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Effect of age, diet and tissue type on PCr response to creatine supplementation

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ABSTRACT

Creatine/phosphorylcreatine (PCr) responses to creatine supplementation may be modulated by age, diet and tissue, but studies assessing this possibility are lacking. Therefore, we aimed to determine whether the PCr responses vary as a function of age, diet, and tissue. Fifteen children, 17 omnivorous and 14 vegetarian adults, and 18 elderly participated in this study. Participants were given placebo and subsequently creatine (0.3 $g \cdot kg^{-1} \cdot day^{-1}$) for 7 days in a single-blind fashion. PCr was measured through phosphorus magnetic resonance spectroscopy (^{31}P-MRS) in muscle and brain. Creatine supplementation increased muscle PCr in children ($p<0.0003$) and elderly ($p<0.001$), whereas the increase in omnivores did not reach statistical significant difference ($p=0.3348$). Elderly had greater PCr increases than children and omnivores ($p<0.0001$ for both), whereas children experienced greater PCr increases than omnivores ($p=0.0022$). In relation to diet, vegetarians ($p<0.0001$), but not omnivores, had significant increases in muscle PCr content. Brain PCr content was not affected by creatine supplementation in any group, and delta changes in brain PCr (-0.7 to +3.9%) were inferior than muscle PCr content (+10.3 to +27.6%; $p<0.0001$ for all comparisons). PCr responses to a standardized creatine protocol (0.3 $g \cdot kg^{-1} \cdot day^{-1}$ for 7 days) may be affected by age, diet and tissue. While creatine supplementation was able to increase muscle PCr in all groups, although to different extents, brain PCr was shown to be unresponsive overall. These findings demonstrate the need to tailor creatine protocols to optimise creatine/PCr accumulation both in muscle and in brain, enabling a better appreciation of the pleiotropic properties of creatine.

KEY-WORDS: phosphorylcreatine, skeletal muscle, brain, children, elderly, adults.

INTRODUCTION

Creatine supplementation has long been used in sport, although its use in clinical settings has increased in the last years (4, 15, 17). There is compelling evidence showing that creatine can improve high-intensive exercise capacity and lean body mass in recreationally trained individuals and athletes (13, 15, 45). Additionally, the therapeutic application of creatine has been a topic of increasing interest (13, 15, 50). In this context, there is evidence that creatine supplementation, particularly along with exercise training, partially offsets physical disabilities in elderly individuals as well as in pediatric and adult patients suffering from muscle weakness/wasting diseases (13, 14, 41).

Recently, growing attention has also been given to the potential therapeutic role of creatine in diseases characterized by brain bioenergetics dysfunction, such as neurodegenerative and psychiatric disorders (20, 23, 24). In such conditions, creatine is believed to optimize brain energy provision, ultimately rescuing brain energy homeostasis, thereby improving disease-related symptoms (6, 25, 35). It has been proposed that the central mechanism by which creatine exerts its physiological effects is through increased tissue creatine/phosphorylcreatine (PCr) content, thereby enhancing ATP re-synthesis via PCr degradation (51).

Harris et al., (17) were the first to show that creatine supplementation is able to increase total muscle creatine content (i.e., free creatine plus PCr) by approximately 20% in young healthy individuals. The most effective protocol used in this seminal study (~0.3 $g \cdot kg^{-1} \cdot day^{-1}$ for 5-7 days), commonly referred to as "creatine loading", has been frequently applied to other populations (e.g., diseased individuals, children, elderly) in an attempt to increase muscle creatine (9, 10, 32, 33, 37), or even brain creatine content (25, 31, 49). However, it is not fully understood whether age (children *vs.* adults *vs.* elderly) and tissue (brain *vs.* muscle)

71 are factors influencing the responsiveness to creatine supplementation. In fact, there is
72 evidence suggesting that elderly and adults may experience distinct increases in muscle
73 creatine/PCr content following supplementation (8, 30), although conflicting results exist (21,
74 33). There is no study examining the effects of creatine supplementation on muscle
75 creatine/PCr accretion in healthy children, hence no direct comparison between children,
76 adults and elderly has been possible so far, precluding any firm conclusion regarding the role
77 of age on creatine accumulation. In relation to tissue, greater doses of creatine seem to be
78 necessary to yield brain creatine/PCr accretion in comparison to those used for muscle
79 loading (46). However, studies directly assessing the differences between brain and muscle
80 creatine/PCr accumulation after a standardized creatine protocol are currently lacking.

81 Another unsolved question is whether (and, if so, to what extent) diet behavior can
82 affect creatine/PCr accumulation in response to creatine loading. From a muscle perspective,
83 data suggest that low meat eaters (who normally show reduced creatine/PCr content)
84 experience greater creatine/PCr accretion following supplementation (17). Nonetheless, the
85 influence of diet on brain creatine/PCr remains essentially unknown. One solitary study
86 showed similar brain creatine content between vegetarians and omnivores (38); however,
87 there is no study comparing brain creatine/PCr responses to creatine supplementation in these
88 populations.

89 Since most studies that have examined the ergogenic or therapeutic effects of creatine
90 supplementation did not measure tissue creatine/PCr to confirm the efficacy of
91 supplementation, it is possible that "negative" outcomes arising from these studies could be
92 due to the inability of the supplementation regime to increase tissue creatine/PCr content.
93 Therefore, we aimed to expand the knowledge on fundamental questions related to responses
94 to creatine supplementation by comprehensively investigating the role of age, tissue and diet

on PCr accumulation following a standardized creatine regimen (relative to individual body mass).

MATERIALS AND METHODS

Participants

Fifteen prepubescent children (10 to 12 years), 17 omnivorous and 14 vegetarian adults (18 to 45 years) and 18 elderly individuals (62 to 84 years) from both sexes took part in this investigation. The main characteristics of the participants are shown in Table 1.

General exclusion criteria were as follows: 1) cognitive and psychiatric disorders, skeletal muscle disturbances or any other disease that could potentially affect creatine metabolism; 2) vegetarian diet for children and elderly; 3) previous use of creatine supplementation; 4) current pregnancy; 5) pacemaker or metal prosthesis that preclude MRS assessment.

Vegetarians had been on a vegetarian diet for at least 1 year (10.2 ± 9.8 years); they were self-identified as lacto-ovo-vegetarians (n=9), ovo-vegetarians (n=1) or vegans (n=4). To ensure an accurate self-classification, all of the subjects were provided with a comprehensive explanation on the definitions regarding vegetarianism and its sub-classifications, according to previously reported criteria (48). No statistical differences between vegetarians and omnivores were shown for any main characteristics, except for dietary intake, especially creatine, as expected.

The study was approved by the local Ethical Committee (School of Medicine, University of São Paulo). All subjects (and their legal guardians, in case of children) signed the written informed consent. The experimental procedures were in accordance with the Helsinki Declaration revised in 2008.

Experimental Design

The participants were given placebo and subsequently creatine for 7 days in a single-blind fashion. PCr was measured through phosphorus magnetic resonance spectroscopy (^{31}P-MRS) in muscle and brain at baseline (i.e., no supplementation) and after both placebo and creatine arms. Baseline and placebo measures were used to calculate the coefficient of variation (CV) for PCr values. Figure 1 illustrates our research questions (i.e., influence of age, diet and tissue on creatine response) and the experimental design.

At baseline, the participants were assessed for BMI, maturational status according to Marshal and Tanner (26) (only children), and physical activity level using the short-version of the International Physical Activity Level Questionnaire (IPAQ) (22). Dietary intake was assessed by 3 non-consecutive 24-h dietary recalls at baseline and during each arm. Energy, carbohydrate, lipids and protein intake were analyzed using the software Avanutri (Rio de Janeiro, Brazil) and creatine intake was estimated based on specific food composition tables (16, 19). All participants were asked to maintain their dietary intake and physical activity levels throughout the experimental intervention.

Creatine supplementation protocol and blinding procedure

The participants received a dose of 0.3 $g \cdot kg^{-1} \cdot day^{-1}$ of placebo (dextrose) for 7 days and, subsequently, creatine monohydrate (Creapure®, AlzChem AG, Germany) for an additional 7 days. Placebo and creatine supplements were given separately in an envelope with 7 packages (one package per day) containing the exact daily amount of the supplement. Participants were instructed to ingest the supplement at breakfast, lunch, dinner and before bedtime. Creatine and placebo supplements were formulated in indistinguishable tablets with identical appearance, taste, and smell. The packages were coded so that the participants were not aware of the contents until completion of the analyses.

Muscle and Brain PCr content

Muscle PCr content was assessed *in vivo* by ^{31}P-MRS using a whole body 3.0T MRI scanner (Achieva Intera, Philips, Best, The Netherlands) and a 14 cm diameter ^{31}P surface coil. In brief, the surface coil was centered on the calf muscle of the left leg. The scanner body coil was used to obtain conventional anatomical T1-weighted magnetic resonance images in 3 orthogonal planes. ^{31}P-MRS was acquired using the image selected *in vivo* spectroscopy (ISIS) sequence with an echo time and repetition time of 0.62 ms and 4500 ms. Spectrum bandwidth was 3000 Hz with 2048 data points and 64 repetitions. Before the intervention, muscle ^{31}P-MRS scans were performed twice on the same day. After the completion of the first test, the patients were asked to leave the machine and, then, to return to it for the re-test. The coefficient of variation (CV) was obtained for children (n = 4), adults (n = 4) and elderly (n = 4) were 14.25%, 6.83%, and 8.63%, respectively.

Brain PCr examination was accomplished using a dual-tune ^{31}P/^{1}H birdcage head coil (AIRI, Cleveland, OH, U.S.A.). A T1-FFE axial sequence was acquired (TR = 7.6 ms; TE = 3.7 ms; flip angle = 8°; isotropic 1-mm^3 resolution) with reconstructions of the sagittal and coronal planes. These images were used for the placement of the ^{31}P-MRS voxel centered in the centrum semiovale. ^{31}P-MRS was acquired using the ISIS sequence with an echo time and repetition time of 0.096 ms and 6000 ms, respectively. Spectrum bandwidth was 6000 Hz with 1024 data points and 128 repetitions. Voxel size varied from 95-120 mm in AP, 70-90 mm in LR, and 40-48 mm in CC direction, as shown in Figure 2. Before the intervention, brain ^{31}P-MRS scans were performed twice as described above for muscle, and the coefficient of variation (CV) obtained for children (n = 4), adults (n = 4) and elderly (n = 4) were 9.99%, 5.93%, and 3.80%, respectively.

Raw spectrum data were analyzed with Java Magnetic Resonance User Interface (jMRUI) software, and processing steps included apodization to 5Hz, Fourier transform, and phase correction. The advanced method for accurate, robust, and efficient spectral fitting (AMARES) algorithm was used to fit the time-domain data (44). Prior knowledge was established to keep some physical parameters constant or constrained; these parameters included line-width constraints, chemical shifts, and J-coupling. Zero- and first-order phase corrections were applied for convergence during the spectral fitting. In addition, for the brain spectra it was necessary to truncate the first two FID data points, which were discarded to reduce the baseline distortion effects produced by the broad-line components (macromolecules). The quality of the fitting was verified by the absence of any residual signals. The PCr signal was quantified relative to the γ-ATP signal and expressed as PCr/γ-ATP ratio both in brain and in muscle, in order to allow for comparison between tissues.

Due to technical issues, brain MRS exams were lost for 1 child, 1 omnivore, 2 vegetarians, and 1 elderly participant, whereas muscle MRS exams were lost for 4 omnivores, 4 vegetarians, and 5 elderly participants.

Statistical analysis

Data were tested by two mixed-models with repeated measures using the software SAS version 9.1. To test the effect of "age", a 3-factor model was performed, with "age" (children, omnivorous adults, and elderly), "supplement" (creatine and placebo) and "tissue" (brain and muscle) as fixed factors. To test the effect of "diet", a 3-factor model was conducted, with "diet" (omnivorous and vegetarian adults), "supplement" (creatine and placebo) and "tissue" (brain and muscle) as fixed factors. In both models, "participants" were defined as random factors with the Tukey-Kramer adjustment being used for multicomparison analyses. We also performed a delta analysis (i.e., PCr values in "creatine"

194 condition subtracted by PCr values in "placebo" condition), having pre-supplementation PCr
195 levels (i.e., following the placebo arm) as a co-variable. A post-hoc analysis using
196 independent samples Student t-test was performed to compare the delta muscle PCr values
197 between males and females within each group, in order to explore the potential influence of
198 sex on creatine loading. Data are expressed as mean ± SD, delta scores, and effect size (ES),
199 unless otherwise stated. The significance level was set at $p<0.05$.
200
201 RESULTS
202 Adult vegetarians showed the lowest creatine intake ($p<0.0001$ *vs.* all others). Adult
203 omnivores had higher creatine intake than children ($p=0.0004$) and elderly ($p<0.0001$), with
204 the latter two groups showing similar creatine consumption ($p=0.8118$). After body mass
205 adjustment, creatine intake remained lower in adult vegetarians in comparison to all other
206 groups ($p<0.0001$); adult omnivores and children had similar creatine intake ($p=0.7393$), but
207 both groups had higher creatine intake than elderly ($p<0.0001$ for both comparisons).
208 In all groups, muscle and brain PCr contents were similar between baseline and after
209 placebo supplementation (i.e., both conditions with no creatine supplementation; $p>0.05$),
210 meaning that PCr content was stable and the measures were repeatable. Therefore, to test the
211 effects of creatine supplementation, all further comparisons will be made between placebo
212 (i.e., before supplementation) vs. creatine (i.e., after supplementation) conditions.
213 Figure 3 depicts the influence of age on tissue PCr content in relation to creatine
214 supplementation. Before creatine supplementation, children had lower muscle PCr content as
215 compared to omnivorous adults and elderly ($p<0.0001$ for both), whereas omnivorous adults
216 and elderly showed similar muscle PCr ($p=0.9535$). Creatine supplementation increased
217 muscle PCr in children ($p<0.0003$) and elderly ($p<0.001$), whereas the increase in
218 omnivorous adults did not reach statistical significance (+10.3%, ES: +1.2; $p=0.3348$).

Muscle PCr responses to supplementation were better in elderly than in children and omnivorous adults ($p<0.0001$ for both), whereas children experienced greater muscle PCr increases than omnivorous adults ($p=0.0022$). Muscle PCr content was superior to brain PCr content, regardless of "age" or "supplement" ($p<0.0001$ for all comparisons). Furthermore, creatine supplementation did not elicit any change in brain PCr in all groups (placebo *vs.* creatine in children: $p=0.8756$; in adult omnivores: $p=0.9595$; in elderly: $p=0.7116$).

Figure 4 illustrates the influence of diet on tissue PCr content in relation to creatine supplementation. Omnivores and vegetarians showed similar muscle PCr content before creatine supplementation ($p=0.2448$). Following creatine supplementation, vegetarians ($p<0.0001$), but not omnivores, had significant increases in muscle PCr content; post-supplementation PCr content was significantly higher in vegetarians than omnivores ($p<0.0001$). Muscle PCr content was higher than brain PCr content, irrespective of "diet" and "supplement" ($p<0.0001$ for all comparisons). Brain PCr content was not affected by placebo or creatine supplementation in any group ($p>0.05$ for all), nor was it significantly different between omnivores and vegetarians before ($p=0.7588$) or after supplementation ($p=0.6826$).

Delta analysis (Figure 5) showed that creatine supplementation promoted greater increases in muscle PCr in vegetarians and elderly when compared with children ($p=0.0204$ and $p=0.0191$, respectively) and omnivores ($p=0.0221$ and $p=0.0212$, respectively). Muscle PCr accretion was comparable between vegetarians and elderly ($p=0.8523$), and children and omnivores ($p=0.9725$). Changes in brain PCr content (-0.7 to +3.9%) were lower than those observed in muscle PCr content (+10.3 to +27.6%; $p<0.0001$ for all groups), and did not differ between children, omnivores, vegetarians and elderly ($p>0.05$ for all comparisons). Co-variation analysis indicated that pre-supplementation PCr content had no influence upon the results ($p>0.05$). In addition, a post-hoc analysis showed no influence of sex on the changes in PCr accumulation in skeletal muscle following supplementation (boys: 0.62 ± 0.42 vs.

girls: 0.32 ± 0.41 PCr/ ATP, p = 0.24; omnivorous men: 0.07 ± 0.75 vs. omnivorous women: 0.45 ± 0.56 PCr/ ATP, p = 0.36; vegetarian men: 1.44 ± 0.48 vs. women: -0.21 ± 2.24 PCr/ ATP, p = 01829; elderly men: 0.60 ± 0.58 vs. women: 0.48 ± 2.14 PCr/ ATP, p = 0.8918).

DISCUSSION

In this study, we examined whether a standardized creatine supplementation protocol (i.e., 0.3 $g \cdot kg^{-1} \cdot day^{-1}$ for 7 days) leads to differential changes in PCr accumulation according to age, tissue and diet. The main findings were as follows: *i)* age did influence muscle PCr accretion, since elderly showed greater PCr increases as compared to children and omnivorous adults; *ii)* diet did influence muscle PCr accretion, since vegetarians had greater PCr increases than omnivores; *iii)* creatine accretion following supplementation did depend on tissue, since PCr accumulation was greater in muscle than in brain; *iv)* brain PCr remained unchanged after creatine supplementation in all groups, suggesting that the classic protocol designed for muscle loading is not effective to significantly increase PCr in brain; *v)* brain PCr content was consistently and markedly lower than muscle PCr content, regardless of age or diet. Collectively, these data indicate that using a single "universal" protocol, originally designed for increasing muscle creatine/PCr content in young individuals, may lead to heterogeneous responses in different populations, since PCr responses were shown to be age-, tissue- and diet-specific.

Muscle creatine/PCr content may fluctuate as a function of lifespan. There are studies showing that muscle creatine/PCr is decreased in older individuals when compared with their younger counterparts (8, 27, 28, 30, 36), despite some evidence suggesting the opposite (21, 33). This possible age-related reduction in muscle creatine/PCr content has been associated with differences in *i)* the distribution of type II fibres, which has slightly greater PCr content than type I fibres (42); and *ii)* the consumption of creatine-based food, which is generally

lower in older than younger individuals (34). To our knowledge, this is the first study examining PCr responses to creatine supplementation in children, adults and elderly individuals simultaneously. Our data showed that all these groups are capable of responding to supplementation with increases in muscle PCr content (main effect of "supplement"), although the ~10% increase in omnivorous adults did not reach statistical significance, corroborating previous findings (4, 34). Importantly, elderly individuals reached greater muscle PCr content than omnivorous adults and children. Likewise, changes in muscle PCr content (Figure 4) were greater in elderly individuals (% change = +22.7; ES = +2.1) than children (% change = +13.9; ES = +1.3) and omnivores (% change = 10.3%; ES = +1.2). These age-related differences could be partially explained by the fact that elderly consumed half of the dietary creatine (on a weight basis) than their younger peers, even though pre-supplementation muscle PCr content was comparable between groups (the influence of diet on PCr content is further discussed in the next paragraph). These data shed light on the unique potential of creatine supplementation in augmenting muscle creatine/PCr content in older populations, possibly leading to gains in physical capacity and lean mass, which may be of great therapeutic relevance (13, 14).

Further to the influence of age on creatine responses, we also showed that diet is a major factor affecting muscle PCr accumulation following supplementation. As expected, muscle PCr increases were dramatically superior in vegetarians than in omnivores. Since pre-supplementation PCr content did not statically differ as a function of diet, which is in agreement with previous data (3), it is possible to speculate that dietary creatine, rather than baseline PCr content, played an important role in contributing to PCr accumulation in response to supplementation. These data support the long-standing notion that chronic low-creatine consumers (e.g., vegetarians) may experience greater muscle responses from creatine supplementation when compared with habitual meat eaters (4, 5, 17). However, it remains

unclear *i)* whether short- or mid-term dietary creatine withdrawal could produce better responses to supplementation; *ii)* whether dietary creatine is also a factor influencing creatine/PCr accumulation in children or older individuals; and *iii)* the molecular mechanisms by which low dietary creatine leads to increased muscle creatine/PCr accretion (e.g., regulation of creatine transporter [CreaT] through diet) (47).

Perhaps the most striking findings from this study was the clear inability of creatine supplementation to increase brain PCr, as opposed to muscle PCr. Northern blot and immunohistochemical experiments revealed the presence of the creatine transporter at the blood-brain-barrier, suggesting that the creatine transporter plays a pivotal role in supplying creatine to the brain (18, 35). In fact, orally ingested creatine (4-8 $g \cdot day^{-1}$ over 2 years) was able to increase brain creatine content in creatine-deficient patients, promoting important therapeutic effects in this condition (39, 40). However, creatine supplementation seems to be much less effective in increasing brain creatine content in healthy individuals. For instance, oral consumption of creatine (20 $g \cdot day^{-1}$ for 4 weeks) yielded an 8.7% increase in total brain creatine, with a considerable intersubject variability (3.5 to 13.3%) (6). More recently, we showed comparable creatine content of the posterior cingulate cortex in vegetarians and omnivores (52), suggesting that brain may primarily rely on its own creatine synthesis rather than creatine uptake. This hypothesis is corroborated by *in situ* hybridization experiments showing brain mRNA expression of the enzymes involved in creatine synthesis (1). The findings herein presented also cast doubt on the ability of creatine supplementation to effectively increase brain creatine/PCr content in healthy individuals, regardless of their age or dietary patterns. At least, it is safe to conclude that the supplementation protocol employed in this study, which is able to promote muscle creatine/PCr loading, failed to produce any increase in brain PCr, indicating that higher-dose and/or longer-duration protocols must be developed to optimize brain creatine/PCr accumulation.

This seems to be a critical step in determining the actual role of increased brain creatine/PCr via supplementation on cognitive function. Importantly, there is evidence that brain creatine/PCr accumulation following supplementation may vary substantially according to the brain region assessed (i.e., from 4.7% in gray matter to 14.6% in the thalamus) (6). Thus, one may argue that studies should employ multi-voxel technologies in an attempt to identify the most (and the least) responsive brain regions to creatine supplementation, since this could better predict the brain functions potentially affected by this supplement. In this respect, however, we recently demonstrated that creatine supplementation at the same dosage employed in the current study failed to increase creatine concentration of the left dorsolateral prefrontal cortex, left hippocampus, and occipital lobe in healthy children (29), suggesting an overall inability of this supplementation protocol to increase brain creatine, at least in this population. In contrast, creatine supplementation (also the same protocol used in this study) resulted in a 9% average increase in total creatine in the hand knob of the left precentral gyrus (sensorimotor cortex) in healthy adults (43).

In fact, the limited number of studies assessing brain creatine/PCr content following creatine supplementation in healthy individuals have shown only minor changes, if any, in these substrates (generally inferior to 10%) (6, 25, 29, 31, 43, 49). Given that these studies have small sample sizes and considerable experimental heterogeneity (e.g., diversified creatine protocols, studied populations, methods to detect creatine/PCr, brain areas of interest), it remains uncertain to what extent creatine supplementation increases brain creatine/PCr content and, more importantly, how this relates to brain functionality. In future, it is also relevant to identify the characteristics of responders and non-responders to creatine supplementation with special reference to brain. As our data provide compelling evidence that diet, which is the major factor influencing creatine/PCr accretion in skeletal muscle following supplementation (as confirmed in the current study), does not affect brain PCr

accumulation (at least in the area assessed), one may suggest that factors underlying creatine responsiveness may be tissue-specific.

This study is not without its limitations. Firstly, we tested only a single creatine protocol, hampering any dose-response analyses; further studies should test different protocols in length and dose to determine the optimal supplementation regime particularly for brain creatine/PCr loading. Secondly, we measured PCr via ^{31}P-MRS instead of total creatine via ^{1}H-MRS technique, since the former showed better reliability in our pilot experiments. While we were not able measure total creatine, PCr itself has been consistently shown to be highly sensitive in response to creatine supplementation (both in muscle (17) and in brain (25, 31, 49)), although it remains controversial whether PCr and free creatine increase to the same extent (2, 7, 11, 12). Thirdly, due to the relatively low number of males and females participants enrolled in this study, it remains to be confirmed whether PCr accumulation is not sex-dependent, as suggested by our sub-analysis. Finally, as elderly individuals exhibited lower dietary creatine intake (on a weight basis) than children and adults, it is impossible to completely separate the effect of diet and age on PCr responses in this study.

In conclusion, this comprehensive study showed that PCr responses to a standardized creatine protocol (i.e., 0.3 $g \cdot kg^{-1} \cdot day^{-1}$ for 7 days) is affected by age, diet and tissue. While creatine supplementation was able to increase muscle PCr in all groups, although to different degrees (i.e., older > younger; vegetarians > omnivores), brain PCr was shown to be unresponsive. These findings demonstrate the need to tailor specific creatine protocols capable of optimising creatine/PCr accumulation both in muscle and in brain, enabling a better appreciation of the pleiotropic properties of creatine, as well as the rational use of creatine supplements in sports and clinical settings.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR'S CONTRIBUTIONS

Author contributions: M.Y.S., G.G.A., and B.G. conception and design of research; M.Y.S., W.A., and R.R.V. performed experiments; M.Y.S., M.G.C.O., and B.G. analyzed data; M.Y.S., M.G.C.O., C.C.L., G.G.A., and B.G. interpreted results of experiments; M.Y.S., and G.G.A. prepared figures; M.Y.S., G.G.A., and B.G. drafted manuscript; W.A., R.R.V., M.G.C.O., and C.C.L edited and revised manuscript; M.Y.S., W.A., R.R.V., M.G.C.O., C.C.L., G.G.A., and B.G. approved final version of manuscript.

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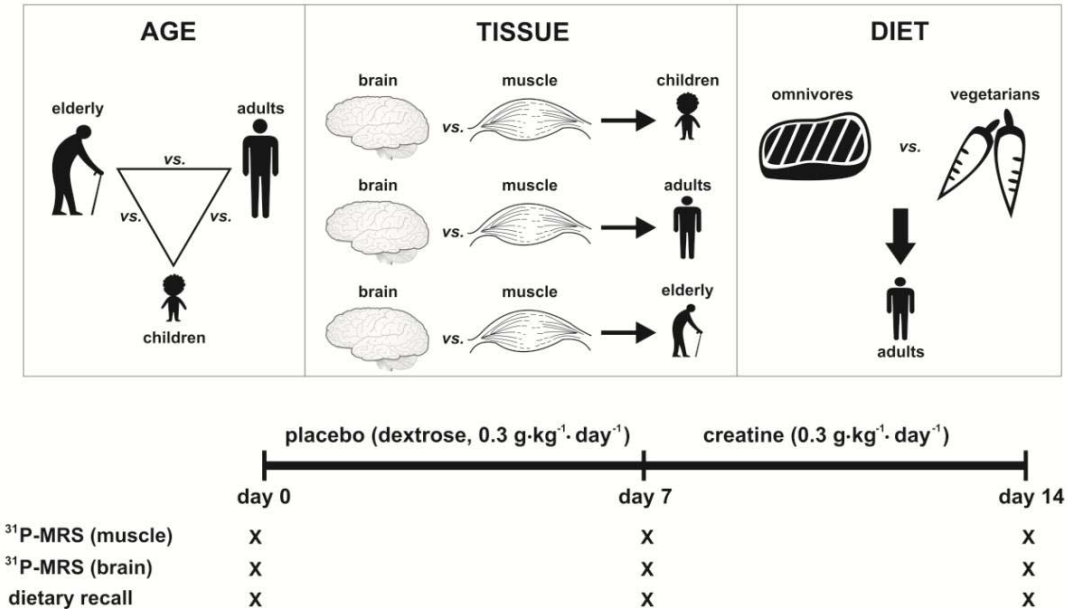
Figure 1. Illustration of the research questions and the studied factors potentially affecting PCr responses to creatine supplementation (top) and the experimental design (bottom).



Abbreviation: ^{31}P-MRS = phosphorus magnetic resonance spectroscopy. Day 0 represents baseline, day 7 and day 14 represent the end of the placebo and creatine arms, respectively.

Figure 2. Magnetic resonance images showing the volume of interest (VOI) selected for phosphorous spectroscopy at the brain and muscle. For brain, a T1-FFE axial sequence was acquired (TR = 7.6 ms; TE = 3.7 ms; flip angle = 8°; isotropic 1-mm^3 resolution) with reconstructions of the sagittal and coronal planes. These images were used for the placement of the ^{31}P-MRS voxel centered in the centrum semiovale. Voxel size varied from 95-120 mm in AP, 70-90 mm in LR, and 40-48 mm in CC direction. For muscle, the surface coil was centered on the calf muscle of the left leg. The scanner body coil was used to obtain conventional anatomical T1-weighted magnetic resonance images in 3 orthogonal planes.

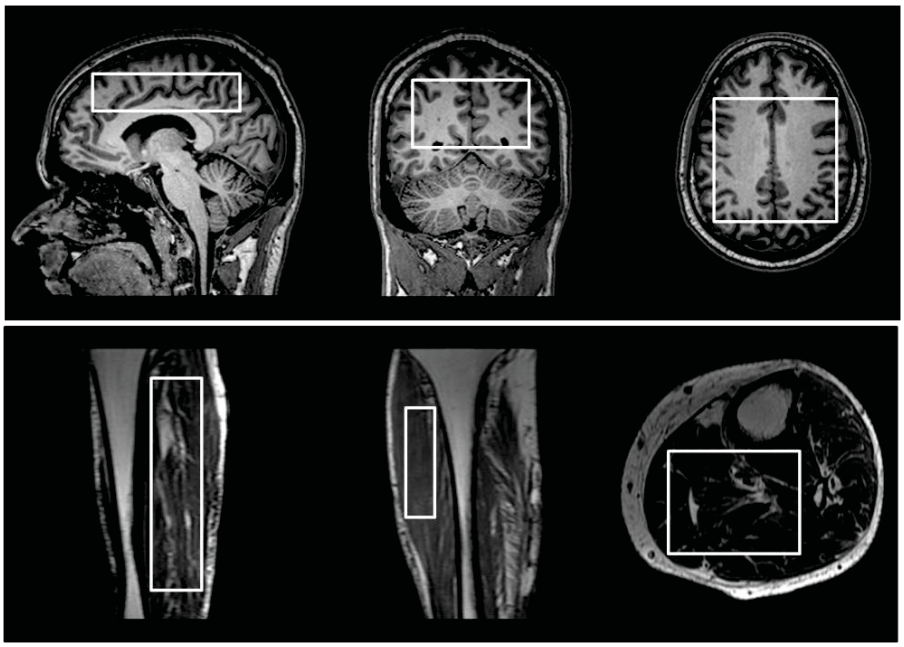
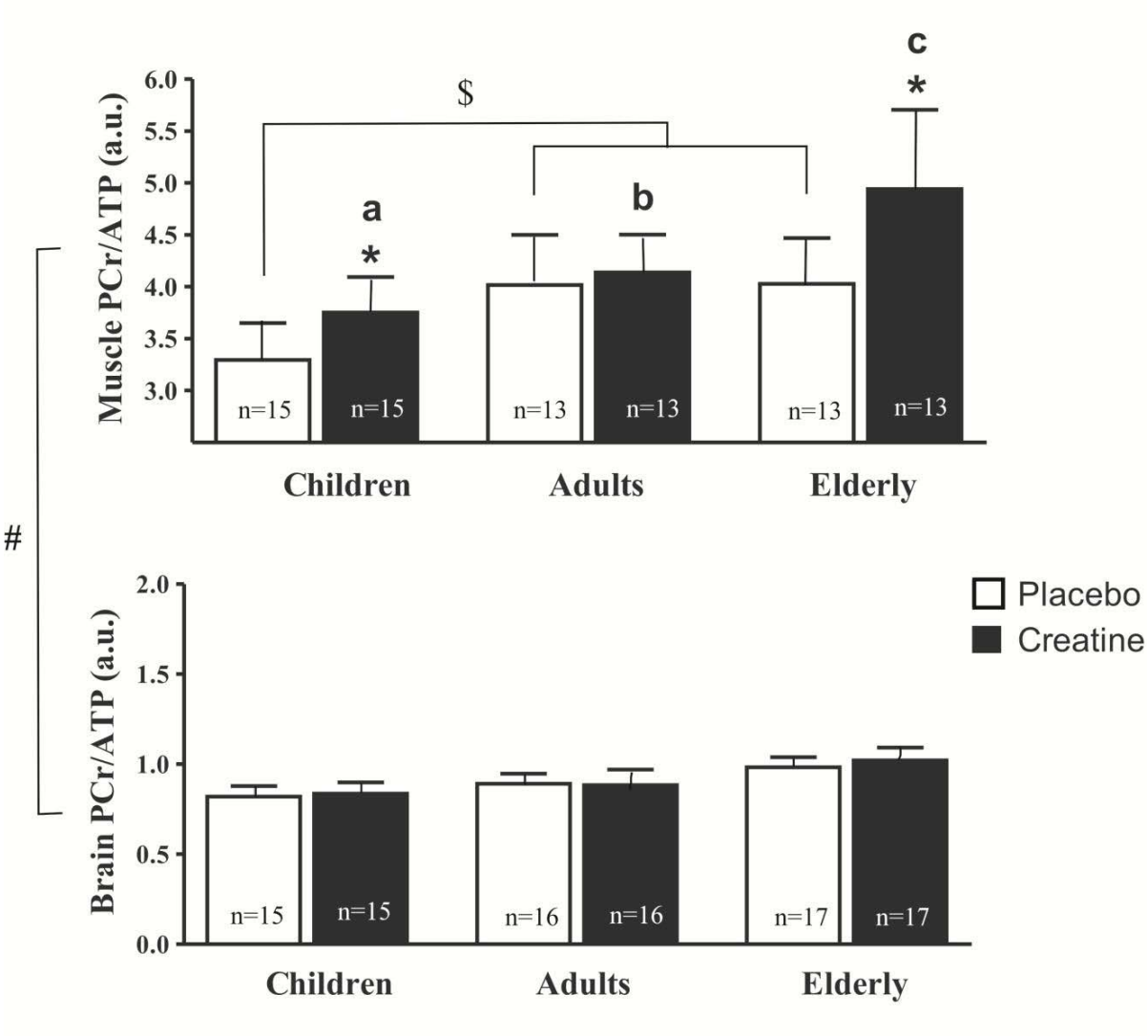


Figure 3. Influence of age on tissue PCr content before and after creatine supplementation.



There were significant main effects of "age" ($p<0.0001$), "supplement" ($p<0.0001$), and "tissue" ($p<0.0001$), and a significant interaction effect of "age" x "supplement" x "tissue" ($p=0.0103$).

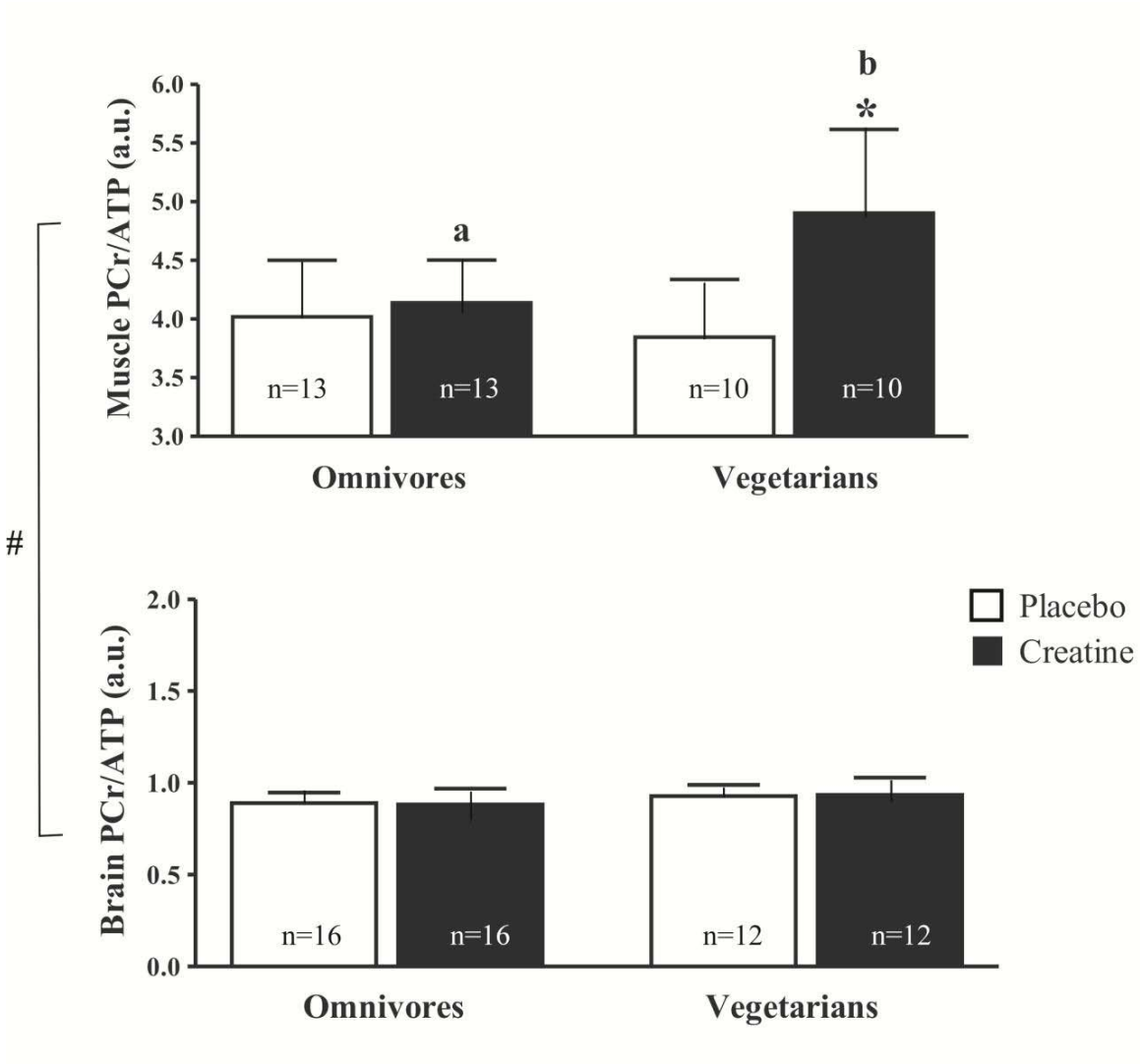
a, b and c are statistically different from one another

*denotes a significant within-group effect (i.e., different from placebo)

$denotes a significant between-group effect (i.e., groups are different before creatine supplementation)

#denotes a significant difference between tissues

Figure 4. Influence of diet on tissue PCr content in relation to creatine supplementation.



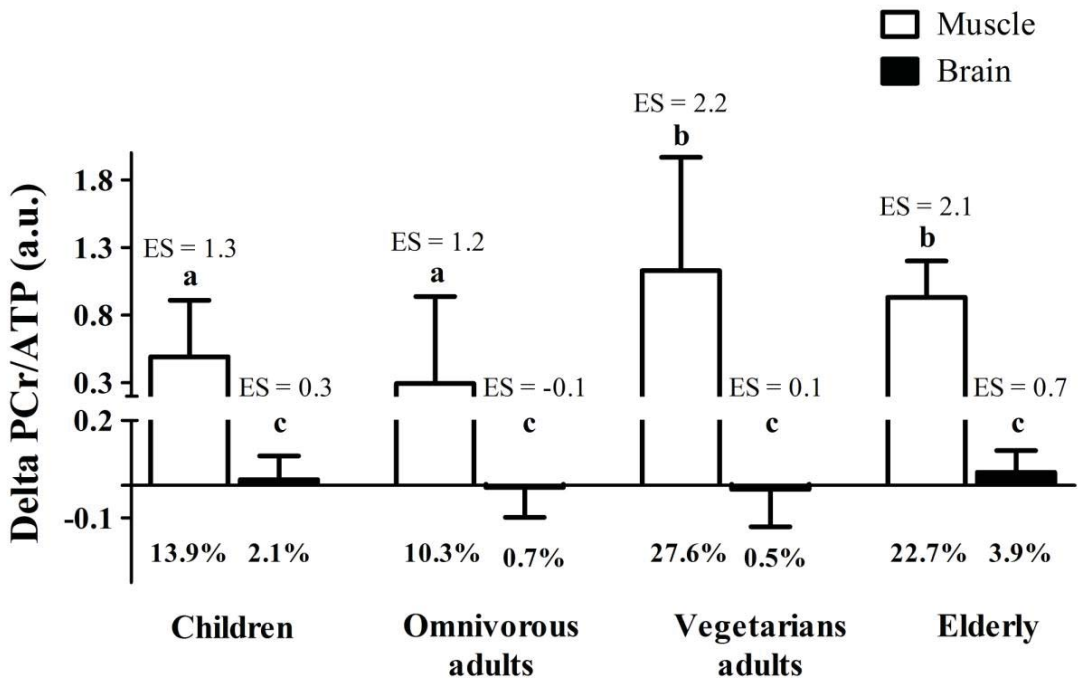
There were significant main effects of "diet" ($p<0.0179$), "supplement" ($p=0.0003$), and "tissue" ($p<0.0001$), and a significant interaction effect of "diet" x "supplement" x "tissue" ($p=0.0037$).

a, b are statistically different from each other

*denotes a significant within-group effect (i.e., different from placebo)

#denotes a significant difference between tissues

Figure 5. Delta changes in PCr in response to creatine supplementation (i.e., creatine subtracted from placebo values) in muscle and brain.



a, b, c are statistically different from each other

Abbreviation: ES = effect size.

Table 1. Participants' demographic characteristics.

Variable	***Children*** *(n=15)*	***Adults***		***Elderly*** *(n=18)*
		Omnivores *(n=17)*	***Vegetarians*** *(n=14)*	
Sex (M / F)	9 / 6	11 / 6	8 / 6	9 / 9
Age (y)	11.20 ± 0.94^{a}	29.18 ± 7.81^{b}	30.21 ± 6.645^{b}	71.78 ± 6.97^{c}
BMI (Kg/h²)	17.73 ± 3.06^{a}	25.27 ± 2.83^{b}	23.40 ± 3.21^{b}	26.19 ± 2.92^{c}
IPAQ (Low / Moderate / High)	4 / 1 / 10	6 / 5 / 6	6 / 3 / 5	13 / 4 / 1
Total energy (Kcal)	2224.30 ± 488.57^{a}	2354.15 ± 762.30^{a}	1670.85 ± 598.14^{b}	1834.00 ± 107.44^{b}
Carbohydrates (g)	321.10 ± 103.73^{a}	290.17 ± 91.97^{a}	238.25 ± 95.05^{b}	235.36 ± 107.87^{b}
Lipids (g)	67.65 ± 21.34^{a}	84.59 ± 49.64^{b}	49.27 ± 27.12^{a}	58.21 ± 30.16^{a}
Protein (g)	80.32 ± 28.02^{a}	102.96 ± 53.06^{a}	64.14 ± 36.20^{b}	84.83 ± 3456^{a}
Protein (g/Kg)	1.60 ± 0.91^{a}	$1.40 \pm 0.73^{a,c}$	0.92 ± 0.48^{b}	$1.24 \pm 0.44^{b,c}$
Dietary creatine (g)	1.03 ± 0.39^{a}	1.73 ± 1.07^{b}	0.01 ± 0.0^{c}	0.92 ± 0.44^{a}
Dietary creatine (g/Kg)	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.00 ± 0.00^{b}	0.01 ± 0.01^{c}

Data are mean ± SD. Abbreviations: M=male; F=female; y=years; BMI=body mass index; IPAQ=International Physical Activity Questionnaire (short-version); g=grams. Different letters mean statistically significant difference between groups (i.e., children, omnivore and vegetarian adults, and elderly).