Clinical Nutrition xxx (2015) 1–6

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Amino acid supplementation in L-dopa treated Parkinson's disease patients

A. Cucca [a], S. Mazzucco [b], A. Bursomanno [c], L. Antonutti [c], F.G. Di Girolamo [b], G. Pizzolato [c], N. Koscica [c], G.L. Gigli [a], M. Catalan [c], G. Biolo [b, *]

[a] University of Udine, Clinica Neurologica, Department of Experimental and Clinical Medical Sciences, Italy
[b] University of Trieste, Clinica Medica, Department of Medical, Surgical and Health Sciences, Italy
[c] University of Trieste, Clinica Neurologica, Department of Medical, Surgical and Health Sciences, Italy

ARTICLE INFO

Article history:
Received 27 August 2014
Accepted 13 December 2014

Keywords:
Parkinson's disease
L-Dopa treatment
Amino acid supplementation
Insulin sensitivity
Motor fluctuations

SUMMARY

Background: The correlation between Parkinson disease and malnutrition is well established, however a protein-restricted diet is usually prescribed because of potentially negative interactions between dietary amino acids and L-dopa pharmacokinetics. This strategy could increase the risk of further nutritional deficits.

Methods: A monocentric, prospective, randomized, double-blind pilot study was performed on two groups of Parkinson-affected, protein-restricted, patients: Intervention (n = 7; amino acid supplementation twice daily) and Placebo (n = 7; placebo supplementation twice daily). At enrolment, after 3- and 6-month supplementation, neurological evaluations (UPDRS III, Hoenh–Yahr scale, L-dopa equivalent dose assessment) were performed and blood sample was collected to define insulin sensitivity (QUICKI index) and oxidative stress (oxidized and reduced glutathione). Repeated measure ANCOVA was applied to define time effect and time × treatment interaction.

Results: Participants were comparable at baseline for all assessed parameters. Neurological outcomes and L-dopa requirement were comparable in both group after 6-month of supplementation, without time × treatment interaction. The decrease in insulin sensitivity, as assessed by QUICKI index, observed after 6 months in both groups, was greater in Placebo than in Intervention (time effect $p < 0.001$; time × treatment interaction $p = 0.01$). Moreover, despite no changes in total erythrocyte glutathione concentrations, oxidized glutathione levels decreased by 28 ± 17% in the Intervention while increased by 55 ± 38% in Placebo (time effect $p = 0.05$; time × treatment interaction $p = 0.05$), after 6-month supplementation.

Conclusions: Amino acid supplementation, assumed with shrewd temporal distribution, did not show detrimental effects on neurological and pharmacological control in protein-restricted Parkinson-affected patients, chronically treated with L-dopa. Furthermore, daily amino acid supplementation partially counteracted insulin resistance development and the loss in antioxidant availability.

© 2015 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

Malnutrition has relevant consequences on some metabolic parameters closely related to Parkinson's disease (PD) pathogenesis, among which chronic systemic inflammation [1], oxidative stress [2], plasma homocysteine levels [3], and, especially, insulin resistance [4]. An increasing number of evidence suggests a close relationship between insulin resistance and dopaminergic degeneration [4]. Also, it should be considered that some drugs used to treat PD, like L-dopa itself, can induce both hyperglycemia and hyperinsulinemia [5]. Growing experimental evidence points to a pathogenic link between insulin resistance and loss of mitochondrial function [6]. Inflammatory background with impaired redox homoeostasis and mitochondrial dysfunction are well recognized factors which contribute to the onset of the dopaminergic damage in PD and its amplification [1,7]. It has been largely demonstrated that amino acids (AAs) represent a useful nutritional approach to

* Corresponding author. Clinica Medica, Department of Medical, Surgical and Health Sciences, University of Trieste, Cattinara Hospital, Strada Di Fiume 447, 34149 Trieste, Italy. Tel.: +39 040 399 4532.
E-mail address: biolo@units.it (G. Biolo).

http://dx.doi.org/10.1016/j.clnu.2014.12.007
0261-5614/© 2015 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

Please cite this article in press as: Cucca A, et al., Amino acid supplementation in L-dopa treated Parkinson's disease patients, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2014.12.007

guarantee a correct functioning of the main endogenous antioxidant systems [8] – such as the reduced glutathione one [9,10]. Furthermore, nutritional supplementation with essential AAs has positive effects on insulin resistance [11,12]. Nevertheless, such nutritional intervention is not commonly performed in PD patients because of negative interactions between dietary AAs and L-dopa pharmacokinetics [13,14]. In accordance with these observations, dietary regimens that shift protein intake to the evening and restrict daily protein intake have been proposed to increase the efficacy of L-dopa which is normally taken 30–60 min before meals. Indeed, protein intake reduction during the first part of the day along with a strict control of the total protein amount to the recommended daily allowance is associated to a reduction in both postprandial and total off time in fluctuating PD patients [15]. On the other hand, a long lasting low-protein regimen rises the question of potential detrimental effects on protein energy balance in a population with an intrinsic tendency toward chronic protein malnutrition [16]. It is believed that long-lasting PD affected patients display a higher risk of developing malnutrition and sarcopenia [17]. The estimated overall prevalence within the Parkinson's population ranges from 0% to 24% [18]. The risk of developing malnutrition increases with the disease progression [17]. Causes of malnutrition in PD are various: motor disability, with mastication and deglutition difficulties, especially in the advanced stage, together with some non-motor symptoms like anxiety, depression and apathy can lead to a reduction in caloric intake [19]. Some motor symptoms such as increased muscular tone, tremor and dyskinesias may increase the energy expenditure [20].

Furthermore, reduced gastro-intestinal motility causes a delayed gastric emptying with a risk of early satiety, nausea and vomiting [21]. These symptoms can also be enhanced by the dopaminergic therapy [22]. Indeed, patients with a long-lasting disease often show a significant loss in body size and changes in their micronutrient composition [17–20]. A high-protein diet may be proficiently administered in hypercatabolic states, such as chronic inflammations, sepsis, cachexia and senile sarcopenia, as it could be helpful in both counteracting anabolic resistance and preventing further muscle loss [23,24]. Thus, the aim of the study was to evaluate whether AA supplementation has not only positive effects on some systemic parameters involved in PD's progression but also to verify the impact of AA supplementation on the patients' nutritional status and motor performances, as well as on the pharmacological control of the disease. Therefore, in this pilot study, we investigated the effect of 6 months of AA supplementation in PD-affected patients chronically treated with L-dopa showing fluctuations in their therapeutic response.

2. Methods

2.1. Study design

This is a monocentric, prospective, randomized, double-blind study carried out in 2010–2013 on two groups of PD-affected patients. The diagnosis of PD was made by a Neurologist specialized in movement disorders according to the UK PD Brain Bank criteria. We enrolled patients (aged from 50 to 90 years, with a body mass index lower than 30 kg/m^2) on L-dopa therapy for at least two years with a suggested protein redistribution diet. All patients showed fluctuations in their therapeutic response (Table 1). Meanwhile the following exclusion criteria were established: diabetes, kidney failure (glomerular filtration rate lower than 30 mL/min), heart failure (NYHA III or more), liver cirrhosis or any other relevant systemic comorbidity (e.g. infectious, neoplastic or immune-mediated diseases). The active treatment group (Intervention group) received AA supplementation with *Aminotrofic*® (Errekappa Euroterapici, SPA) for 6 months. Each bag contained 4 g of free essential, water-soluble AAs (pharmaceutical composition reported in supplemental materials). Patients took 2 bags of essential AA mixture 60 min after lunch and 60 min after dinner, for a total daily dose of 16 g, each time at least 60 min before the following L-dopa administration. Every administration of amino acid mixture corresponds to 28 g of proteins, since leucine concentration in muscle protein, as previously reported [25] is estimated to be around 9%. The control group (Placebo group) received placebo according to the same procedure. The two groups consisted of patients followed by the Centre for Parkinson's disease and Movement Disorders at the Cattinara University-Hospital, Trieste, Italy.

Table 1
Participants general characteristics at baseline.

	Intervention	Placebo	p
Number	7	7	
Sex (F/M)	3/4	4/3	
Age (y)	74 ± 1	74 ± 4	0.97
BMI (kg/m^2)	25 ± 1	26 ± 1	0.30
Waist circumference (cm)	95 ± 3	100 ± 2	0.28
Disease duration (y)	5.6 ± 1.5	6.0 ± 1.4	0.84

Data expressed as mean ± SEM. Statistical analysis performed using Student t test. BMI, body mass index.

Patients who agreed to participate signed a written informed consent. The study complies with the Declaration of Helsinki and its amendments and was approved by the Institution's Ethics committee. Patients were randomly allocated on a consecutive basis, according to their enrolment order and their gender.

2.2. Study population

A total of 22 patients were enrolled in this study (10 females and 12 males, see also the flow-chart in supplemental materials). Five patients included into the Intervention group dropped-out. Two of them showed side-effects potentially related to AA supplementation such as early satiety, pyrosis and nausea. Two patients were dropped-out because of low adherence to the study. One dropped-out because of concurrent morbidity (bronchial pneumonia). Among the Placebo group, three patients dropped out: one showed low adherence to the study, whereas the other two were due to concurrent medical problems (major surgery, neoplastic pathology). The overall most frequent cause of dropping-out was low or no adherence to the protocol. In all cases, patients explained the lack of compliance with their difficulty in taking the product twice daily, since an already complex polytherapy consisting of various medications was taken at different times per day. Fourteen patients completed the study correctly. General characteristics of this sample are detailed in Table 1.

2.3. Assessments

Patients underwent a baseline anamnestic and neurological assessment together with an analysis of clinical and metabolic parameters of interest (T0). After three (T3) and six (T6) months of treatment, patients were re-assessed, according to the same methods. Patients were assessed in the “on” state, 1 h after taking the morning dose of L-dopa. After each evaluation, 12 mL of overnight fasting blood were collected in EDTA tubes, immediately centrifuged and stocked at −80 °C, until analyses. Blood analyses were performed at the laboratory of the Department of Medical, Surgical and Health Sciences of the University of Trieste. During each week before the three scheduled visits, patients were asked to complete a specific diary where the “on” and “off” periods had to be reported each day.

Please cite this article in press as: Cucca A, et al., Amino acid supplementation in L-dopa treated Parkinson's disease patients, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2014.12.007

2.3.1. Neurological assessment

At each evaluation, complete medical history was collected, including concomitant medications. The disease severity and progression were assessed by internationally validated clinical scales (Unified Parkinson's Disease Rating Scale): UPDRS III, Hoenh–Yahr (H&Y). The average duration of "off period" was calculated according to the self-compiled diary delivered by the patient at each examination. For analytic purposes, the total daily L-dopa equivalent dose (LED) was recorded and calculated according to the conversion factors used by Tomlinson and Colleagues [26].

2.3.2. Anthropometric and nutritional assessments

Anthropometric data were collected with patients in the "on" phase when involuntary movements were rare and did not hamper assessments. Body weight was measured to the nearest 0.1 kg using a digital scale. Height was measured to the nearest 0.5 cm using a stadiometer. Body mass index was calculated as ratio between body weight, in kg, and height squared, in metre squared. Waist circumference measurements in cm were performed with a flexible measuring tape. At each evaluation a Mini Nutritional Assessment test (MNA) was performed. This screening tool contains 18 questions regarding anthropometry, diet and health. MNA scores between 24 and 30 points indicate optimal nutritional status, MNA scores between 17 and 23.5 indicate a risk for malnutrition, and MNA scores <17 indicate malnutrition.

2.3.3. Blood analysis

The following parameters were determined: insulin sensitivity, lipid profile, systemic inflammatory status, oxidative stress. Insulin sensitivity was measured with the QUICKI index, by determining fasting insulin and glucose levels. Lipid profile was determined by measuring total cholesterol, LDL cholesterol (calculated by Friedewald formula), HDL cholesterol and triglycerides. Measurement of C-reactive protein (CRP) was performed to assess the inflammatory status. Plasma insulin, glucose and CRP concentrations as well as lipid profile were assessed by standard procedures. We also assessed the erythrocyte glutathione system as a marker of oxidative stress, measuring the ratio between reduced (GSH) and oxidized (GSSG) glutathione by a commercially available kit (Oxford Biomedical Research Inc., MI). Plasma AA concentrations were assessed by gas chromatography–mass spectrometry (HP 5890; Agilent Technologies, Santa Clara, CA), using the internal standard technique, as previously described [27].

2.4. Statistics

Results are presented as mean ± SEM. Repeated measure ANCOVA was applied to define the effect of time and time × treatment interaction. Student t test was used to compare baseline characteristics between groups. Data were log-transformed where appropriate. Statistical analysis was performed using SPSS statistical software (version 12; SPSS, Inc., Chicago, IL). $p \leq 0.05$ was considered statistically significant.

3. Results

All clinical, metabolic and nutritional parameters, as well as inflammation and oxidative stress-related parameters did not show any significant difference between the two groups at baseline (Table 3).

3.1. Neurological outcomes

Subjects who received AA supplementation did not require an increase in L-dopa doses to maintain a satisfying therapeutic response as no significant changes occurred in their LED after six month follow-up. Comparable results emerged in the Placebo group (Fig. 1). According to our data, AA supplementation did not worse either motor performances or fluctuations. Indeed, UPDRS III assessment as well as daily average of "off" periods did not significantly change after 6 months of treatment, in both groups (Table 2). No time × treatment interaction ($p = 0.47$) was observed for the H&Y scores.

3.2. Nutritional and metabolic outcomes

A significant ($p < 0.001$) increase in MNA score has been observed in both Intervention (from 26.1 ± 0.6 to 28.7 ± 1.0) and Placebo (from 24.8 ± 2.8 to 25.9 ± 0.6) groups, with no time × treatment interaction ($p = 0.31$). No other significant variations emerged between the two groups by comparing all anthropometric and muscle strength parameters (Table 3).

Insulin sensitivity, as assessed by QUICKI index, significantly decreased in both groups. By comparing mean T6-T3 QUICKI index to baseline, a greater decrease in insulin sensitivity emerged in the Placebo group (−1.5 ± 2.8%) as compared to the Intervention group (−0.4 ± 2.1%). With regard to lipid profile assessment, neither time effect nor time × treatment interaction has been observed by ANCOVA analysis. After 6 months of observation, changes in triglycerides were greater in the Placebo than in the Intervention group (+11 ± 7 mg/dL and −7 ± 4 mg/dL, respectively), even though statistical significance was not achieved ($p = 0.07$). No other significant variations emerged by comparative assessment of the other metabolic parameters included in our analysis. Total erythrocyte glutathione concentrations did not change within both groups. Significant changes after six months of treatment were observed in GSSG concentrations. GSSG decreased by 28 ± 17% in the Intervention group while an increase of 55 ± 38% was observed in the Placebo when T6-T3 mean and T0 data were considered (Fig. 2).

In both groups no significant variations occurred in the CRP assessment as well as in plasma homocysteine concentration. Plasma AA concentrations are reported in supplemental materials.

4. Discussion

In this study we investigated the effect of 6 months of AA supplementation in protein-restricted PD patients chronically treated with L-dopa, showing fluctuations in their therapeutic response. No changes in all neurological parameters such as motor global performances, average daily off-period, disease progression and LED have been observed. Among metabolic parameters, both groups

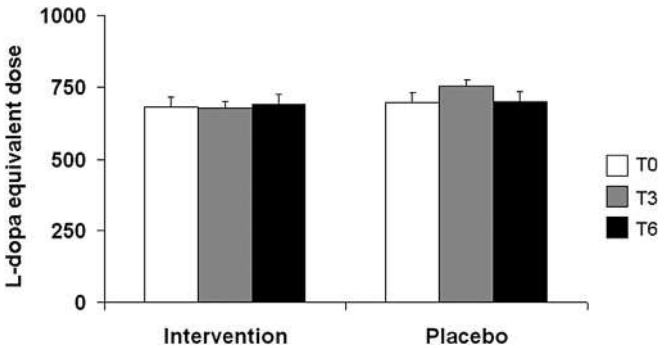

Fig. 1. L-dopa equivalent dose assessed at baseline (T0), after 3 (T3) and 6 (T6) months of treatment with daily amino acid (Intervention group) or placebo (Placebo group) supplementation. Time effect ($p = 0.73$) and time × treatment interaction ($p = 0.33$) did not reach the statistical significance, as assessed by repeated measure ANCOVA.

Please cite this article in press as: Cucca A, et al., Amino acid supplementation in L-dopa treated Parkinson's disease patients, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2014.12.007

Table 2
Neurological parameters at baseline (T0), after 3 (T3) and 6 (T6) months of treatment with daily amino acid (Intervention group) or placebo (Placebo group) supplementation.

ANCOVA						
	Treatment	T0	T3	T6	Time effect	Time × treatment interaction
UPDRS III	Intervention	12.6 ± 1.4	15.3 ± 2.9	16.3 ± 2.9	0.08	0.54
	Placebo	12.7 ± 3.6	12.9 ± 2.3	13.1 ± 1.9		
Average daily "off" time	Intervention	1.6 ± 0.4	1.7 ± 0.6	2.4 ± 0.7	0.33	0.65
	Placebo	1.5 ± 0.2	1.6 ± 0.5	1.6 ± 0.3		

Data expressed as mean ± SEM. For each reported parameter, basal values were not statistically different between the two groups, as assessed by Student t test. Statistical analysis performed using repeated measure ANCOVA. UPDRS, Unified Parkinson's Disease Rating Scale.

displayed an increase in insulin resistance which was significantly counteracted in the Intervention group by the AA supplementation. Furthermore, according to our study, daily AA supplementation prevented the loss in antioxidant protection observed in the Placebo group as it was associated to an overall increase in antioxidant glutathione availability.

4.1. Effects of amino acid supplementation on neurological parameters in PD patients

It is known that the products of gastric proteolysis inside the intestinal lumen increase the intestinal transit time and favour L-dopa degradation by the peripheral dopa-decarboxylase, thus reducing its enteral absorption [28,29]. Furthermore, it has been demonstrated by Pincus and Colleagues [13] that in conditions of markedly elevated plasma levels of large neutral AAs, the increase in L-dopa plasma concentration has no significant effect in motor control while parkinsonian symptoms tend to be more pronounced. Indeed, an inverse relationship between plasma levels of large neutral AAs and the parkinsonian's clinical performance emerged [14]. The best explanation for this phenomenon is that large neutral AAs derived from dietary protein compete with L-dopa for the same carrier in both enteral mucosa and blood–brain barrier. This is also consistent with post-prandial motor blocks often reported by patients on L-dopa treatment. To minimize the possible detrimental effects of AA supplementation we designed

Table 3
Clinical and metabolic parameters at baseline (T0), after 3 (T3) and 6 (T6) months of daily treatment with amino acid (Intervention group) or placebo (Placebo group) supplementation.

ANCOVA						
	Treatment	T0	T3	T6	Time effect	Time × treatment interaction
Nutritional status						
Body weight (kg)	Intervention	64.1 ± 2.3	64.6 ± 2.3	64.6 ± 2.6	0.12	0.35
	Placebo	68.9 ± 2.2	70.5 ± 1.9	71.1 ± 2.6		
Handgrip (kg)	Intervention	29 ± 5	28 ± 5	27 ± 4	0.42	0.88
	Placebo	30 ± 3	31 ± 2	28 ± 3		
Glicometabolic assessment						
Glucose (mg/dL)	Intervention	101 ± 3	104 ± 3	110 ± 8	0.07	0.90
	Placebo	101 ± 3	102 ± 2	100 ± 3		
Insulin (microU/mL)	Intervention	5.3 ± 0.6	5.0 ±0 .4	5.7 ± 0.4	0.04	0.16
	Placebo	8.7 ± 1.9	8.9 ± 1.3	8.7 ± 1.6		
QUICKI	Intervention	0.372 ± 0.009	0.370 ± 0.005	0.360 ± 0.006	<0.001	0.01
	Placebo	0.351 ± 0.014	0.343 ± 0.008	0.344 ± 0.006		
Lipid metabolism						
Total cholesterol (mg/dL)	Intervention	187 ± 12	188 ± 15	182 ± 13	0.69	0.22
	Placebo	198 ± 15	209 ± 16	212 ± 20		
LDL (mg/dL)	Intervention	112 ± 13	117 ± 14	108 ± 12	0.77	0.23
	Placebo	126 ± 17	136 ± 18	138 ± 21		
Triglycerides (mg/dL)	Intervention	62 ± 7	65 ± 9	54 ± 4	0.72	0.15
	Placebo	75 ± 11	81 ± 14	86 ± 14		
HDL (mg/dL)	Intervention	62 ± 4	59 ± 4	62 ± 4	0.45	0.46
	Placebo	57 ± 5	57 ± 6	57 ± 5		
Systemic inflammation and oxidative stress						
CRP (mg/dL)	Intervention	0.66 ± 0.54	0.26 ± 0.10	0.72 ± 0.63	0.06	0.25
	Placebo	0.26 ± 0.07	0.29 ± 0.09	0.21 ± 0.05		
Plasma homocysteine concentration (μmol/mL)	Intervention	19.5 ± 2.5	18.7 ± 2.1	16.9 ± 2.4	0.50	0.39
	Placebo	20.8 ± 1.1	25.1 ± 3.4	27.1 ± 5.4		
Totale glutathione (μmol/L)	Intervention	2305 ± 53	2379 ± 74	2376 ± 101	0.58	0.70
	Placebo	2423 ± 49	2449 ± 69	2465 ± 63		
GSH (μmol/L)	Intervention	1558 ± 84	1622 ± 60	1585 ± 68	0.15	0.29
	Placebo	1678 ± 112	1472 ± 200	1765 ± 102		
GSSG (μmol/L)	Intervention	5.2 ± 1.8	2.1 ± 0.4	2.6 ± 0.5	0.04	0.10
	Placebo	3.7 ± 0.7	4.2 ± 1.1	6.8 ± 2.9		
GSH-to-GSSG ratio	Intervention	465 ± 89	981 ± 194	779 ± 165	0.45	0.43
	Placebo	520 ± 82	649 ± 213	741 ± 236		

Data expressed as mean ± SEM. For each reported parameter, basal values were not statistically different between the two groups, as assessed by Student t test. Statistical analysis performed using repeated measure ANCOVA. Changes from baseline analysed by unpaired Student t test. Data were log-transformed were appropriate. BMI, body mass index. CRP, C-reactive protein. GSH, reduced glutathione. GSSG, oxidised glutathione. HDL, high-density lipoprotein. LDL, low-density lipoprotein. MNA, Mini Nutritional Assessment.

Please cite this article in press as: Cucca A, et al., Amino acid supplementation in L-dopa treated Parkinson's disease patients, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2014.12.007

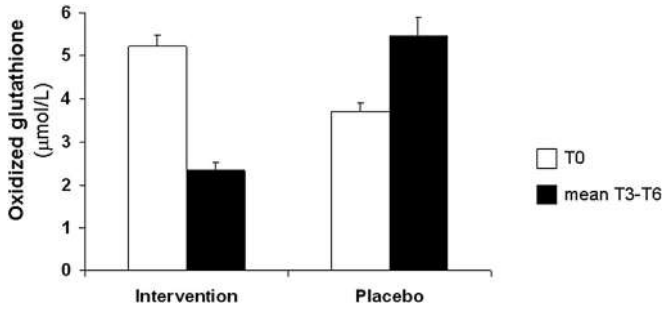

Fig. 2. Erythrocyte oxidized glutathione (GSSG) concentrations was reported at baseline (T0) and as mean of data collected after 3 and 6 months of treatment (mean T6-T3) with daily amino acid (Intervention group) or placebo (Placebo group) supplementation. There was a statistically significant time effect ($p = 0.05$) over the observation period as well as a statistically significant time × treatment interaction ($p = 0.05$), as determining using the repeated measure ANCOVA.

our study so that there was an interval of at least two hours between AA assumption and L-dopa administration. In fact, patients assumed the AA mixture at least one hour after lunch and one hour after dinner while the L-dopa administration was rigorously prescribed 60 min before meals. Moreover, for the same reason, we decided to supplement patients with a free-AA mixture that, as compared to dietary protein, has a more rapid systemic kinetics, due to faster intestinal absorption [30]. The overall prevalence of fluctuations in the clinical response to L-dopa, among patients following several years of disease, is estimated to be more than 50% [31]. The causes of fluctuation and loss in L-dopa response are not fully known. Diminished availability of dopamine in the central nervous system, impaired gastrointestinal absorption of L-dopa and alterations in striatal dopamine receptors sensitivity have been proposed as possible causal mechanisms. Gradual increase in the off duration is one other challenge in the therapeutic management of such patients and it may lead to an escalation in L-dopa dosage with further detrimental effects on motor control and a higher risk of developing dyskinesias. Therefore, in order to increase our sensitivity in finding out all potential pharmacological interactions, we specifically set-up our cohorts with long-lasting L-dopa treated patients, already showing fluctuations in their clinical response. According to our study AA supplementation does not hamper the pharmacological control of the disease, as evidenced by the absence of significant changes in LED, UPDRS, average off-duration and H&Y between the two groups. Indeed, in spite of the possible negative interaction between L-dopa and AAs, there has been no need to increase L-dopa doses to maintain a satisfying therapeutic response as confirmed by no changes in dopamine requirements in patients who received AA supplementation. According to our data, 6-month AA supplementation shows a safe profile in all assessed neurological parameters related to disease progression and motor control. Therefore, as it is already proficiently used in many chronic conditions sharing common nutritional and metabolic patterns, we believe that PD affected patients may take advantage of AA supplementation as well. Indeed, the exploitation of a proper time lapse between L-dopa administration and AA load may be critical for both the efficacy and safety of such intervention.

4.2. Effects of amino acid supplementation on metabolic profile in PD patients

In our cohort, a progressive decline in insulin sensitivity emerged in both groups over the 6-month follow-up, as it could be easily expected in such a population. Nevertheless, patients who received AA supplementation have shown a reduced trend towards an increase in insulin resistance, as compared to the Placebo group. The mechanisms responsible for such effect need to be fully clarified. AA supplementation may have a positive effect on insulin resistance by means of both insulinotropic and non-insulinotropic effects [8,9]. In fact, along with the well-known anabolic effects on muscle protein balance, essential AAs such as leucine, show considerable insulinotropic effects both *in vivo* and *in vitro* [8,9,32]. Moreover, some positive effects of cysteine on insulin sensitivity could be partially explained with a decrease in the oxidative stress which is a well established contributor in both PD [1,7] and type 2 diabetes [33] pathogenesis.

Cysteine, in fact, is the limiting substrate for the synthesis of glutathione, the main ubiquitous antioxidant in human body. An increase in oxidized glutathione is detectable in the Placebo Group, with no changes in total glutathione. This data is consistent with a progression in the oxidative stress among these patients. Interestingly, this negative event is prevented in patients receiving AA supplementation. This could be partly explained by the increased intake of cysteine with AA supplementation as well as by the beneficial effects of the supplementation on insulin sensitivity, that can be directly associated to the amelioration of the oxidative stress status.

4.3. Limitations of the study

In our opinion, the main limitation of this pilot study was the small sample size of our population as well as the brevity of the follow-up (6 months). Furthermore, a significant rate of drop-outs occurred. In most cases this was attributable to objective difficulties experienced by patients in taking the AA oral suspension twice daily in the context of a very complex polytherapy. More palatable and manageable formulations may help improve the compliance to the intervention. Such limitations intuitively restrict the overall impact of the metabolic data provided by this pilot study. Nevertheless, similar positive effects on glucose metabolism trough amino acid supplementation have been clearly reported in previous literature. As a case in point, the same amino acid mixture showed a positive metabolic modulation toward insulin sensitivity in patients with acute brain haemorrhage [34]. Another limitation we have to acknowledge concerns the outcomes chosen to investigate the impact of amino acid load on nutritional status. We determined the effects of amino acid supplementation on muscle function but, for logistic and technical reasons, we did not measure body composition, and in particular muscle mass, during the observational study.

5. Conclusions

We tested AA supplementation as an interventional effective option in fluctuating PD-affected patients. The overall analysis of our preliminary data suggests that AA supplementation, when assumed with shrewd temporal distribution, has no detrimental effects in L-dopa chronically treated patients. Positive effects in both insulin sensitivity and oxidative status have been observed in patients receiving 6 months of AA supplementation. These data are ultimately consistent with previous literature regarding general population. Further prospective studies on large-cohorts of fluctuating patients are warranted to fully elucidate all possible effects of long-lasting AA supplementation in the advanced stage of the disease.

Funding sources

None to declare.

Statement of authorship

AC, organization and execution of the research project; writing the manuscript; review and critique of the manuscript. SM, execution of the research project; execution of statistical analysis; review and critique of the manuscript. AB, execution of the research project. LA, execution of the research project. FGDG, execution of the research project. GP, conception of the research project; design of the statistical analysis; general evaluation of the statistical results. NK, review and critique of the manuscript. GLG, review and critique of the manuscript. MC, execution of the research project; design of the statistical analysis; review and critique of the manuscript. GB, conception of the research project; design of the statistical analysis; review and critique of the manuscript.

Conflict of interest statement

None to declare.

Acknowledgements

We would like to acknowledge Errekappa Euroterapici, SPA for its unrestricted support to this study.

The Authors wish to remember the invaluable contribution of the late Professor Gilberto Pizzolato (1950–2013), distinguished scholar and passionate Neurologist, who devoted his life to the study and treatment of Parkinson's Disease and Movement Disorders.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2014.12.007

References

[1] Collins LM, Toulouse A, Connor TJ, Nolan YM. Contributions of central and systemic inflammation to the pathophysiology of Parkinson's disease. Neuropharmacology 2012;62:2154–68.
[2] Johnson WM, Wilson-Delfosse AL, Mieyal JJ. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients 2012;4:1399–440.
[3] Isobe C, Murata T, Sato C, Terayama Y. Increase of total homocysteine concentration in cerebrospinal fluid in patients with Alzheimer's disease and Parkinson's disease. Life Sci 2005;77:1836–43.
[4] Aviles-Olmos I, Limousin P, Lees A, Foltynie T. Parkinson's disease, insulin resistance and novel agents of neuroprotection. Brain 2013;136:374–84.
[5] Van Woert MH, Mueller PS. Glucose, insulin, and free fatty acid metabolism in Parkinson's disease treated with levodopa. Clin Pharmacol Ther 1971;12:360–7.
[6] Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes 2007;56:1376–81.
[7] Hwang O. Role of oxidative stress in Parkinson's disease. Exp Neurobiol 2013;22:11–7.
[8] Manders RJ, Little JP, Forbes SC, Candow DG. Insulinotropic and muscle protein synthetic effects of branched-chain amino acids: potential therapy for type 2 diabetes and sarcopenia. Nutrients 2012;4:1664–78.
[9] Newsholme P, Brennan L, Rubi B, Maechler P. New insights into amino acid metabolism, beta-cell function and diabetes. Clin Sci (Lond) 2005;108:185–94.
[10] Lyons J, Rauh-Pfeiffer A, Yu YM, Lu XM, Zurakowski D, Tompkins RG, et al. Blood glutathione synthesis rates in healthy adults receiving a sulfur amino acid-free diet. Proc Natl Acad Sci U S A 2000;97:5071–6.
[11] van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, Keizer HA. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. Diabetes Care 2003;26:625–30.
[12] Solerte SB, Fioravanti M, Locatelli E, Bonacasa R, Zamboni M, Basso C, et al. Improvement of blood glucose control and insulin sensitivity during a long-term (60 weeks) randomized study with amino acid dietary supplements in elderly subjects with type 2 diabetes mellitus. Am J Cardiol 2008;101:82E–8E.
[13] Pincus JH, Barry KM. Plasma levels of amino acids correlate with motor fluctuations in parkinsonism. Arch Neurol 1987;44:1006–9.
[14] Nutt JG, Woodward WR, Hammerstad JP, Carter JH, Anderson JL. The "on–off" phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. N Engl J Med 1984;310:483–8.
[15] Barichella M, Marczewska A, De Notaris R, Vairo A, Baldo C, Mauri A, et al. Special low-protein foods ameliorate postprandial off in patients with advanced Parkinson's disease. Mov Disord 2006;21:1682–7.
[16] Sheard JM, Ash S, Mellick GD, Silburn PA, Kerr GK. Malnutrition in a sample of community-dwelling people with Parkinson's disease. PLoS One 2013;8:e53290.
[17] van der Marck MA, Dicke HC, Uc EY, Kentin ZH, Borm GF, Bloem BR, et al. Body mass index in Parkinson's disease: a meta-analysis. Parkinsonism Relat Disord 2012;18:263–7.
[18] Sheard JM, Ash S, Silburn PA, Kerr GK. Prevalence of malnutrition in Parkinson's disease: a systematic review. Nutr Rev 2011;69:520–32.
[19] Kashihara K. Weight loss in Parkinson's disease. J Neurol 2006;253(Suppl. 7):VII38–41.
[20] Markus HS, Cox M, Tomkins AM. Raised resting energy expenditure in Parkinson's disease and its relationship to muscle rigidity. Clin Sci (Lond) 1992;83:199–204.
[21] Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. Lancet Neurol 2003;2:107–16.
[22] Woitalla D, Goetze O. Treatment approaches of gastrointestinal dysfunction in Parkinson's disease, therapeutical options and future perspectives. J Neurol Sci 2011;310:152–8.
[23] Biolo G, Ciocchi B, Lebenstedt M, Barazzoni R, Zanetti M, Platen P, et al. Short-term bed rest impairs amino acid-induced protein anabolism in humans. J Physiol 2004;558:381–8.
[24] Burd NA, Gorissen SH, van Loon LJ. Anabolic resistance of muscle protein synthesis with aging. Exerc Sport Sci Rev 2013;41:169–73.
[25] Biolo G, Fleming RY, Maggi SP, Wolfe RR. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. Am J Phys 1995;268:75–84.
[26] Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010;25:2649–53.
[27] Biolo G, Agostini F, Simunic B, Sturma M, Torelli L, Preiser JC, et al. Positive energy balance is associated with accelerated muscle atrophy and increased erythrocyte glutathione turnover during 5 wk of bed rest. Am J Clin Nutr 2008;88:950–8.
[28] Djaldetti R, Baron J, Ziv I, Melamed E. Gastric emptying in Parkinson's disease: patients with and without response fluctuations. Neurology 1996;46:1051–4.
[29] Melamed E, Zoldan J, Galili-Mosberg R, Ziv I, Djaldetti R. Current management of motor fluctuations in patients with advanced Parkinson's disease treated chronically with levodopa. J Neural Transm Suppl 1999;56:173–83.
[30] Daenzer M, Petzke KJ, Bequette BJ, Metges CC. Whole-body nitrogen and splanchnic amino acid metabolism differ in rats fed mixed diets containing casein or its corresponding amino acid mixture. J Nutr 2001;131:1965–72.
[31] Schrag A, Quinn N. Dyskinesias and motor fluctuations in Parkinson's disease. A community-based study. Brain 2000;123:2297–305.
[32] Xu G, Kwon G, Cruz WS, Marshall CA, McDaniel ML. Metabolic regulation by leucine of translation initiation through the mTOR-signaling pathway by pancreatic beta-cells. Diabetes 2001;50:353–60.
[33] Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 2011;50:567–75.
[34] Laviano A, Aghilone F, Colagiovanni D, Fiandra F, Giambarresi R, Tordiglione P, et al. Metabolic and clinical effects of the supplementation of a functional mixture of amino acids in cerebral hemorrhage. Neurocrit Care 2011;14:444–9.