

Behavioral/Cognitive

## Creatine Supplementation Enhances Corticomotor Excitability and Cognitive Performance during Oxygen Deprivation

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**Table S1**

*Neuropsychological responses to hypoxia and creatine supplementation*

	Baseline	Creatine	Placebo	Effect of hypoxia <sup>a</sup>		Effect of supplementation <sup>b</sup>	
	Raw scores	Raw scores	Raw scores	t	p	t	p
<b>Verbal memory</b>							
Initial correct hits	12.9 ± 1.5	12.6 ± 1.8	12.7 ± 1.6	-0.11	0.46	-0.30	0.39
Initial correct passes	14.8 ± 0.4	14.4 ± 0.8	14.1 ± 0.7	-3.09	< <b>0.01*</b>	1.03	0.16
Initial target reaction time (ms)	727.9 ± 111.4	723.2 ± 98.1	737.2 ± 84.4	0.82	0.21	-0.55	0.30
Delayed correct hits	11.3 ± 3.1	10.9 ± 3.0	11.0 ± 2.4	0.24	0.41	0.13	0.45
Delayed correct passes	14.8 ± 0.4	13.9 ± 0.8	14.0 ± 0.9	-3.54	< <b>0.01*</b>	-0.15	0.44
Delayed target reaction time (ms)	724.5 ± 84.6	797.1 ± 108.5	806.7 ± 111.9	3.40	< <b>0.01*</b>	-0.07	0.47
<b>Visual memory</b>							
Initial correct hits	12.0 ± 2.0	11.2 ± 3.2	10.6 ± 1.9	-1.64	0.06 <sup>†</sup>	0.25	0.40
Initial correct passes	13.2 ± 1.7	12.8 ± 2.0	13.3 ± 1.6	0.46	0.33	-0.65	0.26
Initial target reaction time (ms)	834.7 ± 116.7	812.7 ± 116.9	851.9 ± 89.8	0.93	0.19	-1.25	0.12
Delayed correct hits	10.7 ± 2.4	10.4 ± 2.4	9.6 ± 2.4	-0.90	0.19	1.06	0.15
Delayed correct passes	12.8 ± 1.3	12.1 ± 1.8	12.6 ± 1.9	-0.12	0.45	-1.24	0.12
Delayed target reaction time (ms)	846.8 ± 97.4	846.1 ± 123.6	903.9 ± 84.6	1.93	<b>0.04*</b>	-1.66	0.06 <sup>†</sup>
<b>Finger tapping test</b>							
Right taps average	65.1 ± 7.9	59.6 ± 7.9	59.7 ± 7.2	-3.66	< <b>0.01*</b>	-0.05	0.48
Left taps average	58.9 ± 7.2	53.1 ± 7.4	53.8 ± 8.4	-4.71	< <b>0.01*</b>	-0.37	0.36
<b>Symbol digit coding</b>							
Correct responses	84.8 ± 28.4	88.1 ± 30.4	83.4 ± 30.2	0.25	0.41	0.66	0.26
Errors responses	2.0 ± 1.7	3.2 ± 2.8	4.1 ± 2.4	3.34	< <b>0.01*</b>	-1.83	<b>0.04*</b>
<b>Stroop test</b>							
Simple reaction time (ms)	246.5 ± 34.2	288.6 ± 54.8	278.5 ± 28.2	2.73	< <b>0.01*</b>	0.57	0.29
Congruent reaction time (ms)	534.7 ± 71.5	553.3 ± 78.5	578.4 ± 97.2	-1.05	0.16	0.49	0.32
Congruent correct responses	12.0 ± 0.0	11.9 ± 0.3	11.9 ± 0.5	1.52	0.08 <sup>†</sup>	-0.74	0.24
Congruent commission errors	0.5 ± 0.6	0.5 ± 0.6	0.8 ± 1.3	0.96	0.18	-1.05	0.16
Incongruent reaction time (ms)	644.5 ± 65.5	654.2 ± 89.0	645.6 ± 64.5	0.31	0.38	0.26	0.40
Incongruent correct responses	24.0 ± 0.0	23.8 ± 0.6	23.2 ± 2.3	-1.32	0.10	0.96	0.18
Incongruent commission errors	1.9 ± 1.8	2.1 ± 1.8	2.0 ± 1.5	1.49	0.08 <sup>†</sup>	0.89	0.20

<b>Shifting attention test</b>							
Correct responses	55.5 ± 9.0	56.6 ± 8.4	52.9 ± 12.4	-0.78	0.23	1.31	0.11
Errors responses	5.4 ± 3.8	6.4 ± 4.4	8.3 ± 6.9	2.37	<b>0.02*</b>	-0.78	0.23
Average correct reaction time (ms)	1005.2 ± 168.3	946.1 ± 137.5	981.8 ± 140.7	-0.43	0.34	-1.03	0.16
<b>Continuous performance test</b>							
Correct responses	39.9 ± 0.3	39.1 ± 1.9	38.2 ± 4.6	-1.44	0.09 <sup>†</sup>	1.13	0.14
Omission errors	0.1 ± 0.3	0.9 ± 1.9	1.8 ± 4.6	1.44	0.09 <sup>†</sup>	-1.13	0.14
Commission errors	0.7 ± 1.2	1.1 ± 1.7	3.0 ± 5.6	2.51	<b>0.01*</b>	-2.21	<b>0.02*</b>
Choice reaction time correct (ms)	392.2 ± 62.0	403.5 ± 38.8	434.6 ± 77.4	2.18	<b>0.02*</b>	-1.60	0.07 <sup>†</sup>

**Table S1.** Neuropsychological responses to hypoxia and creatine supplementation.

Primary outcome scores for all neuropsychological tests collected under baseline and hypoxic conditions during CrM and PLA. Note: for primary scores, higher error response scores indicate worse performance and higher correct response scores indicate better performance. a – Comparisons with baseline were one-sample t-tests of normalised scores compared to baseline (0) to assess the effect of hypoxia. b – Between treatment comparisons were paired t-tests of normalised scores for CrM compared to PLA to assess the effect of CrM supplementation. Descriptive data are mean ± SD; \* p < 0.05; † p ≥ 0.05 and < 0.1. Bold typeface highlights statistically significant comparisons.

*Domain score calculations:*

<b>Clinical domain</b>	<b>Domain score calculation</b>
Composite memory	Verbal memory initial correct hits + verbal memory initial correct passes + verbal memory delayed correct hits + verbal memory delayed correct passes + visual memory initial correct hits + visual memory initial correct passes + visual memory delayed correct hits + visual memory delayed correct passes
Verbal memory	Verbal memory initial correct hits + verbal memory initial correct passes + verbal memory delayed correct hits + verbal memory delayed correct passes
Visual memory	Visual memory initial correct hits + visual memory initial correct passes + visual memory delayed correct hits + visual memory delayed correct passes
Processing speed	Symbol digit coding correct responses – symbol digit coding error responses
Executive function	Shifting attention test correct responses – shifting attention test error responses
Psychomotor speed	Finger tapping test right taps average + finger tapping test left taps average + symbol digit coding correct responses
Reaction time	(Stroop test congruent reaction time + stroop test incongruent reaction time correct) / 2
Complex attention	Stroop incongruent commission errors + shifting attention test error responses + continuous performance test commission errors + continuous performance test omission errors
Cognitive flexibility	Shifting attention test correct responses – shifting attention test error responses – stroop test commission errors

**Table S2***Analysis of variance for neuropsychological responses to hypoxia and creatine supplementation*

	Baseline	Creatine	Placebo	ANOVA statistics		
	Standard scores	Standard scores	Standard scores	F	dof	p
<b>Neurocognitive index</b>	104.2 ± 10.6	99.7 ± 14.3	92.2 ± 23.0	3.402	2, 28	<b>0.048*</b>
<b>Composite memory</b>	106.0 ± 13.1	97.8 ± 21.2	96.1 ± 16.7	1.979	2, 28	0.157
<b>Verbal memory</b>	103.9 ± 15.6	97.1 ± 17.8	96.5 ± 15.4	1.785	2, 28	0.186
<b>Visual memory</b>	105.9 ± 12.1	99.3 ± 18.8	98.1 ± 15.1	1.263	2, 28	0.298
<b>Processing speed</b>	126.5 ± 33.8	128.9 ± 34.6	124.4 ± 39.8	0.225	2, 28	0.800
<b>Executive function</b>	100.5 ± 17.9	100.9 ± 17.9	91.9 ± 28.9	1.556	2, 28	0.229
<b>Psychomotor speed</b>	118.8 ± 18.5	114.5 ± 23.0	112.0 ± 22.9	2.068	2, 28	0.145
<b>Reaction time</b>	103.2 ± 10.6	100.7 ± 12.6	98.9 ± 13.8	0.450	2, 28	0.642
<b>Complex attention</b>	93.7 ± 16.8	86.4 ± 22.7	70.7 ± 51.5	3.747	2, 28	<b>0.036*</b>
<b>Cognitive flexibility</b>	98.8 ± 18.2	98.9 ± 19.3	88.9 ± 31.7	1.821	2, 28	0.180

**Table S2.** ANOVA results assessing the neuropsychological responses to hypoxia and creatine supplementation.

Results of the repeated measures ANOVA assessing the effect of hypoxia and creatine supplementation. One within subjects factor was defined as TREATMENT, with three levels: normoxic baseline, CrM and PLA. Abbreviations: dof, degrees of freedom. Descriptive data are standard score group mean ± SD; \* p < 0.05. Bold typeface highlights statistically significant effects.

**Table S3***Neurophysiological responses to hypoxia and creatine supplementation*

	Creatine			Placebo			Effect of hypoxia <sup>a</sup>		Effect of supplementation <sup>b</sup>	
	Baseline	Hypoxia	$\Delta$ (hypoxia – baseline)	Baseline	Hypoxia	$\Delta$ (hypoxia – baseline)	t	p	t	p
<b><math>\Sigma</math>MEP</b>	2.33 $\pm$ 0.76	3.42 $\pm$ 0.78	1.09 $\pm$ 1.16	2.57 $\pm$ 1.02	2.79 $\pm$ 0.85	0.21 $\pm$ 1.33	0.57	0.29	1.85	<b>0.04*</b>
<b>RMT (% MSO)</b>	50.4 $\pm$ 5.1	50.3 $\pm$ 6.0	0.1 $\pm$ 2.9	53.3 $\pm$ 4.6	54.1 $\pm$ 3.9	0.8 $\pm$ 3.3	0.93	0.19	-0.78	0.22
<b>M wave amplitude (mV)</b>	17.2 $\pm$ 6.8	18.2 $\pm$ 7.3	1.0 $\pm$ 1.8	15.2 $\pm$ 5.3	15.7 $\pm$ 5.5	0.5 $\pm$ 2.3	0.78	0.23	0.83	0.21

**Table S3.** Neurophysiological responses to hypoxia and creatine supplementation.

Raw  $\Sigma$ MEP, RMT and M wave amplitude measures collected under baseline and hypoxic conditions during CrM and PLA. a – Comparisons with baseline were one-sample t-tests of delta scores compared to baseline (0) to assess the effect of hypoxia. b – Between treatment comparisons were paired t-tests of delta scores for CrM compared to PLA to assess the effect of CrM supplementation. Descriptive data are mean  $\pm$  SD; \* p < 0.05. Bold typeface highlights statistically significant comparisons.

**Table S4***Analysis of variance for  $\Sigma$ MEP responses to hypoxia and creatine supplementation*

	Creatine		Placebo	
	Baseline	Hypoxia	Baseline	Hypoxia
$\Sigma$ MEP	2.33 $\pm$ 0.76	3.42 $\pm$ 0.78	2.57 $\pm$ 1.02	2.79 $\pm$ 0.85

	ANOVA STATISTICS		
	F	dof	p
SUPPLEMENTATION	0.714	1, 12	0.415
INTERVENTION	7.710	1, 12	<b>0.017*</b>
SUPPLEMENTATION * INTERVENTION	4.950	1, 12	<b>0.046*</b>

**Table S4.** ANOVA results assessing the  $\Sigma$ MEP responses to hypoxia and creatine supplementation.

Results of the two-way repeated measures ANOVA assessing the effect of hypoxia and creatine supplementation. Two within-subjects factors were defined; SUPPLEMENTATION (two levels: creatine & placebo) and INTERVENTION (two levels: baseline & hypoxia). Abbreviations: dof, degrees of freedom. Descriptive data are mean  $\pm$  SD; \*  $p < 0.05$ . Bold typeface highlights statistically significant effects.

**Table S5*****N-acetylaspartate and choline responses to creatine supplementation***

	Creatine (mmol/L)	Placebo (mmol/L)	Between treatment comparison		Linear regression with $\Delta$ corticomotor excitability			
			t	p	R <sup>2</sup>	F	dof	p
<b>NAA</b>	16.96 ± 3.00	16.45 ± 1.95	0.76	0.23	0.13	1.62	1, 12	0.23
<b>Cho</b>	1.70 ± 0.42	1.52 ± 0.33	1.64	0.06 <sup>†</sup>	0.06	0.74	1, 12	0.41

**Table S5.** Neurometabolite responses to creatine supplementation and supplementation-induced changes in corticomotor excitability.

Seven days of dietary CrM supplementation did not change the concentration of other important neural metabolites measured with <sup>1</sup>H- MRS. Similarly, there was no dependence between the change in corticomotor excitability and the change in neurometabolite concentration with CrM supplementation. Between treatment comparisons were paired t-tests of metabolite amplitudes for CrM compared to PLA to assess the effect of CrM supplementation. Abbreviations: NAA, N-acetylaspartate; Cho, choline; dof, degrees of freedom. Descriptive data are mean ± SD.

**Table S6**  
*Ventilatory responses to hypoxia*

	Normoxia	Hypoxia	Between treatment comparison	
	FiO <sub>2</sub> = 0.21	FiO <sub>2</sub> = 0.10	t	p
V <sub>t</sub> (L)	1.1 ± 0.7	1.2 ± 0.7	-3.16	<b>0.01*</b>
V <sub>f</sub> (breaths/min)	11.5 ± 2.7	14.9 ± 8.5	-1.20	0.14
V <sub>E</sub> (L/min)	9.8 ± 2.9	11.8 ± 2.9	-2.49	<b>0.03*</b>
PetCO <sub>2</sub> (mmHg)	34.8 ± 3.3	30.5 ± 5.3	3.30	<b>0.01*</b>
PiO <sub>2</sub> (mmHg)	150.3 ± 1.9	73.8 ± 5.6	34.36	<b>&lt; 0.01*</b>

**Table S6.** Ventilatory responses to hypoxia.

Hypoxic gas (FiO<sub>2</sub> = 0.10) increased minute ventilation via an increase in tidal volume. The hypoxic ventilatory response elicited mild hypocapnia shown by depressed end tidal CO<sub>2</sub>. Between treatment comparisons were paired t-tests of normoxic scores compared to hypoxic scores to assess the effect of hypoxia. Abbreviations: V<sub>t</sub>, tidal volume; V<sub>f</sub>, breathing frequency; V<sub>E</sub>, respiratory minute volume; PetCO<sub>2</sub>, partial pressure of end tidal CO<sub>2</sub>; PiO<sub>2</sub>, partial pressure of inspired oxygen. Descriptive data are mean ± SD collected from a subset of participants; \* p < 0.05. Bold typeface highlights statistically significant paired comparisons.