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Dietary supplementation of creatine monohydrate reduces the human fMRI BOLD signal

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ABSTRACT

Creatine monohydrate is an organic acid that plays a key role in ATP re-synthesis. Creatine levels in the human brain vary considerably and dietary supplementation has been found to enhance cognitive performance in healthy individuals. To explore the possibility that the fMRI Blood Oxygen Level Dependent (BOLD) response is influenced by creatine levels, BOLD responses to visual stimuli were measured in visual cortex before and after a week of creatine administration in healthy human volunteers. The magnitude of the BOLD response decreased by 16% following creatine supplementation of a similar dose to that previously shown to increase cerebral levels of phosphocreatine. We also confirmed that cognitive performance (memory span) is increased. These changes were not found in a placebo group. Possible mechanisms of BOLD change are considered. The results offer potential for insight into the coupling between neural activity and the BOLD response and the more immediate possibility of accounting for an important source of variability during fMRI analysis in clinical studies and other investigations where between-subjects variance is an issue.

Creatine monohydrate (Cr) is a naturally occurring organic acid that acts as a buffer for cytosolic and mitochondrial pools of adenosine triphosphate (ATP). It increases an organism's ability to re-synthesise ATP from ADP by using the energy provided by the phosphate bond from phosphocreatine (PCr). Oral administration of Cr has been found to enhance performance on memory and intelligence tests in both a young healthy vegetarian cohort and in sleep-deprived subjects and it has been suggested that this effect is related to an enhancement in cerebral energetics [22,20]. Cr is known to increase oxidative metabolism in skeletal muscle [8] and oral supplementation has been demonstrated to increase cerebral ATP and PCr concentrations [21]. However, Cr is an ergogenic compound that enhances the ability of all cells to re-synthesise ATP. Thus, it is not clear whether its effect on cognition is due to a general enhancement of metabolic function throughout the body or a direct enhancement of cerebral metabolism. Nevertheless, the results are striking and if Cr has a direct effect upon cerebral metabolism then the implications, particularly for functional Magnetic Resonance Imaging (fMRI), are far reaching. An underlying assumption of fMRI is that neural activity is tightly coupled to the BOLD signal [13] but the precise nature of such coupling is uncertain and remains an area of active research [27,23]. Whilst it is known that the turnover of creatine kinase increases in visual cortex during visual stimulation [9], there is as yet no evidence that Cr levels directly affect the magnitude of the BOLD response.

To establish whether the magnitude of the BOLD response is influenced by Cr levels, we have measured responses to visual stimuli in the primary visual cortex (V1) of 22 healthy human volunteers using fMRI, before and after oral administration of Cr or a placebo (11 in the Cr group and 11 in the placebo group). We chose to measure responses in V1 since it is the only cortical area in which an increase in creatine kinase turnover during stimulation has been documented [9] and we chose a dosing regime that is known to lead to an increase in PCr in occipital cortex [21]. Despite reports of large increases in cognitive performance in healthy young and older adults [22,20], one recent study failed to find cognitive enhancement [25]. We therefore also measured cognitive performance to verify previous reports of cognitive enhancement.

The experiment was a standard fMRI event-related design. Each trial lasted 2 s and consisted of a presentation of a counter-phasing checkerboard. Inter-trial intervals (ITI) were determined by a Poisson distribution [15] with a range of 2–10 s and a mean of 5.5 s. Each run included 40 trials of each stimulus contrast and a 10 s buffer period at the beginning and end. Two sessions were conducted, separated by seven days, before and after Cr/placebo supplementation. For six subjects in each group, retinotopic data were acquired in a previous session. These subjects completed three runs of 604 s in each session. A further five subjects in each group completed their retinotopic scans at the beginning of their first session in week 1.

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These subjects completed two runs of 604 s in each session. With this exception, all scanning sessions were identical.

MRI images were acquired with a 3-Tesla Siemens Magnetom Trio scanner with an 8-channel array head coil. Anatomical (T1-weighted) images were obtained at the start of each scanning session (MPRAGE, 160 axial slices, in-plane resolution 256×256, 1 mm isotropic voxels, TR = 1830 ms, TE = 4.43 ms, flip angle = 11°, bandwidth = 130 Hz/pixel), followed by functional data acquisition with a gradient echo, echoplanar sequence (TR = 2 s, 28 contiguous axial slices, interleaved acquisition order, 3 mm isotropic voxels, in-plane resolution of 64×64 voxels, flip angle = 90°, TE = 30 ms, bandwidth = 1396 Hz/pixel). Functional scanning runs consisted of 304 volumes and lasted 604 s. Pre-processing and analysis was performed with BrainVoyager QX (version 1.9; Brain Innovation, Inc., The Netherlands). Functional data were pre-processed to correct for head-motion and slice-timing, and filtered with a temporal high-pass filter (0.014 Hz). No spatial smoothing was performed on the functional data. Functional images from both sessions were co-registered to a high-quality anatomical image (MPRAGE) of the same subject, acquired in the first session. A standard GLM analysis was performed with regressors related to the two stimulus conditions and regressors derived from each subject's head-motion parameters. The retinotopic data were analysed by fitting an appropriate model. The phase of the fitted model at each voxel was projected onto an inflated and flattened representation of each subject's hemispheres enabling identification of the boundaries of early visual areas. An area corresponding to V1 was defined on the 2D surface representation of each hemisphere and back-projected into 3D space. ROI-based GLM analyses were subsequently conducted for each subject yielding signal amplitude (beta) values for each time-point, group and contrast condition. These were converted to % signal change using the values of constant (session-related) regressors as denominators.

Stimuli were back-projected onto a screen mounted in the rear of the scanner bore by a computer-controlled LCD projector. Subjects viewed the stimuli at a distance of 65 cm via a mirror mounted on the head coil that provided a horizontal view of approximately 30°. The stimuli consisted of low (10%) and high (nominally 100%) contrast counter-phase checkerboards whose contrast reversed at 8 Hz. The check size subtended approximately 2° and the entire stimulus subtended approximately $20° \times 20°$. On half the trials the checkerboard was presented at high contrast and half of the trials were presented at low contrast. The central 2° and background of the stimulus was medium grey and contained a small black fixation, present throughout the experiment. Retinotopic data were acquired using a counterphase (8 Hz) checkerboard "wedge" stimulus (a 24° sector) of radius 12°. Check size was scaled in approximate accordance with the cortical magnification factor. The wedge rotated clockwise at a rate of 64 s/cycle, eight cycles were presented.

Creatine supplementation (Sci-Mx: Gloucestershire, UK) was provided at a dose of 20 g/day for five days, followed by two additional days at a dose of 5 g/day. Subjects returned to the lab once per day and (for the first five days) were issued with two 10 g doses, one that was taken immediately, and one that they were instructed to take later the same day. Subjects were provided with additional doses to cover weekends, and any other days when it was impossible for them to attend the lab. All subjects reported compliance with the dosing regimen. The placebo group was given the same dosage of maltodextrin. All subjects were informed that they were receiving Cr. The final dose was administered at least 1 h before the testing and scanning sessions began. We used maltodextrin, a polysaccharide, as a placebo since it is similar in appearance to Cr (and similarly flavourless) and has a high glycemic index. It thus rapidly raises and then lowers insulin levels. Therefore, even following a large physiological dose, preprandial levels would be restored rapidly. The doses administered in this experiment were small in comparison to typical dietary intake (e.g. around 20% of the glucose content of a typical chocolate bar). Similarly, the rapid peak (45 min) and graceful degradation in Cr concentration known to occur after 5 g (the dose we used on the final day) of oral administration [19] precluded any acute modulation of the BOLD response. The mean and median age of the Cr group was 30.18 and 27 years (SD = 8.37) respectively and the mean and median age of the placebo group was 25 years (SD = 4.82). There was no significant difference between the age of the groups ($t = 1.779$, df = 20, $p = 0.091$). All were students or employees at Royal Holloway University of London who reported that they had not used Cr supplementation within the last three years. Experiments were conducted in accordance with the Declaration of Helsinki, approved by a local ethics committee at Royal Holloway, and written informed consent was obtained. Standard MRI screening procedures were followed.

In order to verify previous reports of cognitive enhancement following Cr supplementation we also measured performance on the Backwards Digit Span (BDS) [28] and Raven's Advanced Progressive Matrices (RAPM) [24] prior to each scan. The BDS comprises a set of number sequences of increasing length with two different sequences of each length. Subjects were required to repeat each sequence backwards. The test was terminated when the subject failed to repeat two sequences of the same length. Different number sequences were used for the two testing sessions. Subjects were required to complete as many items of the RAPM as possible in 5 min. Since the RAPM tests are ordered in terms of difficulty, odd-numbered and even-numbered tests were administered on weeks 1 and 2 respectively. Whilst we did not counterbalance administration of odd and even-numbered tests, the differential difficulty between contiguous tests is small and our analyses revealed that there was no significant difference in performance in the placebo condition across weeks ($t = 0.773$, df = 10, $p = 0.457$), nor was there a significant difference in performance between creatine and placebo groups in week 1 ($t = 0.187$, df = 20, $p = 0.854$).

There was a clear reduction in BOLD amplitude following Cr supplementation that was not seen in the placebo condition. The results are summarized in Fig. 1 (left panel). They are expressed in terms of the $\log_2$ ratio of BOLD response obtained after Cr/placebo to that obtained, in exactly the same region of interest, in the initial scan. Thus, a value of zero represents no change, positive numbers indicate an increase and negative numbers a decrease. The very large reduction in BOLD response following Cr supplementation for one subject in one (low contrast) condition may be considered an outlier ($z = -4.22$). However, the subject's response in this condition was consistent across hemispheres and is graphed for completeness. Removal of this subject's data does not affect the significance of our main finding of reduced BOLD signal following Cr supplementation.

There was no significant difference between Placebo and Cr BOLD responses to visual stimuli in V1 before Cr/placebo supplementation for either low ($t = 0.458$, df = 20, $p = 0.652$) or high contrast ($t = 0.204$, df = 20, $p = 0.84$). There was no significant difference in the effect of Cr with respect to stimulus contrast ($t = 0.5643$, df = 20). However, the mean BOLD response was 16.4% lower following Cr supplementation and 9.6% higher following placebo (Maltodextrin) supplementation. A Group × Week ANOVA revealed a significant interaction effect ($F(1, 42) = 4.81$, $p = 0.034$) between compound (Cr/placebo) and week (pre-/post-supplementation) and individual t tests revealed a significant reduction in BOLD response following Cr supplementation ($t = 2.791$, df = 21, $p = 0.0109$) but no significant change in BOLD response following placebo supplementation ($t = 0.659$, df = 21, $p = 0.517$). One-sample t tests, averaged across contrast conditions, similarly revealed a significant reduction in BOLD relative to no change for the Cr group ($t = 2.551$, df = 21, $p = 0.0186$) but no significant difference for

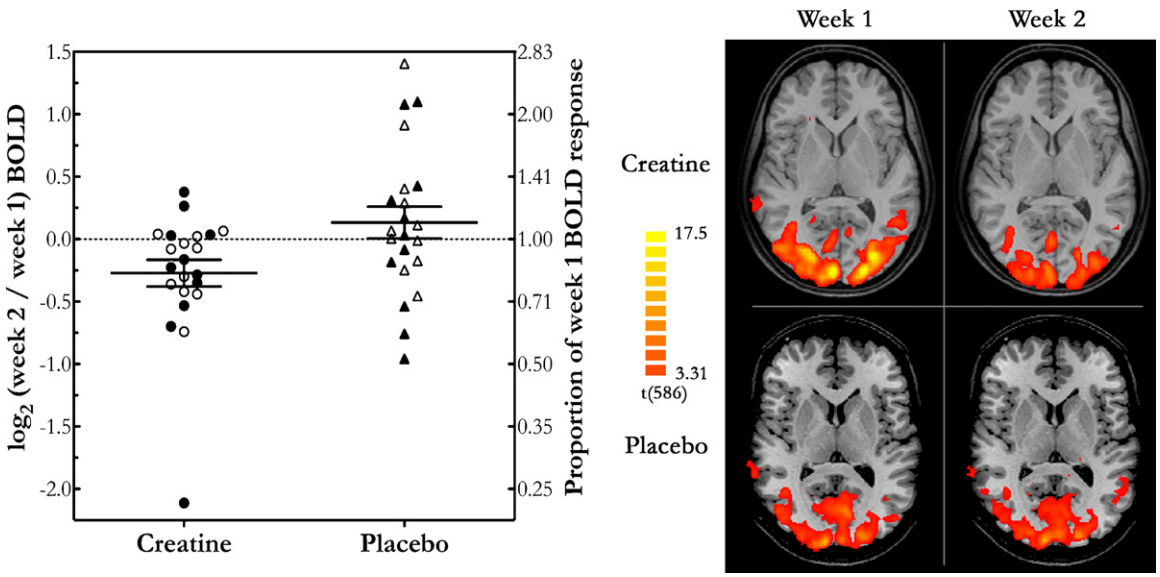

Fig. 1. Left panel: The ratio of the BOLD response before and after Cr/placebo. The ratio for each subject, averaged across the two hemispheres, is represented by a circle (Cr) or triangle (placebo). Open symbols represent high contrast and closed symbols represent low contrast stimuli. Long horizontal lines represent the mean, error bars represent ± 1 S.E.M. ($n = 11$). A value of zero (left y axis) indicates no change after supplementation. Right panel: BOLD response to visual stimuli before (left) and after (right) administration of Cr (top panels) or placebo (bottom panels) for a typical subject from each group (the one whose individual results are closest to the group mean). Activation is shown as an overlay coloured according to the t value obtained for each voxel. Activation maps were thresholded at $p = 0.001$ (uncorrected for multiple comparisons).

the placebo group ($t = 1.038$, df = 21, $p = 0.3105$). The difference in response between week 1 and week 2 is also significantly different between Cr and placebo groups ($t = 2$, df = 42, $p = 0.0192$). Discarding the potential oulier (see Fig. 1) revealed the same pattern of results ($t = 2.192$, df = 41, $p = 0.0341$, two-tailed). The variance in response difference between week 1 and week 2 was, however, signifcantly lower for the Cr group ($F = 4.165$, df = 21,20, $p = 0.0023$) once the potential outlier was removed. In order to estabish whether Cr also had an effect on the dynamics of the haemodynamic response we also conducted a 3×2 mixed design ANOVA on the time-to-peak of the BOLD signal (determined by the maximum value for each condition and each subject of the averaged event-related time courses). Week and contrast were evaluated as within groups variables and compound (Cr vs placebo) as a between groups variable. The results indicate no significant main effect of week ($F(1, 20) = 2.531$, $p > 0.05$), contrast ($F(1, 20) = 1.998$, $p > 0.05$) or drug ($F(1, 20) = 1.045$, $p > 0.05$) on the time-to-peak of the BOLD signal.

The effect of Cr is also illustrated in Fig. 1 (right panel) for a typical subject from each group as a functional overlay on a horizontal slice through the visual cortex. The decrease in BOLD response following creatine but not placebo is clearly visible.

Following Cr, mean Backward Digit Span (BDS) increased significantly by 26.9% ($t = 3.39$, df = 10, $p = 0.0069$) whilst there was no significant change in BDS following placebo ($t = 0.43$, df = 10, p 0.6761). Performance on the RAPM increased non-significantly by 9.6% following Cr ($t = 1.882$, df = 10, $p = 0.0745$) and reduced non-significantly by 4.5% ($t = 0.7733$, df = 10, $p = 0.4572$) following placebo. A Group × Week ANOVA revealed a main effect of week ($F(1, 20) = 5.75$, $p = 0.026$, two-tailed) and a significant interaction between week and compound ($F(1, 20) = 8.58$, $p = 0.008$, two-tailed) for BDS performance. No significant effects were found for RAPM performance.

Our principal result demonstrates that the BOLD response in V1, generally considered to be coupled to metabolic demand and neural activity, is reduced following ingestion of Cr for one week. Our results also show an increase in backward memory span consistent with previous reports using similar doses [22,20] and a non-significant increase in RAPM performance that may possibly be due to insufficient power since a significant increase in RAPM performance has previously been found in a large behavioural study [22]. One recent study [25] failed to find any cognitive enhancement post-Cr but used a dose that was far smaller than previous studies and was typically around 10% of the dose used here. It is possible that these large differences in dose account for the discrepancy. Why should the BOLD response be reduced following Cr supplementation? One possibility is that the effect is related to elevation of cerebral PCr which allows for more efficient synthesis of ATP. Creatine is known to cross the blood-brain barrier and oral supplementation at a very similar dose and duration to that which we have used increases ATP and PCr concentrations within the brain [21]. Thus, the energetic advantage conferred by elevated PCr may lead to the substantial reduction in the BOLD response we find. However, the signalling pathways that underlie neuro-metabolic and neuro-vascular coupling are not well understood. Traditionally, the increase in CBF (and thus BOLD response) attendant upon local cortical activity has been thought to reflect a response to O_2 depletion and CO_2 increases, whilst the cerebral metabolic rate of glucose and O_2 (CMRglu and $CMRO_2$, respectively) have been considered to reflect oxidative glycolysis during periods of high energy demand. However, local PET measurements *in vivo* and global cerebral measurements of these parameters in human primary visual cortex indicate that whilst CBF and CMRglu increase by circa 50% during visual stimulation, O_2 consumption is raised by around 5% [12,18]. Thus the majority of the extra O_2 available during stimulation is not usually uptaken.

There is a number of plausible mechanisms by which increased Cr levels may reduce the BOLD response. Three possibilities are (1) Cr elevation reduces metabolic demand (by elevating PCr) and the stimulus-related change in CBF is consequently reduced. However, there is considerable evidence to suggest that such a straightforward coupling of CBF and neuro-metabolic activity is unlikely [1,2]. (2) Cr influences the mechanisms of the vascular response with no attendant change in metabolic demand. This is akin to suggesting that Cr increases basal perfusion whilst not affecting neural activity. It is possible that Cr's effect on astrocyte metabolism could yield relatively direct vascular changes [14] and there is good evidence that the BOLD response is modulated by basal state [6]. Thus we cannot rule out the possibility that our results reflect an increase in basal

perfusion and no, or a proportionately smaller, change in BOLD response. However, studies of the relation between basal state and BOLD magnitude have used hypercapnic and other manipulations that exclusively modulate perfusion. Cr's ubiquity and physiological properties render it unlikely to have an exclusively vascular effect. (3) Cr enhances the normally relatively low uptake of available O_2 (thus reducing the ratio of oxy- to deoxy-haemoglobin). Cr may lead to an increase in $CMRO_2$ by providing a more direct and abundant energetic pool for oxidative glycolysis, reducing O_2 levels and therefore the BOLD response. If this is the case, our results represent an *increase* in aerobic metabolic activity coupled with a *decrease* in the BOLD response and imply that the amount of freely available ATP in the brain is not tightly coupled with the haemodynamic response. This interpretation of the BOLD reduction is plausible since (1) Cr is known to increase oxidation in skeletal muscle [8] and (2) there is considerable evidence for a decoupling of CBF and metabolic rate [1,2]. However, other plausible possibilities exist such as a more direct neuromodulatory effect, since Cr has recently been implicated in modulation of both glutamatergic and GABAergic function [26,17], and further work will be required to resolve the issue.

Regardless of the underlying cause, the substantial reduction in the BOLD response following Cr supplementation may have general consequences for the interpretation of fMRI data. Endogenous levels of PCr and ATP vary across the brain and oral Cr supplementation has been shown to yield region-dependent elevation in Cr levels [21,10]. The coupling of BOLD to Cr supplementation that we find suggests that meaningful comparison of responses in different areas of the brain requires consideration of the relative levels of PCr across regions. Indeed, it has recently been reported that similar changes in energy metabolism in the lentiform nuclei and visual cortex result in substantially lower BOLD response in the former sub-cortical region [1] and that levels of PCr in the striatum are substantially lower than in cortical areas [21]. Moreover, dietary dependent [11] and independent [3] variations in Cr levels may well be a major source of between-subjects variance in fMRI. If so, careful control could potentially yield real benefits in terms of across-subject statistical signal detection, particularly in studies that compare BOLD responses in different populations. Differences in the shape of the BOLD signal across subjects have often been observed [16] and, given that Cr acts as both a spatial and temporal buffer for ATP, it is conceivable that dietary intake of Cr might contribute to such differences in temporal dynamics as well as response amplitude.

The Cr-induced reduction in BOLD may also bear upon clinical intervention and interpretation in Alzheimer's disease (AD). It has previously been suggested that Cr may hold promise in the treatment of AD [7] and our current results are certainly encouraging in this respect. The effects of Cr administration we find in healthy humans – reduced BOLD response and enhanced memory – are the reverse of those found in pre-symptomatic humans at high risk of developing AD by virtue of carrying the APOE e4 allele [5,4]. Whether Cr supplementation will have any significant therapeutic effect in Alzheimer's is unknown. However the current results, together with Cr's lack of toxicity and low cost make it an attractive potential therapeutic agent, and investigation of this therapeutic potential and its ability to inform clinical interpretation is clearly a priority.

In summary, we have shown that Cr supplementation reduces the fMRI BOLD response by 16% whilst increasing memory span by 26%. The cause is unknown, but a possible mechanism is an increase in cerebral glucose oxidation or basal perfusion. Whilst it is generally assumed that the larger the BOLD response, the greater the underlying neural activity, our reasoning suggests that a reduction in BOLD may occur in response to an increase in oxidative glycolysis. If so, the amount of freely available ATP in the brain may not be tightly coupled with the haemodynamic response. Regardless of the precise mechanism of action, variation in brain Cr may explain some of the between-subjects variance in BOLD responses across a wide range of studies and holds great promise as a probe compound for investigating the nature of the BOLD signal.

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