

Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein

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Abstract

To counteract the debilitating progression of sarcopenia, a protein supplement should provide an energetically efficient anabolic stimulus. We quantified net muscle protein synthesis in healthy elderly individuals (65–79 yrs) following ingestion of an isocaloric intact whey protein supplement (WY; $n=8$) or an essential amino acid supplement (EAA; $n=7$). Femoral arterio-venous blood samples and vastus lateralis muscle biopsy samples were obtained during a primed, constant infusion of L-[ring-²H₅]phenylalanine. Net phenylalanine uptake and mixed muscle fractional synthetic rate (FSR) were calculated during the post-absorptive period and for 3.5 h following ingestion of 15 g EAA or 15 g whey. After accounting for the residual increase in the intracellular phenylalanine pool, net post-prandial phenylalanine uptake was 53.4 ± 9.7 mg phe leg^{-1} (EAA) and 21.7 ± 4.6 mg phe leg^{-1} (WY), ($P < 0.05$). Postabsorptive FSR values were $0.056 \pm 0.004\% \text{ h}^{-1}$ (EAA) and $0.049 \pm 0.006\% \text{ h}^{-1}$ (WY), ($P > 0.05$). Both supplements stimulated FSR ($P < 0.05$), but the increase was greatest in the EAA group with values of $0.088 \pm 0.011\% \text{ h}^{-1}$ (EAA) and $0.066 \pm 0.004\% \text{ h}^{-1}$ (WY), ($P < 0.05$). While both EAA and WY supplements stimulated muscle protein synthesis, EAAs may provide a more energetically efficient nutritional supplement for elderly individuals.

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1. Introduction

Sarcopenia is an insidious process characterized in part by the progressive loss of muscle mass and functional capacity. Numerous interventional strategies including exercise, androgen therapy and nutritional supplementation have been explored, yet the results have been mixed (Ferrando et al., 2003; Parise and Yarasheski, 2000; Fiatarone et al., 1990; Frontera et al., 1990; Fiatarone et al., 1994; Meredith et al., 1992). There are compelling data supporting the efficacy of resistance exercise in aging populations (Parise and Yarasheski, 2000; Fiatarone et al., 1990; Frontera et al., 1990; Fiatarone et al., 1994; Sheffield-Moore et al., 2005). However, in some circumstances, the ability to exercise or move freely may be compromised by physical disability or disease. In such instances, nutritional support may be one of the few remaining alternatives.

Dietary protein supplementation is easy to administer, the cost is relatively low, it can be used in almost all aging populations and it is intuitively and theoretically appropriate. Many nutritional supplement regimens are well tolerated by elderly individuals and can result in increased daily protein and energy intake (Lauque et al., 2000). Although these data are promising, a possible confounding effect is that a protein/nutritional supplement may reduce voluntary food consumption and therefore act primarily as a food replacement (Fiatarone Singh et al., 2000). If indeed this is the case, then strategies aimed at optimizing the anabolic effect of a supplement while reducing the caloric load should be explored.

Ingestion of essential amino acids (EAA) has been shown to effectively stimulate muscle protein synthesis in the elderly (Paddon-Jones et al., 2004; Volpi et al., 1999). Nevertheless, the majority of commercially available supplements are comprised of intact proteins such as whey. Further, while there are compelling practical considerations such as palatability, accessibility and cost that favor the use of whey protein over EAAs, there are no data examining the relative anabolic effect of these two supplements in an aging population.

The purpose of this study was to quantify net muscle protein synthesis in healthy elderly individuals following ingestion of

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an isocaloric supplement containing 15 g of whey protein or 15 g of essential amino acids.

2. Methods

2.1. Subjects

Healthy elderly volunteers were assigned to receive one of two isocaloric supplements: 15 g essential amino acids (EAA) or 15 g whey protein isolate (WY). Subject demographics are presented in Table 1. Volunteers were recruited through The University of Texas Medical Branch (UTMB) Sealy Center on Aging Volunteers Registry. All subjects gave informed, written consent according to the guidelines established by the Institutional Review Board at UTMB. Subject eligibility and exclusion criteria were necessarily strict, given the invasive nature of this study (Paddon-Jones et al., 2004; Volpi et al., 1999). All subjects were physically active and independent but were not athletically trained.

2.2. Experimental protocol

The experimental protocol is depicted in Fig. 1. Stable isotope infusion studies were performed in The General Clinical Research Center at UTMB. Volunteers were instructed to maintain their usual diet during the weeks preceding the metabolic study but refrain from strenuous activity for at least 72 h prior to admission.

Following a 10 h overnight fast, baseline blood samples were drawn for the analysis of background amino acid enrichment. A primed- $(2 \mu\text{mol kg}^{-1})$ continuous infusion $(0.05 \mu\text{mol kg}^{-1} \text{min}^{-1})$ of $[\text{L-ring-}^2\text{H}_5]$ phenylalanine was initiated and maintained for 10 h. 3-Fr 8 cm polyethylene Cook catheters (Bloomington, IN) were inserted into the femoral artery and vein of one leg under local anesthesia. Arterial and venous blood samples were obtained at 15 min intervals before and after supplement ingestion (Fig. 1). Muscle biopsies (approx. 50 mg) were taken under local anesthesia (1% lidocaine) from the lateral portion of the vastus lateralis using a 5 mm Bergstrom biopsy needle as previously described (Paddon-Jones et al., 2004, 2003). The composition of the EAA supplement and the constituent amino acids composition of the whey protein are described in Table 2. An additional 0.19 and 0.08 g of $[\text{ring-}^2\text{H}_5]$ phenylalanine was added to the EAA and WY supplements to maintain the isotopic enrichment (tracer–tracee ratio) in the femoral artery at approximately 0.08 (Paddon-Jones et al., 2004; Patterson, 1997). Supplements

Table 1
Subject characteristics

	15 g WY (n=8)	15 g EAA (n=7)
Sex	4 Male, 3 Female	3 Male, 4 Female
Age (yrs)	69 ± 2	67 ± 2
Height (cm)	168 ± 3	169 ± 1
Weight (kg)	75 ± 3	71 ± 5
Leg blood flow (mL/ min/100 mL leg vol)	3.3 ± 0.3	3.2 ± 0.6

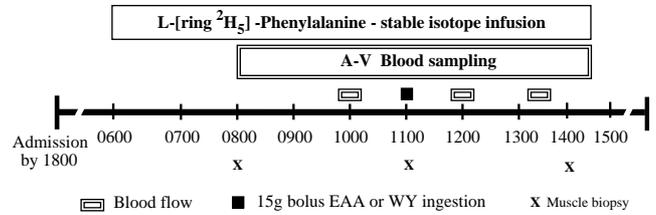


Fig. 1. The infusion Protocol. Femoral A-V samples were obtained at 15 min intervals during an infusion of L-[ring-²H₅]-phenylalanine. Muscle biopsies from the vastus lateralis were obtained at 0800, 1100 and 1400. The supplements were ingested immediately following the 1100 biopsy.

were dissolved in 250 mL of a non-caloric, non-caffeinated soft-drink and consumed as a bolus at 1100.

2.3. Analytical methods

Blood phenylalanine enrichments and concentrations were calculated using gas chromatography mass spectroscopy (GCMS; HP Model 5989. Hewlett-Packard Co., Palo Alto, CA) as previously described (Wolfe, 1992; Patterson et al., 1997). Plasma insulin concentrations were determined by radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA.). Mixed muscle intracellular phenylalanine enrichment and concentrations were determined using the tert-butyldimethylsilyl derivative as previously described (Paddon-Jones et al., 2004; Volpi et al., 2001; Calder et al., 1992). The protein bound L-[ring-²H₅] phenylalanine enrichment was determined using the standard curve approach on a GCMS (HP Model 5989. Hewlett-Packard Co., Palo Alto, CA).

2.4. Calculations

The fractional synthetic rate (FSR) of mixed muscle protein was calculated by measuring the direct incorporation of

Table 2
Composition of the EAA and WY supplement

Amino acid	EAA (g)	WY (g)
Histidine	1.64	0.26
Isoleucine	1.56	0.70
Leucine	2.79	1.75
Lysine	2.30	1.40
Methionine	0.46	0.33
Phenylalanine	2.30	0.44
Threonine	2.20	0.68
Tryptophan	0.10	0.19
Valine	1.73	0.70
Alanine	–	0.72
Arginine	–	0.38
Aspartic acid	–	1.60
Cystine	–	0.25
Glutamic acid	–	2.29
Glycine	–	0.25
Proline	–	0.59
Serine	–	0.59
Tyrosine	–	0.50
Total	15.1 g	13.6 g

L-[ring- $^2\text{H}_5$] phenylalanine into protein, using the precursor-product model (Baumann et al., 1994)

$$\text{FSR} = [(E_{P2} - E_{P1}) / (E_m t)] 60 \times 100$$

where E_{P1} and E_{P2} are the enrichments of bound L-[ring- $^2\text{H}_5$] phenylalanine in the first and second muscle biopsies, t is the time interval between biopsies and E_m is the mean L-[ring- $^2\text{H}_5$] phenylalanine enrichment in the muscle intracellular pool. FSR data in the WY group reflect an $N=7$ as an insufficient muscle sample was obtained from one volunteer. Mixed muscle FSR was calculated as previous research has demonstrated that the pattern of stimulation of myofibrillar, sarcoplasmic and mitochondrial protein was similar following amino acid infusion (Bohe et al., 2003).

Net phenylalanine uptake by the leg was calculated by determining net balance area under the curve during the post-prandial period with a correction for leg volume (Katch and Weltman, 1975) and the residual phenylalanine remaining in the muscle intracellular pool at the completion of the study (Paddon-Jones et al., 2004). Net balance and residual intracellular phenylalanine were calculated as follows:

$$\text{NB} = (C_a - C_v) \text{BF}$$

$$M_{\text{IC residual}} = M_{\text{IC post}} - M_{\text{IC pre}}$$

where C_a and C_v represent the phenylalanine concentrations in the femoral artery and vein respectively. BF represents leg blood flow, as determined by the indocyanine green (ICG) dye dilution method (Jorfeld and Warhen, 1971). $M_{\text{IC post}}$ and $M_{\text{IC pre}}$ represent the intracellular phenylalanine concentration during the pre- and post-supplement periods, respectively.

2.5. Statistical analysis

Data are presented as means \pm SEM. Within and between group comparisons were performed using ANOVA with repeated measures. Differences were considered significant at $P < 0.05$.

3. Results

There were no differences in the physical characteristics of the volunteers in the EAA and WY groups (Table 1), ($P > 0.05$). Neither group demonstrated a change in plasma insulin concentration following supplement ingestion ($P > 0.05$).

Taking the most conservative approach and assuming that all $M_{\text{IC residual}}$ phenylalanine would re-enter the circulation and play no role in protein synthesis ($M_{\text{IC residual}}$ EAA, 81.4 ± 15.3 mg Phe/leg; WY, 2.6 ± 7.4 mg Phe/leg), net phenylalanine uptake was still 3-fold higher in the EAA group, reflecting the greater abundance of phenylalanine in this supplement. In both groups, this value was equivalent to approximately 5% of the phenylalanine contained in each supplement being irreversibly taken up by a single leg during the 3.5 h post-supplement period (Fig. 2).

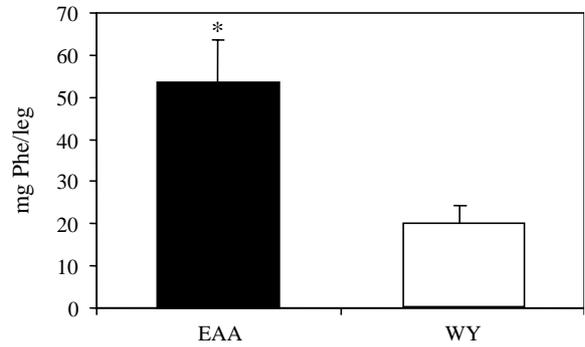


Fig. 2. Net phenylalanine uptake by the leg supplement ingestion. * denotes a significantly higher net uptake in the EAA group ($P < 0.05$).

Mean post-absorptive (EAA, $9.3 \pm 0.5\%$; WY, $8.9 \pm 0.4\%$) and post-prandial (EAA, $8.7 \pm 0.4\%$; WY, $8.3 \pm 0.4\%$) plasma L-[ring- $^2\text{H}_5$] phenylalanine enrichment values were similar in each group did not change in response to supplement ingestion ($P > 0.05$).

Our primary outcome measure, mixed muscle FSR, increased in both groups following supplement ingestion, ($P < 0.05$), but was greatest in the EAA group ($P < 0.05$), (Fig. 3).

4. Discussion

This study demonstrates that ingestion of essential amino acids or whey protein stimulates muscle protein synthesis in elderly. However, compared to isocaloric quantity of whey protein, essential amino acids provide a more energetically efficient nutritional supplement.

While the general finding that both EAA and WY protein ingestion can stimulate muscle protein synthesis in the elderly is certainly a positive result, net accrual of muscle protein is a function of the relationship between muscle protein synthesis and breakdown. In some instances, interpretation of a net change in muscle protein balance may be confounded by the lack of both synthesis and breakdown data. For example,

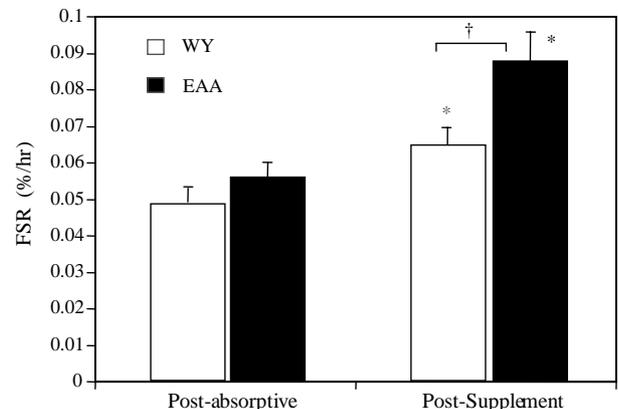


Fig. 3. Mixed muscle fractional synthetic rate (FSR) before and following ingestion of 15 g EAA ($n=7$) or 15 g WY protein ($n=7$). † denotes a significantly higher post-supplement FSR in the EAA group than the WY group ($P < 0.05$). * Denotes a significant increase in FSR following EAA and WY ingestion compared to the post-absorptive period ($P < 0.05$).

following trauma there is a characteristically large increase in mixed muscle FSR, which is counterintuitive if not presented in the context of a concomitant and correspondingly greater increase in fractional breakdown rate (FBR) (Biolo et al., 1997; Wolfe, 2002). However, in healthy populations, acute ingestion of amino acids primarily acts to stimulate muscle protein synthesis, without altering breakdown (Wolfe, 1992). While well suited for providing *proof of concept*, in many acute study protocols, supplements are not provided in a realistic context but rather are typically provided to fasted volunteers with little or no regard for the potential interactive/confounding effect of prior or subsequent meal ingestion. While this has not been addressed in elderly populations, recent data from our laboratory indicates that ingestion of a supplement containing 15 g EAA and 30 g carbohydrate provides a strong anabolic stimulus, yet does not interfere with the response to a mixed nutrient meal provided 3 h later (Paddon-Jones et al., 2005).

An important assumption of protein or amino acid supplementation is that the regular dietary intake of the individual is 'supplemented' by the additional protein load. While this assumption may appear to be self-evident, it has been shown that in some instances, a dietary intervention designed to supplement the protein/caloric intake in elderly individuals may actually function as a meal replacement. Fiatarone Singh et al. (2000) described this phenomenon in a cohort of non-exercising frail elders receiving a 360 kcal mixed nutrient supplement (17% protein, 60% carbohydrate, 23% fat) once a day for 10 weeks. While adherence to the supplement regimen was good, the investigators noted a compensatory, energetically equivalent reduction in ad libitum food consumption with no improvement in functional capacity. Thus, the supplement was in effect a meal replacement.

In the present study, 15 g of EAA and WY protein provided an additional 60 kcal, a relatively minor contribution to a typical daily intake. However, supplements are often consumed 2–3 times per day. Further, given that the WY supplement contained approximately half the essential amino acid content of the EAA supplement (Table 2), and the increase in FSR was 50% that of the EAA group, it is feasible that a 30 g WY dose may be required to produce an equivalent anabolic effect. In this instance, 30 g WY ingested 3 times per day would represent an additional 360 kcal, or the energetic equivalent of the mixed nutrient supplement used by Fiatarone et al. (Fiatarone et al., 1994).

The notion that a greater quantity of whey protein may be necessary to produce a similar stimulatory effect as the EAA supplement is also consistent with recent data from our laboratory. Specifically, while young and elderly individuals respond similarly to ingestion of 15 g of EAA (Paddon-Jones et al., 2004), it appears that the anabolic stimulus afforded by ingestion of a submaximal 7 g dose of EAA is significantly blunted in elderly individuals (Katsanos et al., 2005).

The total amount of phenylalanine taken up by the leg following supplement ingestion was significantly greater in the EAA group (Fig. 2). However, the percentage of phenylalanine in each supplement taken up by the leg was consistent with the relative proportion of phenylalanine in each supplement.

Further, the proportional FSR/essential amino acid dose–response is also consistent with previous research demonstrating that essential amino acids are primarily responsible for stimulating muscle protein synthesis while the addition of non-essential amino acids does not further improve the anabolic stimulus (Volpi et al., 2003).

Whereas EAA supplementation may represent a more energetically efficient supplement for elderly individuals, there are several pertinent practical issues such as cost and palatability that must also be carefully addressed and remedied (Schiffman and Graham, 2000). On the issue of taste, providing the EAAs in capsule form may represent a more viable option. Whey protein, on the other hand, is inexpensive and widely available in an assortment of agreeable flavors.

It is readily apparent that a longer duration study design would be necessary to determine if the acute benefits of supplement ingestion in the elderly translates to improvements in muscle mass or functional capacity. Nevertheless, we have recently demonstrated that repeated acute stimulation of muscle protein synthesis via ingestion of amino acids is cumulative. Specifically, we have found that extrapolation of amino acid uptake/protein accrual measured isotopically over a 24 h period is consistent with actual changes in lean muscle mass (DEXA) occurring over a 28 day period (Paddon-Jones et al., 2004).

In conclusion, ingestion of 15 g of essential amino acids or whey protein stimulates muscle protein synthesis in elderly. This anabolic effect was greater following EAA ingestion. Compared to isocaloric quantity of whey protein, essential amino acids provide a more energetically efficient but less practical nutritional supplement for elderly individuals.

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