

Oral Amino Acids in Elderly Subjects: Effect on Myocardial Function and Walking Capacity

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Key Words

Amino acids · Elderly · Exercise · Walking impairment · Myocardium

Abstract

Background: With advancing age the risk of developing serious nutritional deficiencies increases, and disturbances to the actions of insulin and insulin-like growth factor, coupled with reduced protein/amino acid (AA) intake, impair protein synthesis in muscles. **Objective:** To assess the effects of administering oral AAs on walking capacity and perceived walking impairment, isometric muscular strength, and myocardial function at rest and during isometric exercise. **Methods:** One hundred elderly subjects (aged >65 years) with reduced physical activity were randomized to receive an oral AA mixture (12 g/day) or placebo for 3 months. At baseline and after 3 months of therapy we assessed physical capacity with the 6-min walk test, and perceived physical impairment with the walking impairment questionnaire (WIQ); we assessed maximal isometric muscular strength of the right hand with a handgrip dynamometer, and left ventricular ejection fraction (LVEF) using quantitative two-dimensional echocardiography at rest and during acute overload. **Results:** Three months of AA treatment resulted in significant increases in 6-min walk distance (268.8 ± 34.9 vs. 212 ± 40 m, $p < 0.001$), WIQ scores

(distance: 68.3 ± 12 vs. $53 \pm 14.8\%$, $p < 0.001$; speed: 72.2 ± 14.4 vs. $52.8 \pm 12\%$, $p < 0.001$; stairs: 98.2 ± 24 vs. $72.4 \pm 22\%$, $p < 0.001$), and in maximal muscular isometric strength (20.2 ± 2 vs. 14 ± 2.8 kg, $p < 0.001$). Moreover, peak stress LVEF was higher during AA administration when compared to placebo administration (67 ± 7 vs. $56 \pm 9\%$, $p < 0.01$). Left ventricular response to exercise normalized during AA administration in 24 out of 32 (75%) patients with abnormal LV response at baseline, whereas it remained unchanged in the placebo group. **Conclusion:** An oral AA supply such as that used in this study improves ambulatory capacity, maximal isometric muscle strength and myocardial ability to match an acute overload in elderly subjects without affecting the main metabolic parameters. These functional gains may translate into increased perceived walking capacity.

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Introduction

Complex social and physical environments influence the aging process in many different ways. The identification of mutable risk factors offers the possibility to develop interventions that ameliorate deteriorations in health status that often coincide with aging and perhaps to influence the traditional activities of daily life, and thus

health-related quality of life. Notably, the elderly are a population at high risk of mobility limitation, and participation of elderly people in physical activity and exercise is at a level below that recommended by public health guidelines for the maintenance of physical fitness, health, and function [1, 2]. In the elderly population, the combination of inactivity and the associated high risk of morbidity suggest that interventions able to increase activity and exercise could have a beneficial effect on population health in later life. With advancing age the risk of developing serious nutritional deficiencies also increases, and disturbances of the actions of insulin and insulin-like growth factor, coupled with reduced protein/amino acid (AA) intake, impair protein synthesis in muscles [3]. Moreover, inability to increase adequately cardiac function during exercise may constitute a limiting factor causing physical inactivity even if overall cardiac function in elderly people is adequate to meet the body's requirements at rest.

This study was designed to assess the effects of oral AA administration on walking capacity, maximal isometric muscular strength, and left ventricular (LV) function at rest and during exercise in healthy elderly subjects with reduced physical activity, and to investigate whether the administration of dietary essential AA supplements could modify the self-perceived walking impairment scores.

Methods

Patient Selection and Study Design

We prospectively enrolled 100 healthy elderly subjects (age >65 years) with reduced physical activity [4], and normal resting LV systolic function. In order to avoid bias in the allocation of the subjects, all patients were recruited by outside general physicians and none were enrolled by our institution or by the echocardiography laboratory. The decision was made to study elderly patients as they are encountered in everyday practice.

The amount of physical exercise was assessed by face-to-face interviews with each participant, who completed a validated questionnaire about his/her leisure time physical activities, at baseline and after 3 months of study treatment (AAs or placebo) [5]. Sedentary lifestyle was defined by the number of hours spent sitting down per week during leisure time. Subjects indicated the average amount of time they had been sitting down (watching TV, etc.) on a typical work day and on a typical weekend day. Metabolic equivalents (METs) were then assigned to each activity and a value for total weekly MET-hours was computed: one MET represents the energy expenditure for an individual at rest, whereas a 10-MET activity requires 10 times the resting energy expenditure [6]. Sedentary people were defined as those having a ratio of less than 0.5 for MET-hours of physical activity/week vs. hours spent sitting down during leisure time [7].

Exclusion criteria were the following: LV dysfunction (LVEF <50%), history of exertional angina, exercise tolerance limited by intermittent claudication, arterial hypertension with nonoptimal control of blood pressure (systolic >150 mm Hg, diastolic >90 mm Hg), acute or chronic pulmonary disease, overt nephropathy (creatinine >1.4 mg/dl), and obesity. All subjects gave their informed consent, and the protocol was approved by the ethical committee of our University.

The dietary assessment was performed using a questionnaire including dietary history, 24-hour recall, 7-day recall, 7-day record and food frequency [8, 9]. The estimated daily amount of calories was $1,700 \pm 250$ kcal/day containing 55% of carbohydrates, 30% of lipids, and 15% of proteins. The assignment of placebo or AA treatment to patients was done randomly. After randomization, patients were given the study treatment for 3 months. During the placebo period, the carbohydrate fraction was subtracted from a similar amount of dietary carbohydrate. Subjects were instructed by a registered dietitian to reduce meal-related caloric intake by 450 kcal in order to adjust for the supplements. Both placebo and AAs were ingested as snacks at 10.00 a.m., 4.00 p.m., and 10.00 p.m.

The study consisted of 3 phases: (1) baseline examination with anthropometrical, metabolic and cardiac evaluations; (2) randomization to a 12-week maintenance period on AA or placebo, and (3) anthropometrical, metabolic and cardiac evaluations. During phase 2, to each subject's diet 12 g of AAs plus 12.21 g of glucose, or placebo containing 12.21 g of glucose were added at 10.00 a.m., 4.00 p.m. and 10.00 p.m. The detailed composition of the AA mixture was the following (g/day): *L*-leucine 3.8, *L*-lysine 2, *L*-isoleucine 1.9, *L*-valine 1.9, *L*-threonine 1.1, *L*-cystine 0.4, *L*-histidine 0.4, *L*-phenylalanine 0.3, *L*-methionine 0.2, *L*-tyrosine 0.1, and *L*-tryptophan 0.1. This formulation contains all essential AAs and two non-essential AAs (tyrosine and cystine), in a complex ratio that was designed to match metabolic requirements in conditions of elevated demand [10, 11]. During phase 2 of the study, patients were blinded to treatment (placebo or AAs), with no knowledge of the origin or taste of study medications. In the present study, we cannot entirely exclude that patients could have been able to figure out what they were actually taking because of the very stringent flavor of AAs. In an attempt to prevent this particular bias, placebo and AAs were prepared using an orange flavoring to minimize the taste difference between the two. Furthermore, patients were instructed to withhold any information about their regular medications and comments about the study supplementation (e.g. taste), so as not to influence investigators' (technician, echocardiographer) perceptions. These considerations were made to reduce the potential for bias.

During the baseline and the final visit, information about personal data, habits, (smoking and alcohol intake) and pharmacological therapies were collected by a physician. Height and weight were measured following a standardized protocol. Body mass index (BMI, weight/height²) was used as an estimate of overall adiposity. Blood pressure was measured in the sitting position, with a mercury sphygmomanometer and cuffs of appropriate size; the average of 3 measurements 5 min apart was recorded. A blood sample was collected for the determinations of plasma glucose, total cholesterol, HDL-cholesterol, and triglycerides. A resting standard 12-lead ECG was recorded in each patient.

Ambulatory dysfunction was assessed by a 6-min walk test. A walking impairment questionnaire was used to evaluate the self-

perceived ambulatory dysfunction. Maximal isometric muscular strength of the right hand was measured during isometric exercise by a dynamometer. Finally, LV systolic function at rest and during handgrip was assessed by quantitative two-dimensional echocardiography. Data were collected at baseline and after a 3-month period of oral AA or placebo intake.

Echocardiographic Analysis

Echocardiographic examinations were performed with a Hewlett-Packard cardiology ultrasound system (Sonos 5500). Echocardiographic studies were coded and read by two independent observers in a blinded manner, with no knowledge of the patient's identity and experimental condition. Echocardiographic analysis was performed using the digitized cine loop method (Pre Vue III System, Nova MicroSonics, Inc.). LV volumes were calculated by an ellipsoid biplane area-length method [12]. LVEF was derived as end-diastolic volume minus end-systolic volume divided by the end-diastolic volume.

Isometric Exercise

During handgrip testing, arterial pressure was measured every 30 s by an oscillometric method (Nippon Colin Co. Ltd). LV function was assessed by quantitative two-dimensional echocardiography in apical four- and two-chamber views. Maximal voluntary contraction was determined by means of a handgrip dynamometer. Isometric exercise was performed for 3 min at 40% maximal voluntary contraction of the right hand. Patients were instructed to avoid performing the Valsalva maneuver during handgrip. Heart rate and electrocardiographic tracings were continuously monitored. Normal LV response to handgrip is characterized either by a >0.05 -unit increase or by no changes in LVEF. LV dysfunction induced by isometric exercise is defined as a >0.05 -unit decline in LVEF [13].

Six-Minute Walk Test

Each subject performed a 6-min walk test [4] in the presence of a trained technician, blinded to the patients' medical history and experimental condition, at baseline and after 3 months of treatment. Two cones were placed 20 m apart in a marked corridor. Elderly subjects were instructed to walk as many laps around the cones as possible. Each subject wore a calibrated electronic pedometer (Omron Health Care Corporation, Vernon Hills, Ill., USA) on his/her hip to obtain the total number of steps taken during the test. The technician provided encouragement to continue walking every 2 min and recorded the total distance walked and total number of steps.

Walking Impairment Questionnaire

Self-reported ambulatory ability was assessed using an adapted questionnaire in which the subjects evaluated their walking ability at various speeds and distances and their ability to climb stairs [4, 14, 15]. A scale ranging between 0 and 100 assesses each aspect: a score of 0 represents inability and a score of 100 represents no difficulty in performing the task.

Statistical Analysis

Results are expressed as mean value \pm standard deviation (SD). Multiple comparisons were performed by two-way repeated measures of analysis of variance (ANOVA), followed by the Fischer protected least significant difference test. We determined the num-

ber of patients needed to treat in order to detect a difference of greater than or equal to 6 units in LVEF (%), with a type I error (two-sided p value) of 0.01 and a type II error (beta) of 0.1 (power of 0.9). Using data from a previous pilot study (SD of LVEF equal to 9) where LVEF at peak handgrip in elderly patients decreased by more than 5 units in contrast to the values at rest, we calculated that it would be sufficient to treat 39 patients per group, in a randomized controlled trial design (placebo versus AAs), to detect a significant difference in LVEF at peak handgrip. Assuming that no more than 10% of the patients might be lost to follow-up or might withdraw from the study, a number of 43 patients would suffice to test for our initial hypothesis. For all statistical analyses we used the SPSS package version 10.1 for Windows (Statistical Package for Social Sciences, SPSS Inc., Chicago, Ill., USA). A p value ≤ 0.05 by the two-tailed test was considered to be statistically significant.

Results

Subjects were randomized to receive AA supplementation (group A, $n = 50$) or placebo (group B, $n = 50$). Compliance with the ingestion of the study medication was assessed by face-to-face interviews and by counting the containers of the study medication, at the follow-up visit, and between the visits by weekly telephone calls to encourage the patients to ingest the snacks. Compliance with the study medication was acceptable: 86% of the AAs vs. 87% of placebo was ingested and was in good accordance with the information given by the patients during the interviews. Compliance with the scheduled return visits was very good: only 2 subjects in the AA group (A) and 3 in the placebo group (B) refused the return visits and were excluded from the final analysis. During the study period, patients maintained their habitual sedentary lifestyle. Forty-eight subjects in group A (20 men, 25 women, aged 74 ± 6 years) and 47 in group B (22 men, women, aged 74 ± 5 years) completed the study. The main demographic and baseline characteristics, and concomitant medical treatment of the study population are summarized in table 1.

Body mass index did not change significantly with intervention (baseline versus intervention: 25.2 ± 4 versus 25.1 ± 4 kg/m² for group A, and 25.3 ± 5 versus 25.2 ± 4 kg/m² for group B). Similarly, plasma glucose, total cholesterol, HDL cholesterol, and triglycerides did not significantly change from baseline values in both of the study groups.

There were no complications during the isometric exercise sessions or during the 6-min walk tests. The AA mixture was well tolerated and only a mild dyspepsia was reported by 2 subjects.

Six-Minute Walk Test, Self-Perceived Walking Impairment, and Maximal Isometric Muscular Strength (table 2)

Group A and B patients have similar values in the 6-min walk distance and scores of the walking impairment questionnaire at baseline evaluation. The 6-min walk distance increased from 212.5 ± 34 to 268.8 ± 34.9 m ($p < 0.001$) after AA treatment, but did not change after placebo (212 ± 36 vs. 212 ± 40 m, $p = \text{n.s.}$). The baseline scores on the three subscales of the walking impairment questionnaire (WIQ) changed significantly during AA

treatment. Subjects reported significantly higher scores on the distance (54.2 ± 10.9 vs. $68.3 \pm 12\%$, $p < 0.001$), speed (52.2 ± 12 vs. $72.2 \pm 14.4\%$, $p < 0.001$), and stairs (72.4 ± 20.6 vs. $98.2 \pm 24\%$, $p < 0.001$) subscales on the questionnaire after AA treatment. These parameters did not significantly change after placebo administration. Comparisons of post-intervention values between randomized groups showed significant differences for the 6-min walk distance and the three subscales of WIQ (distance, speed, and stairs; $p < 0.001$, see table 2).

Group A and B subjects had comparable values of baseline maximal isometric muscular strength. Oral AA supplementation significantly increased the maximal muscular capacity for work during isometric exercise (14.6 ± 2.2 vs. 20.2 ± 2 kg, $p < 0.001$), while this parameter did not change after placebo administration (14.4 ± 2.4 vs. 14 ± 2.8 kg) (table 2). As a consequence, maximal isometric muscular strength was significantly higher in the AA-treated than in the placebo group ($p < 0.001$).

Table 1. Main demographic and baseline characteristics of the study population

	Group A (n = 48)	Group B (n = 47)
Sex, M/F	20/28	22/25
Age, years	74 ± 6	74 ± 5
BMI, kg/m ²	27.4 ± 3.6	27.3 ± 3.7
Plasma glucose, mmol/l	4.67 ± 0.4	4.62 ± 0.5
Plasma total cholesterol, mmol/l	5.49 ± 1.3	5.44 ± 1.2
Plasma HDL cholesterol, mmol/l	1.55 ± 0.3	1.50 ± 0.3
Plasma triglycerides, mmol/l	1.53 ± 0.4	1.57 ± 0.5
Diagnosis of hypertension, %	14 (29%)	14 (30%)
Hypolipidemic drugs	4	4
ACE inhibitors	12	10
AT ₂ -receptor blockers	1	–
Calcium channel blockers	4	2
β-Blockers	2	1
Diuretics	4	2
Smoking status:		
nonsmoker/active/former	30/7/11	32/5/10

Values are expressed as mean \pm SD for normally distributed variables.

Resting and Exercise LV Function (table 3)

All study subjects showed a normal resting LVEF (>50%) and groups A and B had comparable values both in LVEF and LV end-diastolic volume index (LVEDVI). Neither resting LVEDVI nor LVEF changed significantly after AA or placebo administration in comparison with baseline evaluation. Heart rate, systolic and diastolic blood pressure did not change in study subjects after oral AA or placebo administration.

In group A, 32 (67%) of the study subjects had a ≥ 0.05 -unit decline in LVEF, and the remaining 16 (33%) had a normal response to handgrip (5 had a significant increase, whereas 11 showed no change in LVEF). At peak handgrip, LVEF was significantly lower than the resting value (55 ± 9 vs. $62 \pm 8\%$, $p < 0.001$). In group B, 32 (68%) of

Table 2. Ambulatory function and maximal isometric muscle strength in elderly subjects after treatment with AAs or placebo

	Group A (n = 48)		Group B (n = 47)	
	baseline	AA	baseline	placebo
6-min walk distance, m	212.5 ± 34	$268.8 \pm 34.9^*$	212 ± 36	212 ± 40
WIQ distance, %	54.2 ± 10.9	$68.3 \pm 12^*$	54 ± 12.9	53 ± 14.8
WIQ speed, %	52.2 ± 12	$72.2 \pm 14.4^*$	53 ± 12.8	52.8 ± 12
WIQ stairs, %	72.4 ± 20.6	$98.2 \pm 24^*$	72.6 ± 22	72.4 ± 22
MIMS, kg	14.6 ± 2.2	$20.2 \pm 2^*$	14.4 ± 2.4	14 ± 2.8

WIQ = Walking impairment questionnaire; MIMS = maximal isometric muscle strength; values are mean \pm SD; * $p < 0.001$ for comparison of post intervention values between groups.

the study subjects had a ≥ 0.05 -unit decline in LVEF, and the remaining 15 (32%) had a normal response to handgrip (5 had a significant increase, whereas 10 showed no change in LVEF). At peak handgrip, LVEF was significantly lower than the resting value (56 ± 8 versus $63 \pm 9\%$, $p < 0.001$).

After 3 months of oral intake of the AA mixture, peak stress LVEF significantly increased in comparison with resting values (67 ± 7 versus $63 \pm 6\%$, $p < 0.01$). In 24 (75%) of the 32 patients with abnormal LV response to handgrip at baseline condition, LVEF did not decline

from resting values (in 8 LVEF increased by >0.05 unit, and in 16 it did not change) (table 4).

After placebo administration there were no significant changes in LVEF values at peak handgrip and values were significantly lower than in AA-treated patients ($p < 0.001$). Moreover, the number of patients with a normal response to exercise in comparison with baseline studies did not change during placebo administration.

Discussion

The inability to adequately increase cardiac function during exercise and the presence of a reduced muscle working ability represent major limiting factors to perform physical activity in elderly people without clinically evident cardiac disease. This study was performed to assess the effects of oral AA supplementation on myocardial function and free-living daily activity in elderly subjects with reduced physical activity and ambulatory disability. This mixture, which contained AAs in a ratio formulated to match a metabolic setting of increased requirement [16–20], improved ambulatory capacity and maximal muscular isometric strength, and did not change metabolic parameters.

There is evidence supporting the beneficial effects of exercise on functional capacity in older people [4, 21–24]. The increases in physical activity parameters, obtained by AA intake in the present study, are of similar magnitude to those reported after exercise training [4, 21, 22]. Exercise also improves cardiac function in elderly people. Unfortunately, previous papers did not assess its capacity to increase LVEF during exercise. However, the relative increases in VO_{2max} of 16–23% are of similar magnitude to increases in LVEF during handgrip evaluated in

Table 3. LV systolic function at rest and during exercise in elderly subjects after treatment with AAs or placebo

	Group A (n=48)		Group B (n=47)	
	baseline	AA	baseline	placebo
<i>Resting</i>				
HR, beats/min	76 ± 10	75 ± 12	74 ± 14	75 ± 10
SBP, mm Hg	139 ± 10	138 ± 15	136 ± 10	138 ± 15
DBP, mm Hg	82 ± 9	82 ± 6	80 ± 12	84 ± 8
LVEDVI, ml/m ²	60 ± 16	58 ± 11	60 ± 18	60 ± 12
LVEF, %	62 ± 8	63 ± 6	63 ± 9	62 ± 10
<i>Handgrip</i>				
HR, beats/min	92 ± 12	89 ± 12	94 ± 14	92 ± 12
SBP, mm Hg	188 ± 21	181 ± 21	185 ± 22	185 ± 20
DBP, mm Hg	95 ± 15	94 ± 14	96 ± 20	96 ± 24
LVEDVI, ml/m ²	64 ± 14	63 ± 14	64 ± 16	64 ± 18
LVEF, %	55 ± 9	67 ± 7*	56 ± 8	56 ± 9

Values are means ± SD. HR = Heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction. * $p < 0.01$ for the comparison of post-intervention values between randomized groups.

Table 4. BMI, plasma creatinine, iron, prealbumin and albumin values at baseline and after treatment in the study population

	Group A (n = 48)		Group B (n = 47)	
	baseline	AA	baseline	placebo
BMI, kg/m ²	27.4 ± 3.6	27.5 ± 3.7	27.2 ± 3.5	27.4 ± 3.7
Creatinine, μ mol/l	87.3 ± 18.9	88.3 ± 17.3	88.3 ± 17.7	87.4 ± 17.1
Iron, μ mol/l	17.4 ± 6.4	18.2 ± 6.4	18.1 ± 6.0	18 ± 5.9
Prealbumin, mg/l	199 ± 82	202 ± 76	198 ± 85	201 ± 73
Albumin, g/l	32.3 ± 4.1	32.0 ± 3.8	32 ± 4.3	32.2 ± 3.4
Mean ± SD.				

the present study (21%). Moreover, training also improves LV fractional shortening and LVEF at rest [24]. In this setting, exercise training seems to be a more powerful intervention because oral AA supplementation did not significantly improve resting LV performance in our patients. This nutritional intervention may be helpful in further improving and, particularly, in maintaining the beneficial effects of training because these latter improvements are precarious, and are completely lost after 4 months of detraining, when elderly subjects are no longer exposed to the constraints and the social stimulation of the imposed protocol [24].

The increase in peripheral muscle working capacity was associated with a significant improvement in myocardial performance and LV ability to match an acute overload. These findings translated into an increased physical function which allowed the patients to increase their capacity to perform the common activities of daily life. In a clinical setting, these results may be relevant because changes in lifestyle which increase levels of activity and exercise could have a great impact in reducing the high risk of illness and disability associated with inactivity.

Advancing age is associated with reduced muscle protein synthesis, altered expression of and chemical modifications to muscle proteins, and reduced muscle strength [19]. Evidence is accumulating that dietary AA supplementation has beneficial effects in the elderly: exogenous

AAs improve muscle protein balance and stimulate muscle protein anabolism despite a significantly higher first-pass splanchnic extraction [3, 20, 25, 26]. Aging also affects myocardial perfusion and function: these resulting alterations lead to abnormalities in coronary vasodilator capacity, and although LV function at rest is preserved, LV reserve capacity declines [27–29]. This latter condition was clearly present in our study patients who had LV dysfunction during exercise. During myocardial ischemia and dysfunction, free fatty acids are transformed from an effective to a harmful fuel. In these conditions, AA supplementation may improve myocardial energetics and limit the damage during LV acute overload which causes high myocardial oxygen expenditure and eventually myocardial ischemia in the aging heart [30].

In conclusion, oral AA supplementation improved ambulatory capacity and maximal muscular isometric strength in elderly subjects without affecting metabolic parameters. Moreover, myocardial performance improved and LV ability to handle an acute overload was restored in a large number of subjects. These functional advantages translated into improved perceived physical function, which enabled these subjects to become functionally more independent. Thus, oral AA supplementation represents a nonpharmacological intervention that can help to maintain muscular and cardiovascular function in elderly subjects.

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