

Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation

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Abstract

While the role of creatine in preventing muscle (peripheral) fatigue for high performance athletes is well understood, its biochemical role in prevention of mental (central) fatigue is not. Creatine is abundant in muscles and the brain and after phosphorylation used as an energy source for adenosine triphosphate synthesis. Using double-blind placebo-controlled paradigm, we demonstrated that dietary supplement of creatine (8 g/day for 5 days) reduces mental fatigue when subjects repeatedly perform a simple mathematical calculation. After taking the creatine supplement, task-evoked increase of cerebral oxygenated hemoglobin in the brains of subjects measured by near infrared spectroscopy was significantly reduced, which is compatible with increased oxygen utilization in the brain. © 2002 Elsevier Science Ireland Ltd and the Japan Neuroscience Society. All rights reserved.

Keywords: Mental fatigue; Central fatigue; Creatine monohydrate; Near infrared spectroscopy; Oxygenated hemoglobin

1. Introduction

Fatigue can be defined as a phenomenon characterized by reduction of performance after continuous workload accompanied by subjective experience of exhaustion. Although mechanisms of muscle (peripheral) fatigue is well understood, biochemical mechanisms of mental (central) fatigue have not been well studied (Newsholme et al., 1992; Davis, 1995).

Creatine, taken from foods, is delivered to muscles and the brain and transformed to phosphocreatine (PCr). PCr transports high-energy phosphates from mitochondria to cytosol and supplies high-energy phosphates into adenosine triphosphate (ATP) when it is consumed (Persky and Brazeau, 2001). Creatine administration has been reported to increase creatine content in the human brain (Dechent et al., 1999). Creatine monohydrate has widely been used as a dietary supplement by athletes to improve performance (Persky and

Brazeau, 2001). However, its effect on the brain function has not been well studied yet (Guerrero-Ontiveros and Wallimann, 1998; Persky and Brazeau, 2001). An anecdotal report suggested that long-term creatine supplement improved neuronal function in a patient with inborn error (Stockler et al., 1996).

In this study, effects of creatine supplement (8 g/day for 5 days) on performance of serial calculation task were examined by double-blind placebo-controlled paradigm. Simultaneously, cerebral hemoglobin oxygenation changes associated with mental fatigue were measured by near infrared spectroscopy (NIRS); a unique method to monitor cerebral oxygenated (oxyHb) and deoxygenated (deoxyHb) hemoglobin non-invasively (Jobsis, 1977). We have been studying cerebral oxygenation changes associated with cognitive activations as well as physiological challenges such as hyperventilation using a single channel NIRS system (HEO-200) and found that oxygenation changes associated with these tasks can reliably be measured (Matsuo et al., 2000).

Here, the authors demonstrated the effects of creatine to reduce mental fatigue and decrease cerebral oxygenated hemoglobin.

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2. Method

2.1. Subjects

Subjects were 24 healthy volunteers (19 men and five women, 24.3 ± 9.1 [mean \pm SD] years old) who did not have any severe general medical diseases or neuropsychiatric diseases. They were not smokers except for one subject. Written informed consent was obtained from all subjects after the purpose and procedure of the experiment were fully explained. Creatine tablets containing 1 g each of creatine monohydrate (Ezaki Glico, Co. Ltd., Osaka, Japan) ($n = 12$) or placebo tablets with similar taste containing no creatine monohydrate ($n = 12$) were randomly numbered and assigned to the subjects in sealed envelopes so that both the researchers and the subjects cannot tell which one was given. They were instructed to take eight tablets per day (8 g creatine monohydrate per day) four times a day, after meals and before sleep, for 5 days. Meals were not controlled. There were no significant differences of age and sex between the two groups.

2.2. Calculation task

Uchida-Kraepelin test (UKT), a unique serial calculation test that can measure mental fatigue, has been standardized and widely used in clinical psychology, psychiatry, and occupational mental health practice in Japan and surrounding countries (Kurahashi et al., 1957; Kato et al., 1999; Yamada, 1996). In this test, a subject is instructed to perform serial calculation of random numbers printed on a paper for 15 min, and another 15-min task is loaded after 5 min rest. Subjects are told to compute as many figures as possible. Time course of the performance of each 1-min is used for the assessment. The time course of performance measured by UKT is almost consistent time-to-time within an individual (Kurahashi et al., 1957; Yamada, 1996). In a typical pattern, the initial performance gradually decreases and re-increases thereafter during the former 15 min. After taking a rest for 5 min, the initial performance becomes higher than before the rest, but it gradually and linearly decreases in the latter 15 min (Fig. 1). The gradual decrease of performance in the latter 15 min has been regarded as caused by mental fatigue.

All subjects underwent UKT twice, before and after administering the tablets. The test was undergone according to the direction recorded by the tape recorder. The performance was standardized by the average performance of each subject in the first trial in order to minimize the effect of inter-individual variation of performance in UKT.

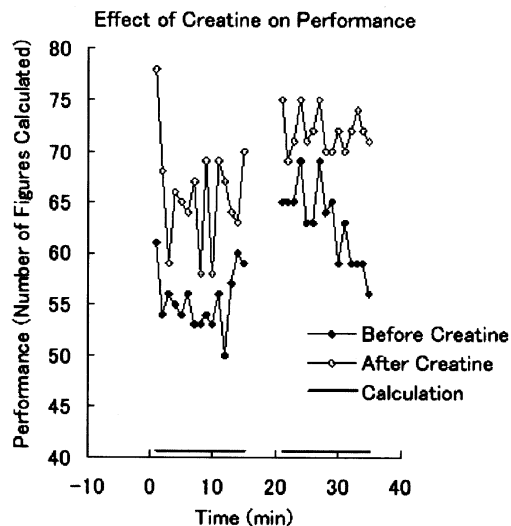


Fig. 1. A representative time course of the performance of serial calculation task (UKT) in each 1-min in one subject (20 years old female) before and after taking creatine.

2.3. NIRS

NIRS measurement was performed by using HEO-200 (OMRON Ltd., Inc., Tokyo, Japan). This instrument consisted of two-wavelength light emitting diodes (760 and 840 nm), so that the two light-absorption characteristics of oxyHb and deoxyHb are different from those at the 805 nm isosbestic wavelength (Shiga et al., 1995). Tissue oximetry using NIRS was based on the modified Beer–Lambert Law (Villringer and Chance, 1997). In the present study, the alterations in oxyHb and deoxyHb from arbitrary baseline values were determined according to the following equations (Shiga et al., 1995); $\Delta\text{deoxyHb} = A[\Delta\text{OD}(760) - B \Delta\text{OD}(840)]$, $\Delta\text{totalHb} = K[\Delta\text{OD}(760) + \Delta\text{OD}(840)]$, and $\Delta\text{oxyHb} = \Delta\text{totalHb} - \Delta\text{deoxyHb}$, where $\Delta\text{OD}(760)$ and $\Delta\text{OD}(840)$ indicate changes in optical density at 760 and 840 nm, respectively. $\Delta\text{deoxyHb}$, ΔoxyHb , and $\Delta\text{totalHb}$, denote changes in the concentrations of deoxyHb, oxyHb, and the totalHb, respectively. The coefficients A , B and K were assumed to be constant.

After the subjects' forehead was cleaned, the optodes were carefully fixed, using a flexible and adhesive fixation pad and an elastic band. The optodes were placed on the left forehead, 1 cm lateral and 3 cm above the supraorbital notch, since the calculation task is reported to activate the left prefrontal cortex (Sakurai et al., 1996). The distance between the optodes was 4 cm. Time resolution was set at 1 s. The average values of oxyHb, deoxyHb, and totalHb in each 1-min were used for further analysis. A researcher checked the subjects' movement of the head during the experiment, so that sudden great changes of NIRS variables could be avoided.

2.4. Statistical analysis

The statistical analyses were performed using SPSS software (SPSS Co. Ltd., Tokyo, Japan). Linear regression analysis and paired *t*-test were used for analysis of the performance in UKT.

In order to assess differences of time courses of hemoglobin changes between in the former and the latter 15 min, two-way repeated measures analysis of variance (RM-AVOVA) was applied with the factors of trial (former and latter) and time (20 min; 1 min before calculation, calculation of 15 min, and 4 min recovery), for oxyHb and deoxyHb in all 24 subjects. When significant interaction between trial and time was observed, multiple comparisons with Dunnett's method were applied with the values, just before the calculation or the final 15th min of the calculation, used as a reference.

In order to analyse the effect of creatine on NIRS data, two-way RM-ANOVA with factors of time (40 data points) and drug (before and after taking creatine or placebo) were used.

3. Results

3.1. Calculation task

A representative time course of the performance in the calculation task in one subject is shown in Fig. 1. Average performance significantly increased on the second UKT ($P < 0.001$) in both groups. Mental fatigue before and after administration of creatine was assessed using linear regression analysis of the second 15-min standardized performance (Fig. 2). The regression coefficient (a , where $y = ax + b$) of the creatine group significantly increased from -0.0115 to -0.0055 ($P < 0.02$, paired *t*-test), while no significant changes were observed in the placebo group (from -0.0078 to -0.0087 , $P > 0.5$).

3.2. Hemoglobin oxygenation changes associated with calculation task

A representative NIRS data in one subject is shown in Fig. 3.

Statistically significant interactions between trial (the former and latter half of calculation) and time (20 min; 1 min before calculation, 15 min calculation, and 4 min recovery), were found for both in oxyHb ($F = 3.3$, $P < 0.01$) and deoxyHb ($F = 5.7$, $P < 0.001$) in the first examination in 24 subjects. Dunnett's multiple comparison (Table 1) revealed that oxyHb significantly increased just after the initiation of calculation and returned to initial level 3 min after the calculation in the former half of the task (Fig. 4, Table 1). In the latter

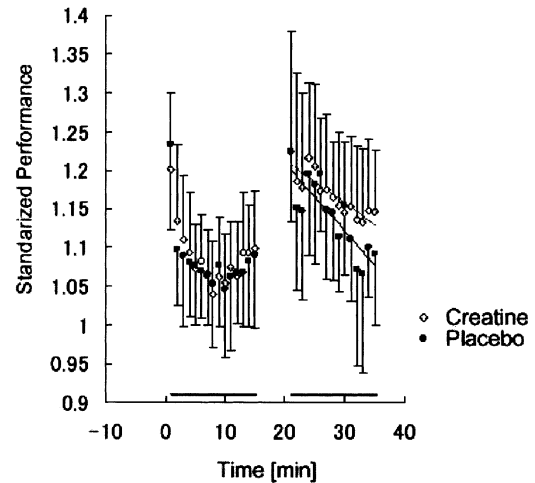


Fig. 2. Time course of the standardized performance of serial calculation task (UKT) in each 1-min. The average standardized performance of two groups (creatine and placebo, $n = 12$ each) at the second examination was plotted against the time. Bars indicate standard deviations. Regression coefficients of the latter 15 min (-0.0055 in the creatine group and -0.0087 in the placebo group), reflecting mental fatigue, significantly increased ($P < 0.02$, paired *t*-test) at the second examination compared with that in the first, only in the creatine group.

half of the task, oxyHb started to increase 1 min after the initiation of calculation, and it did not return to the initial level during 4 min after the calculation.

DeoxyHb decreased just after the initiation of calculation and returned to the initial level 3 min after the calculation in the former half of the task. In the latter half, deoxyHb started to increase 3 min after the initiation of calculation, and it did not return to the initial level during 4 min after the calculation.

In summary, in the latter half of the task, hemoglobin started to change more slowly and these changes lasted longer than that in the former half of the task.

3.3. Effects of creatine on cerebral hemoglobin oxygenation

In the placebo group, no significant interaction between drug and time was found by two-way RM-ANOVA both in oxyHb ($F = 0.59$, $P = 0.97$) (Fig. 5) and deoxyHb ($F = 1.0$, $P = 0.31$) (Fig. 6). On the other hand, significant interaction was found in the creatine group both for oxyHb ($F = 1.76$, $P < 0.005$) (Fig. 5) and deoxyHb ($F = 2.3$, $P < 0.001$) (Fig. 6).

In the creatine supplement group, average oxyHb during the former half of the calculation task was significantly lower on the second examination (0.0217 ± 0.0092 , mean \pm SD, arbitrary unit) against for the first examination (0.0326 ± 0.0073 , $t = 2.21$, $P < 0.05$) (Fig. 5). On the other hand, average deoxyHb during the former half of the calculation task in the creatine group was significantly higher ($-0.0109 \pm$

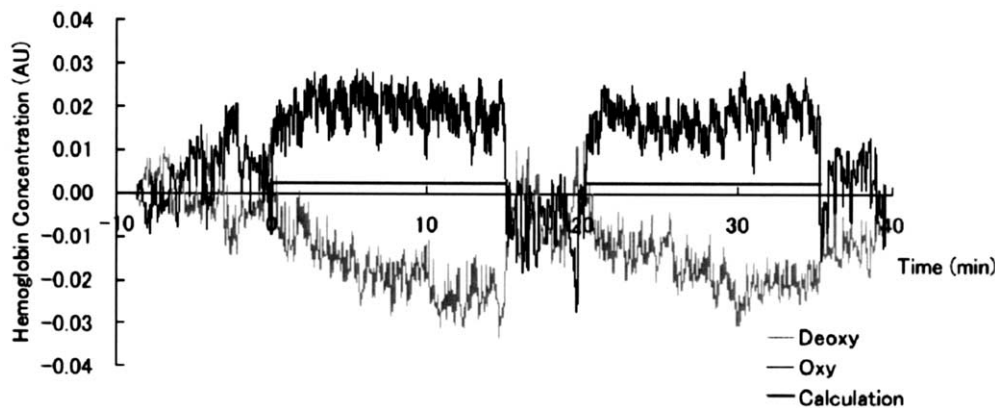


Fig. 3. The time course of oxyHb and deoxyHb during UKT in one subject (20 years old female, whose performance data of calculation task were shown in the Fig. 1). Thick lines indicate oxyHb changes while thin lines deoxyHb. Bars indicate the time when calculation was performed.

0.0071) at the second examination than the first (-0.0143 ± 0.0068 , $t = -3.73$, $P < 0.005$) (Fig. 6). There was no significant difference of oxyHb and deoxyHb between the first and second examinations in the placebo group (Figs. 5 and 6).

4. Discussion

Because oral ingestion of creatine was reported to increase creatine content in the brain measured by proton magnetic resonance spectroscopy (MRS) (Dechent et al., 1999), the effect of creatine on mental fatigue may be mediated by its effect on energy metabolism in the brain. Although it cannot be totally ruled out that reduced muscle fatigue, rather than mental fatigue, contributed to the observed change in the performance of serial calculation task, hemoglobin oxygenation changes associated with creatine supplement suggested that this was mediated by its effect on the brain. However, we should be cautious to interpret the findings.

4.1. The protocol of creatine administration

In this study, 8 g/day creatine was given for 5 days. The dose and duration are different from those used by Dechent et al. (1999), and thus there is no assurance that the creatine levels in the brain were increased after 5 days in the present study. Creatine supplement has been performed for various purposes using different dose and duration. Several reports of creatine supplement in neuromuscular diseases used 10 g or less per day for 5–14 days as initial loading followed by subsequent maintenance phase using lower dose (Hagenfeldt et al., 1994; Tarnopolsky et al., 1997; Vorgerd et al., 2000). We performed the experiment after 5 days loading, because our initial findings in preliminary experiments using proton MRS suggested that creatine concentration in

the brain increased within 5 days after the initiation of 10–30 g/day creatine loading (Murashita et al., unpublished finding). However, there is no evidence indicating that creatine levels increases in the brain after 8 g/day creatine loading for 5 days. It should be examined in the future whether 8 g/day creatine loading for 5 days actually increases creatine concentration in the brain. Difference between placebo and creatine may become much more obvious if creatine loading lasts longer and more calculation tests were done.

4.2. Profiles of hemoglobin oxygenation and calculation performance

In the creatine group, average oxyHb level in the former half of the calculation task was lower and deoxyHb was higher at the second examination than at the first. This is compatible with a hypothesis that only hemoglobin oxygenation was decreased with no change of cerebral blood volume.

By *in vitro* experiments using isolated mitochondria, it was shown that oxygen consumption increased depending on creatine concentration (Jacobus and Diffley, 1986). On the other hand, *in vivo* experiments gave conflicting results. Rico-Sanz and Mendez Marco (2000) reported that oxygen uptake during exercise was increased after creatine loading. However, Stroud et al. (1994) reported that oxygen consumption during exercise did not change after creatine loading. Thompson et al. (1996) also reported that oxyHb changes measured by NIRS during exercise did not change after creatine administration. On the other hand, Nelson et al. (2000) reported that oxygen consumption during exercise decreased after creatine supplement.

It is also puzzling why the oxyHb and deoxyHb were different in the creatine group before and after taking creatine only in the first round of calculation where the fatigue curves of the creatine and placebo group are the same, but no longer differ during the second trial phase

Table 1
Results of Dunnet's multiple comparison

Before calculation		During calculation																		Recovery	
<i>Comparison with the value before the calculation</i>																					
OxyHb																					
Former	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	R	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	NS	NS	
Latter	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	R	NS	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	
DeoxyHb																					
Former	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	R	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	NS	NS	
Latter	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	R	NS	NS	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	
<i>Comparison with the value at the last 15th min of the calculation task</i>																					
OxyHb																					
Former	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	↓	↓	↓	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	R	↓	↓	↓	↓	
Latter	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	↓	↓	↓	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	R	↓	↓	↓	↓	
DeoxyHb																					
Former	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	↑	↑	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	R	↑	↑	↑	↑	
Latter	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	↑	↑	↑	↑	↑	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	R	NS	NS	NS	↑	

R, denotes the value used as the reference in the Dunnet's multiple comparison; NS, non significant; OxyHb, oxygenated hemoglobin; DeoxyHb, deoxygenated hemoglobin.

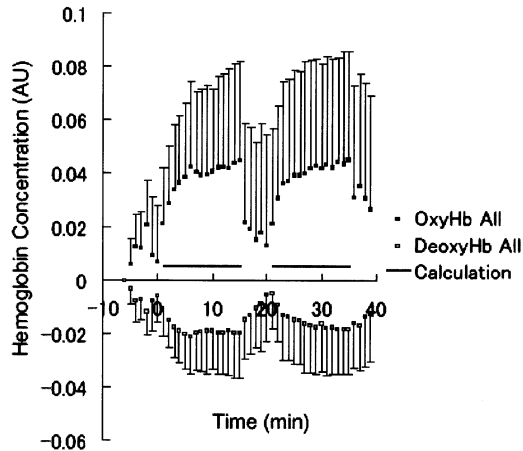


Fig. 4. The time course of averaged oxyHb during UKT in all 24 subjects at the first examination. Ordinate indicates oxyHb and deoxyHb levels measured by NIRS shown as arbitrary unit. Error bars indicate standard deviations. Horizontal bars indicate the time when calculation was performed.

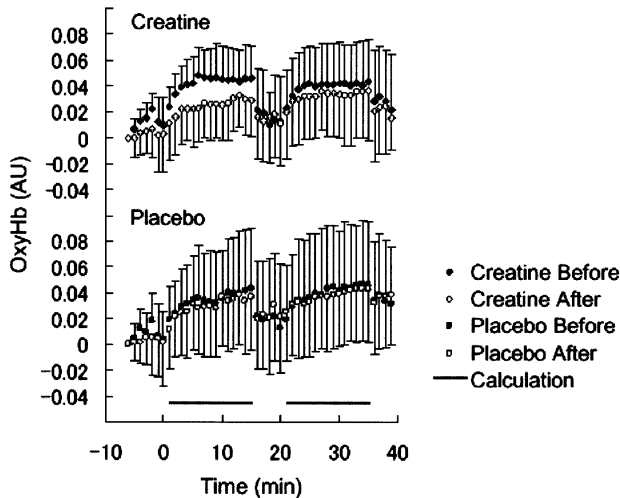


Fig. 5. The time course of oxyHb during the calculation task. Error bars indicate standard deviations. Ordinate indicates oxyHb levels measured by NIRS shown as arbitrary unit. Solid symbols indicate the time course at the first trial and open symbols indicate the time course at the second trial after taking the drugs (8 g/day creatine or placebo) for 5 days. Bottom bars indicate the period of calculation. A significant interaction between drug (before and after taking creatine or placebo) and time (40 data points) was found not for placebo but only for creatine (two-way repeated measures ANOVA, $P < 0.02$). In the creatine group, average of oxyHb in the former half of calculation was significantly lower in the second trial than in the first trial.

when the fatigue parameters start to get different between the two groups. Decline of performance in the second 15-min in UKT may be due to exhaustion caused by the former 15-min calculation. Accumulation of lactate, which causes muscle fatigue, is also known in the brain after photic stimulation (Prichard et al., 1991; Frahm et al., 1996; Sappey-Marinier et al., 1992). It can be hypothesized that increased capacity of the oxidative phosphorylation by creatine (Jacobus and Diffley, 1986;

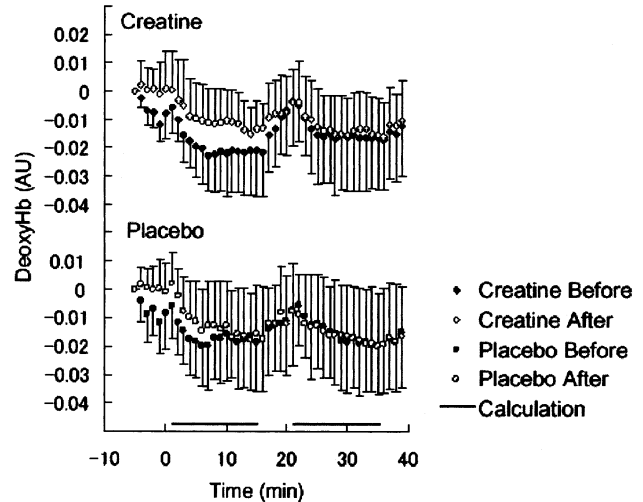


Fig. 6. The time course of deoxyHb during the calculation task. Error bars indicate standard deviations. Ordinate indicates deoxyHb levels measured by NIRS shown as arbitrary unit. Solid symbols indicate the time course at the first trial and open symbols indicate the time course at the second trial after taking the drugs (8 g/day creatine or placebo) for 5 days. Bottom bars indicate the period of calculation. A significant interaction between drug (before and after taking creatine or placebo) and time (40 data points) was found not for placebo but only for creatine (two-way repeated measures ANOVA, $P < 0.005$). In the creatine group, average of deoxyHb in the former half of the calculation task was significantly higher in the second trial than in the first.

Rico-Sanz and Mendez Marco, 2000), shown by the results in NIRS, lead to decreased accumulation of lactate generated by anaerobic glycolysis (Shulman et al., 2001), which caused less mental fatigue in the latter half.

4.3. Time course of hemoglobin oxygenation associated with long calculation task

This study also contains new data of longitudinal observation of hemoglobin oxygenation changes during two 15 min calculation tasks with 5 min interval.

Previous studies using a short task showed that oxyHb increased and reached the plateau within 1 min (Villringer et al., 1997). On the other hand, this study using a long task revealed that oxyHb did not reach the plateau within 1 min but get into the steady state 3 min after the initiation of the calculation. The underlying mechanism of sustained increase of oxyHb in the initial 3 min is of interest, although this finding needs further validation. Differences of time courses of oxyHb and deoxyHb in the former and the latter half of the calculation are also interesting. This may be explained if prolonged mental task decreases extracellular pH due to accumulation of lactate, which causes delayed recovery of increased cerebral blood flow. This hypothesis also needs to be validated by further experiments.

Although the interpretations are speculative and the data are not conclusive, our experiment may suggest a

new line of approach to reduction of mental fatigue involving creatine.

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