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ORIGINAL INVESTIGATION

T. McMorris · R. C. Harris · J. Swain · J. Corbett · K. Collard · R. J. Dyson · L. Dye · C. Hodgson · N. Draper

Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol

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Abstract *Rationale:* Sleep deprivation has a negative effect on cognitive and psychomotor performance and mood state, partially due to decreases in creatine levels in the brain. Therefore, creatine supplementation should lessen the negative effects of sleep deprivation. *Objectives:* The objective of this study was to examine the effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. *Method:* Subjects were divided into a creatine group (n=10) and a placebo group (n=9). They took 5 g of creatine monohydrate or a placebo, dependent on their group, four times a time a day for 7 days, immediately prior to the experiment. The study was double blind. Subjects undertook tests of random movement generation (RMG), verbal and spatial recall, choice reaction time, static balance and mood state pre-test (0 h), after 6, 12 and 24 h of sleep deprivation, with intermittent exercise. They were tested for plasma concentrations of catecholamines and cortisol at 0 and 24 h. *Results:* At 24 h, the creatine group demonstrated significantly less change in performance from 0 h (Δ) in RMG, choice reaction time, balance and mood state. There were no significant differences between groups in plasma concentrations of catecholamines and cortisol. Norepinephrine and dopamine concentrations were significantly higher at 24 h than 0 h, but cortisol were lower. *Conclusions:* Following 24-h sleep deprivation, creatine supplementation had a positive effect on mood state and tasks that place a heavy stress on the prefrontal cortex.

Keywords Stress · Working memory · Prefrontal cortex · Choice reaction time · Balance

T. McMorris (✉) · R. C. Harris · J. Swain · J. Corbett · R. J. Dyson
Centre for Sports Science and Medicine,
University College Chichester,
College Lane, Chichester,
West Sussex PO19 6PE, UK
e-mail: t.mcmorris@ucc.ac.uk
Tel.: +44-1243-816345
Fax: +44-1243-816080

K. Collard
School of Health Professions,
University of Plymouth,
Millbrook House, Topsham Road,
Exeter EX2 6ES, UK

L. Dye
Institute of Psychological Sciences,
University of Leeds,
Leeds LS2 9JT, UK

C. Hodgson · N. Draper
Centre for Research in Adventure Science,
University College Chichester,
College Lane, Chichester,
West Sussex PO19 6PE, UK

Introduction

In this study, the effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, and mood state were examined. Sleep deprivation has been shown to affect physiological and psychological functioning (Kim et al. 2001; Jennings et al. 2003). The energy source for both physiological and psychological performance is the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate and inorganic phosphate. The re-synthesis of ATP is dependent upon phosphorylcreatine, which may become depleted when energy demand is increased. The muscle store of phosphorylcreatine can be increased by dietary supplementation with creatine monohydrate. This has been shown to lessen the negative effects of fatigue on muscle endurance (Greenhaff et al. 1993).

Recent research has found that creatine supplementation can also have a beneficial effect on cognitive performance (Watanabe et al. 2002; Rae et al. 2003). This is probably because creatine monohydrate supplementation results in significant increases in creatine concentrations in the human brain (Dechent et al. 1999). It is, therefore, logical

to hypothesize that changes in performance from baseline (Δ) on cognitive and psychomotor tests, following sleep deprivation with mild exercise, would be less in a group taking creatine supplementation than in a placebo group. Mild exercise was included mainly to ensure that subjects were using similar amounts of energy over the 24-h period. It is, however, accepted that the exercise would add to the physiological stress. Moreover, as the cognitive and emotional centers of the brain interact during stress (Drevets et al. 1995), one would expect creatine supplementation to affect perception of mood state. Therefore, we hypothesized that Δ mood state in the creatine group would be significantly lower than in the placebo group, after 24-h sleep deprivation. Given that creatine supplementation has been shown to have a positive effect on cognitive performance in situations where there was no sleep deprivation (Watanabe et al. 2002; Rae et al. 2003), we decided to also examine the effect of creatine supplementation on Δ scores on cognitive and psychomotor tests and Δ mood state after 6 and 12 h of mild intermittent exercise.

With regard to the biochemical effects of creatine supplementation and sleep deprivation, both physiological and psychological stress have been shown to result in increases in plasma concentrations of the catecholamines, epinephrine (E), norepinephrine (NE), dopamine (DA), and the hormone cortisol (Hoffman et al. 1994; Millan 2004). Animal studies have shown that psychophysiological stress causes increases in brain concentrations of NE and DA (Meeusen and Piacentini 2003), which are neurotransmitters that affect the cognitive and emotional centers in the brain. Due to the action of the hypothalamic–pituitary–adrenal axis, this results in increases of plasma concentrations of these catecholamines (Meerlo et al. 2002). Moreover, research with humans provides strong evidence that hypothalamic–pituitary–adrenal axis activity, in times of stress, also results in increases of plasma concentrations of E and cortisol (Vedhara et al. 2000). If creatine supplementation means that the person is less physically tired, as shown by Greenhaff et al. (1993), and has a positive effect on cognition and mood, as we hypothesize, one would expect individual's taking creatine supplementation to show lower increases in plasma concentrations of E, NE, DA, and cortisol than people not using a supplement. We also hypothesized that Δ catecholamine and cortisol plasma concentrations would be significant predictors of Δ performance on the cognitive and psychomotor tests and Δ mood state.

Method

Subjects

Subjects were paid volunteer male (n=17) and female (n=3) sports science and adventure education majors. Mean (SD) age was 21.11 years (1.85), height 1.81 m (0.02) for males and 1.67 m (0.03) for females, mass 72.20 kg (14.51) for males and 63.22 kg (15.12) for females. All signed informed consent forms and completed a medical questionnaire prior to beginning the experiment. They were informed that they could leave the study at any stage. Ethical approval was obtained from the university ethics committee.

Habituation/learning stage

Subjects completed an incremental exercise test on a cycle ergometer to determine maximum heart rate. Subjects pedaled on a Monark 814E cycle ergometer (Monark Crescent, Varberg, Sweden) at 60 rpm for 5 min with a load of 1 km. After 5 min, 0.4 kg, for the males, and 0.3 kg, for the females, were added every minute until the subject was unable to maintain 60 rpm. Heart rate was taken every minute. Maximum heart rate (HR_{max}) was determined as being the heart rate at the cessation of exercise. Forty-eight hours after undertaking this test, subjects undertook a habituation/learning phase for the experiment. This consisted of the same protocol as in the actual experiment except that no creatine or placebo was taken. Following this stage, subjects were divided into two equal groups (n=10). Group 1 was the creatine supplementation group (n=9 males, 1 female), and group 2 (n=8 males, 2 females) was the placebo group. One male subject, in the placebo group, withdrew during the experiment due to contracting a virus. Subjects took 5 g of creatine monohydrate (Creapure, Deguss AG, Düsseldorf, Germany) or a placebo (Maxijoule, SHS International, Liverpool, UK), dependent on their group, four times a day for 7 days, immediately prior to the experiment. The creatine loading is in line with that used by Harris et al. (1992). The study was double blind. Subjects did not take creatine or placebo on the day of the test. Participants were requested not to eat meat or fish products or to take drinks that contained caffeine 24 h prior to the testing. They were also instructed to undertake their normal weekly exercise routines and their normal diet.

Cognitive tests

Working memory performance was measured by a random movement generation (RMG) test. The subject sat at a display board, holding a stylus. The board was 690× 470×40 mm and contained eight brass circular plates arranged in a semicircular pattern, plus one home plate. The subject started with the stylus placed on the home plate (diameter=30 mm), which was situated centrally on the board. The eight target plates (diameter=30 mm) were positioned 18° apart. Each plate was 300 mm from the home plate. The subject had to move the stylus from the home plate to any of the other plates and back again at the sound of a ringing tone. The speed of response was set at 1.5 Hz. The subjects were instructed to respond in a random fashion, i.e. no patterns must emerge. They were told to imagine how they would have to respond if they were guided by throwing a dice. This is in line with instructions given in other RMG tests (see Brugger 1997). The test lasted for 1 min. The panel was connected to a 486 personal

Cycle 1

HOUR 1	HOUR 2	HOUR 3	HOUR 4	HOUR 5	HOUR 6
Testing* Snack (~ 150 kcals)	First 15 mins cycling at 50% HR_{max}	First 15 mins cycling at 50% HR_{max}	First 15 mins cycling at 50% HR_{max}	First 15 mins cycling at 50% HR_{max}	1 hr cycling at 60% HR_{max} Blood sample†

Cycle 2

HOUR 1	HOUR 2	HOUR 3	HOUR 4	HOUR 5	HOUR 6
Testing‡ Snack (~ 600 kcals)	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	1 hr cycling at 50% HR_{max} Blood sample†

Cycle 3

HOUR 1	HOUR 2	HOUR 3	HOUR 4	HOUR 5	HOUR 6
Testing‡ Meal (~ 1000 kcals)	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	1 hr cycling at 50% HR_{max} Blood sample†

Cycle 4

HOUR 1	HOUR 2	HOUR 3	HOUR 4	HOUR 5	HOUR 6
Snack (~ 600 kcals)	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	First 15 mins walking at 40% HR_{max}	1 hr cycling at 50% HR_{max}

Note. * random movement generation, verbal and spatial recall, choice reaction time and balance tests, and mood state inventory

† a capillary blood sample was taken by finger prick and tested for glucose content

‡ same as Cycle 1 except no balance test

HR_{max} = maximum heart rate

Fig. 1 Diagrammatic representation of procedure

Table 1 Mean (SE) scores for random movement generation (RMG) and adjacency (ADJ) on the random movement generation test, verbal and spatial recall tests, and choice reaction time (CRT) at 0, 6, 12, and 24 h

	0 h		6 h		12 h		24 h	
	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP
RMG	0.405 (0.02)	0.385 (0.01)	0.400 (0.02)	0.372 (0.01)	0.400 (0.02)	0.383 (0.01)	0.396 (0.02)	0.384 (0.01)
ADJ (%)	39.9 (5.72)	30.33 (4.24)	39.6 (5.88)	30.78 (4.18)	34.7 (6.24)	32.22 (4.12)	32.8 (6.27)	29.78 (3.89)
Verbal rcl fwd	7.30 (0.37)	6.44 (0.38)	7.20 (0.39)	6.56 (0.29)	7.40 0.34	6.33 (0.33)	7.90 (0.31)	7.11 (0.31)
Verbal rcl bwd	6.40 (0.54)	5.33 (0.29)	6.00 (0.61)	5.00 (0.44)	6.40 (0.54)	4.89 (0.48)	6.30 (0.65)	5.33 (0.47)
Spatial rcl fwd	7.20 (0.39)	6.56 (0.41)	6.50 (0.37)	6.00 (0.29)	8.80 (0.44)	5.78 (0.22)	6.50 (0.60)	5.78 (0.46)
Spatial rcl bwd	6.70 (0.58)	5.33 (0.33)	5.60 (0.37)	5.33 (0.41)	6.20 (0.44)	5.22 (0.22)	6.30 (0.62)	5.44 (0.34)
CRT (ms)	357 (7)	356 (10)	382 (3)	381 (13)	366 (6)	368 (15)	357 (8)	377 (11)

RMG scores are measured by the random generation index (Evans 1978)
ADJ is the percentage of the frequency of repetition of adjacent responses
Number recall scores are the amount of numbers repeated in the final successful trial
Spatial recall scores are the number of blocks repeated in the final successful trial
CRE GRP Creatine group, *PLA GRP* placebo group, *rcl fwd* recall forward, *rcl bwd* recall backward

computer. The results were analyzed by the computer programme RgCalc (Towse and Neil 1998). The dependent variables were random movement generation (RMG) and adjacency (ADJ). RMG is the index of the frequency of repetition of response alternatives, which is a spatial version of the random number generation measure devised by Evans (1978). ADJ measures the percentage of the frequency of repetition of adjacent responses. The test is a derivation of that of Annoni and Pegna (1997). Neuringer (1986) and Towse and Valentine (1997) have shown that there is no learning effect in random generation tasks when feedback is not supplied. Despite this, we decided that the subjects should become familiar with the test during the habituation/learning period.

Verbal and spatial short-term memory tests were also carried out. There were two tests in each condition, requiring either forward or backward recall. The verbal tests were those of Baddeley et al. (1998), and the spatial tests were variations of the Corsi Block Tapping test (Corsi, unpublished data). In the forward verbal recall test, the experimenter read out a series of numbers, and the subject had to repeat them immediately. The protocol for the backward verbal recall test was identical except that the subject had to recall the numbers in reverse order. The experimenter began with three numbers and increased the amount by one every trial. The dependent variable was the amount of numbers repeated in the final successful trial. In the forward spatial recall test, subjects sat facing a series of blocks (5 cm^3), which were 10 cm apart in a line. The experimenter pointed to several blocks in a given order. The subject was asked to point to the same blocks in the same order. In the backward test, the subject had to point in reverse order. The number of blocks to be remembered began with three and was increased by one every trial. The dependent variable was the number of blocks repeated in the final successful trial.

Psychomotor tests

The first psychomotor test was a classical 4-choice visual reaction time test. Subjects sat facing a digitimer (Queensway Scientific, Fareham, UK), which had four lights in a line and four buttons, one below each light. The subjects sat with the index and second fingers of each hand placed on one of the buttons. They were told to press the appropriate button when one of the lights was illuminated. Subjects undertook 40 trials. There were ten trials on each of the four stimuli as movement time can differ between fingers.

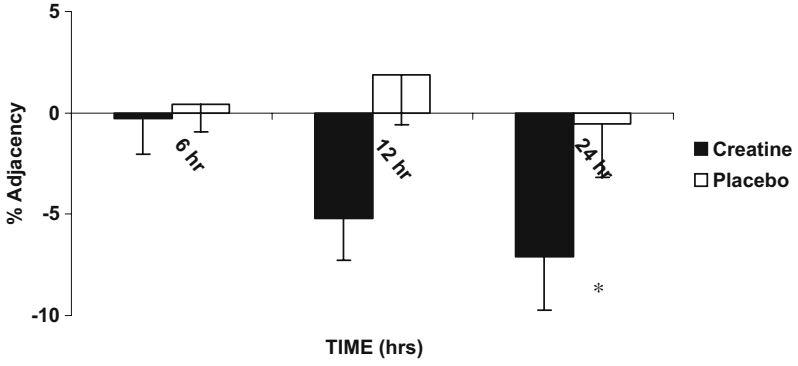


Fig. 2 Mean (SE) Δ ADJ performances for the creatine and placebo groups on the random movement generation task at 6, 12, and 24 h

* $p < 0.05$

Table 2 Mean (SE) Δ scores on the recall tests at 6, 12, and 24 h

	6 h		12 h		24 h	
	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP
Verbal rcl fwd	−0.10 (0.31)	0.11 (0.39)	0.10 (0.31)	−0.11 (0.20)	0.51 (0.34)	0.67 (0.41)
Verbal rcl bwd	−0.40 (0.31)	−0.33 (0.24)	0.00 (0.56)	−0.44 (0.44)	−0.10 (0.55)	0.33 (0.24)
Spatial rcl fwd	−0.70 (0.30)	−0.56 (0.53)	−0.40 (0.40)	−0.78 (0.28)	−0.70 (0.40)	−0.78 (0.43)
Spatial rcl bwd	−0.90 (0.31)	0.00 (0.41)	−0.50 (0.60)	0.00 (0.41)	−0.50 (0.60)	−0.11 (0.37)

CRE GRP Creatine group, *PLA GRP* placebo group, *rcl fwd* recall forward, *rcl bwd* recall backward

Order of presentation differed between tests, and foreperiods were randomized between 0.5 and 2 s. The dependent variable was speed of response. The second psychomotor test was a static balance test. The subject stood balanced on their preferred foot. They stood on a force platform (Kistler, type 9851B, Alton, UK). The subject attempted to maintain balance for 1 min. The amount of sway along the *x*- and *y*-axis was measured. In addition, the number of times the subjects adjusted their position was recorded; this was termed the number of corrections. The amount of sway per correction was calculated by dividing the total amount of sway along both axes by the number of corrections.

Mood state

Mood state was examined by an inventory developed by Hemmings (unpublished data). Hemmings adapted Grove and Prapavessis (1992) the shortened version of the Profile of Mood States (POMS) questionnaire (McNair et al. 1971) for British subjects. The inventory measures the individual's perception of fatigue, vigor, anger, depression and tension. Fatigue refers to feelings of tiredness, weariness and inertia, while vigor represents emotions concerning readiness to undertake physical and mental work. Depression represents feelings of inadequacy and worthlessness, while tension refers to emotions of unease and restlessness. Anger is indicative of feelings of aggression and hostility. It is scored on a 0–4 Likert scale. Internal reliability is high. Cronbach's alpha coefficients of ≥0.72 for each component were found by Hemmings.

Blood sampling

At the beginning and end of the experiment, a 5-ml venous blood sample was taken by venepuncture from a forearm vein. The sample was immediately dispensed into a collection vessel containing 50 μl of potassium ethylene diamine tetracetic acid and placed on ice. The samples were then centrifuged for 5 min at 4°C and 1,900×*g* in a refrigerated centrifuge (8000 series, Centurion Scientific, Ford, UK), after which the plasma was removed and stored at −85°C until analysis. Plasma catecholamine concentrations were analyzed by separation using high-performance liquid chromatography (HPLC), following a standard alumina extraction procedure (see He et al. 1997, for a description). Cortisol was extracted by the method of AbuRaz et al. (2003) and then measured by HPLC.

Procedure

All testing began at 1000 hours on day 1. Subjects arrived 20 min before testing was due to begin. They lay in a supine position for 20 min prior to blood samples being taken by venepuncture. Subjects then undertook four 6-h cycles of testing and exercise (see Fig. 1). Testing at the beginning of Cycle 1 was termed the 0-h condition. Those at the beginning of Cycles 2 and 3 were termed the 6- and 12-h conditions, respectively. During exercise, 5,000 Sports Tester heart rate monitors (Polar Electric, Kempele, Finland) were worn. Heart rates were continually monitored by the experimenters to ensure that the required rate

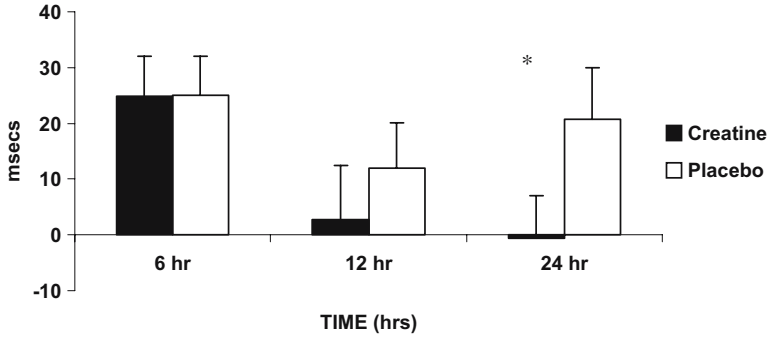


Fig. 3 Mean (SE) Δ times for CRT for the creatine and placebo groups at 6, 12, and 24 h

* $p < 0.05$

Table 3 Mean (SE) performance on the balance test at 0 and 24 h

	0 h		24 h	
	CRE GRP	PLA GRP	CRE GRP	PLA GRP
Sway *x*-axis (cm)	2.80 (0.19)	2.50 (0.25)	2.49 (0.17)	2.34 (0.15)
Sway *y*-axis (cm)	2.33 (0.18)	1.91 (0.10)	1.96 (0.10)	1.88 (0.09)
No. of corrections	44.30 (3.24)	51.00 (2.31)	46.00 (3.15)	41.00 (2.24)
Sway per correction (cm)	0.12 (0.01)	0.09 (0.01)	0.11 (0.01)	0.11 (0.01)

CRE GRP Creatine group, *PLA GRP* placebo group

was maintained. At the end of the first three cycles, a 50-µl capillary blood sample was taken from a finger and analyzed for blood glucose and lactate concentrations with an automated analyzer (2300 StatPlus analyzer, Yellow Springs Industries, USA). The blood glucose test was to ensure that the subjects were not becoming hypoglycemic. The cut-off value for blood glucose concentration was set at ≤3 mmol/l. Lactate was measured to ensure that subjects were exercising aerobically; hence, a cut-off value of >2 mmol/l was chosen. When not exercising, subjects watched television or videos or undertook light reading. Exercise at 40% HR_{max} was walking rather than cycling, because a pilot study had shown that it was difficult to keep heart rates down to 40% HR_{max} while cycling. Moreover, the subjects in the pilot study also suffered from saddle soreness. The slight change in protocol overcame this problem. Following completion of the final cycle, participants lay supine for 20 min before the final blood samples were taken by venepuncture. Then they undertook the final set of cognitive and psychomotor tests, including the balance test, and completed the mood state inventory. This was termed the 24-h condition. Participants were fed at three different times (see Fig. 1), and all ate the same meals.

Statistical analysis

As we were interested in comparing Δ performance at 6, 12, and 24 h between groups on each of the variables but not interaction effects, planned comparisons were used rather than Group × Time analysis of variance (ANOVA). This also guarded against the chances of type II error by reducing the number of comparisons. Δ Scores were calculated instead of actual scores because they take into account individual differences at baseline. RMG, CRT, and sway scores were measured using parametric tests, as the data are ratio measures, and for the mood scores, non-parametric tests were used, as the data are ordinal. Where appropriate, multivariate analyses were used. Effect sizes were measured by Cohen's *d*. The measurement of effect sizes provides statistical information that is additional to probability levels. If probability is >5%, but effect sizes are high, a type II error may be committed. Where probability is <5%, but effect size is low, this indicates a possible type I error (Cohen 1992).

As catecholamine and cortisol concentrations during the habituation period would not be affected by learning, they could be included in the analysis. Catecholamine concentrations were analyzed by a Group × Test × Time

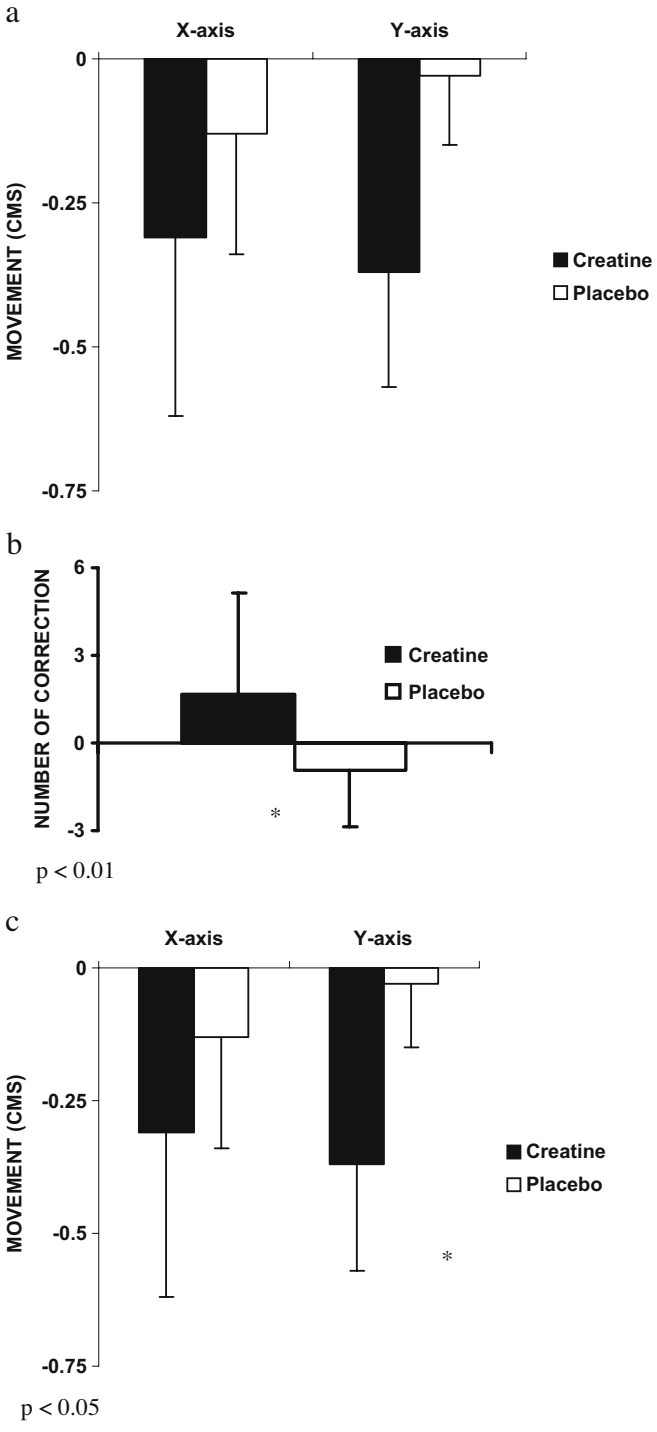


Fig. 4 **a** Mean (SE) Δ sway along the *x*- and *y*-axis for the creatine and placebo groups at 24 h. **b** Mean (SE) Δ number of corrections for the creatine and placebo groups at 24 h. **c** Mean (SE) Δ amount of sway per correction for the creatine and placebo groups at 24 h

Table 4 Mean (SE) scores for mood state at 0, 6, 12, and 24 h

	0 h		6 h		12 h		24 h	
	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP
Fatigue	0.28 (0.17)	0.04 (0.04)	1.00 (0.30)	0.46 (0.15)	0.53 (0.18)	0.86 (0.26)	1.69 (0.40)	3.04 (0.19)
Anger	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)	0.00 (0.00)	0.08 (0.05)	0.11 (0.11)	0.15 (0.08)	1.11 (0.48)
Tension	0.03 (0.03)	0.00 (0.00)	0.10 (0.08)	0.04 (0.04)	0.18 (0.18)	0.07 (0.07)	0.38 (0.22)	0.68 (0.36)
Depression	0.10 (0.10)	0.00 (0.00)	0.18 (0.12)	0.11 (0.11)	0.10 (0.08)	0.00 (0.00)	0.23 (0.15)	0.75 (0.42)
Vigor	2.62 (0.24)	3.03 (0.21)	2.03 (0.25)	2.51 (0.33)	2.18 (0.32)	1.91 (0.28)	1.64 (0.31)	1.20 (0.20)

Scores were measured on a 0–4 Likert scale
CRE GRP Creatine group, *PLA GRP* placebo group

multivariate ANOVA, with follow up analysis by separate univariate ANOVAs. Cortisol concentrations were measured by Group × Test × Time ANOVA. Effect sizes were measured by η^2.

Results

The mean (SE) HR_{max} was 186 bpm (5) for males and 185 bpm (13) for females. Mean heart rates at 40, 50, and 60% were 75, 93, and 112 bpm, respectively, for males, and 74, 93, and 111 bpm, respectively, for females.

Cognitive and psychomotor tests

Table 1 shows the mean (SE) scores for RMG and ADJ on the random movement generation test, scores on the verbal and spatial recall tests and CRT results. It was originally intended to examine Δ scores between groups on the random movement generation task using the multivariate Hotelling's T^2 test, with Δ RMG and Δ ADJ as the dependent variables. Observation of intercorrelations between the variables showed $r>0.70$; therefore, collinearity was a problem. Furthermore, Δ RMG scores demonstrated skewedness. Therefore, Δ performances between groups on the random movement generation task at 6, 12, and 24 h were examined by one-tailed independent samples *t* tests for ADJ only. Figure 2 provides a graphical description. Δ ADJ scores at 24 h for the creatine group were significantly better than those for the placebo group ($t_{17}=1.75$, $p<0.05$, $d=0.76$). There were no significant differences for the other times.

Δ Mean scores (SE) for each group on the recall tests can be seen in Table 2. Δ Performance between groups on the verbal recall tests at each time period was examined by Hotelling's T^2 tests, with forward and backward scores as the dependent variables. There were no significant differences at any time period. Similar analyses were carried out for the spatial recall tests, and again there were no significant effects. Effect sizes ($d<0.50$) do not support the likelihood of a type II error.

Δ Mean times (SE) for choice reaction time (CRT) for each groups at the different time periods can be seen in Fig. 3. One-tailed independent samples *t* tests showed that Δ CRT at 24 h was significantly better for the creatine group ($t_{17}=1.81$, $p<0.05$, $d=0.78$). There were no significant differences at 6 and 12 h.

Mean performance (SE) on the balance test can be seen in Table 3. Figure 4a–c shows the mean (SE) Δ performances. Δ Performance between groups was examined by a series of one-tailed independent samples *t* tests. There were no significant differences between groups for sway along either axis. The Δ number of corrections for the creatine group was significantly higher than that for the placebo group ($t_{17}=2.75$, $p<0.01$, $d=1.42$). The Δ amount

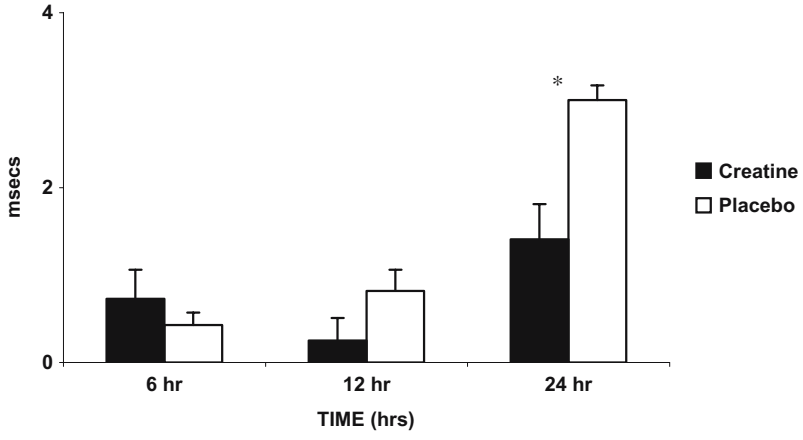
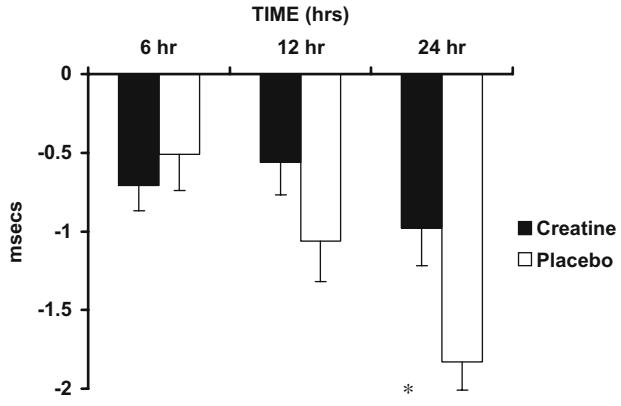


Fig. 5 Mean (SE) Δ scores for perceptions of fatigue for the creatine and placebo groups at 6, 12, and 24 h

* p < .005



*p < 0.02

Fig. 6 Mean (SE) Δ scores for perceptions of vigor for the creatine and placebo groups at 6, 12, and 24 h

of sway per correction for the creatine group was significantly lower than for the placebo group (t_{17}=1.92, p<0.05, d=0.82).

Mood state

Table 4 shows the mean (SE) scores on the mood state inventory for fatigue, vigor, depression, tension and anger. Δ Scores for fatigue and vigor are shown in Figs. 5 and 6, respectively. Δ Scores between groups were compared using a series of one-tailed Mann–Whitney U tests. At 24 h, the Δ scores for fatigue (Z_{17}=2.69, p<0.005, d=1.25) and vigor (Z_{17}=2.30, p<0.02, d=1.09) for the creatine group were significantly better than those for the placebo group. As only ≤3 subjects altered their anger, depression, and tension scores at any time, analysis of Δ scores on these variables was not undertaken.

Catecholamines and cortisol concentrations

Table 5 shows the mean (SE) plasma concentrations of catecholamines and cortisol at 0 and 24 h in the habituation and experimental conditions. Results for two of the subjects in the placebo group could not be included in the analyses, as insufficient blood was collected from them to allow an analysis to be made. Plasma concentrations of E, NE, and DA were examined by a 2 × 2 × 2 (habituation/experiment Condition × Time × Group) doubly multivariate multiple ANOVA. This showed a main effect for time ($\Lambda_{3,11}$=0.46, p<0.05, η^2=0.54) but no other significant effects. Observation of the separate univariate ANOVAs showed that only NE ($F_{1,13}$=13.42, p<0.005, η^2=0.51) and DA ($F_{1,13}$=6.45, p<0.05, η^2=0.33) contributed significantly to the result. Concentrations of NE and DA at 24 h were significantly higher than at 0 h.

Plasma cortisol concentrations were compared using a 2 × 2 × 2 (habituation/experiment Condition × Time × Group) ANOVA. There was a main effect for condition ($F_{1,16}$=6.95, p<0.02, η^2=0.30), with concentrations during the habituation condition being significantly higher than those in the experimental condition. There was also a main effect for time ($F_{1,16}$=6.51, p<0.05, η^2=0.29). Concentrations after 24 h were significantly lower than those at 0 h.

A series of stepwise multiple regression analyses with Δ plasma concentrations of E, NE, DA, and cortisol as the predictor variables and Δ performance on each of the cognitive and psychomotor tests, and scores on the mood state inventory as the dependent variables, were carried out. There were no significant correlations.

Although the published literature does not suggest any gender effects, it was decided to carry out all of the statistical analyses with the female subjects removed. This did not make any significant differences to the results.

Discussion

The results of this study provide support, although not unequivocally, for the claim that creatine supplementation would limit the negative effects of 24-h sleep deprivation on cognitive and psychomotor performance and mood state. There were no performance or mood effects after 6 and 12 h. After 24 h, Δ performance on the random movement generation, CRT and balance tasks by the creatine group were significantly better than those by the

Table 5 Mean (SE) plasma concentrations of epinephrine (E), norepinephrine (NE), dopamine (DA), and cortisol at 0 and 24 h

	Habituation (0 h)		Habituation (24 h)		Experiment (0 h)		Experiment (24 h)	
	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP	CRE GRP	PLA GRP
E (nmol/l)	1.29 (0.13)	1.15 (0.10)	1.31 (0.18)	1.06 (0.07)	1.36 (0.13)	1.06 (0.06)	1.34 (0.10)	1.14 (0.18)
NE (nmol/l)	3.78 (0.29)	3.55 (0.22)	5.32 (0.53)	4.25 (0.53)	4.41 (0.44)	3.85 (0.12)	5.27 (0.53)	4.31 (0.27)
DA (pmol/l)	51.00 (9.12)	37.88 (6.70)	79.10 (15.74)	59.61 (12.06)	74.17 (18.38)	33.48 (6.06)	83.05 (19.87)	42.44 (6.53)
Cortisol (pmol/l)	90.53 (21.78)	115.73 (35.47)	63.34 (11.67)	83.74 (17.72)	64.14 (13.69)	71.79 (13.77)	40.02 (5.19)	64.39 (15.59)

CRE GRP Creatine group, *PLA GRP* placebo group

placebo group. Moreover, Δ mood state scores demonstrated the same effect. There were no significant differences in Δ scores between groups for the number and spatial recall tests.

From these results, it would appear that creatine supplementation is only beneficial following a substantial period of sleep deprivation. It could be argued that at 6 and 12 h, the individuals in the placebo group were still fresh. At these stages, it would appear that the brain is capable of supplying sufficient creatine without recourse to supplementation. However, after 24 h, this does not seem to be the case. The subjects in this experiment had eaten normally throughout the 7 days prior to undertaking the experiment, with the exception of no meat or fish products on the 7th day. In addition, given their calorific intake during the experiment, one would not expect any undue pressure on creatine stores in the brain at 6 and 12 h. The mood state scores show that there was no perception of any problems at these stages.

The situation at 24 h appears to be different. Although the brain uses energy while the person is sleeping, the amount is less than when they are awake, although not by a great amount (Braun et al. 1997). Nevertheless, the individual would be more stressed than under normal circumstances. The mood state scores suggest that the situation may have been exacerbated by negative emotions. Both groups demonstrated significant negative changes in feelings of vigor and fatigue. Attempting to overcome negative effects of stress during cognitive and psychomotor performance is effortful (Eysenck and Calvo 1992); thus, the tests would place an even greater strain on the subjects. It is only at this stage that creatine supplementation proved to be beneficial.

The fact that the random movement generation task and CRT were positively affected by creatine supplementation, but the recall tests were not, is difficult to understand. According to Baddeley (1986), working memory consists of three parts, the central executive, the phonological loop and the visuospatial sketchpad. The phonological loop is responsible for holding verbal information in short-term memory, while the visuospatial sketchpad has the same role but for visual information. The role of the central executive is to recall the information held in the phonological loop and visuospatial sketchpad, recall similar information from long-term memory and coordinate this information to make decisions and solve problems. Research using positron emission tomography (Deiber et al. 1991; Frith et al. 1991) has shown that the central executive tasks take place in large areas of the prefrontal cortex. Moreover, other parts of the brain are also thought to be active during random movement generation (Brugger 1997; Heuer et al. 2005). Functional neuroimaging research has shown that phonological loop tasks depend on the activation of Broca's area and the left hemisphere premotor cortex, while visuospatial tasks activate the right hemisphere premotor cortex (Smith and Jonides 1999).

It has been argued that central executive tasks are more susceptible to stress than phonological loop and visuospatial sketchpad tasks (Dietrich and Sparling 2004), while Heuer et al. (2005) demonstrated negative effects on a random number generation task following sleep deprivation. Exactly how and why creatine supplementation would benefit the performance of these tasks but not the phonological loop and visuospatial sketchpad tasks cannot be determined from this study. Future research could examine the differences in energy output by the different parts of the brain that are activated during these tasks. If central executive tasks are shown to induce more brain activity, this would provide something of an explanation for the beneficial effects of creatine supplementation during fatigue. At this moment, however, this is merely conjecture. Moreover, this argument is not fully supported by the fact that CRT results showed the same effects as the central executive tasks and CRT does not stress the prefrontal cortex as much as do random generation tasks (Bares et al. 2003).

The results of the balance test were not exactly as we had anticipated. After 24 h, the creatine group demonstrated a positive Δ score for number of corrections, while the creatine group's score was negative. The difference was statistically significant. Similarly, the creatine group showed significantly less Δ amount of sway per correction compared to the placebo group. Thus, under stress, the creatine group demonstrated less negative effects in perceptual awareness of changes in the center of gravity than the placebo group. This meant that changes in their amount of sway per correction were much smaller than those of the placebo group. However, there were no significant differences for Δ scores on movement along the *x*- and *y*-axis. This latter finding was surprising as, given the beneficial effects of creatine supplementation on physical performance (Greenhaff et al. 1993), one would have expected creatine supplementation to affect not only perception but also motor performance. This does not appear to have been the case.

With regard to results concerning changes in plasma concentrations of catecholamines and cortisol, the data do not support the hypothesis that creatine supplementation would have a significant effect. If we accept that creatine supplementation results in increases in brain (Dechent et al. 1999) and muscle (Harris et al. 1992) concentrations of creatine, it would appear that these increases are insufficient to affect catecholamine and cortisol responses to sleep deprivation.

Given that there has been a limited amount of research into the effect of sleep deprivation on catecholamine and cortisol plasma concentrations, the present results deserve some comment. As expected, catecholamine concentrations rose after 24 h. Observation of the separate univariate ANOVAs showed that NE and DA contributed significantly to the results, but E did not. During stress E plasma concentrations rise due to the action of the hypothalamic–pituitary–adrenal axis (Meerlo et al. 2002). Moreover, they have been shown to be better indicators of changes in cognitive performance than changes in plasma concentrations of NE and DA (Peyrin et al. 1987). Correlations between plasma concentrations of E and NE tend to be high (McMorris et al. 2000). It should be noted, however, that

psychophysiological stress does not necessarily affect E and NE in the same way (e.g. Melin et al. 1997). Moreover, there is some evidence that sleep deprivation disrupts the workings of the hypothalamic–pituitary–adrenal axis mechanism (Meerlo et al. 2002).

Cortisol concentrations showed a significant decrease over 24 h, which was the opposite to what we expected. von Treuer et al. (1996) examined plasma cortisol concentrations before, during, and after a night of sleep deprivation and in a control condition. Although, in general, they found that cortisol concentrations were elevated following a period of no sleep, there was some evidence that sleep can actually induce increases in cortisol concentrations. This was also demonstrated by Opstad (1991). In our experiment, it is possible that, at 0 h, cortisol concentrations were abnormally high as anxiety was heightened due to apprehension at undertaking the test. After 24 h, the subjects felt relieved rather than anxious and, therefore, the decrement in cortisol concentrations. To some extent, this may be supported by the fact that cortisol concentrations during the habituation session were significantly higher than in the experimental condition. It looks as though the habituation session helped to somewhat ease anxiety. However, the mood state data do not support this explanation. The possibility that sleep deprivation somehow affected the normal daily pattern of changes in cortisol concentrations, similar to those demonstrated by Opstad (1991), should not be ruled out.

The hypotheses concerning catecholamines and cortisol concentrations were based on their responses to stress in general. When the stressor has both physical and emotional characteristics, the effect will be compromised by the roles that E, NE, DA, and cortisol play peripherally. Peripherally, catecholamines and cortisol are secreted by the adrenal cortex to aid glycolysis and lipolysis, and, thus, their plasma concentrations may be poor indicators of what is happening in the brain. This brings into question the validity of the argument that, due to the action of the hypothalamic–pituitary–adrenal axis, plasma concentrations of catecholamines and cortisol are good predictors of changes in brain concentrations. The fact that catecholamines do not readily cross the blood brain barrier may mean that they are poor indicators of what is happening in the brain. Therefore, rather than examining relationships between cognition and changes in plasma concentrations of catecholamines, which do not cross the blood brain barrier, it may be more beneficial to examine changes in plasma concentrations of metabolites of NE and DA, such as 3,4-methoxyhydroxyphenylglycol and homovanillic acid, which do cross the blood brain barrier and are more indicative of brain usage (Peyrin et al. 1987).

In conclusion, it can be said that the results of this experiment provide evidence of a positive effect of creatine supplementation on cognitive and psychomotor performance and mood state, following 24 h of sleep deprivation, although somewhat selectively. Central executive and CRT tasks were positively affected; however, tasks stressing the phonological loop and visuospatial sketchpad were not. It would appear that creatine supplementation is an aid to overcoming brain fatigue. There was no significant effect of creatine supplementation on plasma concentrations of catecholamines and cortisol. This brings into question the use of plasma concentrations of neurotransmitters, which do not cross the blood brain barrier, as indicators of brain activity.

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Appendix

Appendix A

A.1 Cycle 1

Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Testing[a], snack (~150 kcal)	First 15 min cycling at 50% HR_{max}	First 15 min cycling at 50% HR_{max}	First 15 min cycling at 50% HR_{max}	First 15 min cycling at 50% HR_{max}	1 h cycling at 60% HR_{max}, blood sample[b]

HR_{max} Maximum heart rate

[a]Random movement generation, verbal and spatial recall, choice reaction time and balance tests, and mood state inventory

[b]A capillary blood sample was taken by finger prick and tested for glucose content

Appendix B

B.1 Cycle 2

Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Testing[a], snack (~600 kcal)	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	1 h cycling at 50% HR_{max}, blood sample[b]

HR_{max} Maximum heart rate

[a]Same as Cycle 1 except no balance test

[b]A capillary blood sample was taken by finger prick and tested for glucose content

Appendix C

C.1 Cycle 3

Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Testing[a], meal (~1,000 kcal)	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	1 h cycling at 50% HR_{max}, blood sample[b]

HR_{max} Maximum heart rate

[a]Same as Cycle 1 except no balance test

[b]A capillary blood sample was taken by finger prick and tested for glucose content

Appendix D

D.1 Cycle 4

Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Snack (~600 kcal)	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	First 15 min walking at 40% HR_{max}	1 h cycling at 50% HR_{max}-HPS50269

HR_{max} Maximum heart rate

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