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The effects of oral amino acid intake on ambulatory capacity in elderly subjects

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ABSTRACT. ***Background and aims:*** *The combination of high prevalence of inactivity in the older population, and high risk of ill-health and disability associated with inactivity, suggests that interventions that are successful in increasing levels of activity may have a great impact on population health in later life. With advancing age, the risk of developing serious nutritional deficiencies also increases. This study was designed to assess the effects of dietary amino acid supplementation on effort tolerance in healthy elderly subjects with reduced physical activity.* ***Methods:*** *Forty-four subjects (age >65 years) with sedentary life-style and lower health-related quality of life were studied. Subjects, in an open-label fashion, received an oral amino acid mixture (AAM, 12 g/day) containing essential and non-essential amino acids for a 3-month period. Ambulatory dysfunction resulting in sedentary life-style was assessed by a 6-min walk test. A walking impairment questionnaire (WIQ) was used to evaluate self-perceived ambulatory dysfunction. Maximal isometric muscular strength of the right hand was measured during isometric exercise by a handgrip dynamometer.* ***Results:*** *The 6-min walk distance increased from 214.5±32 to 262.8±34.8 m (p<0.001) after AAM treatment. The baseline scores on the three subscales of WIQ changed significantly during treatment: WIQ distance increased from 56.2±12.9 to 66.3±12.8% (p<0.001); WIQ speed from 52±12.6 to 69.2±14.8% (p<0.001) and WIQ stairs from 74.4±22.6 to 94.2±25% (p<0.001), as did maximal isometric muscular strength (16.6±2.4 vs 19.2±2.2 kg, p<0.001). Changes in plasma glucose (+11±11 mg/dL), total cholesterol (0±39 mg/dL), HDL cholesterol (0±17 mg/dL), and triglycerides (-11±58 mg/dL) were not significant between baseline and AAM.* ***Conclusions:*** *An oral amino acid supplement, as used in this pilot study, improves ambulatory capacity and maximal isometric muscle strength in elderly subjects without affecting the main metabolic parameters. Amino acid supplementation may thus represent useful non-pharmacological intervention to maintain physical fitness in these subjects.*

(Aging Clin Exp Res 2004; 16: 00-00)

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INTRODUCTION

The world's elderly population (65 years of age and over) is currently growing at a rate of 2.4 per cent per year, considerably faster than the total world population (1). This has resulted from reduced parity, improved health, and increased longevity. Individuals age in many different ways as a result of complex social and physical environments. Identifying variability and determining mutable risk factors raise the potential for positive intervention in the process of aging, and are perhaps most influential in the domain of traditional activities of daily life, and the health-related quality of life.

Elderly people are known to represent a population at high risk of mobility limitation. 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 (2-4). The capacity of an individual to perform physical work depends on several factors including: cardiovascular stamina, muscle strength/power, flexibility, psychomotor skills (5) and other physiological systems or common chronic conditions (pulmonary function, nervous control, joint range of motion). These attributes tend to decline with advancing age, but it remains unclear to what extent this is the result of a sedentary life-style, chronic subclinical or clinical diseases, or intrinsic biological processes of aging.

The combination of the high prevalence of inactivity in the older population, and the high risk of ill-health and disability associated with inactivity, suggest that interventions that are successful in increasing levels of activity and ex-

Key words: Amino acids, elderly, muscle strength, walking capacity.

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Received May 30, 2003; accepted in revised form April 2, 2004.

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ercise may have a great impact on population health in later life. With advancing age the risk of developing serious nutritional deficiencies also increases, and disturbances in the actions of insulin and insulin-like growth factor, coupled with reduced protein/amino acid intake, impair protein synthesis in muscle (6).

In the light of these premises, this study was designed to assess the effect of administering dietary essential amino acid supplementation on effort tolerance in healthy elderly subjects with reduced physical activity.

METHODS

Subjects

We enrolled 50 elderly (age >65 years) subjects with sedentary life-style and lower health-related quality of life (7). Health-related quality of life (QoL) was assessed at baseline by the Medical Outcomes Study Short-Form 36 (MOS SF-36), Chinese version. The cut-off values for the following four subscales, used to define lower health-related QoL in the study population and for our purposes, were set as follows: general health ≤40, vitality ≤40, social function ≤60, mental health ≤60. Each domain of these scales was scored from 0 (poorest health) to 100 (optimal health). The main demographic and baseline characteristics of the study population are listed in Table 1. All subjects lived independently at home. Subjects were excluded from participation for the following reasons: 1) left ventricular (LV) dysfunction (LV ejection fraction ≤50%) secondary to previous myocardial infarction, severe valvular heart disease, or cardiomyopathy; 2) history of exertional angina; 3) exercise tolerance limited by intermittent claudicatio; 4) hypertensive heart disease with left ventricular dysfunction and/or non-optimal control of blood pressure; 5) acute or chronic pulmonary disease; 6) overt nephropathy (creatinine >1.4 mg/dL); 7) potential sources of gastrointestinal bleeding, anemia, and other endocrine or major organ diseases; 8) obesity; 9) cancer; 10) infectious diseases.

During the study, all subjects followed their habitual diet verified by a registered dietician: the estimated daily number of calories was 1700±250 Kcal/day, containing 55% of carbohydrates, 30% of fat, and 15% of proteins. To maintain the same amount of daily energy intake, they were instructed to reduce carbohydrates by the same energy amount corresponding to that of the amino acid supplement. The amino acid mixture was ingested as a snack at 10:00 am, 4:00 pm and 10:00 pm.

The study consisted of 3 phases: 1) baseline examination with anthropometrical and physical evaluations; 2) 12-week maintenance period on high amino acid intake; 3) anthropometrical and physical evaluations. During phase 2, 12 grams of the amino acid mixture were added to each subject's diet at 10:00 am, 4:00 pm and 10:00 pm, in an open-label fashion. The subjects were informed of the nature of the oral mixture to be taken, the taste of the supplement, and the fact that there were no important side-effects apart from possible mild digestive discomfort in some subjects already affected by gastro-esophageal diseases. Subjects were also told that elderly people frequently exhibit protein deficiencies due to low protein intake, and that the proposed amino acid mixture could effectively close this gap and enhance protein synthesis. To minimize the extent of a placebo effect, subjects were not told that the amino acid mixture could potentially and positively influence muscle strength or augment the capacity to do physical activity. The detailed composition of the amino acid mixture is listed in Table 2. The formulation contains all indispensable amino acids and two non-es-

Table 1 - *Main demographic and baseline characteristics of study population.*

Sex (M/F)	16/28
Age (years)	74±6
Plasma glucose (mg/dL)	85±8
Total cholesterol (mg/dL)	225±50
HDL-cholesterol (mg/dL)	60±11
Triglycerides (mg/dL)	144±101
Diagnosis of hypertension (%)	31 (70%)
Hypolipidemic drugs	4
ACE-inhibitors	13
AT_2-receptor blockers	3
Calcium-channel blockers	14
β-blockers	3
Diuretics	8
Antiplatelet agents	7
α-blockers	2
Oral anticoagulant therapy	1
Antiarrhythmic drugs	4
Smoking status: none/active/former	26/7/11

Table 2 - *Composition of amino acid mixture.*

	Per 100 g	**Grams per day**	**Biosynthesis**
KJ	1666	450	-
Protein (N x 6,25)	0.67	0.18	-
Carbohydrates	45.29	12.21	-
Fats	2.38	0.64	-
L-leucine	13.889	3.8	essential
L-lysine	7.222	2	essential
L-isoleucine	6.944	1.9	essential
L-valine	6.944	1.9	essential
L-threonine	3.889	1.1	essential
L-cystine	1.667	0.4	non-essential
L-istidine	1.667	0.4	essential
L-phenylalanine	1.111	0.3	essential
L-methionine	0.556	0.2	essential
L-tyrosine	0.333	0.1	non-essential
L-triptophane	0.222	0.1	essential

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sential amino acids (tyrosine and cystine), in a complex ratio which was planned to match metabolic requirements in conditions of elevated demand (8, 9). It is known that 75% of human nitrogen needs are covered by only 5 amino acids: leucine, isoleucine, valine, threonine and lysine (8). The study protocol was approved by the local Ethical Committee of the School of Medicine, and written informed consent was obtained from all participants after the nature of the procedure had been explained.

Study design

During the baseline and final visit, information about personal data, life habits (smoking and alcohol intake) and any pharmacological therapies were collected by a physician. Anthropometrical, laboratory and clinical data were also collected. Height and weight were measured following a standardized protocol. Body mass index (BMI, weight/height^2) was used as an estimate of overall adiposity. Blood pressure was measured in the sitting position, with a standard clinical mercury sphygmomanometer and cuff of appropriate size; the average of 3 measurements 5 minutes apart was recorded. A blood sample was collected for determination of plasma glucose, total cholesterol, HDL-cholesterol and triglycerides. A resting standard 12-lead ECG was recorded in each subject.

Ambulatory dysfunction resulting in sedentary lifestyle was assessed by a six-minute walk test. A walking impairment questionnaire (WIQ) was used to evaluate self-perceived ambulatory dysfunction. Maximal isometric muscular strength was measured during isometric exercise by a dynamometer. Data were collected before and after a 3-month period of oral amino acid intake. On a separate occasion, the baseline concentration of each amino acid present in the mixture and their plasma level excursion after oral intake of 12 grams were determined, in a fasting state. Determinations were performed in 10 subjects. Blood samples were obtained at 0, 20, 40, 60, 90, and 120 minutes after the amino acid load.

Six-minute walk test

Each subject performed a 6-min walk test by a trained technician, blind to the experimental condition, before and after 3 months of oral amino acid intake. 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. A calibrated electronic pedometer (Omron Health Care Corporation, Vernon Hills, IL, USA) was worn on each subject's hip to count 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 the total number of steps (10, 11).

Walking impairment questionnaire (WIQ)

Self-reported ambulatory ability was assessed using an adapted questionnaire in which subjects evaluated their walking ability at various speeds and distances and their ability to climb stairs (7, 11). A scale ranging between 0 and 100 assessed each aspect: a score of 0 represented inability, and 100 no difficulty in performing the task.

Isometric muscular strength

Maximal voluntary contraction of the right hand was determined during isometric exercise by means of a handgrip dynamometer, before and after taking the amino acid supplement. During isometric contraction of the right hand, subjects were in a sitting position, and were instructed to breath normally and to avoid performing the Valsalva manoeuvre.

Biochemical assays

Plasma glucose was measured with a glucose oxidase method on a Beckman Glucose Analyzer. Plasma insulin was determined by standard radioimmunoassay. Total cholesterol, HDL cholesterol, and triacylglycerols were determined by enzymatic techniques. Amino acids were determined by a standard HPLC method (12).

Statistical analysis

Results are expressed as mean values ±standard deviations (mean±SD). Comparisons were made with the paired or unpaired Student's *t*-test, as appropriate. All statistical analyses were performed with the SPSS package, version 10.1 for Windows (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois). A p-value ≤ 0.05 by the two-tailed test was considered to indicate statistical significance.

RESULTS

Six subjects (12%) refused return visits and were excluded from the final analysis. Our analysis was thus based on the results of 44 subjects (16 men, 28 women, aged 74±6 years) who completed the study. Body mass index did not change with intervention (baseline *vs* intervention: 25.2±4 *vs* 25.1±4 kg/m^2).

Similarly, changes in plasma glucose (+11±11 mg/dL), total cholesterol (0±39 mg/dL), HDL cholesterol (0±17 mg/dL), and triglycerides (-11±58 mg/dL) were not significant between baseline and intervention.

There were no complications during the exercise sessions. The amino acid mixture was well tolerated, and only mild dyspepsia was reported by two subjects.

As shown in Table 3, following amino acid supplement intake, there was a significant increase in plasma branched-chain lysine, threonine, istidine and phenylalanine amino acid concentrations.

Six-minute walk test and ambulatory ability

The 6-min walk distance increased from 214.5±32 to 262.8±34.8 m ($p<0.001$) after amino acid treatment (Table 4). The baseline scores on the three subscales of the WIQ changed significantly during treatment. Sub-

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Table 3 - *Baseline amino acid concentrations and maximal percentage increase in blood plasma after 12 g of oral intake in 10 subjects.*

	Baseline concentration (μmol/L)	Maximal increase (%)
L-leucine	127±25	451±159**
L-lysine	169±26	217±66**
L-isoleucine	48±7	454±148**
L-valine	228±28	237±68*
L-threonine	126±39	154±37*
L-cystine	13±16	111±34
L-istidine	79±14	138±31*
L-phenylalanine	45±6	136±20*
L-methionine	23±4	140±41
L-tyrosine	50±10	113±23
L-triptophane	NA	NA

Values are means±SD.
*$p<0.05$; **$p<0.01$ maximal value after oral ingestion *vs* baseline value.

jects reported significantly higher scores on the distance, speed and stairs subscales on the questionnaire after treatment: WIQ distance increased from 56.2±12.9 to 66.3±12.8% ($p<0.001$); WIQ speed from 52±12.6 to 69.2±14.8% ($p<0.001$), and WIQ stairs from 74.4±22.6 to 94.2±25% ($p<0.001$).

Isometric muscle strength

Maximal isometric muscular strength of the right hand (expressed in kg) increased from 16.6±2.4 to 19.2±2.2 kg ($p<0.001$; baseline *vs* amino acid treatment) (Table 4).

DISCUSSION

This study was performed to assess the effects of amino acid supplementation on community-living daily activity in elderly subjects with sedentary life-styles and ambulatory disability. The amino acid mixture, which contained all essential and two non-essential amino acids (tyrosine and cystine) in a ratio planned to match a metabolic setting of increased requirement, improved ambulatory capacity and maximal muscular isometric strength, and did not change the most important metabolic parameters in elderly subjects.

As extensively shown by several studies, the administration of essential amino acids in conjunction with small amounts of glucose promotes muscle anabolism by increasing muscle protein synthesis (13). A chronic nutritional approach is also considered not only more physiological but even superior to an aggressive approach in preventing protein loss (8). These findings translate into increased physical function, which allows these subjects to increase their capacity to perform the common activities of daily life and to become more functionally independent. There is some evidence supporting the positive effects of regular exercise on functional capacity in elderly people (14-16). In this context, the nutritional intervention used in our study may be helpful in maintaining the advantages obtained by training, because there are data supporting that benefits obtained by exercise are lost after 4 months training, when elderly subjects lose the constraints and social stimulation of the imposed protocol (17).

In a clinical setting, our results may be important because changes in life-style which increase levels of activity and exercise may have a great impact in reducing the high risk of ill-health and disability associated with inactivity.

Advancing age is associated with reduced muscle protein synthesis, altered expression of and chemical modifications to muscle proteins, reduced muscle strength, and muscle power (18). These age-related impairments in the quantity and quality of contractile proteins contribute to physical disability and frailty, loss of independent function, the risk of falling and fractures, and escalating health-care costs (19). Although this study did not directly assess the mechanisms which account for improved physical stamina, evidence is accumulating that dietary amino acid supplementation has beneficial effects in the elderly: exogenous amino acids improve muscle protein balance and stimulate muscle protein anabolism, despite a significantly higher first-pass splanchnic extraction (20-22).

Moreover, other mechanisms supported by experimental studies may contribute to improve ambulatory function. In adult rats made osteoporotic by estrogen deficiency and reduced protein intake, the administration of

Table 4 - *Ambulatory function and maximal isometric muscle strength before and after amino acid mixture supplementation.*

Variables	Baseline	Amino acid mixture	*p*-value
Ambulatory function			
6-min walk distance (m)	214.5±32	262.8±34.8	<0.001
Self-reported ambulatory ability			
WIQ distance (%)	56.2±12.9	66.3±12.8	<0.001
WIQ speed (%)	52±12.6	69.2±14.8	<0.001
WIQ stairs (%)	74.4±22.6	94.2±25	<0.001
Maximal isometric muscle strength (kg)	16.6±2.4	19.2±2.2	<0.001

WIQ= walking impairment questionnaire. Values are means±SD.

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essential amino acid supplements increased bone strength through modifications of bone mineral density, trabecular architecture, and cortical thickness (23). Similar applications to humans may result in further improvement in ambulatory function in healthy elderly subjects.

Some limitations of the present study must be taken into consideration before the results can be accepted.

First, the design of the study is certainly not the "gold standard" for interventions, because it is not controlled by placebo and not blind. Hence, as in all uncontrolled studies, a placebo effect cannot be excluded but, as mentioned in the methods, we tried to reduce its influence. Further studies controlling for this sources of bias and with larger numbers of subjects, possible during a longer period of amino acid supplementation, should be undertaken to confirm the present results. In the light of this issue, our work should be considered as a pilot study.

In addition, because of the design, the possible role of the training effect on distance walking and muscle strength may be a serious issue. To minimize cofounding from training, subjects were instructed to continue their habitual life-style and to maintain their usual degree of physical activity/inactivity, as before the intervention.

Furthermore, the possible increase in daily energy intake because of the amino acid mixture may alter our conclusions. Increases in energy supply may partly explain enhanced muscle strength and/or better walking capacity in elderly subjects. To avoid this source of bias, patients were instructed to reduce their energy intake (by reducing carbohydrates) by approximately the same energy amount corresponding to that of the amino acid mixture (450 KJ per day). This measure probably greatly reduces misinterpretation of the results, favoring our conclusion that amino acids, and not energy increase in daily diet, are probably responsible for better ambulatory capacity in the elderly.

In conclusion, oral amino acid supplementation, as used in this pilot study, improves ambulatory capacity and maximal muscular isometric strength in elderly subjects without affecting metabolic parameters. Thus, amino acid supplementation may represent a useful non-pharmacological intervention to maintain physical fitness in these subjects.

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