The effects of creatine supplementation on thermoregulation and physical (cognitive) performance: A review and future prospects

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Abstract

Creatine (Cr) is produced endogenously in the liver or obtained exogenously from foods such as meat and fish. In the human body, 95% of Cr is located in the cytoplasm of skeletal muscle either in a phosphorylated (PCr) or free form (Cr). PCr is essential for the immediate rephosphorylation of adenosine di-phosphate (ADP) to adenosine tri-phosphate (ATP). PCr is rapidly degraded at the onset of maximal exercise at a rate that results in muscle PCr reservoirs being substantially depleted. A well-established strategy followed to increase muscle total Cr content is to increase exogenous intake by supplementation with chemically pure synthetic Cr. Most Cr supplementation regimens typically follow a well-established loading protocol of 20 $g \cdot d^{-1}$ of Cr for approximately 5-7 days; followed by a maintenance dose at between 2–5 $g \cdot d^{-1}$ for the duration of interest, although more recent studies tend to utilise a 0.3 $g \cdot kg^{-1} \cdot d^{-1}$ supplementation regimen. Some studies have also investigated long-term supplementation of up to 1 year. Uptake of Cr is enhanced when taken together with carbohydrate and protein and/or whilst undertaking exercise. Cr supplementation has been shown to augment muscle total Cr content and enhance anaerobic performance; however, there is also some evidence of indirect benefits to aerobic endurance exercise through enhanced thermoregulation. While there is an abundance of data supporting the ergogenic effects of Cr supplementation in a variety of different applications, some individuals do not respond; the efficacy of which is dependent on a number of factors such as dose, age, muscle fibre type and diet; although further work in this field is warranted. Cr is increasingly being used in the management of some clinical conditions to enhance muscle mass and strength. The application of Cr in studies of health and disease has widened recently with encouraging results in studies involving sleep deprivation and cognitive performance.

Keywords: creatine supplementation, thermoregulation, physical performance, health and disease, cognitive function, sleep deprivation

Abbreviations

AGAT	L-arginine:glycine aminotranferase
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
BW	Body weight (kg)
CK	Creatine Kinase
COPD	Chronic Obstructive Pulmonary Disease
Cr	Creatine
CrT	Total Creatine
$CreaT^{-/y}$	Cr transporter (SLC6A8) knock-out mice
FFM	Fat Free Mass (kg)
GAMT	Guanidinoacetate methytransferase
ICW	Intracellular Water (L)
IMP	Inosine Monophosphate
HR	Heart Rate ($bt \cdot min^{-1}$)
HR_{max}	Maximal Heart Rate ($bt \cdot min^{-1}$)
LBM	Lean Body Mass
miRNA	MicroRNA
PCr	Phosphocreatine
RE	Running Economy
RNA	Ribonucleic acid
RM	Repetition Maximum
RPE	Rate of Perceived Exertion
TBW	Total Body Water (L)
T_{core}	Core Body Temperature (ºC)
T_{re}	Rectal Temperature (ºC)

T_{skin}	Skin Temperature (ºC)
f-TRP	Free Tryptophan
$\dot{V}O_2$	Oxygen Uptake ($ml \cdot min^{-1}$; $ml \cdot kg^{-1} \cdot min^{-1}$)
$\dot{V}O_{2max}$	Maximal Oxygen Uptake ($ml \cdot min^{-1}$; $ml \cdot kg^{-1} \cdot min^{-1}$)
$\dot{V}O_{2peak}$	Peak Oxygen Uptake ($ml \cdot min^{-1}$; $ml \cdot kg^{-1} \cdot min^{-1}$)
WR_{max}	Maximal Work Rate (W)

Introduction

Methyl guanidino acetic acid, commonly known as creatine (Cr), is a naturally occurring compound found predominantly in skeletal muscle (Myers and Fine. 1915; Hunter 1922). Cr supplementation has been reported to improve physical performance during a variety of different exercise modalities, intensities and durations and to promote greater gains in strength, muscle mass, bone mineral density and neuromuscular function in populations ranging from trained healthy individuals to the elderly with sarcopenia undergoing exercise rehabilitation (Bosco et al. 1997; Grindstaff et al. 1997; Mihic et al. 2000; Metzl et al. 2001; Mihic et al. 2000; Hespel et al. 2001; Volek et al. 2004; Pearlman and Fielding, 2006; Bazzucchi et al. 2009; Bemben et al. 2010; Devries and Phillips, 2014; Gualano et al. 2011; Gualano et al. 2014; Candow et al. 2015; Chillibeck et al, 2015; Griffen C et al. 2015; Martone et al. 2015; Ramirez-Campilo et al, 2015; Wilkinson et al. 2015, Phillips, 2015) (see Table 1). The mechanism(s) responsible for these effects range from Cr-induced increases in intramuscular phosphocreatine (PCr) levels and the PCr/ATP energy charge ratio, as well as a greater resynthesis rate of PCr following intense exercise, leading to a higher efficiency of ATP utilisation (Wallimann et al. 2011) and to attenuated cardiovascular and thermoregulatory responses during prolonged exercise in the heat (Demant and Rhodes, 1999; Terjung et al. 2000; Lopez et al. 2009). The significant ergogenic effects of Cr during exercise in the heat seem more dependent on thermoregulatory benefits rather than modest cardiovascular effects (Dalbo et al. 2008). In particular, it is hypothesised that a Cr-induced increase in intracellular water (ICW) enhances the specific heat capacity of the body, resulting in a greater capacity to store heat (Kilduff et al. 2004). These cardiovascular and thermoregulatory effects of Cr can be further enhanced when Cr is co-ingested with hyperhydrating agents such as glycerol (Gly) (Riedesel et al. 1987; Kern et al. 2001; Magal et al. 2003; Easton et al. 2007; Polyviou et al. 2012).

Recent studies have also demonstrated a significant effect of Cr on the restoration of physical and mental performance following sleep deprivation, suggesting Cr as a neuroprotective supplement especially when cellular energy provision is compromised such as during severe oxygen deficit and these effects seem to be enhanced when Cr is co-ingested with stimulants such as caffeine (McMorris et al. 2006; McMorris et al. 2007; Cook et al. 2011). In summary, Cr has worthwhile performance benefits owing to separate but also likely synergistic peripheral and central effects. Given these effects, research using this inexpensive and safe dietary supplement should be intensified so as to yield new clinical applications (Persky and Brazeau, 2001; Evangeliou et al. 2009; Strumia et al. 2012). New studies should also involve the very latest approaches in molecular biology (i.e. the “omics” technologies) in order to resolve the issue of “responders” and “nonresponders” to Cr supplementation, thereby harnessing the full potential of this supplement for use in personalised medicine; also a factor for conflicting reports in the Cr literature.

Cr Supplementation and High Intensity (Anaerobic) Exercise Performance

The effect of Cr supplementation on strength and muscular performance has been investigated extensively, with the International Society of Sports Nutrition (ISSN) stating Cr to be the most effective food supplement available for improving high intensity (anaerobic) performance and increasing lean muscle mass (Kreider et al. 2010). A recent meta-analysis of 63 studies showed that Cr supplementation confers beneficial effects on squat and leg press with 1 repetition maximum (RM) increases of 8% and 3%, respectively (Lahners et al. 2015). This

conclusion is warranted despite this meta-analysis including studies with significant methodological limitations. Some specific highlights include the acute effects of 9 $g \cdot d^{-1}$ of Cr supplementation on 1 RM deadlift performance in 13 trained powerlifters were studied by Rossouw et al. (2000). Compared to a placebo group, deadlift 1RM increased significantly in the Cr group suggesting beneficial effects on performance in anaerobically trained athletes. Becque et al. (2000) measured the effect of 20 $g \cdot d^{-1}$ Cr supplementation for 5 days followed by a 2 $g \cdot d^{-1}$ maintenance dose on arm flexor 1RM following 6 weeks of periodised resistance training. While both Cr supplementation and placebo groups showed an increase in 1RM following the resistance training protocol, only the Cr group showed a difference in fat-free mass (FFM), albeit changes in cross sectional area was inferred through anthropometric measures rather than through direct measures. Reported strength increases in collegiate footballers following 1 week of supplementation with either a low (3 $g \cdot d^{-1}$) or high (20 $g \cdot d^{-1}$) dose Cr regimen followed by a 5 $g \cdot d^{-1}$ maintenance dose for 9 weeks (Wilder et al. 2001, Wilder et al. 2002), which seems the result of training *per se* rather than due to Cr supplementation. However, Bemben et al. (2001) demonstrated significant improvements in Wingate anaerobic power output measures such as peak torque and upper and lower body strength following 20 $g \cdot d^{-1}$ of Cr for 5 days followed by 2 $g \cdot d^{-1}$ of Cr for 8 weeks compared to placebo. All three previously mentioned studies (Bemben et al. 2001; Wilder et al. 2001; Wilder et al. 2002) recruited college football players at the early stages of their athletic careers, which may confound the functional outcomes reported.

Cr has also been shown to improve lower body strength in older men but had no effect on upper body strength (Chrusch et al. 2001). As with Becque et al. (2000) and Bemben et al. (2001), significant increases were reported in body mass and lean tissue mass in Cr supplemented subjects compared to placebo (Chrusch et al. 2001). Kilduff et al. (2002) designed a randomised, double-blind, controlled trial whereby 32 resistance-trained men (Cr group n = 21; placebo group n = 11) were administered 20 $g \cdot d^{-1}$ of Cr + 180 $g \cdot d^{-1}$ dextrose solution or a placebo (200 $g \cdot d^{-1}$ dextrose) for 5 days in accordance with Harris et al. (1992) and subjects performed a set of 5 maximal 30 seconds isometric bench press repetitions. Peak force per repetition and overall force per repetition was greater in the Cr group with respect to placebo. Within the Cr group, a subset of 4 subjects were classified as nonresponders on the basis of reduced Cr uptake (estimated to be equal or less than 21 $mmol \cdot kg^{-1}$ dry muscle weight increase in Cr concentration following Cr supplementation) and the remaining 17 subjects were classed as responders (estimated to be equal or greater than 32 $mmol \cdot kg^{-1} \cdot$dry weight muscle in Cr concentration following Cr supplementation); evidence of an "ergogenic threshold" phenomenon as described by Kilduff et al. (2003). These estimated thresholds in Cr uptake are very similar to those directly determined by Greenhaff et al. (1994) whereby the nonresponders had a Cr uptake of approximately 10 $mmol \cdot kg^{-1}$ dry weight muscle and all but one of the responders had a Cr uptake greater than 25 $mmol \cdot kg^{-1}$ dry weight muscle. Impaired Cr uptake or nonresponse to Cr supplementation seems to affect 30% of individuals and is defined as having less than a 10 $mmol \cdot kg^{-1}$ dry weight muscle increase in Cr uptake after a short loading period (Greenhaff et al. 1994) although co-ingestion of Cr with carbohydrates has been shown to substantially increase muscle Cr stores by up to 60% in responders (Green et al. 1996). Nevertheless, there seems to be a spectrum in Cr uptake response rather than a dichotomy (Becque et al. 2000; Kreider et al. 1998). Classification of subjects into responders and nonresponders should therefore not be made on the basis of strength gains but on physiological reasoning. Kilduff et al. (2003) tested the influence of training on Cr uptake in previously non-resistance trained males. A significant correlation was found between change in body mass and change in $180^{o} \cdot s^{-1}$ isokinetic force and

isometric force as measured by isokinetic dynamometry. Cr uptake was also positively correlated with the change in body mass, $60^{o} \cdot s^{-1}$ isokinetic force, $180^{o} \cdot s^{-1}$ isokinetic force and isometric force, whereas no changes were observed in the placebo group. These results suggest that Cr supplementation, combined with 4 weeks of strength training, can increase muscle strength but only in subjects whose estimated Cr uptake and body mass are significantly increased. Therefore, the greater the Cr uptake and associated body mass changes, the greater the performance gains. Estimated Cr uptake in responders was 28.8 $mmol \cdot kg^{-1}$ dry muscle weight. Cr uptake of less than 21 $mmol \cdot kg^{-1}$ indicates a nonresponse and 2 of the 9 subjects given Cr were estimated to have Cr uptakes of 18.7 and 14.8 $mmol \cdot kg^{-1}$ dry muscle weight, respectively. This ratio of responders to nonresponders (Kilduff et al. 2003) is in agreement with our previous study (Kilduff et al. 2002) and these results implicate other factors in addition to diet, Cr dose, training status and training experience. Muscle Cr uptake has been reported to increase from 118.1 $mmol \cdot kg^{-1}$ dry muscle to 182.8 $mmol \cdot kg^{-1}$ dry muscle in exercised muscle and linked to the increase in peripheral blood flow (Harris et al. 1992). The ingestion of 20 $g \cdot d^{-1}$ of Cr typically results in an increase in total muscle Cr concentration of up to 20%, of which 20% will typically be stored as PCr (Harris et al. 1992). However, 20 $g \cdot d^{-1}$ loading followed by 4 weeks of 2 $g \cdot d^{-1}$ supplementation results in Cr becoming trapped in skeletal muscle and may result in Cr stimulated protein synthesis (Greenhaff et al. 1996). However, Hultman et al. (1996) showed that low dose ingestion of 3 $g \cdot d^{-1}$ for 28 days resulted in a similar magnitude of elevation in PCr in muscle as with a higher loading dose of 20 $g \cdot d^{-1}$ followed by a further 28 days of 2 $g \cdot d^{-1}$ ingestion.

Cr Supplementation and Endurance (Aerobic) Exercise Performance

The coupling between PCr and $\dot{V}O_2$ kinetics at the onset of exercise until steady state has been well characterised (Binzoni et al. 1992; Barstow et al. 1994; McCreary et al. 1996; Rossiter et al. 1999; Rossiter et al. 2001; Rossiter et al. 2002; Korzeniewski and Zoladz 2006). The ratio of ATP/ADP and PCr/Cr are linked in the mitochondria to the creatine kinase (CK) reaction (Chance and Williams 1955; Meyer et al. 1984; Mahler 1985; Balaban 1990). An increase in the total creatine pool [PCr + Cr] through Cr loading has been shown to influence the $\dot{V}O_2$ on-kinetics (Jones et al. 2002; Jones et al. 2009). In particular, Jones et al. (2002) studied the effects of a Cr loading regimen (involving oral consumption of 20 $g \cdot d^{-1}$ of Cr monohydrate for 5 days, followed by a maintenance dose of 5 $g \cdot d^{-1}$) on the pulmonary O_2 on-kinetics during moderate, heavy exercise and maximal exercise. Despite the fact that the amplitude of the primary component of the O_2 kinetics after Cr supplementation was significantly reduced during heavy exercise, no effect of Cr supplementation on the rate of O_2 uptake at the onset of exercise was found. In a second Cr supplementation study involving a similar Cr loading regimen that assessed muscle [PCr] kinetics directly using ^{31}P magnetic resonance spectroscopy, Jones et al. (2009) found a slowing of phase II (on transient, mono-exponential stage) $\dot{V}O_2$ kinetics during moderate and high intensity knee extension exercise thus implicating the CK reaction as a critical component in the regulation of oxidative phosphorylation.

There has been a limited focus of the Cr literature on endurance exercise performance given the purported primary mechanism of action of Cr is to enhance the capacity for PCr resynthesis during recovery from repeated bouts of intense exercise. In one of the first studies to investigate the effects of 21g Cr monohydrate + 21g dextrose for 5 days during intense endurance exercise lasting approximately 1 hour, no effect on performance

was reported although the accumulation of inosine monophosphate (IMP, a marker of muscle energy balance) at the end of the performance ride was significantly lower on the Cr trial. In a more recent study, Tan et al (2014) measured a battery of physiological markers including total body water (TBW), lactate, branched chain amino-acids (BCAAs), free tryptophan (f-TRP), uric acid and hypoxanthine in endurance trained subjects who performed 60 minutes of running at 65–70% maximal heart rate (HR_{max}) and 2 × 100 m sprint trials pre and post 15 days of Cr supplementation. Cr loading increased body weight (BW) in all athletes and reduced post exercise plasma lactate levels and the f-TRP/BCAA ratio and increased urinary hydroxyproline concentration. These results and the tendency for plasma purine metabolites (the sum of hypoxanthine and uric acid), glutamine, urinary 3-methylhistidine, and urea nitrogen concentrations to decrease before the running trials with Cr prompted these authors to conclude that Cr ingestion may reduce muscle glycogen and protein degradation in aerobic, as well as anaerobic activity. These putative metabolic effects during aerobic exercise warrant further investigation using more direct measures.

Muscle wasting and impaired muscle function associated with chronic obstructive pulmonary disease (COPD) are predictors of disability and increased mortality (Gosselink et al. 1996). There have been numerous strategies designed to attenuate muscle atrophy such as the use of nutritional supplements, recombinant human growth hormone, anabolic steroids and appetite stimulants (Burdet et al. 1997; Creutzberg et al. 2003; Schols 2003; Steiner et al. 2003; Weisberg et al. 2002). Positive effects of these applications have been reported including weight gain and improvements in quality of life, however none of the aforementioned strategies have reported improvements in whole body exercise performance. Muscle performance has been reported to be improved following Cr administration in chronic heart failure (Andrews et al. 1998), in mitochondrial myopathies (Tarnopolsky et al. 1997) and following rehabilitation from disuse atrophy (Hespel et al. 2001). Notably, beneficial effects of Cr have been reported in COPD patients, although Cr supplementation did not affect exercise capacity (Fuld et al. 2005). These authors recruited 41 COPD patients and randomly assigned them to receive either Cr with glucose polymer (5.7 g creatine monohydrate, equivalent to 5 g Cr + 35 g glucose) or glucose polymer only (40.7 g per dose) for 3 doses per day for 14 days, followed by one dose per day for 10 weeks (Fuld et al. 2005). All subjects then undertook a series of measures including the assessment of lung function, quadriceps strength (as measured by isokinetic dynamometry), incremental and endurance shuttle walk tests and assessment of body composition at baseline, after 14 days of supplemental loading and once again after 10 weeks of pulmonary rehabilitation. Both loading and maintenance dose of Cr resulted in an increase in FFM, muscle strength and endurance, and improved the effectiveness of pulmonary rehabilitation in the COPD patients, however, no overall change was seen in whole body exercise performance (Fuld et al. 2005). Critically, quality of life, as measured using St George's Respiratory Questionnaire, showed Cr supplementation when combined with rehabilitation resulted in clinically meaningful improvements (Fuld et al. 2005). These results are in line with previous findings using alternative strategies (Burdet et al. 1997; Creutzberg et al. 2003).

Temperature regulation and performance in the heat are critically dependent on hydration status (Sawka and Pandolf 1990) and hence strategies aimed at minimising sweat loss will help preserve hydration status and potentially enhance thermoregulation and cardiovascular function (Sawke et al. 2007). Numerous strategies have therefore been designed to reduce the detrimental effects of dehydration on thermoregulation during exercise including precooling, fluid ingestion and plasma volume expansion (Olschewski and Bruck 1988; Lee

and Haymes 1995; Galloway and Maughan 2000; Watt et al. 2000). The increased body mass associated with Cr loading has been attributed to osmotic effects resulting in cell swelling and increased protein synthesis (Haussinger et al. 1993). As oral Cr supplementation has consistently been shown to increase TBW and ICW (Francaux and Poortmans 1999; Saab et al. 2002), several studies have hypothesised Cr may confer thermogenic effects through augmented hydration. Kilduff et al (2004) investigated whether these hydrating effects of Cr loading could be harnessed to influence cardiovascular, metabolic and thermoregulatory responses during exercise in the heat. To test this hypothesis, 21 endurance-trained males performed 2 constant-load exercise tests to exhaustion at 63 ± 5 % of maximal oxygen uptake ($\dot{V}O_{2max}$) in a climatic chamber set at 30.3 ± 0.5 ºC before and after 7 days of either Cr or placebo. As hypothesised, Cr supplementation attenuated these responses during exercise in the heat and these effects were attributed to an increase in TBW and ICW. Watson et al (2006) investigated whether 7 days of Cr supplementation would impair hydration status or induce symptoms of heat illness during prolonged exercise in heat. Physically active males performed an 80-minute exercise heat tolerance test performed at 33.5 ± 0.5ºC after 7 days of Cr supplementation. Cr supplementation resulted in increased plasma volume and increased urine colour and specific gravity; markers used to determine hydration status of athletes. As Gly has also been shown to increase hydration status during exercise (Riedesel et al. 1987; Kern et al. 2001; Magal et al. 2003) and in light of previous findings, it was hypothesised that a combination of Cr and Gly may further increase water compartments. Easton et al (2007) tested the effects of either Cr, Gly or a combination of Cr/Gly on endurance-trained subjects who performed prolonged exercise consisting of 40 minutes of constant-load exercise at 63% WR_{max} followed by a 16.1 km (10 mile) time trial in a hot humid environment (30ºC and 70% humidity). While no overall performance effect was found either with Cr supplementation alone or in combination with Gly, there was an attenuation of heart rate (HR), rectal temperature (T_{rec}) and rating of perceived exertion (RPE) with both interventions (Easton et al. 2007). Similarly, Beis et al. (2011) investigated the effects of Cr/Gly supplementation for 7 days on running economy (RE) and exercise performance in male runners. In agreement with Easton et al. (2007), BM and TBW increased and while HR and core temperature (T_{core}) were attenuated, no overall difference were seen in $\dot{V}O_2$, RE or in serum osmolarity and plasma volume. Polyviou et al. (2012) extended this research and reported that Cr combined with Gly and α-lipoic acid (Ala) had similar effects as the Cr/Gly/glucose combination used by Easton et al. (2007) when performing constant load exercise in the heat followed by a 10-mile time trial in a hot humid environment. Despite the fairly consistent effects of Cr-induced hyperhydration (with and without enhancement by other hyperhydrating compounds) on thermoregulatory and cardiovascular responses in the previous studies described, exercise performance in the heat was not enhanced (Kilduff et al. 2004; Easton et al. 2007; Beis et al. 2011; Polyviou et al. 2012). It is possible that the exercise trials were not of sufficient duration and intensity for Cr-induced hyperhydration to have a significant effect on performance. Although generally positive effects of Cr have been reported on thermoregulation, there are a number of other studies that fail to observe any effects of Cr on thermoregulation (Oopik et al. 1998; Terjung et al. 2000; Bennett et al. 2001; Branch et al. 2007; Rosene et al. 2015). Future Cr-induced hyperhydration studies should, therefore, concentrate on longer duration and more strenuous protocols in ambient conditions sufficiently extreme to induce greater levels of dehydration and thermal stress in order to more comprehensively examine the role of Cr on exercise performance in extreme conditions. A focus on the role of Cr loading during intermittent strenuous exercise conducted in hot ambient

conditions (such as when playing football and rugby during sporting events conducted in extreme conditions) is particularly warranted.

New Frontiers in Cr Performance Research

Personalised Medicine

As highlighted above, the individual response to Cr supplementation is highly variable with as much as 30% of the population classified as nonresponders to Cr (Harris et al. 1992; Greenhaff, 1996). With this issue in mind, the application of individualised/personalised medicine approaches are needed to identify the underlying mechanism(s) as to why some people respond to Cr supplementation whereas others do not. Resolving this important issue will require approaches such as those being developed and applied by Bouchard and colleagues in the classic Heritage Family Study to deal with the heterogeneity in the training response i.e., a mean increase in $\dot{V}O_{2max}$ of approximately 400 $ml \cdot min^{-1}$ following 20 weeks of exercise training in subjects recruited from 98 two-generational families (Bouchard et al. 1999). For example, the between family variance was 2.5 times greater than within family variance following statistical model fitting, thereby determining a 47% heritability estimate for $\dot{V}O_{2max}$ response to exercise. Since then, Bouchard and colleagues have continued to develop methods to differentiate the individual response by adopting state-of-the-art omics approaches as these emerge (see Bouchard 2015 for recent commentary); a particular emphasis on collaboration and avoidance of small underpowered studies is particularly pertinent to the Cr literature. Given the issue of responder/nonresponders to Cr supplementation, this type of investigation has yet to be conducted within the Cr literature. Hamel et al. (1986) had earlier shown that identical twins that undertook 90-minute maximal cycle ergometer tests pre and post 15 weeks of exercise training produced similar total power output within twin pairs. More recently, the significance of microRNA (miRNA) in the regulation of the cell phenotype (Grimson et al. 2007; Baek et al. 2008; Selbach et al. 2008) and cell development and differentiation (Rao et al. 2006; Baek et al. 2007) has been demonstrated. As skeletal muscle contains a high concentration of tissue specific miRNA species (Lee et al. 2007), this implies metabolic regulation (Esau et al. 2006; Baek et al. 2007).

miRNA has also been shown to influence skeletal muscle protein synthesis *in vivo* in humans (Gallagher et al. 2010). Several miRNA species regulate muscle differentiation, specifically miR-133a/b, miR-206 and miR-1 (Chen et al. 2006; Rao et al. 2006). Davidsen et al. (2011) showed that high responders to resistance training demonstrate differential regulation of skeletal muscle miRNA expression. In that study, fifty-six males undertook resistance training at a frequency of 5 $d \cdot wk^{-1}$ for 12 weeks and muscle biopsies were obtained from the top and bottom 20% of responders to determine the expression of 21 species of miRNA. Of the 21 miRNAs studied, miR-26a, miR-29a, and miR-378 were downregulated, whereas miR-451 was upregulated in low responders to resistance exercise. In contrast, high responders to resistance training, that is, subjects who increased lean body mass (LBM) with respect to the low responders, displayed a higher concentration of miR-378. These findings seem to mirror the responder/nonresponder issue seen in the Cr supplementation literature in terms of response to resistance training and effects on exercise performance. Some of this variability in response will be due to the process that controls both the influx and efflux of Cr across the cell membrane (see Schoch et al. 2006 for review). For example, Tarnopolsky et al. (2000) assessed the Cr transport protein (CreaT) and mitochondrial Cr kinase protein (mtCK) in patients with numerous myopathies (i.e., inflammatory,

mitochondrial, muscular dystrophy and congenital myopathies). These patients exhibited reduced CreaT, whereas mtCK varied between patients suggesting lower CreaT concentration as a primary factor responsible for the reduced muscle PCr stores. Another pertinent example is the finding that muscle Cr concentration is not affected by a mutation of the X-linked Cr-transporter gene but adversely affects brain Cr concentration, indicating that transport mechanisms for brain and muscle Cr stores differ (Pyne-Geithman et al. 2004).

The influence of diet on the CreaT and total Cr (CrT) store was investigated by Watt et al. (2004) by contrasting the effects of 5 days of Cr supplementation in vegetarians and non-vegetarians. As meat is the primary exogenous source of muscle Cr, unsurprisingly vegetarians had significantly lower CrT and a higher response to Cr supplementation than non-vegetarians. However, there was no difference in CreaT gene expression between groups, suggesting diet does not play a major role in CreaT activity. Future Cr studies involving both human and animal models are required to expand these preliminary studies.

The majority of research looking at the efficacy of Cr supplementation on performance has highlighted the responders versus nonresponders issue. However, very little of this research addressing the responders issue has involved human studies and has focused primarily on animal models. For example, Russell et al. (2014) measured ATP, Cr, PCr and CrT as well as upregulation of Cr synthesis in Cr transporter (SLC6A8) knock-out mice ($CreaT^{-/y}$). All substrates were suppressed in $CreaT^{-/y}$ mice, however, Cr and PCr was detected in $CreaT^{-/y}$ mice muscle. Given the lack of CreaT in $CreaT^{-/y}$ mice, these authors argued in favour of non-specific mechanisms for transport of Cr into myofibres. While CreaT gene expression was not present in $CreaT^{-/y}$ mice, there was differential expression of the Cr biosynthesis genes L-arginine:glycineaminotranferase (*AGAT*); *AGAT* gene expression increased in $CreaT^{-/y}$ mice while guanidinoacetate methytransferase (*GAMT*) gene expression remained unchanged. These results suggest an auto-initiated biosynthesis of AGAT in conditions where CreaT is suppressed (Cullen et al. 2006; McClure et al. 2007).

The issue of responders and nonresponders to Cr supplementation has been well documented (Greenhaff et al. 1994, Kilduff et al. 2002, Kilduff et al. 2003) however, in comparing responders against nonresponders, responders have lower pre-supplementation CrT (Harris et al. 1992) and potentially a longer history of resistance training (MacDougall et al. 1977). Novel approaches are needed to resolve this important issue such as the use of investigations conducted in particular clinical patient groups. For example, patients with gyrate atrophy have noticeable but clinically nonrelevant type II muscle fibre atrophy which can be reversed following Cr supplementation (Sipilä et al. 1981). This observation raises the possibility that higher CrT could be linked with a higher percentage of type II fibres. Responders to Cr also tend to be younger suggesting a perturbed mechanism of Cr uptake with aging (Becque et al. 2000; MacDougall et al.1977). Syrotuik and Bell (2004) provided some support for this assertion and applied a favourable biological profile, comprising of [Cr+PCr], percentage of type II fibres, muscle fibre cross sectional area and FFM, that could differentiate between responders, nonresponders and a third category of "quasiresponders" to Cr supplementation. Such approaches when combined with rapid developments in genomics and other high-throughput omics technologies will allow individuals to be classified into probable responders and nonresponders to Cr supplementation and in the process also enhance our understanding of energy metabolism and exercise performance.

Cr supplementation and Cognitive Enhancement – Effects on Sleep Deprivation and Hypoxia

There is growing evidence to support the role of Cr in negating the effects of mild sleep deprivation and in cognitive enhancement. Sleep deprivation has significant impact on catacholamines and brain function (Hoffman et al. 1994, Millan 2004, Kim et al. 2001, Jennings et al. 2003). Since Cr supplementation has been shown to alleviate fatigue (Greenhaff et al. 1993), it was hypothesised by McMorris at al (2006) that Cr supplementation would attenuate the increase in plasma catacholamines with positive impact on cognitive performance. To test this hypothesis, catacholamines were measured in 19 subjects (Cr = 10, placebo = 9) who undertook a battery of psychomotor tests at 0, 6, 12 and 24 hours of sleep deprivation after 7 days of either 20 $g \cdot d^{-1}$ Cr ingestion or 20 $g \cdot d^{-1}$ placebo. Significant effects of Cr on psychomotor performance were observed following 24 hours of sleep deprivation, although there appeared to be no difference between group performance at 6 and 12 hours. Following on from this study, McMorris et al (2007) measured the effect of 20 $g \cdot d^{-1}$ Cr supplementation on moderate intensity exercise performance following either 18, 24 or 36 hours of sleep deprivation. Two groups of 10 subjects undertook 36 hours of central executive tasks, working memory tests, and other cognitive and mood state tests with intermittent bouts of stair climbing, step ups and walking performed at 65% HR_{max} every 2 hours. There was an effect of Cr supplementation on the central executive task where a group × time interaction was seen. Although these findings need replication, Cr supplementation appeared to impact positively on complex cognitive performance following sleep deprivation. Both previous studies recruited subjects who were students and therefore these findings are not specific to athletic performance. Cook et al. (2011) looked at rugby pass accuracy in 10 elite level players who had either normal sleep (7 – 9 hours) or deprived sleep (3 – 5 hours) and whether there was an effect on skill performance due to either Cr (50 or 100 $mg \cdot kg^{-1}$) or caffeine (1 or 5 $mg \cdot kg^{-1}$) supplementation. Compared to placebo, both acute Cr supplementation and caffeine supplementation were associated with increased passing accuracy following reduced sleep for both dominant and non-dominant passing sides. Both Cr and caffeine supplementation appeared to negate sleep deprivation induced decreased accuracy. However, these authors used a single (acute) dose of Cr rather than the conventional, longer-term, supplementation protocol. The concentration of Cr within tissues may therefore not have been sufficiently enhanced after this single small dose. Previous studies report 5-6 days of dietary supplementation with larger doses is necessary to achieve significant increases in muscle Cr levels (Harris et al. 1992; Hultman et al. 1996). Nevertheless, these are potentially important findings needing replication as sleep deprivation in athletes seems to affect skill performance more than other components of performance (Blumert et al. 2007; Edwards and Waterhouse 2009; Oliver et al. 2009).

Reduced cranial oxygen supply brought about through hypoxia, ischaemia or apnea is associated with neurodegenerative changes to brain tissue through degradation of cellular tissue and mitochondrial disruption (Beal 2005; van den Bogaard et al. 2011; Martin 2012). Cr supplementation has been shown to prevent hypoxic damage to rat hippocampal slices *in vitro* (Carter et al. 1995; Balestrino et al. 1999). Given such findings, Turner et al. (2015) assessed the effects of Cr supplementation on neurophysiological and neuropsychological function using magnetic resonance and spectroscopy in adults during acute O_2 deprivation. During a familiarisation session, 15 subjects performed a psychometric evaluation to assess cognitive performance in normoxic conditions. Following 7 days of supplementation with either 20 $g \cdot d^{-1}$ Cr or a placebo, cognitive tests were repeated in hypoxic conditions. In this study, Cr supplementation was associated with increased neural Cr stores in the left precentral gyrus by 9.2%, increased corticomotor excitability determined by the sum of motor evoked potentials and reduced cognitive decline, thus providing exciting new evidence to suggest that as well as

enhancing physical performance, Cr supplementation could potentially have a neuroprotective role (Turner et al. 2015). These preliminary findings warrant further Cr supplementation studies in the general area of cognitive enhancement with particular focus on memory-related disorders such as dementia and Alzheimer's.

Finally, hypohydration has been shown to impair cognitive functions such as alertness, short-term memory, concentration, arithmetic efficiency, psychomotor processing and attention and increase headaches and tiredness (Cian et al. 2000; Cian et al. 2001; Neave et al. 2001; Shirreffs et al. 2004; Suhr et al. 2004; Ritz and Berrut 2005). The Cr/Gly hyperhydrating protocol developed by Easton et al. (2007) has been successfully applied to counter orthostatic intolerance in male subjects undertaking a postural tilt test whereby subjects lay supine for 30 minutes before being tilted head-up to 70º for a further 30 minutes or until the limit of tolerance (Easton et al. 2009). While cardiovascular parameters were monitored, indices of cognitive performance were not measured. Given the link between Cr, hydration and now cognitive performance, there is now the justification needed to develop studies to investigate whether Cr loading could enhance cognitive performance in conditions of extreme stress such as during fatiguing exercise in extreme conditions.

Conclusions

There is overwhelming evidence that Cr supplementation is a safe supplement with significant performance benefits ranging from a variety of primarily anaerobic, strength and power related activities, especially involving repeated bouts of exercise, and in nearly all populations investigated from athletes to clinical populations. Effects of Cr supplementation on LBM, TBW and ICW have also consistently been demonstrated. Cr supplementation alone, or in combination with other hydrating agents, may therefore have worthwhile performance benefits especially during endurance performance in high ambient heat conditions. Additionally, clinically important benefits of Cr supplementation have been reported in a variety of clinical populations and conditions ranging from improved muscle performance in chronic heart failure, COPD, mitochondrial myopathies, and disuse atrophy. There is also recent evidence showing effects of Cr supplementation on both physical and mental performance following mild sleep deprivation and during hypoxia. No other nutrition-related supplement, with exception of caffeine, has such profound ergogenic effects in numerous environmental conditions and involving almost all populations ranging from healthy individuals and athletes to ageing and clinical populations. Nevertheless, the issue of responders versus nonresponders to Cr loading remains the major limiting factor preventing the more effective use of this ergogenic supplement. It is timely, therefore, that new international research consortia are formed and properly financed to conduct the necessary large and carefully designed Cr loading studies using human and animal models and involving state-of-the-art omics technologies such as genomics, transcriptomics, metabolomics and proteomics.

Conflict of Interest

The authors state that they have no conflict of interest

Ethical Statement

This review does not include original data from animal or human studies

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Table 1. Summary of highlighted effects of creatine on thermoregulation and physical (cognitive) performance

Anaerobic performance	
Increase in 1 repetition maximum following acute supplementation	Rossouw et al. 2000
Increase in lean body mass, lean tissue mass and improved lower body strength in older men	Chrusch et al. 2001
Increase in peak athletic performance following 5 days of low or high dose supplementation	Harris et al. 1992; Bemben et al. 2001; Wilder et al. 2001; Wilder et al. 2002
Increase in peak force per repetition and overall force production during isometric bench press performance	Kilduff et al. 2002
Increase in isokinetic force and torque	Kilduff et al. 2003
Maintenance of isokinetic muscle performance following 3.0-4.3% reduction in body mass compared to placebo in resistance trained subjects	Oopik et al. 1998;
Aerobic performance and thermoregulation	
Slowing of on-transient oxygen ($\dot{V}\mathbf{O_2}$) kinetics	Jones et al. 2009
Increase in body mass, reductions in post-exercise plasma lactate, ratio of f-TRP(tryptophan)/BCAA(branched-chain amino acids) and urinary hydroxyproline	Tan, 2014
Osmotic effects associated with creatine supplementation leads to cell swelling and an increase in protein synthesis	Haussinger et al. 1993
Increase in total body water and intracellular water	
Increase cardiovascular, metabolic and thermoregulatory responses to exercise in the heat	Francaux and Poortmans 1999; Saab et al. 2002
	Kilduff et al. 2003; Kilduff et al. 2004; Easton et al. 2007; Beis et al. 2011; Polyviou et al. 2012
Improve exercise performance in patients with chronic heart failure, mitochondrial myopathy and following rehabilitation from disuse atrophy	Tarnopolsky et al. 1997; Andrews et al. 1998; Hespel et al. 2001
Increase in fat free mass and peripheral muscle strength but did not influence exercise capacity in patients with chronic obstructive pulmonary disease	Fuld et al. 2005

Creatine-induced hyperhydration improves orthostatic intolerance in healthy young male volunteers	
Increase in body mass and strength performance. No effects on core temperature compared to placebo	Easton et al. 2009
Reduced plasma volume loss during 1 hour of hyperthermic submaximal exercise	Bennett et al. 2001
Cognitive performance and neuroprotection	Branch et al. 2007
Increase in psychomotor, complex cognitive task and motor skill performance post-sleep deprivation	McMorris et al. 2006; McMorris et al. 2007; Cook et al. 2011
Some evidence of neuroprotection	Turner et al. 2015
Reduced hypoxic brain damage	Carter et al. 1995; Balestrino et al. 1999

Footnote: All studies presented in Table 1 are human studies with the exception of Carter et al. 1995 and Balestrino et al. 1999 who used *in vitro* animal models