Creatine transporter (CrT)-deficiency, the most common form of the cerebral creatine deficiency syndrome, is known to cause cognitive 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 cognitive deficit in CrT-deficient children remains 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, skew the balance of mitochondrial and cytosolic pathways, and contribute to the plasma pool of Cr, which is at 15–44 μM at fasting in the intestine and also contributes to the plasma micromolar concentrations of ADP to be fully utilized with a sudden energy demand without an extra energy supply. The CrT-deficient children have a critical role of Cr/PCr for maintaining brain energetic and suggest potential therapies of CrT-deficiency.

**CrT and the Cr biosynthesis pathway:** Creatine (from Greek kreas; means “flesh, meat”) was assimilated from meat and adipose tissues and 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, such as the brain. Guanidoacetate is synthesized in the kidney and pancreas. Guanidoacetate is then reduced to guanidine by guanidoacetate 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 is absorbed into the bloodstream. Cr has a short half-life (40–60 minutes), and is stored in skeletal muscle (50 mmol/kg weight, heart, and brain ~10 mmol/g weight). Thus, Cr cannot enter the energy-demanding tissues by passive diffusion. Instead, a high-affinity Na+/K+-dependent CrT facilitates Cr uptake across the steep concentration gradient (Wyss and Kaddurah-Daouk, 2000). Patients with arginine: glycine amidinotransferase- or argininosuccinate synthetase- deficiency showed diminished Cr-uptake across the blood-brain barrier so that intranasally-applied Cr can bypass the blood-brain barrier to reach brain parenchyma. This effects of intranasal Cr-treatment are even more striking when compared to CrT-deficient children, whose high-energy phosphorpyruvate group and thus cannot regenerate ATP directly. So what accounts for the protective effects of post-stroke Cr supplement? We suggest that the higher-affinity CrT of CrT-null mice is amplified after external insults, while chronic autophagy activation may also impair the formation of dendritic spines and synapses, as indicated in the CrT-deficient children (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 intracerebroventricular delivery of Cr conferred no benefits (Chen et al., 2021). These findings support a critical role of Cr in assisting CrT-null mice to counter the shift 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 nucleotide-activated mTORC1 and mTORC2 regulatory complexes associate with the inner mitochondrial membrane and often associates with voltage-dependent anion channel in the outer mitochondrial membrane plus adenosine nucleotide transporter in the inner mitochondrial membrane (Figure 1D). The ATP generated by FOF1-ATPase during OXPHOS is transported to the intermembrane space via adenosine nucleotide transporter in exchange for ADP. The small component of ATP directly diffuses to the cytosol through voltage-dependent anion channel, the activity of ATP released from the intermembrane space via 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 mitochondrial 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 implicated that 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-
stimulated respiration” phenomenon is that it matches OXPHOS with the cellular need for energy while avoiding futile electron transfer that may produce oxygen radicals. Conversely, if mTCK is inhibited or Cr absent, mitochondrial respiration needs a high diffusive concentration of ADP to the matrix, as well as the diffusion 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 transfers the trigger of high-energy phosphate group from ATP to produce PCr that returns to the cytosol, while the resultant ADP re-enters 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 Wallmann 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.

The results in our recent study with CrT-null mice highlight critical functions of CrPCCR in maintaining brain energy homeostasis and stress-response signaling. Our findings also raise an intriguing question on whether intrasynaptic Cr-supplement could be a treatment of Cr 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 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 transfers the trigger of high-energy phosphate group from ATP to produce PCr that returns to the cytosol, while the resultant ADP re-enters 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 Wallmann 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.

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