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Original Article

Plasma Amino Acid Profile in Severely Frail Elderly Patients in Japan

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SUMMARY

Background: Severe frailty, which is the highest level of frailty, leads to multiple health impairments that may individually affect the plasma-free amino acid (PFAA) profile. However, the PFAA profile of severely frail patients has not been clarified. The aim of this study was to describe the PFAA profile of severely frail elderly patients.

Methods: Elderly patients (aged ≥65 years) who were admitted to the Nukada Institute for Medical and Biological Research (Chiba, Japan) were included. Severe frailty was defined using the Canadian Study of Health and Aging Clinical Frailty Scale. Subjects were divided into non-frail and severely frail groups. The PFAA profile and clinical characteristics of the subjects were analyzed.

Results: Compared to the non-frail group (n = 31), the severely frail group (n = 28) had lower body mass index (BMI), serum albumin, serum prealbumin, hemoglobin, and blood pressure and higher C-reactive protein. Seventy-nine percent of severely frail patients had cognitive impairment. Severely frail patients had significantly lower essential amino acid (EAA) plasma concentrations than non-frail patients. Multiple linear regression analysis identified that valine ($p = 0.005$) was strongly associated with BMI. Valine ($p = 0.004$), leucine ($p = 0.004$), tryptophan ($p = 0.006$), lysine ($p < 0.001$), and total EAA ($p < 0.001$) levels were significantly associated with serum prealbumin levels.

Conclusion: Severely frail patients had multiple health impairments. BMI and nutritional status were most significantly associated with low EAA levels.

1. Introduction

Frailty is a common multidimensional geriatric syndrome that is characterized by increased vulnerability and declined physiological reserves.[1] Frailty can predict a patient's risk of death and the need for institutional care.[1–4] According to the Canadian Study of Health and Aging Clinical Frailty Scale (CSHA-CFS),[2] which has strong predictive validity and prognostic power based on clinical judgment, "severe frailty" is the highest level of frailty. It is categorized by terminal illness or complete dependency on others for activities of daily living. Within a few years of being classified as severely frail, patients show a markedly higher morbidity rate.[2,5,6] Thus, intervention programs designed to improve frailty are critical to improve quality of life and to extend longevity.

Amino acids play important roles in both physical and mental functions as nutrients and regulators. Plasma-free amino acids (PFAAs) circulate abundantly and are affected by metabolic disturbances. Recent studies identified abnormal PFAA profiles in age-related diseases,[7–9] indicating that abnormal PFAA metabolism may contribute to the pathophysiology of severe frailty. There is substantial evidence that supplementation of amino acids, particularly essential amino acids (EAAs), has beneficial effects on both physical and mental functions in the vulnerable elderly.[10–12] Therefore, PFAA profiling of severely frail patients could be beneficial in designing nutritional care programs. However, there is no evidence regarding the PFAA profile in severe frailty. Severely frail patients frequently suffer from a combination of multiple health problems, such as malnutrition and physical and psychiatric disabilities.[2] Since these impairments individually affect the PFAA

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profile,[7–9] frail patients could have unique PFAA patterns. Thus, we aimed to profile PFAAs in severely frail elderly patients.

2. Materials and methods

2.1. Study design and subjects

We conducted a cross-sectional study between 2008 and 2009 in Chiba, Japan. The study protocol was approved by the Ethics Committees of the Nukada Institute for Medical and Biological Research (Chiba, Japan) and the Wayo Women's University (Chiba, Japan). All subjects or their next-of-kin provided informed consent. Subjects who were inpatients or outpatients at the Nukada Institute for Medical and Biological Research (Chiba, Japan) and were aged ≥65 years were enrolled. The exclusion criteria were as follows: (1) patients who were unwilling to participate, (2) patients who were not evaluable due to insufficient clinical information and the fact that a judgement of severe frailty could not be made, and (3) patients who were diabetic and had uncontrolled blood glucose levels (hemoglobin A_{1c} >8.0% or fasting blood glucose levels >160.0 mg/dL). The study participants were divided into non-frail (NF) and severely frail (SF) groups. The PFAA profile and clinical characteristics of the patients were analyzed. All data were analyzed anonymously.

2.2. Clinical assessment

Frailty was evaluated using the CSHA-CFS,[2,13] a global clinical measure of biological age that consists of a 7-point scale with good predictive validity for mortality and prognostic power that relies on clinical judgment. This evaluation is based on a bedside judgement of frailty and other clinical information in the patient's medical record. Severely frail patients were bedridden and were fed orally. Subjects in the NF group were identified based on their clinical features and degree of independence, which was assessed using the activities of daily living classification,[14] established by the Japanese Ministry of Health, Labor, and Welfare. SF subjects were categorized as rank C/B2 (the lowest ranks) according to the activities of daily living classification, whereas NF subjects were categorized as rank J (the highest rank), which indicated that the NF group was completely independent. The NF group received scores of 1–3 based on the CSHA-CFS, which indicated that these subjects were NF patients. Cognitive impairment was judged according to the Nishimura Mental Scale[15] and Karasawa's criteria.[16]

Table 1
Clinico-demographic characteristics and plasma-free amino acid profiles of severely frail (SF) and Non-frail (NF) Patients (n = 59).

Characteristic	Patients		p-value
	SF	NF	
	(n = 28)	(n = 31)	
Sex (F/M)	18/10	16/15	0.325
Age (years), mean ± SD	85.9 ± 9.6	80.3 ± 9.0	0.023*
BMI (kg/m^2), mean ± SD	17.7 ± 2.7	22.9 ± 3.6	<0.001***
Albumin (g/dL), mean ± SD	3.4 ± 0.3	4.2 ± 0.3	<0.001***
Prealbumin (mg/dL), mean ± SD	17.1 ± 5.7	27.1 ± 6.9	<0.001***
Hb (g/dL), mean ± SD	11.3 ± 1.4	12.8 ± 2.0	0.003**
hs-CRP (mg/L), mean ± SD	0.9 ± 1.4	0.2 ± 0.3	0.008**
SBP (mmHg), mean ± SD	121.5 ± 14.9	130.5 ± 18.6	0.045*
DBP (mmHg), mean ± SD	68.5 ± 9.8	66.9 ± 10.5	0.549
Cognitive impairment, n (%)	22 (78.6)	0 (0.0)	<0.001***
Calorie intake (kcal/kg/day), mean ± SD	31.1 ± 8.4	31.3 ± 8.4	0.931
Daily nutritional intakes (g/kg/day), mean ± SD			
Protein	1.3 ± 0.4	1.2 ± 0.4	0.304
Carbohydrate	4.3 ± 1.3	4.7 ± 1.4	0.328
Lipid	0.9 ± 0.2	0.8 ± 0.3	0.556
Non-EAAs (μM), mean ± SD			
Alanine	401.0 ± 109.0	407.0 ± 75.0	0.813
Arginine	92.0 ± 21.0	101.0 ± 18.0	0.100
Asparagine	44.0 ± 6.0	43.0 ± 6.0	0.717
Aspartic acid	2.1 ± 0.8	2.2 ± 0.7	0.468
Glutamic acid	29.0 ± 12.0	31.0 ± 14.0	0.577
Glutamine	636.0 ± 83.0	593.0 ± 61.0	0.028*
Glycine	285.0 ± 84.0	255.0 ± 62.0	0.131
Proline	172.0 ± 45.0	158.0 ± 51.0	0.249
Serine	108.0 ± 25.0	108.0 ± 19.0	0.979
Tyrosine	54.0 ± 9.0	65.0 ± 14.0	0.001**
TNEAA[a]	1823.0 ± 195.0	1762.0 ± 156.0	0.193
EAAs (μM), mean ± SD			
Histidine	67.0 ± 11.0	75.0 ± 10.0	0.012*
Isoleucine	51.0 ± 10.0	60.0 ± 14.0	0.004**
Leucine	82.0 ± 17.0	108.0 ± 20.0	<0.001***
Lysine	178.0 ± 22.0	209.0 ± 29.0	<0.001***
Methionine	21.2 ± 3.5	25.2 ± 3.8	<0.001***
Phenylalanine	55.7 ± 7.9	62.1 ± 8.7	0.004**
Threonine	91.0 ± 28.0	122.0 ± 37.0	0.001**
Tryptophan	33.0 ± 9.0	49.0 ± 9.0	<0.001***
Valine	163.0 ± 29.0	220.0 ± 39.0	<0.001***
TEAA[b]	742.0 ± 99.0	930.0 ± 110.0	<0.001***

AbbreviationsBMI, body mass index; DBP, diastolic blood pressure; EAA, essential amino acid; F, female; Hb, hemoglobin; hs-CRP, high-sensitivity C-reactive protein; M, male; SBP, systolic blood pressure; SD, standard deviation; TEAA, total EAA; TNEAA, total non-EAA.
*p < 0.05, **p < 0.01, ***p < 0.001 (Welch's t-test).
[a] TNEAA = alanine + arginine + asparagine + aspartic acid + glutamic acid + glutamine + glycine + proline + serine + tyrosine.
[b] TEAA = histidine + isoleucine + leucine + lysine + methionine + phenylalanine + threonine + tryptophan + valine.

2.3. Biochemical parameters and plasma-free amino acid concentrations

Blood samples were taken after an overnight fast. Serum biochemical and hematological parameters were measured by SRL Inc. (Tokyo, Japan). For the amino acid analysis, blood samples were collected from the forearm vein into disodium ethylenediaminetetraacetate containing tubes and immediately placed on ice. Plasma was prepared by centrifugation at 800 *g* at 4 °C for 15 min and then stored at −80 °C until analysis. The plasma samples were deproteinized using acetonitrile at a final concentration of 80.0% before the measurements were made. Plasma amino acid concentrations were measured by high-performance liquid chromatography electrospray ionization mass spectrometry, followed by precolumn derivatization, as previously reported.[17]

2.4. Statistical analyses

Categorical data were analyzed using Fisher's exact test and continuous data were analyzed using Welch's *t*-test. Multiple linear regression (MLR) analysis was used to evaluate the contributions of clinical factors to the PFAA profile. Statistical analyses were conducted using JMP software version 13.0.0 (SAS Institute Inc., Cary, NC, USA). A $p < 0.05$ was considered statistically significant.

3. Results

Of the 79 subjects enrolled in this study, 20 were not included because they could not be categorized as NF or SF ($n = 18$) or they had uncontrolled diabetes ($n = 2$). In total, 59 subjects were included in the analysis. The mean age of the subjects was slightly higher in the SF group than in the NF group ($p = 0.023$). Body mass index (BMI), albumin, prealbumin, and hemoglobin levels were significantly lower in the SF group than in the NF group ($p < 0.05$). High-sensitivity C-reactive protein levels were higher in the SF group than in the NF group ($p = 0.003$). Cognitive impairment was identified in 79% of the subjects in the SF group, but none of the subjects in the NR group. Macronutrient intake did not differ significantly between the two groups ($p > 0.05$; Table 1).

There were significant differences between the SF and NF groups for 11 PFAAs (glutamine, tyrosine, and all EAAs). For non-EAAs, glutamine and tyrosine levels were higher and lower in the SF group, respectively. In contrast, all plasma EAA levels were significantly lower in the SF group ($p < 0.05$). There was a 20.2% reduction in the total EAA level in the SF group compared to the NF group ($p < 0.001$).

Although several PFAAs indicated significant correlations with clinical variables, most correlation coefficients were low (Table 2). However, valine, leucine, lysine, tryptophan, and the total EAA levels were strongly correlated with BMI, albumin, and prealbumin levels ($r > 0.500$). To investigate the contributions of age, sex, BMI, and nutritional status to PFAA levels, MLR analysis was performed (Table 3). Because prealbumin is a more sensitive nutritional marker than albumin,[18] we used prealbumin for the MLR analysis, which indicated that valine was strongly associated with BMI (Table 3). Valine ($p = 0.004$), leucine ($p = 0.004$), lysine ($p < 0.001$), tryptophan ($p = 0.006$), and the total EAA ($p < 0.001$) levels were significantly associated with prealbumin levels.

4. Discussion

We detected low plasma EAA levels in SF patients. Such patients frequently suffer from multiple health impairments.[2] Decreased BMI, which is associated with mortality, is a hallmark feature of severe frailty.[19] According to some reports,[20,21] the potential mechanisms for decreased body composition (muscle and fat mass) in SF patients include abnormal absorption of nutrients and high energy expenditure, and disuse muscle atrophy. Therefore, SF patients are more prone to malnutrition than NF patients. In agreement with these reports, our study showed that nutritional

Table 2
Pearson's correlation coefficients for the relationship between clinical characteristics and plasma-free Amino acid levels.

Variable[a]	Age	BMI	SBP	DBP	hs-CRP	Hb	ALB	Prealbumin	Dietary Nutritional Intake			
									Calorie	Protein	Carbohydrate	Lipid
Non-Essential Amino Acids												
Alanine	−0.097	0.159	−0.040	−0.141	−0.208	0.111	0.126	0.218	−0.255	−0.267*	−0.189	−0.173
Arginine	−0.031	0.032	0.260*	0.057	−0.150	−0.042	0.179	0.169	0.054	0.055	0.036	−0.060
Asparagine	0.175	−0.239	0.077	0.068	−0.085	−0.031	−0.051	−0.005	0.047	0.054	−0.062	−0.105
Aspartic acid	−0.145	0.312*	−0.020	0.044	0.112	0.151	0.107	0.079	−0.215	−0.162	−0.240	−0.091
Glutamic acid	−0.067	0.286*	−0.054	−0.048	0.243	0.070	0.104	0.050	−0.149	−0.088	−0.152	−0.005
Glutamine	0.065	−0.359**	0.151	0.348**	0.153	0.060	−0.114	−0.186	−0.008	0.027	−0.035	−0.061
Glycine	0.075	−0.154	−0.043	0.164	−0.176	−0.144	−0.137	−0.141	0.053	0.067	0.045	−0.096
Proline	0.178	−0.072	0.041	0.034	0.134	−0.091	−0.152	−0.069	−0.240	−0.302*	−0.212	−0.208
Serine	−0.099	−0.026	0.129	0.213	−0.096	0.236	0.106	−0.029	−0.159	−0.152	−0.214	−0.262*
Tyrosine	0.012	0.191	0.091	−0.226	−0.027	0.336**	0.364**	0.212	−0.056	−0.140	−0.047	−0.171
TNEAA	0.041	−0.151	0.081	0.153	−0.097	0.046	−0.007	0.010	−0.179	−0.178	−0.174	−0.232
Essential Amino Acids												
Histidine	−0.160	0.190	0.255	0.060	−0.108	0.210	0.460***	0.458***	−0.021	−0.149	0.017	−0.028
Isoleucine	−0.192	0.377**	0.051	−0.309*	−0.162	0.155	0.246	0.356**	−0.127	−0.067	−0.101	−0.013
Leucine	−0.227	**0.592***	0.174	−0.178	−0.106	0.354**	**0.541***	**0.565***	−0.184	−0.198	−0.133	−0.151
Lysine	−0.085	0.287*	0.129	−0.018	−0.221	0.302*	0.426***	**0.518***	−0.007	−0.094	0.020	−0.232
Methionine	−0.091	0.286*	0.203	−0.086	−0.225	0.346**	0.393**	0.372**	−0.084	−0.210	−0.021	−0.216
Phenylalanine	0.152	0.383**	0.192	−0.332*	0.112	0.124	0.240	0.255	−0.166	−0.200	−0.161	−0.151
Threonine	−0.083	0.226	0.149	−0.078	−0.295*	0.215	0.415**	0.467***	0.044	−0.088	0.029	−0.028
Tryptophan	−0.120	0.415**	0.266*	−0.156	−0.241	0.370**	**0.578***	**0.512***	0.012	−0.082	−0.001	−0.100
Valine	−0.237	**0.623***	0.199	−0.206	−0.142	0.302*	**0.535***	**0.561***	−0.150	−0.152	−0.082	−0.124
TEAA	−0.182	**0.534***	0.232	−0.189	−0.234	0.348**	**0.597***	**0.670***	−0.083	−0.161	−0.051	−0.147

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[a] Amino acid concentrations were transformed to z-scores for each sex. Z-scores were used for the correlation analysis. High correlation coefficients are shown in bold ($r > 0.500$); Abbreviations: ALB, albumin; BMI, body mass index; DBP, diastolic blood pressure; Hb, hemoglobin; hs-CRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; TNEAA, total non-essential amino acid; TEAA, total essential amino acid.

Table 3
Multivariate linear regression analysis of the association between clinical characteristics and plasma-free amino acid levels.

Variable	Age	Sex	BMI	Hb	Prealbumin
Valine	0.844	0.465	0.005**	0.392	0.004**
Leucine	0.614	0.100	0.025	0.075	0.004**
Tryptophan	0.258	0.833	0.306	0.098	0.006**
Lysine	0.107	0.325	0.845	0.141	<0.001***
TEAA	0.138	0.094	0.127	0.124	<0.001***

**p < 0.01, ***p < 0.001 (Bonferroni corrected).
Abbreviations: BMI, body mass index; Hb, hemoglobin; TEAA, total essential amino acid.

marker levels (serum albumin and prealbumin) were significantly lower in the SF group than in the NF group, despite no differences in food and nutrient intakes between the two groups. This suggests that the SF group may require more energy and nutrition to maintain nutritional status and body composition.

Previous studies have demonstrated that body composition parameters positively correlated with plasma branched-chain amino acids.[8,22] We observed that BMI was reduced in the SF group and that it was significantly correlated with plasma valine level. Thus, the reduced plasma valine levels in SF patients may be due to altered body composition. Conversely, we showed that reduced plasma EAA levels in SF patients could result from their poor nutritional status (defined by serum albumin and prealbumin levels). A previous study reported that protein-energy malnutrition in elderly subjects resulted in reduced plasma levels of all EAAs,[7] which was comparable to our findings. Thus, the low EAA profile in SF patients can mainly be explained by alterations in body composition and malnutrition.

However, altered amino acid metabolism could also affect body composition and nutritional status (and *vice versa*). EAAs, especially branched-chain amino acids, regulate protein synthesis and nutritional status by activating the mammalian target of rapamycin complex.[23,24] Furthermore, EAAs act as precursors of important neurotransmitters, such as catecholamines (phenylalanine), histamine (histidine), and serotonin (tryptophan). Our findings consider the hypothesis that low EAA levels could affect health conditions and nutritional status, because EAAs have several physiological functions. In fact, there is substantial evidence that EAA supplementation improves physical and mental functions through direct and indirect effects.[10–12,25–30] Therefore, the supplementation of EAAs could exert beneficial effects in SF patients with a low plasma EAA profile. However, this study has an important limitation. Due to its cross-sectional design, direct associations between health conditions and PFAA profiles were not sufficiently proven. Future intervention studies of EAAs are needed.

In conclusion, we found that SF patients have low plasma EAA levels, which may be due to low a BMI and insufficient nutritional intake. Therefore, the supplementation of EAAs could have a beneficial effect in SF patients.

Declarations of interest

Y.A., N.O., A.I., T.M., T.A., Y.S., K.N., Y.K., M.M., and Y.N. are employees of Ajinomoto Co., Inc. (Tokyo, Japan).

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References

1. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56:M146–M156.
2. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ (Can Med Assoc J)*. 2005;173:489–495.
3. Pel-Littel RE, Schuurmans MJ, Emmelot-Vonk MH, et al. Frailty: defining and measuring of a concept. *J Nutr Health Aging*. 2009;13:390–394.
4. Fairhall N, Langron C, Sherrington C, et al. Treating frailty—a practical guide. *BMC Med*. 2011;9:83.
5. Matusik P, Tomaszewski K, Chmielowska K, et al. Severe frailty and cognitive impairment are related to higher mortality in 12-month follow-up of nursing home residents. *Arch Gerontol Geriatr*. 2012;55:22–24.
6. Ekerstad N, Swahn E, Janzon M, et al. Frailty is independently associated with short-term outcomes for elderly patients with non-ST-segment elevation myocardial infarction. *Circulation*. 2011;124:2397–2404.
7. Polge A, Bancel E, Bellet H, et al. Plasma amino acid concentrations in elderly patients with protein energy malnutrition. *Age Ageing*. 1997;26:457–462.
8. Lustgarten MS, Price LL, Chale A, et al. Branched chain amino acids are associated with muscle mass in functionally limited older adults. *J Gerontol A Biol Sci Med Sci*. 2014;69:717–724.
9. Trushina E, Dutta T, Persson XM, et al. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. *PLoS One*. 2013;8, e63644.
10. Rondanelli M, Opizzi A, Antoniello N, et al. Effect of essential amino acid supplementation on quality of life, amino acid profile and strength in institutionalized elderly patients. *Clin Nutr*. 2011;30:571–577.
11. Ferrando AA, Paddon-Jones D, Hays NP, et al. EAA supplementation to increase nitrogen intake improves muscle function during bed rest in the elderly. *Clin Nutr*. 2010;29:18–23.
12. Børsheim E, Bui QU, Tissier S, et al. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. *Clin Nutr*. 2008;27:189–195.
13. Rockwood K, Stadnyk K, MacKnight C, et al. A brief clinical instrument to classify frailty in elderly people. *Lancet*. 1999;353:205–206.
14. Sakamoto K, Nakamura T, Hagino H, et al. Report on the Japanese Orthopaedic Association's 3-year project observing hip fractures at fixed-point hospitals. *J Orthop Sci*. 2006;11:127–134.
15. Nishimura T, Kobayashi T, Hariguchi S, et al. Scales for mental state and daily living activities for the elderly: clinical behavioral scales for assessing demented patients. *Int Psychogeriatr*. 1993;5:117–134.
16. Maki N, Ikeda M, Hokoishi K, et al. The validity of the MMSE and SMQ as screening tests for dementia in the elderly general population– a study of one rural community in Japan. *Dement Geriatr Cognit Disord*. 2000;11:193–196.
17. Shimbo K, Kubo S, Harada Y, et al. Automated precolumn derivatization system for analyzing physiological amino acids by liquid chromatography/mass spectrometry. *Biomed Chromatogr*. 2010;24:683–691.
18. Unal D, Orhan O, Eroglu C, et al. Prealbumin is a more sensitive marker than albumin to assess the nutritional status in patients undergoing radiotherapy for head and neck cancer. *Contemp Oncol (Pozn)*. 2013;17:276–280.
19. Hubbard RE, Lang IA, Llewellyn DJ, et al. Frailty, body mass index, and abdominal obesity in older people. *J Gerontol A Biol Sci Med Sci*. 2010;65:377–381.
20. Rémond D, Shahar DR, Gille D, et al. Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. *Oncotarget*. 2015;6:13858–13898.
21. Klipstein-Grobusch K, Reilly JJ, Potter J, et al. Energy intake and expenditure in elderly patients admitted to hospital with acute illness. *Br J Nutr*. 1995;73:323–334.
22. Jourdan C, Petersen AK, Gieger C, et al. Body fat free mass is associated with the serum metabolite profile in a population-based study. *PLoS One*. 2012;7, e40009.
23. Chung J, Bauer DE, Ghamari A, et al. The mTORC1/4E-BP pathway coordinates hemoglobin production with L-leucine availability. *Sci Signal*. 2015;8. ra34.
24. Kim DH, Sarbassov DD, Ali SM, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*. 2002;110:163–175.
25. Ohtani M, Kawada S, Seki T, et al. Amino acid and vitamin supplementation improved health conditions in elderly participants. *J Clin Biochem Nutr*. 2012;50:162–168.
26. Jeevanandam M, Young DH, Ramias L, et al. Effect of major trauma on plasma free amino acid concentrations in geriatric patients. *Am J Clin Nutr*. 1990;51:1040–1045.
27. Solerte SB, Gazzaruso C, Bonacasa R, et al. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. *Am J Cardiol*. 2008;101:69E–77E.
28. Paddon-Jones D, Wolfe RR, Ferrando AA. Amino acid supplementation for reversing bed rest and steroid myopathies. *J Nutr*. 2005;135:1809S–1812S.
29. Børsheim E, Bui QU, Tissier S, et al. Amino acid supplementation decreases plasma and liver triacylglycerols in elderly. *Nutrition*. 2009;25:281–288.
30. Paddon-Jones D, Børsheim E, Wolfe RR. Potential ergogenic effects of arginine and creatine supplementation. *J Nutr*. 2004;134:2888S–2894S.