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Oral creatine monohydrate supplementation improves brain performance: a double-blind, placebo-controlled, cross-over trial

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Creatine supplementation is in widespread use to enhance sports–fitness performance, and has been trialled successfully in the treatment of neurological, neuromuscular and atherosclerotic disease. Creatine plays a pivotal role in brain energy homeostasis, being a temporal and spatial buffer for cytosolic and mitochondrial pools of the cellular energy currency, adenosine triphosphate and its regulator, adenosine diphosphate. In this work, we tested the hypothesis that oral creatine supplementation (5 g d^{-1} for six weeks) would enhance intelligence test scores and working memory performance in 45 young adult, vegetarian subjects in a double-blind, placebo-controlled, cross-over design. Creatine supplementation had a significant positive effect ($p < 0.0001$) on both working memory (backward digit span) and intelligence (Raven's Advanced Progressive Matrices), both tasks that require speed of processing. These findings underline a dynamic and significant role of brain energy capacity in influencing brain performance.

Keywords: creatine; oral supplementation; intelligence; memory; brain bioenergetics

1. INTRODUCTION

The induction of work in the brain induces a cascade of biochemical and physiological sequelae to provide the active brain area with the major fuels of glucose and oxygen. When the induced workload is heavy, the activated brain area may be temporarily fuel-limited (Fox *et al.* 1988; Silver & Erecinska 1994; Dienel & Hertz 2001). Neuronal depolarization results in the restoration of Na^+/K^+ gradients via activation of the Na^+/K^+ATPase. The hydrolysis of adenosine triphosphate (ATP) by this pump is a major driver of energy metabolism in the active brain (Mata *et al.* 1980), and an association of creatine kinase with this and other ATP-dependent ion pumps has been demonstrated (Sappey-Marinier *et al.* 1992; Wallimann *et al.* 1992).

ATP is strongly buffered in the brain by conversion via creatine kinase catalysed reaction to phosphocreatine, the phosphorylated analogue of the guanidino amino acid creatine. ATP can be resynthesized from phosphocreatine 12 times faster than via oxidative phosphorylation and more than 70 times faster than *de novo* pathways (Wallimann *et al.* 1992). Phosphocreatine levels can decrease acutely upon brain activation (Sappey-Marinier *et al.* 1992; Rango *et al.* 1997) while ATP levels remain relatively constant. This pattern of acute decrease in local levels is also displayed by glucose (Merboldt *et al.* 1992; Silver & Erecinska 1994) and oxygen (Lowry & Fillenz 1997; Madsen *et al.* 1999).

These data indicate that the brain may be temporarily fuel-limited upon activation and suggest that brain performance might benefit from an increased supply of fuel in the initial stages of the workload. This has been demonstrated to be so for glucose (Benton *et al.* 1994; Parker & Benton 1995; Kennedy & Scholey 2000; Scholey *et al.* 2001) and oxygen (Moss *et al.* 1998). Owing to its pivotal role in energy homeostasis, creatine may similarly boost brain performance. Indeed, creatine has been shown to be neuroprotective in various neurological conditions (Wyss & Schulze 2002). Recently, brain creatine levels in humans have been shown to increase in response to mental training (Valenzuela *et al.* 2003), and acute oral creatine supplementation has been shown to reduce mental fatigue and decrease task-responsive oxygen delivery (demand) to activated areas on performance of a serial calculation task (Watanabe *et al.* 2002).

We sought to determine whether a beneficial effect on mental performance could occur if the creatine reservoir were increased by oral supplementation. Oral creatine (5 g d^{-1} for six weeks) has previously been shown to increase (by *ca.* 9%) creatine levels in the brains of omnivores (Dechent *et al.* 1999). Brain creatine is derived from both synthesis and diet (Wyss & Kaddurah-Daouk 2000). The dietary source is restricted to animal sources, such as meat. However, supplementation can only increase levels to a saturated value, beyond which excess creatine is excreted. To obtain maximal increase by supplementation, we chose to examine the effect of oral creatine monohydrate supplementation on young adults with vegetarian diets, in whom creatine levels are lower than they are in omnivores (Delanghe *et al.* 1989). We note here that creatine supplementation in vegetarians results in the same ergonomic increases in muscle performance as it does in omnivores, despite vegetarians having lower tissue creatine prior to supplementation (Shomrat *et al.* 2000).

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2. MATERIAL AND METHODS

(a) *Subjects*

Forty-five vegan or vegetarian subjects (12 males (median age of 27.5, range of 19–37 years), 33 females (median age of 24.9, range of 18–40 years); 18 vegan (median duration of 4.6 years, range of 0.7–17 years) and 27 vegetarian (median duration of 14.3, range of 1–23 years)) were recruited with informed consent from among the student population of The University of Sydney. Subjects with a medical history of drug or alcohol abuse, diagnosed psychiatric disorders or diabetes or who showed evidence of renal insufficiency were excluded from the study. This study was approved by the University of Sydney and Macquarie University Human Ethics committees.

(b) *Study design*

The study followed a double-blind, placebo-controlled, crossover design. Subjects were seen on four separate occasions, at six-week intervals, following an overnight fast to minimize any fluctuations in blood glucose. At each visit, a blood sample was taken to measure red cell and plasma creatine levels and blood glucose concentration. Red blood cells contain no creatine transporter so their creatine content represents creatine content in the tissue where the red blood cells are made. Red cells live for an average of 120 days and so red cell creatine content is a delayed, averaged measure of tissue creatine levels and represents a sample of tissue creatine when tissue biopsy is not possible.

A cognitive test battery was also administered. At the end of the first and third test sessions, subjects were given an envelope marked with their study number and containing 5 g doses of supplement (creatine monohydrate ((2-methylguanido)acetic acid); Pan Pharmaceuticals, Australia) or placebo (maltodextrin; Manildra Starches, Australia) in plastic vials. Subjects were asked to consume this supplement at the same time each day for the next six weeks and received advice on how best to take this supplement to ensure maximum solubility and absorption. Subjects returned the envelope with unused vials at the end of each six-week period and the number of vials remaining was used to assess compliance, validated against increases in red cell (tissue) creatine. Between visits 2 and 3, the subjects consumed no supplement. Note: six weeks has been shown to be an adequate 'wash-out' period (Harris *et al.* 1992).

(c) *Cognitive tests*

Subjects completed timed (10 min) parallel versions of Raven's Advanced Progressive Matrices (RAPMs) constructed to have equal levels of difficulty based on the published normative performance data and verified by us on an independent sample of 20 subjects. This intelligence test is well validated as a measure of general ability with minimal dependence on cultural factors (Raven *et al.* 1988). Versions of these tests were assigned evenly across the four visits. Subjects also completed the Wechsler Auditory backward digit span (BDS) task (Wechsler 1955). This subtest of the Wechsler Adult Intelligence Scales loads on both short-term storage and verbal working memory functions supported by mid-ventrolateral and dorsolateral prefrontal cortex, respectively (Owen *et al.* 2000).

(d) *Creatine and glucose measurements*

Creatine concentrations in both blood plasma and red blood cells were measured (Griffiths 1968) on the morning of testing. Blood glucose levels were measured randomly, by finger prick using a hand-held glucometer, to monitor compliance with the request for an overnight fast prior to presenting for testing. None of the blood glucose levels recorded suggested that any of the subjects had violated this request.

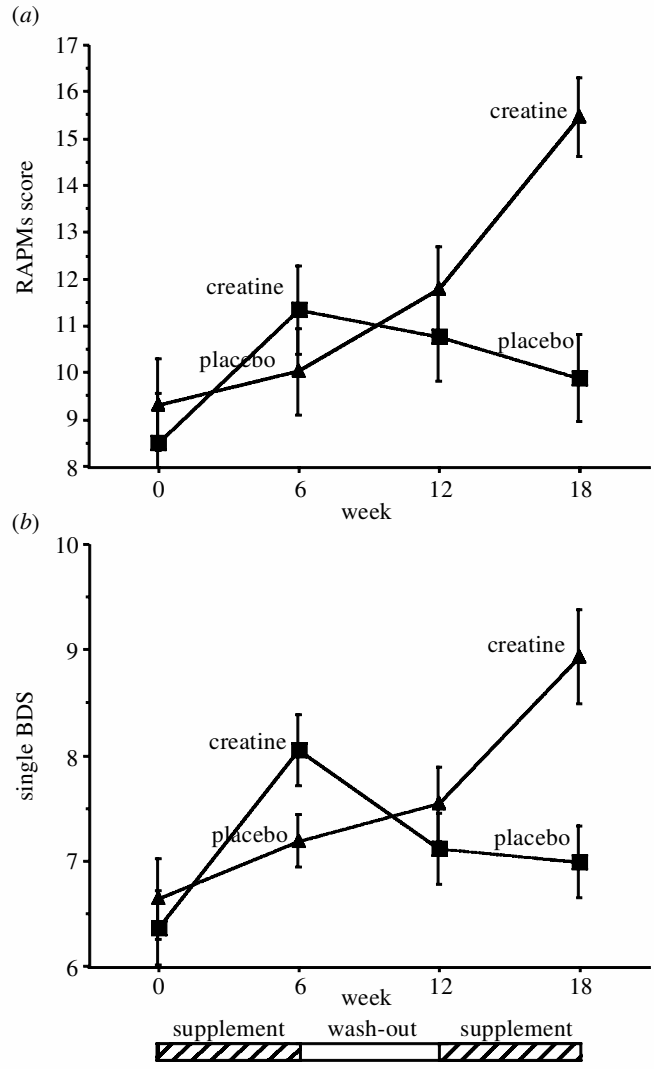

Figure 1. Variation in mean test scores with the supplement and the placebo. (*a*) RAPMs under time pressure and (*b*) BDS. Squares represent group 1 and triangles group 2 subjects. Bars represent standard errors.

3. RESULTS

Measured by the number of vials of creatine returned, mean compliance for creatine supplement was 91% (range of 85–100%) and 97% (range of 88–100%) for placebo. Red blood cell creatine levels, indicative of tissue creatine levels, increased significantly with supplementation ($p = 0.001$) compared with placebo indicating that tissue creatine levels had been increased by the supplement. Plasma creatine levels, more indicative of acute creatine ingestion, did not vary significantly with supplementation.

Supplementation with oral creatine monohydrate significantly increased intelligence (as measured by RAPMs done under time pressure, figure 1*a*) compared with placebo ($F_{3,33} = 32.3$, $p < 0.0001$; repeated-measures ANOVA). There was no significant effect of treatment order ($F_{1,33} = 1.62$, $p = 0.21$), although there was a signifi-

cant interaction with treatment order ($F_{3,99} = 6.7$, $p = 0.0004$). The mean RAPMs raw score under placebo was 9.7 (s.d. = 3.8) items correct in 10 min versus 13.7 (s.d. = 4.1) items correct under the experimental treatment. Supplementation with oral creatine monohydrate (figure 1*b*) significantly affected performance on BDS ($F_{3,34} = 29.0$, $p < 0.0001$), with no effect of order ($F_{3,102} = 0.98$, $p = 0.40$). Mean BDS under the placebo was 7.05 items (s.d. = 1.19), compared with a mean of 8.5 items under creatine treatment (s.d. = 1.76).

4. DISCUSSION

This study showed that increasing creatine intake by oral supplementation resulted in improved brain function, similar to effects shown previously in muscle and heart. These results are in agreement with observations showing that brain creatine levels correlate positively with recognition memory (Ferrier *et al.* 2001) and that creatine supplementation reduces mental fatigue on a serial calculation task (Watanabe *et al.* 2002). This latter study also showed reduced activation-stimulated oxyhaemoglobin delivery to the activated area following creatine supplementation. This suggests that creatine supplementation is acting to smooth fluctuations in the blood oxygen level dependent response curve which results from brain activation (Gjedde *et al.* 1999; Madsen *et al.* 1999), possibly by altering rates of ATP synthesis in the mitochondrion through the mitochondrial creatine kinase–adenine nucleotide translocase–porin complex (Wallimann *et al.* 1992; Saks *et al.* 2000). Most recently, deletion of cytosolic brain-type creatine kinase in mice was shown to result in slower learning of a spatial task and diminished open-field habituation as well as increased intra- and infra-pyramidal hippocampal mossy fibre area suggesting that the creatine–creatine kinase network is also involved in brain plasticity in addition to metabolism (Jost *et al.* 2002).

It is not currently known whether brain creatine levels are lower in vegetarian subjects than omnivores although consideration of factors affecting brain creatine levels suggests that this ought certainly to be the case. It is known that plasma levels are lower in vegetarians (Delanghe *et al.* 1989), but creatine supplementation of vegetarians has been shown to produce similar increases in muscle performance to that seen in omnivores (Shomrat *et al.* 2000). Creatine levels in the brain are controlled by both the rates of synthesis and transport of creatine into the brain and into cells in the brain. This is illustrated by the fact that deficits in the creatine transporter or in the enzymes which synthesize creatine result in similar neurological outcomes (Stöckler *et al.* 1996; Salomons *et al.* 2001). It has also been suggested that a 'creatine cycle' exists between cells in the brain so that a combination of synthesis and transport routes would be required to maintain brain creatine levels (Möller & Hamprecht 1989; Dringen *et al.* 1998). In vegetarians it is likely that the synthesis route is upregulated. Chronic ingestion of creatine has been shown to result in downregulation of the creatine transporter (Guerrero-Ontiveros & Wallimann 1998). We would therefore expect to see a beneficial effect of creatine supplementation on brain performance in most omnivores apart from those who consume very high amounts of meat (*ca.* 2 kg d^{-1}).

Given that RAPMs are a rather pure measure of *g*, or general ability, the data suggest that general ability was improved by creatine supplementation. These results support efficiency models of intelligence (Bates & Stough 1998). With this view, differences in intelligence test performance reflect individual differences in underlying biochemical and structural factors influencing the energetic and temporal resources of the central nervous system. Increasing the energy available for computation increases the speed and (in a distributed computational system such as the brain) power of computational resource, reflected directly in improved general ability.

Long-term supplementation with creatine has yet to be declared truly safe, with reported effects on glucose homeostasis (Rooney *et al.* 2003) and other side effects (Terjung *et al.* 2000). This trial of creatine supplementation showed beneficial effects of creatine on mental performance. These effects may add to the physical enhancement gained by athletes supplementing creatine levels and may be of use to those requiring boosted mental performance in the short term.

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