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Protein supplementation may enhance the spontaneous recovery of neurological alterations in patients with ischaemic stroke

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Objective: To determine whether protein supplementation could enhance neurological recovery in subacute patients with ischaemic stroke.
Design: Alimentation-independent patients with ischaemic stroke were randomly allocated to either 21 days of protein supplementation (protein-supplemented group; $n=20$) or to a spontaneous diet only (control group; $n=21$) in order to investigate the recovery of neurological changes (measured using the National Institute of Health (NIH) Stroke Scale).
Setting: Tertiary care rehabilitation in Italy.
Participants: Forty-two patients (27 male and 15 female; 66.4 ± 11 years) 16 ± 2 days after the acute event.
Intervention: Supplementation with a hyperproteic nutritional formula (10% protein).
Main outcome measures: NIH Stroke Scale and protein intake.
Results: At admission to rehabilitation, both groups of patients were homogeneous for demographic, clinical and functional characteristics. After 21 days from the start of the protocol, the NIH Stroke Scale was found to be enhanced in the group with supplemental proteins (-4.4 ± 1.5 score versus -3 ± 1.4 of control group; $P<0.01$). When expressed as difference (Δ) between baseline and 21 days, the NIH Stroke Scale correlated negatively with change in protein intake (g/day) ($r=-0.50$, $P=0.001$) and positively with change in carbohydrate/protein ratio ($r=+0.40$, $P=0.01$)
Conclusions: Protein supplementation may enhance neurological recovery in subacute patients with ischaemic stroke.

Introduction

Experimental and human studies have shown the importance of protein turnover in cerebral ischaemia. Focal cerebral ischaemia induces suppression of protein synthesis in still viable cerebral neurons

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(penumbra) which, if not reversed, can lead to cell death.[1] This is confirmed by the observation that isolated blocking of protein synthesis is lethal to cells.[2] There is a precise correspondence between cerebral area with inhibited protein synthesis and final infarct size.[3] The suppression of protein synthesis is an important factor limiting the post-ischaemic recovery of neurons.[4]

In humans, the importance of brain protein synthesis for neuronal survival was documented for the first time by a study showing a trend towards the upregulation of the growth arrest and DNA damage protein (GADD34) in the ischaemic brain following cardiac arrest. GADD34 is thought to play a crucial role in the modulation and restoration of protein synthesis.[5] Brain neurons of patients who survived 0–24 hours after cardiac arrest had limited increase in GADD34 localized to the cytoplasm, while the expression of GADD34 in neurons of patients surviving between 24 hours and seven days was markedly increased in cytoplasm and perinuclear regions.[4]

On the basis of these studies, we hypothesized that protein supplementation might improve derangements in brain protein synthesis, and therefore recovery of neuron function, in patients with subacute stroke (>14 days from index event). Following our reasoning, this might enhance rehabilitation efficacy. Our hypothesis was based on the following: (1) different types of dietary proteins influence cerebral proteins[6] and therefore amino acid profile in the brain[7]; (2) increased cell amino acid availability drives protein synthesis[8]; (3) the mean half-lives of brain proteins are relatively short, varying between 3 and 9 days.[9]

Therefore, this investigation was set up to find out whether protein supplementation can enhance the recovery of neurological sequelae in patients with subacute stroke.

Methods

Patients

Forty-two alimentation-independent patients (27 male and 15 female) admitted to our rehabilitation unit 16 ± 2 days (range 12–20 days) after an acute cerebrovascular stroke were enrolled for this study. They came from stroke units (70.7%), neurological departments (24.4%) or general medicine wards (4.9%). Patients with chronic renal failure, diabetes or who were taking hypoglycaemic drugs were excluded. All patients and/or their relatives gave their informed, written consent to enter this study and it was approved by the local technical, ethical committee.

Table 1 reports the patients' demographic, clinical and functional data, and the location of the cerebral lesions (determined by computed tomography scanning or nuclear magnetic resonance imaging) on admission to our rehabilitation unit. Within 72 hours from admission, patients were allocated to treatments according to a complete randomized design. A list of randomization was derived through a random generator procedure using SAS statistical software (SAS Statistical Institute, Cary, NC, USA), A and B being 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.

One group of 20 patients received 21 days of protein supplementation from a nutritional formula (protein supplemented group; PS group) whereas the other group of 21 patients (control group) continued their spontaneous alimentation. The formula (200 mL, Cubitan; Nutricia, Milan, Italy) provided 250 Kcal energy, 20 g proteins, 28 g carbohydrates and 7 g lipids and was sipped between meals. In order to ensure more homogeneous assimilation of nutrients, not linked to the patients' differences in taste and digestion function, we supplied these additional proteins to the patients using a nutritional formula instead of natural food.

The patients/caregivers and physician who evaluated the neurological test, apart from the one who prescribed the supplementation, were blinded to the supplements themselves.

Before and 21 days after the beginning of the experimental protocol, the following variables were measured in all patients:

- Neurological alterations by National Institute of Health Stroke Scale.[10]
- Body weight (BW) measured in kilograms using a mechanical weight lifter (Pabish, Pero,

Table 1 Demographic data and clinical–functional characteristics of patients with ischaemic stroke ($n = 41$) on admission

Variable	PS group	Control group	*P*-value
Age (years)	71 ± 6.9	68 ± 9.1	n.s.
Gender (M/F)	14/6	12/9	n.s.
Co-morbidities, no. of patients (%)			
Arterial hypertension	10 (24.4%)	11 (26.8%)	n.s.
Atrial fibrillation	6 (14.6%)	5 (12.2%)	n.s.
Chronic coronary artery disease	1 (2.4%)	2 (4.9%)	n.s.
Dyslipidaemia	3 (7.3%)	5 (12.2%)	n.s.
Erythrocyte sedimentation rate at 1 hour	30 ± 19 (NV 3–11 mm)	26 ± 17 (NV 3–11 mm)	n.s.
Serum albumin (g/dL)	3.3 ± 0.5 (NV 3.5–5.5)	3.18 ± 0.7 (NV 3.5–5.5)	n.s.
Drugs being taken (no. of patients)			
ACE inhibitors	8 (19.5%)	6 (14.6%)	n.s.
Calcium channel blockers	5 (12.3%)	7 (17%)	n.s.
Beta-blockers	1 (2.4%)	3 (7.3%)	n.s.
Aspirin	5 (12.2%)	3 (7.3%)	n.s.
Antibiotics	6 (14.6%)	4 (9.7%)	n.s.
Lipid-lowering agents	5 (12.2%)	3 (7.3%)	n.s.
Stroke artery zone (CT or NMR) (no. of patients)			
Temporoparietal	4 (9.7%)	5 (12.2%)	n.s.
Parietal	5 (12.2%)	8 (19.5%)	n.s.
Frontal	5 (12.2%)	4 (9.7%)	n.s.
Cerebellar	2 (4.87%)	–	–
Pontine	1 (2.4%)	–	–
Frontoparietal	2 (4.9%)	3 (7.3%)	n.s.
Pontomesencephalic	1 (2.4%)	–	–
Cerebellar pedunculus	1 (2.4%)	–	–
Activities of daily living (ADL) score	1/6 (NV 6/6)	1/6	n.s.
Total Functional Independence Measure (FIM) score	54.9 ± 7.3 (NV 118)	53.3 ± 5.5	n.s.

Values are means ± standard deviation.
ACE, angiotensin-converting enzyme; NV, normal value; CT, computed tomography; NMR, nuclear magnetic resonance.

Milan, Italy). Height (in cm) was calculated from knee height. Body mass index (BMI) was calculated from the body weight and height, according to the formula BMI = BW (in kg)/height (in meters)^2.

- Daily calorie and macronutrient intake (three-day diary): the type and weight of cooked and uncooked food, before and after meals, were recorded. When necessary, these data were converted into raw equivalents, using an appropriate table. Average daily calorie intake (Kcal-I) was expressed in absolute values (kcal-I/day and kcal-I/kg per day). Average protein, carbohydrate (CHO) and lipid intakes were considered in grams/day, in grams per body weight (g/kg) and as percentages of daily calorie intake (% kcal-I). In patients receiving supplements, the amounts of energy and macronutrients contained in the nutritional formula were added to calculate the total nutrients ingested over a day. Moreover, the ratio of CHO (in g/day) to protein (also in g/day) (CHO/protein) was calculated for all patients. The CHO/protein ratio was considered because an experimental study has documented cerebral metabolic dysfunction in rats[11] following diets rich in carbohydrates but low in proteins.
- Urinary nitrogen excretion and nitrogen balance. For this study 24-hour urine samples were collected from an 24-hour indwelling catheter, to determine daily urinary nitrogen excretion, expressed in grams (using the micro Kjeldahl technique). To estimate total nitrogen loss, 2 g was added to the 24-hour urinary nitrogen excretion to compensate for faecal and transcutaneous nitrogen loss.

- Nitrogen balance (NB) was defined as dietary nitrogen intake minus total nitrogen loss:

$$NB(g/day) = N_I - (N_V + 2\,g)$$

where N_I is the nitrogen intake (g/day) obtained as protein intake (g/day): 6.25. In the experimental group, N_I = nitrogen from spontaneous alimentation +3.2 g from supplied proteins. N_V is the urinary nitrogen excretion (g/day) +20% N_V for non-urea nitrogen excretion; 2 g is the nitrogen lost in faeces and sweat.

Twenty-four hours after measuring the above variables, the two groups of patients followed standard rehabilitation therapy consisting of propioceptive neuromuscular facilitation (90 min/day over five days weekly).

Criterion for considering protein supplementation effective

Protein supplementation was considered effective if the NIH Stroke Scale reduced by ≥ 5 points. We chose this score reduction given that in our rehabilitation unit the improvement in NIH Stroke Scale score three weeks after admission to rehabilitation was -3 ± 1.1 (data not published).

Statistical analysis

The χ^2 test was used to compare baseline demographic data and clinical–functional characteristics between the two groups. The same test was used to assess differences in the percentage of patients achieving a 5 NIH Stroke Scale reduction 21 days from when the protocol started. Within each single group, differences between baseline and 21 days were assessed by using paired *t*-test; between-group differences were tested by unpaired *t*-test. Simple linear regression analysis was used to evaluate the role of nutritional variables in predicting NIH Stroke Scale; significantly related variables were tested with multiple regression analysis to examine the simultaneous effect of predictors. A stepwise procedure was applied to identify the final model. Data are given as mean ± standard deviation (SD). Statistical significance was set at $P < 0.05$.

Results

The total number of randomized patients was 42; 21 of them were allocated to the control group and 21 to the supplemented group. Unfortunately, a few hours after having performed randomization, one patient in the latter group had a myocardial infarct and was moved to the intensive care unit. Given that he did not start the protocol (no protein supplementation was provided to him), we decided to exclude this subject from our study. A flow diagram indicating the number of patients recruited and registered is shown in Figure 1.

Table 2 shows the mean and standard deviations of both absolute values and changes over time of neurological test and nutritional variables of the two groups of patients (with and without dietary protein supplementation) at their admission to the rehabilitation unit and 21 days after

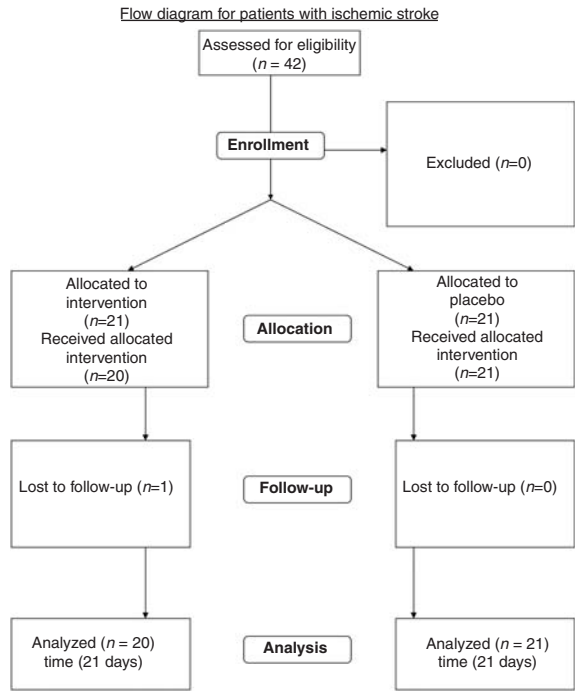

Figure 1 Flow diagram indicating the number of patients recruited and registered.

Table 2 Nutritional variables and neurological test score (NIH Stroke Scale) in patients with stroke at admission to the rehabilitation unit and 21 days after starting the protocol

	PS group ($n=20$)		Control group ($n=21$)	
	Admission	Change	Admission	Change
Body weight (kg)	65.9 ± 11.0	+0.36 ± 0.63	66.8 ± 11.2	−0.5 ± 1.5
Body mass index (kg/m^2)	24.7 ± 3.1	+0.2 ± 3.45	23.0 ± 3.7	0 ± 3.1
Daily energy intake				
kcal	1253 ± 295	+404 ± 257	1689 ± 449	−18.4 ± 379
kcal/kg	19.7 ± 6.0$^{\bullet}$	+5.8 ± 3.6*	25.3 ± 5.5	+0.46 ± 5.5
Daily protein intake				
g	50.8 ± 18.3	+28.3 ± 24.9	71.4 ± 23.0	−1.24 ± 22.5
g/kg	0.8 ± 0.3	+0.43 ± 0.40***	1.07 ± 0.3	−0.002 ± 0.3
%kcal	16.0 ± 3.6	+3.35 ± 4.90*** $^{\bullet}$	16.8 ± 2.9	+0.13 ± 3.5
Daily carbohydrate intake				
g	145.0 ± 44.0	+34.3 ± 39.7	204.0 ± 84.0	+11.6 ± 56
g/kg	2.3 ± 0.9$^{\bullet}$	+0.48 ± 0.64*	3.0 ± 1.0	+0.17 ± 0.88
%kcal	46.4 ± 8.5	−3.3 ± 8.8	46.9 ± 10.0	+4.2 ± 10.8
Daily lipid intake				
g	52.0 ± 1.9	+16.2 ± 8.8	69.0 ± 16.0	−7.4 ± 17.6
g/kg	0.79 ± 0.3	+0.11 ± 0.3	1.03 ± 0.26	−0.08 ± 0.26
%kcal	37.5 ± 7.9	−0.8 ± 7.1	37.2 ± 8.7	−4 ± 7.9
CHO/protein ratio (%)[a]	3.2 ± 1.0	−0.92 ± 1.08** $^{\bullet\bullet}$	2.9 ± 1.0	+0.05 ± 1.2
Nitrogen balance (g/24 h)	+2.84 ± 3.1	+5.76 ± 3.1** $^{\bullet\bullet}$	+1.32 ± 4.3	−1.25 ± 3.6
NIH Stroke Scale score:	12.6 ± 2.7	−4.4 ± 1.50*** $^{\bullet}$	10.1 ± 3.4	−3.0 ± 1.4***

PS group, protein-supplemented group.
Values are mean ± standard deviation; Change is the difference between 21 days and admission.
[a]The normal value of the CHO/protein ratio in age-matched healthy subjects is 3.17 ± 0.8 (data from the authors' laboratory).
Statistical analysis:
Intergroup differences (unpaired *t*-test):
At admission (ADM): $^{\bullet}P<0.01$; 21 days: $^{\bullet\bullet}P<0.001$.
Within-group differences (paired *t*-test, 21 days versus admission):
$*P<0.05$; $**P<0.01$; $***P<0.001$.

starting the protocol. The statistical analysis is also reported.

The results show that 21 days of protein supplementation was associated with enhanced neurological recovery. Indeed, in patients receiving supplements, NIH Stroke Scale improved by an average of 4.4 points, whereas in controls the improvement in NIH Stroke Scale was 3.0 points ($P<0.01$ between the two groups). In particular, there was enhanced recovery in supplemented patients in the motor performances of the paretic arm and leg, which were found more able to resist gravity for 10 seconds (with limb outstretched). The score for both arm and leg was 2.8 ± 0.1 at admission and 1.4 ± 0.2 at 21 days ($P<0.01$). The improvement in limb performance against gravity was less pronounced in control patients (from baseline 2.7 ± 0.2 to 2.2 ± 0.4, $P<0.05$). This difference in improved limb motor performance was highly significant between supplemented and control patients ($P<0.02$). According to our criterion for considering protein supplementation effective, 70% ($n=14$) of supplemented patients and 14.3% ($n=3$) of control patients improved in NIH Stroke Scale by ≥5 points ($P<0.001$ between the two groups).

The study found that supplemented patients, after 21 days of the protocol, significantly improved their baseline energy, carbohydrate, protein intake and nitrogen balance. The CHO/protein ratio decreased significantly. In contrast to those supplemented, control patients had no noteworthy change in their baseline nutritional parameters, including CHO/protein ratio.

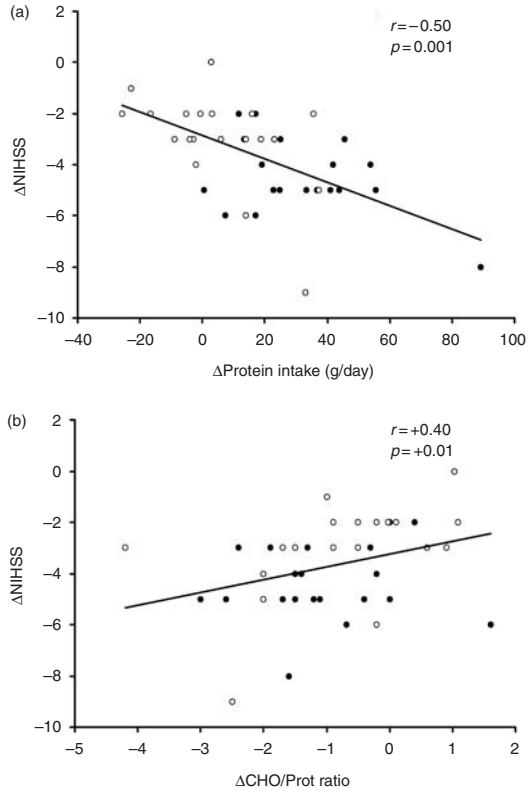

Figure 2 (a) Correlations of change (Δ in protein intake between 21 days and baseline versus change (Δ) in neurological test (NIH Stroke Scale). (b) Correlations of change (Δ in CHO (g)/protein (g) ratio between 21 days and baseline versus change (Δ) in NIH Stroke Scale. Open circles, control group; filled circles, supplemented group.

In this study, when expressed as differences (Δ) between 21 days treatment and baseline, NIH Stroke Scale correlated negatively with change in protein intake ($r=-0.50$, $P=0.001$) and positively with change in CHO/protein ratio ($r=+0.40$, $P=0.01$) (Figure 2a,b).

NIH Stroke Scale correlated with energy intake (kcal; $r=-0.42$, $P=0.009$). No other variables (CHO, lipids) correlated with NIH Stroke Scale.

The same variables, tested with a stepwise multiple regression analysis, showed the major role of proteins in predicting NIH Stroke Scale, with CHO/protein and kcal excluded from the final model.

Discussion

Biochemical and nutritional aspects

This study shows that 21 days of supplementation with proteins may enhance the neurological recovery in patients with subacute stroke.

Protein supplementation may achieve this by both increasing whole body net protein synthesis (reflected by nitrogen balance) and reducing the dietary CHO/protein ratio. This suggests an increased amino acid use counteracting an altered glucose use.[12,13] The patient nutrition survey differentiates the present study from the 'FOOD' trial results,[14] whose primary outcomes were actually death or poor outcome and overall survival over six months. In the 'FOOD' trial, the lack of evidence for effects on nutrition-sensitive outcome such as hospital complications, length of stay, residence at follow-up or quality of life, may have been due to the lack of information on patients' current calorie and macronutrient intakes (carbohydrates, proteins, fats).

Given that: (1) brain neurons process more amino acids in the Krebs cycle than do tissues outside the brain,[15] (2) alimentary proteins/amino acids influence brain protein/amino acid content[6,7] and (3) the patient's whole body metabolism is oriented towards protein synthesis, it is reasonable to assume that an increased rate of protein synthesis in stroke patients with protein supplementation probably also occurred within brain structures.

Potential action of protein supplementation on cortical plasticity and motor recovery

Supplemented proteins may contribute to post-stroke cortical plasticity and improvement of motor activity of paretic limbs by means of multiple mechanisms. These may include amino acid-induced brain reactivation of protein synthesis, energy production and neurotransmitter formation/activity.

Improved protein synthesis may induce axonal sprouting and formation of new cortical connections in perilesional zones[16] and in regions remote from the site of ischaemic lesions.[17] The recruitment of spared networks may improve motor control and skill because in patients with stroke, reactivated networks are associated with the best

gains in the use of the affected arm/leg and in skilled behaviour.[18] Furthermore, reactivated perilesional tissue reduces the risk of a shift of motor activation towards the unaffected hemisphere, thus favouring functional recovery.[19,20]

Another way by which brain protein synthesis may improve motor recovery is working together with rehabilitation procedure to enhance both training-induced plasticity[21] and the production of brain-derived neurotrophic factor. This is an important peptide for lasting long-term potentialization, synaptic efficacy and connectivity.[22–24] The enhanced motor control observed in supplemented patients could also be due to amino acid-induced reactivation of both energy metabolism in penumbra cells and regions distant from ischaemic lesions[1,17] and neurotransmitter system.[8,25–27] With regard to this latter, for example, the activities of norepinephrine, dopamine and adrenaline are important for activity-dependent plasticity and skill learning,[28] even in patients in whom training alone is ineffective in improving performance.[29]

Amino acid-induced reactivation of brain protein synthesis, energy production and neurotransmitter formation/activity could explain why in our study, patient neurological recovery positively correlated with protein intake and negatively with CHO/protein. Theoretically, the enhancement of neurological retrieval in supplemented patients could be partly attributable to the general improvement of nutritional intake.

It cannot be totally excluded that an improved energy intake somehow influences neurological recovery as suggested by simple regression in the present paper. Even if energy intake plays a role, protein intake still remains the major determinant of NIH Stroke Scale as confirmed by stepwise multiple analysis. Improved carbohydrate ingestion very probably did not enhance neurological recovery given the ischaemia-induced alterations in neuronal glucose metabolism previously mentioned. Supporting this, increased dietary carbohydrates with even marginal reduction in proteins have been found to be deleterious for brain function in both epidemiological and experimental investigations (116).[11] In our study, the inefficacy of improved carbohydrate intake on neurological recovery seemed to be supported by the absence of any correlation between carbohydrate intake and the performance of the neurological test.

Lipid intake can be reasonably excluded as a factor enhancing neurological recovery as it was similar in both groups. Therefore, it was protein supplementation that might have enhanced neurological recovery in our patients.

The study has several weaknesses. The small sample size precluded an analysis of the effect of protein supplementation on the brain regions which normally are characterized by a specific metabolism, for instance regions with high (medial temporal lobe) or low (parietal and frontal lobes) glucose metabolism.[30] This would have allowed us to better understand the impact of amino acids on brain function retrieval.

Patients were not monitored in their nutritional intake/supplementation beyond the scheduled 21 days. Long-term monitoring of patient nutrition is an important issue because the cerebral response to nutrients could change over time in relation to brain repair and functional recovery. The knowledge of cerebral response to nutrients over time might allow us to modulate the types of nutrients the patients should take in order to contribute more to the recovery of motor learning, control and skill.

We think that monitoring patient nutritional intake and determining nitrogen balance over a 21-day protocol strengthened the discussion. Indeed, knowledge of patients' nutrition allowed us to reasonably attribute the enhanced neurological recovery to improved protein ingestion as well as point out a plausible underlying mechanism.

Clinical messages

- Protein supplementation is associated with an enhancement of neurological recovery in subacute stroke.
- A reduction of CHO/protein ratio from protein supplementation may contribute to enhanced neurological recovery.

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References

1 Yao H, Ginsberg MD, Eveleth DD *et al*. Local cerebral glucose utilization and cytoskeletal proteolysis as indices of evolving focal ischemic injury in core and penumbra. *J Cereb Blood Flow Metab* 1995; **15**: 398–408.
2 Srivastava SP, Kumar KU, Kaufman RJ. Phosphorylation of eukaryotic translation initiation factor 2 mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase. *J Biol Chem* 1998; **273**: 2416–23.
3 Hata R, Maeda K, Hermann D, Mies G, Hossmann KA. Dynamics of regional brain metabolism and gene expression after middle cerebral artery occlusion in mice. *J Cereb Blood Flow Metab* 2000; **20**: 306–15.
4 Mies G, Ishimaru S, Xie Y, Seo K, Hossmann KA. Ischemic thresholds of cerebral protein synthesis and energy state following middle cerebral artery occlusion in rat. *J Cereb Blood Flow Metab* 1991; **11**: 753–61.
5 Doutheil J, Althausen S, Gissel C, Paschen W. Activation of MYD116 (gadd34) expression following transient forebrain ischemia of rat: implications for a role of disturbances of endoplasmic reticulum calcium homeostasis. *Brain Res Mol Brain Res* 1999; **63**: 225–32.
6 Bourre JM. The role of nutritional factors on the structure and function of the brain: an update on dietary requirements. *Rev Neurol (Paris)* 2004; **160**: 767–92.
7 Choi YH, Chang N, Fletcher PJ, Anderson GH. Dietary protein content affects the profiles of extracellular amino acids in the medial preoptic area of freely moving rats. *Life Sci* 2000; **66**: 1105–18.
8 Fafournoux P, Bruhat A, Jousse C. Amino acid regulation of gene expression. *Biochem J* 2000; **351**: 1–12.
9 Chee PY, Dahl JL. Measurement of protein turnover in rat brain. *J Neurochem* 1978; **30**: 1485–93.
10 Brott T, Adams HP Jr, Olinger CP *et al*. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; **20**: 864–70.
11 al-Mudallal AS, Levin BE, Lust WD, Harik SI. Effects of unbalanced diets on cerebral glucose metabolism in the adult rat. *Neurology* 1995; **45**: 2261–65.
12 Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications after acute stroke. *Stroke* 1996; **27**: 415–20.
13 Layman DK. Role of leucine in protein metabolism during exercise and recovery. *Can J Appl Physiol* 2002; **27**: 646–63.
14 The FOOD trial collaboration. Routine oral nutritional supplementation for stroke patients on hospital (FOOD). A multicentre randomized controlled trial. *Lancet* 2005; **365**: 755–63.
15 Clarke DD, Sokoloff L. Circulation and energy metabolism of the brain. In Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD. eds. *Basic neurochemistry*. Lippincott-Raven, 1988, 637–69.
16 Nudo R, Plautz E, Milliken G. Adaptive plasticity in primate motor cortex as a consequence of behavioral experience and neuronal injury. *Semin Neurosci* 1997; **9**: 13–23.
17 Stroemer RP, Kent TA, Hulsebosch CE. Neocortical neural sprouting, synaptogenesis, and behavioral recovery after neocortical infarction in rats. *Stroke* 1995; **26**: 2135–44.
18 Dobkin BH. Strategies for stroke rehabilitation. *Lancet Neurol* 2004; **3**: 528–36.
19 Rossini PM, Calautti C, Pauri F, Baron JC. Post-stroke plastic reorganisation in the adult brain. *Lancet Neurol* 2003; **2**: 493–502.
20 Liepert J, Storch P, Fritsch A, Weiller C. Motor cortex disinhibition in acute stroke. *Clin Neurophysiol* 2000; **111**: 671–76.
21 Lindberg PG, Skejø PH, Rounis E *et al*. Wallerian degeneration of the corticofugal tracts in chronic stroke: a pilot study relating diffusion tensor imaging, transcranial magnetic stimulation, and hand function. *Neurorehabil Neural Repair* 2007; **21**: 551–60.
22 Finkbeiner S. Neurotrophins and the synapse. *Neuroscientist* 1996; **2**: 139–42.
23 Dobkin BH. Activity-dependent learning contributes to motor recovery. *Ann Neurol* 1998; **44**: 158–60.
24 Doyon J, Benali H. Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol* 2005; **15**: 161–67.
25 Fernstrom JD, Faller DV. Neutral amino acids in the brain: changes in response to food ingestion. *J Neurochem* 1978; **30**: 1531–38.
26 Dobkin BH. Clinical practice. Rehabilitation after stroke. *N Engl J Med* 2005; **352**: 1677–84.
27 Aquilani R, Verri M, Iadarola P *et al*. Plasma precursors of brain catecholaminergic and serotonergic neurotransmitters in rehabilitation patients

with ischemic stroke. *Arch Phys Med Rehabil* 2004; **85**: 779–84.
28 Nudo RJ, Plautz EJ, Frost SB. Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* 2001; **24**: 1000–19.
29 Sawaki L, Cohen LG, Classen J, Davis BC, Bütefisch CM. Enhancement of use-dependent plasticity by D-amphetamine. *Neurology* 2002; **59**: 1262–64.
30 Kaplan RJ, Greenwood CE, Wineocur G, Wolever TM. Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates. *Am J Clin Nutr* 2000; **72**: 825–36.