JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS Vol. 34, no. 2, 721-731 (2020)

LETTER TO THE EDITOR

EFFECTS OF ESSENTIAL AMINO ACID SUPPLEMENTATION ON PAIN IN THE ELDERLY WITH HIP FRACTURES: A PILOT, DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMISED CLINICAL TRIAL

M. RONDANELLI[1,2], D. GUIDO[3], M.A. FALIVA[4], C. GASPARRI[4], G. PERONI[4], G. IANNELLO[5], M. NICHETTI[4], M. NASO[4], V. INFANTINO[6], D. SPADACCINI[4], S. PERNA[7] and R. AQUILANI[8]

[1]IRCCS Mondino Foundation, Pavia, Italy; [2]Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy; [3]Epidemiology Unit, Agency for Health Protection of Milan, Milan, Italy; [4]Endocrinology and Nutrition Unit, Azienda di Servizi alla Persona "Istituto Santa Margherita", University of Pavia, Pavia, Italy; [5]General Management, Azienda di Servizi alla Persona "Istituto Santa Margherita", Pavia, Italy; [6]University of Bari, Department of Biomedical Science and Human Oncology, Bari, Italy; [7]University of Bahrain, Department of Biology, College of Science, Sakhir Campus, Kingdom of Bahrain; [8]Department of Biology and Biotechnology University of Pavia

Received October 24, 2019 – Accepted March 24, 2020

To the Editor,

The main three pain-neuromodulators are gamma-amino-butyric acid, serotonin, and endorphin; even a diet that is low in protein can reduce these pain fighters, because they are produced from amino acid (AA) (1). In fact, effective pain control is not consistently achievable without adequate physiological body levels of essential AA (EAA), and of the hormones and neurochemicals they produce (2). Recently, the effectiveness of 8 g/day EAA supplementation versus placebo during rehabilitation in 60 patients following elective hip arthroplasty were investigated. After treatment, there was a similar decrease in inflammation rate for both groups; only EAA patients showed significant improvement in levels of alanine, glycine, tyrosine, and total AA and in their rate of hip function recovery, measured by Harris hip score, that included domains relative to pain (3). A previous study has demonstrated the efficacy of 8g/day EAA on quality of life determinants in institutionalized elderly patients. EAA patients showed improvement of nutritional status and mental and physical components of SF-36, which included 2 items related to bodily pain (4). Furthermore, it has been demonstrated that EAA can improve walking recovery rate of subjects after hip fracture surgery (5). The benefits derived from EAA can be explained by multiple mechanisms. Primarily, the anabolic activity promoted by EEA enhances wound repair, a process which requires the availability of EAA for *de novo* synthesis of proteins and peptides (6). Given the above, this study aims to assess the efficacy on pain (assessed by VAS) of a supplement with a specific formulation of EAA *versus* placebo in elderly patients admitted for rehabilitation management after surgical treatment of hip fracture. The EAA formulation was built on the basis of AA deficits found in a population of subjects similar to that of

Key words: amino acids; pain; hip fracture; dietary supplement

Corresponding Author:
Dr. Gabriella Peroni,
Endocrinology and Nutrition Unit,
Azienda di Servizi alla Persona
"Istituto Santa Margherita",
University of Pavia, Pavia, 27100, Italy
e-mail: gabriella.peroni01@universitadipavia.it.

0393-974X (2020)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

the present study: the elderly post-femur fracture (5). The secondary endpoints are the evaluation of body composition, nutritional status, quality of life, muscle function, and hip functionality.

MATERIALS AND METHODS

This pilot study was parallel, randomized, double-blind and placebo-controlled, and sample size had not been determined a priori because the subjects were more than 12 per arm, as previously suggested. Subjects who met the admission criteria, and who had signed and given informed consent for the study were randomized to one of the two treatments (experimental treatment or placebo).

The study was approved by the Ethics Committee of the Department of Internal Medicine of the University of Pavia and written consent was given by participants to take part in this study (NCT02402608). All procedures performed were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Elderly people were evaluated after admission to the geriatric physical medicine and rehabilitation division at Santa Margherita Hospital, Azienda di Servizi alla Persona of Pavia, Italy. Every subject underwent complete medical screening before participation, which included vital signs, urine tests, blood tests, and a 12-lead electrocardiogram. Any evidence of kidney, liver or heart disease, or of any other disease that could influence the study results resulted in the subject being excluded. Data gathering took place from the end of January 2019 to the end of June 2019. Eligible subjects were 65 years or older and their appendicular skeletal FFM divided by height squared was 2 SD below the mean for young adults. Thus, the relative skeletal muscle mass (RSMM) for men was 7.26 kg/m2 and for women 5.5 kg/m2 (7). None suffered from severe heart, liver, or kidney dysfunction or acute illness, and their body weight was required to have been stable for at least 6 months. Exclusion criteria were: thyroid disorders, altered glycometabolic control, other endocrinopathies, cancer, people treated with steroids and heparin or who had total walking incapacity. The physical ability of the participants selected had to be similar, as assessed by the activities of daily living (ADL) score. Their cognitive function needed to be normal or only mildly disturbed, as defined by a Mini-Mental State Examination.

Body composition, nutritional status, and food intake

Body composition (fat free mass, fat mass, together with gynoid and android fat distribution) was determined by dual-energy X-ray absorptiometry (DXA), using a Lunar Prodigy DXA (GE Medical Systems). *In vivo* coefficients of variation (CVs) were 0.89% for whole body fat (fat mass) and 0.48% for FFM. RSMM was calculated as the sum of the fat-free soft tissue mass present in arms and legs, normalized for height^2 (Kg/m^2). Body weight was measured to the nearest 0.1 kg, using a precision scale; participants wore light clothing, no shoes, and a standardized method was used. The waist was measured at the midpoint between the top of the hip bone (iliac crest) and lowest rib, using a standardized method.

Hydration was assessed with bioelectrical impedance, as fluid status changes would affect the soft tissue composition estimated by DXA. With the patient in supine position on a nonconductive surface, whole-body reactance and resistance were measured using a single-frequency phase-sensitive impedance plethysmograph [400-mA, 50-kHz alternating current (BIA-101; RJL/Akern Systems)]. Adhesive surface electrodes were attached to the right foot and hand, with measurements taken based on NIH Technology Assessment Conference Statement guidelines. Reactance and resistance were standardized using each individual's standing height (i.e., reactance divided by height and resistance divided by height), given in ohms/m and plotted on the resistance-reactance graph. Bioelectrical impedance vector analysis (BIVA) provides a measure of tissue hydration status and body cell mass that only considers the impedance vector in relation to a population of healthy individuals; this method is valid for detecting hydration changes (divided in under-, normal or over-hydration) and changes in body fluid volume. Sex-specific bivariate reference intervals for the Italian healthy population were available as 50%, 75%, and 95% tolerance ellipses on the resistance-reactance graph.

All participants underwent a mini nutritional assessment (MNA). MNA is a test for classifying malnutrition, based on simple measurements and a brief questionnaire divided into 4 sections: anthropometric assessment (height, weight, and weight loss), general assessment (medication, lifestyle, and mobility), dietary assessment (food and fluid intake, number of meals, appetite, autonomy of eating) and perception of health

and nutrition. Patients had 3 meals daily.

A balanced diet was provided by the hospital kitchen (with standard caloric and macro- and micronutrient content). This consisted of a 4-week rotating menu, therefore diet remained similar throughout the study. A trained dietitian was involved for 3 consecutive days at the beginning and end of the study, using a calibrated dietetic spring scale to weigh all foods served. Any foods served to participants between meals by nurses were recorded together with the amount eaten, using household measurements. The energy and macronutrient content of the food consumed was calculated by a computer software (DR3 v3.1.0; Sintesi Informatica).

Muscle function was assessed using the JAMAR Hand Dynamometer (Jamar 5030J1; Sammons Preston Rolyan; accuracy 0.6 N), by means of a standardized procedure.

Biochemical analyses

Venous blood samples were taken during fasting and collected in vacuum tubes, maintained for 1 h at room temperature, and then centrifuged at 1500 3 g at 20°C for 15 min. Thereafter, serum was transferred to plastic tubes, frozen rapidly, and stored at -80°C while awaiting analysis (1 month later). Whole blood (using EDTA as an anticoagulant) was used to assess hematologic variables. Clinical chemistry markers were measured using the Roche Cobas Integra 400 plus analyzer (Roche Diagnostics), together with custom commercial kits supplied by the manufacturer. Cobas Integra 400 is a continuous-access, random, sample-selective analyzer which provides absorbance photometry to measure substrates and enzymes, ion-selective electrode potentiometry for serum electrolytes, and turbidimetry for specific proteins. Total serum and LDL cholesterol, HDL cholesterol, triglycerides, total bilirubin, total proteins, creatinine, iron, uric acid, glucose, and liver enzymes such as aspartate transaminase, alanine transaminase, and g-glutamyltransferase were measured employing enzymatic-colorimetric methods. A nephelometric high-sensitivity CRP was used to determine C-reactive protein (CRP) (Dade Behring). Erythrocyte, platelet counts and white blood cell; mean cell volumes; hemoglobin concentrations; and mean cell hemoglobin concentrations were determined with a Coulter automated cell counter (MAX-M; Beckman Coulter). Serum albumin was determined by a nephelometric method (Behring Nephelometric Analyzer II, Behring Diagnostics), with a 2% CV. Serum samples required to assay for insulin-like growth factor I (IGF-I) were collected upon admission and after 12 weeks of treatment; samples were pretreated so that IGF-I would be released from binding proteins and then assayed with quality controls. A solid-phase quantitative ELISA kit (R&D Systems) was used to measure serum IGF-I concentrations; the IGF-I minimum detectable dose was 0.026 ng/ml. Intra-assay CV was 4% and the inter-assay CV 7.9%.

Health-related quality of life

In order to assess their quality of life, participants completed the Short-Form 36-Item Health Survey (SF-36) before and after the treatment period. This questionnaire is used for rating health-related quality of life in several fields of research due to its validity, and high test–retest reliability and high internal consistency. Two dimensions were used to summarize the SF-36 scales: "physical health", comprising the first five scales, and "mental health", comprising the last five scales. General health scales and vitality are part of both, i.e. each dimension includes 3 specific and 2 overlapping scales. Standardised summary scores for mental and physical components were determined and used separately as measures of outcome.

The ability of participants to care for themselves was measured using the Katz Index of Independence in Activities of Daily Living. To assess cognitive function, the participants were tested with the "Mini Mental State Examination". This test comprises two parts: the first part only requires vocal responses, covering memory, orientation, and attention; the maximum score being 21; the second tests the ability to name, follow written and verbal commands, write a spontaneous sentence, and copy a complex polygon like a Bender-Gestalt Figure; with a maximum score of nine. Given that Part II involves reading and writing, patients with severely impaired vision may experience additional difficulties; usually these can be alleviated by use of large writing and are allowed for in the scoring. A total score of 30 is the maximum value achievable. There is no timing of the test.

We also evaluated the presence of a geriatric depression with the "Geriatric Depression Scale": a 30-question test with yes/no answers. The scale was designed to avoid most of the symptoms associated with the measurement of geriatric depression.

We also tested the participants' balance with the

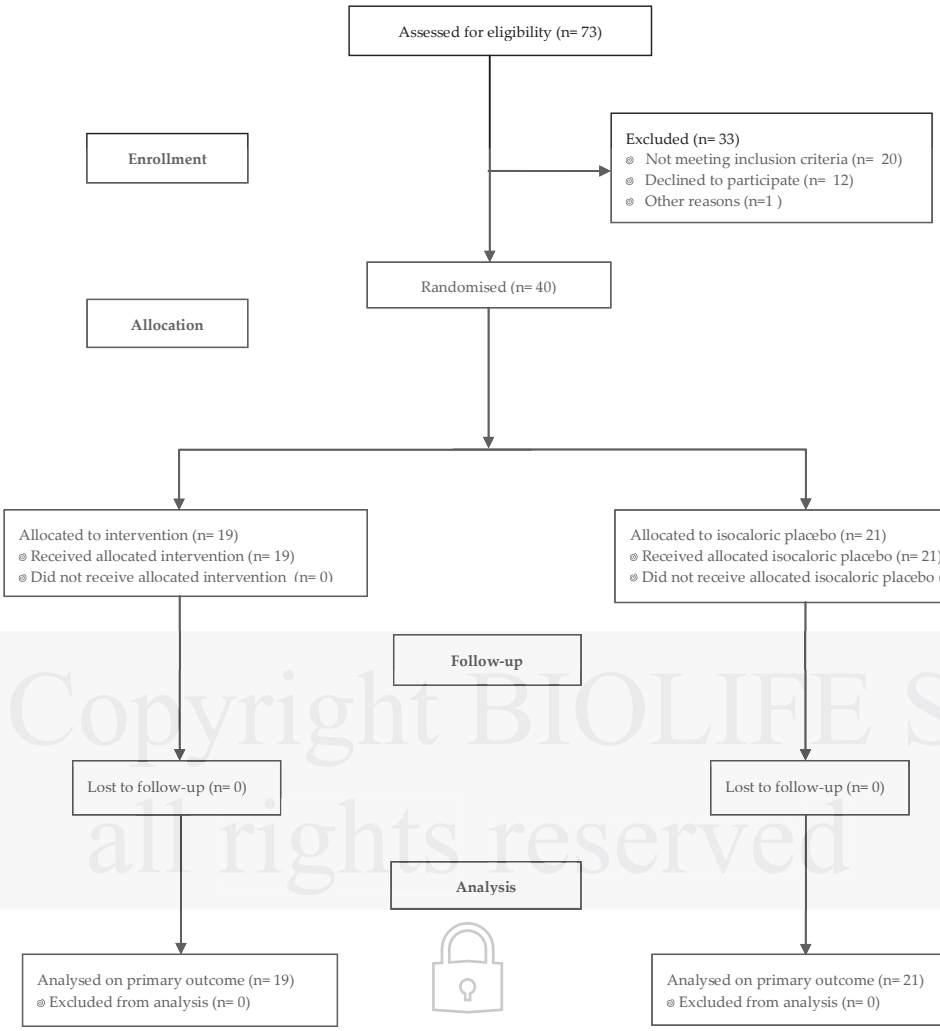

Fig. 1. *Flow diagram of the study.*

Tinetti balance scale (scale from 1 to 40): a simple clinical balance measures characteristics relating to falls. The test uses 14 items to assess balance (with a score out of 24) and 10 items to assess gait (score out of 16). The maximum total score is 40, and the higher the score is, the better the participant's performance.

Hip pathology and hip surgery outcome in participants were assessed using a reliable and valid Italian version of the Harris hip score. The maximum score is 100 points (best possible outcome) and the assessment comprises 10 items, spanning function (7 items, 0–47 points), pain (1 item, 0–44 points), range of motion (2 items, 5 points), absence of deformity (1 item, 4 points). Data analysis uses computer-based algorithms and is performed automatically as part of data processing, taking 5 minutes to complete.

Pain

We evaluated the presence or absence of pain in all participants with the NRS (Numerical Rating Scale) (8), a unidimensional measure of pain intensity in adults. Pain NRS is a single 11-point numeric scale: 0 represents one pain extreme (e.g., "no pain") and 10 represents the other pain extreme (e.g., "pain as bad as you can imagine" and "worst possible pain"). Pain NRS was administered by the

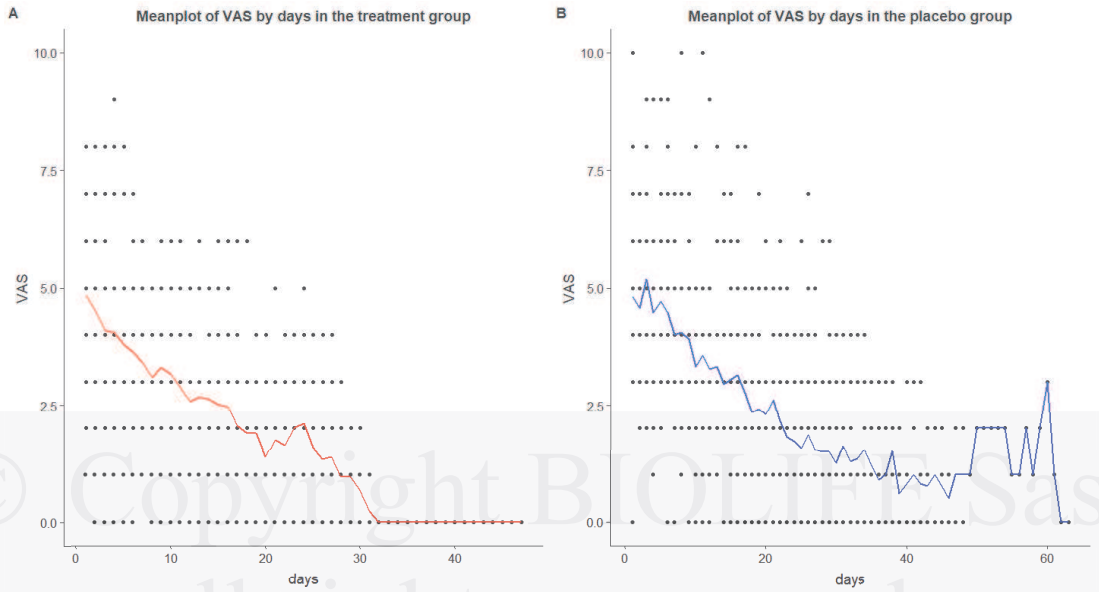

Fig. 2. *Trend of the means of the VAS by day and treatment*

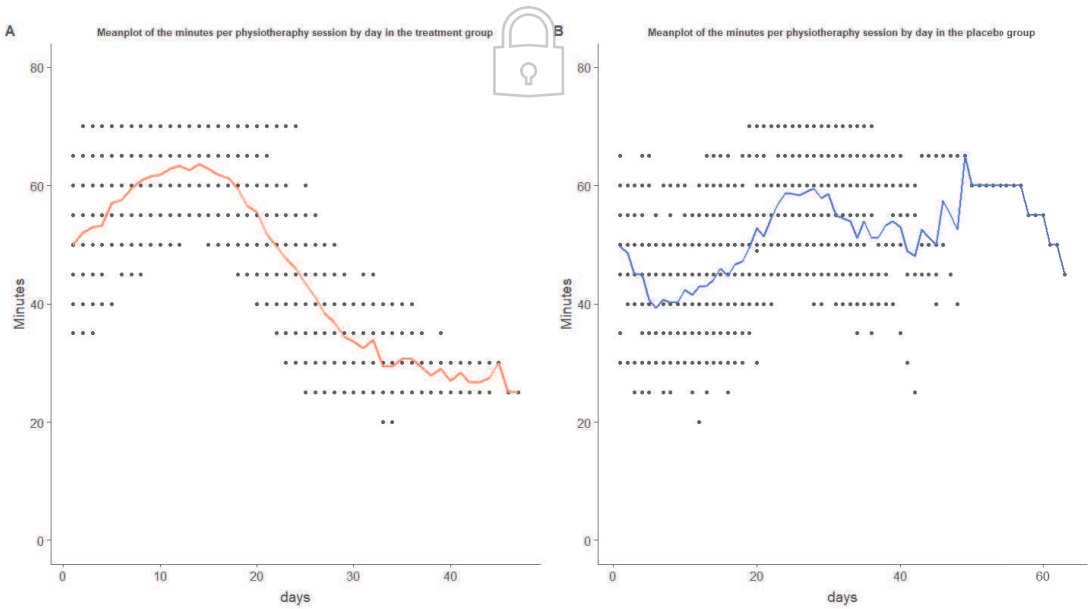

Fig. 3. *Trend of the means of the minutes per daily physiotherapy session by treatment*

physiotherapist every day at the same time, at the end of the morning physiotherapy session, at 11 a.m.

Supplementation

Essential amino acids were administered to participants every day twice a day. Powdered amino acid supplement sachets were provided, and these were taken with water or milk, and the participants were instructed to take the 6.6-gram supplement (1500 mg leucine, 1000 mg lysine, 750 mg glutamine, 550 mg valine, 450 mg isoleucine, 450 mg glycine, 350 mg serine, 250 mg threonine, 250 mg phenylalanine, 350 mg tyrosine, 350 mg histidine, 200 mg

cysteine, 125 mg methionine, 75 mg tryptophan) twice a day, every day, for four weeks. A dietitian checked every day to make sure that every patient complied with the supplementation therapy and took the supplement.

In our clinical practice, we adopt the recommendations for essential amino acids by WHO. On this basis, after considering combined requirements for male and female (mg/Kg BW/d) , the supplemented mixture in the study, relative to the recommended daily amounts, provided leucine + 75.7%; valine -18%; isoleucine + 35.7%; lysine - 27%; threonine + 25.7%; phenylalanine +241.5%.

The control group was administered a placebo consisting in an isocaloric amount of maltodextrin, with the same appearance and flavour as the intervention product, as used in previous studies. As with the case group, a dietitian checked every day to make sure that that every patient took the supplement and complied with the supplementation therapy.

Statistical analysis

Descriptive statistics were expressed as mean (± standard deviation) for numerical variables and the frequencies were computed for the categorical variables. Attrition was addressed through complete cases. Baseline associations between treatment (1= experimental treatment, 0=placebo) and numerical variables were analyzed by two-sample *t*-test, while the associations with gender were analyzed by χ^2 (*chi*-squared) test.

Generalized estimating equation (GEE) models for repeated measures were employed to ascertain the differences in outcomes among individuals across time and treatments. For the outcomes of VAS and minutes per daily physiotherapy session, measured for each hospitalization day, we fitted a GEE model where the time was hospitalization days, and the treatment (i.e. experimental treatment or placebo, with placebo as reference) and their interaction terms were ''focus'' predictors. In addition, the model on the VAS was adjusted for the whole hospitalization period (in terms of total of the days) and the minutes per daily physiotherapy session, whereas the model on the minutes per daily physiotherapy session was adjusted for the hospitalization period and the VAS measured the day before (i.e., in the afternoon or evening of the day before, after the physiotherapy sessions). For the other outcomes, we used a GEE model where the time – considered as a categorical two-level, three-level or four-level factor, based on the number of time points such as baseline (t0) (as reference), at 15 days (t1), at one month (t2), at 45 days or at discharge (t3) – the treatment (i.e. experimental treatment or placebo, with

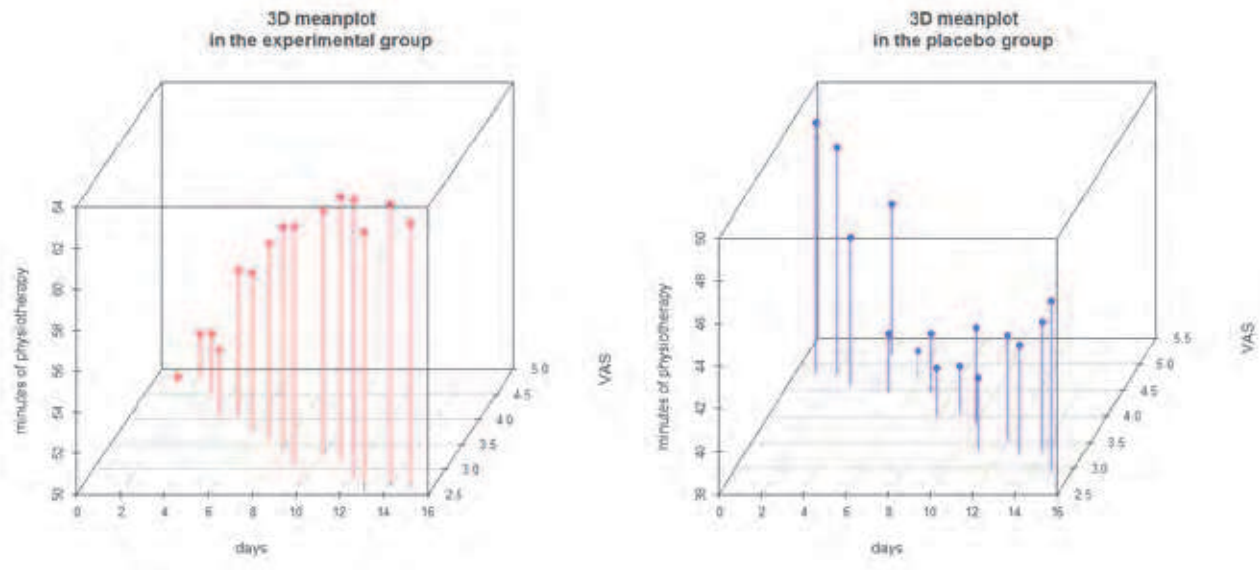

Fig. 4. *Trends of the minutes per daily physiotherapy related to VAS within the first 15 days of treatment*

placebo as reference), and their interaction terms were ''focus'' predictors. An ''identity'' link, an exchangeable correlation structure, and a robust sandwich estimator to consider model misspecifications were used to fit the Gaussian GEE models.

Concerning the GEE models on the VAS and the minutes per daily physiotherapy session, the time parameters (ΔT) were interpreted as adjusted average variation of the outcome for daily increase in the placebo group; the interaction parameters (ΔTxTR) were interpreted as adjusted mean differences of the outcome between treatments for daily increase. Finally, the parameters associated to the treatment (ΔTR) were interpreted as adjusted baseline mean differences between treatments.

For the GEE models on the other outcomes, time parameters (ΔT) were interpreted as adjusted mean differences of the outcome at the time point (t1 or t2 or t3 or t4) from baseline in the placebo group; the interaction parameters (ΔTxTR) were interpreted as adjusted mean differences of the outcome between treatments at a given time point (from baseline). Finally, treatment-associated parameters (ΔTR) were interpreted as baseline mean differences between treatments. GEE models were selected due to their robustness to missing values, and because they allow to manage the intra-subject variability resulting by the three separate measurements performed on each patient.

Wald tests were performed and 95% confidence intervals computed in order to assess statistical significance on model parameters. Two-tailed p-values below 0.05 were considered to be significant.

RESULTS

During the study period, 40 patients met the inclusion criteria: 19 (5 males/14 females, mean age 81.9±8.3) in the experimental group, 21 (5/16, 84.8±8.6) in the placebo group. Fig. 1 shows the patient flow in the two groups. Baseline demographic and clinical data are shown in Table I: the mean of VAS was 4.84±2.09 in the experimental group and 4.81±2.18 in the placebo group. The means of the minutes per daily physiotherapy session were 50±8.82 and 49.8±8.29. Finally, the means of the Tinetti scale were 4.82±6.1 and 2.5±5.3, and 355±153 and 286±130 for the total scores of the SF questionnaire. When examining the baseline associations between treatment and variables, no significant result was found. The mean of the number of hospitalization days was 34.7±5.91 in the experimental group and 37.1±7.91 in the placebo group, but they did not differ statistically.

Table I provides longitudinal outcome details, in terms of mean and standard deviation stratified by treatment. The means of the VAS decreased from baseline until discharge through all considered time points (i.e., 15 days, 3 weeks and 1 month) in both groups, but the decrease in the experimental group was more significant: -47.5% *vs* -37.1% at 15 days, -63.9% *vs* -45.9% at three weeks, and -86.1% *vs* -74.4% at 1 month. Regarding the minutes of physiotherapy session per day, the mean in the experimental group increased at 15 days (from 50 to 62.9) and then decreased until discharge (27.6). Differently, in the placebo group, the mean values were more irregular: they decreased slightly at 15 days (from 49.8 to 46) and then increased at both 3 weeks (51.4) and at 1 month (58.6); finally, at discharge, the daily average minutes were 42.4. In detail, (Figs. 1, 2) the mean plots for the VAS and the minutes per daily physiotherapy session across day and treatment groups (A: experimental treatment, B: placebo). The average trend of the VAS in the experimental group decreased almost constantly compared with the placebo group, where it wavered in the final days (Fig. 2). On the contrary, the trends of the minutes of physiotherapy were fluctuating (Fig. 3): in the experimental group it increased until the 14th day of hospitalization and then steadily decreased, whereas in the placebo group it was very oscillating. Notably, the 11th day of hospitalization reported the biggest difference in average minutes per session (within the first three weeks) between treatments, 21.5 ($P<0.001$), 62.9±6.1 in the experimental group and 41.4±8.8 in the placebo one.

Concerning GEE modelling, in the placebo group the VAS significantly decreased on average per day ($\Delta T = -0.099$, $P<0.001$), but in the experimental treatment the decrease was significantly stronger ($\Delta TxTR = -0.058$, $P<0.001$) (Table II). On the other hand, the result of the model on the minutes of physiotherapy per day was different: in the placebo group, a significant increase was detectable, on average

Table I. *Primary and secondary outcomes across time of treatment* (n.b. values are expressed as means ± standard deviations)

Dietary supplementation (experimental) Group							Placebo Group					
Variable		Baseline	15 days (n=19)	3 weeks (n=19)	1 month (n=15)	At discharge		Baseline (n=21)	15 days (n=21)	3 weeks (n=21)	1 month (n=21)	At discharge
(n=19)	15 days		2.53±1.98	1.74±1.37	0.67±0.72	0.11±0.32		4.81±2.18	3.05±1.91	2.62±1.88	1.24±1.09	1±1.18
(n=19)	3 weeks		62.9±6.52	51.8±8.85	33.7±5.16	27.6±4.82		49.8±8.29	46±9.17	51.4±9.24	58.6±8.68	42.4±6.64
(n=19)	1 month					34.7±5.91						37.1±7.91
(n=15)	At discharge											
(n=21)	15 days		15 days	1 month	45 days or at discharge		n	Baseline	15 days	1 month	45 days or at discharge	
(n=21)	3 weeks		NA	63.1±18.9	63.1±18.4		20	63.3±11.5	NA	62.4±11.1	61.3±11	
(n=21)	1 month		NA	23.7±5.59	23.7±5.54		20	25.8±5.02	NA	25.4±4.68	24.9±4.62	
(n=21)	At discharge		NA	26.1±4.26	25.7±3.87		19	27.8 ±3.17	NA	27.1±3.72	26.4±3.96	
VAS (units)			NA	31±5.18	31.1±4.58		19	32.3±3.97	NA	31.4±3.71	30.6±3.99	
(primary outcome)		4.84±2.09	NA	NA	17.2±3.86		16	2.5±5.3	NA	NA	15.3±4.8	
Daily physioterapy session (minutes)		50±8.82	383±155	NA	NA		21	286±130	284±120	NA	NA	
Days of hospitalization			50.4±14.6	NA	NA		21	40.6±17.8	41.3±18.2	NA	NA	
			58.1±21.2	NA	NA		21	56.9±19.1	57.7±17.7	NA	NA	
Variable	n	Baseline	30.4±32.3	NA	NA		21	12.9±23.7	10.8±17.4	NA	NA	
at discharge	n	Baseline	NA	8.94±8.07	9±8.15		19	9.95±6.55	NA	10.6±6.76	11.2±7.07	
at discharge			NA	18.2±3.69	18.2±3.67		19	15.9±3.37	NA	15.7±4.31	15.7±4.38	
Weight (kg)	18	64±19.1	4.17±0.96	NA	4.193±1.06		12	4.28±1.13	4.31±1.2	NA	4.733±1.45	
BMI (kg/m2)	18	24±5.39	6.68±1.97	NA	6.68±1.92		12	7.56±2.15	7.42±2.07	NA	7.85±2.1	
Mid-Arm Circumference (cm)	18	26.8 ±3.96	NA	NA	6.10 ± 1.25		11	7.46 ± 1.2	NA	NA	6.85 ± 0.946	
Calf Circumference (cm)	18	31.4±4.3	NA	NA	18.99 ± 6.33		10	22.29 ± 7.40	NA	NA	22.16 ± 7.69	
Tinetti (units)	17	4.82±6.1	NA	NA	37.28 ± 8.74		11	39.51 ± 7.06	NA	NA	38.19 ± 5.98	
SF 36 (units)	18	355±153	NA	NA	2.144±0.752		10	1.964±0.861	NA	NA	1.946±0.885	
SF PHS (units)	18	46.2±15.4	NA	NA	3.57 ± 0.91		11	4.14 ± 1.06	NA	NA	4.01 ± 0.85	
SF MHS (units)	18	56.4±21.6	NA	NA	12.45 ± 3.23		11	14.56 ± 3.16	NA	NA	14.08 ± 3.04	
SF Physical activity (units)	18	22.6±31.1	NA	90.2 ± 13.1	NA		20	87 ± 9.09	NA	87.8 ± 9.23	NA	
GDS (units)	16	7.44±7.16	NA	8.40 ± 4.15	NA		20	10.1 ± 7.48	NA	9.62 ± 7.07	NA	
MNA (units)	16	17.9±4.33	NA	1.89 ± 0.98	NA		20	2.20 ± 1.59	NA	2.09 ± 1.48	NA	
Phase Angle (degree)	15	3.91±0.8	NA	6.96 ± 0.36	NA		15	7.06 ± 0.49	NA	6.94 ± 0.52	NA	
BCMI (kg/m2)	15	6.49±1.97	NA	4.42 ± 0.32	NA		15	4.34 ± 0.371	NA	4.20 ± 0.25	NA	
RSMM (kg/m2)	13	6.31 ± 2.11	NA	0.187 ± 0.147	NA		15	0.229 ± 0.379	NA	0.313 ±0.432	NA	
Fat mass (kg)	13	20.57 ± 7.04	NA	28.4 ± 15	NA		15	28.8 ± 11.2	NA	31.0 ± 14	NA	
Free fat mass (kg)	13	37.74 ± 9.27	NA	75.9 ± 18.6	NA		21	84.7 ± 37.9	NA	85.6 ± 40	NA	

(n.b. values are expressed as means ± standard deviations). ET group: experimental treatment group; n: sample size; VAS: Visual Analogue Scale; BMI: Body Mass Index; SF: Short form; PHS: Physical health status; MHS: Mental health status. GDS: Geriatric Depression Scale; MNA: Mini Nutritional Assessment; BCMI: Body Cell Mass Index; RSMM: Relative Skeletal Muscle Mass; HOMA: homeostasis model assessment; CRP: C-reactive protein. Values are expressed as means ± standard deviations; NA: Not Available.

Table II. *Results of the Generalized Estimating Equation models on VAS and minutes per daily physiotherapy session*

Outcome	MD between treatments at baseline (ref. placebo) (Δ_{TR})	Δ/ day in placebo group (Δ_T)	MD between treatments per day (ref. placebo group) (Δ_{TxTR})
VAS *(primary outcome)*	**Δ_{TR} = 0.586 P=0.005 95%CI= 0.180; 0.993**	**Δ_T = -0.099 P<0.001 95%CI= -0.109; -0.088**	**Δ_{TxTR} = -0.058 P<0.001 95%CI= -0.076; -0.040**
Minutes per daily physiotherapy session	**Δ_{TR} = 24.18 P<0.001 95%CI= 21.922; 26.452**	**Δ_T = 0.263 P<0.001 95%CI= 0.188; 0.339**	**Δ_{TxTR} = -1.285 P<0.001 95%CI= -1.382; -1.189**
Minutes per daily physiotherapy session (only for the first fifteen days)	**Δ_{TR} = 5.463 P<0.001 95%CI= 2.424; 8.502**	**Δ_T = -0.384 P=0.002 95%CI= -0.626; -0.143**	**Δ_{TxTR} = 1.193 P<0.001 95%CI= 0.876; 1.509**
Minutes per daily physiotherapy session (quadratic model with centered time predictor)	**Δ_{TR} = 3.060 P<0.001 95%CI= 1.751; 3.667**	**Δ_T = 0.311 P<0.001 95%CI= 0.237; 0.384**	**Δ_{TxTR} = -1.285 P<0.001 95%CI= -1.379; -1.189**
		Δ_T^2 = -0.011 P<0.001 95%CI= -0.015; -0.007	**$\Delta_T^2{}_{xTR}$ = -0.028 P<0.001 95%CI= -0.036; -0.020**

*MD: Mean difference; Δ: average variation of the outcome. In **bold** the statistically significant evidences (P<0.05). P=P-value. 95%CI: 95% confidence interval. VAS: Visual Analogue Scale. T^2: quadratic centered time predictor.*

by ΔT=0.263 (P<0.001), whereas in the experimental group the mean outcome decreased (ΔTxTR =-1.285, P<0.001) (Table II). However, as pointed out above, the mean of the minutes of physiotherapy did not have a linear trend (not even approximately), but the curves in both groups were a bit fluctuating. Regarding that, firstly, we fitted a further GEE model on the subset of data referring to the first fifteen hospitalization days, whose trends were approximately linear: in this time lag, the results were inverted, and we got a significant decrease in the placebo group (ΔT =-0.394, P=0.002) and a stronger increase in the experimental group (ΔTxTR =1.193, P<0.001) (Table II). Secondly, we fitted a further model where we also included a quadratic term for the predictor time (both as main effect and interaction with treatment) due to the curved trend on the whole hospitalization period. Briefly, if the means were monotonically increasing or decreasing over time, but in a curvilinear way, a model with quadratic terms could be appropriate. In this model, the rate of change in the mean response is not constant, but depends on time, and is expressed by two parameters of main effects (ΔT andΔT2) and two others of interaction (ΔTxTR and ΔT2xTR). At the interpretative level, if there is a positive effect of time and a negative effect of time squared, that means that in patients with a greater number of hospitalization days from the acute event, the effect of time is lessened. On the contrary, a positive effect of the time and a positive effect of the time at squared means that in patients with a greater number of hospitalization days, the effect is stronger. Finally, in order to avoid problems of collinearity we have centered the time predictor, considering it as deviation from the mean. Given that, the last row of Table II shows that in the placebo group the minutes of physiotherapy increased (ΔT= 0.311, P<0.001), but when the number of hospitalization days was increased, the effect tended to lessen (ΔT2= -0.011, P<0.001). On the other hand, in the experimental group, the mean outcome decreased in both linear (ΔTxTR= -1.285, P<0.001) and quadratic form (ΔT2xTR= -0.028, P<0.001), i.e. during long hospitalizations. The significance of the last quadratic term was likely due to the increase in minutes in the first ten days that consequently softened the overall linear decrease.

In addition, in order to detect the direct effects of the VAS on the minutes of physiotherapy within 15 days across treatments, we fitted two further GEE models. This considered, the effect was more

negative in the experimental group (-1.254, P<0.001) than in the placebo group (-0.898, P<0.001), but taking time into account, the trends were opposite: in the experimental group the time effect was positive (0.779, P<0.001), whereas in the placebo group it was negative (-0.358, P=0.004) (Fig. 4).

Regarding the modelling on the other outcomes, only the Tinetti scale reported a significant value, as expected, increasing by 12.81 units (P<0.001) at discharge or after 45 days of hospitalization in the placebo group. Analogously, the expected increase in the experimental treatment was comparable and was represented by the lack of significance of the interaction term (ΔT3xTR= -0.46, P=0.850).

Finally, it is worth emphasizing that the all markers of quality of life, such as SF MHS, SF PHS and SF PA, showed a slight (non-significant) improvement of the means at 15 days in the experimental group, that were more noticeable than the changes seen in the placebo group (Table I). Moreover, in a supplemental analysis carried out by using two-way ANOVA for repeated measures, the interaction of the SF-36 outcome was significant (P=0.036).

DISCUSSION

The study demonstrates the effectiveness of supplemented amino acids in reducing pain levels and the time of its resolution in the experimental patient subgroup after surgical treatment for hip fracture. Pain reduction may be very important for the continuation of physiotherapy and the retrieval of function (3). Pain decrease was associated with a significant increase in the duration of daily physical therapy session. The time needed for physiotherapy was 35% lower in the experimental group than in the placebo one. Of note, at discharge, patients on amino acids did not complain of pain. It is probable that essential amino acid acted both centrally (brain) and peripherically by wound repairing in the operated limb. Centrally, the essential BCAAs i) reduce the excitotoxic extracellular glutamate (9), ii) increase brain contents in GABA, histamine and serotonine (10). Peripherally, essential amino acids favor wound repairing in the operated limb (3) and accelerate post-injury muscle regeneration (11). Amino acid-associated effectiveness and efficiency of physical therapy not observed in the placebo group, were supported by three factors. Firstly, during the first 15 days of treatment, the quicker decline of pain was linked to a more prolonged time dedicated to each session of physical therapy. Secondly, from 15th day to discharge, each session of physical therapy required progressively less time. Thirdly, at discharge, patients on amino acids virtually did not complain of pain. These benefits were associated with enhanced improvement, although not statistically significant, in physical health and mental health dimensions of the SF-36 test. Likely, improved test performance was due to reduced pain levels. Considering the interaction between treatment and 1-month CRP levels, it can be accounted for by the reduction of proinflammatory markers by essential amino acids, in particular branched-chain ones (12). In conclusion, the results of this study indicated that, during the first 15 days of hospitalization, the decrease of average VAS was associated with the increased average duration of the physio-session, therefore indicating the effectiveness of EAA supplementation on pain in the elderly with a hip fracture.

REFERENCES

1. Ross J, Tennant F. Amino acids and diet in chronic pain management. Prat Pain Manag 2009; 9(3):34-40.
2. Ross J, Adema D. Nutritional supplements in pain practice. Prat Pain Manag 2011; 9(9).
3. Baldissarro E, Aquilani R, Boschi F, et al. The hip functional retrieval after elective surgery may be enhanced by supplemented essential amino acids. Biomed Res Int 2016; 2016:9318329.
4. 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(5):571-67.
5. Aquilani R, Zuccarelli Ginetto C, Rutili C, et al. Supplemented amino acids may enhance the walking recovery of elderly subjects after hip fracture surgery. Aging Clin Exp Res 2019; 31(1):157-60.
6. Stechmiller JK. Understanding the role of nutrition and wound healing [Internet]. Vol. 25, Nutrition in Clinical

Practice. John Wiley & Sons, Ltd; 2010. p. 61-68.
7. Janssen I, Baumgartner RN, Ross R, Rosenberg IH, Roubenoff R. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. Am J Epidemiol 2004; 159(4):413-21.
8. Farrar JT, Young JP, LaMoreaux L, Werth JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 2001; 94(2):149-58.
9. Bastone A, Micheli A, Beghi E, Salmona M. The imbalance of brain large-chain aminoacid availability in amyotrophic lateral sclerosis patients treated with high doses of branched-chain aminoacids. Neurochem Int 1995; 27(6):467-72.
10. García-Espinosa MA, Wallin R, Hutson SM, Sweatt AJ. Widespread neuronal expression of branched-chain aminotransferase in the CNS: implications for leucine/glutamate metabolism and for signaling by amino acids. J Neurochem 2007; 100(6):1458-68.
11. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. Physiol Rev 2001; 81(1):209-37.
12. 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(11 suppl):69E-77E.