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PLASMA AMINO ACID CONCENTRATIONS ARE ASSOCIATED WITH MUSCLE FUNCTION IN OLDER JAPANESE WOMEN

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Abstract: *Background*: Although several previous studies have found benefits for amino acid supplementation in terms of muscle function, the role of plasma amino acid concentrations on sarcopenia are not well addressed yet. *Objective:* The aim of this study was to compare the amino acid concentrations at each stage of sarcopenia (normal, pre-sarcopenia, dynapenia, and sarcopenia) in community-dwelling older Japanese adults. *Setting and Subjects:* Community-dwelling older Japanese women (n=232, 79.4±7.0 years) participated in this study. *Measurements:* We measured plasma amino acid concentrations, 5-m walking speed, grip strength, and skeletal muscle mass using a bioelectrical impedance data acquisition system and compared them among participants at each stage of sarcopenia. *Results:* The proportions of normal, pre-sarcopenia, dynapenia, and sarcopenia patients were 40.5% (n=94), 12.1% (n=28), 26.3% (n=61), and 21.1% (n=49), respectively. Significant differences were observed for concentrations of leucine, branched-chain amino acid (BCAAs), and essential amino acid (EAAs) among the four groups ($p<0.05$), and the dynapenia and sarcopenia groups showed significantly lower concentrations of leucine than the normal group ($p<0.05$). *Conclusions:* This study indicated a positive relationship between plasma leucine, BCAA and EAA concentrations and muscle function. A longitudinal study is needed to determine the causal relationship between leucine/BCAA concentrations and muscle function.

Key words: Leucine, BCAA, EAA, sarcopenia, dynapenia.

Introduction

Degeneration of skeletal muscle leading to sarcopenia and dynapenia is a serious health care problem in an aging society. The functional decline of skeletal muscle in older adults leads to adverse health outcomes, such as disability and mortality (1, 2). Therefore, numerous studies have examined the effects of various programs on skeletal muscle function and have found beneficial effects of physical exercise and nutritional supplementation in sarcopenic older adults (3).

Recently, our study showed that the qualitative changes in skeletal muscle in sarcopenia are similar to those in dynapenia (4). Sarcopenia is defined as age-related loss of both muscle strength and muscle mass according to two popular criteria: the European Working Group on Sarcopenia in Older People (EWGSOP) and the Asian Working Group for Sarcopenia (AWGS) (5, 6). In contrast, Clark and Manini defined dynapenia as an age-related decrease in muscle strength (7, 8). The thigh muscles in both sarcopenic and dynapenic older adults show poor qualitative changes according to echo intensity and muscle density (4), which indicate similar histological changes in sarcopenia and dynapenia.

Better physical function is linked to high blood amino acid concentrations in older adults, and high levels of serum branched-chain amino acids (BCAA) are associated with a higher fat-free mass in older adults (9). Similarly, plasma amino acid concentrations are lower in older persons with protein energy malnutrition (PEM) than in persons without PEM (10). Furthermore, plasma BCAA concentrations can predict mortality in patients with end-stage liver disease (11). These findings suggest that keeping high amino acid concentrations in the plasma is remarkably important to maintain physical function in frail and disabled older adults.

Higher protein intake with a high biological value would maintain higher BCAA concentrations and improve skeletal muscle function in older adults. Among the BCAAs, leucine plays a more important role in regulating muscle protein because leucine-rich essential amino acids (EAAs) have been shown to activate mammalian target of rapamycin (mTOR) and thereby to enhance muscle protein synthesis (12, 13). Additionally, the effect of a small amount of leucine-rich EAA intake on the activation of muscle protein synthesis is equivalent to a large amount of whey protein intake in older adults (14). Furthermore, combining leucine-rich EAA supplementation and physical exercise can improve muscle strength and skeletal muscle mass volume in sarcopenic older adults (15). Although these studies found benefits for the intake of leucine and EAA in terms of muscle function, the role of plasma leucine and EAA concentrations in sarcopenia is still unknown.

The aim of this study, therefore, was to evaluate plasma amino acid concentrations in each classification of muscle function, i.e., normal, pre-sarcopenia, dynapenia, and sarcopenia. Additionally, we explored the relationships between plasma amino acid concentrations and several skeletal muscle parameters.

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Table 1
Comparison of plasma amino acid concentrations in each stage of sarcopenia adjusted for age

	Normal n=94		Pre-sarcopenia n=28		Dynapenia n=61		Sarcopenia n=49		Overall n=232		General linear model				Posthoc test
											Univariate		Multivariate		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F-value	P-value	F-value	P-value	
Characteristics															
Age	76.0	6.5	78.4	5.7	82.1	6.3	83.1	6.2	79.4	7.0	18.87	<0.01			2,3,5
Height, cm	151.1	5.2	152.8	4.9	146.8	6.4	147.0	6.2	149.3	6.2	13.16	<0.01	6.02	<0.01	2,3,4,5
Weight, kg	52.0	6.9	45.6	4.2	50.6	7.8	41.2	6.5	48.6	8.1	30.32	<0.01	29.30	<0.01	1,3,4,5,6
BMI	22.8	3.1	19.6	2.1	23.5	3.7	19.0	2.5	21.8	3.6	28.22	<0.01	33.10	<0.01	1,3,4,5,6
Plasma															
Non-essential amino acids															
Alanine, nmollmL	323.1	64.5	304.8	75.5	329.4	93.9	305.7	78.4	318.9	77.5	1.25	0.29	1.54	0.20	
Arginine, nmollmL	84.9	16.4	89.1	14.9	84.6	14.1	85.2	18.1	85.4	16.0	0.57	0.64	0.74	0.53	
Asparagine, nmollmL	41.5	8.2	40.2	7.8	41.1	9.0	41.0	8.0	41.1	8.3	0.19	0.90	0.20	0.90	
Aspartate, nmollmL	4.0	2.2	4.8	2.2	3.8	1.8	3.5	1.6	3.9	2.0	2.23	0.09	1.86	0.14	
Cystine, nmollmL	18.9	10.9	13.6	9.7	16.9	9.5	15.3	9.7	17.0	10.3	2.58	0.05	2.13	0.10	
Glutamate, nmollmL	77.7	70.9	83.0	57.9	69.4	50.6	58.1	41.5	72.0	59.3	1.57	0.20	1.11	0.35	
Glutamine, nmollmL	525.1	99.3	491.7	73.1	534.8	99.1	536.1	65.1	525.9	90.6	1.75	0.16	1.54	0.21	
Glycine, nmollmL	234.5	70.5	220.9	71.7	244.8	65.8	234.1	69.9	235.5	69.2	0.80	0.49	0.69	0.56	
Proline, nmollmL	130.7	38.8	132.3	61.7	151.7	58.9	138.4	63.1	138.0	53.4	2.06	0.11	1.23	0.30	
Serine, nmollmL	104.4	15.4	103.6	17.3	105.0	22.5	102.8	19.1	104.1	18.4	0.15	0.93	0.14	0.94	
Tyrosine, nmollmL	63.1	13.0	62.9	14.7	60.7	13.8	57.5	9.7	61.3	12.9	2.24	0.08	2.57	0.06	
Essential amino acids															
Histidine, nmollmL	70.3	8.7	68.2	6.8	69.3	9.0	67.0	6.9	69.1	8.3	1.89	0.13	1.40	0.25	
Lysine, nmollmL	171.5	24.1	177.0	31.5	163.3	25.3	167.0	26.8	169.1	26.2	2.24	0.08	1.55	0.20	
Methionine, nmollmL	22.4	4.4	23.0	5.1	21.7	4.9	21.4	3.8	22.1	4.5	1.08	0.36	1.48	0.22	
Phenylalanine, nmollmL	56.7	8.6	58.6	13.4	55.3	9.1	54.9	7.5	56.2	9.2	1.25	0.29	1.59	0.19	
Threonine, nmollmL	105.7	20.7	111.9	23.0	105.7	25.4	101.9	16.9	105.6	21.7	1.27	0.29	1.06	0.37	
Tryptophan, nmollmL	49.0	8.3	50.1	9.0	46.4	7.7	45.5	8.2	47.7	8.3	3.18	0.02*	2.05	0.11	
Isoleucine, nmollmL	56.6	16.2	54.4	15.9	53.2	12.2	52.1	9.2	54.5	13.9	1.38	0.25	1.88	0.13	
Leucine, nmollmL	104.7	19.8	101.7	21.9	94.1	17.1	94.9	14.6	99.5	18.9	5.46	<0.01**	4.34	<0.01*	2,3
Valine, nmollmL	198.6	36.7	192.8	36.8	186.2	34.6	185.5	32.5	191.9	35.6	2.22	0.09	2.37	0.07	
BCAA, nmollmL	360.0	69.4	348.9	72.6	333.4	60.6	332.4	52.6	345.8	65.2	3.00	0.03*	2.98	0.03	
EAA, nmollmL	835.5	106.3	837.7	132.7	795.2	95.4	790.1	86.3	815.6	104.8	3.38	0.02*	2.70	0.05*	

SD: standard deviation, BCAA: branched-chain amino acids, EAA: essential amino acids; The number indicates a significant difference by the posthoc test: 1, normal vs. pre-sarcopenia; 2, normal vs. dynapenia; 3, normal vs. sarcopenia; 4, pre-sarcopenia vs. dynapenia; 5, pre-sarcopenia vs. sarcopenia; 6, dynapenia vs. sarcopenia.

Methods

Participants

Participants were recruited by an advertisement in the local press and by public advertisements. We recruited community-dwelling older women in Japan. The inclusion criteria were aged 65 years or older and the ability to walk independently (including with a cane). The exclusion criteria were history of stroke, Parkinson's disease, severe psychiatric impairment, severe cognitive impairment, and severe pulmonary, cardiac, or musculoskeletal disorders. In addition, we excluded older persons with artificial implants, such as cardiac pacemakers, that did not permit measurement of bioelectrical impedance. This study was conducted in accordance with the guidelines proposed by the Declaration of Helsinki, and the study protocol was reviewed and approved by the Ethics Committee of Tsukuba University Graduate School of Comprehensive Human Sciences.

Biochemical measurements

For all the participants, the following 20 amino acid concentrations were measured: alanine, arginine, asparagine, aspartate, cystine, glutamate, glutamine, glycine, proline, serine, tyrosine, histidine, lysine, methionine, phenylalanine, threonine,

tryptophan, isoleucine, leucine, and valine. In addition, the BCAA and EAA concentrations were calculated by using each amino acid concentration. Blood samples were drawn in the morning after a fasting period of at least 8 hours. The sample tubes were stored immediately in the cube cooler to maintain the blood temperature at 4°C to prevent hemolysis and degradation of amino acids. Plasma was collected after centrifugation, and the collected plasma samples were transported to the laboratory while cooling with frozen carbon dioxide. All the analyses were performed in the same laboratory.

Skeletal muscle mass index (SMI)

A bioelectrical impedance data acquisition system (MC-780A; TANITA Co, Ltd, Tokyo, Japan) was used to determine bioelectrical impedance. This system uses electrical current at different frequencies to directly measure the amount of water in the body. Using the segmental skeletal muscle mass, a value for the appendicular skeletal muscle mass (kg) was determined, and this appendicular muscle mass was converted into the skeletal muscle mass index (SMI) by dividing the muscle weight by height squared (kg/m^2). Low muscle mass was defined as an SMI less than 5.7 kg/m^2 in women according to the AWGS definition (4).

Measurements of physical performance

For physical performance, we measured the 5-m walking speed and grip strength. Both measurements were performed by physical therapists who received a lecture on the correct protocols for both measures included in the study from a well-trained investigator before the study started. In the 5-m walking test, participants walked independently at their usual speed over a distance of 5 m. If a walking aid was normally used at home, the aid was permitted during the 5-m walking test. This test was recorded twice and was averaged to yield the representative value. Grip strength was measured using a Smedley hand dynamometer with the arm at right angles and the elbow by the side of the body. The participant squeezed the dynamometer with maximum effort without other body movements. The representative value was defined as the better performance of 2 trials with each hand. A slow walking speed was defined as a walking speed less than 0.8 m/sec, and low muscle strength was defined as a handgrip strength less than 18 kg in women according to the AWGS definition (4).

Definition of sarcopenia staging

We defined sarcopenia using the diagnostic algorithm of the AWGS based on the presence of both low muscle function (slow walking speed or low grip strength) and low muscle mass (5). Pre-sarcopenia was defined as low muscle mass without low muscle function according to the EWGSOP definition (6). Finally, dynapenia was operationally defined as low muscle function without low muscle mass. This categorical definition was used in our previous study (4).

Statistical analysis

The general linear model (GLM) was used for univariate and multivariate analyses to assess the differences in each amino acid concentration among the 4 groups (normal, pre-sarcopenia, dynapenia, and sarcopenia). Then, a post-hoc test was performed using a Bonferroni test to assess which group differed significantly from the others. We also performed multivariate analyses for each measure by adjusting for age. Similarly, to assess the differences in each EAA concentration among the groups according to the quartiles of SMI, walking speed and grip strength were used by GLM and Bonferroni test.

The data were managed and analyzed using SPSS (Statistical Package for the Social Sciences, Windows version 21.0; SPSS, Inc., Chicago, IL, USA). A p value less than 0.05 was considered to indicate statistical significance for all analyses.

Results

The characteristics of the study participants are shown in Table 1. The mean age was 79.4±7.0 years (n=232). The proportions of normal, pre-sarcopenia, dynapenia, and sarcopenia patients were 40.5% (n=94), 12.1% (n=28), 26.3% (n=61), and 21.1% (n=49), respectively.

Significant differences were observed for leucine, BCAA, and EAA concentrations among the four groups ($p<0.05$), and the dynapenia and sarcopenia groups showed significantly lower leucine concentrations than the normal group ($p<0.05$) (Table 1). In contrast, non-significant differences were observed for non-EAA concentrations among the four groups ($p>0.05$). Non-significant differences were also observed for all EAA concentrations except for BCAA among the four groups. Figure 1 shows each essential amino acid proportion in the pre-sarcopenia, dynapenia and sarcopenia groups compared with the normal group. The dynapenia and sarcopenia groups in particular showed lower BCAA concentrations than the normal and pre-sarcopenia groups (Figure 1).

Similarly, significant differences were observed for the leucine, valine, BCAA, and EAA concentrations among the 4 groups stratified by grip strength ($p<0.05$), and the lowest quartile showed significantly lower concentrations of leucine, valine, BCAAs, and EAAs than the highest quartile ($p<0.05$) (Table 2). Furthermore, significant differences were observed for isoleucine concentrations among the 4 groups stratified by SMI ($p<0.05$), and the second quartile showed significantly lower isoleucine concentrations than the highest quartile ($p<0.05$) (Table 2).

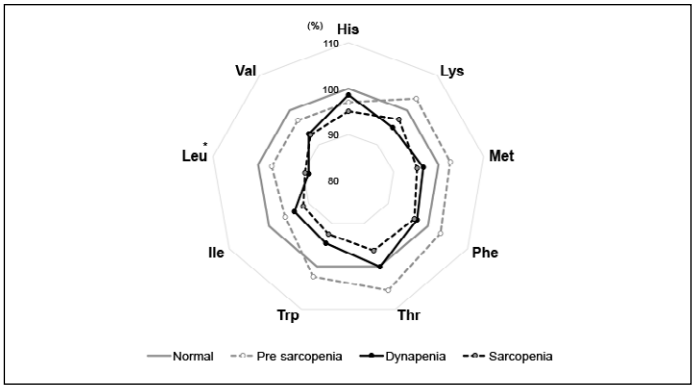
Discussion

In this study, we divided the participants into four groups according to our previous study (4) and found that the dynapenia and sarcopenia groups showed lower plasma leucine, BCAA, and EAA concentrations than the normal and pre-sarcopenia groups. These plasma amino acid concentrations were well correlated with grip strength in particular but not

with SMI or walking speed. Thus, lower plasma amino acid concentrations may be related to lower muscle function in older women.

Figure 1

Schematic representation of each essential amino acid's proportion in the pre-sarcopenia, dynapenia and sarcopenia groups compared to the normal group. Dynapenic and sarcopenic older women showed lower concentrations of valine, leucine and isoleucine than normal and pre-sarcopenic older women



His: histidine, Lys: lysine, Met: methionine, Phe: phenylalanine, Thr: threonine, Trp: tryptophan, Ile: isoleucine, Leu: leucine, Val: valine

The concentrations of leucine, BCAA and EAA in both sarcopenic and dynapenic older women were relatively lower than those in pre-sarcopenic and normal older women. The mean plasma amino acid concentrations of leucine, BCAA and EAA were 94.9 nmol/mL, 332.4 nmol/mL, and 790.1 nmol/mL in sarcopenia and 94.1 nmol/mL, 333.4 nmol/mL, and 795.2 nmol/mL in dynapenia, respectively (Table 1). Yamamoto and colleagues reported the reference value for plasma amino acid concentrations in healthy Japanese middle-aged populations (16). These reference values are relatively higher than our results for sarcopenia and dynapenia. Moreover, Polge reported the plasma amino acid concentrations in French patients with PEM, and these values were even lower than our results for sarcopenia and dynapenia (10). Thus, the leucine, BCAA and EAA concentrations in community-dwelling sarcopenic and dynapenic older women may be lower than those in normal middle-aged and older women but higher than those in patients with PEM.

Our previous study showed that the quality of skeletal muscle in sarcopenic and dynapenic older adults is lower than that in normal or pre-sarcopenic older adults (4). These qualitative changes in skeletal muscle indicate a decrease in the number of fast twitch fibers (17-18) and an increase in fat infiltration within skeletal muscle (19-20). The results of this study suggested that the lower plasma amino acid concentrations were related to low muscle strength but not muscle mass. Thus, leucine, BCAAs and EAAs are associated with real muscle fiber and may help improve muscle function.

In the present study, the plasma levels of leucine, BCAAs and EAAs in sarcopenic and dynapenic older women were lower than those in normal and pre-sarcopenic older women. The lower amino acid concentrations may be due to the following two hypotheses: the first is decreased protein intake, and the second is decreased muscle protein degradation. A previous study indicated that a lower level of EAAs is strongly associated with poor protein intake (10). In contrast, BCAA concentrations are proportionally high according to the fat-free mass because these amino acid concentrations are affected proportionally by the amount of muscle protein degradation (9). Thus, amino acid concentrations may be determined by both dietary habits and current skeletal muscle status.

Several basic studies and clinical trials have shown that leucine-rich EAA supplementation has a positive impact on skeletal muscle function. Leucine-rich EAAs lead to the activation of mTOR signaling and muscle synthesis (12, 13) and improvement of muscle strength and skeletal muscle mass (15). Such effects are remarkable in sarcopenic older adults in particular. Interestingly, some systematic reviews have indicated that the effect of EAAs and protein supplementation on skeletal muscle appears to be limited in well-nourished healthy older adults (21). Our results indicated an association between low amino acid concentrations and poor skeletal muscle function in older women, and the effectiveness of amino acid supplementation may be affected by having adequate or insufficient amino acid concentrations at baseline.

This study shows that lower plasma amino acid concentrations are associated with sarcopenia and dynapenia in community-dwelling older women, and these findings indicate that amino acid behavior is closely related. Our previous study also indicated that the skeletal muscle characteristics in sarcopenic and dynapenic older adults show the same trends (4). Thus, leucine-rich EAA supplementation may be useful to improve skeletal muscle function for both sarcopenic and dynapenic older adults.

There were several limitations of this study that warrant mention. First, daily protein intake was not measured. Therefore, we were not able to explore the details of the cause of the low amino acid concentrations. Second, these findings were provided by a cross-sectional study. Thus, a longitudinal study is required to clarify whether each amino acid concentration influences the incidence of sarcopenia and dynapenia.

Conclusion

Plasma leucine, BCAA and EAA concentrations and skeletal muscle function are positively associated in older Japanese women. A longitudinal study and clinical trials are needed to determine the causal role of these amino acids in muscle function.

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Table 2
Comparison of plasma amino acid concentrations in quartiles of each skeletal muscle indicator adjusted for age

	Q1		Q2		Q3		Q4		General linear model				
									Univariate		Multivariate		Posthoc test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F-value	P-value	F-value	P-value	
Grip strength, kg	<15.8		15.8-18.7		18.8-21.5		≥21.6						
	n=56		n=58		n=59		n=59						
Isoleucine, nmollmL	52.4	11.2	54.0	11.7	55.1	14.4	56.4	17.5	0.83	0.48	1.28	0.28	
Leucine, nmollmL	90.9	16.7	99.8	15.8	100.9	18.1	106.1	21.7	6.89	<0.01**	5.82	<0.01**	1,2
Valine, nmollmL	181.6	34.6	194.7	32.3	191.9	35.2	199.2	38.6	2.58	0.05	2.85	0.04*	2
BCAA, nmollmL	324.9	59.5	348.5	55.5	347.9	64.9	361.7	75.2	3.25	0.02*	3.37	0.02*	2
EAA, nmollmL	777.5	91.0	820.4	95.0	813.5	97.3	850.2	122.6	4.91	<0.01**	4.31	0.01*	2
Walking speed, m/sec	<0.93		0.93-1.17		1.18-1.35		≥1.36						
	n=56		n=58		n=59		n=59						
Isoleucine, nmollmL	53.0	10.7	54.1	14.3	56.1	14.3	54.6	16.0	0.50	0.68	0.69	0.56	
Leucine, nmollmL	93.0	15.9	99.1	18.1	102.5	20.3	103.0	19.8	3.45	0.02*	2.38	0.07	
Valine, nmollmL	186.6	34.7	192.2	36.8	193.9	34.2	194.6	37.0	0.59	0.62	0.55	0.65	
BCAA, nmollmL	332.6	57.6	345.5	65.9	352.5	65.1	352.1	70.7	1.17	0.32	1.02	0.39	
EAA, nmollmL	785.2	90.7	819.0	98.0	820.0	103.8	836.5	119.8	2.46	0.06	1.79	0.15	
SMI, kg/m²	<5.57		5.57-5.95		5.96-6.44		≥6.45						
	n=57		n=59		n=58		n=58						
Isoleucine, nmollmL	53.1	11.8	51.9	11.9	54.0	13.3	58.9	17.4	2.86	0.04*	2.97	0.03*	3
Leucine, nmollmL	96.1	17.1	98.5	17.6	99.7	19.8	103.6	20.7	1.58	0.19	1.19	0.31	
Valine, nmollmL	186.7	33.6	188.5	32.3	192.1	36.5	200.3	39.1	1.67	0.17	1.61	0.19	
BCAA, nmollmL	335.9	59.2	338.9	58.9	345.7	65.8	362.7	74.0	1.99	0.12	1.85	0.14	
EAA, nmollmL	805.3	102.1	810.0	107.9	811.1	89.2	835.8	117.8	1.00	0.39	0.77	0.51	

SD: standard deviation, BCAA: branched-chain amino acids, EAA: essential amino acids; The number indicates a significant difference by the posthoc test: 1, Q1 vs Q3; 2, Q1 vs Q4; 3, Q2 vs Q4.

Conflicts of Interest: The authors have no potential conflicts of interest to disclose

Ethical standard: The Ethics Committee approved the study.

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