EXAMINATION OF WORKOUT SUPPLEMENTS: THE PHYSIOLOGICAL PATHWAYS AND MECHANISMS IN RELATION TO EXERCISE PERFORMANCE

By

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Abstract

Nutritional supplementation to enhance exercise performance has become a growing interest in the athletic industry. The purpose of this thesis was to investigate how the workout supplements β-Alanine and creatine monohydrate function at the physiological level by studying their cellular pathways and mechanisms in order to determine their overall effect on an individual's exercise performance and health. To research information on these topics, I employed various engines such as the University of Arizona's database, PubMed, and NCBI using the keywords "nutritional supplementation," "workout," "β-Alanine," "creatine monohydrate," "exercise," and "performance" to gather research articles pertaining to my thesis topic. A review of the available literature and clinical studies demonstrate that the ergogenic supplement of β-Alanine has a positive marginal improvement on high intensity exercise performance with no negative health effects by increasing the buffering capacity of the muscle cell. The ergogenic supplement of creatine monohydrate has a positive improvement on high intensity exercise performance with a gain in fat-free mass with no negative health effects by improving the phosphorylation of ATP within the muscle cell.

Introduction

In recent years, the use of nutritional supplements to improve athletic performance has increased dramatically in the fitness world, at all levels of competition. There are various factors that contribute to achieving elite physical performance: having a well-balanced diet, getting enough sleep, and drinking plenty of water. However, it is a common idea that by taking additional nutritional supplements, an individual can achieve greater physical performance improvements in a shorter period of time. Nutritional supplements are commonly associated with the athlete demographic, but research has shown that the supplements commonly used by athletes have a wide range of uses for all demographics. Due to this large customer base, there are a multitude of nutritional supplements in the market today, with a variety of potential physiological benefits. In this thesis, I will be researching the ergogenic aid supplements β-Alanine and creatine monohydrate and examining their physiological mechanisms in order to determine their relationship with exercise performance.

β-Alanine has become an increasingly popular workout supplement based on the predicted physiological mechanism that it increases intramuscular L-carnosine. This increase in concentration will create an enhanced buffering capacity of H^+ within the cell, allowing greater and longer performance during high-intensity exercise. Creatine is one of the most widely used supplements across all demographics as the predicted physiological mechanism increases creatine and phosphocreatine within the muscle cells which may allow for an increase in strength, gain in fat-free mass, and some neurological benefits. Through this research, I aim to determine which of these supplements are beneficial to take, how to maximize their efficacy, and educate users on how they affect the body at a physiological level.

β-Alanine

The oral ingestion of β-Alanine as a dietary workout supplement has become a widely used and mainstream method of increasing carnosine levels within skeletal muscle. The potential physiological mechanisms of loading β-Alanine within the skeletal muscle cells is to increase the buffering capacity of H^+ during high intensity exercise to delay onset of muscular fatigue and increase exercise performance. Carnosine is a cytoplasmic histidine dipeptide that is present in mammalian skeletal muscle cells, where its main physiological role is controlling muscular acidosis by acting as a H^+ – or pH – buffer. While carnosine's main function is homeostatic buffer control within the muscle cell, overall, carnosine only contributes around 8-15% of the entire body's buffering capacity, with the main buffer being bicarbonate (HCO_3^-) (Baguet et al., 2009). Carnosine is one of the most abundant metabolites in skeletal muscle cells with approximately 99% of the body's carnosine being produced and stored within muscle cells; it is almost completely absent in other organs within the mammalian body (Derave et al., 2010). Within the skeletal muscular system, carnosine is present within slow twitch muscle cells – used for endurance or aerobic exercise – at a low concentration: 5 mmol/L (Derave et al., 2010). In fast twitch muscle cells – used for high intensity or anaerobic exercise – carnosine concentrations are significantly higher: 20-50 mmol/L (Derave et al., 2010). This supports the proposed physiological mechanism that carnosine acts as a H^+ buffer during high intensity exercise to delay fatigue.

Carnosine levels can vary based on age and sex of an individual. Males produce and store approximately 20-25% more carnosine within the muscle cells than females do, which allows for greater anaerobic performance (Derave et al., 2010). As an individual gets older, there is a steady decrease in carnosine content within the muscle cells, which is consistent with the physiology

behind “muscle age” that states that we lose a higher ratio of fast twitch muscle fibers compared to slow twitch muscle fibers (Derave et al., 2010). This decrease in fast twitch muscle fibers is considered part of the physiological mechanisms behind the decrease in strength and power as an individual gets older.

Carnosine is synthesized by the enzyme carnosine synthase from the amino acids L-histidine and β-Alanine – with β-Alanine being the rate-limiting precursor – via the use of ATP. The enzyme carnosinase degrades carnosine into its amino acid precursors. L-histidine is an essential amino acid which means that the body cannot produce it naturally and must be ingested in the diet to maintain normal physiological levels. L-histidine is present in foods like meat, poultry, fish, and dairy products. β-Alanine can be produced via hepatic breakdown of thymine and uracil, or the ingestion of dietary dipeptides, present in foods with high protein content. The highest concentration and rates of the enzyme carnosine synthase are present in skeletal muscle cells, which support the proposed functionality loading muscle cells with carnosine. There are very low rates of enzymatic degradation of carnosine via the enzyme carnosinase, which allows for synthesized carnosine to remain at high levels within the skeletal muscle cells for extended periods of time. The overall goal of β-Alanine supplemention is to increase carnosine levels within the muscle cell to delay muscle fatigue from acidosis during exercise. While supplementing directly with carnosine may seem more efficient, supplementing with the amino acid β-Alanine has proven to be more physiologically stable and increased carnosine production at sustainably higher rates by increasing the bioavailability of the rate limiting precursor (Blancquaert et al., 2015). Due to the low degradation levels of carnosine, there is a very high loading ceiling present

within muscle cells for carnosine saturation to occur – other skeletal muscle buffer pools are not as expansible.

β-Alanine is a non-proteogenic amino acid that has no ergogenic potential within the muscle cell on its own (Hoffman et al., 2018). When β-Alanine is enzymatically combined with L-histidine to form carnosine, the pK_a of the imidazole ring present on the L-histidine is increased to 6.83 (Hoffman et al., 2018). The normal physiological pH level of a cell is within the range of 7.0-7.1, the pH level of the muscle cell approaches 6.3-6.5 during high intensity exercise due to the increase in H^+ produced by the cell (Hoffman et al., 2018). During high intensity or anaerobic exercise, the primary energy system being used is the metabolism of carbohydrates and current ATP storage within the cell in order to produce fast energy. While this energy system takes seconds to produce high levels of energy, it also produces high levels of H^+ as a byproduct. As the muscle cell continues to produce H^+, the pH of the cell decreases which begins to negatively affect various metabolic processes occurring within the muscle cell. When the pH of a cell drops, there is a decrease in the glycolytic rate and contractile ability of the muscle cell (Huerta Ojeda et al., 2020). This results in a dramatic decrease in the amount of power the muscle is able to produce and induces faster muscle fatigue. The increase in pH from the imidazole ring of the L-histidine residue allows for carnosine to act as a highly effective intracellular pH buffer within the skeletal muscle cells, allowing for increased tolerance to H^+ during high intensity or anaerobic exercise (Hoffman et al., 2018). This physiological buffer mechanism is also currently being researched as having antioxidant potential within the skeletal muscle cell via direct interaction with reactive oxygen species produced during exercise (Baguet et al., 2009).

As supplementation with β-Alanine increases among all populations, there have been many research and clinical studies conducted in order to investigate its direct or indirect role on physical exercise performance. In order to effectively carnosine load the muscle cells to have an ergogenic effect, supplementation of β-Alanine must be consistently conducted for several weeks. Ingestion of 100 milligrams per kilogram body weight or 4-6 grams a day has proven to be enough to elevate muscle carnosine content by 60% in 4 weeks and 80% in 10 weeks (Derave et al., 2010). While β-Alanine can be increased by eating dipeptide rich foods, in order to achieve the 4-6 grams a day, an individual would have to consume approximately 1,000-1,500 grams of chicken a day (Hoffman et al., 2018). Oral β-Alanine supplementation in the form of powder or pills is the most effective means to increase muscle carnosine content. Acute or short-term supplementation of β-Alanine shows minimal increases in carnosine levels shortly after β-Alanine ingestion and is washed out before any effect on physical performance can be measured. There is a linear relationship between β-Alanine supplementation intake and the level of carnosine loading with no significant plateau due to the very high loading ceiling present for carnosine within skeletal muscle cells (Blancquaert et al., 2015). Due to the low rates of carnosine enzymatic degradation, cessation of β-Alanine supplementation after 4-10 weeks of chronic supplementation will result in a washout of approximately 0.03 mmol/L, allowing for the ergogenic effects of β-Alanine to be present even after supplementation cessation (Derave et al., 2010). Skeletal muscle carnosine levels will gradually return to base levels in the slow washout process which can take up to approximately 6-20 weeks (Blancquaert et al., 2015). Various studies have showed that β-Alanine supplementation has caused a direct and measurable decrease in muscle acidosis and was supported by identical levels of bicarbonate (HCO_3^-) in β-

Alanine and placebo groups (Baguet et al., 2009). This supports the proposed physiological mechanisms that β-Alanine does not enhance the buffering capacity of the skeletal muscle cell by sparing bicarbonate (HCO_3^-) but rather carnosine and bicarbonate work as a buffer additive to decrease muscular acidosis and fatigue. Due to carnosine being primarily produced and stored within the skeletal muscle system, chronic β-Alanine supplementation has showed to have no direct effect on resting blood pH but just enhanced the buffering capacity of the skeletal muscle cell during high intensity exercise.

There are many various types of exercise and different physiological mechanisms that occur within the body's cells in order to efficiently create the energy necessary to perform, sustain, and recover from exercise. Using the physiological pathway and mechanisms β-Alanine supplementation follows within the skeletal muscle system, there have been various clinical exercise studies conducted to determine which type of exercise is most enhanced by β-Alanine supplementation and to what degree it is enhanced. Various clinical exercise studies have yielded results that support that the greatest ergogenic potential from carnosine loading the skeletal muscle cells occurs during high intensity exercise that lasting approximately 60-240 seconds (Hoffman et al., 2018). After 4-10 weeks of chronic β-Alanine supplementation at 4-6 grams a day, studies have concluded the following: there is no significant benefit in β-Alanine supplementation groups measured in exercises lasting less than 60 seconds, no significant change in VO2 max – ventilatory threshold – throughout cycling exercises, no significant change to the total oxygen deficit produced after aerobic exercise, no significant change to endurance physical performance, and a measurable increase in achievement during short high intensity exercise lasting less than 4-5 minutes. In 10 different time trial test studies conducted in a meta-analysis,

7 declared a beneficial effect of β-Alanine on physical performance during a high intensity effort in a specific exercise for a set amount of time, while 3 declared a neutral effect after β-Alanine supplementation (Huerta Ojeda et al., 2020). This data supports the potential benefits of taking β-Alanine as a supplement but also shows that there is more research to be conducted on how to effectively take β-Alanine in terms of improving physical exercise performance. Although there has been no direct positive physiological benefits associated with β-Alanine supplementation on endurance exercise, it is theorized that β-Alanine could indirectly affect aerobic exercise capacity and performance by increasing cardiovascular fitness by improving the quality and duration of high intensity exercise performed (Hoffman et al., 2018). Recent studies have provided evidence that shows that chronic supplementation with β-Alanine improves high intensity exercise performance in a similar ratio in both trained and untrained clinical participants. The level of carnosine loading measured within the skeletal muscle cells showed similar increased carnosine values, despite the starting muscular carnosine levels – due to the high ceiling for carnosine loading (Derave et al., 2010). Therefore, the effectiveness of muscular carnosine loading is not impaired or affected by initial carnosine levels or training status. This result allows for all demographics of people to benefit from taking β-Alanine as a supplement to improve the duration and achievement of high intensity exercise. Effects of β-Alanine supplementation on exercise performance are measurably small and supplementation would be most optimal for the elite athlete demographic who participate in high intensity exercise who have already optimized their training and are seeking minor improvements in performance – as a small improvement in performance at that level of competition can make a huge difference in results. For example, elite level sprinters and rowers would highly benefit from β-Alanine supplementation. The older

demographics could also potentially benefit from β-Alanine supplementation in order to get carnosine levels back up and increase the amount of power and endurance they can produce in their everyday life.

Based on the current studies, β-Alanine supplementation and carnosine loading has shown to have no long-term physiological damage or health-related side effects to the body's cells or organs. There has been only (1) reported and consistent side effect from β-Alanine supplementation which is paresthesia – sensation of tingling or numbing of the skin. This side effect was reported at higher rates when β-Alanine supplementation exceeded the recommended amount of 4-6 grams a day but generally disappeared after 60-90 minutes after ingestion (Hoffman et al., 2018). Studies that reported the paresthesia side effect due to β-Alanine declared that by splitting the 4-6 grams a day supplement dosing across smaller amounts throughout the day has decreased the amount of paresthesia experienced. There is also clinical evidence that demonstrates the ingestion of high amount of carbohydrates helps avoid the paresthesia side effect. These results and studies have provided evidence that β-Alanine is an effective ergogenic supplemental aid that could be used to improve high intensity exercise performance by any demographic.

Creatine Monohydrate

Creatine monohydrate is one of the most popular ergogenic supplements taken today and the most researched supplement in terms of its effect on exercise performance and possible side effects on both short- and long-term supplementation. The potential physiological mechanisms of loading creatine monohydrate within the skeletal muscle cell is to increase strength, gain in lean fat-free body mass, and decrease muscle recovery time. Creatine

monohydrate is a non-essential nutrient that is ingested through the diet via high-protein foods such as red meat and fish. It is also endogenously produced by the kidneys and liver in a 2-step reaction composed of 3 amino acids: glycine, arginine, and methionine. Skeletal muscle stores approximately 95% of the body's creatine, with approximately 66% of intramuscular creatine being stored as phosphocreatine and approximately 34% of intramuscular creatine being stored as free creatine (Kreider et al., 2017). While skeletal muscle contains the largest amount of creatine, muscle cells have no capacity for creatine synthesis and therefore must be absorbed into the muscle from circulating blood (Brosnan & Brosnan, 2007). The main goal of supplementing with creatine monohydrate is to saturate the skeletal muscular system with creatine and phosphocreatine in order to increase the capacity of ATP phosphorylation and energy buffer to replenish ATP during exercise which will allow for greater production and maintenance of contractile force. Creatine monohydrate physiological levels vary based on sex and age. The average amount of total creatine stored within the body is approximately 120 mmol/kg (Loon et al., 2003). Males contain approximately 20% more creatine than females, allowing for greater strength and muscle mass size. Evidence has showed that the rate of creatine loss is almost linear with age increase, which correlates with the decrease in strength and muscle mass size as an individual gets older (Loon et al., 2003).

Creatine monohydrate synthesis occurs in the kidney and liver via 2 enzymatic reactions. The first reaction, which mainly occurs in the kidney, is catalyzed by the enzyme glycine amidino transferase [AGAT] which transfers the amidino group from L-arginine to glycine to produce guanidinoacetate [GAA] and L-ornithine (Kreider et al., 2017). The second reaction, which mainly occurs in the liver, is catalyzed by the enzyme guanidinoacetate methyltransferase [GAMT] which

uses S-adenosylmethionine to methylate the produced guanidinoacetate [GAA] – from the first reaction – to produce the final products of creatine monohydrate and S-adenosylhomocysteine (Kreider et al., 2017). The liver releases the final product of creatine monohydrate into the circulating blood and is taken up by skeletal muscle cells via a plasma membrane creatine transporter [CRT] (Kreider et al., 2017). The uptake of creatine monohydrate into all body cells – skeletal muscle, cardiac muscle, brain, and kidneys – occurs against a big concentration gradient. Normal creatine levels in the blood plasma being 50-100 μm and only 5-10 μm in the skeletal muscle (Loon et al., 2003). Due to the large concentration gradient, the creatine transporter uses secondary active transport to transport creatine into the muscle cell, driven by the Na^+/K^+ ATPase sodium gradient. This physiological mechanism indicates that the driving force for creatine transport into the skeletal muscle is increased when the Na^+/K^+ ATPase gradient is high, which occurs when insulin levels increase – after food and carbohydrate consumption – and during high intensity exercise (Brosnan & Brosnan, 2007).

Once creatine is in the muscle cell, it enters the creatine/phosphocreatine cycle that occurs in the cytosol and mitochondria of the cell. The creatine/phosphocreatine cycle serves as an intracellular buffer for ATP and acts as an energy shuttle for high rates of ATP molecules being moved from the mitochondria to the cytoplasm for muscle cell utilization during exercise (Brosnan & Brosnan, 2007). The conversion between creatine and phosphocreatine is catalyzed by the enzyme creatine kinase, in both directions of the reaction. Creatine present in the cytosol of the cell is shuttled into the mitochondria where it is used in oxidative phosphorylation to produce large amounts of ATP, with ADP and phosphocreatine as a byproduct (Brosnan & Brosnan, 2007). Creatine present within the cytosol can also be converted to phosphocreatine

and ADP via creatine kinase during glycolysis. The chemical reaction of the shuttle is as follows: ADP + phosphocreatine + $H^+ \leftrightarrow$ ATP + creatine (Loon et al., 2003). The creatine/phosphocreatine shuttle system effectively connects 2 different sites of fast and large ATP production, oxidative phosphorylation, and glycolysis, which are both utilized during high intensity exercise (Kreider et al., 2017). With the supplementation of creatine monohydrate, there is an intracellular increase in phosphocreatine within the muscle cell. This increase in phosphocreatine enhances the cycle and shuttle of ATP molecules between the mitochondria and cytosol which allows for a greater bioavailability of ATP energy during muscle cell contraction. During high intensity exercise, there is rapid ATP hydrolysis occurring within the muscle cell to produce large amounts of energy and must be buffered in order to keep ADP levels low and ATP levels high to maintain duration of exercise (Cribb et al., 2007). With the creatine/phosphocreatine cycle having the ability to buffer both ADP and ATP within the cell, this reaction physiologically maintains the ratio between ADP:ATP levels within normal levels and protects the efficiency of ATP hydrolysis or breakdown during high intensity exercise (Brosnan & Brosnan, 2007). This reaction coupling also indirectly reduces the production of reactive oxygen species (ROS) within the muscle cells and is currently being investigated as playing a physiological role as a direct/indirect antioxidant (Kreider et al., 2017). While the creatine/phosphocreatine cycle occurs in every skeletal muscle cell in the body, different functions of the cycle predominate in different muscle cells. In fast twitch muscle cells – used in anaerobic or high intensity exercise – the ATP buffer function of the cycle dominates to ensure high contractile function of the cell. In slow twitch muscle cells – used in aerobic or endurance exercise – the ATP energy transport function of the cycle dominates to ensure duration of the exercise (Brosnan & Brosnan, 2007).

As creatine is ingested and endogenously produced within the body, at the same time approximately 1-2% of intramuscular creatine and phosphocreatine is degraded within the body every day (Kreider et al., 2017). During the creatine/phosphocreatine cycle, there is a spontaneous breakdown of creatine and phosphocreatine into creatinine. This breakdown occurs as the muscle cell begins to degrade naturally, creatinine is released into the blood which is filtered by the kidneys and excreted via urine. Due to this spontaneous breakdown of creatine and phosphocreatine, creatine must be continuously replenished in the body with diet and endogenous synthesis from the kidneys and liver. Based on the rate of creatine degradation, the body must replenish approximately 1-3 grams of creatine to maintain normal creatine levels – this value may vary based on total body mass, sex, and age (Kreider et al., 2017). Unlike β-Alanine supplementation, the skeletal muscle does have a physiological ceiling for creatine and supplemental loading is finite. A proposed physiological mechanism behind the creatine loading ceiling is the downregulation or decrease in the total number of creatine transporters in the skeletal muscle cell membrane or a decrease in the overall function of the creatine transporters (Arazi et al., 2021).

Another major goal for creatine supplementation is enhanced recovery time of the muscle cell – due to muscular degradation. A proposed physiological mechanism that is currently being investigated is that creatine promotes glycogen replenishment within the muscle which enhances glycogen loading and thus decreases muscle fatigue during high intensity exercise (Kreider et al., 2017). Another potential physiological mechanism of creatine supplementation that is being researched is that it creates an enhanced tolerance to heat due to the osmotic

properties of creatine which allow for increased water retention and hydration. This mechanism would be particularly beneficial for athletes who train in extreme heat (Kreider et al., 2017).

Creatine monohydrate has been a popular workout supplement for many years and has proven to be one of the most effective supplements on the market that every demographic could benefit from. There are a variety of ways to supplement with creatine monohydrate but to effectively optimize the creatine loading of the muscle cells, long term creatine monohydrate supplementation is necessary by following a specific dosing schedule: high dose short term loading followed by lower dose maintenance loading. Short term creatine monohydrate supplementation – approximately 5-7 days – has shown to statistically increase the muscle free creatine, phosphocreatine, and total creatine content. When initially beginning to supplement with creatine monohydrate, an individual must saturate their skeletal muscle cells with high doses of creatine monohydrate – approximately 15-20 grams a day for the first 5-7 days (Loon et al., 2003). Studies have shown that after day 5 of high creatine loading, there is a statistical increase in average peak power and total work produced by individuals during short but high intensity duration exercise (Loon et al., 2003). Long term creatine monohydrate supplementation – approximately over 6-10 weeks – has shown to statistically increase muscle free creatine, but phosphocreatine and total creatine content levels returned back to baseline levels. After fully saturating the skeletal muscle cells with creatine monohydrate and effectively hitting the loading ceiling for creatine and phosphocreatine, an individual must continue to take a lower maintenance dose of creatine monohydrate to prevent washout and maximize ergogenic effects of supplementation – approximately 3-5 grams a day for 6-10 weeks (Loon et al., 2003). An individual may require a higher maintenance dose based on total muscle mass and size of the

individual; creatine monohydrate maintenance dosing can vary within 3-10 grams a day. Studies have shown that the enhanced performance during high intensity exercise – seen on day 5 – continues to increase after 6 weeks of creatine loading despite phosphocreatine and total creatine content returning to baseline levels. The proposed mechanism that supports this finding is that the initial increase in muscle free and total creatine levels within the body resulted in an increased phosphocreatine phosphorylation rate that remains in effect even as total creatine levels decrease (Loon et al., 2003). This increased phosphorylation rate increased the capacity to use ATP during exercise which allows for the increased potential to maintain high performance during high intensity exercise.

Creatine and phosphocreatine levels experience a finite loading ceiling within the skeletal muscle cells but by increasing the driving force of creatine transport within the skeletal muscle cells, an individual can optimize creatine absorption. The driving force responsible for creatine transport into the muscle cell is the Na^+/K^+ ATPase sodium gradient. Creatine monohydrate supplements are frequently taken with carbohydrates in order to trigger the insulin pathway within cells and increase the Na^+/K^+ ATPase sodium gradient, effectively increasing creatine monohydrate uptake into the muscle as a secondary mechanism (Arazi et al., 2021). Using the insulin pathway alone, approximately 100 grams of carbohydrates would be needed for a 5-gram creatine monohydrate supplementation – an unrealistic amount to be ingested (Brosnan & Brosnan, 2007). Another physiological pathway to increase the Na^+/K^+ ATPase sodium gradient is through exercise. Exercise is a potent stimulant for creatine monohydrate uptake into skeletal muscles, which helps avoid such a high carbohydrate intake during supplement dosing. Clinical studies have shown that creatine monohydrate supplements on their own increased the total

muscle creatine content by 25% while creatine monohydrate supplements plus regular exercise increased total muscle creatine content by 37% (Brosnan & Brosnan, 2007). Creatine monohydrate supplementation is optimized when ingested with a form of carbohydrates and must be accompanied with an exercise program – with progressive overload – in order to effectively creatine load the muscle cells to produce an increase in strength and gain in fat-free body mass.

Creatine monohydrate is one of the most studied and evidence based ergogenic supplements being used as an exercise performance enhancer by a variety of demographics. There have been numerous clinical and research studies completed on how the physiological mechanisms and pathways of creatine monohydrate function within the body in order to determine the overall benefits it provides as a nutritional supplement. Studies have shown that the effect of creatine monohydrate supplementation has shown noticeable increases in performance for short duration high intensity exercise – approximately 30 seconds or repeated bouts of 30 second exercise – but no real effect on long duration endurance exercises that exceeded 2 minutes (Brosnan & Brosnan, 2007). Various clinical studies have demonstrated that control groups who supplemented with creatine monohydrate during a resistance training program showed a greater gain in fat-free lean body mass, increase in strength in every exercise, hypertrophy of Type II fast twitch fibers, and increase in contractile ability of the skeletal muscle cell when compared to the placebo groups who didn't supplement with creatine monohydrate (Loon et al., 2003). It is important to note that supplementation with creatine monohydrate did not affect the body's total oxidative capacity, no statistical difference in VO_2 max – ventilatory

threshold – during aerobic or endurance exercise, and no change in substrate utilization during submaximal or endurance exercise (Loon et al., 2003).

Based on current studies, supplementation with creatine monohydrate shows no adverse effects or long-term health issues with either short- or long-term supplementation. In the initial years of creatine monohydrate being used as a workout supplement, there were concerns about how creatine loading specifically affected the kidneys. Multiple studies have demonstrated the creatine supplementation does not affect blood plasma creatinine levels, creatinine clearance, urea clearance, or albumin clearance (Davani-Davari et al., 2018). These results support the use of creatine monohydrate with no adverse or long-term effect on the kidneys when creatine monohydrate is supplemented appropriately. As creatine continued to become more popular as a workout supplement, there were numerous clinical studies that studied other health effects of creatine monohydrate supplementation. Multiple clinical studies done over the years have concluded that creatine monohydrate loading of the skeletal muscle does not increase musculoskeletal injuries, dehydration, or is directly involved with any gastrointestinal issues (Kreider et al., 2017). These studies support the use of creatine monohydrate as an effective ergogenic supplement that demonstrates an increase in strength and gain in fat free lean body mass that could be used by any demographic.

Conclusion

Nutritional supplementation has become prominent within the athletic demographic as athletes search for strategies to increase and optimize their exercise performance. In this paper, I examined the most current evidence and studies regarding the ergogenic use of β-Alanine and

creatine monohydrate as workout supplements and their link to improving exercise performance on a physiological scale.

β-Alanine has a potential physiological mechanism as a muscle buffer of H^+ during high intensity exercise. By loading the muscle with β-Alanine, the buffering capacity increases and delays the onset of muscular fatigue in order to increase high intensity exercise performance. β-Alanine as a supplement has substantial clinical evidence that supports this physiological mechanism as a nutritional supplement without having any negative health effects. The most optimal way to load β-Alanine into the muscle is by committing to a long-term supplementation schedule of the appropriate dose based on an individual's body mass. The athletic demographic that would most benefit from supplementing with β-Alanine is elite level athletes who specialize in short high intensity exercise and want to boost their performance on a small marginal scale. Those who are focusing on a weight training program would not benefit as greatly from β-Alanine supplementation and could achieve boosted exercise performance in a different way.

Creatine monohydrate has a potential physiological mechanism of regulating the phosphocreatine cycle which maintains ATP levels with muscle cells. By loading the muscle with creatine monohydrate, the creatine and phosphocreatine levels increase which allows for a greater bioavailability of ATP energy for muscle cell contraction during exercise. There is considerable clinical evidence that supports the use of creatine monohydrate as a nutritional supplement without any negative health effects. The most optimal way to load the muscle with creatine is by long-term supplementation of the appropriate dose based on an individual's body mass. By understanding the physiological pathways and mechanisms of creatine monohydrate supplementation, those who desire gains in fat-free lean body mass and increase in strength will

benefit the most by taking creatine monohydrate – as long as it taken with adequate carbohydrate intake and in conjunction with an exercise program. Those who only desire an increase in strength with minimal increase in body mass will not benefit from taking creatine monohydrate and should achieve their desired goals by using supplemental or dietary protein.

Citations

1. Kendall, K. L., Moon, J. R., Fairman, C. M., Spradley, B. D., Tai, C.-Y., Falcone, P. H., Carson, L. R., Mosman, M. M., Joy, J. M., Kim, M. P., Serrano, E. R., & Esposito, E. N. (2014). Ingesting a preworkout supplement containing caffeine, creatine, β-alanine, amino acids, and B vitamins for 28 Days is both safe and efficacious in recreationally active men. *Nutrition Research*, *34*(5), 442–449. https://doi.org/10.1016/j.nutres.2014.04.003
2. Derave, W., Everaert, I., Beeckman, S., & Baguet, A. (2010). Muscle carnosine metabolism and β-alanine supplementation in relation to exercise and training. *Sports Medicine*, *40*(3), 247–263. https://doi.org/10.2165/11530310-000000000-00000
3. Huerta Ojeda, Á., Tapia Cerda, C., Poblete Salvatierra, M. F., Barahona-Fuentes, G., & Jorquera Aguilera, C. (2020). Effects of beta-alanine supplementation on physical performance in aerobic–anaerobic transition zones: A systematic review and meta-analysis. *Nutrients*, *12*(9), 2490. https://doi.org/10.3390/nu12092490
4. Blancquaert, L., Everaert, I., & Derave, W. (2015). Beta-alanine supplementation, Muscle Carnosine and exercise performance. *Current Opinion in Clinical Nutrition and Metabolic Care*, *18*(1), 63–70. https://doi.org/10.1097/mco.0000000000000127
5. Baguet, A., Koppo, K., Pottier, A., & Derave, W. (2009). B-alanine supplementation reduces acidosis but not oxygen uptake response during high-intensity cycling exercise. *European Journal of Applied Physiology*, *108*(3), 495–503. https://doi.org/10.1007/s00421-009-1225-0
6. Hoffman, J. R., Varanoske, A., & Stout, J. R. (2018). Effects of β-alanine supplementation on carnosine elevation and physiological performance. *Advances in Food and Nutrition Research*, 183–206. https://doi.org/10.1016/bs.afnr.2017.12.003

7. Davani-Davari, D., Karimzadeh, I., Ezzatzaegan-Jahromi, S., & Sagheb, M. M. (2018). Potential Adverse Effects of Creatine Supplement on Kidneys in Athletes and Bodybuilders. *Iranian Journal of Kidney Diseases*, *12*(5), 253–261.
8. Brosnan, J. T., & Brosnan, M. E. (2007). Creatine: Endogenous metabolite, dietary, and therapeutic supplement. *Annual Review of Nutrition*, *27*(1), 241–261. https://doi.org/10.1146/annurev.nutr.27.061406.093621
9. Cribb, Paul J., Williams, Andrew, & Hayes, Alan (2007). A creatine-protein-carbohydrate supplement enhances responses to resistance training. *Medicine & Science in Sports & Exercise*, *39*(11), 1960–1968. https://doi.org/10.1249/mss.0b013e31814fb52a
10. Loon, L. J. C., Oosterlaar, A. M., Hartgens, F., Hesselink, M. K. C., Snow, R. J., & Wagenmakers, A. J. M. (2003). Effects of creatine loading and prolonged creatine supplementation on body composition, fuel selection, sprint and endurance performance in humans. *Clinical Science*, *104*(2), 153–162. https://doi.org/10.1042/cs1040153
11. Kreider, R. B., Kalman, D. S., Antonio, J., Ziegenfuss, T. N., Wildman, R., Collins, R., Candow, D. G., Kleiner, S. M., Almada, A. L., & Lopez, H. L. (2017). International Society of Sports Nutrition Position Stand: Safety and efficacy of creatine supplementation in exercise, sport, and medicine. *Journal of the International Society of Sports Nutrition*, *14*(1). https://doi.org/10.1186/s12970-017-0173-z
12. Arazi, H., Eghbali, E., & Suzuki, K. (2021). Creatine supplementation, physical exercise and oxidative stress markers: A review of the mechanisms and effectiveness. *Nutrients*, *13*(3), 869. https://doi.org/10.3390/nu13030869