Does brain creatine content rely on exogenous creatine in healthy youth? A proof-of-principle study

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Running Head: Creatine and brain

ABSTRACT

It has been hypothesized that dietary creatine could influence cognitive performance by increasing brain creatine in developing individuals. This double-blind, randomized, placebo-controlled, proof-of-principle study aimed to investigate the effects of creatine supplementation on cognitive function and brain creatine content in healthy youth. The sample comprised 67 healthy participants aged 10 to 12 years. The participants were given creatine or placebo supplementation for 7 days. At baseline and after the intervention, participants undertook a battery of cognitive tests. In a random sub-sample of participants, brain creatine content was also assessed in the regions of left dorsolateral prefrontal cortex, left hippocampus, and occipital lobe, by proton magnetic resonance spectroscopy (1H-MRS) technique. The scores obtained from verbal learning and executive functions tests did not significantly differ between groups at baseline or after the intervention (all $p > 0.05$). Creatine content was not significantly different between groups in left dorsolateral prefrontal cortex, left hippocampus, and occipital lobe (all $p > 0.05$). In conclusion, a 7-day creatine supplementation protocol did not elicit improvements in brain creatine content or cognitive performance in healthy youth, suggesting that this population mainly rely in brain creatine synthesis rather than exogenous creatine intake to maintain brain creatine homeostasis.

Key words: cerebral metabolism; children; dietary supplement; neuropsychological assessment; nuclear medicine; phosphoryilcreatine.

Introduction

Creatine (α-methyl-guanidine-acetic acid) is an amine mainly found in fish, and red and white meat. It is also synthesized endogenously by a two-step mechanism involving L-arginine and S-adenosyl-L-methionine in the liver, kidneys, pancreas, and brain (Wyss and Wallimann 1994). Creatine plays an important role in rapid energy provision during muscle contraction involving the transfer of N-phosphoryl group from phosphorylcreatine to ADP to regenerate ATP through a reversible reaction catalyzed by creatine kinase (CK) (Wyss and Kaddurah-Daouk 2000). In addition to its function as a temporal energy buffer, phosphorylcreatine can also act as a spacial energy buffer, "shuttling" high-energy phosphates between mitochondria and cellular ATP utilization sites (Wyss and Wallimann 1994). Although approximately 95% of total creatine content is present in skeletal muscle, this amine can be also found in other "excitatory" tissues, such as the brain (Andres et al. 2008).

It is appropriate to assume that creatine plays a relevant physiological role in brain, which is mainly supported by *(i)* the presence of CK isoforms in central nervous system, *(ii)* the mental disturbances (e.g., intellectual disability, autism, speech delay) provoked by brain creatine depletion in creatine-deficient syndromes, and *(iii)* the partial reversal of these symptoms by creatine supplementation (Kaldis et al. 1996, Salomons et al. 2003, Stockler et al. 1994). In fact, it has been shown that oral creatine supplementation is able to penetrate the blood-brain barrier and improve brain energy metabolism in humans (Dechent et al. 1999). However, most of the studies showing increase in brain creatine content following creatine supplementation involve healthy adult subjects (Dechent et al. 1999, Lyoo et al. 2003, Pan and Takahashi 2007), whereas the investigations with pediatric populations are confined to children with creatine-deficient-syndromes (Stockler-Ipsiroglu and van Karnebeek 2014).

The putative physiological outcome resulting from increased brain creatine levels is improvement in cognitive function. In fact, some (Hammett et al. 2010, Ling et al. 2009, McMorris et al. 2007, Stockler et al. 1994, Turner et al. 2015), but not all (Alves et al. 2013a, Alves et al. 2013b, Rawson et al. 2008), studies have shown a beneficial effect of creatine supplementation on numerous cognitive aspects particularly in subjects under stressing conditions (e.g., sleep deprivation, exhausting exercise) and in patients with inborn diseases (e.g., X-linked creatine transporter defect) or psychiatric disorders (e.g., severe depression, post-traumatic disorders, fibromyalgia) that potentially lead to brain creatine depletion. There is also evidence that creatine supplementation can improve memory (as assessed by the word recall test) in vegetarian, but not in omnivorous, adults (Benton and Donohoe 2011). To our knowledge, studies assessing the ability of oral creatine intake to increase brain creatine content and, hence, to improve cognitive performance in healthy pediatric populations are currently lacking. Improving the understanding on the brain creatine metabolism in the physiological state by studying healthy individuals is of upmost importance to advance the knowledge on the potential applications of dietary creatine in pathological conditions (i.e., those characterized by brain creatine reduction).

Creatine supplementation has been thought to energetically “buffer” brain metabolic processes, ultimately preventing energy exhaustion and neuronal death (Andres et al. 2008). Furthermore, a high consumption of ATP and phosphoryilcreatine seems to occur during ordinary mental tasks (e.g., mathematical calculus), in which creatine supplementation was shown to improve cognitive performance, at least in adults (Watanabe et al. 2002). In theory, this nutrient could play a role in enhancing mental performance particularly in developing individuals, who are believed to show higher cerebral metabolism when compared with mature ones (Erecinska et al. 2004).

Indeed, there is evidence that increased brain energetic demand during developing (i.e., about age 8-12) parallels the peak of cortical grey matter in frontal and parietal lobe (Giedd et al. 1999), further supporting the notion that brain creatine availability could be particularly important to match the increased energy turnover required for in developing individuals (Chugani et al. 1987). Nonetheless, it is still unsure as to what extent brain creatine content is reliant on exogenous (i.e., dietary) creatine intake.

Therefore, this proof-of-principle study aimed to investigate the effects of creatine supplementation on cognitive function and brain creatine content in healthy youth.

Material and Methods

Experimental design and participants

This study was registered at clinicaltrials.gov as NCT01803230 and approved by the local Ethics Committee (Clinics Hospital, School of Medicine, University of Sao Paulo). All of the participants and their legal representatives were fully informed of the risks and discomforts associated with the study before giving their written informed consent. The procedures were in accordance with the Helsinki Declaration revised in 2008. This manuscript is reported according to the CONSORT statement (see supplement S1).

This was a 7-day, double-blind, randomized, placebo-controlled study. The sample comprised 67 healthy male and female participants aged 10 to 12 years. The exclusion criteria were as follows: *i*) diagnosed cognitive disorders (e.g., attention deficit hyperactivity disorder, depression, post-traumatic stress disorder); *ii*) post-

traumatic brain injury; *iii*) any diagnosed infectious or chronic diseases; *iv*) previous use of any dietary supplements; *v*) vegetarian diet; *vi)* ocular diseases that could compromise the visual acuity in the cognitive tests. At baseline, pubertal developmental stage was determined according to the methods described by Marshal and Tanner (Marshall and Tanner 1970), and food intake was assessed by three 24-h dietary recalls as previously reported (Avanutri® online, Rio de Janeiro).

The participants were randomized using a computer generated randomization code (Minitab v.15) in blocks of eight in a 1:1 ratio to compose either one of the following groups: 1) creatine supplementation or 2) placebo. At baseline and after 7 days of intervention, participants undertook a battery of cognitive tests. In a random sub-sample of participants, brain creatine content was also assessed in the regions of the left dorsolateral prefrontal cortex, left hippocampus, and occipital lobe, by proton magnetic resonance spectroscopy (1H-MRS) technique, using a 3-Tesla MRI scanner. Participants were advised to report any side effects throughout the study. Participants' main characteristics are shown in Table 1.

Supplementation protocol

Creatine group received creatine monohydrate (0.3g/Kg body weight; Creapure®, Alzchem, Germany) divided into 4 equal doses for 7 days. Placebo group was given the same dose of dextrose. Supplements were similar in appearance and content, but no in taste, which we believe was not a limitation since none of the participants had taken creatine supplements before. The participants were advised to consume their supplements preferably along with meals (e.g., breakfast, lunch, afternoon snack, and dinner).

The supplement packages were coded so that neither the investigators nor the participants were aware of the contents until completion of the analyses. A staff member of our research team, who had no participation in data acquisition, analyses, or interpretation, provided the supplements.

Cognitive assessments

The cognitive battery did not last longer than 20 minutes. The same experienced examiner applied the tests individually at the same time of the day (08:00 to 11:00 a.m.) in a private office. The cognitive battery comprised the following tests: Stroop Test, Rey Auditory Learning Test, Raven Progressive Matrices and Trail Making Test. This battery has been applied to individuals at similar age, and all the tests have shown high reliability (Strauss et al. 2006). Participants underwent one session of habituation 7 days before the baseline assessment. The cognitive tests are briefly describe below:

Stroop Test: The Stroop Test (Victoria version) has been considered a measure of selective attention, susceptibility to interference from conflicting stimuli, and inhibitory control (Strauss et al. 2006). It includes three conditions that consist in naming the color of dots (i.e., “color”), neutral words (i.e., “noncolor word”), and color words printed in incongruent colors (i.e., “color word”). Performance is assessed based on the time to complete each condition.

Rey Auditory Verbal Learning Test (RAVLT): The purpose of this test is to assess verbal learning and memory. A modified version of the Rey’s auditory verbal learning paradigm was used (Strauss et al. 2006). This version includes: *i)* 4 presentations of a 12 words list (list A), followed by free recall attempts (A1, A2, A3 and A4); *ii)* presentation of another 12 words list - list B - and free recall test of this list (B); *iii)* a

fifth recall, without further presentation, of list A (A5); *iv)* a delayed recall of list A, after 20 minutes (A6). The composed scores are: *(i)* learning (ΣSA1-SA4), *(ii)* short-term memory (free recall A5), and *(iii)* long-term memory (delay recall A6).

Raven Progressive Matrices (RPM): The purpose of RPM is to assess reasoning in the visual modality (Strauss et al. 2006). The version used in this study (Colored Progressive Matrices) consists of 36 problems divided into three sets (A, AB, B), each one containing 12 problems. The participant has to complete a matrix by choosing the missing part from six alternatives given at the bottom of the page, and then has to induce a relation on the completed part of the matrix and apply that relation to the incomplete part (Raven 1995). Problems become progressively more difficult, the easier items serving as learning experience for the later and more difficult items. In this version, the problems are printed on colored backgrounds in order to attract the subject's attention. The scale is arranged so that it is presented in the form of illustrations in a book (Strauss et al. 2006). Results are given as the summation of correct scores in the subsets.

Trail Making Test (TMT): The Trail Making Test has been used to assess executive functions and motor speed. It includes two conditions (i.e., "A" and "B"), where condition "A" reflects both motor and visual control and condition "B" condition reflects the additional executive control needed to switch between number and letter sequences (Arbuthnott and Frank 2000). Performance is assessed based on the time to complete each condition. However the test is discarded if the participant is unable to complete a given trial, or if ≥ 7 errors are made in either "A" or "B" condition.

Brain creatine content

In a sub-sample of participants, *in vivo* 1H-MRS of the left dorsolateral prefrontal cortex, left hippocampus and occipital lobe regions was acquired with a whole body 3.0T MRI scanner (Achieva Intera, Philips, Best, The Netherlands), using an eight-channel head coil at baseline and after 7 days of intervention. We chose to measure left dorsolateral prefrontal cortex and left hippocampus due to the involvement of these brain sites in relevant cognitive functions, such as reasoning, processing, learning, and memory (Henke et al. 1997, Kane and Engle 2002). Additionally, we assessed the occipital lobe, which is involved in visual acuity, because this brain region has been shown to be responsive to creatine supplementation regarding creatine accumulation (Pan and Takahashi 2007).

Brain creatine content was measured using a single-voxel (PRESS) technique (voxel size in dorsolateral prefrontal cortex and occipital lobe: 21 mm x 21 mm x 25 mm; voxel size in hippocampus: 43 mm x 9 mm x 15 mm) with echo time/repetition time (TE/TR) of 35/1500ms and NS= 64. Metabolite quantification was carried out using LCModel software (s-provencher.com/pages/lcmodel.shtml) (Provencher 1993) with the voxel water content used as internal reference. To account for differences in tissue composition, the voxel content was segmented into cerebrospinal fluid (CSF), grey (GM) and white matter (WM). Furthermore, differences in water relaxation and concentration for these different tissue compartments were taken into account in order to obtain the final total creatine content in mmol/L, as suggested previously (Zoelch 1977).

To determine the brain tissue composition contained in the MRS voxel of interest, three-dimensional volumetric images were obtained using the 3D-T1- FFE (fast

field echo) technique (FA=8°; TE/TR/TI=3.2/7/900 ms), with an isotropic voxel size of 1 mm^3. Briefly, the brain tissue was extracted using the brain extraction tool (BET), and segmentation into WM, GM, and CSF was achieved using the automated brain segmentation tool FAST (Zhang et al. 2001). Both tools are part of the FSL suite (http://www.fmrib.ox.ac.uk/fsl). Finally, the MRS voxel was overlaid on the segmented image using a Python-based script developed in-house and percentages of WM, GM and CSF were calculated for each voxel.

Based on a pilot study involving 5 subjects, the coefficient of variation (CV) in the left dorsolateral prefrontal cortex, left hippocampus and occipital lobe regions were 5.7, 7.8 and 4.9%, respectively.

Statistical analysis

Brain creatine content, total energy and macronutrients intake, and Stroop and TMT scores were analyzed by a mixed model analysis (group x time), using the SAS software (version 8.2; SAS Institute Inc., Cary, NC). RPM and RAVLT scores were compared between groups by Kruskal-Wallis *t*-tests, using the software SPSS Statistics 17 (Windows & Mac). Pearson's correlations were performed between dietary creatine intake and brain creatine accumulation after supplementation. Data are reported as mean ± (1SD), delta changes, and 95% confidence interval (95% CI), unless otherwise stated. Statistical significance was previously set at $p < 0.05$.

Results

The flow of participants is illustrated in Figure 1. Of the 140 participants screened for participation, 88 were selected and 21 withdrew due to personal reasons. Therefore, 67 participants completed the intervention. Among these participants, 26 were randomly selected to undertake 1H-MRS analysis, with 5 (2 from creatine and 3 from placebo) being further excluded as they missed pre-trial exam.

The scores in Rey Auditory Learning Test, Stroop Test, Trail Making Test, and Raven Progressive Matrices did not significantly differ between creatine and placebo at baseline or after the intervention (all $p > 0.05$). Pre and post data and delta changes for both groups are expressed in Table 2.

Similarly, creatine content (expressed in mmol/L) was not significantly different between groups in the regions of left dorsolateral prefrontal cortex ($p = 0.50$), left hippocampus ($p = 0.80$), and occipital lobe ($p = 0.70$) (Table 3). The areas of interest of 1H-MRS as well as individual responses to creatine and placebo supplementation in brain creatine content can be found in Figure 2.

There were no correlations between dietary creatine intake and creatine accumulation after creatine supplementation (data not shown). No self-reported side effects were recorded throughout the trial.

Discussion

To our knowledge, this was the first study to investigate the effects of creatine supplementation in cognitive performance and brain creatine content in healthy youth. The main finding of this trial was that a 7-day creatine supplementation protocol was

inefficient to induce improvements in cognition, possibly because of the lack of increase in brain creatine content. Overall, these findings suggest that brain creatine does not mainly rely on exogenous creatine intake in this young population.

Creatine has emerged as a potential nutrient capable of promoting beneficial effects in brain metabolism. Interestingly, there is evidence demonstrating that brain creatine depletion is associated with several neural disorders, such as encephalomyopathies and mitochondrial myopathies (in 't Zandt et al. 2004). Furthermore, creatine deficiency syndromes, which are characterized by dysfunctional creatine synthesis or creatine transport, can lead to intellectual disability, autism, speech delay, and brain atrophy (Item et al. 2001, Salomons et al. 2001, Stockler et al. 1994). Importantly, there is evidence showing the presence of CK in pyramidal cells (localized in numerous brain areas, including those investigated in this study), which are thought to be involved in memory and learning processes (Kaldis et al. 1996). This has led to the speculation that increased brain creatine content could improve cognitive performance, with some data (Hammett et al. 2010, Ling et al. 2009, McMorris et al. 2007), but not all (Alves et al. 2013a, Alves et al. 2013b, Rawson et al. 2008), favouring this possibility. Creatine supplementation resulted in improvements in some cognitive tasks in both vegetarian and omnivore participants, but only the former experienced improvements in memory (Benton and Donohoe 2011). Creatine supplementation was also able to improve cognitive performance in both young (Watanabe et al. 2002) and elderly participants (McMorris et al. 2007). Interestingly, Watanabe et al. used near infrared spectroscopy to show that creatine supplementation can enhance cerebral oxidation, which could partially explain the reduced mental fatigue after a mathematical calculus sequence observed in their study (Watanabe et al. 2002). Considering that brain metabolism appears to be enhanced during childhood (Erecinska et al. 2004), one could

speculate that creatine loading could increase brain creatine at early adolescence, thereby contributing to brain bioenergetics through a faster phosphoryilcreatine re-synthesis, ultimately leading to improvements in cognitive performance. However, in this proof-of-principle study, creatine supplementation failed to increase brain creatine content and, consequently, cognition performance.

The reasons why creatine supplementation did not induce increases in brain creatine content or cognitive function could be various. Firstly, the creatine protocol used in this study (i.e., 0.3 g/Kg/day or ~14 g/d for 7 days) was relatively equivalent in length and amount to others used in previous studies showing benefits of creatine on brain creatine accumulation and/or cognition (Lyoo et al. 2003, Pan and Takahashi 2007, Turner et al. 2015). However, the limiting number of studies involving children with brain creatine deficiency, condition that leads to brain creatine depletion, have generally used 0.4 to 0.8 g/Kg/day for months to year (Stockler-Ipsiroglu and van Karnebeek 2014). Therefore, one could argue that our protocol was not sufficient in length and/or amount to elicit increases in brain creatine content in children, which would be in contrast to previous observations in adult and elderly participants where similar creatine regimes were used (McMorris et al. 2007, Benton and Donohoe 2011, Turner et al. 2015). The actual influence of maturational status on brain creatine accumulation in response to equivalent doses of creatine supplementation (on a body weight basis) requires further investigation.

Differences in experimental protocols could also have contributed to the conflicting results. In fact, some studies (McMorris et al. 2007, Turner et al. 2015, Watanabe et al. 2002) have shown that creatine supplementation can promote improvements in cognitive function in participants who are under mentally stressing conditions, such as sleep deprivation, exhausting exercise or serial calculus, which

could potently affect brain creatine/phosphoryilcreatine homeostasis. Conversely, participants were tested at resting in the current study, which suggests that brain creatine loading may produce a functional effect mainly in conditions of brain biogenetic perturbation. This concurs with previous data from our group evidencing the inability of creatine in affecting cognitive function in older individuals (Alves et al. 2013a) and adult patients with fibromyalgia (Alves et al. 2013b), both tested in a non-mentally stressful situation. Collectively, these findings allow speculating that dietary creatine could be of some value in conditions in which inherent (e.g. creatine deficiency syndromes) or environmental (e.g. acute mental stressors) factors lead to a severe creatine depletion that cannot be compensated by endogenous creatine production.

In agreement with the previous argument, it has been speculated that brain seems to be less sensitive to creatine supplementation compared to skeletal muscle, which is supported by differences in the magnitude of creatine accumulation in response to creatine intake between these tissues (Harris et al. 1992, Pan and Takahashi 2007). One physiological explanation for this observation may rely in the fact that this tissue seems to be less dependent on exogenous creatine intake. In fact, the key enzymes involved in creatine biosynthesis (i.e., arginine:glycine amidinotransferase and S-adenosyl-Lmethionine:N-guanidinoacetate methyltransferase), are present in astrocytes, neurons and oligodendrocytes, indicating that the main cell types of the brain have the ability to synthesize their own creatine (Andres et al. 2008, Braissant et al. 2001). Conversely, brain permeability to circulating creatine is limited, probably due to the absence of creatine transporter expression in the astrocytes involved in the blood–brain barrier (Beard and Braissant 2010). Hence, it has been suggested that brain could be a "near-independent compartment for the synthesis and use of creatine" (Braissant et al. 2011). In support to this hypothesis, we recently demonstrated that omnivores and vegetarians

showed comparable brain creatine content, reinforcing the assumption that brain creatine relies primarily on local endogenous synthesis rather than on creatine dietary intake (Solis et al. 2014). Interestingly, in the present study, dietary creatine intake (which was found to be lower than that generally described in adult and older populations) did not correlate with the observed brain creatine content, further strengthening the hypothesis that dietary creatine is not as important as endogenous creatine synthesis in maintaining brain creatine content (Brosnan and Brosnan 2016). In light of the current results, one could infer that heathy adolescents may have baseline brain creatine close to optimal levels due to an efficient endogenous creatine synthesis. In such a condition, creatine supplementation appears to be unable to further increase brain creatine, supporting the notion that the higher the tissue creatine content, the lower the creatine uptake (Harris et al. 1992, Pan and Takahashi 2007).

This study has limitations. Firstly, even though we applied a multiple-voxel approach to track possible changes in creatine content in diversified brain areas that correlate with cognitive function, increases in brain creatine may have occurred in other regions not assessed in this study. This is unlikely, however, since the targeted areas in this study have been shown to respond to creatine supplementation in adult individuals (Pan and Takahashi 2007). Secondly, as previously discussed, the amount and length of our protocol may have been insufficient to enable increases in brain creatine and improvements in cognition in heathy youth. Time-course and dose-response studies are necessary with this population. Nonetheless, doubling creatine administration from 4 g/day to 8 g/day did not result in greater brain creatine accrual in a single infant with creatine deficiency syndrome followed by 25 months (Stockler et al. 1996). Moreover, these results must be confined to heathy children with no sign of cognitive impairment. Further studies involving pediatric populations suffering from cognitive disturbances

(e.g., attention deficit hyperactivity disorder, depression, post-traumatic disorder, post-traumatic brain injury) must be conducted.

In conclusion, a 7-day creatine supplementation protocol (0.3 g/Kg/day) was shown to be unable to elicit improvements in brain creatine content or cognitive performance in healthy youth. These data suggest that this population, consuming a regular omnivorous diet, mainly rely on brain creatine synthesis rather than exogenous creatine intake to maintain brain creatine homeostasis.

Conflict of interest statement

There are no conflicts of interest for all authors.

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Table 1. Participants' main characteristics.

Variable	***Creatine*** ***(n = 35)***	***Placebo*** ***(n = 32)***
Age (years)	11.5 ± 0.8	11.6 ± 0.9
Weight (Kg)	45.8 ± 14.8	43.1 ± 11.4
Height (m)	1.4 ± 0.2	1.5 ± 0.1
Schooling (years)	6.0 ± 1.0	6.0 ± 0.8
Gender (male/female)	19/16	19/13
Pubertal timing (pre-pubertal/puberty)	10/25	11/21
Food Intake		
Total energy (kcal)	1789.5 ± 419.0	1968.2 ± 453.5
Carbohydrates (g)	247.5 ± 64.8	267.3 ± 72.5
Proteins (g)	69.5 ± 17.2	79.3 ± 22.0
Fat (g)	56.0 ± 18.8	64.3 ± 18.4
Creatine (g)	0.5 ± 0.2	0.4 ± 0.1

Table 2. Cognitive performance before and after 7 days of either creatine or placebo supplementation.

Variable	*Creatine (n = 35)*			*Placebo (n = 32)*		
	Pre	**Post**	**Delta (95% CI)**	**Pre**	**Post**	**Delta (95% CI)**
Stroop test						
Color (s)	15.0 ± 3.9	14.6 ± 5.0	-0.4 (-1.9 to 1.1)	14.1 ± 3.3	12.1 ± 2.2	-1.9 (-2.9 to -0.8)
Non-color word (s)	18.9 ± 5.2	18.0 ± 5.4	-0.8 (-1.8 to 0.1)	17.9 ± 4.5	15.3 ± 2.8	-2.6 (-3.8 to -1.5)
Color word (s)	26.6 ± 7.4	24.1 ± 6.4	-2.5 (-4.1 to -0.9)	23.1 ± 5.4	19.4 ± 4.2	-3.6 (-4.8 to -2.5)
Rey Auditory Rey Auditory Learning Test						
Learning (0 – 48)	40.9 ± 6.7	44.4 ± 4.7	3.5 (2.3 to 4.7)	41.5 ± 5.0	45.0 ± 3.4	3.5 (1.9 to 5.0)
Short-term memory (0 – 12)	10.4 ± 1.9	11.2 ± 1.2	0.7 (0.2 to 1.2)	10.2 ± 2.2	11.3 ± 1.0	0.8 (0.1 to 1.4)
Long-term memory (0 - 12)	10.4 ± 1.9	11.1 ± 1.6	0.6 (0.1 to 1.1)	10.5 ± 2.0	11.2 ± 2.2	0.4 (-0.4 to 1.4)
Raven Progressive Matrices						
Total Score (0 – 36)	29.9 ± 4.7	30.4 ± 4.6	0.5 (-0.5 to 1.5)	30.4 ± 4.2	31.8 ± 3.8	1.3 (0.3 to 2.3)
Trail Making Test						
Part A (s)	12.7 ± 4.2	10.8 ± 3.3	-1.8 (-3.4 to -0.3)	10.9 ± 4.6	10.3 ± 5.8	-0.6 (-2.1 to -0.9)
Part B (s)	24.8 ± 14.5	19.3 ± 9.8	-5.5 (-8.0 to -2.0)	19.3 ± 11.5	14.7 ± 7.6	-4.5 (-7.2 to 1.7)

There were no significant difference within or between groups ($p > 0.05$).

Table 3. Creatine content (expressed in mmol/L) in left dorsolateral prefrontal cortex, left hippocampus, and occipital lobe before and after 7 days of either creatine or placebo supplementation.

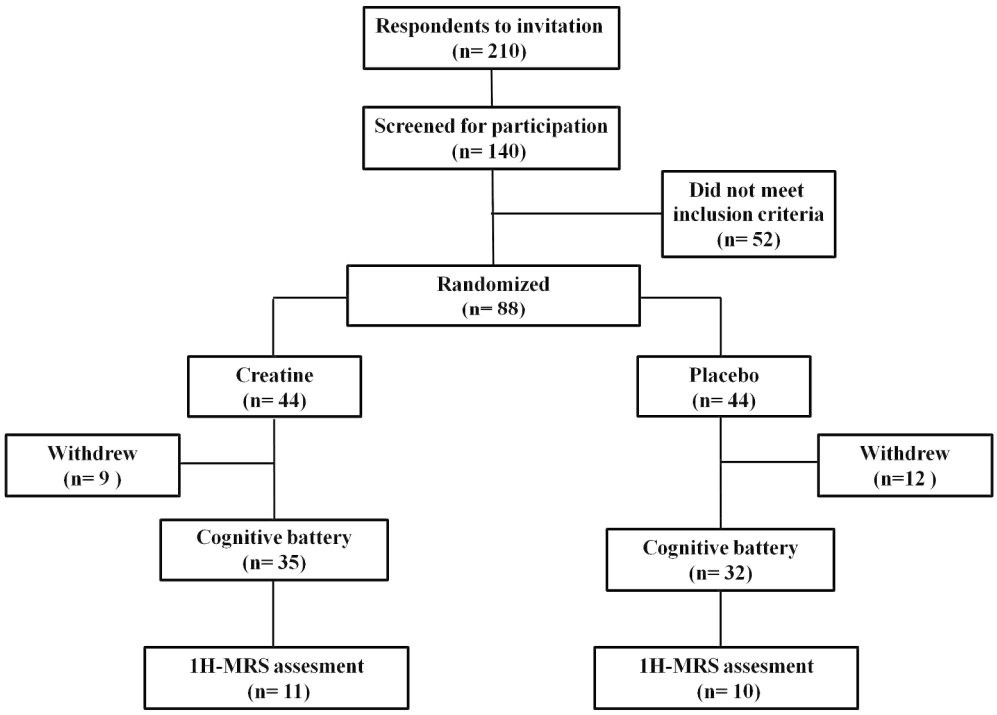
Brain region (mmoL/L)	***Creatine (n = 11)***			***Placebo (n = 10)***		
	Pre	**Post**	**Delta (95% CI)**	**Pre**	**Post**	**Delta (95% CI)**
left dorsolateral prefrontal cortex	6.9 ± 0.8	6.7 ± 0.5	-0.9 to 0.6	6.7 ± 0.8	6.9 ± 0.09	0.6 to 0.9
left hippocampus	7.0 ± 0.5	6.8 ± 0.7	-0.6 to 0.2	6.7 ± 1.0	6.6 ± 1.9	-1.3 to 1.1
occipital lobe	7.9 ± 0.9	8.1 ± 0.8	-0.4 to 0.8	7.7 ± 0.4	7.8 ± 0.5	0.5 to 0.6

There were no significant difference within or between groups ($p > 0.05$).

Figure legends

Figure 1. Flow of participants. Abbreviation: 1H-MRS: proton magnetic resonance spectroscopy.

Figure 2. Creatine content in (A) left dorsolateral prefrontal cortex, (B) left hippocampus, and (C) occipital lobe before and after 7 days of either creatine or placebo supplementation. Individual responses are presented in left panels. Right panels represent T1-weighted magnetic resonance images showing the volume of interest (VOI) selected for proton magnetic resonance spectroscopy at the targeted areas. Note: Missing data were due to poor image acquisition before or after the intervention in left dorsolateral prefrontal cortex (n = 2), left hippocampus (n = 5), and occipital lobe (n = 3).



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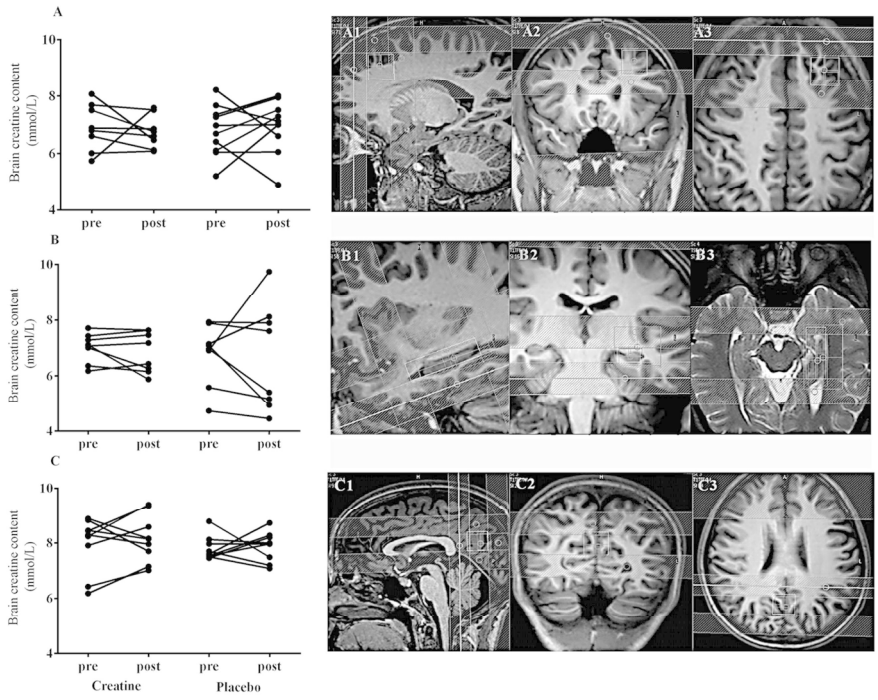

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