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SHORT COMMUNICATION

Plasma kinetics of essential amino acids following their ingestion as free formula or as dietary protein components

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Abstract This investigation compares the levels of plasma kinetics of plasma essential amino acids (EAAs) after ingestion as free-form EAAs (FEAAs) or EAAs as components of dietary protein (DPEAAs), in eighteen healthy individuals, nine elderly (85 ± 6.7 years; 4 male) and nine young (28.7 ± 7 years; 3 males). For two consecutive days, each subject ingested EAAs in the form of (FEAAs) or (DPEAAs) in a random alternate pattern. Five minutes before EAA ingestion (baseline) and 30, 60, 90, 150 and 270 min after, venous blood samples were taken to determine the concentrations of EAAS (micromol/L). In both groups, ingested FEAAs compared to DPEAAs led to faster increase in plasma EAA levels at 30–150 min ($p < 0.0001$). Moreover, the increased plasma EAAs disappeared faster after FEAA compared to DPEAA. These results may be important in those subjects who have high requirement both for EAAs substrates and anabolic efficiency.

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Introduction

Skeletal muscle availability of essential amino acids (EAAs), the main promoter of muscle protein anabolism [1], is of paramount importance to prevent and potentially reduce sarcopenia for elderly patients. A study compares the effects of supplements containing only EAAs and a supplement containing balanced EAAs and non-essential amino acids, suggested that EAAs are primarily responsible for the amino acid-induced stimulation of muscle protein synthesis [1]. Randomized controlled trials tested the effectiveness of EAAs (8 g/day) given as free formula (FEAAs) and reported a significant increase in grip strength for older adults [2, 3] and in lean body mass for sarcopenic individuals [4].

However, the amount of EAAs taken up by muscle could potentially be impaired when EAAs are assumed as dietary protein components (DPEAAs). The co-presence in the diet of complex carbohydrates and fats could slow the rate of intestinal absorption of EAAs [5]. In elderly patients, intestinal absorption of amino acids modulates protein anabolism [6, 7]. It has been reported that elderly subjects may have altered kinetics of intestinal absorption even when EAAs were ingested as FEAAs [8]. In addition to altering kinetic of intestinal absorption another factor limiting muscle, EAA availability may be the reduced rate of disappearance of EAAs after their ingestion in free-form (FEAAs) [8].

To the best of our knowledge, so far no study has compared the plasma kinetics of EAA levels both after DPEAA and FEAA ingestion, in both elderly and young people.

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In this study, we hypothesize that the rate of increase in plasma EAA levels, the achievement of plasma peak values and subsequently, the post-peak disappearance of increased EAAs from plasma could be faster after FEAA rather than after DPEAA ingestion, for both young and elderly individuals. If we are correct, the results of the study could be useful to treat high-need individuals for whatever reason, independent of age, for more efficient metabolic EAA use.

Materials and methods

Nine voluntary healthy elderly (85 ± 6.7 years; 4 male) and nine younger subjects (28.7 ± 7 years; 3 males) with normal body weight (body max index 22.2 ± 2.2 kg/m^2 in elderly; 22.6 ± 1.74 kg/m^2 in young individuals) were enrolled onto the study. The population gave their informed written consent, and the local scientific committee approved the study.

In 2 consecutive days, at 12 a.m, 5 h after a light breakfast (tea 200 mL + biscuits 20 g providing 1.4 g protein), each subject ingested in a random alternate manner, either FEAA or DPEAA. FEAA (8 g) (Aminotrofic, Erre kappa, Milan) was diluted in 100 mL water at room temperature.

Eight grams EAAs contained leucine 2.50 g, lysine 1.3 g, isoleucine 1.25 g, valine 1.25 g, threonine 0.7 g, cysteine 0.3 g, histidine 0.3 g, phenylalanine 0.2 g, methionine 0.1 g, tyrosine 0.06 g and tryptophan 0.04 g. DPEAA was given as a standard meal providing nutritional intake as reported in Table 1. Before and after EAA ingestion, both for FEAAs and DPEAAs, the subjects were asked to lay down in a supine position.

Five minutes before FEAA or DPEAA ingestion (baseline) and 30, 60, 90, 150, 270 min after EAA ingestion, blood samples were taken with an inserted catheter from the ante-cubital vein to determine the concentrations of all amino acids (Amino Quant HPLC method). For the purpose of our study, we only considered from the DPEAA group the same amino acids as the FEAAs.

As EAAs in meal proteins (DPEAAs) were quantitatively higher than FEAAs, we normalized the resulted plasma EAA levels for mg of ingested EAA (as FEAAs or DPEAAs, respectively). Plasma EAA concentrations were expressed in μmol/L and μmol/L per mg of EAAs ingested. The increases in plasma EAAs were expressed both as percentages of baseline values set at zero and as absolute values.

Data are expressed as mean ± SD. The differences in the rate of plasma EAA increases, as well as the achievement of peaks after FEAAs or DPEAAs ingestion were analysed by unpaired t test. The level of significance was set at $p < 0.05$.

Table 1 Nutritional analysis (X ± sd) conducted on test—meal foods

Cal	Carboidrates		Fats		Proteins		EAAs		BCAAs	
	g	%Cal	g	%Cal	g	%Cal	g	%Proteins	g	%Proteins
Young	586.625 ± 191.4	82.898 ± 43.2	50.479 ± 12.2	12.129 ± 0.83	20.749 ± 7.8	41.708 ± 17.7	28.840 ± 8.4	21.94 ± 8.2	48,52 ± 1,2	7,95 ± 3.4	19,04 ± 0,2
Old	412,375 ± 92,6	59,493 ± 23,9	52,531 ± 12,5	10,981 ± 0,3	24,899 ± 5,0	22,691 ± 7.9	22.629 ± 9.0	12.25 ± 2.3	47.93 ± 1.4	4.76 ± 0.95	19.04 ± 0.3

EAAs essential amino acids, *BCAAs* branched-chain amino acids

Results

The study found that, for both young and elderly individuals, ingested EAAs led to different kinetics of plasma EAA increases depending on whether EAAs were assumed as free-form (FEAAs) or as dietary protein (DPEAAs) (Table 2). For each time intervals from fasting values (zero time), the plasma increases in EAAs were significantly higher after FEAAs compared to DPEAAs (Table 2; Fig. 1a, b). The peak achievement was faster after FEAAs (30 min) than after DPEAAs (150 min) ($p < 0.0001$) in younger subjects. Similarly, elderly subjects had peak achievement in 90 min after FEAAs and 150 min after DPEAAs ($p < 0.0001$). Furthermore, the peak values of EAAs were significantly higher in after FEAAs then after DPEAAs for both young and old people (Table 2).

There were important differences also observed in the post-peak EAA disappearance from the plasma, depending on whether EAAs were ingested as FEAAs or as DPEAAs. Indeed, the disappearance was faster and nearly complete at 150 min for FEAA ingestion by younger subjects and in 270 min for older subjects [8]. In contrast, after DPEAA ingestion, disappearance was not observed either in younger or older subjects because the peak EAA plateaued over 3 h of observation (Fig. 1a, b).

The kinetics of plasma BCAAs overlapped with those of EAAs in both groups, both in relation to the plasma level slope towards the peak and the disappearance of amino acids from plasma (Fig. 2a, b).

Discussion and conclusions

This study shows that how EAAs are being assumed, as free-form (FEAAs) or as dietary protein components (DPEAAs), conditions both the kinetics of plasma EAA level increases and the disappearance rate of increased

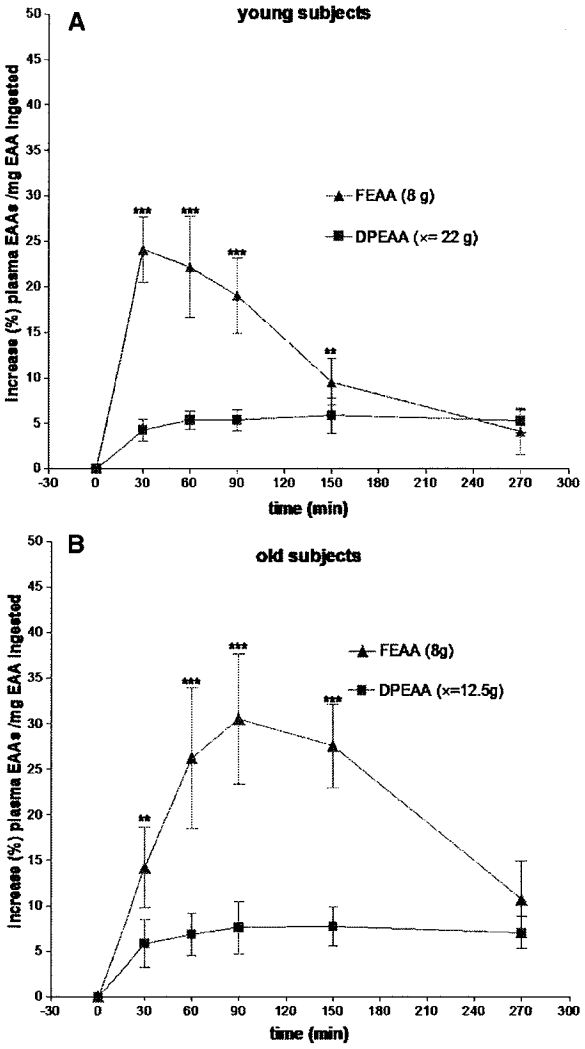

Fig. 1 Comparison of plasma EAA kinetic following FEAAs (*red triangle*) and DPEAAs (*blue square*) ingestion in young (**a**) and in old (**b**) subjects. *p*-values reported * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$

Table 2 Plasma kinetics (μmol/L) ($X \pm$ sd) of essential amino acid increases after DPEAA or FEAA ingestion

Time (min)	Young			Old		
	DPEAA	FEAA	*p* value	DPEAA	FEAA	*p* value
0	303.0 ± 81.76	848.7 ± 168.4	$p < 0.0001$	517.1 ± 157.3	953.7 ± 224.0	$p = 0.0073$
30	340.9 ± 97.5	1822.2 ± 472.3[§]	$p < 0.0001$	469.9 ± 213.0	2521.3 ± 808.1	$p = 0.0006$
60	428.8 ± 80.9	1774.9 ± 447.0	$p < 0.0001$	550.6 ± 186.2	3918.0 ± 1684.6	$p = 0.0022$
90	427.9 ± 92.2	1522.3 ± 330.5	$p < 0.0001$	608.4 ± 232.6	4298.3 ± 1061.5[§]	$p < 0.0001$
150	469.0 ± 153.8[§]	814.6 ± 186.1	$p = 0.0026$	638.4 ± 227.3[§]	2310.1 ± 1490.2	$p = 0.0381$
270	436.3 ± 98.5	365.9 ± 77.4	$p > 0.1$	572.1 ± 190.8	771.5 ± 193.5	$p > 0.1$(N.S.)

Statistical analysis: unpaired student *t* test. The level of significance refers to the differences between the values at each point of time and baseline

DPEAA dietary protein essential amino acids, *FEAA* free-form essential amino acids

[§] Peak values

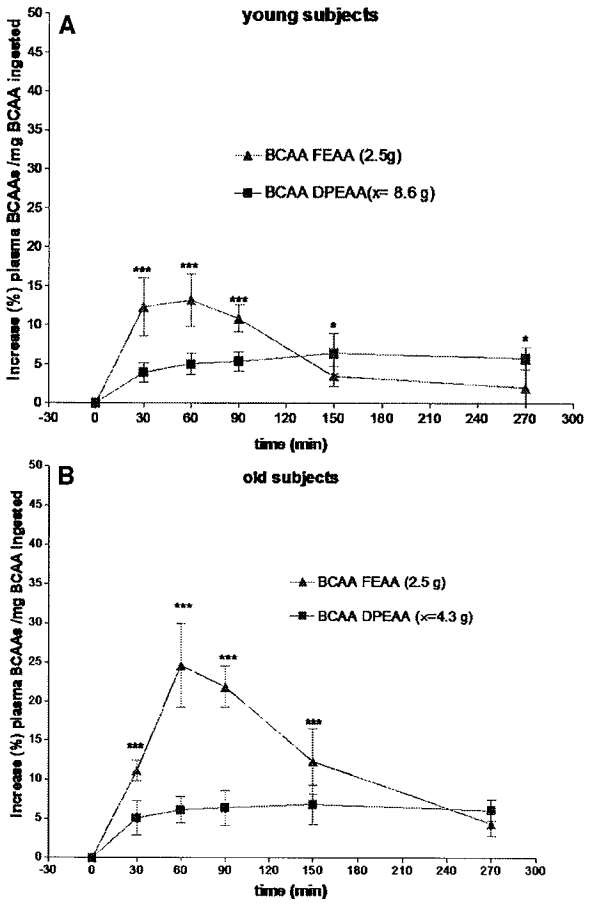

Fig. 2 Comparison of plasma BCAA kinetic following FEAAs (*red triangle*) and DPEAAs (*blue square*) ingestion in young (**a**) and in old (**b**) subjects. *p*-values reported * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$

EAAs from the plasma. EAA increased in the plasma much slower after DPEAAs than after FEAAs, both in younger and older subjects. Two mechanisms may explain this difference. The first is the interference on intestinal EAA absorption by co-present carbohydrates and fats, for ingested DPEAAs [5]. Indeed, these macronutrients are able to slow down gastric protein digestion velocity and their dismissed by-products from the stomach to the intestine. This slows down the rate of amino acid intestinal absorption [9]. The second mechanism may be explained by how EAAs are assumed, as a "bolus" with FEAAs, as a time-consuming meal in case of DPEAAs.

The slowing down of intestinal absorption with DPEAAs might account for the post-peak amino acid plateau and the similar behaviour between younger and older individuals. The EAA plateau probably indicated a balance between the rate of EAA release into the blood stream from intestinal lumen and the rate of tissue/organ uptake of EAAs.

In regard to the post-peak EAA disappearance from the plasma, only FEAA ingestion is followed by a nearly full EAA clearance from plasma although this was less rapid in elderly subjects than in younger ones [5]. The plasma EAA clearance reflects a different negative balance of the substrates between EAA entry into the bloodstream and their uptake by extra-intestinal tissue/organs. The results indicate that the quicker EAA plasma increases, the quicker their uptake by extra-intestinal tissue/organs is. That skeletal muscle is the main tissue of EAA uptake is suggested by the kinetics of BCAAs that overlaps that of total EAAs. BCAAs are largely consumed by skeletal muscle, and their plasma clearance reflects mainly muscle uptake [10].

Our results could have some practical implications for nutritional intervention, especially in a clinical setting. As anabolic muscle response to protein ingestion depends on the velocity of achieved plasma EAA peak [11], the ingestion of EAAs in a free form is a more efficient anabolic stimulus than the ingestion of similar amount of DPEAAs. This aspect is particularly important in those subjects with difficulties in increasing protein-rich food.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Statement of human and animal rights The local scientific committee approved the study.

Informed consent The population gave their informed written consent.

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