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REVIEW ARTICLE

Potential of creatine or phosphocreatine supplementation in cerebrovascular disease and in ischemic heart disease

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Abstract Creatine is of paramount importance for maintaining and managing cellular ATP stores in both physiological and pathological states. Besides these "ergogenic" actions, it has a number of additional "pleiotropic" effects, e.g., antioxidant activity, neurotransmitter-like behavior, prevention of opening of mitochondrial permeability pore and others. Creatine supplementation has been proposed for a number of conditions, including neurodegenerative diseases. However, it is likely that creatine's largest therapeutic potential is in those diseases caused by energy shortage or by increased energy demand; for example, ischemic stroke and other cerebrovascular diseases. Surprisingly, despite a large preclinical body of evidence, little or no clinical research has been carried out in these fields. However, recent work showed that high-dose creatine supplementation causes an 8–9 % increase in cerebral creatine content, and that this is capable of improving, in humans, neuropsychological performances that are hampered by hypoxia. In addition, animal work suggests that creatine supplementation may be protective in stroke by increasing not only the neuronal but also the endothelial creatine content. Creatine should be administered before brain ischemia occurs, and thus should be given for prevention purposes to patients at high risk of stroke. In myocardial ischemia, phosphocreatine has been used clinically with positive results, e.g., showing prevention of arrhythmia and improvement in cardiac parameters. Nevertheless, large clinical trials are needed to confirm these results in the context of modern reperfusion interventions. So far, the most compelling evidence for creatine and/or phosphocreatine use in cardiology is as an addition to cardioplegic solutions, where positive effects have been repeatedly reported.

Keywords Creatine · Phosphocreatine · Creatine kinase · Stroke · Myocardial infarction · Treatment · Prevention

Creatine supplementation in cerebrovascular disease

Background

Creatine (Cr) effects in the brain can be divided into (1) effects concerning the generation and the distribution of energy (so-called "ergogenic" effects) and (2) effects relatively independent on energy generation and distribution (so-called "pleiotropic" effects) (Wallimann et al. 2011).

To affect energy production and distribution, creatine is reversibly phosphorylated to phosphocreatine (P-Cr). Cr and P-Cr are in equilibrium inside the cells. The ergogenic effects of this phosphocreatine/creatine system are twofold (Andres et al. 2008).

Firstly, under physiological conditions the phosphocreatine/creatine system enables the so-called "P-Cr shuttle". Creatine near the mitochondria is phosphorylated to P-Cr by creatine-kinase (CK), using newly mitochondrion-generated ATP. P-Cr then passively diffuses into the cytoplasm, reaching cytosolic ATPases, such as the membrane Na/K-ATPase that regulates intra- and extra-cellular ion concentrations. Here, due to functional coupling of the ATPase

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with a cytosolic CK isoform, P-Cr cedes its phosphate group to ADP, to generate locally (in the vicinity of the ATPase) ATP for enzyme fuelling. In so doing P-Cr reverts back to Cr which then diffuses back to the mitochondrion, where the cycle begins again (Wallimann et al. 2011).

Moreover, under pathological conditions, where energy production is hampered (anoxia or ischemia), P-Cr rapidly transfers its phosphate group to ADP, thus reconstituting the ATP store that could not otherwise be replenished due to ischemia or anoxia. In this way, the phosphocreatine/creatine system prevents or delays the exhaustion of the ATP store that would otherwise be caused by the lack of oxidative glycolysis consequent to anoxia or ischemia.

While nature developed Cr as an endogenous molecule to fulfill the above-described "ergogenic" duties, Cr has a number of additional effects, which are independent of its ergogenic role and are known as "pleiotropic" effects (Wallimann et al. 2011). These effects include among else an antioxidant action (Sestili et al. 2011), a stimulating effect on muscle differentiation and growth (Deldicque et al. 2007; Sestili et al. 2015), neuronal differentiation (Andres et al. 2005; Ducray et al. 2007), inhibition of the apoptosis-inducing mitochondrial permeability transition pore (Dolder et al. 2003), expression of transcription factors and other genes (Allen et al. 2015), stabilization of cell membranes (in its phosphorylated form: Tokarska-Schlattner et al. 2012), prevention of excitotoxicity (Genius et al. 2012), and more (Wallimann et al. 2011). A neuromodulator role of Cr has also been hypothesized (Almeida et al. 2006).

Creatine supplementation as a medicinal therapeutic intervention for neurological diseases

Based on the above considerations, Cr supplementation has been proposed as an adjuvant therapy for a number of brain diseases and pathological conditions, including neurodegenerative diseases (e.g., Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis) (Ferrante et al. 2000). It is important to realize that use of Cr in neurodegenerative diseases does not primarily exploit the "ergogenic" properties of Cr. In fact, although hampered or damaged mitochondrial function has been demonstrated in several neurodegenerative diseases (Chaturvedi and Beal 2013; Cozzolino et al. 2013) in neurodegenerative diseases, there is no major energy shortage for Cr to correct, or at least this is not usually considered the main pathophysiological change leading to disease (Choonara et al. 2009). Thus, the rationale for using Cr in neurodegenerative diseases is usually considered to lay in the "pleiotropic" effects of Cr (Beal 2011).

As an example, it was suggested that Cr might be used in the treatment of Parkinson's disease (Matthews et al. 1999). The suggestion was raised because of the antioxidant properties of Cr, because of its broadly defined "neuroprotective" effects, and because of the protection it afforded to mitochondria in some preclinical models (O'Gorman et al. 1997). While these Cr effects certainly provided a rationale for Cr use in Parkinson's disease, it is obvious that energy shortage may not represent the only pathogenic entity of this disease. Unfortunately, a large randomized and placebo-controlled clinical trial failed to demonstrate a therapeutic role of Cr for Parkinson's disease (Kieburtz et al. 2015). Similar negative or partially negative results were reported for Huntington's disease (Wild Ed 2015) and amyotrophic lateral sclerosis (Rosenfeld et al. 2008; Klivenyi et al. 1999).

The negative or partially negative results in the treatment of these diseases in humans should not be interpreted to mean that Cr has no role whatsoever in the treatment of neurodegenerative diseases. It probably does play a role, but this role is likely confined to very specific conditions. For example, while it was recently reported that Cr failed to prove efficacy for Huntington's disease (Wild 2015), it was suggested that it might be capable to slow down disease progression in pre-symptomatic patients (Rosas et al. 2014). Thus, Cr may have some role in neurodegenerative disease, but this role is probably not a prominent one and is limited to specific conditions, e.g., to pre-symptomatic Huntington's disease patients. This problem (Cr being only marginally indicated for diseases that do not have energy deprivation or imbalance as a major pathogenic mechanism) may be even more important, in clinical practice, because Cr uptake by the brain is slow and limited (see below). From this point of view, it is a pity that most of the human studies of Cr in neurodegenerative diseases did not measure brain levels of Cr after supplementation. It is theoretically conceivable that is we had ways to more efficiently carry Cr across the blood–brain barrier (e.g., lipophilic Cr-derived drugs) we could have better success.

We should, thus, be careful in hypothesizing a general therapeutic role for Cr, since repeated failures might lead to widespread, general skepticism on the potential of this very interesting compound. As a warning, we should not forget that the repeated failures of other compounds on neuroprotection in stroke generated skepticism, despite scores of preclinical positive trials (Röther 2008). Thus, creatine should be first and above all used in those diseases where its potential is maximal, i.e., those diseases where energy failure is the main cause of the disease itself. In this regard, we agree with Turner et al. (2015), who recently wrote: "the CK/PCr system is of greater potential relevance in disease states where there is disruption to cellular energy metabolism and a diminished capacity to meet neuronal energy needs".

In contrast, clinical research has still not tapped the potential of Cr in the treatment of neurological conditions where energy shortage is the main pathogenic mechanism. This is of concern as energy generation and management are probably the most reliable and powerful effects of Cr.

Prevention by creatine of ischemic or anoxic brain damage: preclinical evidence

Given the ergogenic role of Cr, its possible use in preventing brain damage due to energy shortage (brain ischemia or anoxia) has been investigated in animal models. For example, Krivánek and coworkers were the first to demonstrate a positive effect of Cr, showing in in vitro brain slices that the content of P-Cr was related to the maintenance of tissue polarization during oxygen deprivation (Krivanek et al. 1958). Later, the neuroprotective effect of Cr against anoxic damage was repeatedly confirmed, e.g., it was shown by Whittingham and Lipton (1981) that addition of 25 mM Cr to the incubating medium for brain slices increased the Cr and P-Cr content in the tissue, and that this in turn protected cerebral synaptic transmission during anoxia by delaying or preventing P-Cr or ATP disappearance, respectively. The same authors also showed (Lipton and Whittingham 1982) that the higher the P-Cr content of brain slices was, then the longer the evoked potential during anoxia was maintained. Yoneda et al. (1983, 1989) confirmed these findings. They also showed that increase in P-Cr could be obtained with a Cr concentration in the incubating medium as low as 5 mM. Moreover, they showed that recovery of P-Cr after reoxygenation, too, was improved by Cr pretreatment. Carter et al. (1995) moved the evidence to the biochemical field, showing that Cr countered anoxia-induced failure of protein synthesis in brain tissue. Wilken et al. (1998) demonstrated that Cr preincubation maintained the function of the bulbar respiratory center kept in vitro. One of us (MB) and others confirmed these findings; moreover, they added that Cr preincubation delayed anoxic depolarization (Balestrino 1995) and that its neuroprotective effect was maintained even by a Cr concentration in the incubating medium as low as 1 mM (Balestrino et al. 1999). Creatine is able to delay the disappearance of the population spike during anoxia (Adriano et al. 2011), an effect that is due to longer-lasting maintenance of ATP levels during anoxia (Whittingham and Lipton 1981). Figure 1 illustrates this effect.

Given these promising findings, Cr pre-administration was investigated in vivo, too. Several workers were concerned that the notoriously poor crossing by Cr of the blood–brain barrier might be an obstacle to the full exploitation of its neuroprotective effect in vivo. In fact, to enter brain cells, circulating creatine needs to cross both the blood–brain barrier and the brain cells' plasma membrane. Since creatine crosses poorly lipid-rich membranes, both

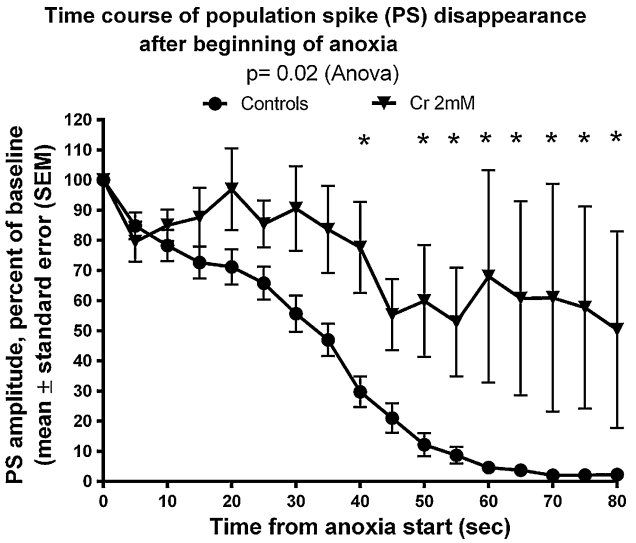

Fig. 1 Time course of population spike (PS) disappearance after beginning of anoxia. The abscissae represent time (seconds) from the beginning of anoxia (nitrogen instead of oxygen in the gas phase of the slices) and the ordinate represent the amplitude of population spike (normalized as a percentage of pre-anoxia value). Data are shown as mean ± standard error (SEM). Creatine is capable of delaying population spike disappearance ($p = 0.02$, ANOVA). *Asterisks* show points of significant difference between controls and creatine-treated slices ($p < 0.01$). $N = 25$ for controls, $N = 19$ for creatine. From Adriano et al. 2011 (modified)

uptakes need a dedicated transporter (Ohtsuki et al. 2002; Lunardi et al. 2006). Despite the transporter, the blood-to-brain transport of creatine is rather slow (Perasso et al. 2003). This problem was by-passed by injecting Cr directly into the brain, via an implanted osmotic pump. In this case, the osmotic pumps were used to continuously inject Cr into the lateral ventricle of rats for several days before and after transient global ischemia. In this way, exogenous Cr only had to cross the brain cells' plasma membrane, not the blood–brain barrier. Through this means, neuroprotection by Cr was demonstrated in three separate experiments (Otellin et al. 2003; Lensman et al. 2006; Korzhevskii et al. 2005). Figure 2 (from Otellin et al. 2003) and Fig. 3 (from Lensman et al. 2006) exemplify the effects of creatine pretreatment in vivo.

Another way of circumventing the poor crossing of blood–brain barrier by Cr was to modify the Cr molecule, making it more lipophilic. One such molecule was cyclocreatine, which in in vivo brain ischemia showed both positive (Woznicki and Walker 1980) and negative (Artru and Michenfelder 1982) results before being abandoned for brain ischemia and repositioned as a possible treatment for Cr transporter deficiency (Enrico et al. 2013; Kurosawa et al. 2012). Another such molecule was phosphocreatine (P-Cr) magnesium complex acetate, which showed neuroprotection in rats against transient middle cerebral artery

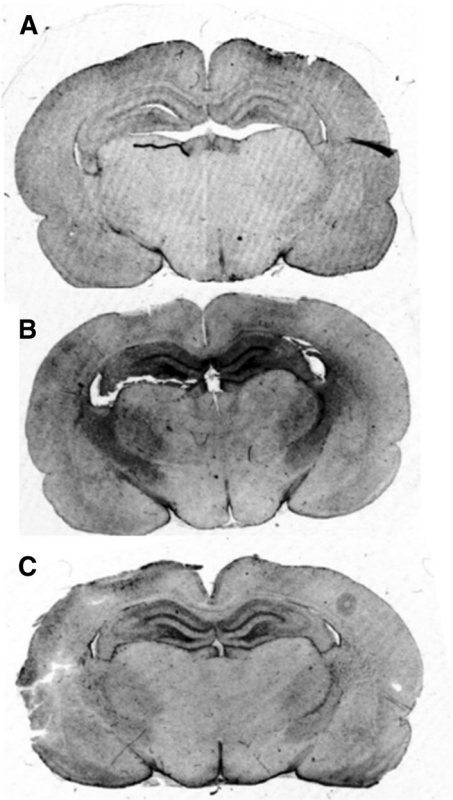

Fig. 2 Glial astrocyte reactivity **a** in control rats, **b** after transient global brain ischemia, and **c** in rats with ischemia treated with creatine. Transient brain ischemia was induced by compression of both carotid arteries for 12 min. Creatine was administered intra-cerebro-ventricularly (ICV) using an osmotic pump (Alzet) for 5 days before ischemia, at the 50 mM concentration. Immunocytochemical staining for glial acid fibrillar protein. Intense staining of the sections indicates the activation of the glial astrocyte functioning, a parameter that correlates with post-ischemic damage. Magnification, 5×. From Otellin et al. 2003 (modified)

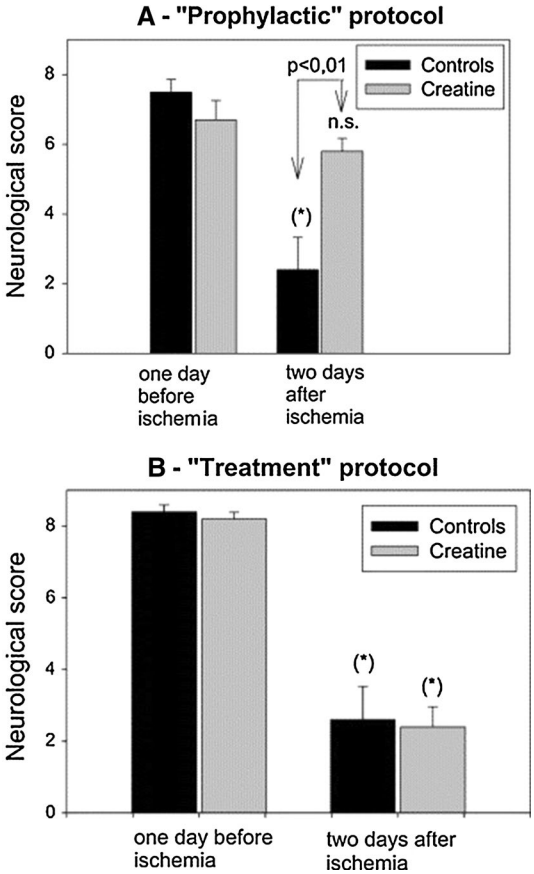

Fig. 3 Neurological scores of rats (after Combs and D'Alecy 1987) subjected to global transient ischemia with the two-vessel occlusion model and infused intra-cerebro-ventricularly (ICV) with saline solution (controls) or with 50 mM creatine. "Prophylactic" means that animals were treated with creatine before ischemia, "Treatment" means that the animals were treated after ischemia. The lower the score, the more severe the neurological impairment. Values are expressed as means and SEM. *Asterisks* show statistically significant difference as compared to one day before ischemia ($p < 0.05$, Wilcoxon test); *ns* not significant as compared to one day before ischemia. A significant difference was observed between the two groups at 2 days after ischemia in the "prophylaxis" protocol (*bracketed arrows*, Mann–Whitney test). The data show that creatine administration was effective in protecting from ischemic damage when administration occurred before ischemia, not when it occurred after it. Number of rats was as follows: **a** "Prophylaxis" protocol: $N = 18$ in the controls group, $N = 9$ in the creatine group. **b** "Treatment" protocol: $N = 13$ in the controls group, $N = 27$ in creatine group. From Lensman et al. 2006

occlusion (Perasso et al. 2009). Recently, Kratirova et al. (2012) showed neuroprotection against transient occlusion of the middle cerebral artery by creatine glycine ethylic ether fumarate.

Nevertheless, some researchers have used Cr as such by systemic administration before transient brain ischemia. Wick et al. (1999) found in Cr-pretreated rats a non-significant reduction in diffusion-weighted changes in rats subjected to transient global ischemia (12 min) that had been pretreated with dietary Cr. Zhu et al. (2004) found that a Cr-fortified diet increased the P-Cr content in brain and reduced ischemic area and caspase activation in rats subjected to transient focal ischemia.

Perhaps the most intriguing paper was by Prass et al. (2007). They found that Cr pretreatment improved both histological and behavioral damage following middle cerebral artery occlusion despite the fact that Cr supplementation was not able to increase the level of Cr in brain. In other words, Cr added to the diet did not increase brain Cr content yet it improved neurological outcome after transient focal ischemia. These authors tried to solve this paradox by proposing that exogenous Cr may be taken up by vascular endothelial and/or smooth muscle cells. In agreement with this hypothesis, they found that Cr increased both survival of cultured endothelial cells after oxygen-glucose deprivation and dilation of isolated middle cerebral artery induced by potassium or by acidosis. As a result, in Cr-pretreated

rats, cerebral blood flow upon reperfusion was higher than in controls. Prass et al. (2007) hypothesized that Cr exerted its neuroprotective effect by entering endothelial cells and increasing their survival and vasodilatory responses. This hypothesis provides a further rationale for the use of Cr in cerebrovascular diseases.

It should be noted that in all of the above studies, Cr had been administered before hypoxia or ischemia. This enables a build-up of creatine–phosphocreatine before hypoxia or ischaemia to delay ATP exhaustion after oxygen or blood deprivation. Less encouraging results have been obtained when Cr was administered after stroke onset. For instance Lensman et al. (2006) found much less protection in this condition compared to the protection they found with administration of Cr before stroke and Prass et al. (2007) found no protective effect when Cr was given after stroke onset.

Finally, besides the above-summarized beneficial effects on models of stroke and of generalized ischemia, pre-administration of Cr has demonstrated to improve survival and neurological outcome in animal models of neonatal hypoxia–ischemia (Adcock et al. 2002; Dickinson et al. 2014). In this case, Cr is given to the mother before hypoxia–ischemia of the pups or neonates. Interestingly, in neonatal animals Cr may provide neuroprotection (including infarct size reduction) even when administered after hypoxic–ischemic insult (Allah Yar et al. 2015; Iqbal et al. 2013).

Prevention by creatine of ischemic or anoxic brain damage: evidence in humans

Despite the wealth of preclinical supportive evidence, Cr has not so far been used in humans to treat or prevent anoxic or ischemic damage, with two notable exceptions.

First, Skřivánek et al. (1995) (also quoted by Strumia et al. 2012) administered P-Cr (as opposed to Cr) to 119 ischemic stroke patients within 8 h after the onset of symptoms, finding a statistically non-significant improvement of symptoms. This paper is noteworthy because it is, to the best of our knowledge, the only attempt to use P-Cr in human stroke patients. Results, however, were negative as the authors found only a statistically non-significant difference. The negative results can easily be explained both by the fact that the treatment was administered a long time after stroke (up to 8 h after stroke onset). As detailed above, in most of the preclinical studies a robust effect of Cr was found only when Cr was administered before stroke, while little or no effect was found when creatine was administered after stroke.

Turner et al. (2015) investigated whether or not Cr supplementation was capable of reverting the harmful effects of hypoxia in humans. In their investigation, healthy human volunteers breathed hypoxic air for 90 min, a condition that worsened their performance on a number of neuropsychological tests. Using a placebo-controlled design, they investigated whether or not Cr (20 g/day for 7 days before the experiment) was able to revert such hypoxia-induced impairment. Using H magnetic resonance spectroscopy (MRS) the investigators verified that the supplementation regime was able to increase the cerebral Cr content. The average increase in the brain creatine content was 9.2 %, comparable to that shown earlier by Dechent et al. (1999) and by Lyoo et al. (2003). Although all authors conclude that the increase they saw occurred in the brain parenchyma, one may wonder whether endothelial uptake may contribute to such an increase, in a way similar to what was suggested by Prass et al. (2007). Further research is needed to fully clarify this issue; nevertheless in vitro (where no endothelium obviously exists) we and others showed that the beneficial effects of creatine against anoxic damage were linked to the increase in the intracellular content of Cr or PCr (Balestrino et al. 1999; Whittingham and Lipton 1981). Turner et al. (2015) considered nine cognitive domains, some of which were negatively affected by hypoxia in a statistically significant way. When hypoxia was preceded by Cr administration, performance was significantly restored in the "complex attention" domain, while trends for improvements were observed in "executive function", "cognitive flexibility" and "neurocognitive index". The study confirms that high-dose Cr supplementation effectively increases the cerebral Cr content in humans. Secondly, it confirms that such an increase is functionally relevant to human performance. Similarly, Andres et al. (2008) had demonstrated usefulness of creatine administration under conditions where energy provision in the brain was hampered by ischemia or hypoxia. McMorris et al. (2006, 2007) showed that creatine administration improved cerebral function when the brain was challenged by a heavy workload.

In an accompanying editorial comment to the paper of Turner et al. (2015), Engl and Garvert (2015) noted that it would not have been possible for P-Cr to buffer ATP shortage for such a long period of time (90 min), as with the high rate of ATP hydrolysis in hypoxia or ischemia P-Cr could only delay its exhaustion by a matter of seconds. This is a valid argument; however, it refers to conditions of total oxygen deprivation. Under such conditions, ATP exhaustion quickly leads to anoxic depolarization, a spreading depression-like condition that causes severe and often irreversible damage to brain tissue (Kaminogo et al. 1998; Balestrino and Somjen 1986; Balestrino 1995). In humans, cortical spreading depression causes a severe loss of function of the brain areas it affects, leading to such symptoms as loss of vision, hemiparesis, and the like (Lauritzen et al. 2011), none of which were suffered by Turner et al.'s (2015) patients. Thus, it is unlikely that hypoxia in Turner et al.'s subjects was so profound as to cause anoxic depolarization. Instead,

it should be noted that a lesser degrees of anoxia or ischemia can disrupt neuronal functioning without causing anoxic depolarization (Schiff and Somjen 1987), and this was probably the case in Turner et al.'s subjects. Further research is needed to understand exactly the cellular pathophysiology of the hypoxia-induced loss of function that Turner et al. (2015) found. However, we can already conclude that it involved energy shortage, but did not involve anoxic depolarization.

Prevention by creatine of ischemic or anoxic brain damage: future perspectives

The above-reviewed experimental evidence allows for several conclusions.

Firstly, it is by now evident that oral administration of Cr is indeed able to increase in humans, albeit to a limited extent, the Cr content of the brain. Very recently, Turner et al. (2015) reported a 9.2 % increase of Cr in the cerebral cortex. This confirms previous findings by Dechent et al. (1999) who found an average increase of 8.7 % and by Lyoo et al. (2003) who found an 8.1 % and 9.3 % increase in the Cr/N-acetyl aspartate and Cr/choline ratios, respectively. Thus, all three papers reported a strikingly similar Cr increase. We should note that in all papers at least one week of Cr administration was required, and that the daily dose given (20 g/day) corresponds to the amount athletes take during the high-loading phase (7 days), which then is usually followed by a maintenance phase at a lower dosage (about 2g/day) (Hall and Trojian 2013). However, this 20 g Cr dose was apparently safe, and is only twice the daily dose that Parkinson's disease patients received (10 g/day) for about 5 years with no adverse effects (Kieburtz et al. 2015). Moreover, the recent paper by Turner et al. (2015) shows that the albeit limited increase in brain Cr that was obtained with this dose is functionally relevant. In fact, the paper by Turner et al. (2015) shows that this albeit limited increase was sufficient to counter at least some of the harmful effects of hypoxia on brain function. One possible way to further improve this result may be finding Cr-derived molecules that are able to better cross the blood–brain barrier while retaining Cr's biological effects. Such molecules would have better brain penetration and for this reason they may be even more effective than Cr itself.

Secondly, the paper by Prass et al. (2007) strongly suggests that the mechanism of action by Cr in stroke is probably not only an increase of energy charge in neuronal and glial cells, but also an improvement in the endothelial function of cerebral arteries. In this case, Cr is thought to increase both survival during ischemia and vasodilation, thus leading to an improvement in cerebral blood circulation.

Thirdly, pretreatment is advised. Although we cannot fully rule out that Cr may have some effects when administered after stroke, the evidence suggests that Cr is much more effective when administered before ischemia or anoxia than when it is administered afterwards. While it is not possible to foresee the occurrence of a stroke, we can identify conditions (for example, transient ischemic attacks or high-risk carotid or cardiac surgery) where a high risk of stroke exists. Prophylactic therapy in such cases may make sense. This represents the "prophylactic neuroprotection" that Savitz and Fisher (2007) first suggested and that was discussed in an earlier paper (Perasso et al. 2013).

Fourth, Cr is already available, and its use is widespread among, for example, athletes (Volek and Rawson 2004). It has already been used in clinical settings where, as we noted above, its long-term use at high dosages has proven quite safe (Kieburtz et al. 2015). As we propose, it should be used for preventing ischemic damage in high-risk patients ("prophylactic neuroprotection", see Savitz and Fisher 2007) rather than for patients who already had a stroke. Thus, we do not propose Cr for curing stroke, and the latter is already commercially and safely available. This in our opinion should allow researchers to moderate the importance of the lengthy and costly preclinical investigations that are recommended for experimental drugs that are developed by the industry as neuroprotectant to be used after stroke occurred (Stroke Therapy Academic Industry Roundtable (STAIR) 1999).

In conclusion, a strong rationale exists for Cr use against anoxic or ischemic brain damage. To this aim, Cr must be administered at high dosage (20 g/day for at least 1 week) before ischemia occurs. The latter fact implies that Cr should be used as a prophylactic treatment in patients who carry a very high risk of stroke in the short term ("prophylactic neuroprotection", Savitz and Fisher 2007). We hope that pilot clinical trials reflecting all of the above may be proposed in the near future.

Creatine supplementation in ischemic heart disease

Background

Myocardial cells specifically depend on aerobic metabolism, given their typical "unrest" working condition. Moderate ATP reduction during hypoxia causes inotropic impairment; this reflects in exercise limitations but represents an important mechanism of protection, preventing further ATP depletion that could lead to irreversible contracture and myocardial loss (Kubler and Katz 1977). Acting as an energy (read "ATP") buffer and regulator, the creatine system grants stability to the ATP concentration in myocardial cells.

Experimental models show that P-Cr/Cr plays a substantial role in the myocardium during stressing condition. Transgenic mice unable to synthesize Cr, given the lack of the enzyme guanidinoacetate N-methyltransferase (GAMT), showed a reduced inotropic reserve and increased susceptibility to ischemia–reperfusion injury associated with P-Cr/Cr deficiency. While P-Cr seemed to protect the myocardium from anoxic stress, its role seems to be marginal for cardiac function at rest (Hove et al. 2005). Branovets et al. showed that cardiomyocytes from resting animals with GAMT deficiency has preserved mitochondrial organization and intracellular compartmentation even in the absence of an active P-Cr/Cr system (Branovets et al. 2013). A reasonable hypotheses is that the deficiency in P-Cr/Cr causes metabolic-contractile impairment only during stressing conditions (with increased or variable ATP demand), but subsequent results from Lygate et al. made such a hypothesis more questionable: their GAMT-deficient mice has unaltered maximal exercise capacity and response to chronic myocardial infarction (survival and remodeling), and no obvious metabolic adaptations (Lygate et al. 2013). In another model of acquired P-Cr/Cr depletion induced by chronic administration of β-guanidinopropionate (β-GP, a Cr analog) the compromised P-Cr system induced minor contractile dysfunction in the resting healthy rat (Neubauer et al. 1999). In a subsequent study in a murine model of post-myocardial infarction (MI) heart failure, treatment with β-GP lead to a lack of ATP homeostasis, even if the functional data of left-ventricle developed pressure was not additionally reduced by MI and β-GP. On the other side, when β-GP treatment was initiated before MI, no animals survived. (Horn et al. 2001).

Even if P-Cr/Cr has minor impact on myocardial function in the normal heart at resting workload, the failing heart typically shows reduced P-Cr and P-Cr/ATP ratio, revealing a "low-energy" status (Neubauer 2007). Interestingly, some years ago different authors discussed the question whether an altered energy status in the myocardium is a cause or an "adaptive" consequence of altered load conditions. (Ingwall 1993; Neubauer et al. 1999). Anyway, even if P-Cr reduction may be a cause, an indirect marker or even an adaptation, it seems to be an unfavorable sign, given that a low P-Cr/ATP ratio is a strong predictor of mortality in dilated cardiomyopathy (Neubauer et al. 1997).

The so-called "pleiotropic" effects of Cr are at work in the heart, as well. For example, PCr, but much less so Cr, was shown recently to bind directly to biological membranes and to exert a strong membrane-stabilizing effect against mechanical or oxidative membrane stress in erythrocyte membranes (Tokarska-Schlattner et al. 2012). On the other hand, earlier date with myocardial cells demonstrated that Cr not only stabilized cell membranes (Zucchi et al. 1989) but also in liver cells over-expressing mitochondrial CK it reduced oxidative-stress and maintained the mitochondrial permeability transition pores (mPTP) closed (Dolder et al. 2003). Lygate et al. (2012) showed in mouse heart that creatine reduces mPTP opening. In addition, Cr supplementation reduced some cardiovascular risk factors such as homocysteine and lipid peroxidation (Deminice et al. 2009). However, it seems that Cr supplementation in the heart mainly exploits the ergogenic effects of Cr, which plays its most powerful and physiological role for energy provision in the form of P-Cr.

Creatine in heart disease: preclinical evidence

Creatine (Cr) and phosphocreatine (P-Cr) have been tested for potential medical applications, based on the aforementioned rationale derived from biological studies.

Several models of myocardial injury were investigated. Studies used "in vitro" anoxia, "ex vivo" and "in vivo" ischemia–reperfusion models such as transient coronary occlusion and cardiac arrest with cardioplegia.

Parratt and Marshall observed P-Cr effects on isolated guinea-pig cardiac muscle preparations. Hearts perfused with P-Cr were more resistant to the effects of anoxia as manifested by a gradual decrease both in developed tension and in the rate of tension development. In the presence of P-Cr, the time taken to reach 25, 50, and 75 % of the control resting heart performance values was significantly increased by approximately 60 %. Of note, free Cr or inorganic phosphate did not improve these parameters (Parratt and Marshall 1974), and thus it must have been the effect of P-Cr itself that preserved cardiac function under ischemia–reperfusion.

The same authors also observed an antiarrhythmic effect of P-Cr in an in vivo model of myocardial ischemia. Pretreatment with P-Cr significantly reduced ventricular arrhythmias following acute coronary ligation in the dog (Marshall et al. 1974). This antiarrhythmic effect was confirmed again some years later by other authors in a rat model of ischemia or ischemia–reperfusion (Fagbemi et al. 1982; Hearse et al. 1986).

More recently, Woo et al. (2005) showed that intravenous P-Cr administration successfully prevents ventricular dysfunction in a rodent model of a transient coronary occlusion. In P-Cr-treated animals a significantly greater preservation of myocardial ATP levels correlated with improved hemodynamic parameters at multiple time points, such as maximum pressure, maximum left ventricular dP/dt ejection fraction, and stroke work (Robinson et al. 1984).

Further results on P-Cr cardioprotection came from its experimental use in cardioplegia. Since its introduction, the cardioplegic solution was progressively developed to target three different mechanisms of cardioprotection: cardiac arrest, hypothermia and energy preservation. P-Cr has

been proposed as a new compound to be added to cardioplegic solutions in addition to the more usual constituents, as preservation of cardiac P-Cr and ATP concentrations directly relate to faster cardiac recovery following cardiac surgery.

Robinson et al. (1984) added P-Cr to the St. Thomas' Hospital cardioplegic solution, then tested it in a rat heart model of cardiopulmonary bypass and ischemic arrest. Dose–response studies indicated that 10.0 mmol/L of P-Cr was the optimal concentration. In this study, improved recovery of aortic flow and cardiac output was found after a 40-min period of normothermic (37 °C) ischemic arrest increased from 21.2 to 32.8 % in the PCr-free control group to 82.5 and 82.6 % ($P < 0.001$), respectively, in the P-Cr-treated group. In addition, the P-Cr group showed a decreased CK release from the myocardium into the blood serum, as well as a decrease in reperfusion arrhythmias, and a significantly shorter time between crossclamp removal and return of regular rhythm, thus completely obviating the need for electrical defibrillation (Woo et al. 2005).

31P-NMR spectrometry was used by Sharov et al. (1987) to study the effects of P-Cr on the isolated, perfused rat heart. The hearts were chemically arrested using St. Thomas' Hospital solution and made totally ischemic for 35 min at 37 °C. In the presence of P-Cr (10 mmol/L), almost complete recovery of heart function and myocardial P-Cr content was observed, as well as a 61 % recovery of ATP content measured after 30 min of reperfusion. In addition, sarcolemma protection and integrity were also improved (Robinson et al.1984).

Antiarrhythmic effects of P-Cr in post-ischemic reperfusion were in accordance with electrophysiological studies.

In an animal model, P-Cr pretreatment induced a significant difference in the action potential occurring in the main left papillary muscles subjected to a 40-min period of hypoxia compared to control animals: *vis à vis* a significant (11 %) decrease in the maximum rate of depolarization and a significant (14 %) increase in the duration of the action potential (Sharov et al. 1987).

Of note, an unfavorable result has been reported in a study by Webster et al. (2012) with Cr administration. They compared sedentary and exercised rats, both divided into two groups with and without a Cr supplement in the diet. Rats were then tested for their resistance to a typical ischemia–reperfusion injury. The heart from sedentary, untreated rats performed better in recovering cardiac output, while creatine supplementation induced a worsening in both sedentary and exercised rats. (Webster et al. 2012) Another study on oral administration by Neubauer found that a dietary supplementation of Creatine is ineffective in preventing ventricular remodeling after myocardial infarction. The authors hypothesized a reduced Cr uptake in dysfunctional myocardium, due to Cr transporter down-regulation (Neubauer et al. 1998).

Ever since a poor uptake of exogenous extra Cr by myocardial cells was first demonstrated, Cr-derived molecules with an improved myocardial uptake have been sought (Neubauer et al. 1998; Horn et al. 1998). For example, cyclocreatine is a synthetic analog of Cr that possibly can reach higher concentrations in the myocardium. We should underline that cyclocreatine does not get converted into creatine. Instead, it is phosphorylated as such but with a slower CK reaction velocity. This may still be beneficial in preserving ATP during ischaemia, but may limit the rapid availability of energy reserve during times of peak energy demand. It has been demonstrated that ischemic hearts of rats previously fed with 1 % cyclocreatine were able to maintain their total ATP much better than controls after ischemia. In addition, the same hearts showed a marked delay of rigor-contracture onset and glycolysis cessation. These results were confirmed later using nuclear magnetic resonance spectroscopy with 31P (MRS) (Loike et al. 1988; Roberts and Walker 1982). A subsequent study by Osbakken et al. (1992) provided a direct comparison between Cr and cyclocreatine administration, in addition to a control group. They tested a rat model of myocardial ischemia with 31P-MRS, looking at the time needed for a 50 % depletion of high-energy phosphates, as a measure of ischemic endurance. The time to halve the concentration of ATP and P-Cr was twofold and threefold, respectively, in cyclocreatine-treated rats, in comparison to the Cr-treated ones which did not differ from the controls. Moreover, among the three groups a similar time was required for cardiac mechanical function to recover, even if given the experimental design the cyclocreatine-treated group had undergone a longer time of ischemia (Jacobstein et al. 1989).

Creatine in heart disease: evidence in humans

To date intervention studies of the P-Cr/Cr system in humans during myocardial ischemia have used only P-Cr and not Cr, probably with the rationale that P-Cr was the "active" high-energy compound of Cr. In addition, intravenous administration of P-Cr better fits in the acute clinical setting of myocardial infarction. Despite some evidence for a potential benefit of non-phosphorylated Cr, animal models of myocardial ischemia and human studies on heart failure have been unsuccessful with Cr alone (Osbakken et al. 1992).

Some of the best evidence of an antiarrhythmic action of P-Cr is the work by Ruda et al. (1988), who carried out a clinical trial with 60 randomized patients presenting with acute myocardial infarction. To these patients, P-Cr was administered intravenously within 6 h of symptom onset (2 g of P-Cr i.v. bolus, followed by a 2-h infusion at a rate

of 4 g/h). Twenty-four-h Holter monitoring showed a significant decrease in the frequency of ventricular premature beats and of the number of ventricular tachycardia paroxysms in the treatment group.

A biomarker study by Reimers et al. (1994) observed markedly reduced CK and MB-CK release after myocardial infarction when a prolonged treatment with intravenous P-Cr was administered in the immediately post-acute phase. Reduced enzyme release and/or reduction of arrhythmias have been observed in P-Cr-treated patients in other trials (Reimers et al. 1994; Coraggio et al. 1987).

Even if most of the results on myocardial ischemia have been acquired in the so-called "pre-reperfusion" era, Iosseliani et al. (2004) have confirmed the effects of P-Cr in the setting of primary angioplasty. These authors randomized patients to receive intracoronary injections of P-Cr during percutaneous coronary intervention for acute myocardial infarction. The treated group showed a significantly lower extent of myocardial necrosis, as indicated by the significantly lower peaks in troponin I, which in other studies correlated with a significantly greater left ventricular ejection fraction (Raisaro et al. 1989; Iosseliani et al. 2006).

Other significant results on cardioprotective efficacy of P-Cr have come from experience gained in cardiac surgery. The effects of supplementing cardioplegic solution with P-Cr were evaluated in 40 patients undergoing mitral valve replacement. It was shown that in the P-Cr-treated group the Vmax of hexokinase, malate dehydrogenase, glutamate dehydrogenase and total NADH cytochrome c reductase (evaluated in samples of papillary muscle obtained from the removed valve) was significantly greater, and a better cardiac functional state was observed after cardiopulmonary bypass (Pastoris et al. 1991).

Subsequently, another group studied the effect of PCr supplementation of the St. Thomas' Hospital cardioplegic solution on 50 patients undergoing valve replacement. While they did not find any differences in recovery of function, myocardial high-energy phosphate content, and ultrastructure, they showed a higher incidence of spontaneous sinus rhythm in the treated group compared to controls. Furthermore, they showed in the PCr-treated patients a reduction of the incidence of postoperative arrhythmias and a better response to inotropic support (Chambers et al. 1996).

These findings have been supported by other studies. Specifically, Cossolini et al. (1993) tested the addition of P-Cr as cardioprotection in newborn infants and pediatric patients from 9 days to 13 years (mean age 33.9 months) undergoing open heart surgery for congenital heart disease.

Recently, the clinical use of P-Cr in cardioplegic solution was tested during coronary artery bypass graft in elderly patients (Guo-han et al. 2013). Use of P-Cr significantly increased the concentration of superoxide dismutase in the blood after the release of aortic clamping, decreased the blood concentration of CK, CK-MB, lactate dehydrogenase, and cardiac troponin T after clamp release, and improved the morphological appearance of cardiac mitochondria. These results were in agreement with a previous study, showing improved "electrical" recovery and reduced inotropic support requirements (Cossolini et al. 1993).

Creatine in heart disease: future perspectives

A Cochrane meta-analysis (Horjus et al. 2011) on Cr and Cr analogs in cardiac disease and hypertension defined as "unclear" the effects on mortality, progression of myocardial infarction and ejection fraction, while it found some evidence that Cr supplementation might improve dysrhythmia and dyspnoea. The authors summarized the results as "inconclusive evidence to decide on clinical application of Cr analogs for hypertension and heart diseases", and they underlined the limitations related to heterogeneity of the studies using different analog, dose and route of administration.

Furthermore, a review of the literature suggests some interesting considerations. There is only limited information concerning actual uptake of PCr by the human heart. However, we should underline that, while we ourselves did not find PCr uptake by brain cells in vitro (Perasso et al. 2008), Preobrazhenskiĭ et al. (1986) found, on the contrary, that 32P-phosphocreatine was indeed taken up by isolated perfused rat hearts, the more so after they were made ischemic. Moreover, Soboll et al. (1997) found that phosphocreatine was taken up by both isolated rat heart mitochondria and liposomes. Thus, we cannot rule out that phosphocreatine may, at least to some extent, be actually taken up as such by heart cells and in this way increase the creatine/phosphocreatine system. However, additional protective mechanisms by phosphocreatine in the heart have also been suggested. Specifically, phosphocreatine may protect myocardial cells by inserting itself into the sarcolemma and modifying its physical properties (Saks et al. 1992). It inhibits platelet aggregation (Saks et al. 1992; Panchenko et al. 1994), thus probably exerting a beneficial effect in clinical conditions of coronary thrombosis.

Parenteral administration of P-Cr has, instead, some practical limitations especially in a "pre-medication" setting, as P-Cr is unstable in solution being quickly hydrolysed. Not surprisingly, the best clinical results with heart so far were obtained with PCr in cardioplegic solutions,

probably because of the combination of favorable time of administration (before cardiac ischemia) and the high bioavailability due to the route of direct intra-venous administration.

From a pharmacological perspective, a potentiation of the P-Cr/Cr content could possibly be obtained with two alternative strategies. One may consider either to use a premedication with Cyclocreatine (Osbakken et al. 1992); or to boost the Cr transporter (Lygate et al. 2012). A reduction in the endogenous Cr-synthesis or Cr dietary intake is unlikely to underly the fall in P-Cr/Cr depletion in heart dysfunction. More likely it is a combination of down-regulation of the cardiac Cr transporter, reducing Cr uptake, as well as a modifications in the cardiac CK isoform expression pattern, all of which will influence the local levels of Cr and the PCr/Cr ratio, as well as CK function in general (Horjus et al. 2011; Neubauer et al. 1999).

In summary, our current knowledge of the phosphocreatine/creatine system provides the rationale for further clinical investigations. A key point is the identification of the best Cr-derived molecule to be effective for each clinical setting: acute "coronary" ischemia, chronic ischemia during heart failure, ischemia during cardiac surgery. Probably, a better choice than Cr is P-Cr, when parenteral administration is possible for a limited time, remembering that its effects probably depend more on "pleiotropic" effects such as sarcoplasmatic membrane stabilization.

Cyclocreatine, hypothetically could be considered for preconditioning and treatment (instead of Cr), but up to date we have found only positive results in preclinical studies of heart disease. We believe that future results could come from these directions, if systematically explored. Nevertheless, cyclocreatine is not converted to creatine being instead phosphorylated as such but with a slower CK reaction velocity. This may limit its capability to rapidly provide energy at times of energy failure.

Summing up, direct intra-venous administration of phosphocreatine (PCr) to patients is still the most promising intervention for reinforcing the cardiac P-Cr/Cr system in the acute–subacute clinical phase of myocardial ischemia. Since P-Cr is chemically unstable, it has to be dissolved from either a solid PCr powder that is stored frozen or from a frozen sterile PCr-containing stock solution, which, however, is somewhat more complicated and demanding for the clinical personnel than just oral Cr supplementation (which itself appears to be much less effective). It is important that the results reviewed here on the use of P-Cr in myocardial reperfusion will be confirmed in future larger clinical trials.

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Compliance with ethical standards

Conflict of interest Maurizio Balestrino and Enrico Adriano were among the founders of NovaNeuro Srl, a spin-off of the University of Genova whose aim is, among others, the invention and commercialization of creatine-based nutritional supplements.

References

Adcock KH, Nedelcu J, Loenneker T, Martin E, Wallimann T, Wagner BP (2002) Neuroprotection of creatine supplementation in neonatal rats with transient cerebral hypoxia-ischemia. Dev Neurosci 24(5):382–388

Adriano E, Garbati P, Damonte G, Sali A, Armirotti A, Balestrino M (2011) Searching for a therapy of creatine transporter deficiency: some effects of creatine ethyl ester in brain slices in vitro. Neuroscience 29(199):386–393. doi:10.1016/j.neuroscience.2011.09.018

Allah Yar R, Akbar A, Iqbal F (2015) Creatine monohydrate supplementation for 10 weeks mediates neuroprotection and improves learning/memory following neonatal hypoxia ischemia encephalopathy in female albino mice. Brain Res 21(1595):92–100

Allen PJ, DeBold JF, Rios M, Kanarek RB (2015) Chronic high-dose creatine has opposing effects on depression-related gene expression and behavior in intact and sex hormone-treated gonadectomized male and female rats. Pharmacol Biochem Behav 130:22–33

Almeida LS, Salomons GS, Hogenboom F, Jakobs C, Schoffelmeer AN (2006) Exocytotic release of creatine in rat brain. Synapse 60(2):118–123

Andres RH, Ducray AD, Huber AW, Pérez-Bouza A, Krebs SH, Schlattner U, Seiler RW, Wallimann T, Widmer HR (2005) Effects of creatine treatment on survival and differentiation of GABA-ergic neurons in cultured striatal tissue. J Neurochem 95(1):33–45

Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR (2008) Functions and effects of creatine in the central nervous system. Brain Res Bull 76(4):329–343

Artru AA, Michenfelder JD (1982) Cyclocreatine phosphate, an analogue of creatine phosphate, does not improve hypoxic tolerance in mice. J Neurochem 39(4):1198–1200

Balestrino M (1995) Pathophysiology of anoxic depolarization: new findings and a working hypothesis. J Neurosci Methods 59:99–103

Balestrino M, Somjen GG (1986) Chlorpromazine protects brain tissue in hypoxia by delaying spreading depression-mediated calcium influx. Brain Res 385(2):219–226

Balestrino M, Rebaudo R, Lunardi G (1999) Exogenous creatine delays anoxic depolarization and protects from hypoxic damage: dose-effect relationship. Brain Res 816:124–130

Beal MF (2011) Neuroprotective effects of creatine. Amino Acids 40(5):1305–1313

Branovets J, Sepp M, Kotlyarova S, Jepihhina N, Sokolova N, Aksentijevic D et al (2013) Unchanged mitochondrial organization and compartmentation of highenergy phosphates in creatine-deficient GAMT−/− mouse heart. Am J Physiol Heart Circ Physiol 305(4):H506–H520

Carter AJ, Müller RE, Pschorn U, Stransky W (1995) Preincubation with creatine enhances levels of creatine phosphate and prevents anoxic damage in rat hippocampal slices. J Neurochem 64(6):2691–2699

Chambers DJ, Haire K, Morley N, Fairbanks L, Strumia E, Young CP, Venn GE (1996) St. Thomas' hospital cardioplegia: Enhanced

protection with exogenous creatine phosphate. Ann Thorac Surg 61:67–75

Chaturvedi RK, Beal MF (2013) Mitochondria targeted therapeutic approaches in Parkinson's and Huntington's diseases. Mol Cell Neurosci 55:101–114

Choonara YE, Pillay V, du Toit LC, Modi G, Naidoo D, Ndesendo VM, Sibambo SR (2009) Trends in the molecular pathogenesis and clinical therapeutics of common neurodegenerative disorders. Int J Mol Sci 10(6):2510–2557

Coraggio F, Spina M, Scarpato P (1987) Analysis of creatine phosphate on the evolution of ischemic lesion in acute myocardial infarction. Farmaci Terapia 4:91–93

Cossolini M, Sonzogni V, Di Dedda G et al (1993) D'Alessandro LC, ed. Heart Surgery. Paediatric cold heart surgery: experience with creatine phosphate added to cardioplegic solution. Roma: Casa Editrice Scientifica Internazionale, pp 442–443

Cozzolino M, Ferri A, Valle C, Carrì MT (2013) Mitochondria and ALS: implications from novel genes and pathways. Mol Cell Neurosci 55:44–49

Dechent P, Pouwels PJ, Wilken B, Hanefeld F, Frahm J (1999) Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. Am J Physiol 277(3 Pt. 2):R698–R704

Deldicque L, Theisen D, Bertrand L, Hespel P, Hue L, Francaux M (2007) Creatine enhances differentiation of myogenic C2C12 cells by activating both p38 and Akt/PKB pathways. Am J Physiol Cell Physiol 293(4):C1263–C1271

Deminice R, Portari GV, Vannucchi H, Jordao AA (2009) Effects of creatine supplementation on homocysteine levels and lipid peroxidation in rats. Br J Nutr 102:110–116

Dickinson H, Ellery S, Ireland Z, LaRosa D, Snow R, Walker DW (2014) Creatine supplementation during pregnancy: summary of experimental studies suggesting a treatment to improve fetal and neonatal morbidity and reduce mortality in high-risk human pregnancy. BMC Pregnancy Childbirth 27(14):150

Dolder M, Walzel B, Speer O, Schlattner U, Wallimann T (2003) Inhibition of the mitochondrial permeability transition by creatine kinase substrates. Requirement for microcompartmentation. J Biol Chem 278:17760–17766

Ducray AD, Schläppi JA, Qualls R, Andres RH, Seiler RW, Schlattner U, Wallimann T, Widmer HR (2007) Creatine treatment promotes differentiation of GABA-ergic neuronal precursors in cultured fetal rat spinal cord. J Neurosci Res 85(9):1863–1875

Engl E, Garvert MM A (2015) Prophylactic role for creatine in hypoxia? J Neurosci 24;35(25):9249–51

Enrico A, Patrizia G, Luisa P, Alessandro P, Gianluigi L, Carlo G, Maurizio B (2013) Electrophysiology and biochemical analysis of cyclocreatine uptake and effect in hippocampal slices. J Integr Neurosci 12(2):285–297

Fagbemi O, Kane KA, Parratt JR (1982) Creatine phosphate suppresses ventricular arrhythmias resulting from coronary artery ligation. J Cardiovasc Pharmacol 4:53–58

Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, Kaddurah-Daouk R, Hersch SM, Beal MF (2000) Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. J Neurosci 20(12):4389–97. PubMed PMID: 10844007

Genius J, Geiger J, Bender A, Möller HJ, Klopstock T, Rujescu D (2012) Creatine protects against excitoxicity in an in vitro model of neurodegeneration. PLoS One 7(2):e30554

Guo-han C, Jian-hua G, Xuan H, Jinyi W, Rong L, Zhong-min L (2013) Role of creatine phosphate as a myoprotective agent during coronary artery bypass graft in elderly patients. Coron Artery Dis 24(1):48–53

Hall M, Trojian TH (2013) Creatine supplementation. Curr Sports Med Rep 12(4):240–244

Hearse DJ, Tanaka K, Crome R, Manning AS (1986) Creatine phosphate and protection against reperfusion-induced arrhythmias in the rat heart. Eur J Pharmacol 131:21–30

Horjus DL, Oudman I, van Montfrans GA, Brewster LM (2011) Creatine and creatine analogues in hypertension and cardiovascular disease. Cochrane Database Syst Rev 9:11

Horn M, Frantz S, Remkes H et al (1998) Effects of chronic dietary creatine feeding on cardiac energy metabolism and on creatine content in heart, skeletal muscle, brain, liver and kidney. J Mol Cell Cardiol 30:277–284

Horn M, Remkes H, Strömer H, Dienesch C, Neubauer S (2001) Chronic phosphocreatine depletion by the creatine analogue beta-guanidinopropionate is associated with increased mortality and loss of ATP in rats after myocardial infarction. Circulation 104(15):1844–1849

Hove M, Lygate CA, Fischer A, Schneider JE, Sang AE, Hulbert K et al (2005) Reduced inotropic reserve and increased susceptibility to cardiac ischemia/reperfusion injury in phosphocreatine-deficient guanidinoacetateN-methyltransferase-knockout mice. Circulation 111(19):2477–2485

Ingwall JS (1993) Is cardiac failure a consequence of decreased energy reserve? Circulation 87:58–62

Iosseliani DG, Koledinsky AG, Kuchkina NV (2004) Does intracoronary injection of phosphocreatine prevent myocardial reperfusion injury following angioplasty of infarct-related artery in acute-stage of myocardial infarction? J Intervent Cardiol 6:10–14

Iosseliani DG, Koledinsky AG, Kuchkina NV (2006) The possibility to limit reperfusion injury of cardiomyocytes using intracoronary cytoprotectors during endovascular reperfusion of the infarct-related artery. J Intervent Cardiol 11:10–16

Iqbal S, Nazir N, Gillani Q, Akbar A, Iqbal F (2013) Effect of creatine monohydrate supplementation on various hematological and serum biochemical parameters of male albino mice following neonatal hypoxia-ischemia encephalopathy. Sci World J 19(2013):286075

Jacobstein MD, Gerken TA, Bhat AM, Carlier PG (1989) Myocardial protection during ischemia by prior feeding with the creatine analog: Cyclocreatine. J Am Coll Cardiol 14:246–251

Kaminogo M, Suyama K, Ichikura A, Onizuka M, Shibata S (1998) Anoxic depolarization determines ischemic brain injury. Neurol Res 20(4):343–348

Kieburtz K, Tilley BC, Elm JJ, Babcock D, Hauser R, Ross GW, Augustine AH, Augustine EU, Aminoff MJ, Bodis-Wollner IG, Boyd J, Cambi F, ChouK Christine CW, Cines M, Dahodwala N, Derwent L, Dewey RB Jr, Hawthorne K, Houghton DJ, Kamp C, Leehey M, Lew MF, Liang GS, Luo ST, Mari Z, Morgan JC, Parashos S, Pérez A, Petrovitch H, Rajan S, Reichwein S, Roth JT, Schneider JS, Shannon KM, Simon DK, Simuni T, Singer C, Sudarsky L, Tanner CM, Umeh CC, Williams K, Wills AM (2015) Writing Group for the NINDS Exploratory Trials in Parkinson Disease (NET-PD) Effect of creatine monohydrate on clinical progression in patients with Parkinson disease: a randomized clinical trial. JAMA 313(6):584–593

Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, Mueller G, Wermer M, Kaddurah-Daouk R, Beal MF (1999) Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. Nat Med 5(3):347–350

Korzhevskii DE, Otellin VA, Grigor'ev IP, Kostkin VB, Polenov SA, Lentsman MV, Balestrino M (2005) Structural organization of astrocytes in the rat hippocampus in the post-ischemic period. Neurosci Behav Physiol 35(4):389–392

Kratirova NV, Veselkina OS, Kolpakova ME, Chefu SG, Korzhevskiĭ DE, Daĭneko AS, Prosvirina MS, Piskun AV, Vlasov TD (2012) Effect of intragastric creatine glycine ethylic ether fumarate

administration in a rat model of occlusive ischemia. Ross Fiziol Zh Im I M Sechenova. 98(10):1258–1263

Krivanek J, Bures J, Buresova O (1958) Evidence for a relation between creatine phosphate level and polarity of the cerebral cortex. Nature 182(4652):1799

Kubler W, Katz AM (1977) Mechanism of early "pump" failure of the ischemic heart: Possible role of adenosine triphosphate depletion and inorganic phosphate accumulation. Am J Cardiol 40:467–471

Kurosawa Y, Degrauw TJ, Lindquist DM, Blanco VM, Pyne-Geithman GJ, Daikoku T, Chambers JB, Benoit SC, Clark JF (2012) Cyclocreatine treatment improves cognition in mice with creatine transporter deficiency. J Clin Invest. 122(8):2837–2846

Lauritzen M, Dreier JP, Fabricius M, Hartings JA, Graf R, Strong AJ (2011) Clinical relevance of cortical spreading depression in neurological disorders: migraine, malignant stroke, subarachnoid and intracranial hemorrhage, and traumatic brain injury. J Cereb Blood Flow Metab 31(1):17–35

Lensman M, Korzhevskii DE, Mourovets VO, Kostkin VB, Izvarina N, Perasso L, Gandolfo C, Otellin VA, Polenov SA, Balestrino M (2006) Intracerebroventricular administration of creatine protects against damage by global cerebral ischemia in rat. Brain Res 1114(1):187–194

Lipton P, Whittingham (1982) *R*educed ATP concentration as a basis for synaptic transmission failure during hypoxia in the in vitro guinea-pig hippocampus. TSJ Physiol 325:51–65

Loike JD, Zalutsky DL, Kaback E, Miranda AF, Silverstein SC (1988) Extracellular creatine regulates creatine transport in rat and human muscle cell*s*. Proc Natl Acad Sci USA 85:807–811

Lunardi G, Parodi A, Perasso L, Pohvozcheva AV, Scarrone S, Adriano E, Florio T, Gandolfo C, Cupello A, Burov SV, Balestrino M (2006) The creatine transporter mediates the uptake of creatine by brain tissue, but not the uptake of two creatine-derived compounds. Neuroscience 142(4):991–997

Lygate CA, Bohl S, ten Hove M, Faller KM, Ostrowski PJ, Zervou S, Medway DJ, Aksentijevic D, Sebag-Montefiore L, Wallis J, Clarke K, Watkins H, Schneider JE, Neubauer S (2012) Moderate elevation of intracellular creatine by targeting the creatine transporter protects mice from acute myocardial infarction. Cardiovasc Res 96:466–475

Lygate CA, Aksentijevic D, Dawson D, ten Hove M, Phillips D, de Bono JP, Medway DJ, Sebag-Montefiore L, Hunyor I, Channon KM, Clarke K, Zervou S, Watkins H, Balaban RS, Neubauer S (2013) Living without creatine: unchanged exercise capacity and response to chronic myocardial infarction in creatine-deficient mice. Circ Res 112(6):945–955

Lyoo IK, Kong SW, Sung SM, Hirashima F, Parow A, Hennen J, Cohen BM, Renshaw PF (2003) Multinuclear magnetic resonance spectroscopy of high-energy phosphate metabolites in human brain following oral supplementation of creatine-monohydrate. Psychiatry Res 123(2):87–100

Marshall RJ, Parratt JR (1974) Reduction in ventricular arrhythmias following acute coronary artery ligation in the dog after the administration of creatine phosphate. Naunyn Schmiedeberg's Arch Pharmacol 281:437–441

Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, Kaddurah-Daouk R, Beal MF (1999) Creatine and cyclocreatine attenuate MPTP neurotoxicity. Exp Neurol 157(1):142–149

McMorris T, Harris RC, Swain J, Corbett J, Collard K, Dyson RJ, Dye L, Hodgson C, Draper N (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, Harris RC, Howard AN, Langridge G, Hall B, Corbett J, Dicks M, Hodgson C (2007) Creatine supplementation, sleep deprivation, cortisol, melatonin and behaviour. Physiol Behav 90:21–28

Neubauer S (1999) High-energy phosphate metabolism in normal, hypertrophied, and failing myocardium. Heart Failure Rev 4:269–280

Neubauer S (2007) The failing heart—an engine out of fuel. N Engl J Med 356(11):1140–1151

Neubauer S, H K. (1999) Functional and energetic consequences of chronic myocardial creatine depletion by b-guanidinopropionate in perfused hearts and in intact rats. J Mol Cell Cardiol 31:1845–1855

Neubauer S, Horn M, Cramer M et al (1997) Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation 96:2190–2196

Neubauer S, Remkes H, Spindler M, Horn M, Wiesmann F, Prestle J et al (1999) Downregulation of the Na(+)-creatine cotransporter in failing human myocardium and in experimental heart failure. Circulation 100(18):1847–1850

O'Gorman E, Beutner G, Dolder M, Koretsky AP, Brdiczka D, Wallimann T (1997) The role of creatine kinase in inhibition of mitochondrial permeability transition. FEBS Lett 414(2):253–257

Ohtsuki S, Tachikawa M, Takanaga H, Shimizu H, Watanabe M, Hosoya K, Terasaki T (2002) The blood-brain barrier creatine transporter is a major pathway for supplying creatine to the brain. J Cereb Blood Flow Metab 22(11):1327–1335

Osbakken M, Ito K, Zhang D, Ponomarenko I, Ivanics T, Jahngen EG, Cohn M (1992) Creatine and cyclocreatine effects on ischemic myocardium: 31P nuclear magnetic resonance evaluation of intact heart. Cardiology 80:184–195

Otellin VA, Korzhevskii DE, Kostkin VB, Balestrino M, Lensman MV, Polenov SA (2003) The neuroprotective effect of creatine in rats with cerebral ischemia. Dokl Biol Sci. May-Jun 390:197

Panchenko E, Dobrovolsky A, Rogoza A, Sorokin E, Ageeva N, Markova L, Titaeva E, Anuchin V, Karpov Y, Saks V (1994) The effect of exogenous phosphocreatine on maximal walking distance, blood rheology, platelet aggregation, and fibrinolysis in patients with intermittent claudication. Int Angiol 13:59–64

Parratt JR, Marshall RJ (1974) The response of isolated cardiac muscle to acute anoxia: protective effect of adenosine triphosphate and creatine phosphate. J Pharm Pharmacol 26:427–433

Pastoris O, Dossena M, Vercesi L, Bruseghini M, Pagnin A, Ceriana P (1991) Biochemical changes induced in the myocardial cell during cardioplegic arrest supplemented with creatine phosphate. J Cardiothorac Vasc Anesth 5:475–480

Perasso L, Cupello A, Lunardi GL, Principato C, Gandolfo C, Balestrino M (2003) Kinetics of creatine in blood and brain after intraperitoneal injection in the rat. Brain Res 974(1–2):37–42

Perasso L, Lunardi GL, Risso F, Pohvozcheva AV, Leko MV, Gandolfo C, Florio T, Cupello A, Burov SV, Balestrino M (2008) Protective effects of some creatine derivatives in brain tissue anoxia. Neurochem Res 33(5):765–775

Perasso L, Adriano E, Ruggeri P, Burov SV, Gandolfo C, Balestrino M (2009) In vivo neuroprotection by a creatine-derived compound: phosphocreatine-Mg-complex acetate. Brain Res 18(1285):158–163

Perasso L, Spallarossa P, Gandolfo C, Ruggeri P, Balestrino M (2013) Therapeutic use of creatine in brain or heart ischemia: available data and future perspectives. Med Res Rev 33(2):336–363. doi:10.1002/med.20255

Prass K, Royl G, Lindauer U, Freyer D, Megow D, Dirnagl U, Stöckler-Ipsiroglu G, Wallimann T, Priller J (2007) Improved reperfusion and neuroprotection by creatine in a mouse model of stroke. J Cereb Blood Flow Metab 27(3):452–459

Preobrazhenskiĭ AN, Dzhavadov SA, Saks VA (1986) Possible mechanism of the protective effect of phosphocreatine on the ischemic myocardium. Biokhimiia 51(4):675–83 (article in Russian)

Raisaro A, Bargiggia CS, Bertucci C (1989) Clinical evaluation of phosphocreatine effect during acute myocardial infarction: A multicenter study: 139147. Proceedings of the International Meeting "Cardioprotection with phosphocreatine in cardiology and cardiac surgery" IRCCS, Policlinico S. Matteo, Università degli studi, Pavia

Reimers B, Maddalena F, Cacciavilani L et al (1994) La fosfocreatina nell'infarto miocardico acuto: studio randomizzato multicentrico. Il Cuore 11:345–354

Roberts JJ, Walker JB (1982) Feeding a creatine analogue delays ATP depletion and onset of rigor in ischemic heart. Am J Physiol Heart C Physiol 12:H911–H916

Robinson LA, Braimbridge MV, Hearse DJ (1984) Creatine phosphate: an additive myocardial protective and antiarrhythmic agent in cardioplegia. J Thorac Cardiovasc Surg 87:190–200

Rosas HD, Doros G, Gevorkian S, Malarick K, Reuter M, Coutu JP, Triggs TD, Wilkens PJ, Matson W, Salat DH, Hersch SM (2014) PRECREST: a phase II prevention and biomarker trial of creatine in at-risk Huntington disease. Neurology 11;82(10):850–7

Rosenfeld J, King RM, Jackson CE, Bedlack RS, Barohn RJ, Dick A, Phillips LH, Chapin J, Gelinas DF, Lou JS (2008) Creatine monohydrate in ALS: effects on strength, fatigue, respiratory status and ALSFRS. Amyotroph Lateral Scler 9(5):266–272

Röther J (2008) Neuroprotection does not work! Stroke 39(2):523–524

Ruda MY, Samarenko MB, Afonskaya NI, Saks VA (1988) Reduction of ventricular arrhythmias by phosphocreatine (Neoton) in patients with acute myocardial infarction. Am Heart J 116:393–397

Saks VA, Dzhaliashvili IV, Konorev EA, Strumia E (1992) Molecular and cellular aspects of the cardioprotective mechanism of phosphocreatine. Biokhimiia 57(12):1763–84. Article in Russian

Savitz SI, Fisher M (2007) Prophylactic neuroprotection. Curr Drug Targets 8(7):846–849

Schiff SJ, Somjen GG (1987) The effect of graded hypoxia on the hippocampal slice: an in vitro model of the ischemic penumbra. Stroke. Jan-Feb 18(1):30–37

Sestili P, Martinelli C, Colombo E, Barbieri E, Potenza L, Sartini S, Fimognari C (2011) Creatine as an antioxidant. Amino Acids 40(5):1385–1396

Sestili P, Barbieri E, Stocchi V (2015) Effects of creatine in skeletal muscle cells and in myoblasts differentiating under normal or oxidatively stressing conditions. Mini Rev Med Chem. 16(1):4–11

Sharov VG, Saks VA, Kupriyanov VV et al (1987) Protection of ischemic myocardium by exogenous creatine phosphate: morphologic and phosphorus 31-nuclear magnetic resonance studies. J Thorac Cardiovasc Surg 94:749–761

Skřivánek O, Kalvach Pavel, Benetin Ján, Brzák M, Kukumberg I, Strnad P, Strnadová V, Traubner Pavol, Turčány P, Vávra P (1995) Waberžinek, Gerhard. Kreatinfosfát v léčbě akutních ischemických iktů. Prakticky Lekar 75:7–8

Soboll S, Conrad A, Eistert A, Herick K, Krämer R (1997) Uptake of creatine phosphate into heart mitochondria: a leak in the creatine shuttle. Biochim Biophys Acta 1320(1):27–33

Stefan Neubauer, Michael Horn, Klaus Schnackerz (1998) Manipulating creatine levels post-myocardial infarction chronic effects on left ventricular remodelling. Magn Reson Mater Phys, Biol Med 6:126–128

Stroke Therapy Academic Industry Roundtable (STAIR) (1999) Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 30(12):2752–2758

Strumia E, Pelliccia F, D'Ambrosio G (2012) Creatine phosphate: pharmacological and clinical perspectives. Adv Ther. 29(2):99–123

Tokarska-Schlattner M, Epand RF, Meiler F, Zandomeneghi G, Neumann D, Widmer HR, Meier BH, Epand RM, Saks V, Wallimann T, Schlattner U (2012) Phosphocreatine interacts with phospholipids, affects membrane properties and exerts membrane-protective effects. PLoS One 7(8):e43178

Whittingham TS, Lipton P (1981) Cerebral synaptic transmission during anoxia is protected by creatine. J Neurochem 37:1618–1621

Turner CE, Byblow WD, Gant N (2015) Creatine supplementation enhances corticomotor excitability and cognitive performance during oxygen deprivation. J Neurosci 35(4):1773–1780

Volek JS, Rawson ES (2004) Scientific basis and practical aspects of creatine supplementation for athletes. Nutrition 20(7–8):609–614

Wallimann T, Tokarska-Schlattner M, Schlattner U (2011) The creatine kinase system and pleiotropic effects of creatine. Amino Acids 40(5):1271–1296

Webster I, Toit E F Du, HuisamenB and Lochner A (2012) The effect of creatine supplementation on myocardial function, mitochondrial respiration and susceptibility to ischaemia/reperfusion injury in sedentary and exercised rats. Acta Physiol 206:6–19

Wick M, Fujimori H, Michaelis T, Frahm J (1999) Brain water diffusion in normal and creatine-supplemented rats during transient global ischemia. Magn Reson Med 42(4):798–802

Wild E (2015) http://en.hdbuzz.net/181. Accessed 18 Jan 2016

Wilken B1, Ramirez JM, Probst I, Richter DW, Hanefeld F (1998) Creatine protects the central respiratory network of mammals under anoxic conditions. Pediatr Res 43(1):8–14

Woo YJ, Grand TJ, Zentko S et al (2005) Creatine phosphate administration preserves myocardial function in a model of off-pump coronary revascularization. J Cardiovasc Surg 46:297–305

Woznicki DT, Walker JB (1980) Utilization of cyclocreatine phosphate, and analogue of creatine phosphate, by mouse brain during ischemia and its sparing action on brain energy reserves. J Neurochem 34(5):1247–1253

Yoneda K, Okada Y (1989) Effects of anoxia and recovery on the neurotransmission and level of high-energy phosphates in thin hippocampal slices from the guinea-pig. Neuroscience 28(2):401–407

Yoneda K, Arakawa T, Asaoka Y, Fukuoka Y, Kinugasa K, Takimoto K, Okada Y (1983) Effects of accumulation of phosphocreatine on utilization and restoration of high-energy phosphates during anoxia and recovery in thin hippocampal slices from the guinea pig. Exp Neurol 82(1):215–22

Zhu S, Li M, Figueroa BE, Liu A, Stavrovskaya IG, Pasinelli P, Beal MF, Brown RH Jr, Kristal BS, Ferrante RJ, Friedlander RM (2004) Prophylactic creatine administration mediates neuroprotection in cerebral ischemia in mice. J Neurosci 24(26):5909–12

Zucchi R, Poddighe R, Limbruno U, Mariani M, Ronca-Testoni S, Ronca G (1989) Protection of isolated rat heart from oxidative stress by exogenous creatine phosphate. J Mol Cell Cardiol 21:67–73