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Beyond muscles: The untapped potential of creatine

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ABSTRACT

Creatine is widely used by both elite and recreational athletes as an ergogenic aid to enhance anaerobic exercise performance. Older individuals also use creatine to prevent sarcopenia and, accordingly, may have therapeutic benefits for muscle wasting diseases. Although the effect of creatine on the musculoskeletal system has been extensively studied, less attention has been paid to its potential effects on other physiological systems. Because there is a significant pool of creatine in the brain, the utility of creatine supplementation has been examined *in vitro* as well as *in vivo* in both animal models of neurological disorders and in humans. While the data are preliminary, there is evidence to suggest that individuals with certain neurological conditions may benefit from exogenous creatine supplementation if treatment protocols can be optimized. A small number of studies that have examined the impact of creatine on the immune system have shown an alteration in soluble mediator production and the expression of molecules involved in recognizing infections, specifically toll-like receptors. Future investigations evaluating the total impact of creatine supplementation are required to better understand the benefits and risks of creatine use, particularly since there is increasing evidence that creatine may have a regulatory impact on the immune system.

1. Introduction

Creatine is naturally synthesized in the liver, kidney, and pancreas of vertebrates from the amino acids arginine, methionine, and glycine [1–3]. *In vivo*, creatine is a product of the arginine biosynthesis pathway and metabolizes to creatinine [3,4]. Individuals who eat meat and/or fish obtain approximately 1 g d^{-1} of creatine from the diet [5], and approximately 1 g d^{-1} is synthesized endogenously. Vegetarians have significantly lower muscle creatine stores and lower creatinine levels as compared to those who eat meat and/or fish products [6,7]. The average creatine pool for a 70 kg individual ranges from 120–140 g and approximately 2 g d^{-1} is lost in the urine in the form of creatinine [1]. Given that daily intake and excretion are approximately equal, the most efficient way to increase creatine stores in the body is through dietary supplementation. Creatine enters the muscle cells via a sodium- and chloride-dependent creatine transporter [3,8,9] and is primarily stored in the skeletal muscle as free creatine or phosphocreatine, which is a major source of energy to the host [3,8,10].

While the majority of creatine in the body is stored in skeletal muscles [3], there is also a significant pool of creatine in the brain [11], which may provide some protection against neurological disorders and trauma. For example, several studies using animal models have shown that oral creatine supplementation provides neuroprotective effects in a variety of neurological conditions including traumatic brain injury [12], Huntington's Disease [13], amyotrophic lateral sclerosis [14], ischemia [15], and Parkinson's Disease [16]. There have also been a small number of studies that suggest that pro-inflammatory responses are reduced following creatine supplementation [17–24]. However, the mechanism of how creatine acts in modulating inflammation remains unclear, although recent work [25] demonstrating that European sea bass fed a diet supplemented with arginine (a precursor of creatine) had reduced disease resistance suggests that the mechanism may be evolutionarily conserved. The potential of creatine as an immunomodulator may have important implications for individuals with certain pro-inflammatory diseases such as arthritis.

The purpose of this review is to examine the potential of dietary creatine supplementation to modulate disease, as well as to discuss potential mechanisms of action of creatine in its ability to function as a neuroprotective or immunomodulatory agent.

2. Creatine use by athletes

Creatine was first discovered as a constituent of meat in the 1800s; however, it was not until the 1970s that it was used as a potential ergogenic aid by athletes in the Soviet Union and Eastern bloc countries, and then gained wide research interest in the 1990s [26]. The phosphagen

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energy system is the metabolic system that produces ATP most rapidly, as compared to glycolysis or the aerobic system [27]. Inside the cell, creatine phosphokinase catalyzes a reversible reaction between the γ-phosphate group of ATP to the guanidino group of creatine resulting in ADP and phosphocreatine (Fig. 1). Cellular stores of phosphocreatine can reach concentrations of up to 40 mM [3,8,28–30] and is essential for replenishing ATP stores that are immediately used during high intensity exercise.

Dietary creatine supplementation increases the phosphocreatine stores in the muscles, and has been shown to enhance performance during high-intensity, short duration activities or repeated bouts of high-intensity exercise with short rest periods such as jumping, sprinting, and strength training [26,31–39]. It is estimated that 27–78% of all college athletes have used creatine supplements [40–43] and the proportion of individuals using creatine is likely much higher in athletes participating in sports such as football, track, wrestling, and soccer [26,31–34,44]. A recent study of body builders in Iran reported that creatine was the most common nutritional supplement used by men (60.8%) [35]. Initially, creatine was primarily utilized by elite athletes; but its use has become increasingly widespread among older adults, recreational athletes, and high school athletes [1,45–51].

The most widely used form of creatine by athletes is creatine monohydrate [41–43]. Oral bioavailability of creatine monohydrate is low due to the rapid conversion of creatine to creatinine in acidic environments, as would be encountered in the stomach [52,53]. While there has been little study examining how creatine crosses the intestine, the small intestine does express the Na+/Cl− creatine transporter [54] which is also expressed in other organs including the brain, kidney, and heart [55,56]. However, some work has suggested that creatine may move across the jejunum by paracellular movement [57]. The contributions of each of these potential mechanisms of transport is unclear although it has been shown that creatine supplementation of individuals deficient in the creatine transporter does improve muscular, but not cognitive and psychiatric symptoms of the condition [58], indicating that paracellular transport may be a sufficient to increase creatine stores in the muscles.

Athletes normally engage in the practice of loading that consists of ingesting 20 g d^{-1} of creatine for five days administered over several (usually four) doses followed by 1–10 g d^{-1} for several weeks or months [3,34]. The loading phase increases muscle stores of phosphocreatine 15 to 40% [59,60]. Minimal side effects as a result of the creatine loading phase have been reported and include cramping, nausea, fluid retention, and diarrhea [43,61]. Although it is typically recommended that individuals creatine load for 4–7 days, it has been reported that creatine uptake into muscle is greatest during the first 2 days of loading [62]. Hultman et al. [63] have also reported that a dose of 3 g d^{-1} for 28 days is as effective as creatine loading for increasing total muscle creatine stores. Therefore, 'slowly loading' the muscle with creatine may result in significant increases in performance and alleviate side effects that are sometimes associated with a 4–7 day loading regimen.

Very few individuals (~20% of users) reach maximal creatine saturation of their skeletal muscles (160 mmol/kg dry mass [10]), thus there is significant interest in developing formulations that have enhanced bioavailability. One currently available form of creatine, creatine ethyl ester, is reported to have a greater degree of stability and bioavailability than creatine monohydrate [53]. It is postulated that because the carboxyl group is no longer available, creatine ethyl ester is not converted to creatinine in the stomach, but can be absorbed in the intestine where the creatine ethyl ester enters the blood. Esterases in the intestinal cells and blood convert the creatine ethyl ester to creatine, which is then stored in the muscle cells as phosphocreatine. Of particular note, creatine ethyl ester is more stable than creatine monohydrate at a lower pH (as would be encountered in the stomach) [52,53]. In addition, *in vitro* studies utilizing Caco-2 cell monolayers have demonstrated increased permeability of creatine ethyl ester compared to creatine monohydrate [53]. Together, these studies [52,53] suggest that creatine ethyl ester may be more bioavailable than creatine monohydrate.

There are multiple mechanisms by which creatine functions to enhance athletic performance. As shown in Fig. 1, creatine is the substrate for the creatine kinase reaction, resulting in the generation of phosphocreatine, which comprises 60% of the creatine in skeletal muscle (~60% of muscle creatine is stored as phosphocreatine and 40% as free creatine) [2]. As previously mentioned, phosphocreatine is responsible for the re-phosphorylation of ADP to ATP during bursts of high intensity movements, thus resulting in an increased availability of energy during short periods of explosive exercise [35–39,64]. As phosphocreatine levels decline due to the re-phosphorylation of ADP, phosphofructokinase production is stimulated, thereby increasing the rate of glycolysis [39]. Creatine can also function to buffer the pH changes that occur due to the accumulation of lactate and hydrogen ions by using the hydrogen ions in the creatine kinase reaction [65,66]. Individuals with creatine or phosphocreatine deficiencies due to genetic defects in proteins involved in creatine synthesis (L-arginine–glycine amidinotransferase or guanidinoacetate methyltransferase) or transport (creatine transporter [SLC6A8]) have reduced levels of ATP in the brain resulting in developmental delays and mental retardation [67–69]. While individuals with defects in creatine synthesis can be treated with exogenous creatine, no treatment exists for individuals with deficits in the creatine transporter [67–69].

3. Creatine as a mediator of neuroprotection

3.1. The mitochondrial permeability transition pore

Because of the high levels of creatine in the central nervous system [11], a considerable number of studies have focused on the potential neuroprotective effects of oral creatine supplementation in a variety of neurological conditions including traumatic brain injury (TBI) [12,70,71], Huntington's Disease (HD) [13,72–76], amyotrophic lateral sclerosis (ALS) [14,77–80], cerebral ischemia [15,81], and Parkinson's Disease (PD) [82–88]. One of the key focuses as to how creatine may work to reduce neuropathology in the central nervous system (CNS) has been the effect of creatine on the mitochondrial permeability transition pore [14,89]. In the CNS, the mitochondrial permeability transition pore is induced in the conditions mentioned above including stroke [90], PD [91,92], HD [93], TBI [94,95], and ALS [14,96,97]. The mitochondrial permeability transition pore is also induced by reactive oxygen species (ROS) [98] and, correspondingly, ROS are released as a result of development of the pore [99]. Conversely, high ATP and ADP levels prevent the mitochondrial pore from being induced [98,100,101].

Excitotoxicity is a major cause of neuronal death that is the result of an influx of calcium into the cell as a result of glutamate receptor

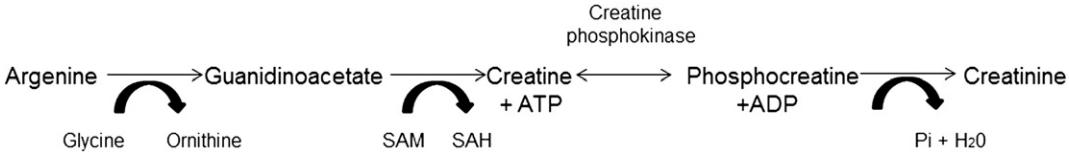

Fig. 1. The arginine biosynthesis pathway. Creatine is generated as part of the arginine biosynthesis pathway. Creatine and phosphocreatine exist in equilibrium in the cell. Creatine phosphokinase catalyzes the reaction between creatine and phosphocreatine, which results in energy (ATP) generation. Hydrolysis of phosphocreatine yields the end product of the pathway, creatinine. SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine