INTRODUCTION

Creatine (Cr), which is synthesized when glycine and methionine are endogenously converted into phosphocreatine (PCr), is crucial for preserving the energy equilibrium throughout the body, particularly in energy-intensive tissues such as the muscular and central nervous systems. Cr is essential for the creatine phosphate shuttle, which moves Pi from mitochondria into the cytosol to form PCr and supports cellular bioenergetics. The endogenous synthesis of Cr provides approximately half of the daily requirement, with the remainder originating from food, primarily red meat, fish, or dietary supplements. Approximately 95% of Cr in the body is stored in the muscle, with the remaining 5% located in the heart, brain, and testes. Approximately 2/3 of this stored Cr is in the form of PCr, with the remaining 5% being free creatinine. The ergogenic role of Cr supplementation in improving muscle strength, lean mass, and exercise performance in both athletes and active leisure participants has been extensively documented since the 1970s. In addition to enhancing muscle performance, Cr is essential for the bioenergetics of the central nervous system because it replenishes adenosine triphosphate (ATP) without the need for oxygen. In addition to its bioenergetics, Cr has been reported to have anti-apoptotic, anti-excitotoxic, and anti-oxidative properties in vivo and in vitro. Therefore, researchers have investigated possible applications of Cr interventions in preclinical and clinical settings. A range of Cr regimens have demonstrated advantageous effects in vivo and in animal models of certain neurodegenerative diseases. Nonetheless, supplementation with Cr significantly benefits Cr-deficient disorders in humans. Therefore, determining the optimal dosage regime to increase brain Cr levels is essential for maintaining brain health. This short review seeks to offer insights into the ideal dosage regimen.
Cr biosynthesis and dietary transportation in the brain

Cr is provided through dietary consumption and endogenous synthesis in the whole body. The primary dietary sources of this nitrogenous organic acid are fish, red meat, and, to a lesser extent, dairy products. Dietary Cr is absorbed by a specific Na⁺/Cr⁺-createine transporter (SLC6A8) via an unidentified mechanism, which subsequently enters the bloodstream and travels throughout the body. Furthermore, two enzymatic processes are involved in the endogenous synthesis of Cr. These processes involve the following: l-arginine glycine aminotransferase (AGAT) converts l-arginine and glycine into guanidinoacetate (GAA) and l-ornithine in the mitochondrial intermembrane space; N-guanidinoacetate methyltransferase (GAMT) transfers a methyl group from S-adenosylmethionine (SAM) to GAA to produce creatine; and a specific creatine plasma membrane transporter, SLC6A8, is observed in the kidney, brain, and liver. Dietary Cr enters the brain through the blood-brain barrier (BBB) which is expressed in the microcapillaries of the BBB, neurons, and oligodendrocytes but not in perivascular astrocytes. In the brain, both AGAT and GAMT are expressed in astrocytes, and released Cr is taken up by neurons expressing SLC6A8, a specific Cr transporter. This suggests that the balance between endogenous production in the brain and dietary uptake may alter intracellular Cr content. Consequently, increasing intracellular Cr content may benefit brain function by increasing bioenergetics and strengthening neuroprotective functions.

Role of brain Cr in mitochondrial function of the neural system

Factors affecting brain Cr levels include aging, reduced physical activity, depression, and psychiatric abnormalities. Cr supplementation elicits beneficial effects under brain Cr deficit-related conditions, including physiological stress such as exercise and sleep deprivation and pathophysiological states such as creatine deficiency syndrome, mild traumatic brain injury, Alzheimer’s disease, and depression. Although the underlying molecular mechanisms are poorly understood, the pleiotropic effects of Cr may be associated with mitochondrial bioenergetics. The higher diffusion capacity and ability to reverse transport its N-phosphoryl group to the ADP of PCr can resolve the issue of the ATP diffusion ratio when the cell is insufficient to maintain energy demands. Due to its high energy requirements, brain tissue is susceptible to mitochondrial damage, reactive oxygen species (ROS), and energy depletion. According to in vitro and in vivo investigations, supplementation with Cr prevents intracellular Ca²⁺ and ROS accumulation, delays membrane depolarization, shields ATP depletion, and delays the opening of the mitochondrial permeability transition pore. ROS-induced nitrification of proteins and mitochondrial DNA and mitochondrial dysfunction characterized by swollen mitochondrial morphology, altered membrane potential, and ATP reduction are implicated in neurodegenerative diseases, aging, and cognitive decline. Therefore, improving mitochondrial function and reducing oxidative stress may be relevant treatments for neurodegenerative diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and amyotrophic lateral sclerosis (ALS).

The beneficial roles of Cr

Cr as a bioenergetics

Cr is necessary for glutamate clearance during excitatory synaptic transmission in the brain. For example, mice lacking CK isoforms display aberrant behaviors, such as impaired spatial learning and deficiencies in establishing and maintaining mossy fiber connections in the hippocampus. Therefore, Cr supplementation may provide powerful cellular bioenergetics by regulating the PCr/ATP system in the brain.

Cr as an anti-oxidant

Cr appears to have direct and indirect anti-oxidant effects. Through an ADP-recycling mechanism, Cr protected rat mitochondrial DNA from oxidative damage in a dose-dependent manner. Moreover, Cr protects against radicals such as superoxide anion (O2⁻), peroxynitrite (ONOO⁻), and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical. Furthermore, creatine has an indirect anti-oxidant effect on differentiated mouse myotube cultures (C2C12) through the upregulation of two significant anti-oxidant enzymes observed in the cytoplasm and mitochondria, peroxiredoxin-4 and thioredoxin-dependent peroxide reductase, respectively. Based on previous findings, Cr has been speculated to possess anti-oxidant properties, thereby protecting the nervous system against oxidative stress.

Cr as an anti-inflammatory factor

In addition to its anti-oxidant role, Cr has an anti-inflammatory function, although its molecular mechanism is poorly understood. For example, creatine reduces endothelial permeability and neutrophil adhesion to endothelial cells by suppressing the adhesion molecules ICAM-1 and E-selectin expression on endothelial cells. According to in vivo research, creatine may decrease the expression of Toll-Like Receptor (TLR) 2, a protein bound to the plasma membrane that identifies acylated bacterial lipoproteins on macrophages, which are important cells involved in the early stages of the immune response; this, in turn, may have contributed to the reduction in experimentally induced inflammation. Additionally, a recent study provided preliminary evidence that supplementation with creatine can prevent tumor-induced skeletal muscle atrophy in rats by reducing the proinflammatory environment caused by the tumor. Based on these studies, Cr supplementation may exert anti-inflammatory effects on the brain.

Cr supplementation and Alzheimer’s disease (AD)

Alzheimer’s disease is the most prevalent neurodegenerative disease, characterized by the accumulation of extracellular plaques, majorly consisting of amyloid-β peptide, and...
intracellular neurofibrillary tangles consisting of hyperphosphorylated tau. Reduced judgment, memory impairment, aphasia, miscalculation, agnosia, and other symptoms are the main clinical symptoms. The primary molecular mechanisms of AD are now understood to be abnormal tau protein phosphorylation and abnormal Aβ deposition. Aβ and tau proteins have a certain relationship and work together to mediate the progression of AD. Aging and neurodegenerative disorders are strongly linked to mitochondrial dysfunction, which produces the ATP required for neurons to survive and function at their best. Hyperphosphorylated tau pathology and typical Aβ deposition in AD can result from neuronal mitochondrial dysfunction. Consequently, tau pathology and Aβ deposition exacerbate this mitochondrial defect. Memory loss and synaptic toxicity are ultimately brought on by intracellular Ca2+ imbalance and energy deficiency brought on by Aβ42 oligomers resulting from mitochondrial dysfunction. In patients with AD, brain CK is profoundly inactivated by oxidation, and toxic aggregates co-reside at the Cr-rich site, suggesting that creatine metabolism is closely related to neuronal loss. Studies have suggested that Cr supplementation exerts positive effects in AD models. For example, this compound supplementation hampered transglutaminase-catalyzed protein aggregation in sedimentation studies, protected hippocampal neurons against amyloid-β neurotoxicity, and produced internalization of NMDA receptors under the presence of amyloid-β peptides in cortical neuronal cultures. Recently, by upregulating the expression of high-molecular-weight species and downregulating the low-molecular-weight 12 kDa mOC87 Aβ oligomer—the only Aβ species where higher concentration was correlated with worse cognitive impairment in this study—Cr supplementation changed the way Aβ was processed in female 3xTg mice. 3xTg mice of both sexes with increased concentrations of Cr in their hippocampi showed reduced levels of pTau/Tau in both sexes. These findings imply that CrM may have bioenergetic effects in AD that affect tau and A-beta protein phosphorylation and processing. Despite the beneficial role of Cr in AD-related experiments, only the neuroprotective effects of Cr in patients with AD have been reported since AD pathophysiology is more complex and multifaceted. Therefore, further research is required to determine how well an optimal Cr regimen protects against AD pathophysiology and pathology in humans.

Cr supplementation and Parkinson’s disease (PD)

Parkinson’s disease is the second most common neurodegenerative disease, characterized by progressive dopaminergic neuronal loss in the substantia nigra par compacta and intracellular inclusion of α-synuclein aggregates termed Lewy body (LB). Symptoms of this disorder include resting tremors, bradykinesia, muscle rigidity, blurred vision, depression, and dementia. Missense mutations in the SNCA genes (A53T, A53E, H50Q, G51D, E46K, and A30P) have been reported through genetic testing in patients with early-onset PD. In the brain, α-Syn can interact with specific cell types through a variety of mechanisms, such as glial cell phagocytosis and degradation of α-Syn, glial cell activation of inflammatory pathways, α-Syn trans-mission between glial cells and neurons, and interactions with peripheral immune cells. These findings have raised the possibility that α-Syn plays a role in the development of familial PD. Additional evidence supporting the role of mitochondrial dysfunction in Parkinson’s comes from genetic studies concentrating on the monogenic forms of the disease. Most proteins encoded by pathogenic mutations in PINK1 (PARK6), PRKN (PARK2), DJ-1 (PARK7), SNCA (PARK1), FBXO7 (PARK15), CHCHD2 (PARK22), and VPS13C (PARK23) are known to be involved in the mitochondrial quality control system. These mutations can cause familial PD. Complex I in the mitochondrial electron transport system is closely associated with PD pathogenesis, in which complex I activity is inhibited by typical PD-mimicking neurotoxins such as 6-hydroxydopamine (6-OHDA), rotenone, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This induced dopaminergic neuronal death. In an MPTP model, Cr supplementation exerted a neuroprotective effect, as indicated by significantly reduced dopaminergic neuronal loss. In addition, Cr co-treatment with rofecoxib (a cyclooxygenase-2 inhibitor) or coenzyme Q10 resulted in a greater reduction in dopaminergic neuronal loss than either treatment alone, suggesting that combination therapy is more effective at improving PD-related features. Moreover, Cr treatment diminished abnormal involuntary movements in an L-dopa-induced dyskinesia rat model. These results imply that supplementation with Cr may have both beneficial and therapeutic effects compared to the adverse effects of conventional treatment. More recently, Cr derivatives showed neuroprotective and anti-oxidant effects in models of 6-hydroxydopamine-induced (on synaptosomes), tert-butylihydroperoxide-induced (mitochondria), and iron/ascorbate-induced (microsomes) oxidative stress. This effect is attributed to the maintenance of low glutathione levels, ROS scavenging, and membrane stabilization against free radicals. Similar to AD, in clinical trials, Cr treatment resulted in improved mood but not motor performance, as measured by the Unified PD rating scale. In contrast, studies showed the neuroprotective impact of Cr treatment in patients with PD, as proven by Cr-treated improvement in upper-body strength and co-treatment with Cr and coenzyme Q10 administered decreased cognitive decline. Consequently, it may be necessary to conduct large-scale clinical trials with long-term follow-up to determine clinical outcomes.

Cr supplementation and Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis is characterized by the progressive loss of motor neurons in the entire CNS and spinal cord, eventually resulting in muscular paralysis and subsequent death. Neurodegenerative pathogenesis is closely associated with gliosis, oxidative stress, glutamate excitotoxicity, mitochondrial defects, and abnormal protein folding. The primary neuropathological characteristic of ALS is the extensive loss of lower motor neurons in the brainstem and anterior horn of the spinal cord. Although...
the precise pathogenesis remains unknown, according to Grad et al. ALS usually affects adults and manifests as a progressive loss of motor function, leading to respiratory failure and death. In SOD1-G93A mutant mice, lower levels of ATP and CK activity were observed before disease onset. This suggests improving the effects of Cr supplementation in patients with ALS. In a mouse model of ALS, the neuroprotective effects of Cr supplementation were demonstrated, in which Cr supplementation reduced neuronal loss in the motor cortex and substantia nigra and reduced ROS-induced damage, along with the induction of behavioral recovery. However, several human studies have shown that the beneficial effects of Cr in animal studies have failed to reproduce motor and respiratory functions in patients. The disparity between research conducted on humans and animals could indicate that Cr treatment started later in humans than in animals before symptoms appeared in the former case.

**Cr supplementation and Huntington’s disease (HD)**

Amplification of the CAG repeats in exon 1 of the huntingtin gene results in a mutant form of the huntingtin protein (mHtt), which causes HD, an autosomal-dominant illness. Disease symptoms include progressive choreoathetotic movements, cognitive impairment, and neuropsychiatric illnesses, which ultimately lead to death. mHtt causes neuronal death, mainly in GABAergic neurons, related to reduced mitochondrial complex II and III activities, facilitating cerebral lactate levels and a PCr/inoorganic phosphate ratio in the muscle. Cr treatment reduces lesion size, ameliorates convulsive behavior, and dampens lactate generation in rats stereotaxically injected with 3-nitropropionic acid (3-NPA, an irreversible succinate dehydrogenase inhibitor) or malonate (a reversible succinate dehydrogenase inhibitor). Moreover, Cr supplementation increases survival and body weight, delays motor symptoms, and reduces brain lesion size. Recent studies have shown that Cr supplementation improves neural progenitor cell survival depending on the developmental stage of HD, implying that promising findings from studies investigating the translational applications of Cr supplementation in NSC and NPC cell replacement therapies suggest that Cr may enhance cell graft survival and promote differentiation toward GABAergic phenotypes in striatal transplantation models. However, in clinical trials, Cr-treated improvement was not observed as measured by the Unified HD Rating Scale used to evaluate cognition, motor function, and functional ability. In contrast, a few clinical trials have reported beneficial effects in patients with HD. For example, a Cr-enhanced diet induces increased brain glutamate levels and decreased serum levels of 8-hydroxy-2-deoxyguanosine, a marker of oxidative insult to DNA, in patients with HD. Moreover, in patients likely to develop HD, high-dose Cr therapy significantly reduces cortical and striatal atrophy. These results suggest that Cr can delay disease progression and symptoms.

**Cr supplementation and creatine deficiency syndromes**

Mutations in AGAT, GAMT, and SLC6A8, which control Cr production and transportation, result in creatine-deficiency disorders. These syndromes manifest as mental retardation, autism, brain atrophy, delayed speech acquisition, and epilepsy. The pathological characteristics of these diseases include Cr depletion and the deposition of arginine and GAA. Among these syndromes, creatine supplementation elicits significant consequences for AGAT and GAMT mutations, suggesting that creatine supplementation can potentially be a therapeutic approach for defects in Cr-synthesizing enzymes. SLC6A8 deficiency is less effective against neurological symptoms because of the impermeability of Cr into the BBB. More recently, a study showed that di-acetyl Cr ethyl ester, a Cr derivative that travels across biological membranes with high lipophilicity, suppresses electrophysiological failure and elevates Cr levels in hippocampal slice systems. Moreover, Cr supplementation restores memory impairment, abnormal CK activity, and dysregulated redox homeostasis in GAA deposition-induced Cr deficiency models. Therefore, Cr supplementation has therapeutic potential against impaired Cr metabolism, such as Cr deficiency.

**CONCLUSION**

Cr is a potent stimulator of cellular bioenergetics, which increases the availability of high-energy phosphate in energy-demanding tissues, including the brain and muscles. In addition to serving as an energy reservoir, Cr possesses secondary effects, including anti-oxidant and anti-inflammatory properties, although the exact mechanism remains unknown. Due to its beneficial effects, Cr has been used to treat several neurological abnormalities. The beneficial effects of Cr are associated with mitochondrial function, and Cr supplementation has significant effects on noxious energy metabolism, such as ATP depletion. Various Cr regimens have demonstrated advantageous effects in in vitro and animal research on several neurodegenerative diseases. However, most clinical trials have been unable to replicate favorable results. These findings suggest that preventive interventions for neuroprotection in at-risk patients are the most promising area. To determine the best Cr supplementation for improving the pathophysiology of neurodegenerative illnesses, a few issues need to be resolved in future research. Future preclinical research must determine the putative molecular mechanisms underlying the beneficial effects of Cr on neurological defects. Future research in the clinical domain must examine more inquiries, taking into account the degree and phases of disease development and the timing and dosage of Cr treatment. In addition, larger sample sizes are required for reproducible clinical studies. Clinical applications for elevating brain Cr levels may consider several issues, including disease severity, timing of supplementation during disease onset and progression, and Cr-derivative enhancement of BBB permeability. Therefore,
identifying the optimal regimen to increase brain Cr levels is essential for maintaining brain health and treating neurodegeneration.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Seoul Women’s University (2023-0089).

REFERENCES

Creatine and neurodegeneration


Creatine and neurodegeneration.


68. Heyes LFF, Fighera MR, Furian AF, Oliveira MS, Myskiw JC, Fiorenza NG, Petry JC, Coelho RC, Mello CF. Effectiveness of creatine monohydrate on seizures and oxidative damage induced by methylmalonate. Pharmacol Biochem Behav. 2006;83:136-44.


