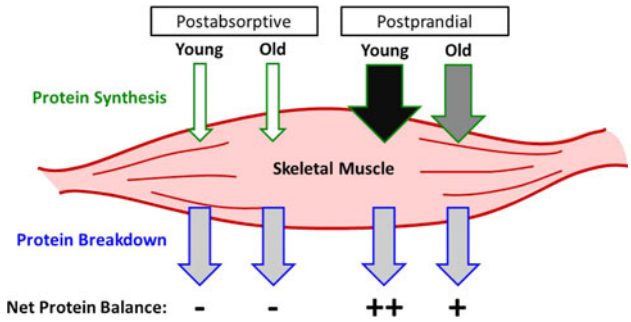

Fig. 1 Skeletal muscle protein turnover balance between protein synthesis and breakdown in skeletal muscle switches from negative (−) net balance in the fasted (postabsorptive) state to positive (+) following AA intake (postprandial), primarily due to an upregulation of protein synthesis. The anabolic sensitivity to AA availability is blunted with age, which can result in a diminished acute synthetic response following meal ingestion in old (+) when compared with young (++) muscle

2012; Dillon et al. 2009) while others have shown no such effects (Verhoeven et al. 2009; Walrand et al. 2008; Yarasheski et al. 2011). The possible reasons for the disparities between these reports regarding fasting rates of muscle protein synthesis during aging and the influence of AA availability remain elusive but may at least in part be due to differences in study methodologies, timing of measurements, subject characteristics, habitual dietary intakes, and supplement dosing regimens (Table 1). For instance, the high protein diets utilized by Walrand et al. (2008) and Tieland et al. (2012), as well as the leucine supplement used by Verhoeven et al. (2009) (2.5 g leucine compared with 4 g leucine used by Casperson et al. (2012)) may have been insufficient to fortify the meals with the amount of leucine necessary to stimulate chronic changes in baseline anabolic activity (Table 1). Secondly, a possible explanation for the observed differences between studies is the dietary protein intakes of the subjects before supplementation. It is possible that habitual intakes close to the current recommendations for adults of 0.8 g protein kg body weight^{-1} day^{-1} do not maximally stimulate protein synthesis in all older adults (Paddon-Jones and Rasmussen 2009). Findings by Walrand et al. (2008) that a high protein diet (3.0 g kg fat free mass^{-1} day^{-1}) did not further stimulate protein synthesis in young or old when compared with a control protein diet containing 1.5 g kg fat free mass^{-1} day^{-1} may have reflected adequate AA intake during the control period whereas the free-living diets of the subjects in the study by Dillon et al. (2009) were not controlled and were possibly lower in protein content. Similarly, the habitual diets of 0.99 g protein kg body weight^{-1} day^{-1} reported by Verhoeven et al. (2009) and 1.0 g reported by Tieland et al. (2012) (vs. 0.8 reported by Casperson et al. (2012)) may have been adequate before supplementation. Finally, regardless of total daily dietary intake, differences in the acute distribution of daily amino acid intake may be a crucial determinant in the observed chronic effects. The effectiveness of a chronic with-meal supplementation approach vs. a between-meal approach likely depends in part on how close dietary AA intake at each meal approximates the threshold necessary to acutely reach circulating leucine concentrations necessary to elicit a maximum anabolic response in the individual. A with-meal approach may be more beneficial if dietary protein intake is not sufficient to reach this threshold but would have no further benefit on net balance across the day if this acute threshold is already met in the absence of supplementation. A between-meal approach may be more beneficial in such a case in order to increase the number of anabolic responses, and amount of time spent in positive net balance, across the day.

It is unclear how improving AA intake may chronically alter baseline rates of skeletal muscle protein synthesis in older individuals. However, increases in baseline protein synthesis do not effect changes in the maximum attainable acute anabolic response. For instance, the maximum acute synthetic response to an EAA load did not increase following 3 months of EAA supplementation despite chronic improvements in basal FSR (Dillon et al., 2009). This is similar to observations in adult rats where 10 days of leucine-enhanced feeding resulted in maximally induced FSR in the postabsorptive state without further induction following acute meal feeding (Rieu et al. 2003). In contrast to adult rats, old rats receiving leucine-enhanced diets in that study did not display these chronic elevations of basal FSR but did have improved anabolic sensitivity to acute meal ingestion. Observations of changes in basal protein synthesis with unchanged acute responses to AA are similar to those following chronic androgen administration in older adults and possibly reflect improvements in synthetic efficiency and increased reutilization of proteolysis-derived AA for protein synthesis. (Ferrando et al. 2003; Sheffield-Moore et al. 2000). In short, these studies may collectively point to the importance of chronically maintaining proper meal distributions and adequate acute AA intakes during aging to prevent declines in baseline muscle protein synthesis. In addition to maintaining adequate baseline rates of protein synthesis, optimization of the acute anabolic response to AA feeding will add toward protection against the onset and progression of sarcopenia.

Postprandial protein synthesis in aging

Increased AA availability enhances skeletal muscle protein synthesis in healthy older adults (Paddon-Jones et al. 2004; Pennings et al. 2012; Rasmussen et al. 2002; Symons et al. 2007, 2009; Volpi et al. 1998, 1999; Yang et al. 2012).

Table 1 Comparison of leucine contents in supplements and concluded anabolic responses in older adults

	Age (yrs)	Habitual protein intake (g protein $kg^{-1}day^{-1}$)	Supplement	Acute dose [Leu] (g)	Peak arterial [Leu] (nmol/ml)	Concluded acutely anabolic in older adults?	Chronic dosing	Concluded chronically anabolic in older adults
Symons et al. (2007, 2009)	68 ± 2	NR	340 g Beef (90 g protein, 30 g EAA)	5.9	NR	Yes	–	–
			113 g Beef (30 g protein, 10 g EAA)	2.0	330	Yes		
Yang et al. (2012)	70 ± 4	1.03	40 g Whey (12 % Leu)	4.8[c]	300[e,v]	Yes	–	–
	70 ± 5	1.04	40 g Soy (8 % Leu)	3.2[c]	225[e,v]	No		
	72 ± 5	1.04	20 g Whey (12 % Leu)	2.4[c]	250[e,v]	Yes		
	72 ± 6	1.03	20 g Soy (8 % Leu)	1.6[c]	220[e,v]	No		
Pennings et al. (2012)	73 ± 1	NR	35 g Whey	NR	575[e,v]	Yes	–	–
	73 ± 2		20 g Whey	NR	475[e,v]	Intermediate		
	73 ± 2		10 g Whey	NR	375[e,v]	No		
Borsheim et al. (2008)	67 ± 6	0.99 ± 0.21	EAA + Arg (22 g/day)	4.0	NR	–	Between meals	Yes (16 weeks)
Casperson et al. (2012)	68 ± 2	0.75–0.85 (range)	Leu (12 g/day)	4	NR	–	With meal	Yes (2 weeks)
			7 g EAA + glucose	1.7	NR	Yes	–	–
Rieu et al. (2006)	70 ± 1	0.8[e]	BCAA (0.052 g Leu/kg BW)	3.8[c]	350[e]	Yes	–	–
Tieland et al. (2012)	78 ± 1	1.0 ± 0.1	Protein (30 g/day)	NR	NR	–	With meal	No (24 weeks)
Koopman et al. (2009)	64 ± 1	NR	35 g Casein	3[c]	250[e,v]	Yes	–	–
Paddon-Jones et al. (2004)	67 ± 2	NR	15 g EAA	2.8	NR	Yes/blunted versus young	–	–
Katsanos et al. (2005, 2006)	67 ± 2	NR	6.7 g EAA (41 % Leu)	2.8	700[e]	Yes	–	–
	67 ± 2		6.7 g EAA (26 % Leu)	1.7	459	No/blunted		
Verhoeven et al. (2009)	71 ± 4	0.99 ± 0.07	Leu (7.5 g/day)	2.5	NR	–	With meal	No (3 months)
Cuthbertson et al. (2005)	70 ± 6	NR	10 g EAA	NR	600[e,v]	Blunted versus young	–	–
			20 g EAA	NR	800[e,v]	Blunted vs. young		
Dillon et al. (2009)	68 ± 2	NR	EAA (15 g/day)	1.4	NR	–	Between meal	Yes (3 months)
			7.5 g EAA	1.4	NR	Yes	–	–
Volpi et al. (1998)	71 ± 2	NR	10 % AA (intravenous)	NR	352 ± 18	Yes	–	–
Volpi et al. (2000)	72 ± 1	NR	40 g AA + 40 g Glucose (2.2 g AA every 10 min for 3 h)	NR	347	Blunted versus young	–	–

Table 1 continued

	Age (yrs)	Habitual protein intake (g protein $kg^{-1}day^{-1}$)	Supplement	Acute dose [Leu] (g)	Peak arterial [Leu] (nmol/ml)	Concluded acutely anabolic in older adults?	Chronic dosing	Concluded chronically anabolic in older adults
Guillet et al. (2004)	72 ± 2	1.1	10 % AA (intravenous)	NR	351[h]	Blunted versus young	–	–
Yarasheski et al. (2011)	70 ± 4	0.9–1.1 (range)	Habitual diets were adjusted ± 0.2 g protein/kg BW/day	NR	NR	–	With meal	No (3 days)

Values are mean ± SEM unless specified otherwise

NR not reported, *NS* not significant

[c] Calculated estimate from dose description provided in the reference

[e] Estimated from graphs in the reference

[h] Arterialized blood from heated hand vein

[v] Venous blood concentrations are given when arterial concentrations were not provided in the reference

Some (Boirie et al. 1997; Volpi et al. 1999), but not all (Koopman et al. 2009), have shown age-related increases in splanchnic extraction of oral AAs, possibly reducing AA availability for skeletal muscle protein synthesis. However, despite increased first-pass splanchnic AA uptake, skeletal muscle protein synthesis was induced similarly in older and young individuals following ingestion of 40 g of mixed AAs (Volpi et al. 1999). Besides the possibility that age-related changes in AA availability may play a role in some individuals, there is a general consensus that there are changes in anabolic sensitivity to AAs at the cellular level with increased age, resulting in subpar anabolic responses under conditions of low AA availability (Breen and Phillips 2011; Cuthbertson et al. 2005; Dardevet et al. 2000).

The term anabolic resistance is generally used to describe reduced anabolic responses to stimuli. Links between reduced anabolic sensitivity to AAs and impaired insulin action have been shown in older individuals (Volpi et al. 2000) and a diminished sensitivity in anabolic signaling through the mTOR pathway in response to leucine with increased age has clearly been demonstrated in rat muscle (Dardevet et al. 2000). Insulin increases capillary recruitment and skeletal muscle perfusion (Coggins et al. 2001; Zhang et al. 2004) and is required for the optimum stimulation of mTOR signaling by AAs (Dennis et al. 2011; Prod'homme et al. 2005). The permissive action of insulin during the anabolic response to protein ingestion in healthy older persons becomes even more apparent when dietary leucine content is below the threshold to reach its maximum anabolic capacity (Katsanos et al. 2008). Furthermore, while maintaining insulin sensitivity during aging is important in order to retain robust anabolic responses through mTOR signaling following meals (Chevalier et al. 2011), some healthy older adults with normal glucose turnover may still have blunted anabolic response to small boluses of EAAs when compared with younger individuals, even under hyperinsulinemic conditions (Volpi et al. 2000).

It has become increasingly evident that a minimum threshold concentration of AAs needs to be reached to exert a robust anabolic response in skeletal muscle protein synthesis (Kobayashi et al. 2003) and that this threshold is increased in aged muscle, requiring higher concentrations of amino acids like leucine to elicit maximum anabolic responses in old muscle comparable with those observed in young muscle (Dardevet et al. 2000; Breen and Phillips 2011). The anabolic resistance may therefore be overcome by ensuring adequate acute intakes of high quality sources of AAs, or leucine, above a minimum threshold necessary to stimulate protein synthesis (Cuthbertson et al. 2005; El-Kadi et al. 2012; Gazzaneo et al. 2011; Katsanos et al. 2006; Pennings et al. 2012; Rieu et al. 2006; Symons et al. 2007, 2009; Yang et al. 2012) (Table 1). It has been

suggested that promoting a general minimum daily protein recommendation of 0.8 g kg^{-1} day^{-1} for adults may not be sufficient to protect older individuals with anabolic resistance by not ensuring that the minimum threshold is met at each meal (Paddon-Jones and Rasmussen 2009). Katsanos et al. (2006) showed that the provision of 6.7 g of EAA increased muscle protein synthesis in older subjects only after the leucine content was raised from ~26 to 41 % (Katsanos et al. 2006). In general, higher acute doses of AA supplements yield more robust anabolic responses in older individuals. For instance, the supplement used by Volpi et al. (2000) was administered as small boluses containing 2.2 g AA every 10 min for 3 h (i.e., totaling 40 g AA), and resulted in a blunted anabolic response in the older vs. younger subjects (Volpi et al. 2000). In contrast, acute ingestion of a single 35 g protein bolus yields skeletal muscle protein synthetic responses that are similar between young and old (Koopman et al. 2009), and such dose responses to oral protein loads have been confirmed in older adults (Pennings et al. 2012). Likewise, Symons et al. (2009) showed that older and younger men had similar increases in FSR in response to either 113 or 340 g of lean beef (containing ~30 and 90 g protein, and ~10 and 30 g EAA, respectively) suggesting that protein synthesis was maximally induced in both age groups with either dose (Symons et al. 2009). Besides absolute EAA content, protein quality may have a large influence on the observed anabolic response to a meal. Yang et al. (2012) recently reported that 40 g soy protein isolate is less effective in stimulating muscle protein synthesis in older men than equal quantities of whey protein, likely due to differences in digestibility/absorption and relative leucine content (~8 and 12 % for soy and whey, respectively) (Yang et al. 2012). Collectively, these studies show that while the maximum rate of protein synthesis that can be induced is similar between old and young, the quantity and quality of AAs necessary to reach this maximum rate can vary. However, it remains unclear exactly which changes in cellular machinery are responsible for the age-related declines in anabolic sensitivity.

EAAs and mechanisms of protein synthesis stimulation

The most widely recognized mechanism of AA-induced protein synthesis involves the mTOR Complex 1 pathway (Dodd and Tee 2012), although mTOR-independent mechanisms exist (Haegens et al. 2012). Activation of mTOR Complex 1 requires interaction between mTOR and several regulatory proteins including Rheb, Rag, raptor, GβL, and LRS which leads to downstream signaling through S6K1 and 4E-BP1 and translation initiation (Han et al. 2012; Kimball and Jefferson 2006b; Roccio et al. 2006) Fig. 2. Insulin facilitates AA-induced stimulation of the mTOR pathway through PI3 K/Akt, involving the inhibition of TSC2 (Kimball and Jefferson 2006b). TSC2, when complexed with TSC1, functions as a GTPase activating protein and results in reduction of Rheb-bound GTP to GDP and the dissociation of Rheb from the mTOR Complex 1. While the presence of insulin is required, signals provided by EAAs are necessary for full activation of protein synthesis (Anthony et al. 2002; Balage et al. 2001; Campbell et al. 1999; Hafen 2004). Association of Rheb to the mTOR Complex 1 appears to require AA, and GTP loading of Rheb has been shown to be blocked by AA depletion in vitro (Long et al. 2005; Roccio et al. 2006). Alterations in regulation of Rheb may be contributing to age-related impairments in AA-induced signaling through mTOR, as the upregulation of Rheb expression following resistive exercise plus ingestion of essential amino acids appears to be blunted in older adults (Drummond et al. 2009).

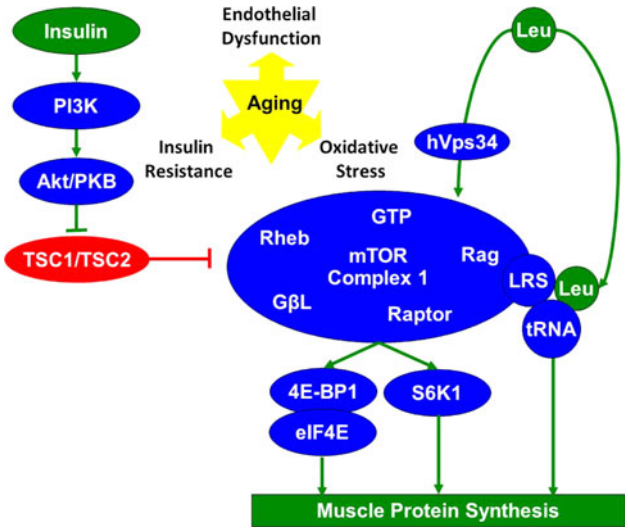

Fig. 2 Regulation of skeletal muscle protein synthesis by essential amino acids. This simplified schematic illustrates some of the key known and hypothesized pathways of mTORC1 regulation by leucine and insulin discussed in this review. Although the exact mechanisms behind the age-related anabolic resistance to leucine-induced skeletal muscle protein synthesis remain unclear, insulin resistance, endothelial dysfunction, and oxidative stress are thought to be among the potential contributors

The BCAA leucine is of particular interest in the discussion of AA-induced anabolism because of its unique potency among AAs in stimulating protein synthesis through the mTOR pathway in adults (Atherton et al. 2009). Interestingly, the BCAAs valine and isoleucine do not appear to share this anabolic potency despite their close structural relationship to leucine (Atherton et al. 2009). However, other AAs such as glutamine and arginine, which are conditionally EAAs for infants (Wu 1998; Wu et al.

2004), appear to play important roles in the regulation of mTOR activity in earlier stages of mammalian development (Gonzalez et al. 2011; Wang et al. 2012; Xi et al. 2011; Yao et al. 2008).

The stimulatory effects of AAs, and leucine in particular, on the initiation of protein synthesis have been described in several tissues including muscle, heart, liver, pancreas, jejunum, and ovary (Dennis et al. 2011; Glynn et al. 2010; Suryawan et al. 2012a, b; Wang et al. 1998; Xu et al. 1998). The regulatory role of EAAs on protein expression enters at the level of initiation of mRNA translation leading to increased synthesis of ribosomal proteins (Deldicque et al. 2005; Kimball and Jefferson 2006a; McKinnell and Rudnicki 2004; Proud 2004a; Tremblay et al. 2005). This stimulatory effect is detected only when EAAs are present and is in particular attributed to the action of the BCAAs (Kimball and Jefferson 2006b). Among these, leucine is the most potent in activating protein synthetic processes in vitro (Buse and Reid 1975) and the stimulatory effect on muscle protein synthesis is also evident in vivo, requiring the presence of either insulin or carbohydrates (Anthony et al. 2002). Single oral loads of leucine, in amounts up to 100 % of the daily requirement of this AA, have been shown to increase skeletal muscle protein synthesis in a concentration-dependent manner in food deprived rats (Crozier et al. 2005), but the anabolic response to leucine feeding changes with age. Older rats show improved acute anabolic responses to leucine-enriched diets meal after 10 days of supplementation (Rieu et al. 2003) and this is sustained through at least 30 days (Rieu et al. 2007). In contrast to old rats, adult rats fed similar leucine-rich diets had maximally stimulated rates of protein synthesis in the postabsorptive state after 10 days (Rieu et al. 2003).

Increasing leucine intakes are positively associated with hyperphosphorylation of 4E-BP1, dissociation of 4E-BP1-eIF4E, phosphorylation of eIF4G Ser^{1108}, association of eIF4G-eIF4E, and phosphorylation of $S6K1^{Thr389}$, and promotion of translation initiation in perfused rat hindlimb (Balage et al. 2001; Nagasawa et al. 2002). However, phosphorylation of S6K1 is blunted following AA infusion in older adults and is associated with a diminished protein synthetic response when compared with responses in young adults (Guillet et al. 2004). In addition to activating translation initiation, leucine and insulin activate elongation through activation (dephosphorylation) of eEF2 through an mTOR-mediated pathway (Browne and Proud 2004; Proud 2004a, b).

The detailed mechanisms through which leucine and other AAs relay signals to the mTOR pathway to regulate protein anabolism remain unclear, and how aging affects these mechanisms is even more elusive. Suggested leucine-mediated mechanisms include promotion of Rheb binding to mTOR, stabilization of the activated mTOR-raptor complex, or mTOR/raptor activation through an alternative class 3 PI3K (hVps34) parallel to that controlled through insulin (Kimball and Jefferson 2006b; Long et al. 2005; Nobukuni et al. 2005). Recent studies have implicated hVps34 (Byfield et al. 2005; Gran and Cameron-Smith 2011; Nobukuni et al. 2005) and LRS (Han et al. 2012), as rate limiting leucine sensors for mTOR-mediated protein synthesis. Aminoacyl-tRNA synthetases, including LRS, have been shown vulnerable to oxidative damage (Takahashi and Goto 1990) providing a possible age-associated mechanism for decreased sensitivity of aging muscle to the anabolic response to leucine.

Physical activity, AAs, and aging

Physical activity provides important direct mechanical anabolic stimuli to skeletal muscle (Dreyer et al. 2010; Goodman et al. 2011) but also induces hemodynamic responses leading to increased AA availability and utilization for skeletal muscle protein synthesis. The delivery and utilization of AA for muscle protein synthesis is facilitated by increases in macrovascular blood flow (Biolo et al. 1995) and by the opening of the capillary beds for nutrient flow within the muscle (Vincent et al. 2006). This increase in nutritive flow allows for an increased exchange of AA and other substrates into, and products of degradation out of, the muscle. Moderate aerobic exercise induces muscle protein synthesis in the old and young (Sheffield-Moore et al. 2004), and while insulin-mediated blood flow is impaired with age (Meneilly et al. 1995), aerobic exercise normalizes insulin-induced vasodilation and skeletal muscle protein synthesis in older subjects to those found in younger subjects for up to 18 h following the bout of exercise (Fujita et al. 2007). These changes are associated with reductions in the vasoconstrictor endothelin-1 suggesting improvements in endothelial function and increased vasorelaxation. However, despite similar responses in FSR between old and young, the anabolic efficiency of older muscle in response to AAs following exercise is reduced when compared with young (Durham et al. 2010). While microvascular blood flow was lower before and after exercise in older vs. younger subjects, the age-related reduction in anabolic sensitivity to AAs during exercise could not be explained by impairments in AA availability as arterial and interstitial concentrations of several amino acids, including leucine, were higher in old when compared with young. In addition, insulin increased marginally in the old but not in the young in response to exercise plus AA infusion. Insulin-mediated vasodilation in skeletal muscle is nitric oxide (NO) dependent (Steinberg et al. 1994) and age-related changes in NO signaling may be central to these impairments during exercise.

Production of NO from Arginine and O_2 by NO synthase (NOS) is regulated by many constitutive and inducing factors including insulin, micronutrients, and macronutrients such as amino acids (Wu and Meininger 2002). Uncoupling of endothelial NOS (eNOS) in arterioles of sedentary older rat muscle results in decreased NO production and increased production of O_2^- in response to in vitro stimulated flow when compared with young muscle (Sindler et al. 2009). In support of a role for reduced endothelial function in skeletal muscle anabolism during exercise in older individuals, blunting in skeletal muscle synthetic efficiency is not evident following induction of microvascular blood flow with an NO donor, sodium nitroprusside (SNP), plus AAs in the absence of exercise (Dillon et al. 2011). Similarly, SNP infusion improves the anabolic response to insulin in absence of exercise in skeletal muscle of older subjects (Timmerman et al. 2010). While these studies suggest that the aging muscle remains responsive to the actions of NO, age-related impairments in endogenous NO production may nevertheless be both caused by (Landmesser et al. 2003), and be a contributing factor to (Sindler et al. 2009), increased oxidative stress and further contribute to the impaired anabolic responses to changes in blood flow during exercise. Whether age-related changes in insulin sensitivity, sarcolemmal integrity, oxidative stress, endothelial function, and/or responsiveness to exercise play significant roles in the decreased anabolic sensitivity to AAs remains to be determined.

Conclusion and perspectives

There is overwhelming evidence suggesting age-related changes in amino acid metabolism contribute to the development of sarcopenia. The basic machinery necessary for anabolic responses appear to remain in place with increased age as fasting rates of protein synthesis and degradation do not change per se and robust skeletal muscle protein anabolism can be induced when certain minimum threshold levels of anabolic stimulation are met. However, several points are emerging from the research regarding the anabolic effectiveness of AA supplementation in older adults: (1) provision of adequate amounts of leucine can acutely stimulate skeletal muscle protein synthesis in older adults equal to that in young, (2) with-meal AA supplementation may have chronic benefit if habitual dietary AA intake is inadequate, (3) between-meal AA supplementation may have chronic benefits by maximizing the frequency of anabolic stimuli, and (4) optimizing the quality of the AA source may reduce the quantity of AAs required to reach the anabolic threshold.

While aging skeletal muscle protein synthesis can be induced to levels found in young, the sensitivity of aging tissues to the presence of the anabolic stimuli decreases due to yet largely unidentified mechanisms. There appear to be clear links between this phenomenon of anabolic resistance and other age-related risks such as decreased nutritional intake, decreased physical activity, decreased endothelial function, increased insulin resistance, increased oxidative stress, and increased inflammation. Successful prevention of the onset of sarcopenia will likely involve a multifactorial approach and ensuring optimum nutritional intake of quality sources of amino acids is paramount.

In the meantime, further research is needed to elucidate (1) how alterations in AA nutrition can chronically improve postabsorptive and postprandial protein synthetic efficiency in older adults, (2) how leucine directly interacts with regulatory factors of the mTOR pathway, (3) whether the anabolic machinery of muscle cells is altered with increased age in a way that explains the reductions in anabolic sensitivity to leucine, and (4) how age-related changes in other anabolic factors including insulin action and responses to exercise affect the anabolic sensitivity to AA.

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