

# (Phospho)creatine: the reserve and merry-go-round of brain energetics

Hong-Ru Chen, Ton DeGrauw, Chia-Yi Kuan\*

Creatine transporter (CrT)-deficiency, the most common form of the cerebral creatine deficiency syndromes, causes cognition impairments and severe reduction of the brain creatine (Cr) and phosphocreatine (PCr) levels, and responds poorly to oral Cr supplement as a treatment option. The causes of cognitive impairments in CrT-deficient children remain unclear. We recently use gene-targeting to create a mouse model of CrT-deficiency to assess the impacts of Cr/PCr deficiency on brain energetics and stress-adaptation responses (Chen et al., 2021). We found that Cr/PCr-deficiency impairs the development of dendritic spines and synapses, skews the balance of mechanistic target of rapamycin (mTOR) and autophagy signaling towards catabolism, and elevates the sensitivity to external stress, including ischemia or hypoxia, to cause greater brain injury. Notably, intranasal delivery of Cr after cerebral ischemia raises the brain Cr/PCr levels and reduces the infarct size in CrT-null mice, despite Cr itself lacking a high-energy phosphoryl group. These findings highlight a critical role of Cr/PCr for maintaining brain energetics and suggest potential therapies of CrT-deficiency.

**CrT and the Cr biosynthesis pathway:** Creatine (from Greek *kreas*; means “flesh, meat”) was isolated from meat in 1832, and has been sold as a dietary supplement for over 150 years. Yet, around 50% of our daily need for Cr comes from *de novo* biosynthesis, which starts with the formation of guanidinoacetate through arginine and glycine via the enzyme arginine: glycine amidinotransferase in the kidney and pancreas. Guanidinoacetate is then methylated in the liver by guanidine-acetate methyltransferase to become Cr and enter blood circulation to reach its target organs (Figure 1A). The Cr from digested food is taken up by the intestine and also contributes to the plasma pool of Cr, which is at 15–44 μM at fasting in humans, far less than the Cr level in higher energy-demanding tissues like the skeletal muscles (~150 mmol/g weight), heart, and brain (~10 mmol/g weight). Thus, Cr cannot enter the energy-demanding tissues by passive diffusion. Instead, a high-affinity Na<sup>+</sup>- and Cl<sup>-</sup>-dependent CrT facilitates Cr-uptake against the steep concentration gradient (Wyss and Kaddurah-Daouk, 2000). Patients with arginine: glycine amidinotransferase- or guanidine-acetate methyltransferase-deficiency showed cognition deficits and reduced brain Cr/PCr levels, but respond well to oral Cr supplement as the treatment (Longo et al., 2011). In contrast, patients of CrT-deficiency—the second most common cause of X chromosome-linked cognitive impairment—showed little improvement of cognitive functions or increase of the brain Cr/PCr levels after oral Cr supplementation (Cecil et al., 2001; Longo et al., 2011). These poor responses to oral Cr treatment highlight the importance of CrT in establishing the brain Cr levels. Further, the kinetics of CrT-mediated Cr-uptake is slow, since it requires two weeks to restore the brain Cr/PCr levels in guanidine-acetate methyltransferase-null mice by daily supplement of 2 g/kg Cr, as judged by sequential proton magnetic resonance spectroscopy (Kan et al., 2007). It is unclear to what degree daily Cr supplement raises the brain Cr/PCr level or enhances cognitive functions in normal subjects. In patients with early and treated Parkinson’s disease, a large clinical trial showed no reduction in clinical symptoms after a daily supplementation of 10 g Cr for 4 years (Kiebert et al., 2015). These findings suggest a critical role of Cr/PCr for brain functions, but the details remain unclear. Nor are there reliable methods to harness the potential of Cr to oppose neurodegeneration

or acute brain injury.

**Cr/PCr as the energy reserve in brain:** Cr/PCr is known to function as an energy shuttle in skeletal muscle cells, travelling between the mitochondria where adenosine triphosphate (ATP) is produced and the cytosolic sites where ATP is utilized, but quickly regenerated by Cr/PCr (Wyss and Kaddurah-Daouk, 2000). The bidirectional energy transfer between ATP-Cr and PCr-adenosine diphosphate (ADP) ( $ADP + PCr \leftrightarrow ATP + Cr$ ) is catalyzed by mitochondrial and cytosolic creatine kinases (CK), respectively (Wallimann et al., 2011). This shuttling system seems superfluous—why not just use ATP as the sole energy currency?—but provides several advantages. First, partially due to the smaller size of PCr than ATP, the muscle cells store up to tenfold higher amount of PCr than ATP as the energy reserve. Second, PCr regenerates ATP 40 times faster than oxidative-phosphorylation (OXPHOS) and 10 times faster than glycolysis, thus fostering muscle cells, and likely neurons, to cope with a sudden energy demand without an extra supply of oxygen and glucose. Third, if without PCr/Cr-mediated ATP regeneration, one proton would be released for every ATP hydrolysis to ADP, which would rapidly cause cellular acidosis and deranged metabolism (Wyss and Kaddurah-Daouk, 2000). Last but not least, an important element of the shuttle scheme is “Cr-stimulated mitochondrial respiration”, which we will discuss in the next section.

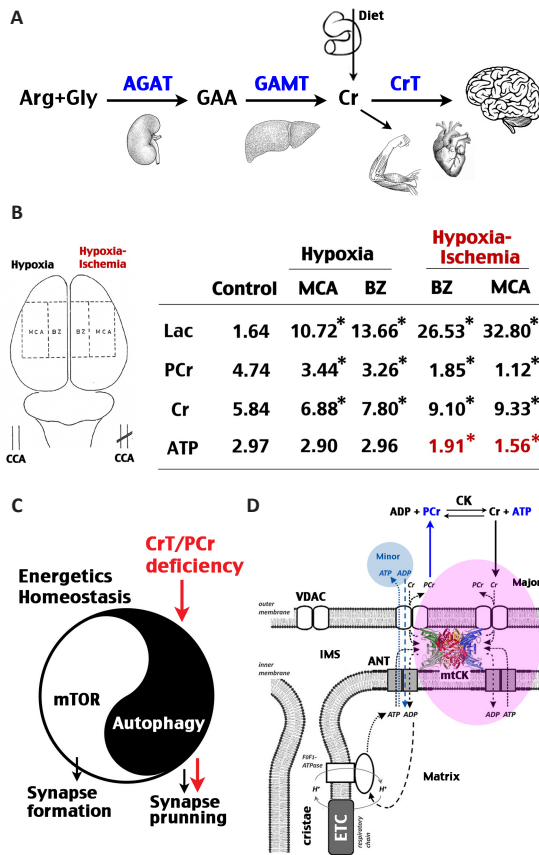
The energy-reserve role of Cr/PCr was cogently demonstrated in a study that compared the effects of hypoxia with unilateral carotid artery occlusion (the Levine procedure) on brain energy metabolism in adult rat brains (Salford and Siesjö, 1974). In the Levine procedure, the common carotid artery (CCA)-ligated cerebral hemisphere will develop infarction, while the contralateral hemisphere shows no palpable injury despite exposure to hypoxia. In Dr. Siesjö’s elegant study, the rat brains were frozen *in situ* at 30 minutes into hypoxia-ischemia to prevent the degradation of cellular chemicals, and processed to enzymatically measure the lactate, PCr, Cr, and ATP levels in the middle cerebral artery territory and the boundary zone between the middle cerebral artery and anterior cerebral artery trees. This analysis showed that the contralateral hemisphere contained lower PCr than untouched controls, but had similar ATP concentrations as untouched rat brains (Figure 1B). In contrast, the ipsilateral hemisphere showed a larger increase of lactate, greater conversion of PCr to Cr, and a severe reduction of ATP, coupled to the subsequent onset of infarction (Figure 1B). These results suggest that when neurons experience energy stress, the first tap into the PCr reserve to maintain ATP in the normal range, but when the PCr reserve is exhausted or unable to keep ATP above a threshold level, cellular injury ensues. Consistent with this scenario, CrT-deficient mice with reduced brain Cr/PCr levels showed greater vulnerability to a multitude of brain insults, such as neonatal hypoxia-ischemia and cerebral ischemia. Further, while CrT-null mice retained a near-normal baseline level of cortical ATP, they showed a larger reduction of cortical ATP after cerebral ischemia than wild-type mice. However, if CrT-null mice received the intranasal application of Cr after ischemia, their brain Cr/PCr levels were elevated and the infarct size was significantly reduced (Chen et al., 2021). Taken together, these results implicate Cr/PCr for maintaining brain energetics to oppose acute brain injury.

Our study of CrT-null mice suggests that Cr/PCr-deficiency not only decreases the energy reserve to cope with stress, but may also skew the balance

of mTOR and autophagy signaling in the brain. The mTOR signaling pathway promotes protein/organelle synthesis when nutrients are sufficient, while autophagy mediates the degradation of cellular contents in lysosomes to remove dysfunctional protein/organelles or regenerate energy in nutrient deficiency (Singh and Cuervo, 2011). The mutually antagonistic mTOR and autophagy signaling pathways converge on critical mediators, including the mammalian autophagy-initiating kinase Ulk1. Under energy starvation, AMP-activated protein kinase (AMPK) promotes autophagy by activating/phosphorylating Ulk1 at the Ser 317 and Ser 777 residues. In contrast, when with sufficient nutrients, the high mTOR activity phosphorylates Ulk1 at Ser 757 residue to disrupt the AMPK-Ulk1 interactions (Kim et al., 2011). Our recent study showed that CrT-deficient neurons and brains have a higher baseline level of AMPK activity and Ulk1 Ser 317-phosphorylation than wildtype counterparts, coupled to reduced mTOR but increased autophagy activities (Chen et al., 2021). These results suggest that constant Cr/PCr-deficiency may skew the balance of mTOR (anabolism) signaling towards autophagy (catabolism) to meet the energy need in the brain (Figure 1C). This signaling imbalance in CrT-null mice is amplified after external insults, while chronic autophagy activation may also impair the formation of dendritic spines and synapses, as detected in the CrT-null mouse brains (Chen et al., 2021). These structural anomalies may be the outcome of chronic Cr/PCr deficiency and the basis for cognition impairments in CrT-deficient patients.

**Cr-stimulated mitochondrial respiration:** As stated above, CrT-null mice developed larger infarction than wildtype controls after cerebral ischemia, correlated with increased autophagy and reduced mTOR activities. We also compared the effects of two routes of post-stroke Cr-supplement in CrT-null mice. Our results showed that intranasal delivery of Cr significantly reduced infarctions and mitigated the pivot to autophagy in CrT-null mice, while intraperitoneal application of Cr conferred no benefits (Chen et al., 2021). These findings support a critical role of CrT in assisting Cr-uptake across the blood-brain barrier so that intranasally-applied Cr can bypass the blood-brain barrier to reach brain parenchyma. These effects of intranasal Cr-treatment are even more striking when one considers that Cr itself lacks a high-energy phosphoryl group and thus cannot regenerate ATP directly. So what accounts for the protective effects of post-stroke Cr supplement? We suggest that these benefits may derive from the “Cr-stimulated mitochondrial respiration” that is closely connected to mtCK (Saks et al., 2000; Wallimann et al., 2011).

After being imported to the mitochondria, the nuclear-encoded mtCK forms octamers in the intermembrane space and often associates with voltage-dependent anion channel in the outer mitochondrial membrane plus adenine nucleotide transporter in the inner mitochondrial membrane (Figure 1D). The ATP generated by F<sub>0</sub>F<sub>1</sub>-ATPase during OXPHOS is transported to the intermembrane space via adenine nucleotide transporter in exchange for ADP. While a small component of ATP directly diffuses to the cytosol through voltage-dependent anion channel, the majority of ATP is captured by octameric mtCK and trans-phosphorylated into PCr in the intermembrane space. Then, PCr in turn leaves the mitochondria via voltage-dependent anion channel to serve as the cytosolic energy shuttle, while the ADP generated in mtCK trans-phosphorylation reaction is transported back to the matrix, which stimulates OXPHOS (Wallimann et al., 2011). It has been shown in permeabilized heart fibers and synaptosomes that mitochondrial respiration in the presence of Cr requires only micromolar concentrations of ADP to be fully stimulated, but needs far higher concentrations of ADP in the absence of Cr (because of the poor direct diffusion of ADP into the matrix) for a comparable respiratory rate (Saks et al., 2000; Monge et al., 2008). An advantage of the “Cr-



**Figure 1 | Important functions of creatine/phosphocreatine and CrT in the brain energetics homeostasis.**

(A) Schematic of the creatine biosynthesis pathway and the role of CrT. (B) The concentrations of lactate (Lac), PCr, Cr, and ATP in the indicated brain regions in adult rats during the Levine/Vannucci procedure (hypoxia plus the unilateral CCA ligation). Values are the means ( $\mu\text{mol/g}$  of wet tissue); \* $P < 0.01$  compared to untouched controls by *t*-test. Data were modified from Salford and Siesjo (1974). (C) The brain energy is maintained, in part, by the mutually antagonistic mTOR (anabolism) and autophagy (catabolism) signaling pathways. While mTOR signaling promotes synaptogenesis, high autophagy activity may stimulate synapse pruning. The results in Chen et al. (2021) suggest that CrT-mutation causes chronic brain energy stress, which tilts the *yin-yang* balance between mTOR and autophagy, leading to reduced synapse formation and/or enhanced synapse pruning. This energy stress-induced structure alterations may be the cause of cognition impairment in CrT-deficient patients. (D) In cells with oxidative metabolism, the mtCK octamers reside in the IMS and associate with VDAC in the outer mitochondrial membrane plus ANT in the inner mitochondrial membrane. ANT mediates the influx of ADP into the matrix, as well as, the efflux of ATP to the IMS, while only a small component of ATP is directly exported to the cytosol via VDAC. Rather, when cytosolic Cr is imported to the mitochondria via VDAC, the octameric mtCK triggers the transfer of high-energy phosphate group from ATP to produce PCr that returns to the cytosol, while the resultant ADP re-entering the matrix to stimulate OXPHOS along the ETC and FOF1-ATPase. This process, described as “creatine-stimulated respiration” in the literature, matches OXPHOS with the cellular need for energy while avoiding futile electron transfer that may produce oxygen radicals. Panel D is modified from Wallimann et al. (2011) with permission. ADP: Adenosine diphosphate; AGAT: arginine: glycine amidinotransferase; ANT: adenine nucleotide transporter; Arg: arginine; ATP: adenosine triphosphate; BZ: boundary zone between the MCA and anterior cerebral artery; CCA: common carotid artery; CrT: creatine transporter; ETC: electron transport chain; GAA: guanidinoacetate; GAMT: guanidinoacetate methyltransferase; Gly: glycine; IMS: intermembrane space; MCA: middle cerebral artery territory; mtCK: mitochondrial creatine kinase; mTOR: mechanistic target of rapamycin; OXPHOS: oxidative-phosphorylation; VDAC: voltage-dependent anion channel

stimulated respiration” phenomenon is that it matches OXPHOS with the cellular need for energy to avoid wasteful or futile electron transfer, which may produce oxygen radicals. Conversely, if mtCK is inhibited or Cr absent, mitochondrial respiration needs a high diffusible concentration of ADP in the cytoplasm to stimulate OXPHOS, which would cause inhibition of muscle contraction or impose cellular injury due to ADP-associated acidosis. As an analogy, the Cr/PCr shuttle makes mitochondrial respiration runs smoothly and efficiently like a “Merry-Go-Round”.

The results in our recent study with CrT-null mice highlight critical functions of Cr/PCr in maintaining brain energy homeostasis and stress-response signaling. Our findings also raise an intriguing question on whether intranasal Cr-supplement could be a treatment of CrT-deficiency or acute brain injury. To our knowledge, no such clinical trials are underway. We suggest that more animal studies are warranted to assess the reversibility of Cr deficiency-induced dendritic spine/synapse and cognition deficits. These studies

may suggest the optimal age of CrT-deficient patients for Cr-supplement treatments. Further, it will be important to compare the efficiency of nose-to-brain transport of Cr between rodents and humans, since the rodents have larger olfactory bulbs and may be uniquely suited for intranasal drug delivery. In conclusion, these additional studies are warranted to harness the cytoprotective potential of Cr/PCr in neurological disorders.

*We thank Dr. Theo Wallimann for insightful discussion and all collaborators who contributed to our research paper upon which the present commentary is based.*

*This work was supported by the NIH grant NS108763 (to CYK).*

**Hong-Ru Chen, Ton DeGrauw, Chia-Yi Kuan\***

Department of Neurosciences, University of Virginia School of Medicine, Charlottesville, VA,

USA (Chen HR, Kuan CY)  
Department of Pediatrics, Division of Neurology, Emory University, Atlanta, GA, USA (DeGrauw T)  
**\*Correspondence to:** Chia-Yi Kuan, PhD, alex.kuan@virginia.edu.  
<https://orcid.org/0000-0002-3453-863X> (Chia-Yi Kuan)  
**Date of submission:** January 7, 2022  
**Date of decision:** February 21, 2022  
**Date of acceptance:** March 21, 2022  
**Date of web publication:** June 2, 2022

<https://doi.org/10.4103/1673-5374.346470>

**How to cite this article:** Chen HR, DeGrauw T, Kuan CY (2023) (Phospho)creatine: the reserve and merry-go-round of brain energetics. *Neural Regen Res* 18(2):327-328.

**Open access statement:** This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

## References

- Cecil KM, Salomons GS, Ball WS Jr, Wong B, Chuck G, Verhoeven NM, Jakobs C, DeGrauw TJ (2001) Irreversible brain creatine deficiency with elevated serum and urine creatine: a creatine transporter defect? *Ann Neurol* 49:401-404.
- Chen HR, Zhang-Brotzge X, Morozov YM, Li Y, Wang S, Zhang H, Kuan IS, Fugate EM, Mao H, Sun YY, Rakic P, Lindquist DM, DeGrauw T, Kuan CY (2021) Creatine transporter deficiency impairs stress-adaptation and brain energetics homeostasis. *JCI Insight* 6:e140173.
- Kan HE, Meeuwissen E, van Asten JJ, Veltien A, Isbrandt D, Heerschap A (2007) Creatine uptake in brain and skeletal muscle of mice lacking guanidinoacetate methyltransferase assessed by magnetic resonance spectroscopy. *J Appl Physiol* 102:2121-2127.
- Kim J, Kundu M, Viollet B, Guan KL (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 13:132-141.
- Longo N, Ardon O, Vanzo R, Schwartz E, Pasquali M (2011) Disorders of creatine transport and metabolism. *Am J Med Genet C Semin Med Genet* 157C:72-78.
- Monge C, Beraud N, Kuznetsov AV, Rostovtseva T, Sackett D, Schlattner U, Vendelin M, Saks VA (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.
- Saks VA, Kongas O, Vendelin M, Kay L (2000) Role of the creatine/phosphocreatine system in the regulation of mitochondrial respiration. *Acta Physiol Scand* 168:635-641.
- Salford LG, Siesjo BK (1974) The influence of arterial hypoxia and unilateral carotid artery occlusion upon regional blood flow and metabolism in the rat brain. *Acta Physiol Scand* 92:130-141.
- Singh R, Cuervo AM (2011) Autophagy in the cellular energetic balance. *Cell Metab* 13:495-504.
- Wallimann T, Tokarska-Schlattner M, Schlattner U (2011) The creatine kinase system and pleiotropic effects of creatine. *Amino Acids* 40:1271-1296.
- Writing Group for the NINDS Exploratory Trials in Parkinson Disease (NET-PD) Investigators, Kiebertz K, Tilley BC, Elm JJ, Babcock D, Hauser R, Ross GW, Augustine AH, Augustine EH, Aminoff MJ, Bodis-Wollner IG, Boyd J, Cambi F, Chou K, Christine CW, Cines M, Dahodwala N, Derwent L, Dewey RB Jr, Hawthorne K, et al. (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* 80:1107-1213.

C-Editors: Zhao M, Liu WJ, Wang Lu; T-Editor: Jia Y