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"Time course of plasma creatine concentrations following oral administration of a novel creatine formulation"

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Resume

1. INTRODUCTION

1.1 History of Creatine: Discovery and Evolution Through Time

Creatine was first discovered in 1832 by the French scientist Michel Eugène Chevreul, who isolated a new nitrogen-containing compound from skeletal muscle and named it after the Greek word *kreas*, meaning "meat" [1].

During the second half of the nineteenth century, several studies identified creatine as a naturally occurring constituent of vertebrate muscle tissue and demonstrated that it could also be obtained through dietary intake, particularly from meat and fish products.

A major breakthrough came during the 1920s, when scientists discovered phosphocreatine, the phosphorylated form of creatine stored in skeletal muscle. Shortly afterward, the creatine kinase reaction was identified, establishing the fundamental role of the creatine-phosphocreatine system in the regeneration of adenosine triphosphate (ATP) during muscular contraction [2]. Interest in creatine increased substantially during the second half of the twentieth century, particularly after studies demonstrated that oral creatine supplementation could increase intramuscular creatine stores [3].

In the 1990s, creatine supplementation became widely popular in sports nutrition following evidence showing improvements in strength, power output, high-intensity exercise capacity, and training adaptations [4]. More recently, research has progressively expanded beyond athletic performance. Increasing evidence has suggested potential therapeutic applications in several clinical conditions, including neurodegenerative diseases, sarcopenia, muscular dystrophies, traumatic brain injury, and metabolic disorders [5]. In parallel, the role of creatine in brain energy metabolism, neuroprotection, healthy aging, and mitochondrial function has attracted growing scientific interest.

As research expanded, creatine became one of the most extensively investigated dietary supplements in sports nutrition. The publication of multiple position stands by the International Society of Sports Nutrition (ISSN) further consolidated the scientific consensus regarding the efficacy and safety of creatine supplementation across different populations, including athletes, older adults, and clinical populations [5,6].

Today, creatine is considered one of the most extensively studied and scientifically supported nutritional supplements in both sport science and clinical nutrition. Despite decades of

research, several aspects of creatine metabolism, tissue transport, pharmacokinetics, and therapeutic potential remain under active investigation.

1.2 Creatine: Structure, Biosynthesis, and Metabolism

Since its discovery, creatine has attracted considerable scientific interest for its roles in ATP regeneration, muscle performance, and metabolic homeostasis. Although commonly associated with sports supplementation, creatine is a naturally occurring molecule continuously synthesized in the human body and participates in numerous physiological processes beyond skeletal muscle function.

Creatine is a guanidino compound that plays a central role in cellular energy homeostasis, particularly in tissues with high and fluctuating energy demands. It is synthesized endogenously and distributed primarily to skeletal muscle and the central nervous system (CNS), where rapid ATP turnover is required to sustain cellular activity [1]. Approximately 95% of total body creatine is stored in skeletal muscle, containing 120–140 g in a healthy 70-kg adult [4], while the remaining fraction is distributed among the brain, heart, testes, and other tissues [7].

From a chemical perspective, its molecular formula is $C_4H_9N_3O_2$, corresponding to a molecular weight of 131.13 g/mol. The molecule contains several functional groups, including a methyl group ($-CH_3$), a carboxyl group (-COOH), and an amidino group attached to a nitrogen atom (Fig. 1). These structural features underlie the biochemical properties of creatine and its role in intracellular phosphate-transfer reactions involved in energy metabolism [1].

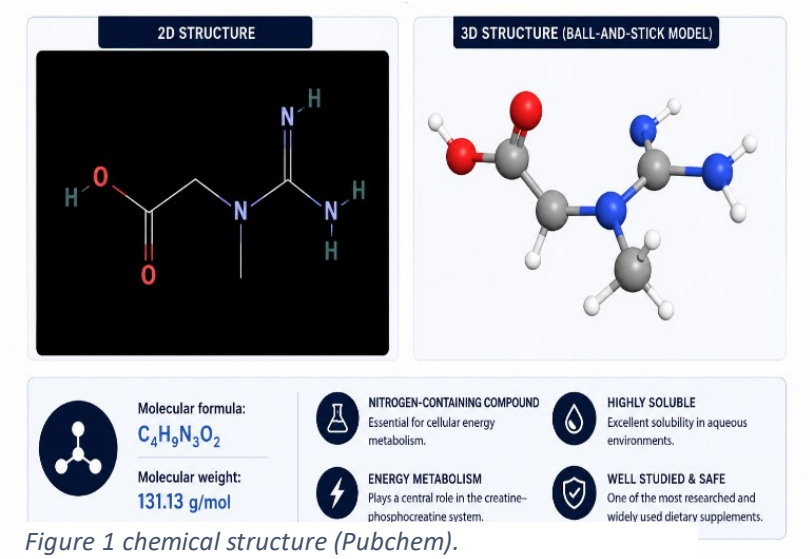

Figure 1 chemical structure (Pubchem).

In physiological aqueous environments, creatine predominantly exists as a zwitterion, a molecular form that carries both positive and negative charges yet remains electrically neutral overall.

Specifically, the guanidino moiety is protonated and carries a positive charge, whereas the carboxyl group is deprotonated and carries a negative charge. This ionic configuration strongly influences the physicochemical properties of creatine, including its solubility, membrane transport, and distribution within biological fluids [1].

The ionization state of creatine is influenced by the pH of the surrounding environment. Under physiological conditions (pH approximately 7.4), the zwitterionic form predominates and represents the most stable species in solution. At lower pH values, such as those encountered in highly acidic environments, protonation of functional groups becomes more pronounced, whereas under more alkaline conditions, deprotonation is favored. These pH-dependent changes can influence the chemical stability of creatine and its conversion into degradation products.

The relationship between pH and creatine stability is particularly relevant in the context of supplementation. In acidic aqueous solutions, especially during prolonged storage, creatine can undergo non-enzymatic cyclization to form creatinine, its principal degradation product. However, under the acidic conditions normally present in the stomach, the rate of this conversion appears relatively limited because gastric emptying occurs before substantial degradation can take place [8]. Consequently, oral creatine supplementation remains highly effective despite exposure to gastric acidity. Overall, the acid-base properties of creatine contribute not only to its chemical behavior in solution but also to its physiological stability, absorption, and bioavailability within the human body.

Endogenous creatine synthesis occurs through a two-step enzymatic pathway involving glycine, arginine, and methionine [9,10]. The first reaction occurs mainly in the kidneys and pancreas, where the enzyme arginine: glycine amidinotransferase (AGAT) catalyzes the transfer of an amidino group from arginine to glycine, generating guanidinoacetate and ornithine.

The guanidinoacetate produced is subsequently transported to the liver, where guanidinoacetate methyltransferase (GAMT) catalyzes a methylation reaction using S-adenosylmethionine (SAM) as the methyl donor, forming creatine and S-adenosylhomocysteine (SAH).

Once synthesized, creatine is released into the bloodstream and distributed to target tissues through the sodium- and chloride-dependent creatine transporter SLC6A8, which mediates

cellular uptake in skeletal muscle, brain, and other energy-demanding tissues [2,11]. Within muscle fibers, approximately two-thirds of total creatine is present as phosphocreatine, whereas the remaining fraction exists as free creatine [1].

The physiological importance of creatine largely depends on its reversible conversion to phosphocreatine via the enzyme creatine kinase. This reaction uses ATP as a phosphate donor and represents a key component of the cellular energy-buffering system. Regardless of their physiological roles, both creatine and phosphocreatine undergo a spontaneous, irreversible, non-enzymatic cyclization reaction, resulting in the formation of creatinine, the principal degradation product of creatine metabolism [1]. Unlike creatine synthesis or phosphorylation, its formation does not require enzymes or metabolic regulation. Instead, it occurs through a spontaneous intramolecular cyclization followed by the irreversible loss of a water molecule, producing creatinine. Creatinine is subsequently released into the bloodstream and excreted in urine. Because its production rate is relatively constant and closely related to muscle mass, circulating and urinary creatinine concentrations are widely used in clinical practice as indirect indicators of renal function and glomerular filtration rate [8].

Overall, the biosynthesis, transport, storage, and metabolism of creatine constitute an integrated physiological network essential for maintaining cellular energy balance.

2. PHYSIOLOGICAL ROLES OF CREATINE

2.1 The Creatine–Phosphocreatine System and Cellular Bioenergetics

The primary physiological function of creatine is to facilitate the rapid regeneration of ATP, the universal energy currency of the cell. Cellular ATP stores are relatively limited and would be depleted within seconds if not continuously replenished. To prevent energetic failure during periods of increased ATP consumption, creatine functions as a readily available reservoir of high-energy phosphate groups through its reversible conversion to phosphocreatine, a reaction catalyzed by creatine kinase (CK).

Within mitochondria, ATP generated through oxidative phosphorylation is utilized by mitochondrial creatine kinase to phosphorylate free creatine, producing phosphocreatine. Once formed, phosphocreatine rapidly diffuses through the cytosol toward regions of elevated ATP utilization. Cytosolic creatine kinase isoforms subsequently transfer the phosphate group back to adenosine diphosphate (ADP), regenerating ATP directly at sites of energy consumption. This process, commonly known as the phosphocreatine shuttle, enables the efficient transport of metabolic energy between intracellular compartments and helps maintain stable ATP concentrations despite substantial fluctuations in energy turnover [2].

An additional physiological function of the creatine-phosphocreatine system involves the regulation of the intracellular ATP/ADP ratio. Maintaining a high ATP/ADP ratio is essential for preserving the thermodynamic conditions required for optimal cellular metabolism. By rapidly buffering increases in ADP concentration during periods of elevated energy demand, phosphocreatine contributes to metabolic stability and supports the activity of ATP-dependent cellular processes [1]. Consequently, alterations in creatine availability can influence mitochondrial performance, cellular resilience to metabolic stress, and overall energetic capacity [2].

Collectively, these mechanisms establish creatine as a central regulator of cellular energy metabolism. Rather than functioning solely as an ATP buffer, the creatine-phosphocreatine system coordinates intracellular energy storage, transport, and utilization, thereby supporting the energetic requirements of numerous tissues under both physiological and pathological conditions.

2.2 Skeletal Muscle Physiology and Exercise Performance

In the muscle, one of the primary physiological roles of phosphocreatine is to rapidly regenerate ATP during the transition from rest to intense exercise. Because ATP stores are limited and maximal energy demand increases dramatically during contraction, phosphocreatine serves as the immediate energy buffer, maintaining ATP availability until glycolytic and oxidative metabolism are fully activated [1].

The importance of this mechanism is particularly evident in type II fast-twitch muscle fibers, which are characterized by high rates of force production, rapid ATP utilization, and a greater reliance on anaerobic metabolism. These fibers contain relatively high phosphocreatine concentrations and depend heavily on the creatine kinase reaction to sustain repeated high-intensity contractions [7].

Beyond its direct role in ATP regeneration, the creatine-phosphocreatine system helps maintain intracellular homeostasis during muscular activity. By buffering ATP concentrations and limiting excessive ADP accumulation, phosphocreatine helps preserve membrane excitability, calcium handling, and ion transport processes that are essential for normal contractile function. Although creatine does not directly prevent fatigue, maintaining cellular energetic stability may delay the physiological disturbances that contribute to force decline during prolonged or repeated high-intensity exercise [2].

The rapid resynthesis of phosphocreatine during recovery periods represents another critical determinant of exercise capacity. Following intense muscular contractions, phosphocreatine stores must be restored before subsequent efforts can be performed at maximal intensity. Studies have demonstrated that individuals with greater intramuscular creatine availability generally exhibit an enhanced capacity to sustain repeated high-intensity exercise, highlighting the importance of phosphocreatine recovery kinetics in sports performance [3].

The physiological significance of creatine in skeletal muscle extends beyond acute energy metabolism. Increasing evidence suggests that creatine availability may influence several cellular processes involved in muscle adaptation and remodeling. One proposed mechanism involves the osmotic properties of creatine. Elevated intracellular creatine concentrations promote water retention within muscle fibers, resulting in cellular swelling. This phenomenon has been proposed as a potential anabolic signal that influences protein turnover, glycogen synthesis, and intracellular pathways involved in muscle growth and adaptation [12].

In addition to its effects on cellular hydration, creatine supplementation has been associated with molecular adaptations related to muscle hypertrophy. Experimental studies have reported alterations in the expression of genes involved in protein synthesis, glucose metabolism, cellular stress responses, and myogenic differentiation following creatine supplementation [12]. Furthermore, creatine supplementation combined with resistance training has been associated with increased satellite cell activation and myonuclear accretion, mechanisms believed to contribute to long-term increases in skeletal muscle mass and functional capacity [13].

Taken together, current evidence supports the view that creatine plays a multifaceted role in skeletal muscle physiology. In addition to functioning as a critical component of cellular bioenergetics, creatine influences recovery capacity, exercise performance, muscle adaptation, and metabolic regulation. These diverse physiological actions largely explain why creatine supplementation remains one of the most extensively studied and consistently effective nutritional interventions for improving high-intensity exercise performance and supporting skeletal muscle function [7].

2.3 Creatine and Cardiac Energetics

The heart is one of the most metabolically active organs in the human body, requiring a continuous and tightly regulated supply of energy to sustain lifelong contractile activity. Unlike skeletal muscle, which alternates between periods of activity and recovery, cardiac muscle functions continuously and therefore depends on highly efficient mechanisms for ATP production, transport, and utilization. Within this context, the creatine–phosphocreatine system plays a central role in maintaining myocardial energy homeostasis and supporting the heart's extraordinary energetic demands [14]. In cardiac tissue, phosphocreatine serves as an immediately available energy reserve that buffers transient mismatches between ATP production and utilization. During acute increases in workload, such as those during exercise or sympathetic stimulation, phosphocreatine concentrations may temporarily decrease to preserve ATP availability and maintain contractile performance [14].

The importance of creatine metabolism in cardiac physiology becomes particularly evident under pathological conditions. Experimental and clinical studies have consistently demonstrated that reductions in myocardial creatine and phosphocreatine concentrations are common features of several cardiovascular diseases, including heart failure, myocardial ischemia, and cardiomyopathies. Notably, decreases in myocardial phosphocreatine content

often precede clinically apparent impairments in cardiac function, suggesting that energetic abnormalities may contribute directly to disease progression rather than merely reflect secondary consequences of cardiac dysfunction.

For instance, heart failure is characterized not only by impaired mechanical performance but also by profound alterations in myocardial energy metabolism. Patients with chronic heart failure frequently exhibit reduced phosphocreatine-to-ATP ratios, diminished creatine kinase activity, and lower total creatine concentrations within cardiac tissue. These changes collectively result in a reduced energetic reserve, limiting the heart's ability to respond to increases in workload and contributing to progressive functional decline [14].

The clinical relevance of these observations has sparked considerable interest in the therapeutic potential of targeting myocardial creatine metabolism. Experimental models have demonstrated that manipulating creatine availability can influence cardiac energy status and resistance to metabolic stress. Nevertheless, despite promising mechanistic evidence, translating these findings into effective clinical therapies remains challenging. The regulation of myocardial creatine uptake, intracellular compartmentalization, and long-term adaptations to altered creatine availability remain poorly understood, and clinical studies have produced heterogeneous results [2]. Collectively, current evidence indicates that creatine plays a pivotal role in maintaining myocardial energy homeostasis under both physiological and pathological conditions.

2.4 Creatine in the Central Nervous System

Although creatine has traditionally been studied in the context of skeletal muscle physiology, growing evidence indicates its importance in the CNS, where it helps maintain cellular energy homeostasis and neuronal function. The brain represents one of the most metabolically demanding organs in the human body, accounting for approximately 20% of total resting oxygen consumption despite representing only about 2% of body mass. This high energetic demand reflects the continuous ATP requirements for maintaining membrane potential, synthesizing and releasing neurotransmitters, transporting axonal cargo, conducting synaptic transmission, and regulating intracellular signaling [1].

As in other highly active tissues, the creatine–phosphocreatine system plays a critical role in supporting cerebral energy metabolism. Creatine is transported across the blood-brain barrier and into neural cells primarily through the sodium- and chloride-dependent creatine

transporter SLC6A8, which is expressed in neurons, oligodendrocytes, and other cell populations within the nervous system [11,15].

The physiological importance of cerebral creatine metabolism is particularly evident when considering the limited energy reserves of neuronal tissue. Unlike skeletal muscle, which can tolerate transient fluctuations in energy supply, neurons are highly sensitive to disturbances in ATP availability. Even brief interruptions in energy production may impair maintenance of ion gradients, alter neurotransmission, disrupt synaptic activity, and ultimately compromise neuronal viability. Consequently, mechanisms that buffer cellular energy fluctuations are essential for preserving normal brain function [16].

Beyond its direct role in ATP buffering, creatine appears to influence several cellular processes involved in neuronal protection and metabolic resilience. Experimental studies suggest that increased creatine availability may support mitochondrial function by facilitating intracellular energy transfer and reducing energetic stress during conditions of increased ATP demand. Since mitochondrial dysfunction represents a common feature of numerous neurological disorders, the interaction between creatine metabolism and mitochondrial physiology has attracted considerable scientific interest. Moreover, neuronal activity is associated with substantial production of reactive oxygen species (ROS), particularly under conditions of metabolic challenge. Although creatine is not considered a classical antioxidant, experimental studies suggest that enhanced phosphocreatine availability may indirectly reduce oxidative damage by preserving mitochondrial efficiency and limiting energetic disturbances that promote excessive ROS generation. These observations support the hypothesis that creatine may contribute to cellular resilience under conditions of oxidative and metabolic stress.

Another proposed neuroprotective mechanism involves regulating intracellular calcium homeostasis. Calcium ions play a fundamental role in neuronal signaling; however, excessive intracellular calcium accumulation may trigger a cascade of pathological events, including mitochondrial dysfunction, activation of degradative enzymes, and apoptotic signaling pathways. Experimental evidence suggests that preserving cellular energy status via the creatine-phosphocreatine system may help maintain calcium homeostasis and reduce susceptibility to calcium-mediated cellular injury.

Particular attention has also been directed toward the potential role of creatine in limiting excitotoxicity, a pathological process characterized by excessive glutamatergic stimulation and neuronal damage. Excitotoxic mechanisms are implicated in a wide range of neurological conditions and are frequently associated with disturbances in cellular energy metabolism. By supporting ATP availability and mitochondrial integrity, creatine may enhance neuronal

tolerance to excitotoxic stress, although the precise molecular mechanisms remain incompletely understood [16].

The potential neuroprotective properties of creatine have stimulated extensive research into its application in both acute and chronic neurological disorders. Experimental studies have investigated creatine supplementation in models of Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), traumatic brain injury, spinal cord injury, muscular dystrophies, and cerebral ischemia [17]. In many preclinical models, creatine administration has been associated with improvements in cellular bioenergetics, attenuation of neuronal damage, and enhanced survival of vulnerable cell populations.

Despite encouraging mechanistic findings, translation into clinical practice has proven more complex. While some studies have reported potential benefits in specific neurological conditions, others have failed to demonstrate significant clinical improvements. Differences in disease stage, supplementation protocols, treatment duration, blood-brain barrier transport, and tissue-specific creatine uptake may partially explain the variability observed across studies [17]. Consequently, although the biological rationale supporting creatine supplementation in neurology remains compelling, its therapeutic efficacy has not yet been conclusively established for most neurodegenerative diseases.

Beyond its role in pathological conditions, creatine metabolism may also contribute to normal cognitive function. Because cognitive processes rely heavily on ATP-dependent neuronal activity, several studies have examined whether increased creatine availability may influence cognitive performance, particularly under conditions of elevated metabolic stress, such as sleep deprivation, hypoxia, or prolonged mental effort. Although the available evidence remains heterogeneous, these findings further support the broader concept that creatine serves as an important regulator of cerebral bioenergetics beyond its traditional role in skeletal muscle physiology [18].

Collectively, current evidence indicates that creatine plays a central role in maintaining neuronal energy homeostasis and supporting brain function. Through its involvement in ATP buffering, mitochondrial support, oxidative stress regulation, calcium homeostasis, and cellular resilience, the creatine–phosphocreatine system represents a fundamental component of cerebral metabolism. Ongoing research continues to investigate the therapeutic potential of creatine in neurological disorders and to further expand our understanding of its physiological functions within the central nervous system.

2.5 Creatine and Healthy Aging

Aging is characterized by a progressive decline in physiological function across multiple organ systems, accompanied by reductions in muscle mass, muscular strength, physical performance, metabolic flexibility, and mitochondrial function. Collectively, these alterations contribute to increased frailty, reduced independence, impaired quality of life, and a greater risk of morbidity and mortality in older adults [19]. Since many age-related functional impairments are closely associated with disturbances in cellular energetics, creatine has emerged as a promising nutritional strategy to support healthy aging and preserve physiological function throughout the lifespan.

One of the most prominent age-related changes is the progressive loss of skeletal muscle mass and strength, a condition known as sarcopenia. Although sarcopenia is a multifactorial process, several contributing mechanisms have been identified, including reductions in physical activity, chronic low-grade inflammation, hormonal alterations, impaired neuromuscular function, decreased anabolic sensitivity, and mitochondrial dysfunction [19].

Advancing age is associated with alterations in muscle energetics that may reduce the capacity to rapidly regenerate ATP during periods of increased demand. In addition, older adults frequently exhibit lower phosphocreatine availability, slower phosphocreatine recovery kinetics, and reduced mitochondrial efficiency compared with younger individuals [20]. These changes may contribute to declines in muscular performance, increased fatigability, and impaired functional capacity. By increasing intramuscular creatine and phosphocreatine stores, creatine supplementation may partially offset these energetic limitations and improve the capacity to perform daily physical activities.

The potential benefits of creatine during aging extend beyond its direct effects on muscle bioenergetics. A growing body of evidence suggests that creatine supplementation, particularly when combined with resistance training, can promote favorable adaptations in body composition and physical function. Numerous intervention studies have reported improvements in lean body mass, muscular strength, muscular endurance, and functional performance in older adults receiving creatine supplementation alongside structured exercise programs. These effects are believed to result from the combined influence of enhanced training capacity, improved recovery, increased cellular hydration, and potentially greater activation of anabolic pathways involved in muscle remodeling.

An additional factor contributing to age-related muscle loss is anabolic resistance, a phenomenon characterized by a diminished responsiveness of skeletal muscle to anabolic

stimuli such as dietary protein intake and resistance exercise. Although the precise mechanisms remain incompletely understood, impaired intracellular signaling, chronic inflammation, and alterations in muscle satellite cell function appear to play important roles. Emerging evidence suggests that creatine supplementation may support muscle adaptation by enhancing training responsiveness and promoting a more favorable environment for muscle protein accretion, although further mechanistic studies are required to fully clarify these effects [19].

Beyond skeletal muscle, aging is also associated with progressive declines in mitochondrial function and increased reactive oxygen species production. Mitochondria become less efficient with age, leading to reduced ATP production and increased reactive oxygen species generation. Since the creatine-phosphocreatine system is functionally integrated with mitochondrial energy metabolism, creatine has been proposed as a potential strategy to improve cellular energy efficiency and enhance resilience to metabolic stress [21]. While these mechanisms remain under investigation, they may contribute to the broader physiological benefits observed in older populations.

Recent research has further expanded interest in the potential role of creatine in healthy aging beyond musculoskeletal health. Because aging affects multiple tissues characterized by high energetic demands, including the nervous and cardiovascular systems, investigators have begun exploring whether creatine supplementation may support cognitive function, neurological health, and overall functional independence in older adults. Although preliminary findings are encouraging, the available evidence remains less robust than that supporting the musculoskeletal benefits of creatine supplementation, and additional large-scale clinical trials are required before definitive conclusions can be drawn [22].

Importantly, creatine is also characterized by a favorable safety profile. Long-term supplementation studies conducted in older adults have generally demonstrated good tolerability and have not identified clinically relevant adverse effects in healthy individuals when consumed at recommended dosages [7]. This safety profile has contributed significantly to the growing interest in creatine as a practical and accessible nutritional intervention for aging populations.

Despite the substantial body of evidence supporting the benefits of creatine supplementation in older adults, several questions remain unresolved. Considerable interindividual variability exists in responsiveness to supplementation, and optimal dosing strategies, supplementation duration, and interactions with different exercise modalities remain under investigation.

Furthermore, many of the proposed mechanisms underlying the non-muscular effects of creatine remain incompletely understood and require further study.

Taken together, current evidence suggests that creatine should be considered not only as an ergogenic aid for athletes but also as a potentially valuable nutritional strategy for promoting healthy aging. Through its effects on cellular energetics, muscle function, physical performance, and possibly other age-sensitive physiological systems, creatine may help preserve functional capacity and quality of life throughout the aging process [19].

3. CREATINE SUPPLEMENTATION BIOAVAILABILITY

3.1 Different Forms of Creatine and Bioavailability

Although creatine monohydrate remains the most extensively studied and scientifically validated form of creatine supplementation, several alternative formulations have been developed over the last two decades to improve solubility, stability, gastrointestinal tolerance, absorption kinetics, and overall bioavailability [23]. The increasing commercial availability of these products has generated considerable scientific interest in their pharmacokinetic behavior and physiological efficacy.

Bioavailability refers to the proportion of an ingested compound that reaches the systemic circulation and becomes available for tissue uptake and biological activity. In the case of creatine supplementation, bioavailability depends on multiple factors, including intestinal absorption, chemical stability, aqueous solubility, plasma transport, tissue uptake through the SLC6A8 transporter, and intracellular retention [8].

Creatine monohydrate (CM) is currently considered the gold standard formulation because of its extensive scientific validation, favorable safety profile, low cost, and consistently demonstrated efficacy (Kreider *et al.*, 2017). Numerous studies have demonstrated that creatine monohydrate exhibits excellent oral bioavailability, with intestinal absorption approaching 100% under normal physiological conditions [8,23]. However, despite its efficacy, creatine monohydrate exhibits relatively low aqueous solubility, especially in cold water, and may partially convert to creatinine under acidic conditions or during prolonged storage in liquid solutions [23].

To overcome these limitations, several novel creatine formulations have been introduced. Among these, creatine hydrochloride (creatine HCl) has received attention for its markedly higher water solubility than creatine monohydrate. Increased solubility may theoretically improve gastrointestinal tolerance and reduce the amount of undissolved creatine remaining in the gastrointestinal tract. Nevertheless, current evidence does not conclusively demonstrate superior muscle creatine accumulation or enhanced performance outcomes compared with creatine monohydrate [23].

Buffered creatine formulations, often marketed as pH-buffered creatine, were developed to reduce the degradation of creatine to creatinine in acidic environments. The rationale behind

these formulations is that greater pH stability may improve systemic availability and reduce gastrointestinal discomfort. However, independent studies have generally failed to demonstrate clear physiological advantages of creatine monohydrate regarding muscle creatine content, body composition, or exercise performance [24].

Creatine pyruvate (CP) is a creatine salt formed by the binding of creatine to pyruvic acid. It was developed to combine the ergogenic properties of creatine with the metabolic functions of pyruvate, a key intermediate in cellular energy metabolism. Compared with creatine monohydrate, creatine pyruvate exhibits greater aqueous solubility, a characteristic that has been proposed to influence its gastrointestinal dissolution and absorption kinetics [25].

Following ingestion, creatine pyruvate dissociates into creatine and pyruvate. The creatine component becomes available for absorption and subsequent uptake by target tissues, whereas pyruvate enters normal intermediary metabolic pathways. Because pyruvate occupies a central position between glycolysis and mitochondrial oxidative metabolism, it has been hypothesized that its presence could contribute additional metabolic effects beyond those attributable to creatine alone. However, the physiological significance of this contribution remains uncertain [25].

Interest in creatine pyruvate largely stems from studies investigating its pharmacokinetic profile. Jäger and colleagues reported that creatine pyruvate produced higher peak plasma creatine concentrations than an equivalent dose of creatine monohydrate, suggesting enhanced systemic availability. These findings were attributed primarily to the improved physicochemical properties of the pyruvate salt, including increased solubility and potentially more rapid dissolution within the gastrointestinal tract [23].

Some evidence has also suggested potential performance benefits. In a controlled supplementation study, four weeks of creatine pyruvate administration improved performance during intermittent handgrip exercise and was associated with physiological adaptations indicative of enhanced aerobic recovery capacity. Nevertheless, the mechanisms responsible for these effects were not clearly established, and it remains difficult to determine whether the observed benefits were attributable to increased creatine availability, the pyruvate moiety itself, or a combination of both factors [23].

Despite these findings, the scientific literature on creatine pyruvate remains limited when compared with the extensive body of evidence available for creatine monohydrate. Existing studies generally involve small sample sizes and short intervention periods, and no consistent evidence demonstrates superior effects on muscle creatine accumulation, strength development, lean body mass, or exercise performance. Consequently, current reviews

conclude that although creatine pyruvate appears to be a safe and bioavailable source of creatine, its practical advantages over creatine monohydrate have not been conclusively demonstrated [25].

An important factor influencing creatine bioavailability is intestinal absorption. Studies have demonstrated that oral creatine supplementation results in rapid increases in plasma creatine concentrations, typically peaking within approximately 1–2 hours after ingestion [8]. Following absorption, creatine is transported through the bloodstream and subsequently taken up by skeletal muscle and other tissues through the SLC6A8 transporter. The efficiency of this uptake process may vary among individuals due to differences in baseline creatine stores, muscle fiber composition, insulin sensitivity, dietary habits, and transporter expression.

Despite ongoing development of novel formulations, current scientific evidence generally indicates that no alternative form has consistently demonstrated superior long-term efficacy compared with creatine monohydrate in increasing intramuscular creatine stores or improving exercise performance under controlled conditions [7]. Nevertheless, differences in solubility, gastrointestinal tolerance, plasma kinetics, and absorption profiles remain areas of active investigation.

For this reason, increasing attention has been directed toward the pharmacokinetic evaluation of new creatine formulations and their systemic absorption characteristics. Understanding potential differences in bioavailability and plasma creatine kinetics may provide important insights into the effectiveness of novel products and their possible applications in both sports nutrition and clinical settings.

3.2 Creatine Supplementation

Creatine supplementation is currently considered one of the most effective and extensively studied nutritional strategies for improving high-intensity exercise performance, muscle strength, and lean body mass [5].

The most commonly used and scientifically validated form of supplementation is creatine monohydrate (CM). Numerous studies have demonstrated that creatine monohydrate supplementation effectively increases total intramuscular creatine content by approximately 10–40%, depending on baseline creatine stores, muscle fiber composition, dietary habits, and individual responsiveness [3,4]. Several supplementation protocols have been proposed in the

literature. The traditional "loading phase" consists of approximately 20 g/day (0.3g/kg/day) of creatine monohydrate, typically divided into four daily doses of 5 g for 5–7 days, followed by a maintenance phase of 3–5 g/day [4]. Alternatively, lower-dose protocols without a loading phase, generally involving 3–5 g/day for several weeks, have also been shown to progressively increase intramuscular creatine concentrations, although at a slower rate [7].

The timing of creatine ingestion has also been investigated. Some studies suggest that post-exercise supplementation may enhance creatine uptake due to increased muscle blood flow and insulin sensitivity, although evidence remains partially inconsistent [19]. Co-ingestion with carbohydrates or with carbohydrates plus protein, has been reported to increase muscular creatine retention, likely through insulin-mediated stimulation of the SLC6A8 creatine transporter [26].

Regarding safety, creatine monohydrate is currently recognized as one of the safest ergogenic supplements available when consumed at recommended dosages in healthy individuals [7]. Long-term studies have not demonstrated clinically significant adverse effects on renal or hepatic function in healthy populations. Nevertheless, caution is generally recommended in individuals with pre-existing kidney disease or other relevant medical conditions, and further long-term data are still needed for some novel creatine formulations.

An important and often underestimated aspect of creatine physiology concerns interindividual variability in responsiveness to supplementation. Not all individuals exhibit the same increase in intramuscular creatine concentrations following supplementation. Subjects with lower baseline muscle creatine stores generally demonstrate larger increases after supplementation, whereas individuals with already elevated baseline concentrations may experience smaller physiological changes [27]. Muscle fiber composition also appears relevant, since type II fibers tend to accumulate greater amounts of creatine compared with oxidative type I fibers. Moreover, subjects with lower baseline intramuscular creatine stores, such as vegetarians or individuals with lower habitual meat intake, generally show greater increases in muscle creatine content following supplementation [28]. Conversely, some individuals appear to be "non-responders" or "low responders," possibly due to already elevated baseline creatine stores, differences in muscle fiber composition, or variability in SLC6A8 transporter expression and activity.

At the molecular level, variability in creatine responsiveness may depend in part on differences in SLC6A8 transporter expression and activity. Since creatine uptake into skeletal muscle and neural tissue is transporter-dependent, alterations in transporter regulation could influence tissue saturation and the efficacy of supplementation. Genetic defects affecting SLC6A8

function are known to cause cerebral creatine deficiency syndromes characterized by intellectual disability, neurological dysfunction, language impairment, and autistic-like behaviors [29]. These observations further highlight the fundamental physiological importance of creatine transport in human metabolism and neurological function.

In recent years, increasing scientific attention has focused on the possible role of creatine as a "conditionally essential" nutrient during specific physiological or pathological conditions. Situations characterized by increased energetic demand, reduced endogenous synthesis, impaired transport, aging, injury, or disease may increase creatine requirements beyond the body's synthetic capacity. This hypothesis has contributed to the expansion of research into clinical creatine supplementation beyond sports nutrition alone [30].

Despite the extensive literature available, several controversies and limitations remain. Clinical outcomes are not always consistent across studies, particularly in neurological diseases and chronic pathological conditions. Differences in study design, supplementation protocols, dosage, treatment duration, disease severity, and interindividual variability complicate the interpretation of results. Furthermore, although creatine monohydrate has demonstrated a strong safety profile in healthy individuals, long-term evidence regarding some novel creatine formulations remains limited.

Nevertheless, the broad physiological involvement of creatine in cellular bioenergetics, mitochondrial function, muscular performance, neurological activity, and metabolic regulation continues to support its relevance as one of the most important and extensively investigated compounds in exercise physiology and clinical nutrition.

4. PROPOSED EXPERIMENTAL DESIGN

4.1 PURPOSE OF THE STUDY

4.1.1 Rationale

Creatine supplementation is widely recognized as one of the most effective nutritional strategies for increasing intramuscular creatine stores and improving performance during high-intensity exercise. In addition to its ergogenic applications, creatine supplementation has also been investigated in several clinical contexts, including neurodegenerative diseases, rehabilitation, aging, and metabolic disorders [7]. Among the available formulations, creatine monohydrate remains the most extensively studied form in the scientific literature [25]. Following oral ingestion, creatine is absorbed into the bloodstream and subsequently taken up by target tissues, particularly skeletal muscle[25]. Plasma creatine concentrations generally peak approximately 60 minutes after oral administration of creatine monohydrate [3]. An initial increase in plasma creatine levels, followed by a progressive reduction, may indirectly suggest tissue uptake of creatine from the systemic circulation [25].

Previous studies have demonstrated that the conversion of creatine into creatinine within the gastrointestinal tract is minimal, resulting in an almost complete intestinal absorption of orally administered creatine [8]. Nevertheless, several novel creatine formulations have recently been developed to improve solubility, stability, gastrointestinal handling, or bioavailability compared with conventional creatine monohydrate [25].

In this context, it is scientifically relevant to evaluate whether these novel formulations differ in absorption kinetics and systemic availability from creatine monohydrate, which currently represents the reference formulation in the literature. The novel creatine formulation Creamix, investigated in the present study, is reported in Tab. 1.

CREAMIX	g / DOSE
Creatine pyruvate	1.55180
Creatine hydrochloride (HCL)	1.52870
Creatine phosphate	1.45161
Maltodextrins	1.03208
Magnesium bisglycinate chelate	0.37500
Silicon dioxide	0.06000
Chromium picolinate	0.00081
TOTAL	**6.00000**

Tab. 1 Composition of the novel creatine formulation Creamix.

4.1.2 Study Hypothesis and Objectives

The primary objective of the present study is to compare the systemic absorption of the novel creatine formulation Creamix with that of creatine monohydrate, the current reference standard for creatine absorption, by evaluating plasma creatine concentrations following oral administration of both formulations. The study hypothesis is that Creamix may exhibit an absorption profile comparable to that of creatine monohydrate. To investigate this hypothesis, plasma creatine concentrations will be measured at multiple time points following ingestion of each formulation in healthy adult subjects under controlled experimental conditions. Comparing plasma creatine kinetics may provide useful information about absorption characteristics and bioavailability of the two investigated formulations.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design

The present study was designed as a monocentric controlled crossover clinical trial conducted under standardized experimental conditions. All participants underwent both experimental conditions in randomized order, receiving the two creatine formulations on separate occasions, with a one-week washout period between sessions to minimize potential carryover effects.

The experimental procedures were performed under controlled laboratory conditions, and all participants followed the same standardized protocol throughout the study. The crossover design was selected to reduce interindividual variability and improve the sensitivity of the treatment comparison.

The primary outcome of the study was the evaluation of plasma creatine concentrations following oral administration of the investigational formulations. Blood samples were collected at multiple time points to assess the systemic absorption kinetics of creatine.

4.2.2 Participants

Healthy adult volunteers aged between 18 and 35 years were recruited for the study. Inclusion criteria included the absence of creatine supplementation during the six months preceding enrollment, absence of diagnosed cardiovascular, metabolic, muscular, or neurological disorders, and a body mass index (BMI) between 18 and 30 kg/m^2. At the beginning of the first experimental session, body weight and height were measured for each participant using standardized procedures. Subjects were excluded if one or more inclusion criteria were not met or if they reported the use of medications or supplements potentially capable of influencing muscle metabolism or creatine absorption.

Before participation, all subjects received detailed information regarding the experimental procedures and signed a written informed consent form. All experimental procedures were conducted in accordance with ethical standards for human research. The characteristics of the participants are illustrated in Table 2.

ID	Age (years)	Weight (Kg)	Height (m)	BMI (kg/m²)
CR1	23	84,5	1,71	28,8978
CR2	27	60,3	1,67	21,6214
CR3	24	79,1	1,82	23,88
CR4	28	66,8	1,63	25,1421
CR5	25	71,3	1,78	22,5035
CR6	23	70,1	1,7	24,2561
CR7	20	81,6	1,88	23,0874
CR8	30	56,8	1,61	21,9127
CR9	24	91,5	1,73	30,5724
MEAN ± SD	24,8 ± 3,01	73,55 ± 11,49	1,72 ± 0,08	24,65 ± 3,11

Tab. 2 Characteristics of participants.

4.2.3 Supplementation Protocol

Participants arrived at the laboratory following an overnight fast of approximately 10–12 hours, having consumed their final meal no later than 20:00 on the evening before testing. Subjects were instructed to refrain from structured physical exercise during the 24 hours preceding each experimental session and to avoid alcohol and caffeine consumption beginning from 20:00 on the previous evening.

During the experimental sessions, the consumption of food, caffeinated beverages, or caloric drinks was not permitted. Water intake was standardized to predefined limits: 250 mL before baseline measurements and a maximum of 700 mL throughout the session.

Creatine was administered orally as a single dose dissolved in 200 mL of water. Participants received either:

- 5 g of creatine monohydrate, or
- 10 g of Creamix,

with dosages selected to provide an equivalent total creatine content between formulations. Both formulations were administered in aqueous solution, and no additional fluid intake was permitted for at least 20 minutes after ingestion to reduce variability in gastrointestinal absorption. The exact time of ingestion was recorded and defined as time zero (T0).

4.2.4 Blood Sampling and Biochemical Analysis

Venous blood samples were collected at baseline (T0) and at 30 minutes (T1), 60 minutes (T2), 90 minutes (T3), 120 minutes (T4), and 180 minutes (T5) following creatine ingestion, according to protocols previously described in the literature.

Participants remained seated for at least ten minutes before each blood collection. Venous blood samples were obtained from an antecubital vein using EDTA-containing collection tubes.

Blood samples were centrifuged within 30 minutes of collection at 2,000 rcf for 15 minutes at 4 °C. Plasma was subsequently aliquoted into pre-labeled microtubes and stored at −80 °C until biochemical analysis. To minimize analytical variability, all samples from the same participant will be analyzed in the same analytical session.

Total plasma creatine concentration will be determined using a commercially available enzymatic assay kit (Creatine Assay Kit, Abcam, ab65339) according to the manufacturer's instructions. The assay is based on an enzymatic reaction converting creatine and phosphocreatine into a detectable product, generating a colorimetric signal proportional to creatine concentration. Absorbance will be measured spectrophotometrically at the recommended wavelength.

A standard calibration curve will be generated using creatine standards supplied with the kit, and sample concentrations will be calculated by interpolation. All plasma samples will be analyzed in duplicate, and quality control samples will be included to ensure assay reliability. Although the assay measures total creatine, the contribution of phosphocreatine in plasma is considered negligible; therefore, measured values predominantly reflected circulating free creatine concentrations.

4.2.5 Statistical Analysis

Statistical power analysis was performed using G*Power, assuming a repeated-measures ANOVA with a within-subject crossover design. Considering a medium effect size (Cohen's f = 0.4), an alpha level of 0.05, a desired statistical power of 0.80, and a correlation among repeated measures of 0.5, the estimated minimum sample size required was eight participants.

Data distribution normality will be assessed using the Shapiro–Wilk test. Variations in plasma creatine concentrations over time will be analyzed using repeated-measures analysis of variance (RM-ANOVA). When significant main effects are identified, post hoc comparisons

will be performed using the Bonferroni correction. Data will be presented as mean ± standard deviation (SD), and statistical significance will be established at $p < 0.05$.

5. CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Expected Outcomes

The proposed study was designed based on previously published pharmacokinetic investigations evaluating the plasma response following oral administration of different creatine formulations, particularly those conducted by Harris and colleagues and Jäger et al [3,23].

Based on these studies, both creatine monohydrate and the Creamix formulation are expected to produce the characteristic pharmacokinetic profile observed after oral creatine ingestion, characterized by a rapid increase in plasma creatine concentration during the first hour after supplementation, followed by a progressive decline toward baseline over the subsequent hours. This pattern has been consistently reported in human pharmacokinetic studies investigating isomolar doses of creatine monohydrate and alternative creatine salts (Fig. 2).

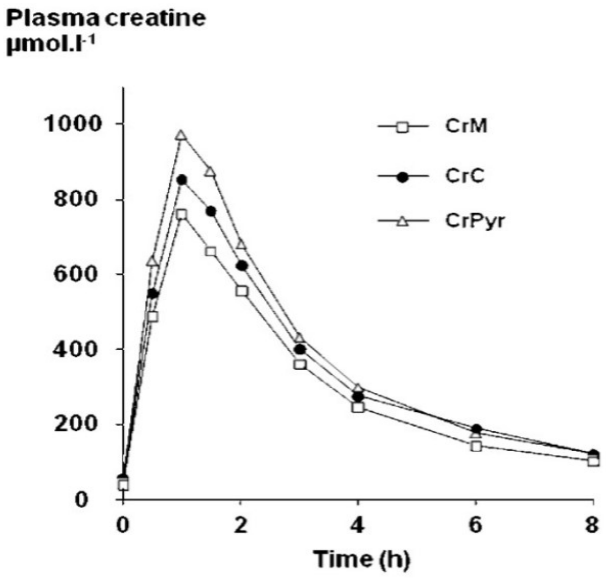

Figure 2 Representative plasma creatine concentration–time curves following oral administration of isomolar doses of creatine monohydrate (CrM), tri-creatine citrate (CrC), and creatine pyruvate (CrPyr), adapted from Jäger et al. (2007).

Given that Creamix contains a combination of creatine pyruvate, creatine hydrochloride, and creatine phosphate, each with distinct physicochemical properties, it may be hypothesized that the formulation could exhibit a pharmacokinetic profile different from that of creatine monohydrate alone. Based on previous comparative pharmacokinetic investigations

evaluating alternative creatine formulations, particularly those conducted by Harris and colleagues and by Jäger et al. [3,25], any differences would most likely be observed during the early post-ingestion phase and may include modest variations in plasma Cmax, AUC, or the initial concentration–time profile. However, the magnitude and direction of these potential differences remain speculative and represent the primary question addressed by the proposed study. However, based on the currently available evidence, including the comparative pharmacokinetic studies by Jäger and colleagues[25], any such differences would be expected to remain relatively small and should be interpreted with caution.

Importantly, given the absence of previous pharmacokinetic studies specifically evaluating the Creamix formulation, no a priori assumption of superiority over creatine monohydrate can be made. Although formulation-dependent differences in plasma concentration–time profiles may reasonably be hypothesized based on the physicochemical characteristics of its components and on previous comparative studies of alternative creatine formulations, the proposed investigation is inherently exploratory. Its primary objective is to determine whether Creamix exhibits a pharmacokinetic profile that differs from that of creatine monohydrate under controlled experimental conditions, rather than to demonstrate superior efficacy.

Furthermore, if modest differences in plasma creatine concentrations are observed between the two formulations, these findings would primarily reflect differences in systemic absorption rather than definitive differences in biological efficacy. Since oral creatine is almost completely absorbed under normal physiological conditions, the present study is not designed to investigate intestinal absorption per se, but rather to evaluate whether the formulation influences the rate and extent of creatine appearance in the systemic circulation. Therefore, plasma creatine concentration represents a useful pharmacokinetic proxy of systemic availability during the early absorption phase. Nevertheless, although a higher or more rapid plasma appearance may indicate improved systemic absorption, it would not necessarily demonstrate greater intracellular creatine uptake, enhanced phosphocreatine synthesis, or superior long-term retention within skeletal muscle. Consequently, additional studies directly assessing tissue creatine accumulation and functional outcomes would be required to establish whether pharmacokinetic differences translate into meaningful physiological advantages.

The proposed crossover design could represent an appropriate methodological approach for generating preliminary pharmacokinetic data while minimizing interindividual variability. The results from such a study would primarily characterize the absorption profiles of the Creamix formulation tested and could provide a rationale for larger investigations specifically designed to evaluate tissue creatine accumulation and functional outcomes.

5.2 Future Directions

Future investigations should extend beyond the evaluation of plasma creatine concentrations and integrate multiple levels of biological assessment. In particular, studies combining pharmacokinetic analyses with direct measurements of skeletal muscle creatine content, magnetic resonance spectroscopy, muscle biopsies, or validated performance outcomes would provide a more comprehensive understanding of formulation-dependent differences in creatine utilization.

In addition to acute pharmacokinetic investigations, future research should also include longer-term supplementation protocols. Chronic intervention studies would allow researchers to determine whether potential differences observed during the early absorption phase result in greater intramuscular creatine accumulation, enhanced phosphocreatine storage, or improved tissue retention following repeated supplementation. Such studies could clarify whether acute pharmacokinetic advantages are maintained over time and whether they translate into meaningful physiological adaptations.

Furthermore, longer supplementation protocols involving athletes, older adults, vegetarians, and clinical populations may help establish whether formulation-dependent differences are associated with measurable improvements in exercise performance, muscle strength, body composition, recovery capacity, or clinically relevant outcomes in populations characterized by impaired energy metabolism.

Ultimately, despite the continuous development of novel creatine formulations, creatine monohydrate remains the current reference standard against which all alternative products should be compared. Therefore, the proposed experimental protocol represents an initial step toward addressing an important gap in the current literature by investigating whether modifications in formulation composition are associated with measurable differences in systemic creatine availability and plasma pharmacokinetics. Establishing whether such pharmacokinetic differences ultimately translate into superior tissue uptake and functional benefits will require adequately powered randomized controlled trials integrating pharmacokinetic, biochemical, physiological, and performance endpoints.

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