Research article

Effect of calorie-protein supplementation on the cognitive recovery of patients with subacute stroke

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Introduction: The objective of this study was to investigate whether protein-calorie supplementation may enhance the cognitive retrieval of patients with stroke.
Patients and methods: A randomized, double-blind, controlled pilot clinical trial was performed comparing diet and diet plus protein-calorie supplementation regimens. The subjects were 48 patients with subacute stroke (≥ 14 days from index event). Anthropometric and nutritional (3-day diary) variables, cognitive function (Mini-Mental State Examination; MMSE) were determined before and 21 days after randomization in control and daily supplemented group (formula providing 250 kcal + 20 g protein).
Results: At day 21 after starting the protocol, only the supplemented group significantly improved their performance to MMSE ($\log_{10}$MMSE +0.6 ± 0.4 score; $P = 0.01$ from baseline).
Conclusions: Protein-calorie supplementation may enhance the recovery of cognitive function in subacute stroke patients.

Keywords: calorie-protein supplementation, cognitive recovery, subacute stroke

Introduction

Cognitive dysfunction is a common complication of stroke, its prevalence being 36% shortly after the stroke[1] and 31% at 12 months after the acute event.[2] Moderate-to-severe levels of cognitive deficit may be an obstacle to learning new abilities during rehabilitation and to regaining a satisfactory social life. Thus, all strategies aimed at improving cognitive alterations following a stroke are highly desirable.

We believe that nutrition is one natural means of improving cognitive recovery in stroke patients. We hypothesized that nutritional manipulation consisting of supplementary energy and proteins may enhance the recovery of cognition in stroke patients undergoing rehabilitation. This hypothesis was based on the results of studies documenting that: (i) the intake of both energy and macronutrients influences cognition in healthy subjects;[3] (ii) the types of dietary proteins influence the types of cerebral proteins[4] and, therefore,

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Table 1 Demographic, clinical, and functional data and the locations of the cerebral lesions (determined by computed tomography scanning or nuclear magnetic resonance imaging) of the two groups of patients at their admission to our rehabilitation unit

	Control group (*n* = 24: 13 M + 11 F)	Supplemented group (*n* = 24: 14 M + 10 F)	*P*-value
Age (years)	71 ± 8.5	73 ± 6.2	ns
Co-morbidities (number of patients)			
Arterial hypertension	14 (59%)	14 (57.6%)	ns
Atrial fibrillation	8 (37.5%)	7 (29.1%)	ns
Dyslipidemia	4 (16.6%)	7 (29.1%)	ns
Chronic coronary artery disease	5 (20.8%)	4 (16.6%)	ns
Erythrocyte sedimentation rate (1 h; mm)	25 ± 13 (N.V. 3–11)	28 ± 16	ns
Serum albumin (g/dl)	3.4 ± 0.5 (N.V. 3.5–5.5)	3.3 ± 0.6	ns
Drugs being taken (number of patients)			
ACE inhibitors	8 (33.3%)	9 (37.5%)	ns
Calcium channel blockers	7 (29.1%)	8 (33.3%)	ns
Antibiotics	3 (12.5%)	4 (16.6%)	ns
β-Blockers	1 (4.1%)	1 (4.1%)	ns
Aspirin	6 (25%)	8 (33.3%)	ns
Lipid-lowering agents	4 (16.6%)	7 (29.1%)	ns
Stroke artery zone (CT or NMR; number of patients)			
Parietal	7 (29.1%)	8 (33.3%)	ns
Temporoparietal	5 (20.8%)	7 (29.1%)	ns
Frontal	8 (33.3%)	9 (37.5%)	ns
Frontoparietal	2 (8.3%)	–	–
Cerebellar	–	1 (4.1%)	–
Pontomesencephalic	–	1 (4.1%)	–
Total functional independence measure (FIM)	52.7 ± 8.4 (N.V. 118)	50.9 ± 10.3	ns

Values are mean ± SD; ns, not significant.
ACE, angiotensin-converting enzyme; N.V., normal value.

the amino acid profile in the brain;[5] (iii) long-term potentiation, a paradigm of learning and memory, is dependent on *de novo* protein synthesis;[6] (iv) protein synthesis is suppressed in ischemic but still viable cerebral neurons (penumbra), which, if not reversed, leads to cell death;[7] and (v) restoration of protein synthesis might, contrariwise, allow cells to repair ischemic damage and recover function.[8]

The aim of this study was, therefore, to determine whether supplementation with energy and proteins in rehabilitation patients with subacute stroke (≥ 14 days from index event)[9] may enhance the recovery of cognitive dysfunction.

Patients and methods

Patients

Forty-eight patients (27 males and 21 females) with subacute stroke (≥ 14 days from index event)[9] due to a cerebrovascular accident were enrolled in this randomized, double-blind, controlled trial. To be included, the patients had to have cognitive dysfunction, as shown by a score of < 20 in the Mini-Mental State Examination (MMSE),[10] and be independent in their alimentation. Patients who were aphasic or had chronic renal failure and/or diabetes on hypoglycemic therapy were excluded. The patients had been transferred to our rehabilitation unit from stroke units (64.6%), neurological departments (20.8%) or general medicine wards (14.6%).

After undergoing routine clinical and biochemical rehabilitation protocols, the patients were randomly allocated into two groups: one group of 24 patients received 21 days of calorie-protein supplementation with a nutritional formula (supplemented group) whereas the other group of 24 patients continued their spontaneous alimentation (control group). The nutritional formula consisted of a 200 ml mixture (Cubitan, Nutricia, Italy) providing 250 kcal of energy, 20 g proteins, 28.2 g carbohydrates, and 7 g lipids to be sipped between meals. Patients were allocated to treatments according to a complete randomized design. A list of randomization was derived through a random generator procedure using the statistical software SAS, being A and B the identifiers of the blinded treatments. The list was available to both the principal investigator and the hospital pharmacist. The principal investigator sequentially allocated a patient to treatment A or B according to the list.

The physician who evaluated the MMSE score was blinded to the supplementation and was different from the physician who prescribed the supplementation.

Table 1 reports the patients' demographic, clinical, and functional data and the locations of the cerebral lesions (determined by computed tomography scanning or nuclear magnetic resonance imaging).

Methods

After the randomization, the following parameters were measured in all patients:

1. Body weight, measured in kilograms using a mechanical weight lifter (Pabish, Pero, Milan, Italy), and height (in cm), calculated from knee height.[11] The body mass index (BMI in kg/m^2) was calculated from the body weight and height.

2. Daily calorie and macronutrient intakes (3-day diary): the types and weights of cooked and uncooked food, before and after meals were recorded. When necessary, we converted these data into raw equivalents, using an appropriate table.[12] Average daily calorie intake (kcal-I) was expressed in absolute values (kcal-I/day and kcal-I/kg/day). Average protein, carbohydrate and lipid intakes were considered in grams/day (g/day), in grams per body weight (g/kg) and as percentages of daily calorie intake (%kcal-I). For patients receiving the supplement, the amounts of energy and macronutrients contained in the nutritional formula were added to calculate the total nutrients ingested over a day.

All the above nutritional measurements and the MMSE were repeated 21 days after starting the protocol. All patients and/or their relatives gave informed, written consent to enter this study, which was approved by the local technical, ethical committee.

Criterion for considering dietary manipulation effective

Supplementary calories and proteins were considered effective if the $\log_{10}$MMSE score improved by ≥ 0.3 points (= score of 2 as the geometric mean). We chose this score increase because the improvement in $\log_{10}$MMSE score over 3 weeks in our rehabilitation unit is 0.204 ± 0.114 (= score of 1.6 ± 1.3 as the geometric mean; range 0–4; unpublished data).

Statistical analysis

As MMSE gives results on an ordinal scale, we transformed the test scores to $\log_{10}$MMSE. We also considered the geometric mean of $\log_{10}$MMSE score, when useful.

Repeated measure of analysis of variance (ANOVA test and Fisher's PLSD test) was applied to test differences over time in nutritional parameters and $\log_{10}$MMSE scores between the supplemented and control groups. The chi-squared test was used to show the distribution of patients relative to demographic, clinical, and functional characteristics as well as to a gain of ≥ 0.3 in the MMSE score. Data are given as mean ± SD. The level of statistical significance was set at $P < 0.05$.

Results

Table 2 records the mean ± SD data for body weight, BMI, nutritional intakes (energy, carbohydrates, proteins and lipids), and cognitive test scores ($\log_{10}$MMSE) of the two groups of patients both at their admission to the rehabilitation unit and 21 days after starting the protocol. Statistical analyses are also shown.

Nutritional parameters

On admission, the two groups of patients were comparable for both anthropometric measures and nutritional intakes.

At day 21 after starting the protocol, supplemented but not control patients had improvements in their daily nutritional intakes, whereas body weight and BMI remained virtually unchanged in both groups. In supplemented patients, calorie intake increased from 20 ± 3.9 to 24.2 ± 3.3 kcal/kg ($P < 0.05$) and protein intake improved from 0.8 ± 0.3 to 1.06 ± 0.32 g/kg ($P < 0.05$). The time-course of calorie-protein ingestion was significantly different between the two groups of patients ($P < 0.005$). Lipid intake normalized for body weight remained practically unchanged in both groups.

Cognitive test (MMSE)

On admission, the cognitive impairment tended to be less pronounced in the control group than in the supplemented group. However, the difference was not statistically significant (Fisher's PLSD test, $P = 0.15$). At day 21 after starting the protocol, only supplemented patients had a significant improvement in their performance in the MMSE. Indeed, the gain in $\log_{10}$MMSE score was 0.6 ± 0.4 (4 ± 2.6 as the geometric mean), from a baseline score of 1.2 ± 0.39 to a score at day 21 of 1.3 ± 0.5 ($P < 0.003$; as a geometric mean from 16.4 ± 2.5 to 20.3 ± 3.3).

Table 2 Nutritional parameters and cognitive test (MMSE) results in patients with stroke at admission to the rehabilitation unit and 21 days after starting the protocol

		Control group (n = 24)		Supplemented group (n = 24)	
		Admission	21-days	Admission	21-days
Body weight	(kg)	66.2 ± 8.7	65.9 ± 8.8	64 ± 9.6	64.4 ± 9
Body mass index	(kg/m^2)	23.7 ± 4.1	23.6 ± 4.4	24.4 ± 3.8	24.6 ± 2.9
Daily energy intake					
	kcal	1070 ± 210	1109 ± 206	1268 ± 241	1548 ± 212[a,e]
	kcal/kg	16.5 ± 4.4	17.3 ± 5	20 ± 3.9	24.2 ± 3.3[a,d]
Daily protein intake					
	g	33 ± 8.5	39 ± 9	51.3 ± 21.6	67 ± 17[b]
	g/kg	0.5 ± 0.2	0.59 ± 0.12	0.8 ± 0.3	1.06 ± 0.3[b]
	%kcal	12.3 ± 3.7	13.9 ± 4.5	16.2 ± 4.5[c]	17.3 ± 3.8[c]
Daily carbohydrate intake					
	g	152 ± 15	160 ± 23	154 ± 44	181 ± 39
	g/kg	2.3 ± 0.4	2.5 ± 0.5	2.4 ± 0.7	2.8 ± 0.7
	%kcal	56.8 ± 9.1	57.7 ± 8.5	48.6 ± 8.5	46.7 ± 9.3
Daily lipid intake					
	g	44.8 ± 15.5	43.6 ± 13.9	47.5 ± 2.9	61.9 ± 15
	g/kg	0.71 ± 0.25	0.68 ± 0.4	0.8 ± 0.18	0.96 ± 0.51
	%kcal	37.7 ± 8.2	35.4 ± 7.7	33.7 ± 9.4	36 ± 6.9
MMSE (score)					
	$\log_{10}$	1.26 ± 0.3	1.27 ± 0.2	1.2 ± 0.39	1.3 ± 0.5[f]
	Geometric mean	18.4 ± 0.5	19 ± 1.2	16.4 ± 2.5	20.3 ± 3.3

Statistical analysis – ANOVA test and Fisher's PLSD test: values are mean ± SD
Inter-group differences: [a]$P < 0.002$, [b]$P < 0.005$, [c]$P < 0.05$.
Intra-group differences: [d]$P < 0.05$, [e]$P < 0.02$, [f]$P < 0.003$.

According to our criterion of effectiveness, all supplemented but only 20% of control patients (chi-squared test, $P < 0.0001$) improved their MMSE score by at least 0.3 (2, as the geometric mean).

Discussion

This study shows that daily supplementation with 250 kcal energy and 20 g protein enhanced cognitive recovery in all rehabilitation patients with subacute stroke and marginally satisfactory nutritional intakes. The average gain in MMSE score was more than 2-fold higher than that observed over the same period of observation in our clinical practice.[13]

Nutritional intakes

The patients in this study had a marginally satisfactory nutritional intake. Indeed, the calorie intake was close to the resting energy expenditure (19 kcal/kg/day), necessary for basal metabolic body requirements, previously reported by our group in patients with stroke.[14] Likewise, the protein intake whose normal value is, at least, 0.8 g/kg/day, was low.[15]

It was not surprising to find that the nutritional intake of these patients with sequelae of a cerebrovascular accident was altered.[14,16,17] What is worthy of note is the observation that after 21 days of the protocol, the alimentation of the patients, at net of the supplementation in supplemented group, did not improve, and yet the patients' body weight did not change significantly. This suggests that, even if marginal, the nutritional intake in clinically stable, bed-ridden patients with subacute stroke (of whom only 14.6% were on antibiotics) may be enough to maintain body weight over 3–4 weeks after the acute injury. Likely, the injury to an organ with intense metabolic activity such as the brain, the hemiparesis, and the absence of physical activity reduce the degree of body energy and protein utilization.

In supplemented patients, the association of an important improvement in cognition and a trend towards an increase in body weight might suggest greater utilization of nutritional substances by brain structures for functional purposes rather than by peripheral tissues for anatomical accrual.

Potential impact of energy-protein supplementation on recovery of brain function

The supplemental energy likely contributed to the improvement in cognitive function of the study patients. Indeed, several studies have documented that energy substances can enhance memory via mechanisms including gut peptides and the vagus nerve.[18–20] With regards to proteins (and, thus, amino acids), these substances may influence the recovery of cognition by acting both directly and indirectly on brain structures.

Increased brain amino acid availability may directly re-activate the synthesis of neural proteins and boost both neuron energy and neurotransmitter formation. Indeed, in ischemic penumbra, amino acids can be taken up and used by resistant cells that have the ability to restore the normal rate of protein synthesis,[8] which is blunted or even suppressed in ischemic regions.[21] That areas of ischemia need more amino acids can be inferred from the finding of increased specific activity of the amino acid leucine within them.[7]

The restoration of protein synthesis is of paramount importance for both neuronal survival and function as protein synthesis may reduce the expansion of brain infarction.[22] Indeed, there is a precise correspondence between the cerebral area with inhibited protein synthesis and the final size of the infarct.[22]

The re-activation of protein synthesis might also affect the formation of neurotrophins[23] (such as NGF, BDNF, NT-3, NT-4/5, bFGF) and interleukins,[24] all molecules that may play a role in improving cognitive function. Neurotrophins have been documented to protect cultured rat hippocampal and cortical neurons[25,26] and interleukins improve the resistance of brain structures to ischemic cell injury.[24]

Another direct mechanism by which amino acids may improve cognitive function is increased neuronal energy formation,[27] which favours neuronal electrical stabilization.[28]

It is important to note that energy is also essential for protein synthesis, hence for sprouting and remodelling of electrical circuitry.[29]

Increased neurotransmitter synthesis[30] is another direct mechanism by which amino acids from supplemented proteins may enhance the recovery of cognition in stroke patients.

As regards indirect mechanism, amino acids may favour the recovery of cognition by inducing an increase of the cerebral content of insulin,[31,32] a hormone involved in brain cognitive functions and dysfunctions.[33] That calorie-protein supplementation may increase brain insulin levels can be inferred from investigations documenting that food-induced circulating insulin can cross the blood–brain barrier[34] and insulin receptors are distributed throughout the brain.[35] Insulin acts on areas of the brain that are important for cognition. For instance, insulin promotes glucose utilization in the hippocampus,[36] and influences both the response of the post-synaptic neurons[37–39] and the firing rates of neurons of the hypothalamus,[40] suprachiasmatic nucleus[41] and hippocampus.[42,43] Interestingly, circulating insulin may influence brain function independently of glucose.[44]

In brief, this study shows that supplemental energy and protein intake in patients with subacute stroke has a positive impact on cognition, confirming findings reported for healthy subjects.[20]

Clinical implications

This study has some practical, clinical implications. Subacute stroke patients at admission to a rehabilitation unit may have low energy-protein intakes that, if not corrected, tend to remain virtually unaltered until the patients' discharge from hospital. In this case, it can be expected that the patients will have impaired nutritional status[14] and plasma amino acid concentrations.[45] Abnormalities in plasma amino acids may reduce cognitive recovery in stroke patients.[45]

In patients with subacute stroke, a MMSE score of < 20 and marginal nutritional intakes, daily ingestion of energy ≥ 24 kcal/kg and proteins > 1 g/kg seems to assure an enhancement of cognitive recovery.

Limitations of the study

The accuracy of the MMSE in detecting cognitive impairment in acute stroke has been questioned.[46] This should not, however, be relevant in our study because the patients enrolled were in a subacute state, *i.e.* with a much better clinical status than that of patients in the acute phase.[47] Furthermore, the patients were not aphasic and were able to understand everything they were asked. A second limitation of this study is that it was not planned to investigate whether reducing carbohydrate intake rather than increasing protein intake would be effective in enhancing cognitive recovery. This would be of clinical importance for patients receiving a diet with a reduced protein intake (those with chronic renal insufficiency) or calorie intake (diabetics, over-weight subjects).

Lastly, future studies performed on both ischemic animals and humans will address the impact of lipid supplementation on cognitive recovery. This is relevant for repairing ischemic brain as it has been shown that the n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA)[48] and its derived mediator neuroprotectin D1[49] confer high-grade neurobehavioral and histological neuroprotection[48] in ischemic brain of rats.

Furthermore it will be interesting to investigate whether protein and n-3 fatty acid supplementation may act synergistically in improving the retrieval of cognition in ischemic brain. In fact, a possible protein-mediated increase in serum albumin might induce an increased brain availability and consumption of free-fatty acids that may be used as an alternative source of energy.[50]

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