

Creatine as a booster for human brain function. How might it work?

Caroline D. Rae^{a, b, *}, Stefan Bröer^c^a Neuroscience Research Australia, Barker St Randwick, NSW 2031, Australia^b School of Medical Sciences, UNSW, High Street, Randwick, NSW 2052, Australia^c Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia

ARTICLE INFO

Article history:

Received 14 July 2015

Received in revised form

4 August 2015

Accepted 15 August 2015

Available online 18 August 2015

Keywords:

Cognition

Neuroprotection

NMDA receptor

Bioenergetics

Phosphocreatine

Creatine kinase

ABSTRACT

Creatine, a naturally occurring nitrogenous organic acid found in animal tissues, has been found to play key roles in the brain including buffering energy supply, improving mitochondrial efficiency, directly acting as an anti-oxidant and acting as a neuroprotectant. Much of the evidence for these roles has been established *in vitro* or in pre-clinical studies. Here, we examine the roles of creatine and explore the current status of translation of this research into use in humans and the clinic. Some further possibilities for use of creatine in humans are also discussed.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Creatine (2-[Carbamimidoyl(methyl)amino]acetic acid, also known as methylguanidoacetic acid) is a naturally occurring nitrogenous organic acid found in animal tissues. Discovered in 1832 by Michel Chevreul and chemically identified in 1847 (Liebig, 1847), the compound, isolated by basification of muscle, was named “creatine”, derived from *kreas* (κρέας) the Greek word for “meat”. The identifier of creatine and one of the fathers of Biochemistry, Justus von Liebig, largely supported his research endeavours by making and selling Liebig's meat extract (*Fleisch-brühe* in German) which contained about 8% creatine (Wallimann, 2007); plainly even the very first scientists working with creatine recognised its potential as a supplement.

1.1. Where does creatine come from?

Humans obtain creatine from two sources; by consumption (of meat or artificial sources such as laboratory-synthesised creatine) and by synthesis. Creatine is taken up from the gut by the sodium dependent creatine transporter (see below for more discussion of

transport mechanisms) but it is not clear how it crosses the basolateral membrane (Orsenigo et al., 2005).

Brain synthesises creatine, although the majority of total body synthesis takes place in kidney, pancreas and liver. Creatine is synthesised in a two-step reaction (Fig. 1); the amidino group is transferred from arginine to glycine in a reaction catalysed by L-arginine-glycine amidino transferase (AGAT: E.C. 2.1.4.1) forming guanidinoacetate. This molecule is subsequently methylated by transferring the methyl group from the donor S-adenosyl-L-methionine, yielding creatine and S-adenosylhomocysteine in a reaction catalysed by guanidinoacetate-methyltransferase (GAMT; E.C. 2.1.1.2). Deficiencies in either of these enzyme activities cause developmental delay with mental retardation and language deficits (Fig. 1). GAMT deficiency (Stockler et al., 1994) results in a more severe phenotype than AGAT deficiency (Bianchi et al., 2000; Item et al., 2001), most probably due to the extra effects of elevated guanidinoacetate (GAA). These effects include intractable (to anti-epileptic medication) seizures and extrapyramidal motor signs. GAA is an antagonist at GABA_A receptors (Rae et al., 2015) as are other guanidino compounds, which are also elevated in GAMT-deficiency (Schulze et al., 2003). These particular symptoms can be reduced by restricting arginine intake and thus reducing GAA levels. Both deficiencies can be remediated by supplementation with significant amounts (300–400 mg/kg/day (Stöckler-Ipsiroglu et al., 2012)) of creatine.

* Corresponding author. Neuroscience Research Australia, Barker St Randwick, NSW 2031, Australia.

E-mail address: c.rae@unsw.edu.au (C.D. Rae).

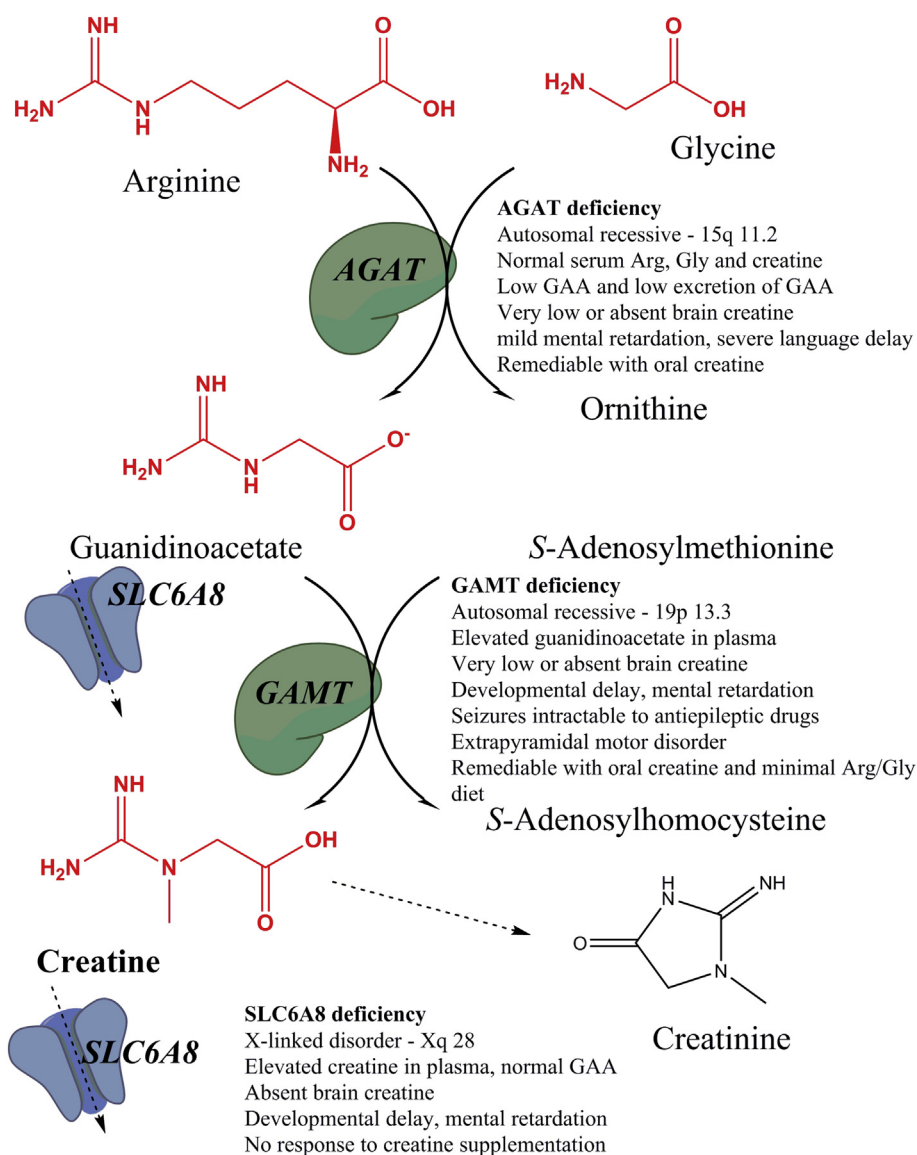


Fig. 1. Synthesis and uptake of guanidinoacetate and creatine and their deficiency syndromes. AGAT, L-arginine-glycine amidino transferase; GAMT, guanidinoacetate-methyltransferase; SLC6A8, solute carrier 6A8.

A third creatine deficiency disorder exists (Salomons et al., 2001) caused by mutations in the sodium-dependent creatine transporter, SLC6A8. While symptoms are in common with those caused by lack of creatine synthesising enzymes, the deficiency is mostly intractable to remediation with oral creatine or with precursors such as guanidinoacetate or combined arginine/glycine therapy (van de Kamp et al., 2012). Guanidinoacetate, the intermediate in creatine synthesis is also a substrate of SLC6A8.

Investigation of these disorders and why or why not they respond to creatine supplementation therapy has proven illuminating for our understanding of brain creatine synthesis and compartmentation in the brain. The creatine transporter SLC6A8 is an active, sodium-dependent creatine uptake mechanism; creatine concentrations in the brain are several fold higher than in plasma (40 μmol in plasma vs 6–12 mM in brain) so there is clearly active accumulation of creatine. However, supplementation with oral creatine has only limited effect on brain creatine; supplementation with 5 g/day for 6 weeks increased brain creatine by an average of only 11% in healthy young men (Dechent et al., 1999), while ~20 g/

day for 7 days followed by 2 g/day for 7 days resulted in increases of ~8% (Lyyo et al., 2003). Studies using a shorter supplementation time frame or doses lower than 5 g have tended to report no significant change in brain creatine levels (Rawson and Venezia, 2011). It has been suggested that the creatine transporter is near saturated meaning its ability to increase uptake under physiological conditions is limited. Transfer of creatine across the blood–brain and blood-CSF barrier appears to be limited (Braissant, 2012) and must be supplemented through creatine synthesis (Fig. 2).

Cells in the brain have been shown to cooperate in creatine synthesis (Fig. 2). While some cells in the brain have both synthesising enzymes and can make their own supply of creatine, some cells have only AGAT and export guanidinoacetate which is taken up via SLC6A8 by cells with GAMT and used to make creatine (Salomons et al., 2001; van de Kamp et al., 2012). Surprisingly GAMT is absent in many neurons (Tachikawa et al., 2004), while expressed at higher levels in oligodendrocytes and astrocytes. This suggests that glial cells may serve as local producers of creatine, which is then accumulated in neurons via SLC6A8. The bulk of brain

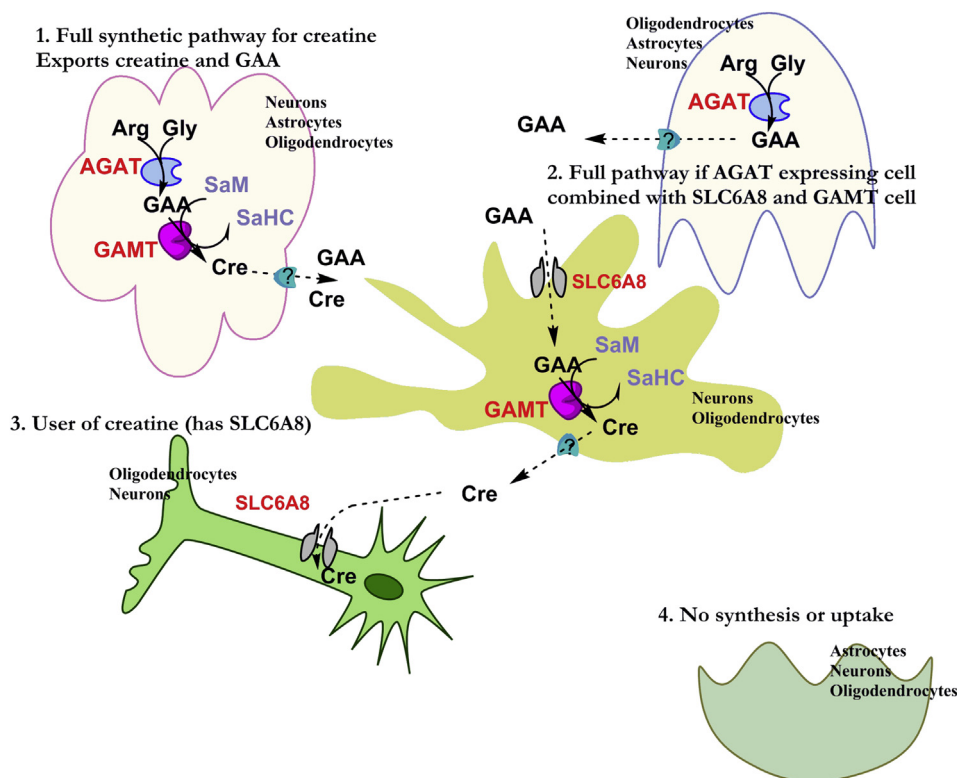


Fig. 2. Current understanding of metabolism, compartmentation and synthesis of creatine and its analogues in the brain. Creatine synthesis and uptake is different in subpopulations of cells in the brain (Béard and Braissant, 2010). Some neuronal, astrocytic and oligodendrocyte subpopulations contain both enzymes of the creatine synthesis pathway (designated 1 in the figure) AGAT (Arginine–glycine aminotransferase) and GAMT (Guanidinoacetate methyltransferase). Others, (2) possess only AGAT, and are therefore net producers of guanidinoacetate (GAA), or possess only GAMT and therefore need the creatine transporter SLC6A8 in order to take up the precursor GAA. A third subpopulation (3), possess none of the synthetic enzymes and are absolutely reliant on SLC6A8 to take up creatine produced by other cells (Type 1 or 2). A fourth subpopulation that does not possess any synthetic enzymes or transporters and does not use creatine (4). SaM; S-adenosylmethionine; SaHC, S-adenosylhomocysteine. Figure adapted from Rae (2014b).

SLC6A8 appears to be expressed in neurons (Mak et al., 2009), small amounts in endothelial cells of the blood–brain barrier, but it appears to be absent from astrocytes (Lowe et al., 2015).

This means that the ability to take up either guanidinoacetate or creatine is crucial to maintain brain creatine supplies even when brain creatine synthesis machinery is intact and explains why dysfunction of the creatine transporter SLC6A8 is so deleterious.

The route by which creatine effluxes from cells – as proposed for astrocytes – is still unclear (marked unknown in Fig. 2). It is plain that creatine effluxes from cells at a steady rate and that normally this efflux sustained by sodium-dependent uptake or by endogenous synthesis. Guanidinoacetate has been reported to leave the brain via the taurine transporter (Braissant, 2012). Other candidate efflux transporters might be the organic cation transporters (OCTs) which are known to transport creatine (hOCT2), a range of guanidine compounds and creatinine (Kimura et al., 2009). These transporters may also be responsible for creatine efflux from the basolateral membrane in the gut (Koepsell, 2004). A recently discovered creatine transporter, SLC16A12, or MCT12 is not expressed in the brain apart from eye (Abplanalp et al., 2013) and is unlikely to be a candidate.

Creatine is also known to efflux from brain following osmotic challenge (Bothwell et al., 2001) but the route by which it does this remains unclear. Astrocytes have been shown to have an efflux mechanism for creatine which is pharmacologically distinct from that for taurine (Bothwell et al., 2002).

It appears that under physiological conditions net import of creatine occurs through the blood brain barrier, while net efflux occurs through choroid plexus (Tachikawa et al., 2012). Expression

of SLC6A8 has been reported on the blood and brain side of blood brain barrier endothelial cells (Ohtsuki et al., 2002), however, for a net blood to brain flux a different exit route must be proposed. Local synthesis in astrocytes adds further creatine. The steady state amount of creatine is then accumulated into neurons through SLC6A8. Thus, there is an equilibrium between accumulation of creatine through SLC6A8 in the blood brain barrier and neurons and efflux of creatine/guanidinoacetate/creatinine from the brain through blood-CSF barriers, for instance via organic cation transporters. Therefore, lack of SLC6A8 would drain the brain of creatine.

1.2. Roles of creatine in the brain

The primary and best recognised role of creatine is as an acceptor of high energy phosphate in the reaction catalysed by creatine kinase (Eq. (1)).



Creatine kinase mediates fast exchange between the energy currency ATP and the phosphorylated form of creatine, phosphocreatine. The reaction also requires a proton and so may change the local pH under conditions of high ATP demand (Rango et al., 1997; Sappey-Marinié et al., 1992).

Phosphocreatine, through the creatine kinase-catalysed reaction, acts in concert with multiple ATP-producing and requiring reactions as a buffer for high energy phosphate bonds. The high energy phosphate bond in phosphocreatine has a higher free energy of hydrolysis than that in ATP (ΔG° kJ/mol = -45.0 cf. -31.8 , respectively; (Wyss and Kaddurah-Daouk, 2000)). Phosphocreatine

is a smaller, less negatively charged molecule than ATP, its diffusion to sites of demand is fast and creatine kinase can quickly interconvert the two. Given ample PCr, the creatine kinase reaction regenerates ATP at a rate 40 times faster than oxidative phosphorylation and 10 times faster than glycolysis (Walliman et al., 1992). Phosphocreatine therefore acts as a quickly accessible “Swiss Bank account” for energy currency, allowing cells to “hide” ATP in an accessible form.

The brain is an organ with high energy demands, using around 20% of resting metabolism in adults despite accounting for only 2% of actual mass (Attwell and Laughlin, 2001). It is an energetically expensive organ to run as a consequence and it has evolved to conserve energy with complex autoregulatory systems in place to rapidly upregulate metabolism once an area of the brain is activated (for detailed discussion of mechanisms and their limiting bounds see (Riera et al., 2008)). These include rapid increases in blood flow and blood volume to deliver the extra oxygen and glucose that is required to fuel brain energy demands. Inspection of the time courses of these regulatory mechanisms shows blood flow peaking several seconds following a stimulus with brain eventually reaching a new steady-state metabolism if the stimulus is continued (Mangia et al., 2007; Apšvalka et al., 2015). This means that energy is delivered to the brain as and when it is required, incurring significant energy savings, but the flipside to this is that the brain is not necessarily operating at maximal capacity when suddenly required to do a task. In the few seconds immediately following stimulation, brain physiological and biochemical responses are characterised by an “initial dip” (Fig. 3). Local brain oxygen supplies dip, as evidenced by reduction in the water signal on fMRI (Hu and Yacoub, 2012) caused by a relative increase in paramagnetic deoxyhaemoglobin (Grinvald et al., 1991). Local glucose levels also dip although the length of the dip and recovery are on a much longer time scale than the localised dip in oxygen levels (Silver and Erecinska, 1994). Studies using ^{31}P magnetic resonance spectroscopy (MRS), and also long echo time ^1H MRS which is susceptible to relative changes in the ratio of PCr to creatine (for discussion of measurement of creatine using MRS see (Rae, 2014b; Mountford et al., 2010)), have shown initial dips in the level of phosphocreatine in the occipital cortex following photic stimulation (Rango et al., 1997; Sappey-Mariniér et al., 1992; Ke et al., 2000; Kato et al., 1996) along with increased local pH, suggesting hydrolysis of PCr through the creatine kinase catalysed reaction (Eq. (1)). It is likely that the pH changes reflect the activity of cytosolic creatine kinase; MRS is spatially a relatively insensitive method and even less so with the ^{31}P nucleus. The relative volume of the cytosolic compartment compared to the mitochondrial one (around 250:1) means that it is unlikely that changes in mitochondrial pH in the absence of changes in cytosolic pH would be detectable (Wang et al., 2011).

Although it is generally accepted that PCr and the creatine kinase reaction buffer ATP levels to the point where they are barely altered by brain activity (Sappey-Mariniér et al., 1992) it is possible that they do initially alter in healthy people if the brain is stimulated hard from a rested state (Rae et al., 2002). It is also apparent that there are disorders and conditions where ATP levels do change with workload (Yuksel et al., 2015; Rae et al., 2013, 2009).

The different isoforms of creatine kinase are key (Fig. 4). Brain contains the cytosolic brain-type creatine kinase (BB-CK) (Eppenberger et al., 1967) and the ubiquitous mitochondrial creatine kinase. Each of these enzymes play key roles in controlling energy availability.

Cytosolic creatine kinase is a dimer that can be composed of muscle (M) or brain (B) type isoforms, creating three possible isozymes, the muscle specific MM-CK, the mixed MB-CK found, for example, in heart and the brain specific BB-CK (Walliman et al.,

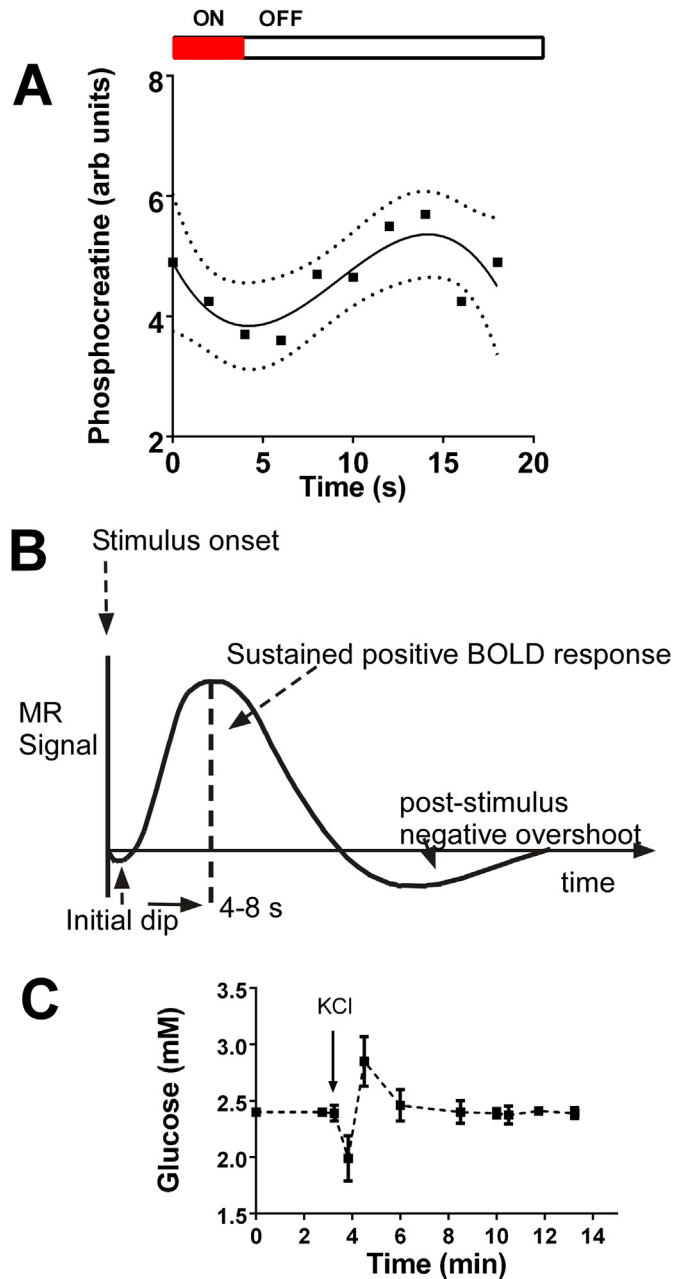


Fig. 3. Time courses of substrate availability following stimulation in brain. Panel A shows time course of phosphocreatine changes in occipital lobe following a 4s flashing checkerboard stimulus. Data were collected from a single human subject at 1.5 T using an 8 cm ^{31}P surface coil with one single transient collected every 2 s. The duty cycle (4 s 16 Hz photic stimulation followed by 26 s of rest) was repeated 8 times and spectra were binned to make a time course with 2 s resolution with each spectrum comprising 8 scans (Rae et al., 2002). Dotted lines show 95% confidence interval of the fitted function. Panel B shows the generic blood oxygen response to a stimulus detected using functional magnetic resonance imaging (adapted from Kornak et al. (2011)). Panel C shows the glucose concentration at a fixed point in the cerebral cortex of rat during and following a single wave of spreading depression (induced by application of KCl) (adapted from Silver and Erecinska (1994) with the permission of the authors).

1992). The cytosolic creatine kinase generates significant amounts of ATP upon brain activation and drives cognitive functions, dendrite formation and cell motility (Jost et al., 2002; Kuiper et al., 2009; In't Zandt et al., 2004; Streijger et al., 2005).

Mitochondrial creatine kinase is the key enzyme to generate PCr from freshly synthesised ATP. Octomeric mitochondrial creatine

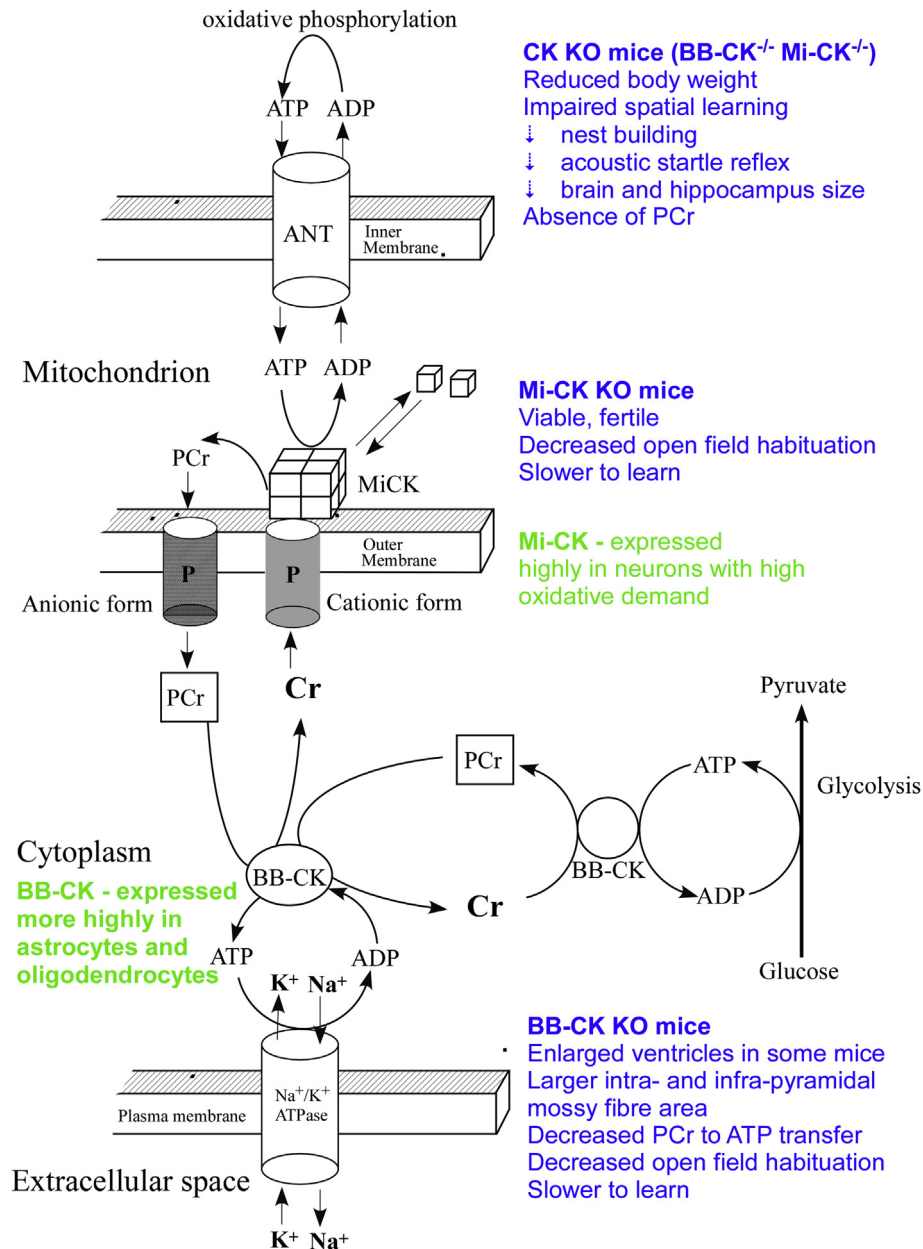


Fig. 4. Creatine, cytosolic and mitochondrial compartments, their respective creatine kinase isoforms and interacting enzymes. Cytosolic creatine kinase (CK) interacts with ATP produced via glycolysis. It acts as a rapid supplier of ATP for hydrolytic cytosolic reactions, including the Na⁺K⁺-ATPase. Mitochondrial creatine kinase (MiCK) forms a metabolon with a porin (a voltage-dependent anion channel), which when combined with mitochondrial creatine kinase prefers a cationic form favouring creatine influx. These proteins also combine with the adenine nucleotide translocase, ATP-synthaseF₀F₁ and an inorganic phosphate carrier. This metabolon is visible on electron micrographs (Kottke et al., 1994). Deletion of one or the other forms of CK has metabolic and behavioural consequences.

kinase assembles in a complex between inner and outer mitochondrial membranes. This includes adenine nucleotide translocase and a porin, which, when entered into the complex, favours transport of creatine over phosphocreatine. The porin also allows efflux of high energy phosphate bonds from the mitochondrion in the form of phosphocreatine, although ATP and ADP can also pass through porin in its anionic form. Creatine itself influences mitochondrial respiration rates, by altering the apparent K_M of the mitochondrial voltage-dependent anion channel for ADP (Monge et al., 2008). Thus it is involved in regulating oxidative phosphorylation (Holtzman et al., 1998, 1997) most likely through controlling the availability of ADP (Saks et al., 2000). So called “creatine stimulated respiration” has been observed in muscle (Kay et al.,

2000), heart mitochondria (Jacobus and Diffley, 1986) and in brain (Monge et al., 2008), likely through reduction in the dissociation constant for Mg-ATP with mitochondrial creatine kinase and creatine (Kay et al., 2000).

In addition to its role in increasing mitochondrial ATP production rates, creatine is also useful directly as an anti-oxidant, proving superior in some cases to glutathione (Lawler et al., 2002; Sestili et al., 2006).

Creatine has been suggested to be active at the GABA receptor (Almeida et al., 2006; Koga et al., 2005) although careful perusal of the original literature shows reported activity only for creatinine (the cyclic breakdown product) not creatine (de Deyn and Macdonald, 1990; Neu et al., 2002). The precursor,

guanidinoacetate, is an agonist at GABA-A receptors and an antagonist at GABA- ρ , in the latter case at least at physiologically relevant concentrations (Rae et al., 2015). Creatine may also be active at the receptor responding to the major excitatory neurotransmitter glutamate, the NMDA receptor (NMDAR). Creatine has been implicated in population spike amplitude modulation (Royes et al., 2008) and in anti-immobility effects in the tail suspension test (Cunha et al., 2015). The NMDAR polyamine binding site may be a possible site of activity (Oliveira et al., 2008). This has led to the suggestion that creatine is a neuromodulator, with release of creatine shown in response to electrical stimulation (Almeida et al., 2006); this release was blocked by absence of Ca^{2+} or by tetrodotoxin, and enhanced by blockade of K^{+} channels, consistent with neurotransmitter behaviour. Addition of creatine enhanced $\text{Na}^{+}\text{K}^{+}\text{ATPase}$ activity via an NMDA-calcineurin pathway (Rambo et al., 2012). Creatine is protective against glutamate toxicity, through a mechanism unrelated to its ability to inhibit the activation of the mitochondrial permeability transition pore (Klivenyi et al., 2004). In summary, the evidence that creatine modulates the NMDA receptor is moderately strong but has not yet been proven conclusively.

2. What is the evidence that creatine provides extra health benefits in the brain?

Creatine (8 g/day for 5 days, administered as 4×2 g each day) was given to 24 healthy young volunteers (24 ± 9.1 y) in a double blind placebo-controlled trial and their performance was assessed on a serial calculation task, where subjects perform mathematical calculations for 15 min, followed by a 5 min rest, then another 15 min of calculation. Performance on the latter 15 min gradually decreases in a manner that has been attributed to mental fatigue. The authors found that the performance decrement on the serial calculation task was decreased by creatine supplementation, indicating that the capacity of the brain to perform a repeated task was enhanced by supplementation (Watanabe et al., 2002). Similarly, 45 young adult vegetarians received 5 g/day of creatine for six weeks in a placebo-controlled double-blind cross-over trial, with testing of their ability at backward digit span (a test of working memory) and Raven's Advanced Progressive Matrices (Rae et al., 2003a). Creatine was found to significantly improve their abilities at both of these tests; in the case of the Raven's, equivalent to one standard deviation or around 15 IQ points. Although tapping different cognitive domains, both of these tasks rely on speed of processing, potentially addressing the energy requirements in the "initial dip". The finding of improved performance on backward digit span was subsequently confirmed in omnivores along with the observation of a decreased Blood Oxygen Level Dependent (BOLD) effect on functional magnetic resonance imaging (fMRI) following a week of creatine supplementation (20 g/day for 5 days followed by 5 g/day for 2 days) (Hammett et al., 2010). Conversely, a study giving ~2 g/day of creatine to 22 healthy young subjects (half received placebo) for six weeks showed no significant change in any of the cognitive domains that were measured, including memory and vigilance (Rawson et al., 2008).

In general, the strength of evidence for creatine having positive effects on brain function in healthy individuals is only moderate and it is plain that there is much further work to do. As pointed out above, transport across the blood brain barrier is limited, reducing the effectiveness of oral supplements. The optimal dosing regimen of creatine remains to be determined, practice effects should be controlled for (Rabbitt et al., 2001) and the studies need to be adequately powered. A cross-over design is helpful for controlling for baseline differences between groups and studies would also benefit from measuring baseline brain creatine and bioenergetics

levels to determine whether baseline bioenergetics status is a significant variable in whether or not creatine supplementation has any efficacy in healthy subjects. It is known that bioenergetic status predicts cognitive performance (Rae et al., 2003b). Vegetarians have been shown to have similar amounts of creatine in the brain as non-vegetarians (Solis et al., 2014), despite having lower muscle creatine (Delanghe et al., 1989).

Studies where baseline bioenergetic status was possibly compromised include a 2006 study (McMorris et al., 2006) which subjected 19 subjects to 24 h sleep deprivation from 10 am, with tests of cognitive, motor and mood function 6, 12 and 24 h into sleep deprivation. Half of the subjects ($N = 10$) had taken creatine (20 g/day in 4×5 g boluses for 7 days) and half ($N = 9$) placebo, with the study designed to examine the effects of creatine on performance after sleep deprivation. Significant effects of creatine pre-supplementation were seen only at 24 h and were confined to choice reaction time, improved perception of fatigue and vigour, and working memory (Table 1). Interestingly, 24 h sleep deprivation has been shown to have no significant effect on brain bioenergetics (Plante et al., 2014) although other authors have suggested that there may be individual differences in the response to sleep deprivation depending on baseline bioenergetics going into the period of sleep deprivation (Murashita et al., 1999) and the creatine kinase system has been shown to react differently according to the level of mental fatigue that is present (Kato et al., 1999). Certainly there is an energetic penalty to sleep deprivation that takes some days to recover (Plante et al., 2014; Rae, 2014a).

The same group of researchers also examined the effect of creatine supplementation in healthy elderly (McMorris et al., 2007). The creatine signal from the brain has been reported to increase with age, including increases in phosphocreatine and ATP (Rae et al., 2003b; Forester et al., 2010; Longo et al., 1993) suggesting that total brain bioenergetics capacity increases rather than decreases with age. With increased creatine improving brain function, the reverse has also been shown, where memory exercises have been shown to increase levels of creatine in the hippocampus of healthy elderly performing training in the Method of Loci task, involving delayed recall, spatial and associative memory training, compared to elderly subjects who were merely undertaking tasks of everyday living (Valenzuela et al., 2003).

The link between creatine and cognitive function in the ageing brain has been further explored in old mice (C57Bl/6J, 24 months of age) which were supplemented with 1% creatine in their standard rodent diet from 12 months of age. Creatine fed mice showed longer "healthy" life span (9%), and longer total life span compared to controls. They also showed better performance on object recognition and decreased latency in initiating exploration of a novel environment (Bender et al., 2008). The animal model also allowed exploration of molecular effects, with lower serum lactate in creatine fed animals. The authors also reported trends towards decreased accumulation of the ageing protein lipofuscin, the accumulation of which has been shown in numerous studies to be due to the oxidative alteration of macromolecules by oxygen-derived free radicals (Terman and Brunk, 1998), and decreased levels of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative damage to DNA (Bender et al., 2008).

3. Creatine supplementation in brain disorders

Creatine is not currently used as a routine supplement in any human brain disorder apart from deficiencies in creatine synthesis, although a number of clinical trials have been undertaken in a range of different disorders, with varying results. Hampering the trials is lack of information on the best dosage regime to increase brain creatine and an incomplete understanding of the longer term

Table 1
Outcomes of studies testing effects of creatine in healthy humans.

Supplement	Subjects	Tests	Outcome	Ref.
8 g/day for 5 days, administered as 4 × 2 g each day. Double blind placebo controlled	24 healthy volunteers (24 ± 9.1y)	Serial calculation task	Significant improvement	Watanabe et al. (2002)
5 g/day for 6 weeks	45 healthy vegetarians, 12, males, 33 females	Backward digit span	Significant improvement	Rae et al. (2003a)
Double-blind, placebo controlled cross-over design		Raven's advanced progressive matrices	Significant improvement	
20 g/day for 5 days followed by 5 g/day for 2 days	22 healthy volunteers Age not specified	Backward digit span	Significant improvement	Hammett et al. (2010)
		Raven's advanced progressive matrices	Non-significant improvement	
		BOLD response to flashing chequerboard	Significant decrease in BOLD response	
0.03 g/kg/day for 6 weeks	22 subjects (21 ± 2y)	Simple reaction time	No significant change in any of the tasks measured	Rawson et al. (2008)
Double-blind, placebo controlled		Code substitution (+delay)		
		Logical reasoning symbolic		
		Mathematical processing		
		Running memory		
		Sternberg memory recall		
20 g/day for 5 days as 4 × 5 g throughout day	121 female subjects 20.3 (SE 2.1) y	Word recall	Vegetarians showed sig improvement	Benton and Donohoe (2011)
Placebo controlled	51 omnivores 70 vegan/vegetarians	Reaction time	Omnivores got worse	
		Vigilance	No effect on reaction time.	
		Verbal fluency	Performance less variable on creatine	
			No effect	
			No effect	
5 g/day for 15 days of creatine ethylester	34 subjects 21 ± 1.38y	Memory scanning	Some significant improvements recorded, depending on the statistical test used	Ling et al. (2009)
Double-blind placebo controlled	No vegetarians	Number pair matching		
		Sustained attention		
		Arrow flankers		
		IQ (modified Raven's)		
5 g × 4/day for 7 days	19 subjects 21.1 ± 1.85y	Sleep deprivation with serial testing at 6, 12 and 24 h of:	Significant improvement at 24 h	McMorris et al. (2006)
		Working memory (random movement generation)	No significant differences	
		Forward/backward digit span	Significantly better at 24 h	
		Spatial memory (Corsi block)	Decreased fatigue and increased vigour at 24 h	
		Psychomotor (choice reaction time)		
		Mood state (POMS)		
7 days, 20 g/day (4 × 5 g)	15 subjects 31y (21–55y) 10 males 5 females	Trial of creatine in oxygen deprivation (10% O₂)	Creatine improved scores under hypoxia compared to placebo	Turner et al. (2015)
Randomised, double-blind, placebo controlled cross-over design		Complex attention	Increased under hypoxia compared to placebo	
		Motor cortical excitability	Not significant	
5 g creatine monohydrate × 4/day or placebo.	Healthy elderly	Tested at baseline, 7 days and 14 days, measured change in performance Δ:	Forward recall improved	McMorris et al. (2007)
Group 1 7 day placebo then 7 day creatine, group 2 14 day placebo.	76.4 (8.48y) N = 15 & 17.	Random number generation	Forward and backward recall improved	
		Forward/backward digit recall	Significant improvement	
		Spatial memory (Corsi block)		
		Delayed memory task		
4 × 5 g for 5 days then 5 g/day for total of 24 weeks.	Healthy elderly women N = 56 assigned to 4 groups (creatine Cr or placebo PL) with or without strength training ST.	Mini mental state exam	Not significant	Alves et al. (2013)
Randomised placebo-controlled, double blind trial of creatine with or without strength training.	Cr N = 13 PL N = 12 Cr + ST N = 12 PL + ST N = 10	Stroop	Not significant	
		Trail making	Not significant	
		Digit span	Not significant	
		Delay recall	Not significant	
		Geriatric depression index	Not significant for creatine but improvement with strength training.	

effects of creatine supplementation on endogenous creatine synthesis and uptake.

4. Neurodegenerative disorders

Creatine, when trialled in human neurodegenerative disorders, has not lived up to the potential displayed in animal models (Table 2). A recent large Phase III trial of 10 g/day in Parkinson's disease was discontinued due to futility (Writing Group for the

N.E.T.i.P.D.I, 2015). Trials of creatine supplementation in Huntington's disease have also proven disappointing (Tabrizi et al., 2003; Verbessem et al., 2003) although creatine use does reduce markers of oxidative damage (Hersch et al., 2006) and lowers glutamate/glutamine levels (Bender et al., 2005). Several possible explanations for the differences between mice and men have been essayed. These include the fact that the human trials did not use as much creatine as trials in mice and failure to fully understand the mechanisms of each disease (Adhihetty and Beal, 2008). The trials

Table 2

Outcomes of studies testing effects of creatine in brain disorders.

Supplement	Subjects	Tests	Outcome	Ref
2 year study 20 g/day for 6 days, then 2 g/day for 6 months, then 4 g/day for total time of 2 years. Placebo controlled, randomised	Parkinson's disease N = 60 40 received creatine, 20 placebo. Study finished with N = 31 and 17, respectively	SPECT Dopamine binding UPDRS Increase over time in dopaminergic therapy	No significant change No significant change No significant change overall - depression score significantly improved in creatine group Threefold smaller increase in dopaminergic therapy over 2 years in creatine group	Bender et al. (2006)
10 g/day for minimum of 5 years. Double-blind, parallel group placebo controlled trial.	Parkinson's disease N = 1741 within 5 years of Parkinson's disease diagnosis. Placebo, 61.5 (9.6) years Creatine, 62.1 (9.7) years.	Global statistical test using 5 measures of Parkinson's disease progression	No change in primary or secondary outcome measures. Trial terminated due to futility	Writing Group for the N.E.T.i.P.D.I (2015)
1 year study 5 g/day (1 g, 3 g then 1 g with each of the three meals) Placebo controlled. Assigned by independent investigator	Huntington's disease. N = 26 creatine (50.1 ± 2.6y) and N = 15 placebo (49.6 ± 1.9y)	UPDRS Battery of cognitive tests	No significant effect of creatine was found	Verbessem et al. (2003)
16 week tolerability trial 8 g/day (2 × 4 g each day) for 16 weeks. Randomised, placebo-controlled.	Huntington's disease. N = 64 Creatine age 44.7, placebo 47.9y.	UPDRS Brain creatine levels Serum 8OH2'dG levels	No change ↑ 13% occipital lobe ↑ 7.5% frontal lobe Significantly less than placebo	Hersch et al. (2006)
10 month trial 10 g/day for 10 months	Huntington's disease. N = 13 creatine, N = 4 spousal controls	Total motor score UPDRS Brain creatine levels	No change No change Significantly elevated	Tabrizi et al. (2003)
8–10 weeks creatine (20 g/day for 5 days) then 6/day with none on Sundays (to prevent augmentation)	Huntington's disease. N = 16	¹ H MRS (Glx resonance) UPDRS MMSE	Significantly lower compared to baseline No change No change	Bender et al. (2005)
10 week trial of creatine vs placebo as augment for escitalopram 3 g/day for one week then 5 g/day for 7 weeks	Major depressive disorder Escitalopram + either: N = 25 creatine 45.7 (127)y N = 27 placebo 47.5 (9.5)y All female	Hamilton depression score Montgomery–Åsberg depression rating scale	Significant improvement over placebo as early as week 2 Significant improvement over placebo as early as week 2	Lyoo et al. (2012)
3 month randomised cross over trial 3 –5 g/day creatine or placebo	Schizophrenia N = 10, 7 males, 5 females 42.8 ± 8y.	Positive and negative syndrome scale (PANSS) Clinical global impressions Cognitive test battery	No significant effect No significant effect No significant effect	Kaptsan et al. (2007)
Open label trial 3 g/day for one week then 5 g/day for 3 weeks.	Post traumatic stress disorder N = 10	Clinician administered PTSD scale Hamilton rating scales for depression & anxiety Clinical global impressions Sleep quality scale Sheehan disability scale	Improvement in all scores, intrusiveness most improved, avoidance the least. Significant improvement No improvement Significant improvement Improved	Amital et al. (2006)
Open label trial of creatine, randomised to study and control groups 0.4 g/kg/day for 6 months or nothing	Traumatic brain injury N = 39, children aged 1–18y	Clinical outcomes Headaches Dizziness Fatigue	No significant change Improvements seen in several variables Significantly less Significantly less Significantly less	Sakellaris et al. (2006, 2008)

in Parkinson's disease which did show some promise were conducted in early stage Parkinson's disease, while the Phase III trial was in patients whose disease was further progressed. Given the extent of damage to the brain that has already occurred in early diagnosed Parkinson's disease (Halliday et al., 2011), it might be more efficacious to trial use of creatine in as yet undiagnosed patients who are at higher risk of Parkinson's disease (Lerche et al., 2014).

Creatine supplementation has been suggested as possibly efficacious in early Alzheimer disease (Wyss and Schulze, 2002); decline of brain creatine levels is associated with conversion from mild cognitive impairment to dementia (Pilatus et al., 2009) and there are known mitochondrial deficits and decrease of BB-CK in frontal lobe in Alzheimer disease (Burbaeva et al., 1992). To date, no trial of creatine in the dementias has been published.

5. Creatine in mental health

Bioenergetic abnormalities are well documented in depression (Moore et al., 1997). They are related to the severity of the depression (Kondo et al., 2011) and are normalised following treatment (Iosifescu et al., 2008). Direct current stimulation, an emerging treatment for major depression, has been shown to modulate brain bioenergetics, with the degree of response related to baseline bioenergetics status (Rae et al., 2013).

Creatine supplementation has shown some promise in treatment of major depressive disorder (Table 2). Several small open label trials have shown promise (Kondo et al., 2011; Roitman et al., 2007) and a randomised 6 week trial of creatine or placebo as an augmentation to escitalopram (serotonin transporter inhibitor (Chen et al., 2005)) showed significant improvements in depression scores as early as two weeks after consumption (Lyoo et al., 2012).

Work in animals has shown a gender effect, with female rats responding to creatine in an anti-depressant type fashion (Allen et al., 2012). Reflecting this, and the tendency of females to suffer more from depression (Piccinelli and Wilkinson, 2000), trials in humans have largely focussed on females.

A small open label trial of 4 weeks creatine supplementation in patients with chronic post-traumatic stress disorder showed significant improvements in ratings of disability, including improvement in sleep quality. The largest improvements were seen in those diagnosed with comorbid depression (Amital et al., 2006).

Creatine did not show efficacy in a small trial of persons with schizophrenia (Kaptan et al., 2007). It is not possible to say from this work whether creatine may or may not have effects in this disorder. Bioenergetic abnormalities have been inconsistently reported in schizophrenia (reviewed in Potwarka et al., (1999)) and, as schizophrenia is a heterogeneous disorder, it is possible that a subset of patients may benefit.

6. Creatine and neuroprotection

There is considerable evidence in animals for neuroprotective effects of creatine against traumatic injury (Sullivan et al., 2000), in ischaemia and in stroke. Creatine has been trialled as an open-label administration of 0.4 g/kg/day in children with traumatic brain injury, with improvements recorded in several clinical indices (Sakellaris et al., 2006) and also in reported headaches, dizziness and fatigue scales (Sakellaris et al., 2008).

7. Areas for translation to the clinic of creatine therapies

Repetitive collapse of the upper airway during obstructive sleep apnea/hypopnea (OSA) exposes the brain of sufferers to frequent, transient, hypoxic episodes. Creatine levels, which are significantly lower in the hippocampus of those with OSA (Bartlett et al., 2004) have been shown to be neurobiomarkers of disease severity and also of cognitive impairment (Bartlett et al., 2004). Sufferers also show altered bioenergetics response to the hypopnea associated with apnea. Extended hypoxia (12% O₂, %satO₂ = 86%) in healthy controls has been shown to have negligible impact on brain bioenergetics as measured with ³¹P magnetic resonance spectroscopy (Vidyasagar and Kauppinen, 2008) but under similar levels of transient hypoxia during apnea, OSA sufferers display large increases in inorganic phosphate with decreases in ATP. Somewhat surprisingly, the creatine kinase system, which in this case is most probably the cytosolic creatine kinase system, played no role, with no significant alteration in brain pH or in phosphocreatine (Rae et al., 2009).

Creatine (and phosphocreatine) levels are known to be low in muscle of those with OSA and are increased by treatment with continuous positive airway pressure (CPAP) (Trenell et al., 2007). A trial of creatine vs placebo on cognitive tasks undertaken in hypoxia (SpO₂ reduced by 19%) showed significant effects of creatine on tasks of complex attention in particular (Table 1). Scores of executive function and cognitive flexibility were improved by creatine although the difference did not reach statistical significance (Turner et al., 2015). These are tasks which are typically impaired in OSA (El-Ad and Lavie, 2005). Taken together, this suggests that creatine may be of benefit in OSA, particularly for the 50% of OSA sufferers who do not tolerate CPAP therapy.

8. Conclusion

In summary, the evidence for creatine as a nutraceutical for the brain is supportive of its use for cognitive enhancement, particularly in conditions where baseline bioenergetics are less than

optimal. Further trials are needed in neurodegenerative conditions, particularly at early stage when creatine may help to retard decline. Creatine may be of use in depression and depression-related disorders. Much work is still to be done in ischaemia, hypoxia and stroke and the potential of creatine has yet to be tested in obstructive sleep apnea.

We have yet to see the full potential of von Liebig's discovery.

Acknowledgements

This work was supported by a grant from the Australian NHMRC (Fellowship to CR #630516).

References

- Abplanalp, J., Laczkó, E., Philp, N.J., et al., 2013. The cataract and glucosuria associated monocarboxylate transporter MCT12 is a new creatine transporter. *Hum. Mol. Genet.* 22, 3218–3226.
- Adhihetty, P.J., Beal, M.F., 2008. Creatine and its potential therapeutic value for targeting cellular energy impairment in neurodegenerative diseases. *Neuromol. Med.* 10, 275–290.
- Allen, P.J., D'Anci, K.E., Kanarek, R.B., et al., 2012. Sex-specific antidepressant effects of dietary creatine with and without sub-acute fluoxetine in rats. *Pharmacol. Biochem. Behav.* 101, 588–601.
- Almeida, L.S., Salomons, G.S., Hogenboom, R., et al., 2006. Exocytotic release of creatine in rat brain. *Synapse* 60, 118–123.
- Alves, C.R.R., Mereghe Filho, C.A.A., Benatti, F.B., et al., 2013. Creatine supplementation associated or not with strength training upon emotional and cognitive measures in older women: a randomized double-blind study. *PLoS ONE* 8, e76301.
- Amital, D., Vishne, T., Roitman, S., et al., 2006. Open study of creatine monohydrate in treatment-resistant posttraumatic stress disorder. *J. Clin. Psychiatry* 67, 836–837.
- Apšvalka, D., Gadie, A., Clemence, M., et al., 2015. Event-related dynamics of glutamate and BOLD effects measured using functional magnetic resonance spectroscopy (fMRS) at 3T in a repetition suppression paradigm. *NeuroImage* 118, 292–300.
- Attwell, D., Laughlin, S.B., 2001. An energy budget for signaling in the grey matter of the brain. *J. Cereb. Blood Flow Metab.* 21, 1133–1145.
- Bartlett, D., Rae, C., Thompson, C.H., et al., 2004. Hippocampal area metabolites relate to severity and cognitive function in obstructive sleep apnea. *Sleep Med.* 5, 593–596.
- Béard, E., Braissant, O., 2010. Synthesis and transport of creatine in the CNS: importance for cerebral functions. *J. Neurochem.* 115, 297–313.
- Bender, A., Koch, W., Elstner, M., et al., 2006. Creatine supplementation in Parkinson disease: a placebo-controlled randomized pilot trial. *Neurology* 67, 1262–1264.
- Bender, A., Beckers, J., Schneider, I., et al., 2008. Creatine improves health and survival of mice. *Neurobiol. Aging* 29, 1404–1411.
- Bender, A., Auer, D., Merl, T., et al., 2005. Creatine supplementation lowers brain glutamate levels in Huntington's disease. *J. Neurol.* 252, 36–41.
- Benton, D., Donohoe, R., 2011. The influence of creatine supplementation on the cognitive functioning of vegetarians and omnivores. *Br. J. Nutr.* 105, 1100–1105.
- Bianchi, M.C., Tosetti, M., Fornai, F., et al., 2000. Reversible brain creatine deficiency in two sisters with normal blood creatine level. *Ann. Neurol.* 47, 511–513.
- Bothwell, J.H., Rae, C., Dixon, R.M., et al., 2001. Hypo-osmotic swelling activated release of organic osmolytes in brain slices: implications for brain oedema in vivo. *J. Neurochem.* 77, 1632–1640.
- Bothwell, J.H., Styles, P., Bhakoo, K.K., 2002. Swelling-activated taurine and creatine effluxes from rat cortical astrocytes are pharmacologically distinct. *J. Membr. Biol.* 185, 157–164.
- Braissant, O., 2012. Creatine and guanidinoacetate transport at blood-brain and blood-cerebrospinal fluid barriers. *J. Inher. Metab. Dis.* 35, 655–664.
- Burbaeva, G.S., Aksenova, M., Makarenko, I., 1992. Decreased level of creatine kinase BB in the frontal cortex of Alzheimer patients. *Dement. Geriatr. Cognitive Disord.* 3, 91–94.
- Chen, F., Larsen, M.B., Sánchez, C., et al., 2005. The S-enantiomer of R, S-citalopram, increases inhibitor binding to the human serotonin transporter by an allosteric mechanism. Comparison with other serotonin transporter inhibitors. *Eur. Neuropsychopharmacol.* 15, 193–198.
- Cunha, M.P., Pazini, F.L., Ludka, F.K., et al., 2015. The modulation of NMDA receptors and L-arginine/nitric oxide pathway is implicated in the anti-immobility effect of creatine in the tail suspension test. *Amino Acids* 47, 795–811.
- de Deyn, P.P., Macdonald, R.L., 1990. Guanidino compounds that are increased in cerebrospinal fluid and brain of uremic patients inhibit GABA and glycine responses on mouse neurons in cell culture. *Ann. Neurol.* 28, 627–633.
- Dechent, P., Pouwels, P.J.W., Wilken, B., et al., 1999. Increase of total creatine in human brain after oral supplementation of creatine-mono-hydrate. *Am. J. Physiol.* 277, R698–R704.
- Delange, J., De Slypere, J., De Buyzere, M., et al., 1989. Normal reference values for creatine, creatinine, and carnitine are lower in vegetarians. *Clin. Chem.* 35,

- 1802–1803.
- El-Ad, B., Lavie, P., 2005. Effect of sleep apnea on cognition and mood. *Int. Rev. Psychiatry* 17, 277–282.
- Eppeberger, H.M., Dawson, D.M., Kaplan, N.O., 1967. The comparative enzymology of creatine kinases: I. Isolation and characterisation from chicken and rabbit tissues. *J. Biol. Chem.* 242, 204–209.
- Forester, B.P., Berlow, Y.A., Harper, D.G., et al., 2010. Age-related changes in brain energetics and phospholipid metabolism. *NMR Biomed.* 23, 242–250.
- Grinvald, A., Frostig, R.D., Siegel, R.M., et al., 1991. High-resolution optical imaging of functional brain architecture in the awake monkey. *Proc. Natl. Acad. Sci. U. S. A.* 88, 11559–11563.
- Halliday, G., Lees, A., Stern, M., 2011. Milestones in Parkinson's disease—clinical and pathologic features. *Mov. Disord.* 26, 1015–1021.
- Hammett, S.T., Wall, M.B., Edwards, T.C., et al., 2010. Dietary supplementation of creatine monohydrate reduces the human fMRI BOLD signal. *Neurosci. Lett.* 479, 201–205.
- Hersch, S.M., Gevorkian, S., Marder, K., et al., 2006. Creatine in Huntington disease is safe, tolerable, bioavailable in brain and reduces serum 8OH²/dG. *Neurology* 66, 250–252.
- Holtzman, D., Meyers, R., Ogorman, E., et al., 1997. In vivo brain phosphocreatine and ATP regulation in mice fed a creatine analog. *Am. J. Physiol.-Cell Physiol.* 41, C1567–C1577.
- Holtzman, D., Brown, M., Ogorman, E., et al., 1998. Brain ATP metabolism in hypoxia resistant mice fed guanidinopropionic acid. *Dev. Neurosci.* 20, 469–477.
- Hu, X., Yacoub, E., 2012. The story of the initial dip in fMRI. *Neuroimage* 62, 1103–1108.
- In't Zandt, H.J.A., Renema, W.K.J., Streijger, F., et al., 2004. Cerebral creatine kinase deficiency influences metabolite levels and morphology in the mouse brain: a quantitative in vivo ¹H and ³¹P magnetic resonance study. *J. Neurochem.* 90, 1321–1330.
- Iosifescu, D.V., Bolo, N.R., Nierenberg, A.A., et al., 2008. Brain bioenergetics and response to triiodothyronine augmentation in major depressive disorder. *Biol. Psychiatry* 63, 1127–1134.
- Item, C.B., Stöckler-Ipsiroglu, S., Stromberger, C., et al., 2001. Arginine:Glycine amidinotransferase deficiency: the third inborn error of creatine metabolism in humans. *Am. J. Hum. Genet.* 69, 1127–1133.
- Jacobus, W., Diffley, D., 1986. Creatine kinase of heart mitochondria. Control of oxidative phosphorylation by the extramitochondrial concentrations of creatine and phosphocreatine. *J. Biol. Chem.* 261, 16579–16583.
- Jost, C.R., der Zee, V., Catharina, E., et al., 2002. Creatine kinase B-driven energy transfer in the brain is important for habituation and spatial learning behaviour, mossy fibre field size and determination of seizure susceptibility. *Eur. J. Neurosci.* 15, 1692–1706.
- Kaptsan, A., Odessky, A., Osher, Y., et al., 2007. Lack of efficacy of 5 grams daily of creatine in schizophrenia: a randomized, double-blind, placebo-controlled trial. *J. Clin. Psychiatry* 68, 881–884.
- Kato, T., Murashita, J., Shioiri, T., et al., 1996. Effect of photic stimulation on energy metabolism in human brain measured by ³¹P-MR spectroscopy. *J. Neuropsychiatry Clin. Neurosci.* 8, 417–422.
- Kato, T., Murashita, J., Shioiri, T., et al., 1999. Relationship of energy metabolism detected by P-31 MRS in human brain with mental fatigue. *Neuropsychobiology* 39, 214–218.
- Kay, L., Nicolay, K., Wieringa, B., et al., 2000. Direct evidence for the control of mitochondrial respiration by mitochondrial creatine kinase in oxidative muscle cells in situ. *J. Biol. Chem.* 275, 6937–6944.
- Ke, Y., Cohen, B.M., Bang, J.Y., et al., 2000. Assessment of GABA concentration in human brain using two-dimensional proton magnetic resonance spectroscopy. *Psychiatry Res.: Neuroimaging* 100, 169–178.
- Kimura, N., Masuda, S., Katsura, T., et al., 2009. Transport of guanidine compounds by human organic cation transporters, hOCT1 and hOCT2. *Biochem. Pharmacol.* 77, 1429–1436.
- Klivenyi, P., Calingasan, N.Y., Starkov, A., et al., 2004. Neuroprotective mechanisms of creatine occur in the absence of mitochondrial creatine kinase. *Neurobiol. Dis.* 15, 610–617.
- Koepsell, H., 2004. Polyspecific organic cation transporters: their functions and interactions with drugs. *Trends Pharmacol. Sci.* 25, 375–381.
- Koga, Y., Takahashi, H., Oikawa, D., et al., 2005. Brain creatine functions to attenuate acute stress responses through gabaergic system in chicks. *Neuroscience* 132, 65–71.
- Kondo, D.G., Sung, Y.H., Hellem, T.L., et al., 2011. Open-label adjunctive creatine for female adolescents with SSRI-resistant major depressive disorder: a ³¹-phosphorus magnetic resonance spectroscopy study. *J. Affect. Disord.* 135, 354–361.
- Kornak, J., Hall, D.A., Haggard, M.P., 2011. Spatially extended fMRI signal response to stimulus in non-functionally relevant regions of the human brain: preliminary results. *Open Neuroimaging J.* 5, 24–32.
- Kottke, M., Wallimann, T., Brdiczka, D., 1994. Dual electron microscopic localization of mitochondrial creatine kinase in brain mitochondria. *Biochem. Med. Metab. Biol.* 51, 105–117.
- Kuiper, J.W.P., van Horssen, R., Oerlemans, F., et al., 2009. Local ATP generation by brain-type creatine kinase (CK-B) facilitates cell motility. *PLoS ONE* 4, e5030.
- Lawler, J.M., Barnes, W.S., Wu, G., et al., 2002. Direct antioxidant properties of creatine. *Biochem. Biophys. Res. Commun.* 290, 47–52.
- Lerche, S., Seppi, K., Behnke, S., et al., 2014. Risk factors and prodromal markers and the development of Parkinson's disease. *J. Neurol.* 261, 180–187.
- Liebig, J., 1847. Kreatin und Kreatinin, Bestandtheile des Harns der Menschen. *J. für Prakt. Chem.* 40, 288–292.
- Ling, J., Kritikos, M., Tiplady, B., 2009. Cognitive effects of creatine ethyl ester supplementation. *Behav. Pharmacol.* 20, 673–679.
- Longo, R., Ricci, C., Dalla Palma, L., et al., 1993. Quantitative ³¹P MRS of the normal adult human brain. Assessment of interindividual differences and ageing effects. *NMR Biomed.* 6, 53–57.
- Lowe, M.T., Faull, R.L., Christie, D.L., et al., 2015. Distribution of the creatine transporter throughout the human brain reveals a spectrum of creatine transporter immunoreactivity. *J. Comp. Neurol.* 523, 699–725.
- Lyoo, I.K., Kong, S.W., Sung, S.M., et al., 2003. Multinuclear magnetic resonance spectroscopy of high-energy phosphate metabolites in human brain following oral supplementation of creatine-monohydrate. *Psychiatry Res.: Neuroimaging* 123, 87–100.
- Lyoo, I.K., Yoon, S., Kim, T.S., et al., 2012. A randomized, double-blind placebo-controlled trial of oral creatine monohydrate augmentation for enhanced response to a selective serotonin reuptake inhibitor in women with major depressive disorder. *Am. J. Psychiatry* 169, 937–945.
- Mak, C., Waldvogel, H., Dodd, J., et al., 2009. Immunohistochemical localisation of the creatine transporter in the rat brain. *Neuroscience* 163, 571–585.
- Mangia, S., Tkac, I., Gruetter, R., et al., 2007. Sustained neuronal activation raises oxidative metabolism to a new steady-state level: evidence from H-1 NMR spectroscopy in the human visual cortex. *J. Cereb. Blood Flow Metab.* 27, 1055–1063.
- McMorris, T., Harris, R.C., Swain, J., et al., 2006. Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. *Psychopharmacology* 185, 93–103.
- McMorris, T., Mielcarz, G., Harris, R.C., et al., 2007. Creatine supplementation and cognitive performance in elderly individuals. *Aging Neuropsychol. Cognit.* 14, 517–528.
- Monge, C., Beraud, N., Kuznetsov, A.V., et al., 2008. Regulation of respiration in brain mitochondria and synaptosomes: restrictions of ADP diffusion in situ, roles of tubulin, and mitochondrial creatine kinase. *Mol. Cell. Biochem.* 318, 147–165.
- Moore, C.M., Christensen, J.D., Lafer, B., et al., 1997. Lower levels of nucleoside triphosphate in the basal ganglia of depressed subjects: a phosphorus-31 magnetic resonance spectroscopy study. *Am. J. Psychiatry* 154, 116–118.
- Mountford, C.E., Stanwell, P., Lin, A., et al., 2010. Neurospectroscopy: the past, present and future. *Chem. Rev.* 110, 3060–3086.
- Murashita, J., Yamada, N., Kato, T., et al., 1999. Effects of sleep deprivation: the phosphorus metabolism in the human brain measured by ³¹P-magnetic resonance spectroscopy. *Psychiatry Clin. Neurosci.* 53, 199–201.
- Neu, A., Neuhoff, H., Trube, G., et al., 2002. Activation of GABA_A receptors by guanidinoacetate: a novel pathophysiological mechanism. *Neurobiol. Dis.* 11, 298–307.
- Ohtsuki, S., Tachikawa, M., Takanaga, H., et al., 2002. The Blood–Brain barrier creatine transporter is a major pathway for supplying creatine to the brain. *J. Cereb. Blood Flow Metab.* 22, 1327–1335.
- Oliveira, M.S., Furian, A.F., Figuera, M.R., et al., 2008. The involvement of the polyamines binding sites at the NMDA receptor in creatine-induced spatial learning enhancement. *Behav. Brain Res.* 187, 200–204.
- Orsenigo, M., Faelli, A., De Biasi, S., et al., 2005. Jejunal creatine absorption: what is the role of the basolateral membrane? *J. Membr. Biol.* 207, 183–195.
- Piccinelli, M., Wilkinson, G., 2000. Gender differences in depression. *Br. J. Psychiatry* 177, 486–492.
- Pilatus, U., Lais, C., de Rochmont, A.D.M., et al., 2009. Conversion to dementia in mild cognitive impairment is associated with decline of N-acetylaspartate and creatine as revealed by magnetic resonance spectroscopy. *Psychiatry Res. Neuroimaging* 173, 1–7.
- Plante, D.T., Trksak, G.H., Jensen, J.E., et al., 2014. Gray matter-specific changes in brain bioenergetics after acute sleep deprivation: a ³¹P magnetic resonance spectroscopy study at 4 Tesla. *Sleep* 37, 1919–1927.
- Potwarka, J.J., Drost, D.J., Williamson, P.C., et al., 1999. A ¹H-decoupled ³¹P chemical shift imaging study of medicated schizophrenic patients and healthy controls. *Biol. Psychiatry* 45, 687–693.
- Rabbitt, P., Diggle, P., Smith, D., et al., 2001. Identifying and separating the effects of practice and of cognitive ageing during a large longitudinal study of elderly community residents. *Neuropsychologia* 39, 532–543.
- Rae, C., 2014a. The energetic cost of a night on the town. *Sleep* 37, 1881–1882.
- Rae, C.D., 2014b. A guide to the function and metabolic pathways of metabolites observed in human brain ¹H magnetic resonance spectra. *Neurochem. Res.* 39, 1–36.
- Rae, C., Bates, T.C., Huard, B., et al., 2002. Real time response of brain phosphocreatine to a 4s workload determined by ³¹P fMRS. *Proc. Aust. Neurosci. Soc.* 13, 140.
- Rae, C., Digney, A.L., McEwan, S.R., et al., 2003. Oral creatine monohydrate supplementation improves cognitive performance: a placebo-controlled, double blind, cross-over trial. *Proc. R. Soc. Lond.-Ser. B Biol. Sci.* 270, 2147–2150.
- Rae, C., Scott, R.B., Lee, M.A., et al., 2003. Brain bioenergetics and cognitive ability. *Dev. Neurosci.* 25, 324–331.
- Rae, C., Bartlett, D., Yang, Q., et al., 2009. Dynamic changes in brain bioenergetics during obstructive sleep apnea. *J. Cereb. Blood Flow Metab.* 29, 1421–1428.
- Rae, C., Lee, V.H.-C., Ordridge, R.J., et al., 2013. Anodal transcranial direct current stimulation increases brain intracellular pH and modulates bioenergetics. *Int. J. Neuropsychopharmacol.* 16, 1695–1706.

- Rae, C., Nasrallah, F., Balcar, V., et al., 2015. Metabolomic approaches to defining the role(s) of GABA_A receptors in the brain. *J. Neuroimmune Pharmacol.* 1–12.
- Rambo, L.M., Ribeiro, L.R., Schramm, V.G., et al., 2012. Creatine increases hippocampal Na⁺, K⁺-ATPase activity via NMDA-calcineurin pathway. *Brain Res. Bull.* 88, 553–559.
- Rango, M., Castelli, A., Scarlato, G., 1997. Energetics of 3.5 s neural activation in humans: a ³¹P spectroscopy study. *Magn. Reson. Med.* 38, 878–883.
- Rawson, E.S., Venezia, A.C., 2011. Use of creatine in the elderly and evidence for effects on cognitive function in young and old. *Amino Acids* 40, 1349–1362.
- Rawson, E.S., Lieberman, H.R., Walsh, T.M., et al., 2008. Creatine supplementation does not improve cognitive function in young adults. *Physiol. Behav.* 95, 130–134.
- Riera, J.J., Schousboe, A., Waagepetersen, H.S., et al., 2008. The micro-architecture of the cerebral cortex: functional neuroimaging models and metabolism. *Neuroimage* 40, 1436–1459.
- Roitman, S., Green, T., Osher, Y., et al., 2007. Creatine monohydrate in resistant depression: a preliminary study. *Bipolar Disord.* 9, 754–758.
- Royes, L.F., Figuera, M.R., Furian, A.F., et al., 2008. Neuromodulatory effect of creatine on extracellular action potentials in rat hippocampus: role of NMDA receptors. *Neurochem. Int.* 53, 33–37.
- Sakellaris, G., Kotsiou, M., Tamiolaki, M., et al., 2006. Prevention of complications related to traumatic brain injury in children and adolescents with creatine administration: an open label randomized pilot study. *J. Trauma Acute Care Surg.* 61, 322–329.
- Sakellaris, G., Nasis, G., Kotsiou, M., et al., 2008. Prevention of traumatic headache, dizziness and fatigue with creatine administration. A pilot study. *Acta Paediatr.* 97, 31–34.
- Saks, V.A., Kongas, O., Vendelin, M., et al., 2000. Role of the creatine/phosphocreatine system in the regulation of mitochondrial respiration. *Acta Physiol. Scand.* 168, 635–641.
- Salomons, G.S., van Dooren, S.J.M., Verhoeven, N.M., et al., 2001. X-linked creatine-transporter gene (SLC6A8) defect: a new creatine-deficiency syndrome. *Am. J. Hum. Genet.* 68, 1497–1500.
- Sappey-Marinié, D., Calabrese, G., Fein, G., et al., 1992. Effect of photic stimulation on human visual cortex lactate and phosphates using ¹H and ³¹P magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab.* 12, 584–592.
- Schulze, A., Bachert, P., Schlemmer, H., et al., 2003. Lack of creatine in muscle and brain in an adult with GAMT deficiency. *Ann. Neurol.* 53, 248–251.
- Sestili, P., Martinelli, C., Bravi, G., et al., 2006. Creatine supplementation affords cytoprotection in oxidatively injured cultured mammalian cells via direct antioxidant activity. *Free Radic. Biol. Med.* 40, 837–849.
- Silver, I.A., Erecinska, M., 1994. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo- and hyperglycaemic animals. *J. Neurosci.* 14, 5068–5076.
- Solis, M.Y., Painelli, V.D., Artioli, G.G., et al., 2014. Brain creatine depletion in vegetarians? A cross-sectional ¹H-¹MRS study. *Br. J. Nutr.* 111, 1272–1274.
- Stockler, S., Holzbach, U., Hanefeld, F., et al., 1994. Creatine deficiency in the brain: a new, treatable inborn error of metabolism. *Pediatr. Res.* 36, 409–413.
- Stöckler-Ipsiroglu, S., Mercimek-Mahmutoglu, S., Salomons, G.S., 2012. Creatine deficiency syndromes. In: *Inborn Metabolic Diseases*. Springer, pp. 239–247.
- Streijger, F., Oerlemans, F., Ellenbroek, B.A., et al., 2005. Structural and behavioural consequences of double deficiency for creatine kinases BCK and UCKmit. *Behav. Brain Res.* 157, 219–234.
- Sullivan, P.G., Geiger, J.D., Mattson, M.P., et al., 2000. Dietary supplement creatine protects against traumatic brain injury. *Ann. Neurol.* 48, 723–729.
- Tabrizi, S., Blamire, A., Manners, D., et al., 2003. Creatine therapy for Huntington's disease: clinical and MRS findings in a 1-year pilot study. *Neurology* 61, 141–142.
- Tachikawa, M., Fukaya, M., Terasaki, T., et al., 2004. Distinct cellular expressions of creatine synthetic enzyme GAMT and creatine kinases uCK-Mi and CK-B suggest a novel neuron-glia relationship for brain energy homeostasis. *Eur. J. Neurosci.* 20, 144–160.
- Tachikawa, M., Ikeda, S., Fujinawa, J., et al., 2012. Gamma-aminobutyric acid transporter 2 mediates the hepatic uptake of guanidinoacetate, the creatine biosynthetic precursor, in rats. *PLoS One* 7.
- Terman, A., Brunk, U.T., 1998. Lipofuscin: mechanisms of formation and increase with age. *Appl. Physiol.* 106, 265–276.
- Trenell, M.I., Ward, J.A., Yee, B.J., et al., 2007. Influence of constant positive airway pressure therapy on lipid storage, muscle metabolism and insulin action in obese patients with severe obstructive sleep apnoea syndrome. *Diabetes Obes. Metab.* 9, 679–687.
- Turner, C.E., Byblow, W.D., Gant, N., 2015. Creatine supplementation enhances corticomotor excitability and cognitive performance during oxygen deprivation. *J. Neurosci.* 35, 1773–1780.
- Valenzuela, M.J., Jones, M., Sachdev, P., et al., 2003. Memory training alters hippocampal neurochemistry and healthy elderly. *Neuroreport* 14, 1333–1337.
- van de Kamp, J.M., Pouwels, P.J.W., Aarsen, F.K., et al., 2012. Long-term follow-up and treatment in nine boys with X-linked creatine transporter defect. *J. Inher. Metab. Dis.* 35, 141–149.
- Verbessem, P., Lemiere, J., Eijnde, B., et al., 2003. Creatine supplementation in Huntington's disease: a placebo-controlled pilot trial. *Neurology* 61, 925–930.
- Vidyasagar, R., Kauppinen, R.A., 2008. P-31 magnetic resonance spectroscopy study of the human visual cortex during stimulation in mild hypoxic hypoxia. *Exp. Brain Res.* 187, 229–235.
- Walliman, T., Wyss, M., Brdiczka, D., et al., 1992. Intracellular compartmentation, structure and function of creatine kinase isoenzyme in tissues with high and fluctuating energy demands: the phosphocreatine circuit for cellular energy homeostasis. *Biochem. J.* 281, 21–40.
- Wallimann, T., 2007. Introduction—creatine: cheap ergogenic supplement with great potential for health and disease. In: *Creatine and Creatine Kinase in Health and Disease*. Springer, pp. 1–16.
- Wang, X., Leverin, A.-L., Han, W., et al., 2011. Isolation of brain mitochondria from neonatal mice. *J. Neurochem.* 119, 1253–1261.
- Watanabe, A., Kato, N., Kato, T., 2002. Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation. *Neurosci. Res.* 42, 279–285.
- Writing Group for the N.E.T.I.P.D.I., 2015. Effect of creatine monohydrate on clinical progression in patients with Parkinson disease: a randomized clinical trial. *JAMA* 313, 584–593.
- Wyss, M., Kaddurah-Daouk, R., 2000. Creatine and creatinine metabolism. *Physiol. Rev.* 80, 1107–1212.
- Wyss, M., Schulze, A., 2002. Health implications of creatine: can oral creatine supplementation protect against neurological and atherosclerotic disease? *Neuroscience* 112, 243–260.
- Yuksel, C., Du, F., Ravichandran, C., et al., 2015 Mar 10. Abnormal high-energy phosphate molecule metabolism during regional brain activation in patients with bipolar disorder. *Mol. Psychiatry*. <http://dx.doi.org/10.1038/mp.2015.13> (Epub ahead of print).